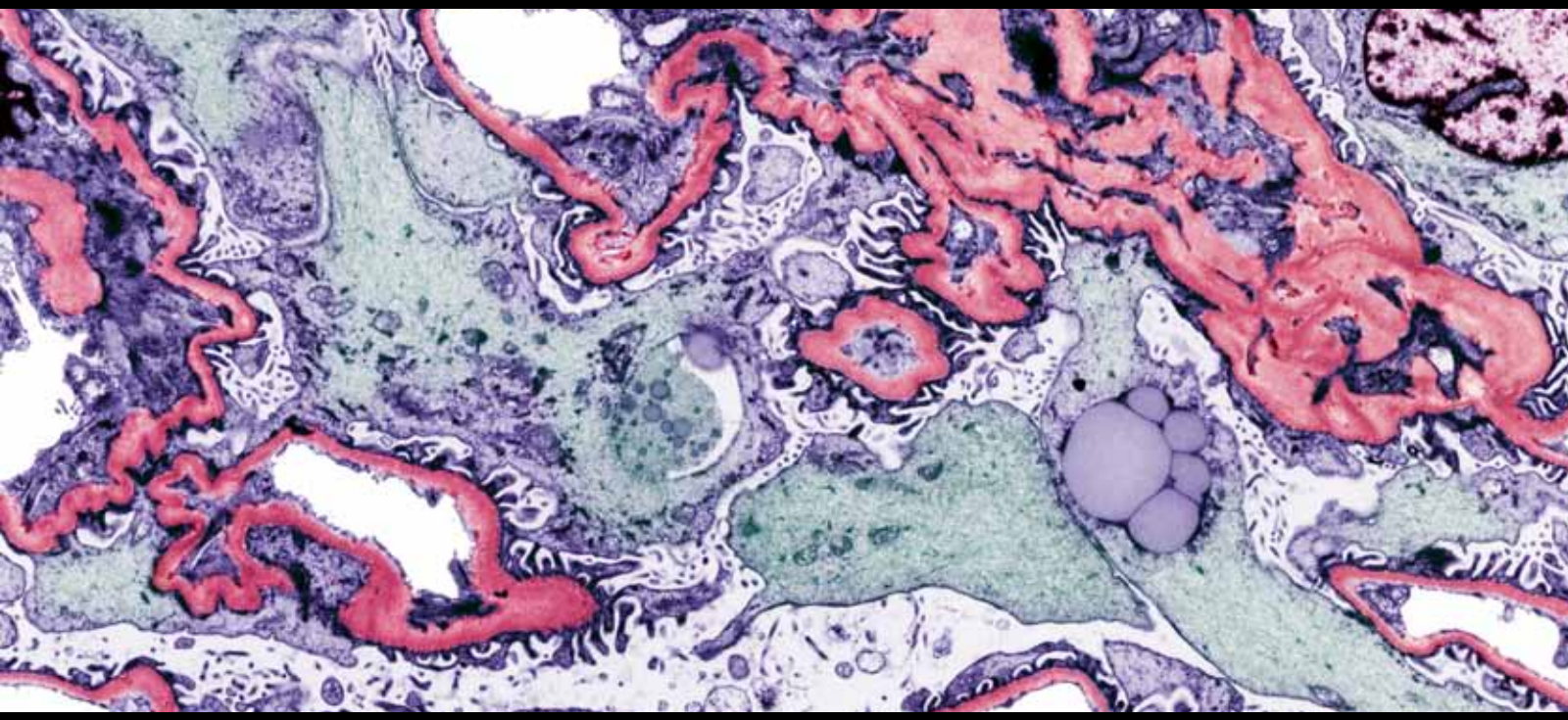
Systemic Lupus Erythematosus

Guest Editors: Hiroshi Okamoto, Ricard Cervera, Tatiana S. Rodriguez-Reyna, Hiroyuki Nishimura, and Taku Yoshio



Systemic Lupus Erythematosus

Systemic Lupus Erythematosus

Guest Editors: Hiroshi Okamoto, Ricard Cervera, Tatiana S. Rodriguez-Reyna, Hiroyuki Nishimura, and Taku Yoshio

Editorial Board

Contents

Systemic Lupus Erythematosus, Hiroshi Okamoto, Ricard Cervera, Tatiana S. Rodriguez-Reyna, Hiroyuki Nishimura, and Taku Yoshio
Volume 2012, Article ID 815753, 2 pages

A Novel Method for Real-Time, Continuous, Fluorescence-Based Analysis of Anti-DNA Abzyme Activity in Systemic Lupus, Michelle F. Cavallo, Anna M. Kats, Ran Chen, James X. Hartmann, and Mirjana Pavlovic
Volume 2012, Article ID 814048, 10 pages

Role of Structure-Based Changes due to Somatic Mutation in Highly Homologous DNA-Binding and DNA-Hydrolyzing Autoantibodies Exemplified by A23P Substitution in the VH Domain, A. V. Kozyr, A. V. Kolesnikov, A. E. Khlyntseva, A. G. Bogun, G. A. Savchenko, I. G. Shemyakin, and A. G. Gabibov
Volume 2012, Article ID 683829, 7 pages

Screening Tests of Reproductive Immunology in Systemic Lupus Erythematosus, Zdenka Ulcova-Gallova, Alice Mockova, and Miroslava Cedikova
Volume 2012, Article ID 812138, 6 pages

Evaluation of ENA-6 Profile by ELISA Immunoassay in Patients with Systemic Lupus Erythematodes, Izeta Aganovic-Musinovic, Jasenko Karamehic, Lamija Zecevic, Faris Gavrankapetanovic, Nesina Avdagic, Asija Zaciragic, Tomislav Jukic, Nerima Grcic, and Suvada Svrakic
Volume 2012, Article ID 321614, 8 pages

Intravenous Immunoglobulin in the Management of Lupus Nephritis, Scott E. Wenderfer and Trisha Thacker
Volume 2012, Article ID 589359, 10 pages

Multiple Autoantibodies Display Association with Lymphopenia, Proteinuria, and Cellular Casts in a Large, Ethnically Diverse SLE Patient Cohort, Rufei Lu, Julie M. Robertson, Benjamin F. Bruner, Joel M. Guthridge, Barbara R. Neas, Swapan K. Nath, Jennifer A. Kelly, Kathy L. Moser Sivils, Eliza F. Chakravarty, Diane L. Kamen, Gary S. Gilkeson, Daniel J. Wallace, Michael H. Weisman, R. Hal Scofield, John B. Harley, and Judith A. James
Volume 2012, Article ID 819634, 11 pages

Neonatal Lupus Erythematosus, Kam Lun Hon and Alexander K. C. Leung
Volume 2012, Article ID 301274, 6 pages

Vascular Disease in Systemic Lupus Erythematosus, Athina Pyrpasopoulou, Sofia Chatzimichailidou, and Spyros Aslanidis
Volume 2012, Article ID 876456, 4 pages

Osteonecrosis in Systemic Lupus Erythematosus: An Early, Frequent, and Not Always Symptomatic Complication, Paola Caramaschi, Domenico Biasi, Ilaria Dal Forno, and Silvano Adami
Volume 2012, Article ID 725249, 7 pages

The Role of Hyaluronan and CD44 in the Pathogenesis of Lupus Nephritis, Susan Yung and Tak Mao Chan
Volume 2012, Article ID 207190, 9 pages

Cutaneous Manifestations of Systemic Lupus Erythematosus, Luís Uva, Diana Miguel, Catarina Pinheiro, João Pedro Freitas, Manuel Marques Gomes, and Paulo Filipe
Volume 2012, Article ID 834291, 15 pages

Identification of TNIP1 Polymorphisms by High Resolution Melting Analysis with Unlabelled Probe: Association with Systemic Lupus Erythematosus, Jie Zhang, Yuewen Chen, Yong Shao, Qi Wu, Ming Guan, Wei Zhang, Jun Wan, and Bo Yu
Volume 2012, Article ID 265823, 5 pages

Ocular Manifestations of Systemic Lupus Erythematosus: A Review of the Literature, Neal V. Palejwala, Harpreet S. Walia, and Steven Yeh
Volume 2012, Article ID 290898, 9 pages

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 815753, 2 pages
doi:10.1155/2012/815753

Editorial

Systemic Lupus Erythematosus

Hiroshi Okamoto,[1] Ricard Cervera,[2] Tatiana S. Rodriguez-Reyna,[3] Hiroyuki Nishimura,[4] and Taku Yoshio[5]

[1] *Minami-Otsuka Clinic, Minami-Otsuka Institute of Technology, 2-41-9 Minami-Otsuka, Toshima-ku, Tokyo 170-0005, Japan*
[2] *Department of Autoimmune Diseases, Hospital Clinic, Calle Villarroel, 170, Barcelona, Catalonia 08036, Spain*
[3] *Department of Immunology and Rheumatology, National Institute of Medical Sciences and Nutrition Salvador Zubirán, Vasco de Quiroga 15, Colonia Sección XVI, 14000 Mexico City, DF, Mexico*
[4] *Faculty of Biomedical Engineering, Toin University of Yokohama, 1614 Kurogane-machi, Aoba-ku, Yokohama 225-8802, Japan*
[5] *Division of Rheumatology and Clinical Immunology, School of Medicine, Jichi Medical University, 3311 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan*

Correspondence should be addressed to Hiroshi Okamoto, hokamoto@parkcity.ne.jp

Received 1 November 2012; Accepted 1 November 2012

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by widespread immunologic abnormalities and multiorgan involvement including the skin, joints, and kidney as well as the peripheral and central nervous systems. The clinical course of SLE is usually dependent on the organ involved such as neuropsychiatric syndromes of systemic lupus erythematosus (NPSLE), lupus nephritis (LN), lupus pancreatitis (LP), and pulmonary hypertension (PH). Early diagnosis and epoch-making therapeutic strategies are prerequisites for improved prognosis of patients with SLE. To fulfill this medical need, a better understanding of the pathogenesis of SLE is required. Research over the course of several years has yielded a basic scientific understanding of SLE pathogenesis. This includes understanding the role of various cytokines and chemokines in disease progression, the chronic activation of plasmacytoid dendritic cells by circulating immune complexes, and the expression of neutrophil-specific genes.

The main focus of this special issue is on the diagnostic tools for organ involvement in SLE and in the development of new therapeutic strategies as well as the basic understanding of SLE pathogenesis. Thankfully, we have several research articles as well as outstanding review articles on this subject. We hope this special issue is an international forum for researchers and clinicians to summarize the most recent developments and ideas in the field.

To understand the pathogenesis of SLE, genetic background, several small molecules, and autoantigens/autoantibodies are extensively studied. In this special issue, we have several articles to introduce the recent topics in this field.

The extracellular matrix (ECM) is not only a structural support that maintains the architecture of tissues and organs, but also plays critical roles during inflammatory processes. Hyaluronan (HA) is a major component of the ECM that can directly regulate inflammatory processes through its interaction with its cell surface receptor CD44. In this special issue there is an outstanding review on the pathogenesis of SLE. S. Yung and T. M. Chan overviewed and discussed the putative mechanisms through which hyaluronan and its cell surface receptor, CD44, contribute to the pathogenesis of SLE, with particular emphasis on lupus nephritis.

Recently, the genetic background of SLE was revealed by a series of genome-wide association studies (GWAS) and several SLE susceptive genes including *MHC*, *BLK*, *ITGAM*, *STAT4*, *IRF5*, *BANK1*, and *ETS1* were identified. The polymorphisms on TNFα-induced protein 3 (TNFAIP3 or A20) interacting protein1 (TNIP1) have been reported to associate with the disease risk of several autoimmune diseases including SLE in Caucasians. J. Zhang et al. investigated the association of TNIP1 with SLE in the Chinese population using high-resolution melting (HRM) analysis. Although they found no association between TNIP1 SNPs with the

disease risk of SLE in the Chinese population, they found a new SNP rs79937737 located on 5 bp upstream of rs7708392.

SLE is characterized by diverse clinical symptoms and autoantibody production against a variety of nuclear and cytoplasmic antigens. The occurrence and prevalence of these autoantibody specificities have been used to characterize the diverse clinical presentations of SLE. The standard screening assay for the detection of autoantibodies, immunodiffusion, and enzyme-linked immunosorbent assays (ELISAs) are often employed. R. Lu et al. introduced newer screening technologies, like the Luminex bead-based assay performed with the Bio-Rad BioPlex 2200, which focus on performing a sensitive multiplex analysis of selecting autoantibody specificities. They studied the association between autoantibody specificities and SLE ACR criteria and subcriteria using conditional logistic regression analysis with sera from European American SLE patients.

I. Aganovic-Musinovic et al. evaluated the concentration values of each antigen of extractable nuclear antigens (ENA)-6 profiles to investigate possible correlation between the concentration of Sm antibodies and circulating immune complexes. Based on calculations from ROC curves, they found that Sm/RNP was clearly a very important marker for diagnosis of SLE.

In lupus nephritis (LN), autoantibodies have been shown to be critical in the initiation and progression of renal injury, via interactions with both Fc-receptors and complement. One approach in the management of patients with LN is the use of intravenous immunoglobulin. This therapy has shown benefit in the setting of many forms of autoantibody-mediated injury; however, the mechanisms of efficacy are not fully understood. S. E. Wenderfer and T. Thacker overview the data supporting the use of intravenous immunoglobulin therapy in LN as well as discuss the potential mechanisms of action.

In this special issue, we have excellent reviews on various manifestations of SLE, such as ocular manifestations, cutaneous manifestations, osteonecrosis as well as vascular diseases, a complication of SLE, and neonatal lupus, a specific form of lupus.

About one-third of patients with SLE have ocular manifestations, such as keratoconjunctivitis sicca, retinal vasculitis, and optic neuritis/neuropathy. Prompt diagnosis and treatment of eye disease are paramount as they are often associated with high levels of systemic inflammation and end-organ damage. N. V. Palejwala et al. clearly reviewed the mechanism, diagnosis, and treatment of ocular manifestations in SLE with a large collection of clinical pictures. They also discuss the use of immunosuppressive agents as well as biologic agents. This review provides a great lesson for all the physicians who treat SLE patients, especially rheumatologists.

The skin is one of the target organs most variably affected and is involved in up to 85% of SLE cases. Cutaneous lesions account for four of these 11 criteria of SLE established by the American College of Rheumatology (ACR). Skin lesions in patients with lupus may be specific or nonspecific. L. Uva et al. clearly reviewed the SLE-specific cutaneous changes: malar rash, discoid rash, photosensitivity, and oral mucosal lesions as well as SLE nonspecific skin manifestations, their pathophysiology, and management. This review includes typical pictures of various types of cutaneous lesions and also provides us a great lesson to learn.

Vascular disease is frequent in patients with SLE and represents the most frequent cause of death in established disease. A. Pyrpasopoulou et al. overviewed the prevalence of the different forms of vasculopathy that can be encountered in a lupus patient, described their pathogenesis, and addressed their impact on disease severity and outcome.

Osteonecrosis may complicate the course of SLE and may contemporaneously affect multiple joints. The major risk factor associated with the development of osteonecrosis is the use of glucocorticoid at high doses. P. Caramaschi et al. discuss the pathogenesis of osteonecrosis in SLE patients, factors associated with the development of osteonecrosis, relationship between osteonecrosis development, and type of corticosteroid use as well as the treatment of osteonecrosis in SLE.

Neonatal lupus erythematosus (NLE) refers to a clinical spectrum of cutaneous, cardiac, and systemic abnormalities observed in newborn infants whose mothers have autoantibodies against Ro/SSA and La/SSB. The condition is rare and usually benign and self-limited but sometimes may be associated with serious sequelae. K. L. Hon and A. K. C. Leung review the pathophysiology, clinical features, and management of infants with this condition. They also provide us the evidence of the prognosis of NLE and also propose a question about the management of future pregnancies of SLE women.

We hope that readers of Autoimmune Diseases will find in this special issue not only attractive data, but also useful reviews on crucial aspects of SLE.

Hiroshi Okamoto
Ricard Cervera
Tatiana S. Rodriguez-Reyna
Hiroyuki Nishimura
Taku Yoshio

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 814048, 10 pages
doi:10.1155/2012/814048

Research Article

A Novel Method for Real-Time, Continuous, Fluorescence-Based Analysis of Anti-DNA Abzyme Activity in Systemic Lupus

Michelle F. Cavallo,[1,2] Anna M. Kats,[1] Ran Chen,[1,3] James X. Hartmann,[1] and Mirjana Pavlovic[1,4]

[1] *Department of Biological Sciences, Florida Atlantic University, Boca Raton, FL 33431, USA*
[2] *Department of Biological Sciences, Florida Atlantic University, Davie, FL 33314, USA*
[3] *Department of Molecular Genetics and Microbiology, University of Texas at Austin, Austin, TX 78712, USA*
[4] *Department of Computer and Electrical Engineering and Computer Science, Florida Atlantic University, Boca Raton, FL 33431, USA*

Correspondence should be addressed to Mirjana Pavlovic, mpavlovi@fau.edu

Received 3 June 2012; Revised 25 September 2012; Accepted 31 October 2012

Academic Editor: Hiroyuki Nishimura

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the production of antibodies against a variety of self-antigens including nucleic acids. These antibodies are cytotoxic, catalytic (hydrolyzing DNA, RNA, and protein), and nephritogenic. Current methods for investigating catalytic activities of natural abzymes produced by individuals suffering from autoimmunity are mostly discontinuous and often employ hazardous reagents. Here we demonstrate the utility of dual-labeled, fluorogenic DNA hydrolysis probes in highly specific, sensitive, continuous, fluorescence-based measurement of DNA hydrolytic activity of anti-ssDNA abzymes purified from the serum of patients suffering from SLE. An assay for the presence and levels of antibodies exhibiting hydrolytic activity could facilitate disease diagnosis, prediction of flares, monitoring of disease state, and response to therapy. The assay may allow indirect identification of additional targets of anti-DNA antibodies and the discovery of molecules that inhibit their activity. Combined, these approaches may provide new insights into molecular mechanisms of lupus pathogenesis.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a chronic, multifactorial, antigen-driven, systemic, autoimmune disease which often presents a broad spectrum of clinical entities. SLE is characterized by the production of an array of inflammation-inducing autoantibodies of IgG and IgM isotypes directed against nuclear antigens, including single-stranded (ss) and double-stranded (ds) DNA. High titers of both antibody classes are involved in disease development and associated with flare [1–4]. Titer of either species allows for differentiation between lupus patients and healthy donors and for monitoring patients in flare and with inactive disease [5–9]. Accordingly, anti-DNA antibody levels in patient sera are used to monitor disease activity and progression [10–12]. However, according to Shoenfeld et al. (1988), although titers vary significantly, anti-DNA antibodies are always detectable in the sera of all healthy mammals [13]. Additionally, methods for quantifying antibody titer can produce greatly varying results from the same serum sample [5] and simply measuring the titer of anti-DNA antibodies does not provide detailed information about antibody functionality or potential pathogenicity. Assaying for antibody hydrolytic activity, in addition to monitoring titer, may allow physicians to better predict changes in disease cycle as well as researchers to illuminate potential roles for abzymes in perpetuation of disease.

Although sophisticated biosensor-based techniques [14] have been developed for simple detection of the presence of a mixture of both anti-ssDNA and anti-dsDNA antibody levels directly from the blood of SLE patients, assay systems for detecting hydrolytic activity of anti-DNA autoantibodies are still mainly at the electrophoretic level. Most published methods for analyzing the hydrolytic activity of anti-DNA

antibody are discontinuous, laborious, and hazardous, often employing radioactivity and/or carcinogenic dyes. For the most part, these assays do not lend themselves to automation nor to study of the reaction kinetics [15–20]. Gololobov et al. [21] have made the only attempt so far at continuous, kinetic analysis of anti-dsDNA antibody hydrolytic activity using the flow linear dichromism technique (FLD).

In contrast, studies of enzymes that hydrolyze DNA (various DNAases) have provided automated, quantitative measurement of DNA hydrolysis. Modern methods for analyzing DNAse activity range from fluorescence-based (intercalating dyes and fluorescently labeled molecular beacon-based technology) to electrospray ionization mass spectrometry [22–25]. Methods for analyzing autoantibody activity—abzymes in lupus patients—should match the level of sensitivity and ease with which DNAse I, the typical standard control, is analyzed. In order to devise an updated method for measuring abzyme activity, we investigated the utility of a dual-labeled, fluorogenic hydrolysis probe as a substrate for continuous measurement of catalytic activity and compared abzyme activity with a DNAse I control.

Anti-dsDNA antibodies are found in 70–90% of SLE patients and are considered the hallmark of lupus disease. However, we have chosen to investigate anti-ssDNA antibodies, which present in 30–70% of patients, based on data indicating that they are hydrolytic, nephritogenic, and may serve as strong predictors of flare [3, 4, 26–28]. The significance of anti-ssDNA antibodies in SLE is further supported by data indicating that they are still present after treatment with immunosuppressive therapy which eliminates anti-dsDNA antibodies, investigations in mouse models of nephritogenic lupus in which only anti-ssDNA antibodies were found, and findings that some anti-dsDNA antibodies are not pathogenic [6, 7, 19, 27, 29, 30].

In summary, our basic premises are (1) anti-ssDNA antibodies produced by normal serum donors and those produced by lupus patients can be differentiated based on whether or not they demonstrate hydrolytic activity; (2) these antibodies may be of clinical significance and could prove useful in facilitating diagnosis of lupus disease; (3) it is possible to use hydrolysis probes to determine whether or not purified antibody can cleave DNA. An assay which makes use of hydrolysis probes affords a less hazardous and less laborious method than those currently in use. Additionally, it provides a more sensitive platform in line with the technologies currently available for analysis of DNA hydrolyzing enzymes.

2. Materials and Methods

2.1. Sample Collection. Sera were separated from fresh, whole blood, drawn intravenously from healthy, normal donors (ND) and Lupus patients (LP) with informed consent and under the guidance of protocols approved by Florida Atlantic University's Institutional Review Board (ref. HO5-278). Serum was harvested by differential centrifugation, aliquoted into 1 mL RNAse/DNAse-free tubes, and stored at −20°C prior to antibody purification.

2.2. Purification of Anti-ssDNA Antibody with Binding Specificity for Poly-(dT) Oligomer. Natural, polyclonal anti-ssDNA antibodies of the IgG isotype with binding specificity for a purchased oligo-(dT) 20-mer target were isolated using a two-step purification method, previously developed in house [7], which allows for efficient isolation of the intact target molecule. Briefly, the method is based on antibody affinity for runs of thymine (T_5) and the ability to isolate total IgG with the use of Protein G coated magnetic dynabeads (Dynal Biotech, now part of Invitrogen, Carlsbad, CA, USA). Substantially less laborious than earlier protocols, this method yields antibody of incomparable purity as confirmed at the nanogram level of sensitivity via SDS-PAGE and silver staining. It is also gentle enough to allow further functional analysis of these proteins, which are prone to denaturation under harsh conditions. Purified antibody was stored at −20°C in storage buffer consisting of 25 mM Tris-HCl and 50% glycerol, pH 7.

2.3. Confirmation of Antibody Purity. Antibody purity was confirmed as described in Pavlovic et al. [7] by nonreducing SDS-PAGE and silver staining using the Pharmacia PhastSystem with PhastGel Gradient 4–15 separation gels, PhastGel buffer strips, and full-range rainbow molecular weight markers (GE Healthcare, Piscataway, NJ; Owner's manual Separation Technique File no. 130).

2.4. Antibody Quantification. Titers of pure samples were determined as described in Pavlovic et al. [7] by Pierce Micro BCA Assay (Thermo Fisher Scientific/Pierce Chemical Co., Rockford, IL, USA) and a modified ELISA kit developed in house. The ELISA uses ssDNA as substrate in the form of oligo-(dT) 20-mer (Eurofins MWG Operon, Huntsville, AL, USA) bound to Streptavidin microplates (Roche Diagnostics, Indianapolis, IN, USA) with a 4-parameter curve fit model for quantification, which is incorporated into the software of the SpectraMax 190 microplate reader used (Molecular Devices, LLC, Sunnyvale, CA, USA). Serial dilutions of purified human anti-DNA antibody were compared with a human IgG standard.

2.5. Antibody Classification. IgG isotype was confirmed as described in Pavlovic et al. [7] by Western Blot, also using the PhastGel system, with HRP-labeled goat anti-human secondary antibody and TMB substrate (PhastSystem Development Technique File no. 220).

Presence of IgG_1, IgG_2, IgG_3, and IgG_4 subclasses was confirmed using a commercial Zymed, human IgG subclass profile ELISA kit (Invitrogen, Carlsbad, CA, USA) [31].

2.6. Probe Design. Probe design was based on sequence preference data obtained by Gololobov et al. [15] and X-ray crystallographic data published by Tanner et al. [32]. According to Gololobov's hydrolysis studies in anti-ssDNA mouse monoclonal antibody BV 04-01, anti-ssDNA antibodies prefer C-C regions in ssDNA [15, 27]. Tanner et al. [32] showed that sequence recognition, binding to target DNA, and coordination of the active site occurs at thymine repetitive sequences in their studies of mouse monoclonal

anti-ssDNA antibodies. Probe design also accounted for AT rich sequence preferences of DNAse I [15, 27, 32]. The following dual-labeled, fluorogenic oligonucleotide (18-mer) probe (Eurofins MWG Operon, Huntsville, AL, USA) was designed for continuous, fluorescence-based analysis of DNA hydrolytic activity of purified anti-ssDNA antibody with binding specificity for an oligo-(dT) 20-mer: 5′-6-FAM-ATATAGCGC$_5$T$_5$-DQ1-3′.

2.7. Confirmation of Antibody Hydrolytic Activity. Prior to continuous, fluorescence-based assay, hydrolytic abilities of anti-ssDNA antibodies purified from SLE patient serum were confirmed by Agilent 2100 Lab-on-Chip digital analysis with a DNA7500 microchip (Agilent Technologies, Santa Clara, CA, USA). Hydrolysis of ss herring sperm DNA (obtained by heating and fast cooling) by anti-ssDNA SLE antibody was compared to hydrolysis by a commercial DNAse I positive control (Applied Biosystems, Austin, TX, USA). Parallel DNAse I and anti-ssDNA antibody reactions were analyzed at 37°C within separate reaction buffers consisting of 25 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.5 and 22 mM Tris-HCl, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.5, respectively, in order to meet requirements of each entity per data published by Odintsova et al. [33]. Enzyme to substrate ratio was 1 : 2 (1 μg of protein = 1 unit of DNAse I) and samples were loaded in volumes of 10 μL.

2.8. Confirmation of DNAse I and Abzyme Ability to Hydrolyze Designed Probe Sequence. Cleavage of the oligo 18-mer hydrolysis probe was first analyzed by UV spectrophotometry (A260) to confirm ability of SLE antibodies and DNAse I control to bind and cleave the probe sequence. SLE antibodies were also compared with antibodies purified from normal healthy donors in this experiment. Lupus antibody or DNAse I was combined with probe in optimized enzyme to substrate ratios of 1 : 0.45 and 1 : 2, respectively, within the same distinct reaction buffers specified in Section 2.7. Total reaction volume was 200 μL with a final probe concentration of 1 μg/mL. DNAse trials were conducted at 37°C. UV spectrometry was performed on antibody samples incubated at 4°C, a temperature at which DNAse I is inactive. A total of four lupus patient serum samples and four normal healthy donor serum samples were analyzed in this preliminary confirmation of hydrolytic ability.

2.9. Real-Time, Continuous, Fluorescence-Based Hydrolysis Assay. Five trials of the anti-ssDNA lupus antibody, normal healthy donor antibody and control DNAse I reactions were run with samples loaded in triplicate within the same distinct reaction buffers as specified in Section 2.7. Black, flat-bottom, polystyrene, 96 well microtiter plates (MTX Lab Systems, Vienna, VA, USA) were used to prevent cross-contamination. Purchased lyophilized hydrolysis probe was reconstituted in 1X reaction buffer. Hydrolysis probe was combined with DNAse I, LP anti-ssDNA antibody, and ND anti-ssDNA antibody to produce final enzyme to substrate ratios of 1 : 2, 1 : 0.45, and 1 : 0.85, respectively, across samples in total reaction mixture volumes of 200 μL with final probe concentrations of 1 μg/mL. Probe hydrolysis was measured using a Finstruments Fluoroskan II plate reader (MTX Lab Systems, Vienna, VA, USA) with a sensitivity range of pmol-μmol and DeltaSoft3 software. Samples were read top to bottom over an 8 hour assay run at 37°C with fluorescence excitation at 485 nm and emission detection at 538 nm (FAM was read as FITC as directed by Finstruments technical support). Kinetic measurements were carried out every 2 minutes over 30 minutes for DNAse I and every 5 minutes over 8 hours for antibody. Results are reported in absolute fluorescent units.

2.10. Statistical Analysis. Triplicate absolute fluorescent data were averaged at each time step. A one-way analysis of variance (ANOVA) was applied to each data set, DNAse I, and anti-ssDNA antibody, against the control (oligo) to test if there was a significant difference ($\alpha = 0.05$) in the occurrence or intensity of fluorescence, a surrogate for hydrolysis. Time steps (5 min and 1 hour, resp.) were treated as repeated measures. Besides both being compared to the oligo control, the fluorescence of the lupus antibody was also compared to the fluorescence of the normal donor antibody with ANOVA.

3. Results

The purity of the isolated anti-DNA antibodies was determined by nonreducing SDS-PAGE and silver staining. Silver staining with sensitivity to the nanogram level of detection showed an apparent single band with a molecular weight matching IgG in lupus patients and two bands of 150 kDa MW in healthy or normal control serum (Figure 1). Western blot analysis confirmed that the proteins present in the bands are IgG isotype (Figure 2), and subclass profiling by ELISA confirmed the presence of four human IgG subclasses in the purified material [7, 31].

Analysis by Agilent 2100 lab-on-chip technology confirmed hydrolytic activity of SLE anti-ssDNA antibody. Results showed hydrolysis of ss herring sperm DNA (500 bp nucleotides) into nucleotides of much smaller MW (25–35–50 bp) by separate addition of both DNAse I and SLE anti-ssDNA antibody to reaction mixtures described in Section 2.7 (Figures 3(a) and 3(b)).

UV spectrophotometry confirmed the ability of DNAse I and hydrolytic antibody from lupus patients to bind and cleave the designed probe sequence. UV spectrophotometry also demonstrated that anti-poly-(dT) specific ssDNA antibody purified from normal serum did not hydrolyze the probe (Figures 4(a) and 4(b)). This preliminary analysis was performed on four lupus patient serum samples (LP 1–4) and four normal donor serum samples (ND 1–4) and produced results consistent with results of analysis by Agilent and of previous studies which have demonstrated that lupus anti-DNA antibodies hydrolyze DNA while normal anti-DNA antibodies do not [6, 15, 17, 20].

Hydrolytic activity of DNAse I and purified anti-DNA antibody was next measured by continuous, fluorescence-based hydrolysis assay (Figures 5 and 6). The DNAse I reaction maxima was reached at 15 minutes and the reaction completed at 30 minutes, (Figure 5) while the slower antibody reaction completed in 8 hours (Figure 6).

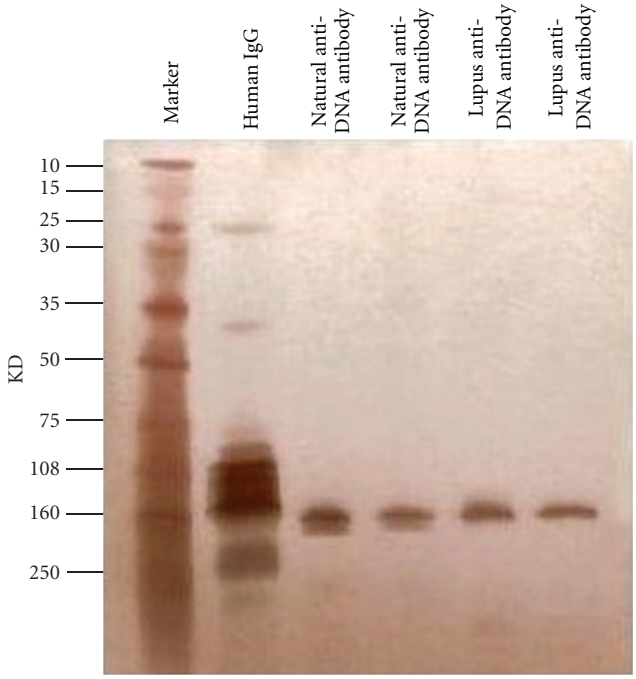


Figure 1: Electrophoretic analysis of the purity of anti-DNA antibody isolated via two-step magnetic bead method. *Purified anti-DNA antibodies from normal healthy serum donors and SLE patients are detected by nonreducing SDS-PAGE and silver staining.

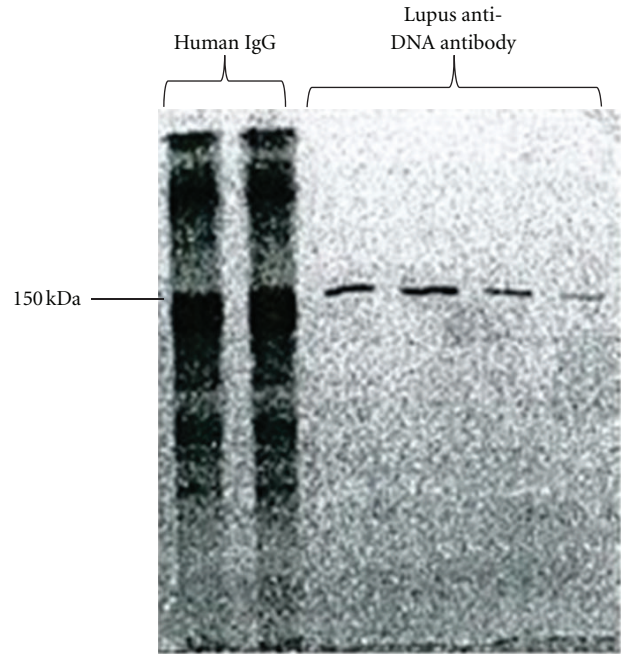


Figure 2: Classification of isolated anti-DNA antibody by western blot. *Western blotting was used to confirm that the proteins isolated using the two-step purification method are IgG.

Hydrolytic activity was maintained in the antibody storage buffer used, 25 mM Tris, 50% Glycerol, pH 7 at −20°C, and, as was seen in analysis by UV spectrophotometry, both DNAse I and lupus antibody hydrolyzed the probe sequence. A distinct difference between activities of anti-ssDNA antibody purified from lupus patient serum versus normal serum is also detected by varying levels of fluorescence output in continuous, fluorescence-based analysis of hydrolysis (Figure 6).

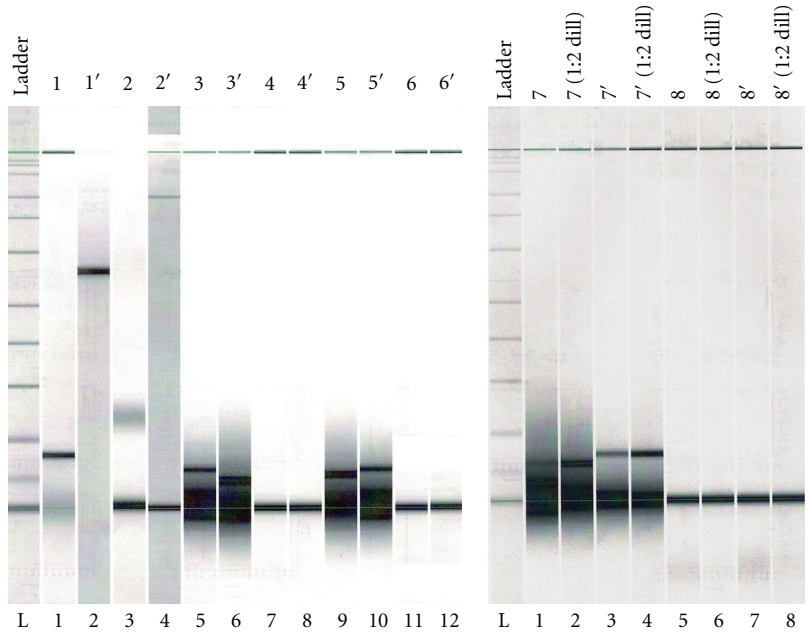
Blank buffer controls, which did not contain probe, did not fluoresce. As visualized in Figures 5 and 6, a baseline level of background fluorescence due to incomplete quenching was observed in control samples containing probe without enzyme. Additionally, an increase in fluorescence above the background produced by uncleaved probe is seen in samples containing antibody purified from normal donor serum. However, as the levels of fluorescence in these normal samples remained constant over time, we assume that natural fluorescence of the protein has contributed to this increase in overall fluorescent output. Despite this, samples containing probe hydrolyzed by DNAse I and SLE antibody show a substantial increase in total fluorescence output (mean = 1.93 ± 0.25) above baseline levels produced by probe alone (mean = 0.78 ± 0.04) or by samples containing probe and antibody purified from normal healthy donors (mean = 1.07).

This semiquantitative measurement indicates that DNAse I, at its optimal ratio, displays much faster activity than antibody (reaching a peak of 3.721 units at 30 min. versus 2.277 units at 180 min. for antibody) (Figure 7). Kinetic parameters have not been measured at this time since the main goal of this initial work was to establish the methodology; however, this apparent difference in timing of the reactions and their peaks indicates that they are two different entities. The differences seen between the DNAse I and lupus anti-ssDNA reaction profiles align with differences in reaction mixtures (requirements for coordinators), reaction duration (30 min. versus 8 hrs.), and temperature (4°C versus 37°C) (Table 1).

4. Discussion

The use of a dual-labeled, fluorogenic hydrolysis probe as a substrate for measuring anti-DNA antibody hydrolytic activity was inspired by the principles of Fluorescence Resonance Energy Transfer (FRET) and their application in real-time PCR [34]. There are a number of parameters that can affect the assay. Molecular bleaching of the labeled substrate can occur over the long reaction period between antibody and DNA, and this is a disadvantage of the methodology [31]. Despite this, the use of hydrolysis probes was deemed preferable to the use of intercalating dyes which are carcinogenic and could negatively affect the binding affinity for and hydrolysis of DNA by antibody.

Tanner et al. [32] demonstrated the specific binding of anti-DNA antibodies to thymine polymers via arginine



(a) DNAse I (b) Lupus anti-ssDNA antibody

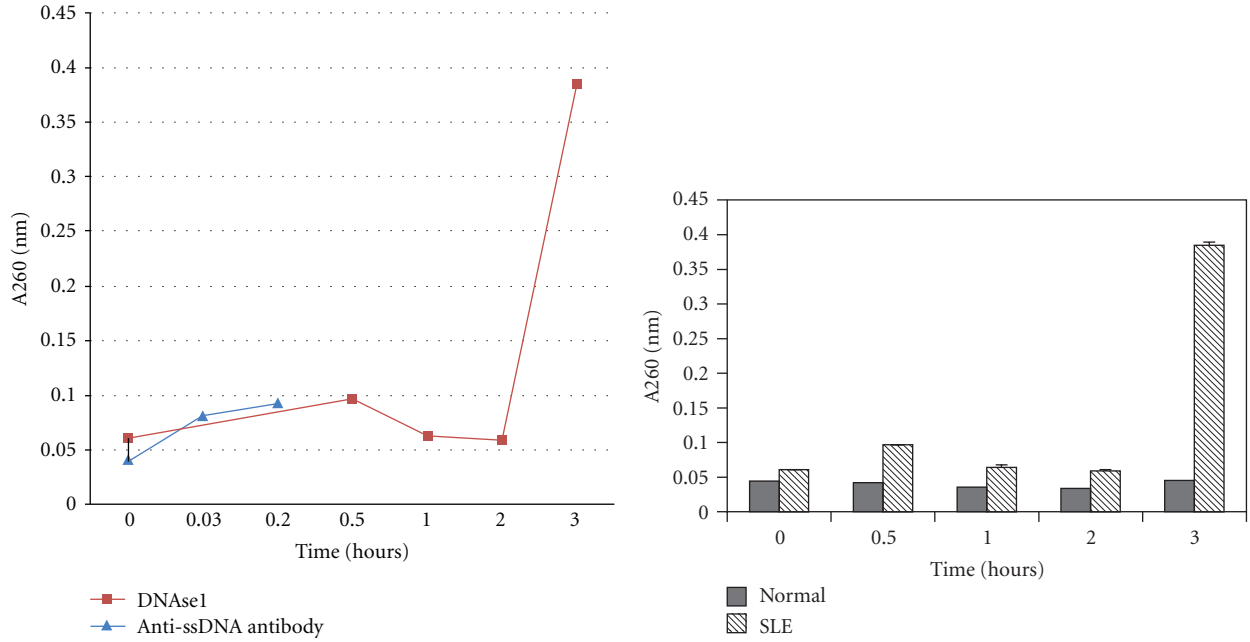
Figure 3: Detection of hydrolysis of ss herring sperm DNA by Agilent 2100 Bioanalyzer (DNA7500 microchip) digital analysis. *Agilent 2100 Lab-on-Chip digital analysis comparison of hydrolysis of ss herring sperm DNA (obtained by heating and fast cooling) by DNAse I control and anti-poly-(dT) ssDNA antibody sample purified from lupus patient serum demonstrates that lupus anti-poly-(dT) ssDNA antibody is hydrolytic. (a) Lane 1: ss herring sperm DNA with DNAse I at T = 0. Lane 2: ss herring sperm DNA, no DNAse I. Lane 3: ss herring sperm DNA with DNAse I at T = 5 min. Lane 4: lambda phage, no DNAse I. Lanes 5, 6: ss herring sperm DNA with DNAse I at T = 30 min. Lane 7: lambda phage with DNAse I at T = 30 min. Lanes 8: lambda phage, no DNAse I. Lanes 9, 10: same as 5, 6. Lanes 11, 12: DNAse I, no substrate. (b) Lanes 1–4: ss herring sperm DNA with lupus anti-ssDNA antibody at T = 3 hrs. Lanes 5–8: lambda phage DNA, no antibody.

Table 1: Summarized comparison of DNAse I (from bovine pancreas, Ambion) and lupus anti-ssDNA antibody hydrolysis reactions.

Parameter/condition	DNAse I	Lupus anti-ssDNA antibody
pH	7.5	7.5
Active temperature range	+22–54°C	+4–37°C
Requirement for Ca^{2+}	—	0.5 mM
Requirement for Mg^{2+}	10 mM	5 mM
Time to reaction completion	30–40 min	8 hrs

groups using X-ray analysis. The arginine groups are responsible for sequence recognition, and the antibodies bind the DNA at the thymine repetitive sequences (5-mer) via tyrosine side chains within a hydrophobic pocket created by tyrosine and tryptophan within the antibody binding site [15, 27, 30]. While binding affinity of antibody for the probe sequence could have been enhanced due to the presence of the FAM and DQ1 labels, our initial goal was to determine whether presence or absence of hydrolytic capability could be distinguished using this methodology. If binding affinity was enhanced, the labels should have affected both lupus and normal samples; however, we still see a difference in hydrolytic abilities of these entities when comparing their activities against labeled probe. Additionally, since our probe was 18 bases in length (in order to meet requirements for quenching), it would only bind to a single antibody reactive site [19], thus allowing for the monitoring of individual hydrolytic events and targeted analysis of specific sequences. The FLD method, which is the only other continuous method for analyzing DNA hydrolytic antibody activity currently published, requires the use of longer polymers [21].

Our lab previously published a novel, two-step method for purification of natural, polyclonal, anti-ssDNA antibodies of the IgG isotype specific for a purchased oligo-(dT) 20-mer target [7]. The method is based on anti-ssDNA antibody affinity for runs of thymine (T_5) as demonstrated by Tanner et al. [32] and the ability to isolate total IgG with the use of



(a) DNAse I and lupus anti-ssDNA antibody

(b) Lupus and normal anti-ssDNA antibody

Figure 4: Discontinuous measurement of hydrolysis of oligo 18-mer by DNAse I, lupus patient, and normal donor anti-ssDNA antibody by UV spectrophotometry. *5′-6-FAM-ATATAGCGC$_5$T$_5$-DQ1-3′ 18-mer hydrolysis probe was incubated with DNAse I or anti-ssDNA antibody purified from lupus patient or normal healthy donor serum. Analysis by UV spectrophotometry demonstrates a change in A260 reading after 3 hours indicating hydrolysis by lupus anti-poly-(dT) ssDNA antibody. Normal healthy donor-derived anti-poly-(dT) ssDNA antibody does not display hydrolytic activity. No change was seen in replicates containing buffer only and those containing substrate only throughout the trials. Endpoint analysis via one-way ANOVA comparing hydrolytic activity revealed a significant difference between activity of antibody purified from lupus patients and normal donors ($F = 5465.06$, $P < 0.0001$). (a) Determination of ability of DNAse I and lupus anti-ssDNA antibody to hydrolyze probe by UV spectrophotometry. The DNAse reaction peaks after approximately 15 minutes and comes to completion in 30 minutes. Activity starts at 0.03 hour, peaks at 0.2 hours, and subsides at 0.5 hours and is much faster than DNA hydrolysis by anti-ssDNA antibody isolated from SLE patient serum. (b) Comparison of ability of purified lupus and normal anti-ssDNA antibodies to hydrolyze probe by UV spectrophotometry. Anti-ssDNA antibodies isolated from normal donors did not demonstrate hydrolytic activity; however, anti-ssDNA antibodies isolated from SLE patients show cleavage ability during the 3 hour incubation time point. These results were consistent across all samples analyzed. *$P < 0.01$ for normal individuals versus SLE patients.

Protein G coated magnetic dynabeads. Streptavidin-coated dynabeads, are incubated with biotynilated oligo-(dT) 20-mer, washed, and then incubated with serum during the first purification step. The beads are washed multiple times prior to elution of bound material. During the second purification step, the eluate is then incubated with protein G beads prior to washing and elution.

This method yielded highly pure antibody as confirmed at the nanogram level of sensitivity via SDS-PAGE and silver staining and was shown to be of IgG isotype as is demonstrated in our previous publication. We believe it unlikely that a nonantibody protein with binding specificity for the poly-(dT) 20-mer would also have been targeted by protein G and show hydrolytic activity against an alternate sequence previously shown to be a hydrolytic target of anti-ssDNA antibodies [15]. For these reasons, we deemed it unnecessary to incorporate into the purification procedure further treatment to destroy potential contaminating enzymes (e.g., "acid shock" [21]) which could also alter antibody structure and consequently functionality. Our method is gentle, quite rapid, and easy to perform in comparison to previous methods. While the yield is low in comparison to purification methods like those used by Gololobov et al. [21], the yield can be increased by increasing the number of beads used; however, the hydrolysis assay that we have developed can be performed with substantially less-purified antibody than was used in the analysis by FLD method [21]. The levels of potential DNA substrate in our assay ranged from 0 to 1000 ng/mL. This range encompasses that found in blood from healthy patients where DNA and oligonucleotide content is very low (in the range of 10–40 ng/mL), and the range within lupus patients that display levels close to 400 ng/mL [33].

The hydrolytic activity of highly purified anti-ssDNA antibodies from lupus patients and the detection of catalytic IgG in other autoimmune diseases, such as Hashimoto's thyroiditis and multiple sclerosis, implicate these antibodies in autoimmune disease pathogenesis [31, 33, 35]. Lupus

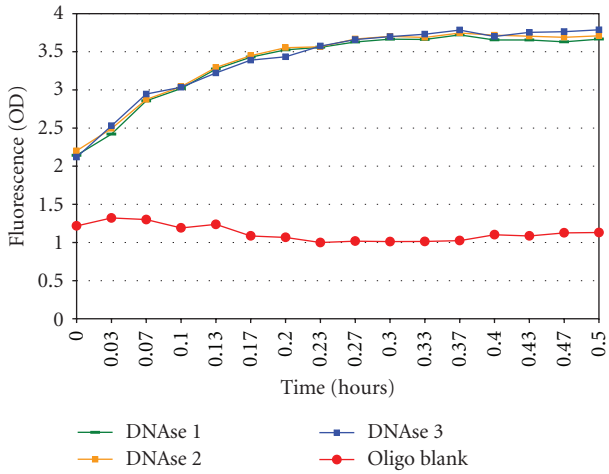


Figure 5: Continuous measurement of oligo 18-mer hydrolysis by DNAse I by fluorescence assay. *Analysis of DNAse I positive control via continuous, fluorescence-based assay on Fluoroskan II (Finstruments) demonstrates that 5′-6-FAM-ATATAGCGC$_5$T$_5$-DQ1-3′ 18-mer hydrolysis probe is cleaved by DNAse I and that adequate quenching to allow differentiation between cut and uncut probe was achieved. Oligo blank contained all reaction components except for DNAse I (25 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.5, and 1 μg/mL 5′-6-FAM-ATATAGCGC$_5$T$_5$-DQ1-3′ 18-mer hydrolysis probe). Results were consistent across all trials. DNAse I trials 1, 2, and 3 are displayed as representative trials. Pooled fluorescence data showed statistical significant difference from oligo blank ($F = 29.78$, $P < 0.0001$).

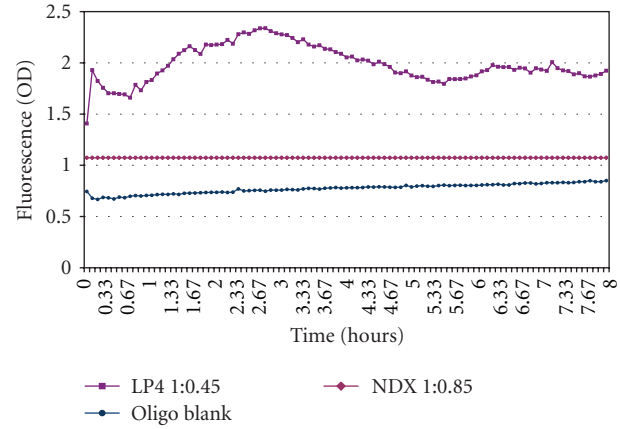


Figure 6: Continuous measurement of oligo 18-mer hydrolysis by lupus patient (LP) versus normal healthy donor (ND) by fluorescence assay. *Comparison of lupus patient-derived anti-poly-(dT) ssDNA antibody (LP4) and normal healthy donor-derived anti-poly-(dT) ssDNA antibody (NDX) via continuous fluorescence-based assay on Fluoroskan II (Finstruments) demonstrates that 5′-6-FAM-ATATAGCGC$_5$T$_5$-DQ1-3′ 18-mer hydrolysis probe is cleaved by lupus patient-derived antibody but not by normal donor-derived antibody. Oligo blank contained all reaction components except for antibody (25 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.5, and 1 μg/mL 5′-6-FAM-ATATAGCGC$_5$T$_5$-DQ1-3′ 18-mer hydrolysis probe). ANOVA indicates all three fluorescence patterns of LP4, NDX, and the oligo control are significantly different from each other at $P < 0.0001$.

anti-DNA antibodies demonstrate the ability to penetrate living cells via receptors such as Myosin I, to enter the nucleus, and to trigger cell death in vitro. Hydrolytic anti-DNA antibody could potentially maintain and perpetuate disease by DNA hydrolysis in the nuclei of penetrated cells [36–42]. Future analyses should include cytotoxicity studies and fluorescence-aided tracking of purified, natural hydrolytic antibodies obtained and investigated by the herein described methods.

The methodologies developed within our lab provide a platform for investigation of potential prognostic roles of hydrolytic anti-ssDNA antibodies in flare. Additionally, assaying antibody hydrolytic activity could assist in disease diagnosis and facilitate monitoring of disease state/cycle and responses to therapy. The assay for hydrolysis also allows for indirect identification of pathogenic targets of anti-DNA antibodies and inhibitors of anti-DNA antibody activity. With modifications to the two-step purification procedure and the hydrolysis probe sequence, these analyses can be adapted for investigation of dsDNA antibodies (which show preference for C-G regions [27]), antibody activity against short viral sequences of potential interest in lupus (e.g., Parvovirus B19, HSV, and EBV gene sequences [6, 43]), or any user-defined sequence, as well as comparison of antibody isotypes and subclasses associated with lupus disease. Combined, these methods are less harsh, less hazardous, more rapid, and equally or more specific than existing methods for purification and analysis of DNA hydrolytic antibodies.

Herein we have demonstrated that hydrolysis probes can be used for nonhazardous, continuous measurement of DNA hydrolytic activity intrinsic to a purified population of anti-ssDNA antibodies produced by SLE patients. We have also shown that, when analyzed by this method, these SLE antibodies are distinguishable from DNAse I based on reaction requirements, speed and duration, and distinguishable from anti-ssDNA antibodies produced by normal donors based on presence versus absence of hydrolytic activity. Further work will include kinetic analyses using the probe methodology and quantification of kinetic parameters as has been done for anti-dsDNA lupus patient antibody by Gololobov et al. [21].

Abbreviations

Ab: Antibody
6-FAM: 6-carboxyfluorescein
DQ: Dark quencher
ds: Double-stranded
EBV: Epstein-Barr virus
ELISA: Enzyme-linked immunosorbent assay
FRET: Fluorescence resonance energy transfer
HSV: Herpes simplex virus

Time (Minutes)	DNAse I (ratio 1 : 2)	Anti-ssDNA antibody (ratio 1 : 0.45)
0	2.154	1.408
10	3.422	1.822
20	3.693	1.703
30	3.721	1.696
60	—	1.832
120	—	2.178
180	—	2.277
240	—	2.053

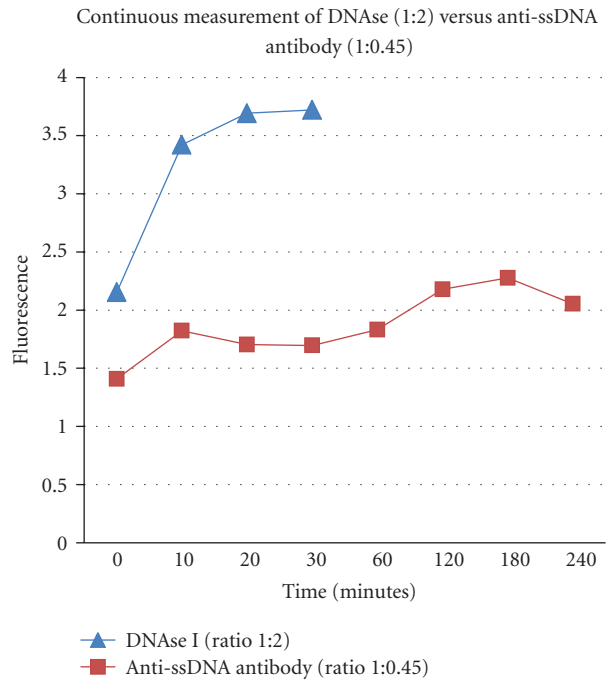


Figure 7: Differences in kinetic behavior of DNAse I and anti-ssDNA lupus antibody in optimized ratios used for detection of ss-DNA hydrolytic activity and expressed in fluorescent units per minutes.

LP: Lupus patient
ND: Normal donor
ss: Single-stranded
SLE: Systemic lupus erythematosus.

Disclosure

The authors of this paper do not have a direct financial relationship with any commercial identity mentioned in this paper. Florida Atlantic University filed US provisional patent application 60/659, 882 (Real time fluorescence assay for anti-DNA hydrolytic activity) for this work on 3/10/2005. The provisional expired on 3/10/2006 and was incorporated into a new US provisional patent application, 61/445, 160 (Compositions, methods and kits for analyzing hydrolytic antibodies and for detecting, predicting, and treating hydrolytic antibody-related diseases), filed on 2/22/2011, which has also since expired. The university and working group are no longer pursuing patents on these methods.

Acknowledgments

This research was supported by funding provided by private donors. Patient samples were provided by Dr. Ira Pardo with Rheumatology Associates of South Florida in Boca Raton, FL, USA. The authors would like express their sincere appreciation to Sheryl R. van der Heiden for her assistance with statistical analyses and editing.

References

[1] D. S. Pisetsky, "Immune response to DNA in systemic lupus erythematosus," *The Israel Medical Association Journal*, vol. 3, no. 11, pp. 850–853, 2001.

[2] J. B. Harley and J. A. James, "Epstein-Barr virus infection induces lupus autoimmunity," *Bulletin of the NYU Hospital for Joint Diseases*, vol. 64, no. 1-2, pp. 45–50, 2006.

[3] S. Aotsuka and R. Yokohari, "A kit for the simultaneous estimation of IgG-class antibodies to double-stranded and single-stranded DNA for clinical purposes," *Ryumachi*, vol. 28, no. 2, pp. 96–101, 1988 (Japanese).

[4] M. Teodorescu, "Clinical value of anti-ssDNA (denatured DNA) autoantibody test: beauty is in the eyes of the beholder," *Clinical and Applied Immunology Reviews*, vol. 2, no. 2, pp. 115–128, 2002.

[5] D. A. Isenberg, C. Dudeney, and W. Williams, "Measurement of anti-DNA antibodies: a reappraisal using five different methods," *Annals of the Rheumatic Diseases*, vol. 46, no. 6, pp. 448–456, 1987.

[6] G. A. Nevinsky and V. N. Buneva, "Natural catalytic antibodies in norm, autoimmune, viral, and bacterial diseases," *The Scientific World Journal*, vol. 10, pp. 1203–1233, 2010.

[7] M. Pavlovic, R. Chen, A. M. Kats et al., "Highly specific novel method for isolation and purification of lupus anti-DNA antibody via oligo-(dT) magnetic beads," *Annals of the New York Academy of Sciences*, vol. 1108, pp. 203–217, 2007.

[8] H. Krippner, S. Merle, and K. Pirlet, "Enzyme immunoassay for IgG and IgM antibodies against dsDNA and ssDNA," *Zeitschrift fur Rheumatologie*, vol. 42, no. 5, pp. 256–260, 1983.

[9] M. Napirei, H. Karsunky, B. Zevnik, H. Stephan, H. G. Mannherz, and T. Möröy, "Features of systemic lupus erythematosus in Dnase1-deficient mice," *Nature Genetics*, vol. 25, no. 2, pp. 177–181, 2000.

[10] D. Alarcón-Segovia, "The predestination of autoantibodies," *Current Rheumatology Reports*, vol. 3, no. 3, pp. 173–174, 2001.

[11] M. P. Madaio and K. Yanase, "Cellular penetration and nuclear localization of anti-DNA antibodies: mechanisms, consequences, implications and applications," *Journal of Autoimmunity*, vol. 11, no. 5, pp. 535–538, 1998.

[12] D. Portales-Pérez, D. Alarcón-Segovia, L. Llorente et al., "Penetrating anti-DNA monoclonal antibodies induce activation of human peripheral blood mononuclear cells," *Journal of Autoimmunity*, vol. 11, no. 5, pp. 563–571, 1998.

[13] Y. Shoenfeld, O. Ben-Yehuda, Y. Messinger et al., "Autoimmune diseases other than lupus share common anti-DNA idiotypes," *Immunology Letters*, vol. 17, no. 3, pp. 285–290, 1988.

[14] F. Ricci, G. Adornetto, D. Moscone, K. W. Plaxco, and G. Palleschi, "Quantitative, reagentless, single-step electrochemical detection of anti-DNA antibodies directly in blood serum," *Chemical Communications*, vol. 46, no. 10, pp. 1742–1744, 2010.

[15] G. V. Gololobov, C. A. Rumbley, J. N. Rumbley et al., "DNA hydrolysis by monoclonal anti-ssDNA autoantibody BV 04-01: origins of catalytic activity," *Molecular Immunology*, vol. 34, no. 15, pp. 1083–1093, 1997.

[16] B. Mouratou, S. Rouyre, and J. L. Guesdon, "A method for the detection and screening of catalytic anti-DNA antibodies," *Journal of Immunological Methods*, vol. 269, no. 1-2, pp. 147–155, 2002.

[17] N. A. Ponomarenko, E. S. Aleksandrova, I. I. Vorobiev et al., "Natural antibody catalytic activities in mice with autoimmune disorders," *Doklady Biochemistry*, vol. 375, no. 1–6, pp. 224–227, 2000.

[18] A. M. Shuster, G. V. Gololobov, O. A. Kvashuk, A. E. Bogomolova, I. V. Smirnov, and A. G. Gabibov, "DNA hydrolyzing autoantibodies," *Science*, vol. 256, no. 5057, pp. 665–667, 1992.

[19] P. C. Swanson, C. Ackroyd, and G. D. Glick, "Ligand recognition by anti-DNA autoantibodies. Affinity, specificity, and mode of binding," *Biochemistry*, vol. 35, no. 5, pp. 1624–1633, 1996.

[20] G. A. Nevinsky, A. A. Breusov, A. G. Baranovskii et al., "Effect of different drugs on the level of DNA-hydrolyzing polyclonal IgG antibodies in sera of patients with hashimoto's thyroiditis and nontoxic nodal goiter," *Medical Science Monitor*, vol. 7, no. 2, pp. 201–211, 2001.

[21] G. V. Gololobov, E. A. Chernova, D. V. Schourov, I. V. Smirnov, I. A. Kudelina, and A. G. Gabibov, "Cleavage of supercoiled plasmid DNA by autoantibody Fab fragment: application of the flow linear dichroism technique," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 1, pp. 254–257, 1995.

[22] S. J. Choi and F. C. Szoka, "Fluorometric determination of deoxyribonuclease I activity with PicoGreen," *Analytical Biochemistry*, vol. 281, no. 1, pp. 95–97, 2000.

[23] J. J. Li, R. Geyer, and W. Tan, "Using molecular beacons as a sensitive fluorescence assay for enzymatic cleavage of single-stranded DNA," *Nucleic Acids Research*, vol. 28, no. 11 article e52, 2000.

[24] G. Tolun and R. S. Myers, "A real-time DNase assay (ReDA) based on PicoGreen fluorescence," *Nucleic Acids Research*, vol. 31, no. 18, article e111, 2003.

[25] R. H. van den Heuvel, S. Gato, C. Versluis, P. Gerbaux, C. Kleanthous, and A. J. Heck, "Real-time monitoring of enzymatic DNA hydrolysis by electrospray ionization mass spectrometry," *Nucleic Acids Research*, vol. 33, no. 10 article e96, 2005.

[26] D. S. Pisetsky and I. A. Vrabie, "Antibodies to DNA: infection or genetics?" *Lupus*, vol. 18, no. 13, pp. 1176–1180, 2009.

[27] L. S. Rodkey, G. Gololobov, C. A. Rumbley et al., "DNA hydrolysis by monoclonal autoantibody BV 04-01," *Applied Biochemistry and Biotechnology*, vol. 83, no. 1–3, pp. 95–105, 2000.

[28] A. M. Cline and M. Z. Radic, "Apoptosis, subcellular particles, and autoimmunity," *Clinical Immunology*, vol. 112, no. 2, pp. 175–182, 2004.

[29] L. Spatz, A. Iliev, V. Saenko et al., "Studies on the structure, regulation, and pathogenic potential of anti-dsDNA antibodies," *Methods*, vol. 11, no. 1, pp. 70–78, 1997.

[30] W. Yumura, S. Suganuma, K. Nitta, Y. Sano, K. Uchida, and H. Nihei, "Prolonged membranous lupus nephritis with change of anti-ssDNA antibody titer and repeated renal relapse," *Clinical and Experimental Nephrology*, vol. 8, no. 4, pp. 363–368, 2004.

[31] A. M. Kats, *Purification and analysis of autoimmune antibody reactive with DNA [M.S. thesis]*, Florida Atlantic University, 2008.

[32] J. J. Tanner, A. A. Komissarov, and S. L. Deutscher, "Crystal structure of an antigen-binding fragment bound to single-stranded DNA," *Journal of Molecular Biology*, vol. 314, no. 4, pp. 807–822, 2001.

[33] E. S. Odintsova, M. A. Kharitonova, A. G. Baranovskiĭ, L. P. Siziakina, V. N. Buneva, and G. A. Nevinskiĭ, "DNA-hydrolyzing IgG antibodies from the blood of patients with acquired immune deficiency syndrome," *Journal of Molecular Biology*, vol. 40, no. 5, pp. 857–864, 2006.

[34] M. Arya, I. S. Shergill, M. Williamson, L. Gommersall, N. Arya, and H. R. H. Patel, "Basic principles of real-time quantitative PCR," *Expert Review of Molecular Diagnostics*, vol. 5, no. 2, pp. 209–219, 2005.

[35] T. A. Nevzorova, D. A. Temnikov, and V. G. Vinter, "Special features of the DNA-hydrolyzing activity of the antibodies in systemic lupus erythematosus," *Biochemistry*, vol. 68, no. 12, pp. 1300–1306, 2003.

[36] M. Pavlovic, M. Cavallo, A. Kats et al., "From Pauling's abzyme concept to the new era of hydrolytic anti-DNA autoatnibodies: a link to the rational vaccine design? A review," *International Journal of Bioinformatics Research and Applications*, vol. 7, no. 3, pp. 220–238, 2011.

[37] M. H. Foster, T. Kieber-Emmons, M. Ohliger, and M. P. Madaio, "Molecular and structural analysis of nuclear localizing anti-DNA lupus antibodies," *Immunologic Research*, vol. 13, no. 2-3, pp. 186–206, 1994.

[38] A. G. Gabibov, A. V. Kozyr, and A. V. Kolesnikov, "Disease association and cytotoxic effects of DNA-hydrolyzing autoantibodies," *Chemical Immunology*, vol. 77, pp. 130–156, 2000.

[39] A. V. Kozyr, A. V. Kolesnikov, E. S. Aleksandrova et al., "Novel functional activities of anti-DNA autoantibodies from sera of patients with lymphoproliferative and autoimmune diseases," *Applied Biochemistry and Biotechnology*, vol. 75, no. 1, pp. 45–61, 1998.

[40] A. V. Kozyr, A. V. Kolesnikov, N. A. Zelenova et al., "Autoantibodies to nuclear antigens: correlation between cytotoxicity and DNA-hydrolyzing activity," *Applied Biochemistry and Biotechnology*, vol. 83, no. 1–3, pp. 255–268, 2000.

[41] A. V. Kozyr, L. P. Sashchenko, A. V. Kolesnikov et al., "Anti-DNA autoantibodies reveal toxicity to tumor cell lines," *Immunology Letters*, vol. 80, no. 1, pp. 41–47, 2002.

[42] K. Yanase, R. M. Smith, A. Puccetti, L. Jarett, and M. P. Madaio, "Receptor-mediated cellular entry of nuclear localizing anti-DNA antibodies via myosin 1," *Journal of Clinical Investigation*, vol. 100, no. 1, pp. 25–31, 1997.

[43] M. Pavlovic, A. Kats, M. Cavallo, and Y. Shoenfeld, "Clinical and molecular evidence for association of SLE with parvovirus B19," *Lupus*, vol. 19, no. 7, pp. 783–792, 2010.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 683829, 7 pages
doi:10.1155/2012/683829

Research Article

Role of Structure-Based Changes due to Somatic Mutation in Highly Homologous DNA-Binding and DNA-Hydrolyzing Autoantibodies Exemplified by A23P Substitution in the VH Domain

A. V. Kozyr,[1] A. V. Kolesnikov,[1,2] A. E. Khlyntseva,[1] A. G. Bogun,[1] G. A. Savchenko,[1] I. G. Shemyakin,[1] and A. G. Gabibov[3]

[1] *State Research Center for Applied Microbiology and Biotechnology, Serpuhov District Obolensk 142279, Russia*
[2] *Institute of Immunological Engineering, Lyubuchany 142380, Russia*
[3] *Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho-Maklaya Street 16/10, Moscow 117997, Russia*

Correspondence should be addressed to A. V. Kozyr, fenya_rys@mail.ru

Received 4 June 2012; Accepted 27 September 2012

Academic Editor: Hiroyuki Nishimura

Anti-DNA autoantibodies are responsible for tissue injury in lupus. A subset of DNA-specific antibodies capable of DNA cleavage can be even more harmful after entering the living cells by destroying nuclear DNA. Origins of anti-DNA autoantibodies are not fully understood, and the mechanism of induction of DNA-cleaving activity remains speculative. The autoantibody BV04-01 derived from lupus-prone mouse is the only DNA-hydrolyzing immunoglobulin with known 3D structure. Identification and analysis of antibodies homologous to BV04-01 may help to understand molecular bases and origins of DNA-cleaving activity of autoantibodies. BLAST search identified murine anti-DNA autoantibody MRL-4 with sequences of variable region genes highly homologous to those of autoantibody BV04-01. Despite significant homology to BV04-01, not only MRL-4 had no DNA-cleaving activity, but also reversion of its unusual P23 mutation to the germline alanine resulted in a dramatic loss of affinity to DNA. Contrary to this effect, transfer of the P23 mutation to the BV04-01 has resulted in a significant drop in DNA binding and almost complete loss of catalytic activity. In the present paper we analyzed the properties of two homologous autoantibodies and mutants thereof and discussed the implications of unusual somatic mutations for the development of autoantibodies with DNA-binding and DNA-hydrolyzing activity.

1. Introduction

Anti-DNA autoantibodies are known as important factors of tissue injury in autoimmune diseases, such as SLE [1]. Immune complexes containing anti-DNA antibodies form tissue deposits primarily in kidney that cause apoptotic cell death and severe tissue injury [2, 3].

Nucleic acid cleaving antibodies represent a subset of autoantibodies, capable of single-stranded and double-stranded DNA and RNA hydrolysis [4, 5]. Anti-DNA autoantibodies penetrate cellular membrane and localize to the nuclei of living cells [6]. Furthermore, certain DNA-cleaving antibodies may also penetrate living cells causing apoptosis in caspase-dependent manner, presumably by introducing nicks in nuclear DNA [7]. Cell death due to entry of DNA-hydrolyzing antibodies can contribute to tissue injury observed in autoimmune diseases.

Despite decades of research, complete picture of development of DNA-binding and DNA-hydrolyzing activities by autoantibodies is lacking. Acquisition of affinity and specificity of autoantibodies to DNA is believed to proceed via antigen-driven selection on the background of impaired

censoring mechanisms, which are normally deleting or silencing autoreactive B-cell clones at the healthy state [1–3]. A long-standing paradigm, which holds that nucleosomes represent the primary (auto)antigen-inducing anti-DNA antibodies, is questioned by the observations that foreign DNA-protein complexes can also serve as efficient antigens for induction of anti-DNA antibodies that cause lupus-like nephritis with proteinuria and glomerular IgG deposits [8, 9]. Other mechanisms such as molecular mimicry may also participate in the induction of anti-DNA autoantibodies [10]. At present, it is not known, if impairment of immune system censorship normally deleting autoreactive clones is required for maturation of high-affinity anti-DNA antibodies. Antibodies against foreign DNA-protein complexes as well as antibodies with DNA specificity induced by other hitherto unknown mechanisms can contribute to the development of autoimmune pathologies.

While studying murine DNA-cleaving autoantibody BV04-01 [11, 12], we identified its close homolog anti-DNA antibody MRL-4 [13]. Heavy chain of MRL-4 contained an unusual somatic mutation and replacement of germline Ala-23 by a proline. In this study, we demonstrated that a single mutation not being involved in direct interaction with the antigen can cause profound effect on the binding and catalytic properties of DNA-specific autoantibody.

2. Materials and Methods

2.1. Chemicals, Materials, Bacterial Strains, and Vector DNA. Unless stated otherwise, chemicals were purchased from Sigma. Bacterial growth media and media supplements were from VWR Scientific (BD Difco). The pET-22b(+) vector and *Escherichia coli* strains *BL21* and *BL21*(*DE3*) were obtained from Novagen. *DH12S E. coli* was from Invitrogen, as well as pBluescript SK(−) plasmid DNA. Oligonucleotides were prepared by Syntol and Evrogen. All solutions used in this study were made using 18 MΩ ultrapure water from a Millipore synthesis station and sterilized by autoclaving.

2.2. Cloning, Expression, and Purification of Single-Chain Antibody Fragments. The pET22b(+) vector was modified by PCR-guided mutagenesis to replace GCG (coding for Ala-20 in the pelB signal peptide) by the synonymous GCC with simultaneous disruption of the original Nco I cite. This permits cloning of single-chain antibody constructs with 5′-appended Nco I site and 3′ terminal pelB codons so that processing of pelB by periplasmic secretion system liberates native mature terminus of the antibody light chain. In addition, sequence encoding c-myc peptide (EQKLISEEDL) flanked by 5′-Xho II and 3′-Sal I recognition sites, was inserted in-frame with the downstream His6 tag into the Xho I site.

Single chain antibody SCA04-01, containing variable regions of BV04-01 heavy and light chain genes, was prepared earlier [12]. It was PCR-amplified using forward VKF (5′-CATTCCATGGCCGATGTTGTGATGACCCAA) and reverse VHR (5′-TTACTCGAGTGCAGAGACAGTGACCAGAGT) primers to introduce upstream Nco I and downstream Xho I cloning sites (underlined portions of primer sequences). Resulting PCR product was digested by Nco I and Xho I, and ligated with the modified Nco I-Xho I digested pET22b(+). cDNAs encoding VH and VL of DNA-binding antibody MRL-4 were amplified from corresponding RNA, isolated from hybridoma cells (kind provision of Dr. A. Theofilopoulos) using TriZol reagent. Primers for VH amplification were MRL1F (5′-AAGAGCTCTGAGGGTAAAGGCGAGGTGCAGCTTGTTGAGACT) as forward primer and VHR as reverse primer.

Amplified cDNA coding for the VL was then modified by appending upstream Nco I and downstream Spe I site with part of the scFv linker sequence, using forward VKF and reverse KLR (5′-AACACTAGTACCAGATTTTATTTCCAGCTTGGTCCCCGA) primers. VH cDNA was amplified using MRLF (5′-TCAACTAGTAGCGGCTCTGGTAAGAGCTCTGAGGGTAAAGGCGAGGTGCAGCTTGTTGAGACT) forward and VHR reverse primers to append downstream Xho I site and upstream Spe I site together with the rest of the linker sequence (GSTSGSGKSSEGKG) [14]. Amplified DNAs corresponding to VH and VL chains of BV04-01 and MRL-4 were digested by corresponding restriction enzymes and cloned into the modified pET22b(+) digested by Nco I and Xho I by 3-fragment ligation using Rapid Ligation DNA kit (Roche).

Light chain of antibody BV04-01 was prepared by joining Vκ derived from SCA04-01 by PCR with VKF and KRR (5′-ATACTCGAGTTATTCAAGCTTGGTCCCCGAATACGGAACA) primers, and Cκ, amplified from the mouse RNA (see above) using CκF (5′-TCGGGGACCAAGCTTGAAATAAAA) and CκR (5′-TCTCTCGAGACACTCATTCCTGTTGAAGCT) primers. Vκ fragment was cloned into modified pET22b(+) vector between Nco I and Xho I sites, and Cκ was next cloned downstream to Vκ using PCR-introduced Hind III site and Xho I site.

DH12S cells were transformed by constructs encoding single-chain antibodies using electroporation, and correct clones were identified by colony PCR and DNA sequencing. Plasmids encoding BV04-01 and MRL-4 scFvs were isolated and used to transform BL21(DE3) cells. Transformants, plated at a density sufficient to form a lawn to the 2xYT medium, contained 1% agar, 1% glucose, and 100 μg/mL ampicillin, were grown overnight, washed out from Petri dishes with 2xYT medium/0.5% glucose, and used to inoculate 4 liters of 2xYT medium, containing 0.1% glucose and 100 μg/mL ampicillin. Cells were grown at 37°C until OD600 reached 0.9, then chilled to 20°C on ice, and IPTG was added to the culture to reach final concentration of 0.2 mM. Protein production continued for additional 10 hours at 20°C with vigorous shaking. Isolation of periplasmic scFvs was performed following earlier described protocol [15]. After growth completion, the culture was chilled on ice for 20 min and pelleted by 15 minutes of centrifugation at 3000 ×g at 4°C. The pellet was washed with the buffer containing 200 mM Tris-HCl, 20% sorbitol, and 1 mM EDTA, pH 8.0, resuspended in 50 mL of the same buffer and incubated on ice for 1 hour with occasional stirring. Cell debris was removed by centrifugation at 30000 ×g for

40 min. Supernatant was extensively dialyzed against the chelating buffer (100 mM NaCl, 50 mM Tris–HCl, and pH 8.0). Protein and DNA purification was conducted using FPLC AKTA system (GE Healthcare) with corresponding software and accessories. Clarified periplasmic fraction was loaded on the column, which has been prepacked with 10 mL of Talon chelating resin (Clontech) and equilibrated by the same buffer. After washing the column, scFvs were eluted with 250 mM imidazole, pH 8.0, diluted 10 times by ion exchange buffer (10 mM Tris-HCl, 10 mM NaCl, and pH 8.0), and loaded on MonoQ column (GE Healthcare). Elution was performed employing 0 to 0.5 M NaCl gradient. Purified samples of recombinant ScFv were analyzed by Laemmli SDS-PAGE and validated by Western blotting with monoclonal antibodies against c-myc peptide (Novus Biologicals).

2.2.1. Site-Directed Mutagenesis. It was performed using QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent) according to the manufacturer's protocol.

2.3. DNA Cleavage Assay. Pure supercoiled form of pBluescript DNA was prepared as described earlier [4]. Various concentrations of purified recombinant scFvs were incubated with 1 μg of plasmid DNA in presence of 1 mM $MgCl_2$ for 12 hours at 37°C. Reaction products were resolved in 1% agarose gel and visualized by ethidium bromide staining. Percent of plasmid conversion from supercoiled into circular and linear form was calculated based on the results of densitometry. Acquisition of agarose gel images and subsequent densitometry measurements was done using GelDoc 2000 gel documentation system (Bio-Rad).

2.4. DNA Enzyme-Linked Immunosorbent Assay. Double-stranded DNA fragment of 254 nucleotides was PCR-amplified from genomic DNA of *Fusarium avenaceum* employing 5′-biotinylated primers (5′Bio-CGAACCATC-GAGAAGTTC and 5′Bio-CCAGTGGTTAGTGACTGC), synthesized by Syntol. PCR products were treated by phenol-chloroform mixture, pelleted by three volumes of ethanol with subsequent centrifugation (10 minutes at 10000 g) and dissolved in buffer A containing 20 мM Tris-HCl pH 7.5 and 100 mM NaCl. Purification of the DNA fragment was conducted using gel filtration column Superdex G75 (flow rate 0.5 mL/min, sample volume 0.2 mL).

Purified PCR fragment was diluted in buffer A and immobilized on HBC NeutrAvidin Strips (Thermo Scientific) in amount of 1 μg of DNA per well. Unbound DNA was removed by three rounds of washing with buffer A supplemented with 0.02% Tween-20. The surface of the wells was blocked with Superblock Blocking buffer (Thermo Scientific). Recombinant scFvs were applied to the wells in series of concentrations ranging from 0.005 to 5 μg of antibody per well in the same buffer, incubated for 1 hour at room temperature, and washed three times with buffer A. Bound single chain antibodies were hybridized to anti-c-myc monoclonal antibody (Novus Biologicals), which, subsequently to above-described washing procedure, was detected by anti-mouse Fc-fragment peroxidase conjugate and soluble TMB substrate (Thermo Scientific). Reaction was stopped by addition of 10% H_2SO_4 and absorbance was measured at 405 nm. Spectrophotometric measurements were done employing Varioskan Flash multimodal plate spectrophotometer (Thermo).

2.5. SPR Measurements. Surface plasmon resonance assay was conducted employing ProteOn XPR 36 analyzer (Bio-Rad). Measurements were carried out in buffer, containing 20 mM Na_2HPO_4/NaH_2PO_4, 137 mM NaCl, 0.01% Tween 20, and 100 mg/L bovine serum albumin. Biotinylated DNA fragment, obtained as described in the previous section, was immobilized on the surface of NLS ProteOn sensor chip. Interaction of single chain antibodies with DNA was determined at flow rate 75 μL/min. Experimental data was processed using ProteOn Manager Software, and calculation of kinetic parameters was performed based on Langmuir model of protein adsorption.

3. Results

In attempt to explore the incidence of DNA-hydrolyzing antibodies among already characterized DNA-specific autoantibodies, we ran BLAST search using sequences encoding VL and VH domains of BV04-01.

BLAST search of homologs of catalytic light chain of antibody BV04-01 revealed relative abundance of identical or highly homologous light chains among sequenced antibodies. At the same time, antibodies contained such L-chains were largely specific to antigens other than nucleic acids. Judging by ELISA data, isolated recombinant L-chain of BV04-01 did not display any DNA-hydrolyzing activity.

Only few antibodies with L-chains highly homologous to that of BV04-01 had specificity to DNA and were listed as autoantibodies. Surprisingly, one of these anti-DNA autoantibodies—MRL-4 [13]—contained VH domain that differed from VH of BV04-01 by only few residues.

We therefore sought to perform comparative analysis of MRL-4 and BV04-01 with respect to DNA-binding and DNA-cleaving activities in order to obtain new information regarding potential origin of catalytic activity in autoimmune anti-DNA antibodies.

Genes encoding MRL-4 VH and VL were cloned from corresponding hybridoma cell line and used to construct scFv as it was done for BV04-01 [14]. Recombinant MRL-4 scFv produced in *E. coli* displayed no DNA-hydrolyzing activity using supercoiled plasmid DNA as substrate. Incubation of MRL-4 scFv with various oligonucleotides did not result in detectable DNA-hydrolyzing activity. At the same time, replacement of BV04-01 VL by MRL-4 VL in single chain antibody BV04-01 yielded in antibody with DNA-hydrolyzing activity indistinguishable from that of BV04-01 scFv (87% and 83% of plasmid cleavage, resp. according to densitometric data).

As it has been inferred from the 3D structure of the complex of BV04-01 Fab fragment with (dT3) [11], and later supported by molecular simulation of the predicted antibody

active site and metal-binding pocket [12], formation of the antibody-DNA complex bends DNA, presumably activating one of the phosphodiester bonds. Subsequent hydrolysis and product release necessary for catalytic turnover require conformational changes in the ligand-antibody complex. From previous studies, and in line with hypothesis on antigen-driven nature of anti-DNA autoantibodies [1–3], it is known that some elements of the heavy chain including CDRH3 directly contact DNA in the antibody combining site [11]. At the same time, little is known about structural determinants of conformational dynamics in DNA-hydrolyzing antibodies. Catalysis implies that the enzyme would have been capable of turnover, in other words, for repeated conformational changes accompanying substrate binding, transition state formation, and product release. Therefore, we attempted to identify the antibody elements whose variation can affect conformational rearrangements of the antibody-antigen complex. To do this, we conducted site-directed mutagenesis at selected sites of MRL-4 and BV04-01 antibody chains, which might affect conformation changes in these antibodies, and screened DNA-binding capacity of the resulting mutants by DNA ELISA. Alignment of primary structure for single-chain antibodies BV04-01, MRL-4, and their mutants obtained is presented in Figure 1.

An unusual somatic mutation, L4P (Kabat numbering) is present in the BV04-01 heavy chain. Reversion of this mutation to the germline gene sequence, as well as replacement of proline by alanine, did not affect binding and hydrolysis of DNA by BV04-01 ScFv. Another unusual mutation found in MRL4 is replacement of germline Ala-23 by Pro. Unlike the L4P reversion in BV04-01, reversion of the P23 in the MRL-4 scFv to germline sequence resulted in significant loss of binding capacity of the antibody to DNA. We then asked if acquisition of P23 by BV04-01 VH fragment can cause any changes in its DNA reactivity and analyzed DNA-binding and DNA hydrolyzing activity of A23P mutant of BV04-01 ScFv. Unexpectedly, not only DNA cleaving activity was abolished, but also DNA binding was dramatically decreased due to the A23P mutation in BV04-01 VH, judging by ELISA data. Results of ELISA are presented in Figure 2. Hydrolysis of supercoiled DNA substrate by mutants is shown in Figure 3.

Taking into account significant loss of DNA binding due to mutation effect of Pro to Ala at VH23 of MRL-4, we next analyzed reversion of Pro to the germline Ala in the MRL-4 VH. DNA binding of MRL-4 scFv containing P23A in the VH domain dropped to the level comparable to that of the SCA04-01 VH A23P. To provide accurate quantitative data on changes in DNA-binding capacity of mutant and wild-type variants of MRL-4 scFv and SCA04-01, we analyzed these antibodies using surface plasmon resonance (SPR) technique. SPR data indicated that the biggest decrease in DNA binding efficiency was found in the SCA04-01 VH A23P mutant. Replacement of alanine to proline resulted in approximately 2.5 orders of magnitude drop in K_d and loss of DNA-hydrolyzing activity to the level undetectable by supercoiled DNA cleavage assay. Reversion of P23 in MRL-4 VH to the germline Ala had less pronounced effect, resulting in 20-fold loss of K_d (Table 1).

4. Discussion

Proline is a unique amino acid that can affect the conformation of a protein domain or even an entire protein. Proline switches play significant role in regulation of enzymatic activity in certain synthetic or regulatory pathways [16]. A protein inactive in certain proline conformation is activated by the conformation switch catalyzed by prolyl cis-trans isomerase. There was no data regarding proline switches in antibodies, until Feige et al. discovered proline switch dependent control of CH1 domain folding induced by CH1-CL assembly [17]. Proline-dependent conformation of CDR loops plays important role in shaping of the antigen-binding site.

Kappa-chain cis-proline conformation in the L95 position helps to maintain canonical structure of CDRL3 loop, which is quite stable despite high degree of variability of other amino acid residues in this loop. Other canonical CDR loops in antibodies can also contain Pro residues [18]. Moreover, in the absence of proline, CDR sequence can adopt non-X-proline cis-peptide conformation required for stabilization of the antibody combining site [19].

It has been noted that the percentage of proline residues increases by 42% in mice and 50% in human antibodies during affinity maturation [20]. Known proline usage changes are mainly confined to turns and kinked regions, providing overall structure stability increase, while reducing energy needed for the turn stabilization [19]. In anti-DNA autoantibodies, this type of proline mutation (T45P) can be found for example, in the heavy chain of 33.C9 monoclonal antibody [21], where it occurs in the turn region between CDRH1 and CDRH2.

Our data suggests that praline residues occurring via somatic mutation process can play an important role in acquisition and modulation of anti-DNA antibody binding and catalytic activity. In MRL-4, P23 is acquired by somatic mutation resulting in 20-fold increase in the autoantibody affinity to DNA comparing to the antibody variant containing reversion to the germline. Mutation of A23P in BV04-01 heavy chain results in 60-fold reduction of DNA binding and completely abolishes catalytic activity. The 23rd position in the heavy chain is immediately downstream to the invariant Cys residue forming the intrachain disulfide bond. Changes in the flexibility of peptide bond rotation at such position during heavy chain folding may affect final conformation of CDRH1 and potentially cause other long-distance effects on the Fv structure.

Discovery of the naturally occurring mutation that can change binding or catalytic properties of anti-DNA autoantibody by affecting the FV structure rather than specific antibody-antigen contacts raises important questions regarding maturation process leading to development of anti-DNA autoantibodies. Available data shows that DNA-binding activity is gradually acquired by antibodies through somatic hypermutation and antigen-driven selection [1–3, 21]. Replaced amino acids, such as positively charged arginines, are responsible for increasing antibody affinity by forming direct contacts with DNA [22]. Whether

```
Light chain
                  <-----------FR1---------><---CDR1--><-------FR2-----><CDR2>
BV04-01           DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVS
MRL-4                 I
MRL4VHP23A            I
BV04-01VHA23P         T

                  <-----------FR3--------------------><-CDR3--><-FR4-->
BV04-01           NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHVPLTFGAGTKLE
MRL-4                                                 Y   S
MRL4VHP23A                                            Y   S
BV04-01VHA23P                                         L   A

Heavy chain
                  <-----------FR1---------><-CDR1-><--------FR2----><---CDR2->
BV04-01           EVQPVETGGGLVQPKGSLKLSCAASGFSFNTNAMNWVRQAPGKGLEWVARIRSKSNNYAT
MRL-4               L                P
MRL4VHP23A          L                A
BV04-01VHA23P       P                P

                  <-----------------FR3----------------><--CDR3-----><--FR4--->
BV04-01           YYADSVKDRFTISRDDSQNMLYLQMNNLKTEDTAMYYCVRDQTG-TAWFAYWGQGTLVTVS
MRL-4                          S                    AANWS
MRL4VHP23A                     S                    AANWS
BV04-01VHA23P                  N                    QTG-T
```

Figure 1: Amino acid sequences alignment of the light and heavy chain variable regions of the antibodies BV04-01, MRL-4, and their mutants.

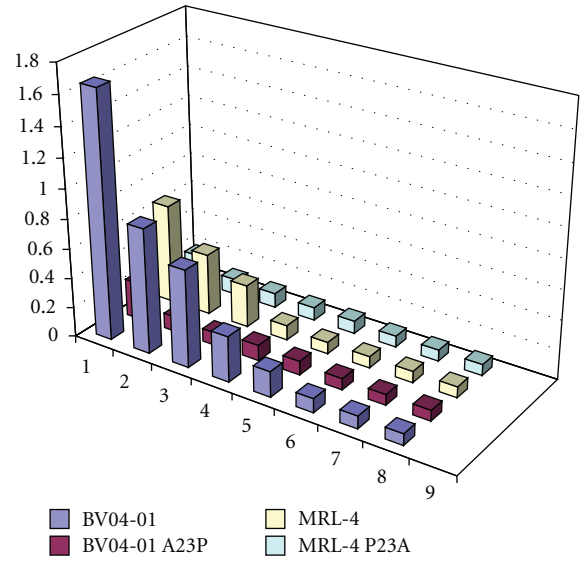


Figure 2: DNA binding by BV04-01 and MRL-4 single-chain antibodies and their mutants assayed by ELISA. Vertical axis represents absorbance measured at 405 nm. Horizontal axis displays amounts of recombinant antibodies per ELISA well: 1: 5 μg, 2: 1 μg, 3: 0.5 μg, 4: 0.1 μg, 5: 0.05 μg, 6: 0.01 μg, 7: 0.005 μg, 8 buffer without antibody. Experimental error for determined absorbance values was calculated within a series of 5-independent ELISA experiments and did not exceed 5% of medium absorbance value.

antigens represent DNA-protein complexes, DNA mimics, or molecules irrelevant to nucleic acids remains to be determined [2, 10]. At the same time, in a number of cases, no correlation between antigen-driven selection and DNA-binding is observed, for example, when an anti-DNA autoantibody reverted to its germline displays no alterations in DNA binding [23].

At present, the physiological significance of P23 mutation in formation of pathogenic anti-DNA autoantibodies cannot be determined due to limited available data. For example, analysis of the literature revealed somatic mutation T24P in the heavy chain of murine cross-reactive anti-pneumococcal/anti-dsDNA autoantibody [24]. Despite extensive analysis of the role of somatic mutations in this

Table 1: Affinity constants of BV04-01 and MRL-4 ScFvs and their mutants determined by SPR.

Wild type and mutant ScFv	ds DNA ELISA*	ds DNA SPR, M[1]**
BV04-01	100 ± 1.21	$(1.8 \pm 0.31) \times 10^{-9}$
BV04-01 A23P	1.2 ± 0.02	$(2.9 \pm 0.67) \times 10^{-7}$
MRL-4	100 ± 1.52	$(1.7 \pm 0.18) \times 10^{-8}$
MRL-4 P23A	4.6 ± 0.61	$(8.5 \pm 0.84) \times 10^{-6}$

* Binding of wild type and mutant single chain antibodies in solid-phase ELISA with double-stranded DNA. Results for mutants are expressed in relative units by using wild types as standards, which were set to 100 relative units.
** Binding kinetics was analyzed by SPR with double-stranded DNA fragments as ligands and antibodies as analytes and calculated K_d values were presented.
[1] Binding of wild type and mutant single chain antibodies to double-stranded DNA.

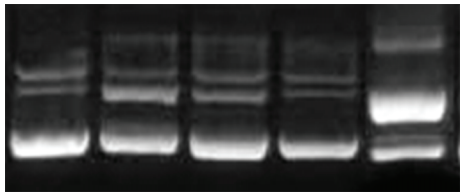


Figure 3: DNA hydrolysis by single-chain antibodies BV04-01, MRL-4 and their mutants. Supercoiled plasmid substrate was incubated overnight with 0.1 mkg of different ScFv in buffer, containing 20 mM Tris-HCl pH 7.5, 100 mM NaCl, and 1 mM $MgCl_2$. Lane 1: control plasmid without an antibody; lane 2: plasmid with ScFv mutant BV04-01 VH A23P; lane 3: plasmid with ScFv mutant MRL-4 VH P23A; lane 4: plasmid with ScFv MRL-4; lane 5: plasmid with ScFv BV04-01.

antibody in DNA binding affinity and pathogenicity, the role T24P mutation was not studied. Earlier, it has been hypothesized that unusual somatic mutations found in lupus autoantibodies, including those occurring in FRs, do not emerge due to antigen-specific activation, but rather reflect lack of negative selection of B cells acquiring autoreactivity through somatic mutation. This can lead to enhanced survival of B-cells with mutations in antibody genes uncoupled from hot spot targeting [25]. With mutations permissible by the impaired clonal deletion mechanism, the route to the high-affinity anti-DNA autoantibody can be very short, as MRL-4 P23A germline revertant displays very low level of binding to DNA.

Why P23 mutation/reversion has such an opposing effect in otherwise structurally similar antibodies? Depending on the P23 isomerization, the structure of CDRH1 of MRL-4 can differ significantly from that of the BV04-01, thus potentially affecting overall conformation of the antigen-binding site of MRL-4. The latter can be significantly more rigid comparing to that of BV04-01, due to restriction imposed by the presence of P23. Bulky W105 residue in the CDRH3 of MRL-4, lacking in BV04-01, may account for increase in rigidity of antibody-DNA complex, either interacting with DNA via stacking mechanism, or by forming hydrophobic contacts with the light chain. Replacement of P23 by alanine in MRL-4 can introduce flexibility in the entire antigen-antibody complex, increasing motion of certain amino acid residues including, for example, W105, and thus weakening antibody-DNA, or VH-VL interaction, or both.

On the contrary, catalytic activity of BV04-01 necessitates the antibody-DNA complex to maintain substantial degree of flexibility, as catalytic reaction implies initial binding of a substrate to an enzyme, conformational rearrangement required for lowering the reaction energy barrier, and subsequent release of reaction product(s). One can speculate, that introduction of proline at the base of CDR1 can reduce its flexibility, by "freezing" it in certain conformation, thus not only disrupting the contact of His31 with DNA [11], but also impeding overall conformational changes required for successful catalysis of phosphodiester bond cleavage. Interestingly, none of the other DNA-hydrolyzing antibodies with known sequences [26, 27] contain Pro or other potentially conformation-restricting mutations at this or adjacent positions. Comparative analysis between 3D structures of MRL-4, BV04-01, and VH23 mutants thereof can help to understand the mechanism of influence of P23 mutation on DNA-binding and DNA-hydrolyzing activity.

Extensive search for anti-DNA autoantibodies containing this or similar type of mutations and their analysis would help to answer whereas the observed phenomenon is an isolated case or an important route to maturation of DNA-binding and DNA-hydrolyzing autoantibodies contributing to pathogenesis in systemic autoimmune disorders.

5. Conclusions

Acquisition of high affinity to DNA in autoantibodies is known to proceed via somatic hypermutation and is believed to be antigen-driven. Corresponding mutated residues are frequently involved in direct contacts with DNA. In this paper, we describe conformation-dependent modulation of DNA-binding capacity of homologous autoantibodies, whereby mutation changing the rigidity of the DNA-binding (MRL-4) antibody Fv or its portion results in significant increase in antibody affinity to DNA. In DNA-hydrolyzing antibody (BV04-01) the same mutation, introduced artificially, decreased binding affinity and abolished catalytic activity.

Difference observed in the mutation effect hints on the importance of conformation flexibility for the antigen-binding site of a catalytic antibody. Thus, pathways of acquisition of DNA binding can differ in structurally similar antibodies, and this difference may reflect specific conditions

under which antibodies become catalytic. Knowledge of such conditions can help to develop catalytic antibodies tailored to target specific epitopes of the antigen of choice. Further structural and statistical data is required to estimate the role of this phenomenon in maturation of anti-DNA autoantibodies.

Acknowledgment

The authors express their gratitude to Professor A. Theofilopoulos for kind provision with hybridoma strain MRL-4.

References

[1] O. P. Rekvig and J. C. Nossent, "Anti-double-stranded DNA antibodies, nucleosomes, and systemic lupus erythematosus: a time for new paradigms?" *Arthritis and Rheumatism*, vol. 48, no. 2, pp. 300–312, 2003.

[2] J. van der Vlag and J. H. M. Berden, "Lupus nephritis: role of antinucleosome autoantibodies," *Seminars in Nephrology*, vol. 31, no. 4, pp. 376–389, 2011.

[3] E. S. Mortensen, K. A. Fenton, and O. P. Rekvig, "Lupus nephritis: the central role of nucleosomes revealed," *American Journal of Pathology*, vol. 172, no. 2, pp. 275–283, 2008.

[4] A. M. Shuster, G. V. Gololobov, O. A. Kvashuk, A. E. Bogomolova, I. V. Smirnov, and A. G. Gabibov, "DNA hydrolyzing autoantibodies," *Science*, vol. 256, no. 5057, pp. 665–667, 1992.

[5] A. V. Vlassov, O. A. Andrievskaya, T. G. Kanyshkova et al., "RNA-hydrolyzing antibodies from peripheral blood of patients with lupus erythematosus," *Biochemistry*, vol. 62, no. 5, pp. 474–479, 1997.

[6] D. Vlahakos, M. H. Foster, A. A. Ucci, K. J. Barrett, S. K. Datta, and M. P. Madaio, "Murine monoclonal anti-DNA antibodies penetrate cells, bind to nuclei, and induce glomerular proliferation and proteinuria in vivo," *Journal of the American Society of Nephrology*, vol. 2, no. 8, pp. 1345–1354, 1992.

[7] A. V. Kozyr, L. P. Sashchenko, A. V. Kolesnikov et al., "Anti-DNA autoantibodies reveal toxicity to tumor cell lines," *Immunology Letters*, vol. 80, no. 1, pp. 41–47, 2001.

[8] D. D. Desai, M. R. Krishnan, J. T. Swindle, and T. N. Marion, "Antigen-specific induction of antibodies against native mammalian DNA in nonautoimmune mice," *Journal of Immunology*, vol. 151, no. 3, pp. 1614–1626, 1993.

[9] G. S. Gilkeson, P. Ruiz, D. Howell, J. B. Lefkowith, and D. S. Pisetsky, "Induction of immune-mediated glomerulonephritis in normal mice immunized with bacterial DNA," *Clinical Immunology and Immunopathology*, vol. 68, no. 3, pp. 283–292, 1993.

[10] H. L. Wun, D. T. M. Leung, K. C. Wong, Y. L. Chui, and P. L. Lim, "Molecular mimicry: anti-DNA antibodies may arise inadvertently as a response to antibodies generated to microorganisms," *International Immunology*, vol. 13, no. 9, pp. 1099–1107, 2001.

[11] J. N. Herron, X. M. He, D. W. Ballard et al., "An autoantibody to single-stranded DNA: comparison of the three-dimensional structures of the unliganded Fab and a deoxynucleotide-Fab complex," *Proteins*, vol. 11, no. 3, pp. 159–175, 1991.

[12] G. V. Gololobov, C. A. Rumbley, J. N. Rumbley et al., "DNA hydrolysis by monoclonal anti-ssDNA autoantibody BV 04-01: origins of catalytic activity," *Molecular Immunology*, vol. 34, no. 15, pp. 1083–1093, 1997.

[13] R. Kofler, R. Strohal, R. S. Balderas et al., "Immunoglobulin κ light chain variable region gene complex organization and immunoglobulin genes encoding anti-DNA autoantibodies in lupus mice," *Journal of Clinical Investigation*, vol. 82, no. 3, pp. 852–860, 1988.

[14] C. A. Rumbley, L. K. Denzin, L. Yantz, S. Y. Tetin, and E. W. Voss, "Construction, characterization, and selected site-specific mutagenesis of an anti-single-stranded DNA single-chain autoantibody," *Journal of Biological Chemistry*, vol. 268, no. 18, pp. 13667–13674, 1993.

[15] S. M. Kipriyanov, "High-level periplasmic expression and purification of scFvs," *Methods in Molecular Biology*, vol. 562, pp. 205–214, 2009.

[16] A. H. Andreotti, "Native state proline isomerization: an intrinsic molecular switch," *Biochemistry*, vol. 42, no. 32, pp. 9515–9524, 2003.

[17] M. J. Feige, S. Groscurth, M. Marcinowski et al., "An unfolded CH1 domain controls the assembly and secretion of IgG antibodies," *Molecular Cell*, vol. 34, no. 5, pp. 569–579, 2009.

[18] V. Morea, A. Tramontano, M. Rustici, C. Chothia, and A. M. Lesk, "Conformations of the third hypervariable region in the VH domain of immunoglobulins," *Journal of Molecular Biology*, vol. 275, no. 2, pp. 269–294, 1998.

[19] J. Luo, G. Obmolova, A. Huang et al., "Coevolution of antibody stability and Vκ CDR-L3 canonical structure," *Journal of Molecular Biology*, vol. 402, no. 4, pp. 708–719, 2010.

[20] L. A. Clark, S. Ganesan, S. Papp, and H. W. T. Van Vlijmen, "Trends in antibody sequence changes during the somatic hypermutation process," *Journal of Immunology*, vol. 177, no. 1, pp. 333–340, 2006.

[21] U. Wellmann, M. Letz, M. Herrmann, S. Angermüller, J. R. Kalden, and T. H. Winkler, "The evolution of human anti-double-stranded DNA autoantibodies," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 26, pp. 9258–9263, 2005.

[22] M. Z. Radic, J. Mackle, J. Erikson, C. Mol, W. F. Anderson, and M. Weigert, "Residues that mediate DNA binding of autoimmune antibodies," *Journal of Immunology*, vol. 150, no. 11, pp. 4966–4977, 1993.

[23] J. Zhang, A. M. Jacobi, T. Wang, and B. Diamond, "Pathogenic autoantibodies in systemic lupus erythematosus are derived from both self-reactive and non-self-reactive B cells," *Molecular Medicine*, vol. 14, no. 11-12, pp. 675–681, 2008.

[24] C. Putterman, W. Limpanasithikul, M. Edelman, and B. Diamond, "The double edged sword of the immune response: mutational analysis of a murine anti-pneumococcal, anti-DNA antibody," *Journal of Clinical Investigation*, vol. 97, no. 10, pp. 2251–2259, 1996.

[25] A. J. Manheimer-Lory, G. Zandman-Goddard, A. Davidson, C. Aranow, and B. Diamond, "Lupus-specific antibodies reveal an altered pattern of somatic mutation," *Journal of Clinical Investigation*, vol. 100, no. 10, pp. 2538–2546, 1997.

[26] Y. R. Kim, J. S. Kim, S. H. Lee et al., "Heavy and light chain variable single domains of an anti-DNA binding antibody hydrolyze both double- and single-stranded DNAs without sequence specificity," *Journal of Biological Chemistry*, vol. 281, no. 22, pp. 15287–15295, 2006.

[27] H. S. Zein, J. A. T. da Silva, and K. Miyatake, "Monoclonal antibodies specific to *Cucumber mosaic virus* coat protein possess DNA-hydrolyzing activity," *Molecular Immunology*, vol. 46, no. 7, pp. 1527–1533, 2009.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 812138, 6 pages
doi:10.1155/2012/812138

Clinical Study

Screening Tests of Reproductive Immunology in Systemic Lupus Erythematosus

Zdenka Ulcova-Gallova,[1] Alice Mockova,[2] and Miroslava Cedikova[3]

[1] *Department of Obstetrics and Gynecology, Faculty of Medicine in Pilsen and University Hospital, University in Pilsen, 30460 Pilsen, Czech Republic*
[2] *Department of Neonatology, Faculty of Medicine in Pilsen and University Hospital, Charles University in Prague, Alej Svobody 80, 30460 Pilsen, Czech Republic*
[3] *Institute of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University in Prague, Karlovarská 48, 30166 Pilsen, Czech Republic*

Correspondence should be addressed to Zdenka Ulcova-Gallova, ulcova-gallova@email.cz

Received 13 June 2012; Revised 30 September 2012; Accepted 30 September 2012

Academic Editor: Hiroshi Okamoto

Female patients in reproductive age with systemic lupus erythematosus and fertility complications together are observed by rheumatologists, gynecologists, and reproductive immunologists. The paper notes the presence of autoantibodies to zona pellucida, to phospholipids (phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl glycerol, phosphatidic acid, annexin V, beta-2 glycoprotein I, and cardiolipin) and of isoantibodies to sperm cells. Isoantibodies to sperm cells are not significantly predominant, but autoimmunity is well expressed in IgG positivity against phosphatidyl inositol, phosphatidyl ethanolamine, phosphatidyl serine, cardiolipin, and beta-2 glycoprotein I, as well as antizona pellucida antibodies in IgG isotype. According to the levels of autoantibodies we have to choose preventive treatment to protect mother and her foetus.

1. Introduction

Although autoimmune diseases (AIDs) are not in general considered a major cause of impaired reproductive capacity some AIDs are associated with infertility or pregnancy wastage. Autoimmune conditions may affect all stages of fertility, from ovarian to implantation failure and pregnancy loss. Systemic lupus erythematosus (SLE) is one of the most common autoimmune disorders that affect women during their childbearing age. Prevalence of SLE in Europe is similar as in USA, ranging from 3.3 to 4.8 per 100.000 person-years [1].

Typical clinical symptoms of SLE include fatigue, fever, arthritis, a photosensitive rash serositis, Raynaud phenomenon, glomerulonephritis, vasculitis, and hematologic abnormalities. Although patients with SLE are as fertile as women in the general population, their pregnancies could be associated with complications [2]. Fertility may be compromised by menstrual irregularities with anovulatory cycles during episodes of active disease or chronic renal failure, use of nonsteroid anti-inflammatory drugs (NSAIDs), high-dose corticosteroids, or cyclophosphamide.

High rate of fetal losses up to 45 percent in SLE women has been described in some studies [2]. Another complication may be implantation failure after in vivo fertilization and embryo transfer or impaired fetal development.

There is a wide variety of autoantibodies associated with SLE. Some of the antibodies are helpful in the diagnostics of the illness, while others are more useful in detecting and monitoring disease activity or potential complications. Antibodies to native double-strand DNA (dsDNA) are relatively specific for the diagnosis of SLE. Serum antinuclear antibodies (ANAs) are found in nearly all individuals with active SLE. Significant for SLE diagnosis is assessment of spectrum extractable nuclear antibodies (Sm, La, Ro), antibodies to ribonucleoprotein (RNP), complement and N-methyl-D-aspartate receptor (ENA panel).

Antiphospholipid antibodies (aPLs) form a large group of antibodies that are detected in patients with SLE as well as with other autoimmune conditions. These antibodies are associated with a wide range of potential complications during pregnancy, including miscarriage, fetal death, intrauterine growth restriction, prematurity, and preeclampsia-especially in the primary antiphospholipid syndrome (APS).

In SLE women, pregnancy should be best planned during periods of disease stabilization and nephritis remission lasting at least six months. Closed collaboration of rheumatologist, obstetrician, and neonatologist is necessary for successful pregnancy and delivery. Fulfilling these prerequisites, there is still around 5% of women with SLE that have fertility or pregnancy problems [3].

A complication in reproduction is very often a reason for thorough immunological examination. We present here our experience with SLE patients from the perspective of reproductive immunology.

2. Objectives

The general aim of our paper is to evaluate the results of screening tests in reproductive immunology in women of childbearing age with SLE. The primary aim is to investigate the occurrence of autoantibodies to zona pellucida and eight various phospholipids and occurrence of isoantibodies to sperm cells in patients with SLE in remission planning pregnancy.

3. Subjects

The study group consists of 52 woman with SLE (mean time from diagnosis 4.62 ± 2.28 years; age 18–42 years, mean 30.4 ± 3.9) that were referred for pregnancy planning to Special Division for Infertility and Immunology of Reproduction at the Department of Obstetrics and Gynaecology, Charles University and Faculty Hospital, Pilsen, Czech Republic. All patients fulfilled the revised criteria for SLE diagnosis [4]. All patients were in remission during the study examinations and during attempts to fertilize, none had acute nephritis or serious renal impairment. None of the patients were treated with corticosteroids in a dose exceeding 10 mg per day, immunosuppressive drugs, only three were on hydroxychloroquine sulfate (plaquenil) medication. Before being examined and treated at our Division, all patients were examined by endocrinologist, gynecologist, and had genetic consultation. No substantial pathology was found in the following hormonal tests that are known to impair fertility: FSH, LH, progesterone, estradiol, prolactin. Pregnancy loss occurred in 38 women from the study group prior to our testing.

The control group consisted of 25 age-matched healthy fertile women (age 24–43 years, mean 31.2 ± 3.2). All control subjects have successfully conceived and have given spontaneous birth to two healthy children, they had prior to our examination regular sexual intercourse and menstrual cycle and did not use hormonal contraceptives. The study was approved by the local research ethics committee at the Charles University Medical Faculty Hospital, Pilsen, and written informed consent was obtained from all participants.

4. Methods

Detection of autoantibodies to zona pellucida, eight phospholipids (phosphatidyl-serine, phosphatidyl-ethanolamine, phosphatidyl-inositol, DL-glycerol, phosphatidic acid, annexin V, beta-2-glycoprotein I, and cardiolipin), and isoantibodies to sperm cells was performed as described below.

Both groups were tested with F-test of equality of variances. For each immunology test percentage values were calculated independently in study and control group, and antibody occurrences were analyzed using standard odds ratio formulas where applicable.

4.1. Sperm Antibodies of Serum (ASA). We have performed tray agglutination test (TAT) [2] and indirect-mixed agglutination test (i-MAR) according to previously published guidelines for examination of the serum ASA [3]. TAT test serves as an initial screening examination. The blood from studied subjects was collected by venipuncture into evacuated tubes and centrifuged. Isolated serum was inactivated by heat (56°C for 30 min) and stored at −20°C until analysis. We performed the TAT test by adding 5 μL of inactivated, geometrically diluted serum and 1 μL of motile donor sperm ($40 \cdot 10^6$/mL), isolated by "swim-up technique" into microchambers covered by paraffin oil. After the incubation (2 h at 37°C), the immunological reaction was evaluated under inverted microscope at 200x magnification. Agglutination of the sperm cells at dilution at least 1 : 64 was considered as a positive result.

The i-MAR test was performed to analyze the antisperm response in IgG and IgA class. One microliter of native sperm suspension, 1 μL of inactivated serum, and 1 μL of glutaraldehyde-fixed sheep erythrocytes precoated with human IgG and IgA were mixed together. Then, 1 μL of the corresponding antiserum anti-IgG and anti-IgA (Behringer, Hannover, Germany) was added. Finally, the mixture was covered with coverslip and incubated in humid Petri chamber for 5–10 min. The result of the sperm agglutination reaction was watched under the inverted microscope at 200x magnification. The i-MAR test was considered as positive, if more than 49% of motile spermatozoa were involved in mixed agglutinates (spermatozoa and sheep erythrocytes coated by the corresponding immunoglobulin).

4.2. Sperm Antibodies in Cervical Ovulatory Mucus. We use ovulatory cervical mucus taken by a special syringe 5 days after condom protected intercourse that is planned to happen during ovulation time. Sperm capillary penetration test serves as screening test, and i-MAR test detects local sperm antibodies of IgG and/or IgA [4].

4.3. Zona Pellucida Antibodies. Classic ELISA tests (Laboserv) for detection of zona pellucida antibodies in isotype of IgG and IgM were used.

Table 1: Prevalence of positive results for each performed antibody analysis with comparison between study and control groups.

Antibody	Number of positive subjects		Odds ratio (95% confidence interval)
	Study group ($n = 52$)	Control group ($n = 25$)	
Antisperm antibodies (ASAs)			
Serum ASA	5/52 (9.6%)	1/25 (4.0%)	2.55 (0.28–23.10)
Cervical ovulatory mucus ASA	6/52 (11.5%)	1/25 (4.0%)	3.13 (0.36–27.52)
Antizona pellucida antibodies (aZP)			
aZP IgG	**12/52 (23.0%)**	**0/25 (0.0%)**	N/A
aZP IgM	1/52 (1.9%)	0/25 (0.0%)	N/A
Antiphospholipid antibodies (aPL) against			
phosphatic acid IgG	1/52 (1.9%)	0/25 (0.0%)	N/A
phosphatic acid IgM	0/52 (0.0%)	0/25 (0.0%)	N/A
phosphatidyl glycerol IgG	2/52 (3.8%)	0/25 (0.0%)	N/A
phosphatidyl glycerol IgM	0/52 (0.0%)	0/25 (0.0%)	N/A
phosphatidyl inositol IgG	**35/52 (67.3%)**	**0/25 (0.0%)**	N/A
phosphatidyl inositol IgM	2/52 (3.8%)	1/25 (4.0%)	0.96 (0.08–11.12)
phosphatidyl ethanolamine IgG	**16/52 (30.8%)**	**0/25 (0.0%)**	N/A
phosphatidyl ethanolamine IgM	3/52 (5.8%)	1/25 (4.0%)	1.47 (0.15–14.88)
phosphatidyl serine IgG	**32/52 (61.5%)**	**0/25 (0.0%)**	N/A
phosphatidyl serine IgM	2/52 (3.8%)	2/25 (8.0%)	0.46 (0.06–3.47)
cardiolipin IgG	**16/52 (30.8%)**	**2/25 (8.0%)**	**5.11 (1.07–24.33)**
cardiolipin IgM	4/52 (7.7%)	0/25 (0.0%)	N/A
beta-2-glycoprotein IgG	**15/52 (28.8%)**	**0/25 (0.0%)**	N/A
beta-2-glycoprotein IgA	9/52 (17.3%)	0/25 (0.0%)	N/A
annexin-V	7/52 (13.5%)	0/25 (0.0%)	N/A

Note: statistically significant values are marked in bold script.

4.4. Panel of Antiphospholipid Antibodies (aPL). ELISA was also used for detection of aPL against phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl inositol, DL-glycerol, and phosphatidic acid. For ELISA, polystyrene microtiter plates were coated with 50 μL of phospholipid (50 μL/mL of methanol) and were allowed to dry overnight at 4°C. The plates were then blocked by solution containing 10% of fetal calf serum (FCS) in 1 M TRIS buffer solution (TBS—250 mM Tris, 1.5 M NaCl), for 30 min at room temperature. Subsequently, polystyrene microtiter plates were washed three times by binding buffer (Na_2CO_3, $NaHCO_3$, NaN_3, pH 9.6).

Serum from patients and controls, diluted to a ratio of 1 : 50 in TBS containing 10% FCS were added to the wells after the wash. The plates were incubated for 2 hours at room temperature and then washed five times with TBS containing 0.05% Tween 20. Fifty microliters of peroxidase-conjugated antihuman immunoglobulin (IgG, IgA, or IgM) was added and incubated for 1 hour at room temperature. The plates were then washed five times in TBS Tween. Fifty microliters of substrate solution were added to each well and incubated in the darkness for 30 min at room temperature. The reaction was stopped by adding 50 μL of 2 M sulphuric acid. The optical density of each well was determined using a Titertek MultisKan MCC/340 (Flow Laboratories, London, UK) at 492 nm. Background optical density was run for all patients' sera diluted 1 : 50 in wells on identical microtiter plates coated with methanol but without phospholipids. The phospholipids used to coat the ELISA plates were: phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl inositol, DL-glycerol, and phosphatidic acid (Sigma, St.Louis, MO, USA).

For detection and quantification of serum antiannexin V (Szabo-Scandic Handels GMBH & Co KG, Vienna, Austria) commercial ELISA kits were used, as well as for detection of IgG and IgA antibodies against beta2-glycoprotein I (beta2GPI) (Immunotech, Prague Division, Czech Republic), cardiolipin levels in IgG and IgM (Millenia, London, UK) (Table 1). Statistical analysis with the use of Statgraphics software was performed to obtain cut-off levels for Ig isotypes of aPLs that were derived as values above 3 standard deviations (SDs).

5. Results

Because equality of variance test proved that both groups were similar in age (as an independent baseline characteristic), we were able to assess individual antibody prevalence percentages as follows: nonzero prevalences of tested antibodies were all higher in the study group compared to

controls with the exception of antiphosphatidyl inositol IgM and antiphosphatidyl serine IgM (3.8% versus 4.0% and 3.8% versus 8.0%, resp.). Difference between study group and control group in antisperm antibodies (ASAs) predominance was only nominal and statistically insignificant in both serum ASA (9.6% versus 4.0%, odds ratio 2.55, 95% confidence interval 0.28–23.10) and cervical ovulatory mucus ASA (11.5% versus 4.0%, OR 3.13, 95% CI 0.36–27.52). Concerning antizona pellucida (aZP) and antiphospholipid (aPL) antibodies, no significant difference was observed in IgM subtypes across all tests. However, in aZP IgG subclass a difference was found to be significant in favor of study group (12 out of 52 subjects, 23.0%, versus zero of 25 subjects). The same was true for almost all individual aPL antibodies in IgG isotype, the exception being antiphosphatic acid IgG and antiphosphatidyl glycerol IgG (detailed results are shown in Table 1 with significant values marked in bold script). Even though we detected anticardiolipin IgG positivity in two of the patients from control group (unlike any other IgG in this study), the difference in prevalence between groups (30.8% in study group versus 8.0% in controls) still yielded statistically significant result (odds ratio 5.11, 95% CI 1.07–24.33).

6. Discussion

Pregnancy is the most significant exception to the immunological rules where the mothers body not only tolerates, but also broadly supports semiallogenic blastocyst, embryo, and fetus. Remarkable tolerance is provided by complex immunoregulatory mechanisms between the mother and fetal trophoblast. Failure of this tolerance is one of the most common causes of fetal loss. As mentioned in many papers, reproductive failure including recurrent pregnancy loss (RPL) and infertility has been linked to presence of various autoantibodies [5].

In SLE as well as in other autoimmune diseases an overproduction of autoantibodies is described, especially against organ-nonspecific antigens. This feature is based on the loss of B-cell tolerance to antigens, nucleic acids, and their binding proteins. Antinuclear antibodies can trigger proliferation of autoreactive lymphocytes in genetically susceptible individuals under the influence of environmental factors (infections, drugs, toxins, smoking, and hormonal factors) leading to expansion of their specificity towards more nuclear antigens or other structures (elements of blood, plasma components, coagulation factors, and complement cascade) [5]. Influenced by many proinflammatory cytokines and chemokines inflammatory cells migrate into target organs, activate their effector mechanisms, and cause chronic inflammation with the help of chronic humoral components.

The aim of our study was to evaluate the occurrence of variety of antibodies possibly responsible for immunological reproduction failure in women with SLE and compare these results with findings in healthy controls.

Many retrospective and prospective studies have proven effect of antisperm antibodies in various human body fluids (serum, semen, cervical ovulatory mucus, follicular, or peritoneal fluid) on fertility of women and men [6]. ASA can influence the mechanisms of transport of spermatozoa within the female genital tract due to decreasing sperm motility, may alter sperm capacitation or the acrosome reaction, can interfere with egg fertilization, or have post-fertilization effects on the zygote and preimplantation early embryo [6].

It seems clear that repeated sperm exposition is not sufficient for most women to start production of antisperm antibodies. Other factors such as mechanical, chemical, or inflammatory lesions of the mucosa in female genital system play an important role [7]. Especially in SLE women the immunosuppressive therapy might affect the likelihood of genital tract infection. The proven higher incidence of CMV, EBV virus, and *Chlamydia trachomatis* infection in SLE patients may contribute to their infertility [8, 9].

Antisperm antibodies (ASAs) in both of our groups were determined to evaluate the difference. In contrast to the above mentioned facts we have found no significant difference between antisperm antibody prevalence in study group and control group. Our prevalence data from control group are in consent with evidence about the presence of these antibodies in healthy women with undisturbed reproductive capability (several authors reported that these antibodies occur in approximately 1 to 2.5% of fertile men and in 4% of fertile women) [6]. Even though our data may suffer from low sample size bias, the benefit of both serum and cervical ovulatory mucus antibody tests seems to be the lowest in our pool of patients and controls.

Zona pellucida (ZP), ten micrometers "strong" oogenetic glycoprotein matrix, is formed during oogenesis and its thickness increases with the growth of the oocyte. It serves basically as a protective layer surrounding the oocyte as it matures and is composed of three major glycoproteins ZP1, ZP2, and ZP3. These glycoproteins seem to be the important ovarian antigens participating in the etiology of some infertility disorders, including autoimmune premature ovarian failure (POF). POF can be seen in approximately 1-2% of healthy women, in 30% is connected with some autoimmune causes and is often presented in women with SLE [10]. Autoimmune POF has been proven in animal studies, where the preantral mouse follicles with both anti-ZP2 and anti-ZP antibodies were cultured and thereafter have shown smaller diameter than controls [11]. Furthermore, antizonal antibodies against glycoproteins of ZP are able to inhibit sperm attachment and penetration into oocyte and may be the cause of natural or artificial fertilization failure. The increased levels of serum AZA are very often detected after repeated unsuccessful IVF as we reported earlier [12]. Some studies confirmed an antigamete antibodies (AGAs) in high percentage of patients with unexplained infertility versus patients with proven etiology of infertility [13].

In the present study we have found borderline significant prevalence of aZP IgG antibodies in the study group in comparison with the control group.

Antiphospholipid antibodies (aPL) form a group of antibodies that is probably associated with compromised fertility

not only in SLE women. Although some retrospective studies reported nonsignificant association between antiphospholipid antibodies and pregnancy loss in patients with SLE other studies have confirmed statistically significant interrelation between them [14]. APL could be present in up to 5% of apparently healthy controls and up to 37% of patients with SLE. Less frequently they also accompany other autoimmune diseases [15, 16]. In animal models the administration of aPL during pregnancy causes placental impairment followed by miscarriage. The thrombosis in uteroplacental vasculature is then the result of endothelial cell activation, inhibition of protein C/S system and fibrinolysis, and annexin V displacement. Another significant mechanism for nonthrombotic fetal damage is straight influence of aPL on the anionic phospholipids and β2-glycoprotein I of trophoblast that very probably impairs the placentary production of chorionic gonadotropin during the early phases of pregnancy [17]. Although the current classification for the antiphospholipid syndrome is based on positive identification of one or more of three standardized laboratory assays: anticardiolipin antibodies (aCL), lupus anticoagulant (LA), and anti β(2)glycoprotein I (anti-β(2)GPI) according to the report of a task force and preconference workshop at the 13th International Congress on antiphospholipid antibodies, Galveston, Texas in April 2010 several other antibodies have been proposed to be relevant to APS. In our laboratory we use the expanded scale of aPL detection of antiannexin V, antiphosphatidylserine, antiphosphatidylethanolamine, antiphosphatidylinositol, antiphosphatidylglycerol, and antiphosphatidic acid. Our results suggest that in women with SLE the aPL IgM screening is unsatisfactory. On the other hand, IgG screening shows a marked difference that may well be associated with reproductive failure. The observed integrity (5 of 7 aPL IgG antibodies are significantly different between study and control group) may be due to hypothetical associations of certain IgG antibodies resulting in notional fixed antibody profiles that carry sufficient potential for infertility or pregnancy wastage only in cooperation.

A review of 10 studies of 554 women with SLE found that fetal demise was more common in those with aPL (38 to 59 percent versus 16 to 20 percent in those without such antibodies), LAs (36 versus 13 percent), or aCL (39 versus 18 percent). Fetal loss typically occurs after 10 weeks gestation [5, 18].

The relationship between autoimmunity and reproduction has long been recognized. Although in one critical review about the impact of abnormal autoimmunity on female fertility doubts about influence of aPL to POF in SLE were expressed SLE remains the autoimmune disease mostly compromised by humoral changes. It seems possible that another unexplained mechanism (e.g. presence of other antibodies as an IgG antilaminin-I antibodies, anticorpus luteum glycoprotein and antilymphocytotoxic autoantibodies, or antimitochondrial M5 type antibodies) could be involved [5]. These antibodies were found to interfere with markers of placental and yolk sac differentiation, invasion, endocytosis, signaling, and others promote thrombotic processes [19, 20].

7. Conclusion

Based on our results and long-term experience, we believe that it is appropriate in women with SLE with recurrent and otherwise unexplained fetal loss to recommend a detailed evaluation by a specialist in reproductive immunology. In the case of positive findings of autoantibodies, preventive treatment is to be considered (e.g. corticosteroids, low molecular weight heparin, low dose of aspirin, intravenous immunoglobulin therapy). Because the evidence supporting association of various individual antibodies with reproductive failure is inconclusive a new meta-analysis of antibody profiles in women at risk may be of benefit.

Conflict of Interests

The authors state no conflict of interests.

Acknowledgments

The authors would like to thank all west Bohemian rheumatologists for close collaboration. The study was supported by the Grant GA UK no. 696212 from the Grant Agency of the Charles University in Prague, and from Charles University Research Fund (Project Number P36).

References

[1] D. J. McCarty, S. Manzi, T. A. Medsger, R. Ramsey-Goldman, R. E. LaPorte, and C. K. Kwoh, "Incidence of systemic lupus erythematosus: race and gender differences," *Arthritis and Rheumatism*, vol. 38, no. 9, pp. 1260–1270, 1995.

[2] R. Andrade, M. L. Sanchez, G. S. Alarcón et al., "Adverse pregnancy outcomes in women with systemic lupus erythematosus from a multiethnic US cohort: LUMINA (LUI)," *Clinical and Experimental Rheumatology*, vol. 26, no. 2, pp. 268–274, 2008.

[3] R. A. Hickman and C. Gordon, "Causes and management of infertility in systemic lupus erythematosus," *Rheumatology*, vol. 50, no. 9, pp. 1551–1558, 2011.

[4] M. Petri, "Review of classification criteria for systemic lupus erythematosus," *Rheumatic Disease Clinics of North America*, vol. 31, no. 2, pp. 245–254, 2005.

[5] Y. Shoenfeld and M. Blank, "Autoantibodies associated with reproductive failure," *Lupus*, vol. 13, no. 9, pp. 643–648, 2004.

[6] W. W. C. Chiu and L. W. Chamley, "Clinical associations and mechanisms of action of antisperm antibodies," *Fertility and Sterility*, vol. 82, no. 3, pp. 529–535, 2004.

[7] S. Mazumdar and A. S. Levine, "Antisperm antibodies: etiology, pathogenesis, diagnosis, and treatment," *Fertility and Sterility*, vol. 70, no. 5, pp. 799–810, 1998.

[8] M. V. Febrônio, R. M. R. Pereira, E. Bonfá, A. D. Takiuti, E. A. G. Pereyra, and C. A. A. Silva, "Inflammatory cervicovaginal cytology is associated with disease activity in juvenile systemic lupus erythematosus," *Lupus*, vol. 16, no. 6, pp. 430–435, 2007.

[9] Y. Berkun, G. Zandman-Goddard, O. Barzilai et al., "Infectious antibodies in systemic lupus erythematosus patients," *Lupus*, vol. 18, no. 13, pp. 1129–1135, 2009.

[10] T. Forges, P. Monnier-Barbarino, G. C. Faure, and M. C. Béné, "Autoimmunity and antigenic targets in ovarian pathology," *Human Reproduction Update*, vol. 10, no. 2, pp. 163–175, 2004.

[11] G. Calongos, A. Hasegawa, S. Komori, and K. Koyama, "Harmful effects of anti-zona pellucida antibodies in folliculogenesis, oogenesis, and fertilization," *Journal of Reproductive Immunology*, vol. 79, no. 2, pp. 148–155, 2009.

[12] Z. Ulcova-Gallova, "Immunological and physicochemical properties of cervical ovulatory mucus," *Journal of Reproductive Immunology*, vol. 86, no. 2, pp. 115–121, 2010.

[13] K. Koyama, A. Hasegawa, Y. Tsuji, and S. Iosjima, "Production and characterization of monoclonal antibodies to cossreactive antigens of human and porcine zonae pellucidae," *Journal of Reproductive Immunology*, vol. 7, no. 3, pp. 187–198, 1985.

[14] K. C. Kalunian, J. B. Peter, H. R. Middlekauff et al., "Clinical significance of a single test for anti-cardiolipin antibodies in patients with systemic lupus erythematosus," *American Journal of Medicine*, vol. 85, no. 5, pp. 602–608, 1988.

[15] R. Cervera, J. C. Piette, J. Font et al., "Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients," *Arthritis and Rheumatism*, vol. 46, no. 4, pp. 1019–1027, 2002.

[16] J. S. Ginsberg, P. Brill-Edwards, M. Johnston et al., "Relationship of antiphospholipid antibodies to pregnancy loss in patients with systemic lupus erythematosus: a cross-sectional study," *Blood*, vol. 80, no. 4, pp. 975–980, 1992.

[17] A. Tincani, L. Spatola, M. Cinquini, P. Merino, G. Balestrieri, and Y. Shoenfeld, "Animal models of antiphospholipid syndrome," *Revue du Rhumatisme*, vol. 65, no. 11, pp. 614–618, 1998.

[18] H. P. McNeil, C. N. Chesterman, and S. A. Krilis, "Immunology and clinical importance of antiphospholipid antibodies," *Advances in Immunology*, vol. 49, pp. 193–280, 1991.

[19] R. Cervera and J. Balasch, "Bidirectional effects on autoimmunity and reproduction," *Human Reproduction Update*, vol. 14, no. 4, pp. 359–366, 2008.

[20] H. J. A. Carp, C. Selmi, and Y. Shoenfeld, "The autoimmune bases of infertility and pregnancy loss," *Journal of Autoimmunity*, vol. 38, no. 2-3, pp. J266–J274, 2012.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 321614, 8 pages
doi:10.1155/2012/321614

Review Article

Evaluation of ENA-6 Profile by ELISA Immunoassay in Patients with Systemic Lupus Erythematodes

Izeta Aganovic-Musinovic,[1] Jasenko Karamehic,[2] Lamija Zecevic,[2] Faris Gavrankapetanovic,[2] Nesina Avdagic,[1] Asija Zaciragic,[1] Tomislav Jukic,[3] Nerima Grcic,[2] and Suvada Svrakic[2]

[1] *Center for Genetics, Medical Faculty, University of Sarajevo, Cekalusa 90, Sarajevo, Bosnia and Herzegovina*
[2] *Department of Clinical immunology, KCUS, Sarajevo, Bosnia and Herzegovina*
[3] *Department of Biomedicine and Public Health, Faculty of Medicine, Osijek University, Osijek, Croatia*

Correspondence should be addressed to Izeta Aganovic-Musinovic, zizanax@gmail.com

Received 27 April 2012; Accepted 8 August 2012

Academic Editor: Hiroshi Okamoto

Autoimmune diseases occur in 3−5% of the population. Study included 30 patients with clinically diagnosed SLE and 30 healthy controls (American college of Rheumatology, 1997). SLE was diagnosed according to criteria issued in 1997 by the American College of Rheumatology (ACR). The aim of this study was to evaluate concentration values of each antigen of ENA-6 profile in SLE, to investigate possible correlation between the concentration of Sm antibodies and CIC, and to test their use as possible immunobiological markers in SLE. Furthermore, the aim of our study was to determine whether there is a correlation between Sm antibodies and CIC and SLE activity. The results revealed that all of these ENA-6 and Sm antibodies as biomarkers complement diagnoses of active SLE but their use as solo markers does not allow classifying patients with SLE. Our study has shown that based on calculations from ROC curves, Sm/RNP was clearly a very important marker for diagnosis of SLE (cut off ≥ 9.56 EU, AUC 0,942). The high incidence of Scl-70 (10%) reactivity suggests that ELISA monitoring of this antibody produces more false positive results than other multiplex assay. An important conclusion that can be drawn from the results of our study is that laboratory tests are no more effective than clinical examination for detecting disease relapse, but are helpful in the confirmation of SLE activity.

1. Introduction

Autoimmune diseases occur in 3–5% of the population [1], as a result from myriad of genetic and environmental factors that lead to altered immune reactivity [2, 3]. The alterations in the immune system initiated by a loss of immunological tolerance to self-antigens lead to the development of autoreactive phenomena that can be detected in the peripheral blood. Defining specific pathogenic mediators that may trigger the development or progression of an autoimmune disease remains a focus of intense research.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by B cell hyperactivity resulting in overproduction of autoantibodies against cytoplasmic, nuclear, and surface antigens and immune complex formation [4, 5].

The majority of autoantibodies found in SLE are targeted at intracellular nucleoprotein particles. 98% of patients have antinuclear antibodies and antidouble-stranded DNA antibodies are found in 50–80% of patients [6]. These autoantibodies are frequently targeted against intracellular antigens of the cell nucleus (double- and single- stranded DNA (dsDNA and ssDNA, resp.,) histones, and extractable nuclear antigens (ENAs). Most of these autoantibodies are not specific for SLE and might be produced nonspecifically as a result of polyclonal B cell activation [7, 8]. ENA-6-Profile is useful for the diagnosis of systemic autoimmune rheumatic diseases such as systemic lupus SLE, Sjögren's syndrome, Sharp syndrome, polymyositis/dermatomyositis, or progressive systemic scleroderma (PSS) [9]. Because antibodies against ENA have a partial marker function for the individual diseases, the isolated detection of these antibodies

with the ENA-6-profile allows serological differentiation of these diseases.

Autoimmune disease detection protocol starts with determination of ANA (antinuclear antigen). Positive ANA test leads to further investigation of extractible nuclear antigens (ENA) [10].

The prevalence (70%) of anti-dsDNA autoantibody is much higher in SLE, giving a higher diagnostic sensitivity than the similarly disease-specific anti-Sm autoantibodies (30%). In some pathological conditions, like SLE, the concentration level of circulating immune complexes (CIC) increases in tissues and causes the activation of humoral immunity effectors' mechanisms, such as of classical complement pathway activation [11].

Six of the most diagnostically useful autoantibodies include those to Ro (SSA), which are found in 40 to 60% of patients with Sjögren's syndrome and in 25 to 35% of patients with ANA-positive SLE. La (SSB) autoantibodies are found in 50 to 60% of patients with Sjögren's syndrome and 5 to 15% of SLE patients [12].

Smith (Sm) antibodies are highly specific for SLE but only occur in 30 to 35% of cases [13]. ELISA monitoring of extractible antinuclear antibodies—Smith antigen is usually used with the concentration value of ds-DNA to control the disease activity [14].

Antibodies to ribonucleoprotein (RNP) are found in 95 to 100% of patients with Mixed Connective Tissue Disease (MCTD) but are also found in up to 45% of patients with SLE [15]. The presence of anti-RNP antibody alone strongly suggests a diagnosis of MCTD [16]. Scleroderma (Scl-70) autoantibodies are the specific markers for scleroderma and are found in up to 60% of patients diagnosed with this disorder [17]. The Jo-1 autoantibody is one of a family of characteristic autoantibodies seen in myositis patients [18, 19].

The aim of this study was to evaluate concentration values of each antibody of ENA-6 profile in SLE, to investigate possible correlation between the concentration of Sm antibody and CIC and to test their use as possible immunobiological markers in SLE. Furthermore, the aim of our study was to determine whether there is a correlation between Sm antibody and CIC and SLE activity.

2. Material and Methods

2.1. Patients. Study included 30 serum samples submitted to our reference laboratory for autoimmune testing from patients with diagnosed SLE diseases and 30 serum samples from healthy individuals. Patients were recruited through input clinical diagnosis according to criteria of SLE diagnosis issued in 1997 by American College of Rheumatology [20].

2.2. ENA-Assay. The detection of anti-nuclear antibodies (ANA's) has long been an important tool in the diagnosis of systemic rheumatic diseases. The antigens used in their detection are purified by the saline extraction of human or animal nuclei, and this has led them being termed as extractable nuclear antigens (ENA's). The most commonly measured ENA specifications are anti-SS-A/Ro, anti-SS-B/La, anti-Sm, anti-Sm/RNP, anti-Jo-1, and anti-Scl-70 [21].

Table 1

Antibodies of ENA-6	μg/mL	CIC	μg/mL
Negative	<10	Negative	<40
Equivocal	10–15	Equivocal	40–50
Positive	>15	Positive	>50

To determine concentrations of Sm-antibody and other ENA 6 antibodies as well as CIC, we have used ELISA method [22].

2.3. Principle of the Procedure. The Autostat II assay is a solid phase immunosorbent assay (ELISA) in which the analyte is indicated by a color reaction of an enzyme and substrate. The Autostat II wells are coated with purified antigens. The device used was Hytec 288.

On adding diluted serum to the wells the antibodies bind to the antigens. After incubating at room temperature and washing away unbound material, horseradish peroxidase conjugated anti-IgG monoclonal antibody was added, which binds to the immobilized antibodies.

Following further incubation and washing, tetra-methyl benzidine substrate (TMB) is added to each well. The presence of the At-Ag complex turns the substrate to a dark blue color. Addition of the stop solution turns the color to yellow.

The color intensity is proportional to the amount of autoantigens present in the original serum sample [23–25].

2.4. Interpretation of Results. For antigens of ENA 6 results below 10 are considered negative, while the results above 15 are considered positive. Results in the range among 10 to 15 are considered equivocal [26, 27]. Referent intervals are as shown in Table 1 [28].

For dsDNA both techniques were applied for determination, ELISA, and immunofluorescence assay (IFA). The basic principle of the procedure is based on the use of slides with epithelial cells (Hep-2 cells) as substrates that are incubated in few steps with diluted serum. The unbound material is removed by aspirating and washing. The drop of the fluorescence conjugate (anti-human IgG fluorescein labeled containing blue dye and 0.099 sodium azid) is added [29]. Depending on the amounts of autoantibodies in specimens, using IF microscope, it is possible to detect different intensity degree of apple-green fluorescence light. Fluorescence grade is determined as 5+; 4+; 3+; 2+, and positive and negative as zero [30].

2.5. Statistical Analysis. The Kolmogorov-Smirnov test of normality was used to test the distribution of variables. Since all variables were skewed they are presented as median and interquartile ranges. Mann-Whitey U-test was used to compare differences between two groups. Since all variables were highly skewed, correlations were assessed by Spearman's

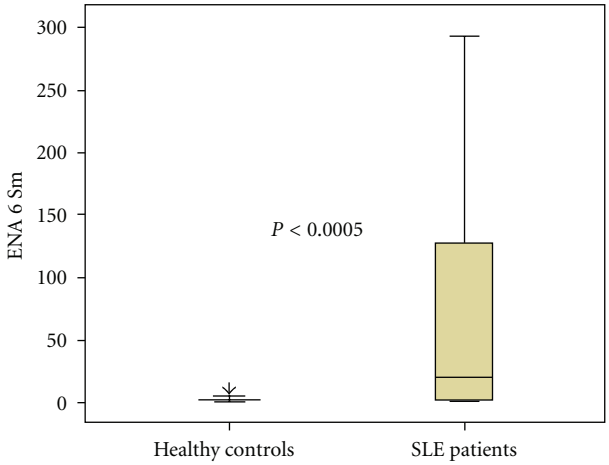


Figure 1: Serum ENA6 Sm concentration in healthy controls and SLE patients. Each bar shows upper and lower quartile, while the square and its central bar indicate interquartile range and median.

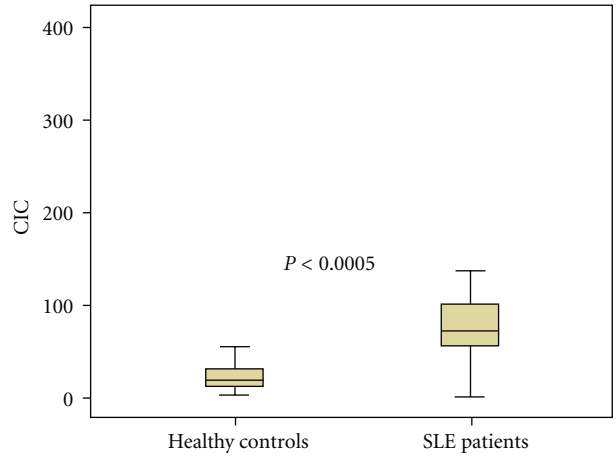


Figure 2: Serum CIC concentration in healthy controls and SLE patients. Each bar shows upper and lower quartile, while the square and its central bar indicate interquartile range and median.

test. A P value of <0.05 was considered statistically significant [31].

Sensitivity, specificity, positive, and negative predictive value were calculated according to the following formula [32]:

$$\begin{aligned} \text{Sensitivity} &= \frac{a}{(a+c)}, \\ \text{Specificity} &= \frac{d}{(b+d)}, \\ \text{Positive predictive value} &= \frac{a}{(a+b)}, \\ \text{Negative predictive value} &= \frac{d}{(c+d)}, \end{aligned} \tag{1}$$

where a = true-positive cases, b = false-positive cases, c = false-negative cases, and d = true-negative cases [32].

Receiver operating characteristic (ROC) curves were constructed by calculating the sensitivities and specificities of ENA 6 SS-A, ENA6 SS-B, ENA6 Sm, Sm/RNP, Jo-1, or SCL 70 assays at several cut-off points [33–35].

The software used was SPSS for Windows (version 17.0; SPSS, Chicago, IL, USA).

3. Results

Figure 1 shows the median and interquartile range of ENA6 Sm serum concentration in the healthy subjects (1, 65; 0, 60–2, 62) and in the SLE patients (19, 07; 1, 97–130,44). Serum ENA6 Sm antibody concentrations in SLE patients were significantly higher compared to healthy controls ($P <$ 0.0005).

Figure 2 shows the median and interquartile range of circulating immune complexes (CIC) serum concentration in healthy subjects (19,00; 12,00–32,00) and in the SLE patients (71,14; 52,99–102,04). Serum CIC concentrations in SLE patients were significantly higher compared to healthy controls ($P < 0.0005$).

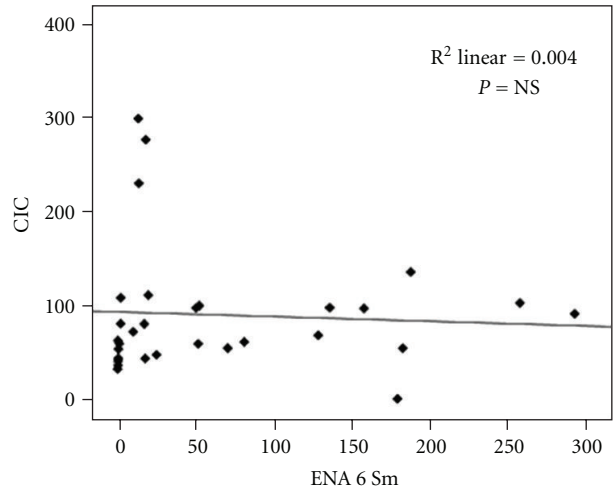


Figure 3: Spearman's correlation analysis between ENA6 Sm and CIC.

Results did not show significant correlation between ENA6 Sm and CIC ($r = 0.29$; P = NS) (Figure 3).

The ROC curves for ENA6 Sm and CIC in the patients with SLE and healthy controls are shown in Figures 4 and 5.

In our study sample 97% of patients were ANA positive and 3% were ANA negative as presented at Figure 6.

Figure 7 presents that 30% of patients were dsDNA negative and 70% were dsDNA positive.

Based on the proposed cut-off values, the sensitivity, and specificity of the ENA6 Sm and CIC were calculated.

Table 2 shows the predictive power of each marker in distinguishing patients with SLE and healthy controls.

Serum concentration of ENA6 SS-A in the SLE patients (11.70; 2.85–183.51) was significantly higher ($P < 0.001$) compared to healthy controls (3.55; 1.20–5.75). Serum concentration of ENA6 SS-B in the SLE patients (6.64; 1.71–46.32) was significantly higher ($P < 0.01$) if compared

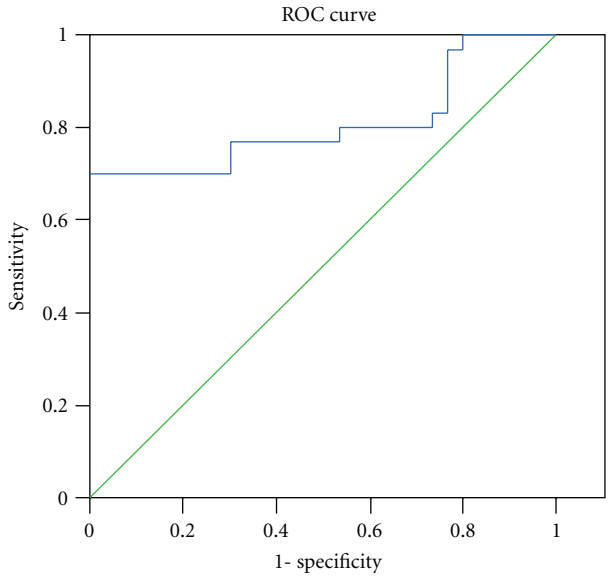


Figure 4: Receiver operating characteristic (ROC) curve of ENA6 Sm for differentiation between SLE patients and healthy control.

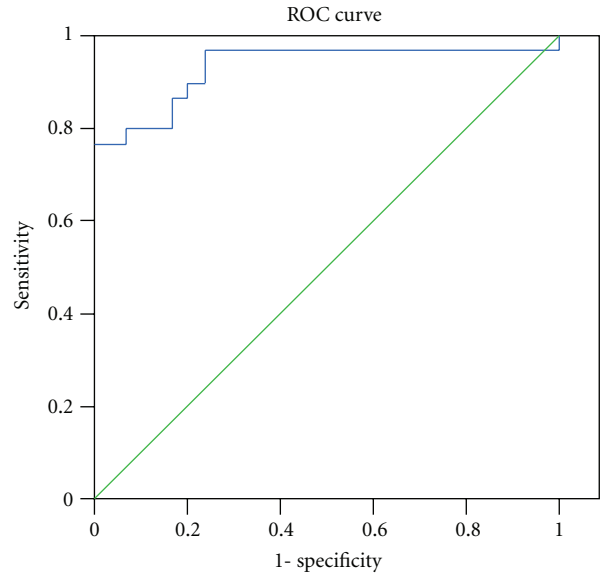


Figure 5: Receiver operating characteristic (ROC) curve of circulating immune complexes (CIC) for differentiation between SLE patients and healthy control.

to healthy controls (3.10; 1.45–5.90). Serum concentration of ENA6 Sm in the SLE patients (19.93; 2.27–135.95) was significantly higher ($P < 0.0005$) compared to healthy controls (1.65; 0.60–2.62). Serum concentration of Sm/RNP in the SLE patients (56.61; 17.70–166.96) was significantly higher ($P < 0.0005$) compared to healthy controls 1.20 (0.50–2.80). Serum concentration of Jo-1 in the SLE patients (2.22; 1.40–4.79) was significantly higher ($P < 0.0005$) compared to healthy controls (0.205; 0.00–0.80). Serum concentration of SCL 70 in the SLE patients (1.10; 0.71–3.33) was significantly higher ($P < 0.0005$) compared to healthy controls (0.155; 0.00–0.28) (Table 3).

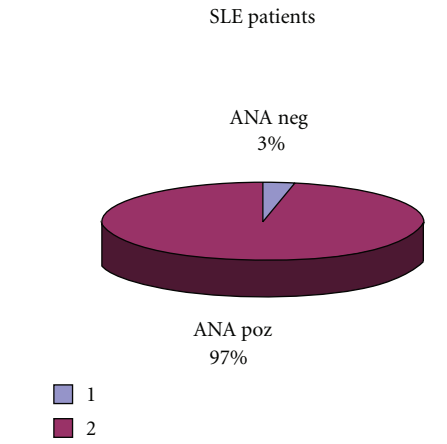


Figure 6: It presents number patients of positive and negative ANA testing's.

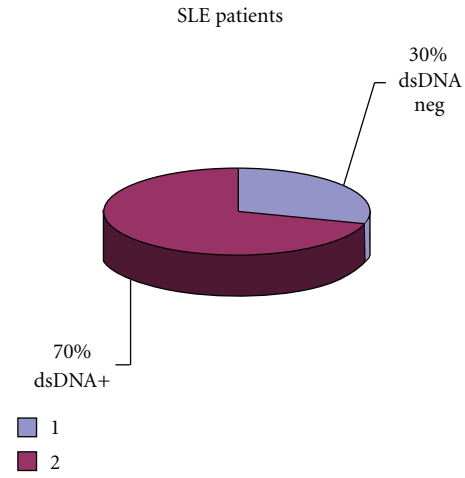


Figure 7: Percentage of positive and negative dsDNA patients.

Results have shown significant correlation between ENA6 SS-A and ENA6 SS-B ($r = 0.99$; $P < 0.01$) (Figure 8.); ENA6 Sm and Sm/RNP ($r = 0.801$; $P < 0.01$) (Figure 9); Jo-1 and SCL 70 ($r = 0.72$; $P < 0.01$) (Figure 10). Results did not show significant correlation between other markers of ENA6 profile.

The ROC curves for ENA6 SS-A, ENA6 SS-B, ENA6 Sm, Sm/RNP, Jo-1, and SCL 70 in the patients with SLE and healthy controls are shown in Figure 11.

In our research according to calculations from ROC curves, Sm/RNP is clearly very important marker for diagnosis of SLE (cut off ≥ 9,56 EU; AUC 0,942). Unexpectedly, the first that follows is Jo-1 (AUC 0,915); then Scl-70 (AUC 0,899); Sm (AUC 0,844); SS-A (AUC 0,740); and SS-B (AUC 0,661).

Based on the proposed cut-off values, the sensitivity, and specificity of the ENA6 markers were calculated. Table 4 shows the predictive power of each markers in distinguishing patients with SLE and healthy controls.

Table 2: Optimal cut-off, area under the curve with 95% confidence interval (AUC, 95% CI), sensitivity, specificity, positive and negative predictive value of ENA6, SM, and CIC in differencing between SLE patients and healthy control.

		SLE patients versus healthy control				
Marker	Optimal cut-off	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
ENA6 Sm	≥9,56 EU	0,809 (0,690–0,928)	70%	100%	100%	76%
CIC	≥54,24 EU	0,931 (0,854–1,00)	76%	100%	100%	81%

SLE: systemic lupus erythematosus; AUC: area under the curve; CI: confidence interval; ENA6 Sm: extractable nuclear antigens 6 Sm; CIC: circulating immune complexes; EU: elisa units.

Table 3: The median and interquartile range of serum concentration of ENA6 profile in the healthy subjects and in the SLE patients.

Marker	Status	Percentiles			P values
		25	50	75	
ENA 6 SS-A	Healthy controls	1.20	3.55	5.75	$P < 0.001$
	SLE patients	2.85	11.70	183.51	
ENA 6 SS-B	Healthy controls	1.45	3.10	5.90	$P < 0.01$
	SLE patients	1.71	6.64	46.32	
ENA 6 Sm	Healthy controls	0.60	1.65	2.625	$P < 0.0005$
	SLE patients	2.27	19.93	135.95	
Sm/RNP	Healthy controls	0.50	1.20	2.80	$P < 0.0005$
	SLE patients	17.70	56.61	166.96	
Jo-1	Healthy controls	0.00	0.205	0.80	$P < 0.0005$
	SLE patients	1.40	2.22	4.79	
SCL 70	Healthy controls	0.00	0.155	0.28	$P < 0.0005$
	SLE patients	0.71	1.10	3.33	

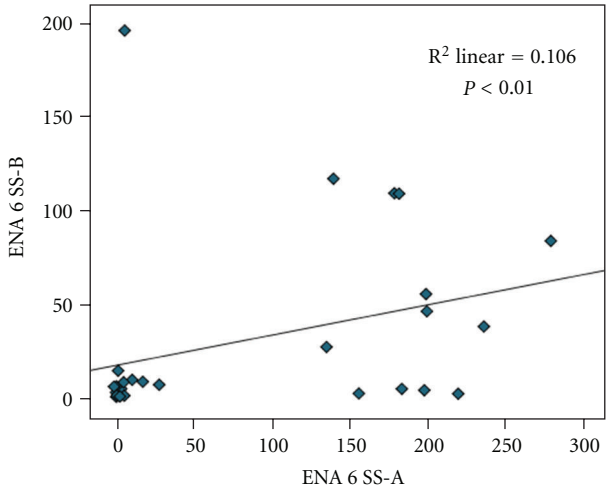


Figure 8: Spearman's correlation analysis between ENA6 SS-A and ENA6 SS-B.

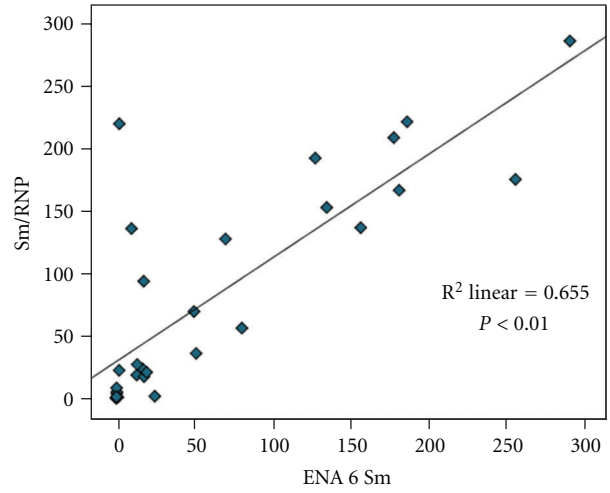


Figure 9: Spearman's correlation analysis between ENA6 Sm and Sm/RNP.

The percentages of patients that had elevated concentration level of ENA antigens were as follows: Sm,/RNP, Sm, SS-A, SS-B, Scl-70 and Jo-1; 73,3%; 66,6%; 50%; 40%; 10%; 6,6%, respectively.

4. Discussion and Conclusion

The aim of this study was to evaluate concentration values of each antibody of ENA-6 profile in SLE and to determine concentration values of CIC and Sm-antibody as potential immunobiological markers in SLE. Furthermore, we aimed to establish whether there is a correlation between Smith antibody in sera and levels of CIC and disease activity [36].

Our results have shown that most valuable marker for SLE activity monitoring is Sm/RNP, than followes Jo-1. Obtained results are not in the accordance with the reports from other authors [37–39]. Possible

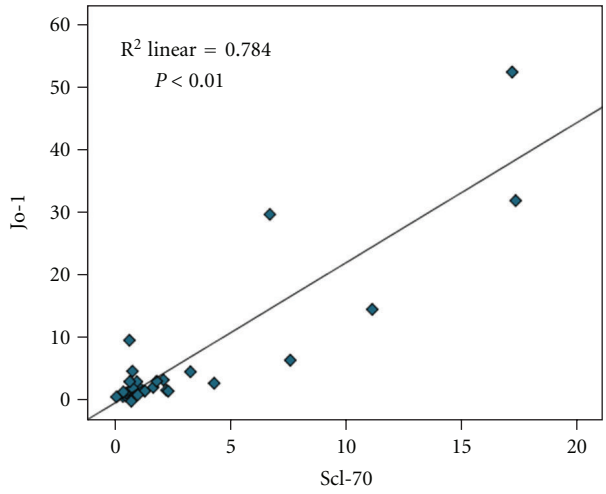


Figure 10: Spearman's correlation analysis between SCL 70 and Jo-1.

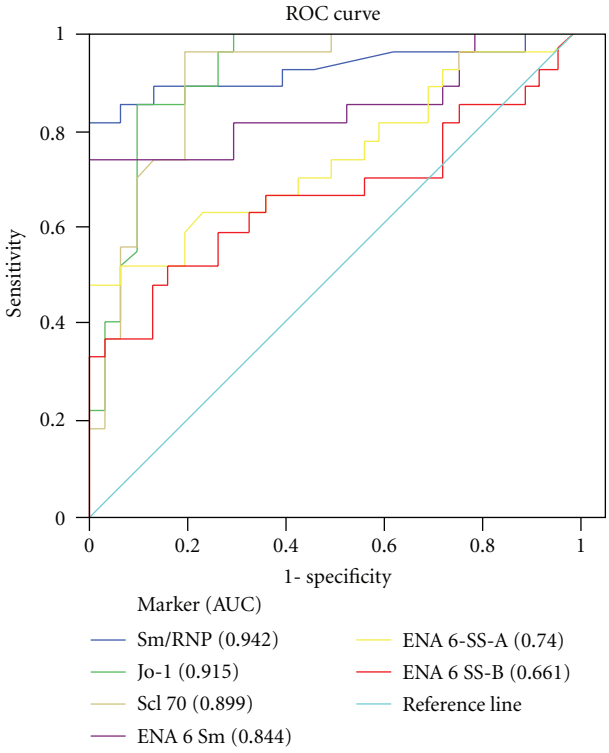


Figure 11: Receiver operating characteristic (ROC) curve of ENA6 profile markers for differentiation between SLE for patients and healthy control.

explanations for our results might be due to small study sample.

High titer of anti-Sm antibody is highly SLE specific although low-titer anti-Sm in ELISA has been reported in other diseases [40]. Anti-Sm antibodies are in fact found without RNP because both proteins associate with common snRNA [40].

For six antigens that comprise ENA-6 profile used in our study (SS-A, SS-B, Sm, RNP, Scl-70, and Jo1) we reported concordances, sensitivity, and specificity in range of 60 to 100%. The highest specificity has been reported in Sm/RNP and Sm (100%); while the highest sensitivity has been reported at Scl-70 (96%) and Jo 1 (83%). There is evidence about correlation among Scl-70 and Jo1; as well as among Sm and Sm/RNP; among SS-A and SS-B; while there is no correlation among those three couples of antibodies [41]. Our results on concentration level and sensitivity of Scl-70 antibody is not in the accordance with other reports [42].

High incidence of Scl-70 (10%) reactivity suggests that ELISA monitoring of this antibody produces more false positive results than other multiplex assay [42, 43]. In our research according to calculations from ROC curves, Sm/RNP is clearly a very important marker for diagnosis of SLE. Surprisingl and interestingly first that follows is Jo-1.

ELISA monitoring of extractible antinuclear antibodies—Sm and CIC made, it possible to identify characteristic changes in serum specimens that are significantly in correlation with disease activity in patients with SLE [43]. Despite some reported prospective studies that suggest no correlation of those immunomarkers and lupus flares and disease activity in this study, we noticed the correlation between Sm antibody as well as CIC and disease activity. It is of note that those two did not show significant correlation among themselves [44–48]. 97% of patients had a positive ANA antibody testing that is usual in some other researches. Anti-dsDNA is less sensitive but more specific for SLE diagnosis [44, 45]. They can be found in the sera of 55% to 80% patients with SLE but not in the sera of the healthy controls which is also confirmed in our study. Most of the investigators indicate that anti-dsDNA antibodies are useful markers of SLE overall activity of SLE [44].

An antibody to Sm, a ribonucleoprotein found in the nucleus of a cell, is almost exclusively present in people with lupus. It is present in 20% of people with the disease but it is rarely found in people with other rheumatic diseases and its incidence in healthy individuals is less than 1%. Therefore, it can be helpful in confirming the diagnosis of systemic lupus [49–56]. Table 2 shows optimal cut-offs, with high confidence interval, sensitivity of 70%, and specificity of 100%.

Detecting ANA, Op De Beéck et al. compared the results obtained using indirect immunofluorescence (IIF) and BioPlex 2200, and discovered that BioPlex test result interval-specific likelihood ratios increased with increasing antibody concentration. BioPlex provided presence of at least three antibodies simultaneously. They concluded that test result specific likelihood ratios and the presence of multiple autoantibodies help with the interpretation of data generated by multiplex immunoassays [57].

Circulating immune complexes are present in many individuals with SLE and rheumatoid arthritis (RA), especially in those with any of the vasculitis complications. Levels of CICs have been reported to show correlation with disease activity, especially during active phases of the disease [45–48, 56]. In this study, results from ROC curve are suggesting that CIC might be even better marker for SLE activity than Smith antigen.

Table 4: Optimal cut-off, sensitivity, specificity, positive and negative predictive value of ENA6 SM, and CIC in between SLE patients and healthy control.

	SLE patients versus healthy control				
Marker	Optimal cut-off	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Sm/RNP	≥8,33 EU	75%	100%	100%	81%
Jo-1	≥1,005 EU	83%	90%	89%	84%
SCL-70	≥0,385 EU	96%	80%	83%	96%
ENA6 Sm	≥9,56 EU	70%	100%	100%	76%
ENA6 SS-A	≥5,90 EU	64%	76%	72%	69%
ENA6 SS-B	≥5,44 EU	60%	73%	69%	65%

SLE: systemic lupus erythematosus; EU: elisa units.

Our results have revealed that all of the used biomarkers do accompany the diagnosis of active SLE but their use as a solo marker does not allow classification of SLE patients. Furthermore, it can be concluded that laboratory tests are no more effective than clinical examinations for detecting disease relapse, but are helpful for confirming the activity of SLE.

Conflict of Interests

The authors declare that they have no conflict of interests with the Autostat.

References

[1] D. L. Jacobson, S. J. Gange, N. R. Rose, and N. M. H. Graham, "Epidemiology and estimated population burden of selected autoimmune diseases in the United States," *Clinical Immunology and Immunopathology*, vol. 84, no. 3, pp. 223–243, 1997.

[2] P. Marrack, J. Kappler, and B. L. Kotzin, "Autoimmune disease: why and where it occurs," *Nature Medicine*, vol. 7, no. 8, pp. 899–905, 2001.

[3] M. R. Salaman, "A two-step hypothesis for the appearance of autoimmune disease," *Autoimmunity*, vol. 36, no. 2, pp. 57–61, 2003.

[4] M. J. Townsend, J. G. Monroe, and A. C. Chan, "B-cell targeted therapies in human autoimmune diseases: an updated perspective," *Immunological Reviews*, vol. 237, no. 1, pp. 264–283, 2010.

[5] M. Tikly and S. V. Navarra, "Lupus in the developing world—is it any different?" *Best Practice and Research: Clinical Rheumatology*, vol. 22, no. 4, pp. 643–655, 2008.

[6] K. Yasutomo, "Pathological lymphocyte activation by defective clearance of self-ligands in sytemic lupus erythematosus," *Rheumatology*, vol. 42, no. 2, pp. 214–222, 2003.

[7] J. C. W. Edwards and G. Cambridge, "B-cell targeting in rheumatoid arthritis and other autoimmune diseases," *Nature Reviews Immunology*, vol. 6, no. 5, pp. 394–403, 2006.

[8] T. Dorner, A. M. Jacobi, and P. E. Lipsky, "B cells in autoimmunity," *Arthritis Research & Therapy*, vol. 11, p. 247, 2009.

[9] S. Yurasov, T. Tiller, M. Tsuiji et al., "Persistent expression of autoantibodies in SLE patients in remission," *Journal of Experimental Medicine*, vol. 203, no. 10, pp. 2255–2261, 2006.

[10] S. M. Orton, A. Peace-Brewer, J. L. Schmitz, K. Freeman, W. C. Miller, and J. D. Folds, "Practical evaluation of methods for detection and specificity of autoantibodies to extractable nuclear antigens," *Clinical and Diagnostic Laboratory Immunology*, vol. 11, no. 2, pp. 297–301, 2004.

[11] M. Mandel, M. Gurevich, R. Pauzner, N. Kaminski, and A. Achiron, "Autoimmunity gene expression portrait: specific signature that intersects or differentiates between multiple sclerosis and systemic lupus erythematosus," *Clinical and Experimental Immunology*, vol. 138, no. 1, pp. 164–170, 2004.

[12] F. Franceschini and I. Cavazzana, "Anti-Ro/SSA and La/SSB antibodies," *Autoimmunity*, vol. 38, no. 1, pp. 55–63, 2005.

[13] P. Migliorini, C. Baldini, V. Rocchi, and S. Bombardieri, "Anti-Sm and anti-RNP antibodies," *Autoimmunity*, vol. 38, no. 1, pp. 47–54, 2005.

[14] B. B. Suh-Lailam, T. R. Chiaro, A. R. Wilson, and A. E. Tebo, "Evaluation of a high avidity anti-dsDNA IgG enzyme-linked immunosorbent assay for the diagnosis of systemic lupus erythematosus," *Journal of Clinical and Experimental Pathology*, vol. 4, no. 8, pp. 748–754, 2011.

[15] R. A. Sinico, B. Bollini, E. Sabadini, L. Di Toma, and A. Radice, "The use of laboratory tests in diagnosis and monitoring of systemic lupus erythematosus," *Journal of Nephrology*, vol. 15, supplement 6, pp. S20–S27, 2002.

[16] S. M. Orton, A. Peace-Brewer, J. L. Schmitz, K. Freeman, W. C. Miller, and J. D. Folds, "Practical evaluation of methods for detection and specificity of autoantibodies to extractable nuclear antigens," *Clinical and Diagnostic Laboratory Immunology*, vol. 11, no. 2, pp. 297–301, 2004.

[17] E. C. LeRoy and T. A. Medsger Jr., "Criteria for the classification of early systemic sclerosis," *Journal of Rheumatology*, vol. 28, no. 7, pp. 1573–1576, 2001.

[18] Registrolesaf, "Registrolesaf. Features of patients with systemic lupus erythematosus and antiphospholipid syndrome registered by rheumatologists and internists," *Reumatología Clinica*, vol. 2, pp. 131–136, 2006.

[19] G. P. Tri, R. C. W. Wong, and S. Adelstein, "Autoantibodies to extractable nuclear antigens: making detection and interpretation more meaningful," *Clinical and Diagnostic Laboratory Immunology*, vol. 9, no. 1, pp. 1–7, 2002.

[20] 1997 Update of the 1982 American College of Rheumatology Revised Criteria for Classification of Systemic Lupus Erythematodes, American College of Rheumatology.

[21] S. Malik, G. R. Bruner, C. Williams-Weese et al., "Presence of anti-La autoantibody is associated with a lower risk of nephritis and seizures in lupus patients," *Lupus*, vol. 16, no. 11, pp. 863–866, 2007.

[22] Blood Tests, The Lupus Site, 2009, http://www.uklupus.co.uk/tests.html.

[23] K. F. Thomson, A. Murphy, M. J. D. Goodfield, and S. A. Misbah, "Is it useful to test for antibodies to extractable

nuclear antigens in the presence of a negative antinuclear antibody on Hep-2 cells?" *Journal of Clinical Pathology*, vol. 54, no. 5, p. 413, 2001.

[24] S. Hiroshi, "Immunity. The measuring method of anti-ENA antibody," *Modern Medical Laboratory*, vol. 31, no. 6, pp. 495–499, 2003.

[25] P. Eissfeller, M. Sticherling, D. Scholz et al., "Comparison of different test systems for simultaneous autoantibody detection in connective tissue diseases," *Annals of the New York Academy of Sciences*, vol. 1050, pp. 327–339, 2005.

[26] R. Lyons, S. Narain, C. Nichols, M. Satoh, and W. H. Reeves, "Effective use of autoantibody tests in the diagnosis of systemic autoimmune disease," *Annals of the New York Academy of Sciences*, vol. 1050, pp. 217–228, 2005.

[27] M. M. A. Fernando and D. A. Isenberg, "How to monitor SLE in routine clinical practice," *Annals of the Rheumatic Diseases*, vol. 64, no. 4, pp. 524–527, 2005.

[28] I. Siddiqui, J. Farooqi, F. Ghani, A. Habib, and D. A. Shariff, "Pattern of anti-ENA (extractable nuclear antigens) antibodies in different clinical desorders, a clinical laboratory based study," *International Journal of Pathology*, vol. 5, no. 2, pp. 67–71, 2007.

[29] H. C. Nossent and O. P. Rekvig, "Is closer linkage between systemic lupus erythematosus and anti-double-stranded DNA antibodies a desirable and attainable goal?" *Arthritis Research and Therapy*, vol. 7, no. 2, pp. 85–87, 2005.

[30] E. Topic, D. Primorac, and S. Jankovic, "Medicinsko biohemijska dijagnostika u klinickoj praksi," *Medicinska Naklada Zagreb*, pp. 295–296, 2004.

[31] H. C. Sox, M. A. Blatt, M. C. Higgins, and K. Marton, *Medical Decision Making*, Butterworth, London, UK, 1989.

[32] X. Zhou, N. A. Obuchowski, D. K. McClish, X. Zhou, N. A. Obuchowski, and D. K. McClish, *Statistical Methods in Diagnostic Medicine*, John Wiley and Sons, New York, NY, USA, 2002.

[33] D. J. Hand, "Measuring classifier performance: a coherent alternative to the area under the ROC curve," *Machine Learning*, vol. 77, no. 1, pp. 103–123, 2009.

[34] M. S. Pepe, *The Statistical Evaluation of Medical Tests for Classification and Prediction*, Oxford University Press, New York, NY, USA, 2003.

[35] B. Hanczar, J. Hua, C. Sima, J. Weinstein, M. Bittner, and E. R. Dougherty, "Small-sample precision of ROC-related estimates," *Bioinformatics*, vol. 26, no. 6, pp. 822–830, 2010.

[36] M. C. Hochberg, "Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 40, no. 9, p. 1725, 1997.

[37] K. Smykał-Jankowiak and Z. I. Niemir, "Structure and function of complement protein C1q and its role in the development of autoimmune diseases," *Postępy Higieny i Medycyny Doświadczalnej*, vol. 63, pp. 134–141, 2009.

[38] M. Olesińska, H. Chwalińska-Sadowska, E. Więsik-Szewczyk, P. Mielnik, and J. Zabek, "Clinical manifestation of systemic lupus erythematosus in patients with antiribosomal P protein antibodies," *Polskie Archiwum Medycyny Wewnetrznej*, vol. 120, no. 3, pp. 76–81, 2010.

[39] S. Agarwal, J. Harper, and P. D. W. Kiely, "Concentration of antibodies to extractable nuclear antigens and disease activity in systemic lupus erythematosus," *Lupus*, vol. 18, no. 5, pp. 407–412, 2009.

[40] T. T. Tran and D. S. Pisetsky, "Systemic lupus erythematosus and related diseases," *Autoimmunity*, vol. 37, no. 4, pp. 301–304, 2004.

[41] S. S. Copple, T. B. Martins, C. Masterson, E. Joly, and H. R. Hill, "Comparison of three multiplex immunoassays for detection of antibodies to extractable nuclear antibodies using clinically defined sera," *Annals of the New York Academy of Sciences*, vol. 1109, pp. 464–472, 2007.

[42] A. Ippolito, D. J. Wallace, D. Gladman et al., "Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity," *Lupus*, vol. 20, no. 3, pp. 250–255, 2011.

[43] M. Mosca, C. Tani, M. Aringer et al., "European league against rheumatism recommendations for monitoring patients with systemic lupus erythematosus in clinical practice and in observational studies," *Annals of the Rheumatic Diseases*, vol. 69, no. 7, pp. 1269–1274, 2010.

[44] D. A. Isenberg, J. J. Manson, M. R. Ehrenstein, and A. Rahman, "Fifty years of anti-ds DNA antibodies: are we approaching journey's end?" *Rheumatology*, vol. 46, no. 7, pp. 1052–1056, 2007.

[45] C. C. Mok, L. Y. Ho, H. W. Leung, and L. G. Wong, "Performance of anti-C1q, antinucleosome, and anti-dsDNA antibodies for detecting concurrent disease activity of systemic lupus erythematosus," *Translational Research*, vol. 156, no. 6, pp. 320–325, 2010.

[46] C. Bigler, H. Hopfer, D. Danner, M. Schaller, M. J. Mihatsch, and M. Trendelenburg, "Anti-C1q autoantibodies do not correlate with the occurrence or severity of experimental lupus nephritis," *Nephrology Dialysis Transplantation*, vol. 26, no. 4, pp. 1220–1228, 2011.

[47] R. A. Sinico, L. Rimoldi, A. Radice, L. Bianchi, B. Gallelli, and G. Moroni, "Anti-C1q autoantibodies in lupus nephritis," *Annals of the New York Academy of Sciences*, vol. 1173, pp. 47–51, 2009.

[48] M. Petri, "Disease activity assessment in SLE: do we have the right instruments?" *Annals of the Rheumatic Diseases*, vol. 66, supplement 3, pp. iii61–iii64, 2007.

[49] A. Rahman and D. A. Isenberg, "Systemic lupus erythematosus," *The New England Journal of Medicine*, vol. 358, no. 9, pp. 929–939, 2008.

[50] L. B. Tucker, "Making the diagnosis of systemic lupus erythematosus in children and adolescents," *Lupus*, vol. 16, no. 8, pp. 546–549, 2007.

[51] D. P. D'Cruz, M. A. Khamashta, and G. R. Hughes, "Systemic lupus erythematosus," *The Lancet*, vol. 369, no. 9561, pp. 587–596, 2007.

[52] M. M. A. Fernando and D. A. Isenberg, "How to monitor SLE in routine clinical practice," *Annals of the Rheumatic Diseases*, vol. 64, no. 4, pp. 524–527, 2005.

[53] F. G. Joseph and N. J. Scolding, "Neurolupus," *Practical Neurology*, vol. 10, no. 1, pp. 4–15, 2010.

[54] L. H. Mackillop, S. J. Germain, and C. Nelson-Piercy, "Systemic lupus erythematosus," *British Medical Journal*, vol. 335, no. 7626, pp. 933–936, 2007.

[55] R. Madhok and O. Wu, "Systemic lupus erythematosus," *American Family Physician*, vol. 76, no. 9, pp. 1351–1353, 2007.

[56] C. C. Mok, "Biomarkers for lupus nephritis: a critical appraisal," *Journal of Biomedicine and Biotechnology*, vol. 2010, Article ID 638413, 11 pages, 2010.

[57] K. Op De Beéck, P. Vermeersch, P. Verschueren et al., "Antinuclear antibody detection by automated multiplexim-munoassay in untreated patients at the time of diagnosis," *Autoimmunity Reviews*. In press.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 589359, 10 pages
doi:10.1155/2012/589359

Review Article

Intravenous Immunoglobulin in the Management of Lupus Nephritis

Scott E. Wenderfer[1] and Trisha Thacker[2]

[1] *Department of Pediatrics, Renal Section, Baylor College of Medicine, 1102 Bates Street, Suite 260, Houston, TX 77030, USA*
[2] *University of Houston, Houston, TX 77004, USA*

Correspondence should be addressed to Scott E. Wenderfer, wenderfe@bcm.edu

Received 5 May 2012; Revised 24 July 2012; Accepted 25 July 2012

Academic Editor: Ricard Cervera

The occurrence of nephritis in patients with systemic lupus erythematosus is associated with increased morbidity and mortality. The pathogenesis of lupus nephritis is complex, involving innate and adaptive cellular and humoral immune responses. Autoantibodies in particular have been shown to be critical in the initiation and progression of renal injury, via interactions with both Fc-receptors and complement. One approach in the management of patients with lupus nephritis has been the use of intravenous immunoglobulin. This therapy has shown benefit in the setting of many forms of autoantibody-mediated injury; however, the mechanisms of efficacy are not fully understood. In this paper, the data supporting the use of immunoglobulin therapy in lupus nephritis will be evaluated. In addition, the potential mechanisms of action will be discussed with respect to the known involvement of complement and Fc-receptors in the kidney parenchyma. Results are provocative and warrant additional clinical trials.

1. Introduction

Intravenous immunoglobulin (IVIg) is a biological agent composed of polyclonal antibodies, derived from the plasma of a large pool of healthy donors [1–5]. It has been primarily used to treat hypogammaglobulinemia but has also shown promise in treating autoimmune diseases, inflammatory diseases, and cancer. It is FDA approved for the treatment of idiopathic thrombocytopenic purpura (ITP) and Kawasaki's vasculitis. Several anecdotal reports and a few studies have shown promising results on the effectiveness of IVIg in the treatment of systemic lupus erythematosus (SLE). Its use has been widespread; however, its efficacy has not been clearly established.

The precise mechanisms by which IVIg functions as an anti-inflammatory agent remains debatable (Table 1) [1, 6–8]. The presence of Ig in the preparations with specificity for variable regions of pathogenic autoantibodies (anti-idiotype responses) can allow for direct binding and neutralization of pathogenic effector functions. The effector functions of autoantibodies are mediated by receptors for constant regions of IgG (FcR) or receptors for complement components that bind to antigen antibody immune complexes. Complement receptors and FcR are expressed on lymphocytes, granulocytes, monocytes, and some parenchymal cells and can induce tissue injury once bound to immune complexes containing pathogenic antibodies. Monomeric IgG in IVIg preparations has been shown to antagonize pathologic immune complexes binding to activating FcR [9]. Alternatively, nonspecific polyclonal Ig can form immune complexes that bind to inhibitory-type Fc-receptors [6]. These inhibitory Fc-receptors then dampen the effector functions of the activating-type FcR and complement receptors [10–12]. IVIg can similarly augment the clearance of pathogenic autoantibodies via the reticuloendothelial system (RES). The RES uses complement receptors and Fc-R on circulating erythrocytes and monocytes to target immune complexes to the spleen and liver, where they are eliminated [13–15]. In addition, the expression of inhibitory FcR can be upregulated by IVIg, tipping the balance of activating and inhibitory responses. This can modulate cellular immune responses in addition to humoral responses [16]. Exogenous

Table 1: Potential mechanisms of action of IVIg.

(i) Anti-idiotypic binding that neutralizes auto-Abs
(ii) Competitive inhibition of binding to activating Fc-receptors
(iii) Upregulation of inhibitory Fc-receptors
(iv) Delayed clearance of Ab-coated blood cells
(v) Increased clearance of auto-Abs by reticuloendothelial system
(vi) Decreased half-life of auto-Abs due to competitive binding to FcRn

Ig can saturate binding sites on the neonatal FcR, a related receptor with widespread expression that usually accounts for the long half-life of albumin and Ig molecules in serum, via protection from lysosomal degradation [17]. This can lead to increased degradation of auto-Abs and reduction of auto-Ab titers. Finally, antibodies with a particular specificity that naturally exist in normal individuals may exert anti-inflammatory effects, such as has been described for the neutralization of basophil and B-cell cytokines BAFF and APRIL by IVIg [18]. It is likely that IVIg works in part through each of these proposed mechanisms.

IVIg preparations can vary in composition depending on the source and method of preparation [19]. This may account for variability in effect when used clinically. The purity of IVIg can vary from 90% to 98%, and the elimination of IgA, IgE, and IgM can vary widely. The range of IgA content extends from 2.9 mcg/mL to 200 mcg/mL. The glycosylation of Ig molecules can vary as well, which has recently been shown to be important for IVIg's effectiveness in immunosuppression [1, 20]. Preparations can also vary according to the preservative used. Brands containing maltose and sucrose are associated with an increased risk of renal toxicity. Therefore, several brands are now available that utilize preservatives such as glycine or L-proline instead of sugars. Moreover, the osmolality can range from 240 to 636 mOsm/kg. All commercial preparations contain some detectable titer of autoantibodies, including antiphospholipid and anti-DNA Abs. They also contain anti-idiotype Abs specific for antiphospholipids, anti-DNA Abs. Overall, the several preparations of IVIg available allow for more individualized therapy depending on the patient's needs.

In order to better understand the risks and benefits of using IVIg for the management of patients with lupus nephritis, a review of the literature was undertaken to abstract the available evidence on efficacy and safety. In addition, the potential mechanisms of action of IVIg that might occur when administered to patients with lupus nephritis are considered by reviewing the literature on parenchymal Fc-receptors in the kidney.

2. Methods

Methods used were modeled from other systematic reviews of treatments for lupus nephritis [21–23]. Briefly, studies of any design in English were sought concerning IVIg in the treatment of patients with complications of lupus nephritis or SLE. Free text searches were undertaken to identify eligible reports from MEDLINE/PubMed (to April 2012) using the terms IVIg or "intravenous immunoglobulin" and the terms "lupus nephritis" or "lupus erythematosus." Additional trials were sought in review articles [2–5] and reference lists of retrieved articles. For completeness, we included randomized trials, cohort studies, and case reports. When it could be determined that the same patients were included in multiple publications, we used the largest body of data, the most informative, or the most recent (as appropriate). Reviews with clinical information published in a fuller form elsewhere were excluded, as were studies in which IVIg was used for treating other conditions.

Two reviewers extracted information from the identified publications independently, and disagreement was resolved by consensus. Information extracted included the number of patients treated, dosing regimens, duration of therapy, additional treatments provided, any demographic information (age, gender, and ethnicity), definitions or classifications of lupus nephritis (WHO was used), study design, efficacy and/or safety outcomes, and drop-out rates. Any definition of lupus nephritis, as provided by authors, was accepted. If studies included patients who did not have lupus nephritis, efficacy information was only used for analysis if reported separately for nephritis patients. Efficacy outcomes sought were those of complete or partial renal response rates as defined by the original authors (mainly urine protein excretion, serum creatinine or creatinine clearance, renal survival, or a combination) and subsequent relapse rates. Adverse events sought included mortality, infections, cytopenias, gastrointestinal problems, amenorrhea, azoospermia, and hospitalization rates. Information of adverse events in all patients receiving IVIg was included as there is no data suggesting that adverse events would be different in patients with or without nephritis [21].

Table 2: Studies identified in review of literature on IVIG therapy for lupus nephritis.

Articles retrieved	630
Articles excluded	598
(i) IVIg use not discussed	144
(ii) Use limited to animal/*in vitro* models	49
(iii) Review article	136
(iv) Use in SLE not discussed	38
(v) Use in SLE limited to non-renal disease	217
(vi) Patients reported in subsequent manuscript	5
(vii) Manuscript not available in English*	11
Articles included	32 (22 efficacy/9 adverse effects/1 both)

* Includes 33 patients with lupus nephritis amongst those reported.

3. Results

An initial literature search identified 630 potential articles for review, of which 32 were found to address the use of IVIg in lupus nephritis (Table 2). Several treatment regimens

using different dosages have been reported, most commonly 400 mg/kg/day for 4-5 daily doses (high dose, standard dosing for ITP), although 400 mg/kg/dose 1 × monthly, or 85 mg/kg/day for 4-5 days (low dose), has also been described. In SLE patients, there is no published data on how long exogenous Ig remains present after administration, and there is a lack of consensus on dosing intervals and the duration of therapy. Most publications fail to specify the source of IVIg administered, which precludes analysis of individual preparation methods. As has been pointed out before, comparison of outcomes between studies is hampered by counting day 1 as first day of treatment, or the day of kidney biopsy, rather than first day of symptoms [24, 25].

The short-term effects of IVIg were reported initially in 1989 in three patients with mild SLE who had been treated with 300–500 mg/kg/dose, 1 dose every 4 weeks [26]. Although this low dose had been effective in some autoimmune neurological diseases, the immediate effects on auto-Ab titers in these SLE patients were modest. When the high-dose regimen (400 mg/kg/day for 5 days) was used, greater reductions in auto-Ab titers were noted [26]. In a cohort study from Germany, 12 patients with mild-to-moderate disease given 2 courses of high-dose IVIG had a decline in anti-dsDNA Abs within 1 week. Antinuclear antibodies and complement protein levels were not affected. Within 6 weeks, improvements were noted in clinical disease activity scores which lasted 5 to 12 months [27]. In an Israeli cohort study, clinical disease scores were also improved in 62 patients receiving low-dose IVIg (~500 mg/kg/dose once every 5 ± 2 weeks for a mean of 6 doses) [28]. Unsatisfactory responses were noted for thrombocytopenia, alopecia, vasculitis, and proteinuria. Based on these reports, dosage appears to be important.

Several case reports were identified describing the use of IVIg for various nonrenal manifestations of SLE. The results described were encouraging (Table 3) but have infrequently led to larger prospective trials [29–37, 39]. Benefits in treating cutaneous lupus have not been reproducible [40]. Cohorts ($n = 26$) with autoimmune hemolytic anemia [41] and with thrombocytopenia ($n = 59$) [42] have been published and showed short-term benefits without sustained responses. Positive effects on achievement of live births have been reported in pregnant women with SLE and antiphospholipid antibodies after treatment with IVIg [38], but randomized controlled trials demonstrated equivalency or even inferiority of IVIg compared to heparin and aspirin [43–45]. However, investigators continue to search for subsets of SLE patients who will benefit from IVIg, and clinicians continue to prescribe it for SLE patients who fail initial therapy regimens. In a cohort study from Israel, 20 patients with SLE and organ specific disease involvement were treated with 1–8 courses of high-dose IVIg [46]. Improvements in clinical disease scores, hypocomplementemia, and autoantibody titers were seen in 80% of patients. However, when looking at organ specific response rates, the improvements were seen more in CNS disease, arthritis, fever, and thrombocytopenia than in proteinuria.

Few studies have followed their patients for long term. A 2012 study from Israel followed patients for a mean of 30 months after initiating therapy [47]. Eleven patients with SLE were treated with high-dose IVIG monthly for 6 months, followed by additional courses given every 2-3 months. At latest followup, 6 patients had complete remission, 3 had partial remissions, and 2 patients were nonresponders, defined by improvements in clinical disease scoring. In responders, IVIG had a significant steroid-sparing effect. Adverse effects were reported in 18% of patients during their first course of IVIg, and 50% of all patients treated. Common adverse effects included headache, fatigue, nausea, visual disturbances, and limb pain. Adverse effects resulted in truncation of 8 courses, and two patients suffered severe effects (seizure, pulmonary embolus).

Table 3: Initial case reports of IVIg therapy of SLE manifestations.

Hjortkjaer Petersen et al. [29]	1990	Pericarditis
Maier et al. [30]	1990	Thrombocyto penia
Tomer and Shoenfeld [31]	1992	Psychosis
Lesprit et al. [32]	1996	Polyneuritis
Aharon et al. [33]	1997	Myelofibrosis
Généreau et al. [34]	1999	Cutaneous lupus
Sherer et al. [35]	1999	Cerebritis
Sherer et al. [36]	1999	Myocarditis
Meissner et al. [37]	2000	Serositis
Sherer et al. [38]	2000	Antiphospholipid syndrome
Hoshi et al. [39]	2004	Pulmonary hemorrhage

3.1. Salvage Therapy of Refractory Lupus Nephritis. Most studies identified that reported efficacy of IVIg in lupus nephritis restricted entry to patients who had failed their initial induction therapy of IV corticosteroids and cytotoxic agents. Twelve case reports were identified (Table 4). The first cases were published in 1982 in Japan, with subsequent cases from Europe and North America [37, 48–55]. Patients received 1 or 2 courses of high-dose IVIg in combination with corticosteroids, with or without plasma exchange or cytotoxic agents. By biopsy, the responders had class II, III, IV, or V nephritis. Patients recovered renal function with reductions in proteinuria and reduced immune deposits on repeat biopsy.

Two cohort studies of high-dose IVIg were identified. In an Israeli cohort, 7 patients with biopsy proven class IV or V nephritis were treated with 1 to 6 courses of high-dose IVIg after failing therapy with IV cyclophosphamide and prednisone [56]. All patients had nephrotic syndrome. All 7 experienced decreases in proteinuria and improvement or resolution of nephrotic syndrome. One patient had a complete remission which persisted at least three years. Only one patient had a relapse, which occurred 4 months after discontinuation of the IVIg [56]. In an Italian cohort study, 12 treatment refractory patients with SLE were treated with 6–24 monthly courses of high-dose IVIg [57]. A progressive clinical improvement was observed in 11 patients, associated with increases complement protein levels and decreases in auto-Abs, and marked improvements in renal function and proteinuria.

Table 4: Studies included in IVIg therapy for lupus nephritis.

Study	N^*	Study design
Sherer et al. 2008 [28]	62	Cohort, 6 courses LD*
Meissner et al. 2000 [37]	1	Case, 1 course HD
Levy et al. 1999 [46]	5	Cohort, 1–8 courses HD*
Akashi et al. 1990 [48]	2	Cohort, 1–3 courses HD
Oliet et al. 1992 [49]	1	Case, 1 course HD
Winder et al. 1993 [50]	2	Cohort, 10–20 courses HD
Arahata et al. 1999 [51]	1	Case RPGN, 1 course HD
Viertel et al. 2000 [52]	1	Case AKI, 2 courses HD
Gan et al. 2002 [53]	4	Cohort, 1 course HD
Kamali et al. 2005 [54]	4	Cohort, 1–6 courses HD
Micheloud et al. 2006 [55]	1	Case pregnancy, 1 course HD
Levy et al. 2000 [56]	7	Cohort, 1–6 courses HD
Francioni et al. 1994 [57]	12	Cohort, 6–24 courses HD
Monova et al. 2002 [58]	58	Cohort, LD up to 7 years
Bridoux et al. 1998 [59]	3	Cohort AKI/TMA, 1 course HD
Becker et al. 1995 [60]	2	ESRD cohort, courses HD
Lin et al. 1989 [61]	9	Pediatric cohort, 1-2 courses HD
Welch et al. 1995 [62]	1	Case, 6 courses HD (induction)
Silvestris et al. 1996 [63]	3	Cohort, 2 courses HD
Boletis et al. 1999 [64]	14	RCT, 18 courses LD (induction)
Corvetta et al. 1989 [65]	3	Cohort, courses HD
Zandman-Goddard et al. 2012 [47]	11	Cohort, 2–17 courses HD
Chacko et al. 2006 [66]	1	Case, 1 course HD
Tan et al. 2008 [67]	1	Case, 1 course HD
Ng 1999 [68]	1	Case, 1 course HD
Ben-Chetrit et al. 1991 [69]	1	Case, 2 courses HD
Pasatiempo et al. 1994 [70]	3	Cohort, 1 course
Barron et al. 1992 [71]	6	Pediatric cohort
CDC MMWR 1999 [72]	120	Registry, includes non-SLE
Sati et al. 2001 [73]	55	Cohort, includes non-SLE
Orbach et al. 2004 [74]	106	Literature review

*Number of patients in study with lupus nephritis receiving IVIg where efficacy or adverse events could be defined based on information provided. LD: low-dose IVIg, HD: high-dose IVIg.

One cohort study was identified that used a longer course of lower-dose IVIg. In a Bulgarian cohort study of patients with all forms of treatment refractory chronic glomerulonephritis, better outcomes were reported in the 58 patients who had SLE [58]. All patients were treated with a low-dose IVIg regimen, 85 mg/kg/day on alternate days, for a total of three days, repeated quarterly for up to 7 years. At the conclusion of the study, 30% of the patients with lupus nephritis achieved full remission (unchanged or improved renal function, resolution of nephrotic syndrome, and proteinuria <0.5 gram/day) and 40% patients achieved partial remission (unchanged or improved renal function, improvement in nephrotic syndrome, and proteinuria <1.5 gram/day). Of nonresponders, nearly all died or survived with end-stage renal disease (ESRD), indicating the severity of disease in this cohort. Reported adverse effects were fever, chills, nausea, vomiting, headache, and rash, and none occurred in more than 10% of individuals.

Additional cohort studies were identified which involved important subsets of patients with SLE. One study demonstrated efficacy of a single course of IVIg in three patients with acute kidney injury from combined inflammatory nephritis and thrombotic microangiopathy [59]. The only study of IVIg treatment in patients with ESRD involved 2 patients with symptomatic SLE on dialysis treated with high-dose IVIg [60]. Both patients demonstrated clinical and serologic improvement and tolerated the IVIg administration well. There were only transient declines in serum albumin concentrations noted, which might reflect saturation of neonatal FcRs that protect albumin from lysosomal degradation [75]. In the only report of IVIg use in children with lupus nephritis, 9 children with biopsy-proven class IV or V nephritis were treated with high-dose IVIg [61]. These children had not responded to pulse methylprednisolone or intravenous cyclophosphamide. Five of 8 with class IV nephritis saw marked improvement in renal function and

decreases in IgG deposits on repeat biopsy, while the remaining three experienced a reduction in their class of nephritis. The sole patient with class V disease had a partial renal response. Occasional fever, chills, hypotension, and rash were reported in these cohorts, but overall prevalence of adverse effects is unclear from the literature.

Overall, the response rates to the various IVIg regimens are promising. However, all of the studies were uncontrolled trials that leave open the possibility that these patients would have done just as well without the IVIg, as there is known to be delayed benefits of IV solumedrol, IV cyclophosphamide, azathioprine, and other immunosuppressants.

3.2. Use of IVIg as Part of Induction Therapy for Lupus Nephritis. Only a few studies were identified that treated lupus nephritis with IVIg as part of the initial therapy. The numbers of patients treated are small, and the doses administered tended to be lower. One case report reported efficacy and safety in a patient with a complement deficiency [62]. As part of the 1999 cohort study from Israel mentioned above, 5 of the 20 patients treated with 1–8 courses of high-dose IVIg had renal involvement, ranging from mild proteinuria or nephrotic syndrome [46]. Improvements in renal disease were noted, but were inferior to those reported in the CNS disease, arthritis, and thrombocytopenia. Patients with nephritis in the 2008 cohort study also showed some response: resolution of urinary casts in 88% of patients, but of proteinuria in only 20% [28]. Conversely, An Italian cohort of three patients with renal flare failed to respond to 2 courses of high-dose IVIg and steroids [63].

In a randomized study of 14 patients with class IV lupus nephritis, IVIg was compared to intravenous cyclophosphamide [64]. Five patients received low-dose IVIg (400 mg/kg) monthly for eighteen months. Nine patients received cyclophosphamide 1 g/m^2 IV every two months for six months, then every three months for twelve months. The method of randomization was not described, and it is unclear if there was any dropout after randomization. Patients could also be treated with prednisone at the physicians' discretion. After the 18-month treatment period, a marked improvement in renal function (both serum creatinine and creatinine clearance) was noted in both groups. IVIg was not shown to be inferior to this dose of IV cyclophosphamide. However, IVIg had a modest steroid sparing effect over cyclophosphamide.

The utility of IVIg as first line therapy for lupus nephritis, therefore, remains unclear. No study has compared IVIg to the most commonly used IV cyclophosphamide regimens (NIH, Eurolupus protocols) or to mycophenolate. In addition, no study of IVIg as add on therapy with any of these induction regimens has been published.

3.3. The Use of IVIg as Maintenance Therapy for Lupus Nephritis. No studies were identified using IVIg as a maintenance therapy for lupus nephritis.

3.4. Adverse Effects of IVIg in SLE. Most patients with SLE and nephritis tolerated their IVIg therapies. However, deterioration of renal function following IVIG treatment has been recognized [65, 66, 70, 72–74, 76]. A CDC report cited 120 cases of nephrotoxicity worldwide, with only 26% of cases occurring in patients with preexisting renal disease [72]. Incidence has been reported in 10–33% of some cohorts [65, 73]. Determining the etiology of acute kidney injury in patients with SLE can be challenging. The majority of nephrotoxicity cases prior to 2000 (90%) have been attributed to IVIg preparations utilizing the stabilizers maltose and sucrose. Intracellular accumulation of these sugars leads to cellular swelling and vacuole formation in tubular epithelial cells of the kidney [74]. Additional risk factors for renal toxicity following IVIG therapy include age >70 years, renal impairment pre-treatment, and diabetes mellitus [73]. In addition to the risk of AKI, IVIg was associated with renal flares [74]. The risk appears to be greater in children, as 3 of 6 patients treated with IVIg in one pediatric cohort from Toronto developed renal flare [71]. Patients with anti-phospholipids or other thrombophilias should receive aspirin therapy to minimize risks for thrombosis associated with infusions of IVIg [67, 74]. Mild adverse reactions of IVIg are common and include infusion reactions, headache, dermatitis, hepatitis, pseudo-hyponatremia, neutropenia, and aseptic meningitis [47, 68, 69, 74]. Infusion reactions typically respond to slowing down the infusion rate, and the other reactions respond to withdrawal of IVIg infusions. Headaches are less frequent when high dose IVIg is given 400 mg/kg/day over 5 days than when given 1 gm/kg/day over 2 days [46].

Finally, the choice of IVIg preparation can be influenced by the SLE patient's comorbidities. In SLE patients with impaired glucose tolerance or diabetes mellitus, preparations with sucrose or maltose should be avoided to minimize risk of hyperglycemia. Patients with nephrotic syndrome or edema should receive more concentrated Ig preparations, whereas patients with renal insufficiency should receive preparations with lower osmolalities. In SLE patients with effective IgA deficiency, the use of IVIg preparations with higher levels is contraindicated as it can result in severe hypersensitivity and anaphylaxis.

4. Discussion

IVIg is an expensive therapy in finite supply as it requires blood donation from healthy donors. Its use in lupus nephritis must be decided upon a case-by-case basis. The treatment of lupus nephritis with IVIg shows promising results in reducing immune deposits in the kidney, reducing proteinuria, improving kidney function, and reducing necessary corticosteroid doses. However, IVIg use has been associated with adverse effects of renal flaring and acute tubular necrosis that can both lead to renal failure. Despite these risks, IVIg treatment appears to be a reasonable option in patients who are refractory to initial induction therapy. Further studies of the use of IVIg as an induction agent for new onset disease as well as renal flares are necessary before its use can be recommended as first line.

The potential mechanisms of action of IVIg are all applicable to patients with lupus nephritis. The pathogenesis of nephritis in lupus includes the accumulation of immune complexes (ICs) between autoantibodies and antigen. IC can form *in situ* or in the circulation. Circulating IC can be passively trapped in the kidney or can be actively bound by renal cells. There is abundant literature demonstrating that IC directly binds to renal parenchymal cells. Heat-aggregated IgG (HA-IgG) localizes to the kidney *in vivo* [77–81] and binds to glomerular mesangial cells [82–91] and glomerular visceral epithelial cells (podocytes) [92] *in vitro* with high specificity. Glomerular IC deposits can be induced in rats and mice by the injection of preformed IC [93–96]. *In vitro*, IC binding to mesangial cells results in IC internalization and activation of specific signaling pathways [83–87], cell proliferation, and release of proinflammatory cytokines and chemokines [90, 91]. Podocytes respond to HA-IgG with altered fibrinolytic activity [97]. Glomerular endothelial cell binding to preformed IC or HA-IgG leads to internalization of autoantibodies [98]. Clearance of pathogenic IC from the kidney by IVIg can act to minimize parenchymal cell activation in lupus patients.

Despite the widely reported findings of IgG accumulation in biopsies of patients with IC kidney diseases, the presence of IC receptors on renal cells has not been firmly established. Binding of IgG by leukocytes is mediated by Fc-receptors (FcγRs) [99]. Named for their ability to bind the Fc-region of IgG, they include FcγRI (activating receptor for monomeric IgG), FcγRII (inhibitory IC receptor), and FcγRIII and FcγRIV (activating IC receptors). Leukocytes internalize IC once bound to FcγR, resulting in IC degradation or cellular activation. FcγR activation plays a role in leukocyte migration, proliferation, cytokine production, hypersensitivity reactions, and peripheral tolerance [87]. There is a paucity of human biopsy studies describing parenchymal expression of FcγRs [100]. There are numerous reports demonstrating expression of receptors for IgG in cultured mesangial cells [91, 97, 101–107]. Constitutive expression of FcγRIII has been reported in rat mesangial cells [102]. Stimulating antibodies to rat FcγRIII activate the same pathways in rat mesangial cells as those activated by preformed IC [102–104]. FcγRIII has also been identified in human mesangial cells, and activation of this receptor induces cytokine production [103]. Other groups have failed to show basal expression of FcγRI or FcγRIII [108], although expression could be measured after stimulation with IFNγ and endotoxin [104, 105]. Mouse mesangial cells constitutively express FcγRII, but FcγRIII requires stimulation with IFNγ [91]. In podocytes, FcγR does not appear to be expressed, but expression of the neonatal FcR has been reported [109, 110]. All of these receptors could theoretically contribute to the accumulation of IgG in the kidney in SLE. IVIg could potentially alter the balance of inhibitory and activating Fc-receptors in the kidney or could saturate neonatal FcR resulting in more degradation or urinary excretion of pathogenic auto-Abs by the kidney.

There have been several advances in understanding the mechanisms of IVIg. Research into effects of parenchymal FcR may lead to new targets for treating renal manifestations of systemic rheumatologic diseases. Efficacy of recombinant Fc-fragments [111, 112] and sialylated Ig [113] in recapitulating the immunosuppressive effects of IVIg in animal models is revolutionizing the field. The potential development of recombinant reagents may allow for the proper prospective randomized controlled trials that have not been possible with IVIg due to its finite supply. For greatest impact, future studies of IVIg and these newer polyclonal Ig-based derivatives need to include standardized response measures and report specifics on the rates of previously identified adverse events. Patients with lupus nephritis would be an ideal population to perform these studies, as new treatments for lupus nephritis are badly needed.

Acknowledgments

This paper was funded by NIH DK081663 and the Victor Chaltiel Young Investigator Grant of the National Kidney Foundation (S. E. Wenderfer P. I.).

References

[1] F. Nimmerjahn and J. V. Ravetch, "The antiinflammatory activity of IgG: the intravenous IgG paradox," *The Journal of Experimental Medicine*, vol. 204, no. 1, pp. 11–15, 2007.

[2] L. Rauova, J. Lukac, Y. Levy, J. Rovensky, and Y. Shoenfeld, "High-dose intravenous immunoglobulins for lupus nephritis—a salvage immunomodulation," *Lupus*, vol. 10, no. 3, pp. 209–213, 2001.

[3] E. Toubi, A. Kessel, and Y. Shoenfeld, "High-dose intravenous immunoglobulins: an option in the treatment of systemic lupus erythematosus," *Human Immunology*, vol. 66, no. 4, pp. 395–402, 2005.

[4] G. Zandman-Goddard, Y. Levy, and Y. Shoenfeld, "Intravenous immunoglobulin therapy and systemic lupus erythematosus," *Clinical Reviews in Allergy and Immunology*, vol. 29, no. 3, pp. 219–228, 2005.

[5] J. Bayry, V. S. Negi, and S. V. Kaveri, "Intravenous immunoglobulin therapy in rheumatic diseases," *Nature Reviews Rheumatology*, vol. 7, no. 6, pp. 349–359, 2011.

[6] A. Samuelsson, T. L. Towers, and J. V. Ravetch, "Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor," *Science*, vol. 291, no. 5503, pp. 484–486, 2001.

[7] R. Clynes, "Protective mechanisms of IVIG," *Current Opinion in Immunology*, vol. 19, no. 6, pp. 646–651, 2007.

[8] F. Nimmerjahn and J. V. Ravetch, "Anti-inflammatory actions of intravenous immunoglobulin," *Annual Review of Immunology*, vol. 26, pp. 513–533, 2008.

[9] E. van Mirre, J. L. Teeling, J. W. M. van der Meer, W. K. Bleeker, and C. E. Hack, "Monomeric IgG in intravenous Ig preparations is a functional antagonist of FcγRII and FcγRIIIb," *Journal of Immunology*, vol. 173, no. 1, pp. 332–339, 2004.

[10] H. U. Lutz, P. Stammler, V. Bianchi et al., "Intravenously applied IgG stimulates complement attenuation in a complement-dependent autoimmune disease at the amplifying C3 convertase level," *Blood*, vol. 103, no. 2, pp. 465–472, 2004.

[11] A. M. Boruchov, G. Heller, M. C. Veri, E. Bonvini, J. V. Ravetch, and J. W. Young, "Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions," *The Journal of Clinical Investigation*, vol. 115, no. 10, pp. 2914–2923, 2005.

[12] S. Konrad, U. Baumann, R. E. Schmidt, and J. E. Gessner, "Intravenous immunoglobulin (IVIG)-mediated neutralisation of C5a: a direct mechanism of IVIG in the maintenance of a high FcγRIIB to FcγRIII expression ratio on macrophages," *British Journal of Haematology*, vol. 134, no. 3, pp. 345–347, 2006.

[13] B. Benacerraf, M. Sebestyen, and N. S. Cooper, "The clearance of antigen antibody complexes from the blood by the reticuloendothelial system," *Journal of Immunology*, vol. 82, no. 2, pp. 131–137, 1959.

[14] J. C. Edberg, G. A. Kujala, and R. P. Taylor, "Rapid immune adherence reactivity of nascent, soluble antibody/DNA immune complexes in the circulation," *Journal of Immunology*, vol. 139, no. 4, pp. 1240–1244, 1987.

[15] J. A. Schifferli and R. P. Taylor, "Physiological and pathological aspects of circulating immune complexes," *Kidney International*, vol. 35, no. 4, pp. 993–1003, 1989.

[16] R. M. Anthony, T. Kobayashi, F. Wermeling, and J. V. Ravetch, "Intravenous gammaglobulin suppresses inflammation through a novel T_H2 pathway," *Nature*, vol. 475, no. 7354, pp. 110–113, 2011.

[17] J. P. Abonia, D. S. Friend, W. G. Austen et al., "Mast cell protease 5 mediates ischemia-reperfusion injury of mouse skeletal muscle," *Journal of Immunology*, vol. 174, no. 11, pp. 7285–7291, 2005.

[18] L. Le Pottier, T. Sapir, B. Bendaoud, P. Youinou, Y. Shoenfeld, and J. O. Pers, "Intravenous immunoglobulin and cytokines: focus on tumor necrosis factor family members BAFF and APRIL," *Annals of the New York Academy of Sciences*, vol. 1110, pp. 426–432, 2007.

[19] J. S. Orange, E. M. Hossny, C. R. Weiler et al., "Use of intravenous immunoglobulin in human disease: a review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology," *Journal of Allergy and Clinical Immunology*, vol. 117, no. 4, supplement, pp. S525–S553, 2006.

[20] Y. Kaneko, F. Nimmerjahn, and J. V. Ravetch, "Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation," *Science*, vol. 313, no. 5787, pp. 670–673, 2006.

[21] R. Andrew Moore and S. Derry, "Systematic review and meta-analysis of randomised trials and cohort studies of mycophenolate mofetil in lupus nephritis," *Arthritis Research and Therapy*, vol. 8, article R182, 2006.

[22] E. Fiore, M. Rizzi, M. Ragazzi et al., "Acute hemorrhagic edema of young children (cockade purpura and edema): a case series and systematic review," *Journal of the American Academy of Dermatology*, vol. 59, no. 4, pp. 684–695, 2008.

[23] M. Ramos-Casals, M. J. Soto, M. J. Cuadrado, and M. A. Khamashta, "Rituximab in systemic lupus erythematosus A systematic review of off-label use in 188 cases," *Lupus*, vol. 18, no. 9, pp. 767–776, 2009.

[24] J. M. Esdaile, L. Joseph, T. Mackenzie, M. Kashgarian, and J. P. Hayslett, "The benefit of early treatment with immunosuppressive agents in lupus nephritis," *The Journal of Rheumatology*, vol. 21, no. 11, pp. 2046–2051, 1994.

[25] J. M. Esdaile, L. Joseph, T. MacKenzie, M. Kashgarian, and J. P. Hayslett, "The benefit of early treatment with immunosuppressive drugs in lupus nephritis," *The Journal of Rheumatology*, vol. 22, no. 6, article 1211, 1995.

[26] M. Ballow and A. Parke, "The uses of intravenous immune globulin in collagen vascular disorders," *The Journal of Allergy and Clinical Immunology*, vol. 84, no. 4, pp. 608–612, 1989.

[27] J. O. Schroeder, R. A. Zeuner, H. H. Euler, and H. Löffler, "High dose intravenous immunoglobulins in systemic lupus erythematosus: clinical and serological results of a pilot study," *The Journal of Rheumatology*, vol. 23, no. 1, pp. 71–75, 1996.

[28] Y. Sherer, S. Kuechler, J. J. Scali et al., "Low dose intravenous immunoglobulin in systemic lupus erythematosus: analysis of 62 cases," *Israel Medical Association Journal*, vol. 10, no. 1, pp. 55–57, 2008.

[29] H. Hjortkjaer Petersen, H. Nielsen, M. Hansen, F. Stensgaard-Hansen, and P. Helin, "High-dose immunoglobulin therapy in pericarditis caused by SLE," *Scandinavian The Journal of Rheumatology*, vol. 19, no. 1, pp. 91–93, 1990.

[30] W. P. Maier, D. S. Gordon, R. F. Howard et al., "Intravenous immunoglobulin therapy in systemic lupus erythematosus-associated thrombocytopenia," *Arthritis and Rheumatism*, vol. 33, no. 8, pp. 1233–1239, 1990.

[31] Y. Tomer and Y. Shoenfeld, "Successful treatment of psychosis secondary to SLE with high dose intravenous immunoglobulin," *Clinical and Experimental Rheumatology*, vol. 10, no. 4, pp. 391–393, 1992.

[32] P. Lesprit, F. Mouloud, P. Bierling et al., "Prolonged remission of SLE-associated polyradiculoneuropathy after a single course of intravenous immunoglobulin," *Scandinavian The Journal of Rheumatology*, vol. 25, no. 3, pp. 177–179, 1996.

[33] A. Aharon, Y. Levy, Y. Bar-Dayan et al., "Successful treatment of early secondary myelofibrosis in SLE with IVIG," *Lupus*, vol. 6, no. 4, pp. 408–411, 1997.

[34] T. Généreau, O. Chosidow, C. Danel, P. Chérin, and S. Herson, "High-dose intravenous immunoglobulin in cutaneous lupus erythematosus," *Archives of Dermatology*, vol. 135, no. 9, pp. 1124–1125, 1999.

[35] Y. Sherer, Y. Levy, P. Langevitz, M. Lorber, F. Fabrizzi, and Y. Shoenfeld, "Successful treatment of systemic lupus erythematosus cerebritis with intravenous immunoglobulin," *Clinical Rheumatology*, vol. 18, no. 2, pp. 170–173, 1999.

[36] Y. Sherer, Y. Levy, and Y. Shoenfeld, "Marked improvement of severe cardiac dysfunction after one course of intravenous immunoglobulin in a patient with systemic lupus erythematosus," *Clinical Rheumatology*, vol. 18, no. 3, pp. 238–240, 1999.

[37] M. Meissner, Y. Sherer, Y. Levy, H. Chwalinska-Sadowska, P. Langevitz, and Y. Shoenfeld, "Intravenous immunoglobulin therapy in a patient with lupus serositis and nephritis," *Rheumatology International*, vol. 19, no. 5, pp. 199–201, 2000.

[38] Y. Sherer, Y. Levy, and Y. Shoenfeld, "Intravenous immunoglobulin therapy of antiphospholipid syndrome," *Rheumatology*, vol. 39, no. 4, pp. 421–426, 2000.

[39] K. I. Hoshi, M. Matsuda, M. Ishikawa et al., "Successful treatment of fulminant pulmonary hemorrhage associated with systemic lupus erythematosus," *Clinical Rheumatology*, vol. 23, no. 3, pp. 252–255, 2004.

[40] O. De Pità, A. M. Bellucci, M. Ruffelli, C. R. Girardelli, and P. Puddu, "Intravenous immunoglobulin therapy is not able to efficiently control cutaneous manifestations in patients with lupus erythematosus," *Lupus*, vol. 6, no. 4, pp. 415–417, 1997.

[41] E. Gomard-Mennesson, M. Ruivard, M. Koenig et al., "Treatment of isolated severe immune hemolytic anaemia associated with systemic lupus erythematosus: 26 Cases," *Lupus*, vol. 15, no. 4, pp. 223–231, 2006.

[42] C. Arnal, J. C. Piett, J. Léone et al., "Treatment of severe immune thrombocytopenia associated with systemic lupus erythematosus: 59 Cases," *The Journal of Rheumatology*, vol. 29, no. 1, pp. 75–83, 2002.

[43] D. W. Branch, A. M. Peaceman, M. Druzin et al., "A multicenter, placebo-controlled pilot study of intravenous immune globulin treatment of antiphospholipid syndrome during pregnancy," *American Journal of Obstetrics and Gynecology*, vol. 182, no. 1, pp. 122–127, 2000.

[44] E. Vaquero, N. Lazzarin, H. Valensise et al., "Pregnancy outcome in recurrent spontaneous abortion associated with antiphospholipid antibodies: a comparative study of intravenous immunoglobulin versus prednisone plus low-dose aspirin," *American Journal of Reproductive Immunology*, vol. 45, no. 3, pp. 174–179, 2001.

[45] G. Triolo, A. Ferrante, F. Ciccia et al., "Randomized study of subcutaneous low molecular weight heparin plus aspirin versus intravenous immunoglobulin in the treatment of recurrent fetal loss associated with antiphospholipid antibodies," *Arthritis and Rheumatism*, vol. 48, no. 3, pp. 728–731, 2003.

[46] Y. Levy, Y. Sherer, A. Ahmed et al., "A study of 20 SLE patients with intravenous immunoglobulin—clinical and serologic response," *Lupus*, vol. 8, no. 9, pp. 705–712, 1999.

[47] G. Zandman-Goddard, A. Krauthammer, Y. Levy, P. Langevitz, and Y. Shoenfeld, "Long-term therapy with intravenous immunoglobulin is beneficial in patients with autoimmune diseases," *Clinical Reviews in Allergy and Immunology*, vol. 42, no. 2, pp. 247–255, 2012.

[48] K. Akashi, K. Nagasawa, T. Mayumi, E. Yokota, N. Oochi, and T. Kusaba, "Successful treatment of refractory systemic lupus erythematosus with intravenous immunoglobulins," *The Journal of Rheumatology*, vol. 17, no. 3, pp. 375–379, 1990.

[49] A. Oliet, E. Hernandez, P. Gallar, and A. Vigil, "High-dose intravenous gamma-globulin in systemic lupus erythematosus," *Nephron*, vol. 62, no. 4, article 465, 1992.

[50] A. Winder, Y. Molad, I. Ostfeld, G. Kenet, J. Pinkhas, and Y. Sidi, "Treatment of systemic lupus erythematosus by prolonged administration of high dose intravenous immunoglobulin: report of 2 cases," *The Journal of Rheumatology*, vol. 20, no. 3, pp. 495–498, 1993.

[51] H. Arahata, K. Migita, H. Izumoto et al., "Successful treatment of rapidly progressive lupus nephritis associated with anti-MPO antibodies by intravenous immunoglobulins," *Clinical Rheumatology*, vol. 18, no. 1, pp. 77–81, 1999.

[52] A. Viertel, E. Weidmann, R. Wigand, H. Geiger, and U. F. Mondorf, "Treatment of severe systemic lupus erythematosus with immunoadsorption and intravenous immunoglobulins," *Intensive Care Medicine*, vol. 26, no. 6, pp. 823–824, 2000.

[53] H. C. Gan, K. Hyoon, and K. Y. Fong, "Clinical outcomes of patients with biopsy-proven lupus nephritis in NUH," *Singapore Medical Journal*, vol. 43, no. 12, pp. 614–616, 2002.

[54] S. Kamali, A. Cefle, M. Sayarlioglu et al., "Experience with monthly, high-dose, intravenous immunoglobulin therapy in patients with different connective tissue diseases," *Rheumatology International*, vol. 25, no. 3, pp. 211–214, 2005.

[55] D. Micheloud, L. Nuño, M. Rodríguez-Mahou et al., "Efficacy and safety of Etanercept, high-dose intravenous gammaglobulin and plasmapheresis combined therapy for lupus diffuse proliferative nephritis complicating pregnancy," *Lupus*, vol. 15, no. 12, pp. 881–885, 2006.

[56] Y. Levy, Y. Sherer, J. George et al., "Intravenous immunoglobulin treatment of lupus nephritis," *Seminars in Arthritis and Rheumatism*, vol. 29, no. 5, pp. 321–327, 2000.

[57] C. Francioni, M. Galeazzi, A. Fioravanti, R. Gelli, F. Megale, and R. Marcolongo, "Long term I.V. Ig treatment in systemic lupus erythematosus," *Clinical and Experimental Rheumatology*, vol. 12, no. 2, pp. 163–168, 1994.

[58] D. Monova, N. Belovezhdov, I. Altunkova, and S. Monov, "Intravenous immunoglobulin G in the treatment of patients with chronic glomerulonephritis: clinical experience lasting 15 years," *Nephron*, vol. 90, no. 3, pp. 262–266, 2002.

[59] F. Bridoux, F. Vrtovsnik, C. Noël et al., "Renal thrombotic microangiopathy in systemic lupus erythematosus: clinical correlations and long-term renal survival," *Nephrology Dialysis Transplantation*, vol. 13, no. 2, pp. 298–304, 1998.

[60] B. N. Becker, H. Fuchs, and R. Hakim, "Intravenous immune globulin in the treatment of patients with systemic lupus erythematosus and end-stage renal disease," *Journal of the American Society of Nephrology*, vol. 5, no. 10, pp. 1746–1750, 1995.

[61] C. Y. Lin, H. C. Hsu, and H. Chiang, "Improvement of histological and immunological change in steroid and immunosuppressive drug-resistant lupus nephritis by high-dose intravenous gamma globulin," *Nephron*, vol. 53, no. 4, pp. 303–310, 1989.

[62] T. R. Welch, A. J. McAdams, and L. S. Beischel, "Glomerulonephritis associated with complete deficiency of the fourth component of complement: response to intravenous immunoglobulin," *Arthritis and Rheumatism*, vol. 38, no. 9, pp. 1333–1337, 1995.

[63] F. Silvestris, O. D'Amore, P. Cafforio, L. Savino, and F. Dammacco, "Intravenous immune globulin therapy of lupus nephritis: use of pathogenic anti-DNA-reactive IgG," *Clinical and Experimental Immunology, Supplement*, vol. 104, supplement 1, pp. 91–97, 1996.

[64] J. N. Boletis, J. P. A. Ioannidis, K. A. Boki, and H. M. Moutsopoulos, "Intravenous immunoglobulin compared with cyclophosphamide for proliferative lupus nephritis," *The Lancet*, vol. 354, no. 9178, pp. 569–570, 1999.

[65] A. Corvetta, R. Della Bitta, A. Gabrielli, P. J. Spaeth, and G. Danieli, "Use of high-dose intravenous immunoglobulin in systemic lupus erythematosus: report of three cases," *Clinical and Experimental Rheumatology*, vol. 7, no. 3, pp. 295–299, 1989.

[66] B. Chacko, G. T. John, N. Balakrishnan, M. G. Kirubakaran, and C. K. Jacob, "Osmotic nephropathy resulting from maltose-based intravenous immunoglobulin therapy," *Renal Failure*, vol. 28, no. 2, pp. 193–195, 2006.

[67] S. Tan, S. Tambar, S. Chohan, R. Ramsey-Goldman, and C. Lee, "Acute myocardial infarction after treatment of thrombocytopenia in a young woman with systemic lupus erythematosus," *Journal of Clinical Rheumatology*, vol. 14, no. 6, pp. 350–352, 2008.

[68] S. K. B. Ng, "Intravenous immunoglobulin infusion causing pseudohyponatremia," *Lupus*, vol. 8, no. 6, pp. 488–490, 1999.

[69] E. Ben-Chetrit, C. Putterman, and M. D. Grebenau, "Transient neutropenia induced by intravenous immune globulin," *The New England Journal of Medicine*, vol. 326, no. 4, pp. 270–271, 1991.

[70] A. M. G. Pasatiempo, J. A. Kroser, M. Rudnick, and B. I. Hoffman, "Acute renal failure after intravenous immunoglobulin therapy," *The Journal of Rheumatology*, vol. 21, no. 2, pp. 347–349, 1994.

[71] K. S. Barron, M. R. Sher, and E. D. Silverman, "Intravenous immunoglobulin therapy: magic or black magic," *The Journal of Rheumatology*, vol. 19, no. 33, pp. 94–97, 1992.

[72] Centers for Disease Control and Prevention, "Renal insufficiency and failure associated with immune globulin intravenous therapy—United States, 1985–1998," *Morbidity and Mortality Weekly Report*, vol. 48, no. 24, pp. 518–521, 1999.

[73] H. I. A. Sati, R. Ahya, and H. G. Watson, "Incidence and associations of acute renal failure complicating high-dose intravenous immunoglobulin therapy," *British Journal of Haematology*, vol. 113, no. 2, pp. 556–557, 2001.

[74] H. Orbach, M. Tishler, and Y. Shoenfeld, "Intravenous immunoglobulin and the kidney—a two-edged sword," *Seminars in Arthritis and Rheumatism*, vol. 34, no. 3, pp. 593–601, 2004.

[75] N. Li, M. Zhao, J. Hilario-Vargas et al., "Complete FcRn dependence for intravenous Ig therapy in autoimmune skin blistering diseases," *The Journal of Clinical Investigation*, vol. 115, no. 12, pp. 3440–3450, 2005.

[76] M. A. Gonzalez-Gay, "The pros and cons of intravenous immunoglobulin treatment in autoimmune nephropathy," *Seminars in Arthritis and Rheumatism*, vol. 34, no. 3, pp. 573–574, 2004.

[77] S. M. Mauer, A. J. Fish, E. B. Blau, and A. F. Michael, "The glomerular mesangium. I. Kinetic studies of macromolecular uptake in normal and nephrotic rats," *The Journal of Clinical Investigation*, vol. 51, no. 5, pp. 1092–1101, 1972.

[78] S. M. Mauer, A. J. Fish, N. K. Day, and A. F. Michael, "The glomerular mesangium. II. Studies of macromolecular uptake in nephrotoxic nephritis in rats," *The Journal of Clinical Investigation*, vol. 53, no. 2, pp. 431–439, 1974.

[79] S. M. Mauer, D. E. Sutherland, R. J. Howard, A. J. Fish, J. S. Najarian, and A. F. Michael, "The glomerular mesangium. 3. Acute immune mesangial injury: a new model of glomerulonephritis," *The Journal of Experimental Medicine*, vol. 137, no. 3, pp. 553–570, 1973.

[80] Y. Shvil, A. F. Michael, and S. M. Mauer, "Uptake of aggregated immunoglobulin by the mouse kidney. I. Effect of endotoxin," *British Journal of Experimental Pathology*, vol. 61, no. 1, pp. 22–29, 1980.

[81] Y. Shvil, S. M. Mauer, and A. F. Michael, "Uptake of aggregated immunoglobulin by the mouse kidney. II. Effect of hydrocortisone," *British Journal of Experimental Pathology*, vol. 61, no. 1, pp. 30–38, 1980.

[82] A. Santiago, T. Mori, J. Satriano, and D. Schlondorff, "Regulation of Fc receptors for IgG on cultured rat mesangial cells," *Kidney International*, vol. 39, no. 1, pp. 87–94, 1991.

[83] P. C. Singhal, A. Santiago, J. Satriano, R. M. Hays, and D. Schlondorff, "Effects of vasoactive agents on uptake of immunoglobulin G complexes by mesangial cells," *American Journal of Physiology*, vol. 258, no. 3, pp. F589–F596, 1990.

[84] R. Neuwirth, P. Singhal, B. Diamond et al., "Evidence for immunoglobulin Fc receptor-mediated prostaglandin2 and platelet-activating factor formation by cultured rat mesangial cells," *The Journal of Clinical Investigation*, vol. 82, no. 3, pp. 936–944, 1988.

[85] J. R. Sedor, S. W. Carey, and S. N. Emancipator, "Immune complexes bind to cultured rat glomerular mesangial cells to stimulate superoxide release. Evidence for an Fc receptor," *Journal of Immunology*, vol. 138, no. 11, pp. 3751–3757, 1987.

[86] A. A. Herrada, F. J. Contreras, J. A. Tobar, R. Pacheco, and A. M. Kalergis, "Immune complex-induced enhancement of bacterial antigen presentation requires Fcγ Receptor III expression on dendritic cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 33, pp. 13402–13407, 2007.

[87] J. V. Ravetch and S. Bolland, "IgG Fc receptors," *Annual Review of Immunology*, vol. 19, pp. 275–290, 2001.

[88] N. Novak, S. Kraft, and T. Bieber, "IgE receptors," *Current Opinion in Immunology*, vol. 13, no. 6, pp. 721–726, 2001.

[89] B. D. Wines and P. M. Hogarth, "IgA receptors in health and disease," *Tissue Antigens*, vol. 68, no. 2, pp. 103–114, 2006.

[90] C. Gomez-Guerrero, M. J. Lopez-Armada, E. Gonzalez, and J. Egido, "Soluble IgA and IgG aggregates are catabolized by cultured rat mesangial cells and induce production of TNF-α and IL-6, and proliferation," *Journal of Immunology*, vol. 153, no. 11, pp. 5247–5256, 1994.

[91] H. H. Radeke, I. Janssen-Graalfs, E. N. Sowa et al., "Opposite regulation of type II and III receptors for immunoglobulin G in mouse glomerular mesangial cells and in the induction of anti-glomerular basement membrane (GBM) nephritis," *The Journal of Biological Chemistry*, vol. 277, no. 30, pp. 27535–27544, 2002.

[92] R. Mancilla-Jimenez, M. D. Appay, B. Bellon et al., "IgG Fc membrane receptor on normal human glomerular visceral epithelial cells," *Virchows Archiv*, vol. 404, no. 2, pp. 139–158, 1984.

[93] A. O. Haakenstad, G. E. Striker, and M. Mannik, "The glomerular deposition of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice," *Laboratory Investigation*, vol. 35, no. 3, pp. 293–301, 1976.

[94] M. Mannik, K. A. David, and V. J. Gauthier, "Preparation and characterization of 4-azido-2-nitrophenyl human serum albumin as an antigen for covalent cross-linking of immune complexes," *Journal of Immunology*, vol. 127, no. 5, pp. 1993–1999, 1981.

[95] V. J. Gauthier, M. Mannik, and G. E. Striker, "Effect of cationized antibodies in preformed immune complexes on deposition and persistence in renal glomeruli," *The Journal of Experimental Medicine*, vol. 156, no. 3, pp. 766–777, 1982.

[96] M. Mannik, L. Y. Agodoa, and K. A. David, "Rearrangement of immune complexes in glomeruli leads to persistence and development of electron-dense deposits," *The Journal of Experimental Medicine*, vol. 157, no. 5, pp. 1516–1528, 1983.

[97] J. P. Haymann, F. Delarue, L. Baud, and J. D. Sraer, "Aggregated IgG bind to glomerular epithelial cells to stimulate urokinase release through an endocytosis-independent process," *Nephron Experimental Nephrology*, vol. 98, no. 1, pp. e13–e21, 2004.

[98] H. Fujii, K. Nakatani, N. Arita et al., "Intenalization of antibodies by endothelial cells via fibronectin implicating a novel mechanism in lupus nephritis," *Kidney International*, vol. 64, no. 5, pp. 1662–1670, 2003.

[99] M. M. Frank, T. J. Lawley, M. I. Hamburger, and E. J. Brown, "Immunoglobulin G Fc receptor-mediated clearance in autoimmune diseases," *Annals of Internal Medicine*, vol. 98, no. 2, pp. 206–218, 1983.

[100] J. C. Jennette and R. H. Heptinstall, *Heptinstall's Pathology of the Kidney*, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 6th edition, 2007.

[101] N. Bagheri, S. R. Chintalacharuvu, and S. N. Emancipator, "Proinflammatory cytokines regulate FcαR expression by human mesangial cells in vitro," *Clinical and Experimental Immunology*, vol. 107, no. 2, pp. 404–409, 1997.

[102] A. Santiago, J. Satriano, S. DeCandido et al., "A specific Fcγ receptor on cultured rat mesangial cells," *Journal of Immunology*, vol. 143, no. 8, pp. 2575–2582, 1989.

[103] M. Morcos, G. M. Hansch, M. Schonermark, S. Ellwanger, M. Harle, and B. Heckl-Ostreicher, "Human glomerular

mesangial cells express CD16 and may be stimulated via this receptor," *Kidney International*, vol. 46, no. 6, pp. 1627–1634, 1994.

[104] H. H. Radeke, J. E. Gessner, P. Uciechowski, H. J. Magert, R. E. Schmidt, and K. Resch, "Intrinsic human glomerular mesangial cells can express receptors for IgG complexes (hFcγRIII-A) and the associated FcεRI γ-chain," *Journal of Immunology*, vol. 153, no. 3, pp. 1281–1292, 1994.

[105] P. Uciechowski, M. Schwarz, J. E. Gessner, R. E. Schmidt, K. Resch, and H. H. Radeke, "IFN-gamma induces the high-affinity Fc receptor I for IgG (CD64) on human glomerular mesangial cells," *European Journal of Immunology*, vol. 28, no. 9, pp. 2928–2935, 1998.

[106] C. Gomez-Guerrero, E. Gonzalez, and J. Egido, "Evidence for a specific IgA receptor in rat and human mesangial cells," *Journal of Immunology*, vol. 151, no. 12, pp. 7172–7181, 1993.

[107] Y. Suzuki, C. Ra, K. Saito et al., "Expression and physical association of Fcα receptor and Fc receptor γ chain in human mesangial cells," *Nephrology Dialysis Transplantation*, vol. 14, no. 5, pp. 1117–1123, 1999.

[108] R. Matre, O. Tonder, and F. Wesenberg, "Human renal glomeruli possess no Fcγ receptors," *Clinical Immunology and Immunopathology*, vol. 17, no. 2, pp. 157–162, 1980.

[109] J. P. Haymann, J. P. Levraud, S. Bouet et al., "Characterization and localization of the neonatal Fc receptor in adult human kidney," *Journal of the American Society of Nephrology*, vol. 11, no. 4, pp. 632–639, 2000.

[110] S. Akilesh, T. B. Huber, H. Wu et al., "Podocytes use FcRn to clear IgG from the glomerular basement membrane," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 3, pp. 967–972, 2008.

[111] M. Debre, M. C. Bonnet, W. H. Fridman et al., "Infusion of Fcγ fragments for treatment of children with acute immune thrombocytopenic purpura," *The Lancet*, vol. 342, no. 8877, pp. 945–949, 1993.

[112] R. M. Anthony, F. Nimmerjahn, D. J. Ashline, V. N. Reinhold, J. C. Paulson, and J. V. Ravetch, "Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc," *Science*, vol. 320, no. 5874, pp. 373–376, 2008.

[113] R. M. Anthony, F. Wermeling, and J. V. Ravetch, "Novel roles for the IgG Fc glycan," *Annals of the New York Academy of Sciences*, vol. 1253, pp. 170–180, 2012.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 819634, 11 pages
doi:10.1155/2012/819634

Research Article

Multiple Autoantibodies Display Association with Lymphopenia, Proteinuria, and Cellular Casts in a Large, Ethnically Diverse SLE Patient Cohort

Rufei Lu,[1,2] Julie M. Robertson,[1] Benjamin F. Bruner,[1,3] Joel M. Guthridge,[1] Barbara R. Neas,[4] Swapan K. Nath,[1] Jennifer A. Kelly,[1] Kathy L. Moser Sivils,[1] Eliza F. Chakravarty,[1] Diane L. Kamen,[5] Gary S. Gilkeson,[5] Daniel J. Wallace,[6] Michael H. Weisman,[6] R. Hal Scofield,[1,2,7] John B. Harley,[8,9] and Judith A. James[1,2]

[1] *Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, USA*
[2] *Department of Medicine and Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA*
[3] *Department of Biology, Harding University, Searcy, AR 72143, USA*
[4] *Department of Biostatistics, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA*
[5] *Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, SC 29425, USA*
[6] *Divison of Rheumatology, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA*
[7] *US Department of Veterans Affairs Medical Center, Oklahoma City, OK 73105, USA*
[8] *US Department of Veterans Affairs Medical Center, Cincinnati, OH 45220, USA*
[9] *Division of Rheumatology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA*

Correspondence should be addressed to Judith A. James, jamesj@omrf.org

Received 3 May 2012; Accepted 13 June 2012

Academic Editor: Taku Yoshio

Purpose. This study evaluates high-throughput autoantibody screening and determines associated systemic lupus erythematosus (SLE) clinical features in a large lupus cohort. *Methods.* Clinical and demographic information, along with serum samples, were obtained from each SLE study participant after appropriate informed consent. Serum samples were screened for 10 distinct SLE autoantibody specificities and examined for association with SLE ACR criteria and subcriteria using conditional logistic regression analysis. *Results.* In European-American SLE patients, autoantibodies against 52 kD Ro and RNP 68 are independently enriched in patients with lymphopenia, anti-La, and anti-ribosomal P are increased in patients with malar rash, and anti-dsDNA and anti-Sm are enriched in patients with proteinuria. In African-American SLE patients, cellular casts associate with autoantibodies against dsDNA, Sm, and Sm/nRNP. *Conclusion.* Using a high-throughput, bead-based method of autoantibody detection, anti-dsDNA is significantly enriched in patienets with SLE ACR renal criteria as has been previously described. However, lymphopenia is associated with several distinct autoantibody specificities. These findings offer meaningful information to allow clinicians and clinical investigators to understand which autoantibodies correlate with select SLE clinical manifestations across common racial groups using this novel methodology which is expanding in clinical use.

1. Introduction

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that is characterized by diverse clinical symptoms and autoantibody production against a variety of nuclear and cytoplasmic antigens [1–3]. The occurrence and prevalence of these autoantibody specificities have been used to characterize the diverse clinical presentations of SLE. The standard screening assay for the detection of autoantibodies, and more specifically anti-nuclear antibodies (ANAs), is an indirect immunofluorescence. However, to identify more specific subsets of autoantibodies, immunodiffusion and

enzyme-linked immunosorbent assays (ELISAs) are often employed. Newer screening technologies, like the Luminex bead-based assay performed with the Bio-Rad BioPlex 2200, are being introduced, which focus on performing a sensitive multiplex analysis of select autoantibody specificities allowing for high-throughput analysis with minimal human time investments or complicated human analysis and interpretation.

Previous work has shown, in many cases, that autoantibodies are present years before SLE diagnosis [4–6] and that autoantibodies usually preceded the onset of clinical symptoms [5, 7–9]. Select lupus autoantibodies are correlated with the occurrence of specific clinical symptoms. Lymphopenia is associated with anti-Ro and anti-dsDNA antibodies; while anti-chromatin antibodies are more commonly found with leukopenia [10–13]. ACR SLE renal classification criteria are strongly correlated with anti-dsDNA and anti-chromatin antibodies. Anti-ribosomal P antibodies associate with increased risk of nephritis in anti-dsDNA positive patients in a juvenile-onset SLE cohort [8, 10, 12, 14, 15]. Interestingly, anti-La antibodies are inversely related with renal and CNS involvement in SLE [11, 16]. Strong associations between anti-Ro and anti-La antibodies in SLE patients with skin manifestations have also been observed [12, 17]. Anti-RNP associates with Raynaud's phenomenon [18]. Interestingly, autoantibodies against proliferating cell nuclear antigen, while present in many systemic autoimmune diseases, are found in high titers in some SLE patients [3]. However, little is known about the clinical significance of those autoantibodies.

Select autoantibodies are also predictive of severe clinical manifestations of ACR criteria. In Canadian First Nations, anti-Sm antibodies are correlated with higher mortality [12]; while the presence of anti-Ro, anti-Sm, and anti-RNP antibodies is associated with increased disease severity in African-American female SLE patients [19]. Antibodies against dsDNA may increase prior to clinical disease flare in SLE patients [20]; while the presence of anticardiolipin antibodies is correlated with a more varied and severe clinical disease course in SLE patients [21], as well as with thrombotic events. These previous studies indicate the importance of examining the specific autoantibody profile in each SLE patient. However, these previous studies relied on precipitating levels of antibodies or historical chart data.

The goal of this study was to examine the detection of autoantibodies in a large ethnically diverse SLE patient cohort using a high-throughput multiplex bead-based assay. Secondarily, we explored whether associations existed between autoantibodies present in patient sera and the occurrence of specific SLE diagnostic criteria.

2. Materials and Methods

2.1. Cohort Selection. A collection of 1,803 SLE patient serum samples were obtained from the Lupus Family Registry and Repository (LFRR) and the Lupus Genetics cohorts at the Oklahoma Medical Research Foundation (OMRF) on the basis of availability of serum, clinical information, and presence of 4 of 11 ACR classification criteria [22, 23] for each patient. Our study was comprised of 836 European-Americans (EA), 618 African Americans (AA), 255 Hispanics (HI), and 93 other races/ethnicities (mixed race/ethnicity, Asian, American Indian, and unknown). Within the AA SLE patients, 127 were of Gullah descent from off the coastal islands of South Carolina and Georgia. Each SLE patient previously had questionnaires, personal interviews, and standardized medical record reviews for documentation of SLE classification criteria and subcriteria [24]. Clinical, demographic, autoantibody, and therapeutic information about each patient was extracted. All participants provided informed consent and the study was approved by the OMRF Institutional Review Board.

2.2. Serologic Autoantibody Testing. ANA antibodies were measured using indirect immunofluorescence with *HEp-2 cells* as the substrate (IIF, INOVA Diagnostics, San Diego, CA) [5, 6, 25]. Detection of ANA at a dilution of 1 : 120 or greater was considered a positive result. The ANA antibody assays were manually read by the CLIA-CAP certified Oklahoma Medical Research Foundation Clinical Immunology Laboratory personnel using a Nikon Optiphot Fluorescence microscope with a HBO blub 100 w mercury lamp under the 20× objective.

2.3. Multiplex Bead-Based Autoantibody Assays. The Bio-Rad BioPlex 2200 (Bio-Rad, Hercules, CA) is a high-throughput, fully automated, serological analysis unit that utilizes multiplex bead technology for antibody detection. Dyed magnetic beads within the BioPlex 2200 ANA kit make possible the simultaneous detection of 13 different autoantibody specificities by using a method that has been previously described [26]. Ten of the detectable autoantibody specificities are commonly associated with SLE and target a variety of antigens including dsDNA, chromatin, ribosomal P, 60 kD Ro (SS-A 60), 52 kD Ro (SS-A 52), La (SS-B), Sm, Sm/RNP complex, nRNP A, and nRNP 68. Three other specificities were assessed (Scl-70, centromere B, and Jo-1) but were excluded from the majority of our analysis based on very low prevalence in this SLE cohort (2.3%, 3.7%, and 0.11%, resp.). Individual autoantibody responses are reported on a semiquantitative scale from 0 to 8, referred to as the Antibody Index (AI). This AI scale is set relative to calibrator, positive and negative control samples provided by the manufacturer. The defined positive cutoff value for each assay is then set to equal an AI of 1.0. However, anti-dsDNA results are reported in IU/mL and have a positive cut-off of 10.0 IU/mL per the manufacturer's recommendation. A sample is designated as ANA positive if detectable levels (AI $\geq$ 1.0 or IU $\geq$ 10.0) of antibody are found for any one of the analytes.

2.4. Statistical Analysis. These data represent a cross-sectional collection of SLE patient samples. Primary analyses used positive and negative classification of the autoantibodies and the SLE clinical criteria or subcriteria. Up to five members of a single family are included in these data with

Table 1: Demographics of study participants.

Race/ethnicity	N(%)	Mean age (SD)	Mean number of ACR criteria (SD)	Female (%)
EA	836 (46.4%)	43.17 (13.63)	5.11 (1.4)	89
AA	618 (34.3%)	40.7 (12.4)	5.2 (1.4)	92
HI	255 (14.1%)	37.92 (12.82)	5.25 (1.55)	89
Other	93 (5.2%)	39.9 (12)	5.04 (1.4)	94
Total	1803 (100%)	51.76 (15.45)	5.15 (1.41)	90

EA: European American, AA: African American, HI: Hispanic, Other: mixed race/ethnicity, American Indian, Asian, and unknown.

all hypothesis testing methods using an adjustment for these clustered family data (1803 patients and 1207 families).

Descriptive statistics included percentages, frequencies, and means for demographic data and percentages and frequencies for autoantibodies and SLE clinical criteria or subcriteria. Heat map visualizations of the prevalence of each of the ACR criteria/subcriteria within each autoantibody positive population were generated using TIBCO Spotfire 4.0. Each distribution (positive or negative) of the collection of autoantibodies and the set of SLE clinical criteria and subcriteria were compared among the four ethnic groups using a generalized linear mixed model method incorporating multiple comparisons with adjustment of multiple testings (Bonferroni method). When ethnicity was found to be significant, multiple comparisons identified the pairwise statistically significant differences between the ethnic groups. Statistical significance was declared when an adjusted P value was less than alpha of 0.05.

Conditional logistic regression modeling was used to examine associations between autoantibody specificities and SLE clinical criteria and subcriteria, with the ACR criteria and subcriteria serving as the outcome and the autoantibodies as the covariates. Univariate conditional logistic regression allowed for identification of associations between an autoantibody and a specific ACR SLE classification criterion.

Multivariate conditional logistic regression modeling was performed to identify models demonstrating associations of covariates, autoantibodies, and sex with a specific outcome or grouped outcome. Interactions between the covariates as well as the potential association or confounding produced by sex were evaluated. Separate analyses were performed for each ethnic group. Odds ratios (ORs) and 95% confidence intervals (CI) were obtained as well as individual covariate statistical significance defined as a P value less than an alpha of 0.05. Confounding was considered present when at least a 20% difference in the OR estimate occurred when including sex in the model. An OR greater than 1.0 indicated a positive association of the antibody with the ACR criterion, where the odds of an outcome were higher for those patients with positive antibody results than for those with negative antibody results. Odds ratios of less than 1.0 indicated a negative association with the odds of the outcome lower for those patients with a positive antibody results compared to those with a negative autoantibody results.

A Cochran-Mantel-Haenszel test (a chi-square test with stratification on family relationship) was used to examine SLE clinical manifestation prevalence in ANA positive compared to ANA negative individuals. All analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC) or Spotfire Decisionsite version 8.2.

3. Results

3.1. Cohort Demographics. This study examined a large, ethnically diverse, clinically heterogeneous cohort of 1,803 SLE patients (Table 1). Study participants met a minimum of 4 of 11 ACR clinical classification criteria [22, 23]. Our cohort consisted of 90% female SLE patients with an average age of 51.8 ± 15.4 and meeting an average of 5.15 ± 1.41 ACR classification criteria. EA SLE patients had the oldest average age at 43.2 ± 13.6 years; while HI SLE patients were the youngest at 37.9 ± 12.8 years. The age of the HI study participants was significantly lower than the EA and AA participants ($P < 0.05$). There was no statistical difference in the number of ACR classification criteria met or in the length of time from diagnosis to sample procurement between the self-reported ethnic groups. The only difference observed in medication usage was between AA and HI SLE patients. AA patients are more likely to be treated with biologic therapy ($P = 0.0194$); while HI patients were less likely to have no current treatment compared to both EA and AA patients ($P = 0.0314$).

3.2. Ethnic Differences in the Presentation of SLE ACR Criteria and Subcriteria. The prevalence of specific ACR classification criteria was examined (Figure 1). The most prevalent criteria were ANA positivity, arthritis, hematological, and immunologic criteria. Ethnic differences in the prevalence of ACR criteria were observed (Figure 1(a)). Renal disorder and immunological disorders were significantly less prevalent in EA patients compared with other ethnic groups ($P < 0.05$). Although discoid rash and hematologic disorder were more prevalent in AA patients compared to both EA ($P < 0.05$) and HI ($P < 0.05$) patients, malar rash, photosensitivity, and oral ulcers were enriched in EA and HI ($P < 0.05$) patients. Immunological disorder was enriched in HI compared to EA ($P < 0.05$) SLE patients. To further dissect the disease profile difference among the different ethnicities (EA, AA, HI, and others) in this study, we also tested the prevalence differences of ACR classification subcriteria in all ethnicity groups (Figure 1(b)). As expected, enrichment of proteinuria was detected in AA and HI compared to EA ($P < 0.05$).

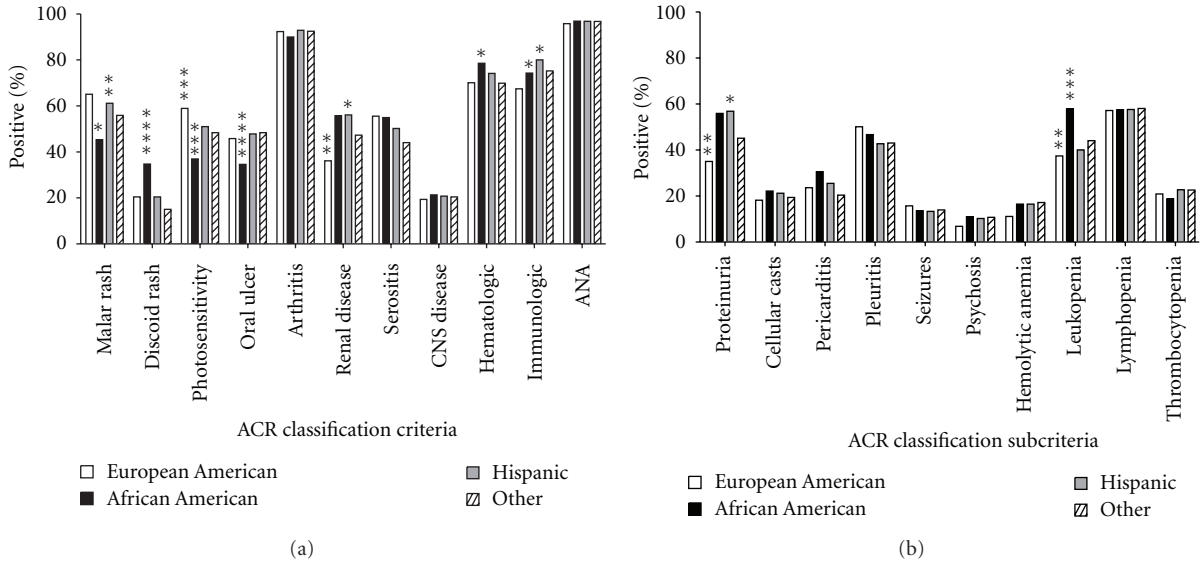


Figure 1: Prevalence of ACR classification criteria and subcriteria. ACR classification criteria (a) and subcriteria (b) prevalence by ethnicity are shown. European American (white bar), African American (black bar), Hispanic (dark grey bar), and other (multiracial/multiethnic, unknown, Asian, and American Indian, striped bar) are displayed. The most common classification criteria met are ANA, arthritis, hematologic, and immunologic. The most common ACR classification subcriteria is lymphopenia. Statistically significant differences ($P < 0.05$) are represented by stars. **** represents statistically different from all other racial groups; * represents statistical difference from EA; ** represents statistical difference from AA; *** represents statistical difference from HI, and the striped star is difference from other.

The analysis also revealed that enrichment of hematologic disorder in AA compared to other two ethnicity groups was mainly due to the higher leukopenia prevalence in AA compared to EA and HI ($P < 0.05$).

3.3. Initial Analysis Identified an Array of Autoantibody and Clinical Criteria Associations. The initial Heat map of prevalence of each autoantibody in relation to each of the 15 ACR SLE clinical criteria is presented (Figure 2). The Heat map illustrated multiple autoantibody enrichments with renal disorder and hematologic disorder in EA, AA, and HI SLE patients. A select few autoantibodies were enriched in patients with mucocutaneous manifestation, within EA and HI patient populations. In particular, noticeable increased prevalence of La and Sm autoantibodies in patients with oral ulcers was observed. A significant enrichment of La and ribosomal P antibodies in patients with psychosis and seizure, respectively, was also observed. Compared to EA, AA and HI patients with discoid rash had increased positivity of Ro/La and Sm/RNP autoantibodies.

Significant results from the initial univariate analyses are listed in Table 2. In EA, the most striking result was the association between hematologic criterion and five autoantibody specificities: anti-60 kD Ro, anti-52 kD Ro, anti-La, anti-Sm/nRNP, and anti-RNP 68 (Table 2). Anti-60 kD Ro, anti-52 kD Ro, and anti-RNP 68 were significantly enriched in patients with lymphopenia; while leukopenia was significantly associated with anti-52 kD Ro and Anti-ribosomal P antibodies. Anti-La responses were more common in SLE patients who did not have malar rash or proteinuria. Development of photosensitivity in EA patients is correlated with female sex (Table 2).

In AA patients, anti-dsDNA, anti-chromatin, and anti-Sm/RNP are more commonly found in SLE patients with cellular casts; while lymphopenia was associated with antibodies against 52 kD Ro. Proteinuria showed significant association with sex with higher odds of development of proteinuria for females. Compared to EA, AA patients displayed a significant association between renal disorder-related subcriteria (proteinuria and cellular casts) and several autoantibodies; whereas EA demonstrated more individual antibody specificities correlated with lymphopenia than did AA.

In the HI patients, the only significant associations between the presence of multiple antibodies and ACR criteria/subcriteria occurred in lymphopenia. In these patients, positive associations with lymphopenia were found with anti-RNP 68, anti-RNP A, and anti-Sm/RNP. Additionally, the presence of anti-chromatin antibodies was negatively associated with the development of hemolytic anemia. Univariate results in other ethnicity groups such as Asian and Native American were not significant at alpha = 0.05 level (not shown) likely due to small sample sizes.

Next we examined prevalence of SLE clinical criteria between ANA positive and ANA negative individuals as reported by the BioRad BioPlex 2200. No significant associations between ANA positive and SLE clinical criteria were observed in EA patients. However, AA ANA-positive patients were more likely to exhibit cellular casts ($P = 0.0021$), hematological disorder ($P = 0.001$), lymphopenia ($P = 0.03$), and immunological disorders ($P = 0.0068$). No difference was observed between the average number of antibodies

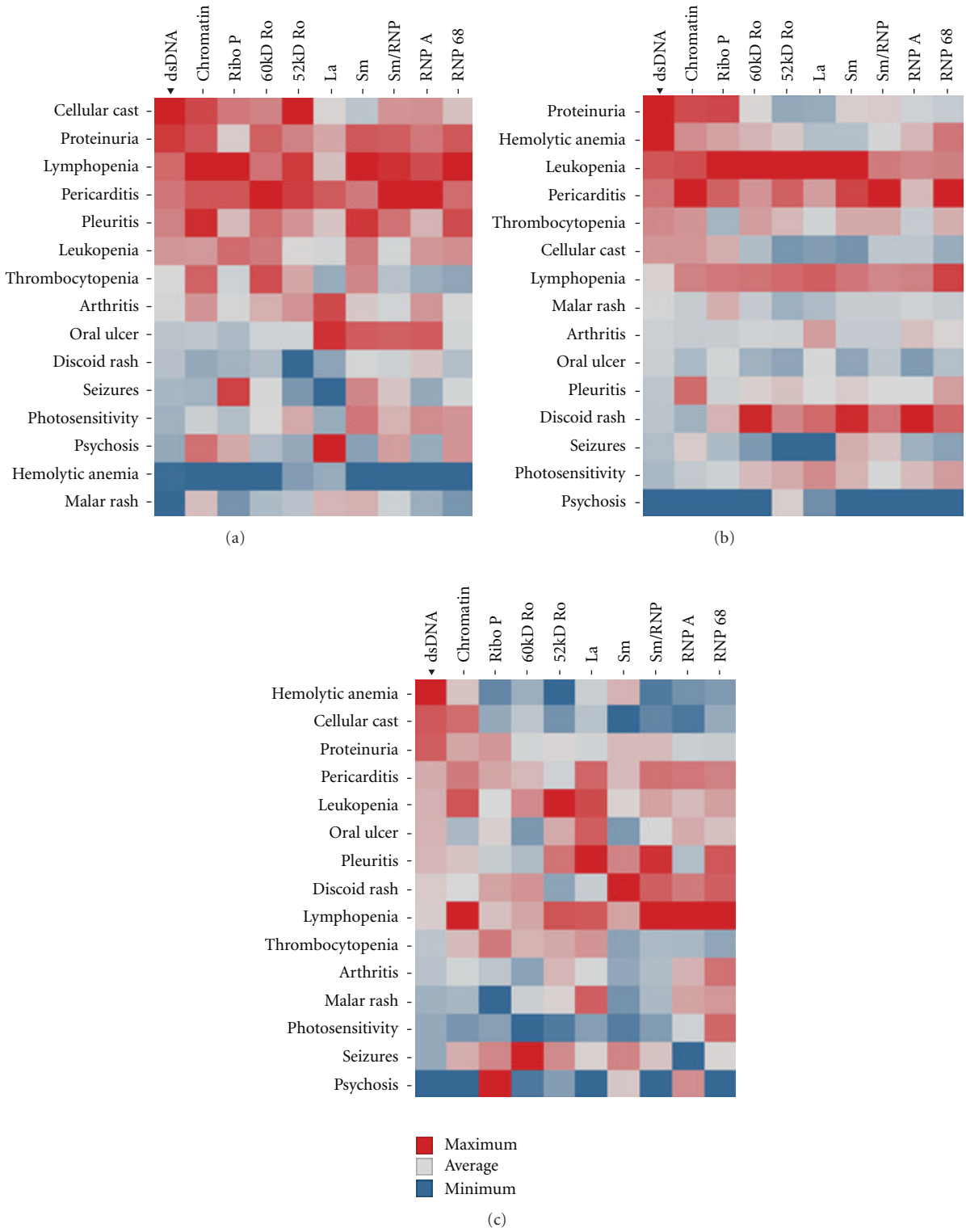


Figure 2: Association of SLE autoantibody prevalence and ACR criteria/subcriteria. Heat maps of the association between SLE autoantibody prevalence and ACR criteria/subcriteria for European Americans (a), African Americans (b), and Hispanic (c) are shown. Data is displayed as highest percent positive represented by the red square, average percent positive (gray square), and the minimum percent positive (blue square). The order of the rows is based on hierarchical clustering outcome of the clinical symptoms.

Table 2: Univariate conditional logistic regression models within European-American, African-American, and Hispanic populations using ACR SLE criteria or subcriteria as the outcome and individual autoantibodies and sex as covariates.

Criteria/subcriteria	Covariates	European American		African American		Hispanic	
		Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Hematological disorder	60 kD Ro	3.19 (1.55, 6.56)	0.0016				
	La	3.84 (1.43, 10.33)	0.0076				
	52 kD Ro	2.99 (1.26, 7.07)	0.0128				
	Sm/RNP	2.28 (1.11, 4.70)	0.0257	2.64 (1.09, 6.37)	0.0315		
	RNP 68	3.92 (1.08, 14.27)	0.0379				
	RNP A					11.93 (1.53, 93.37)	0.0182
Leukopenia	52 kD Ro	2.10 (1.00, 4.38)	0.0489				
	Ribo P	3.77 (1.22, 11.60)	0.0207	3.16 (1.25, 8.00)	0.0154		
	Sm			2.31 (1.33, 6.31)	0.0072		
Lymphopenia	60 kD Ro	2.26 (1.24, 4.15)	0.0082				
	52 kD Ro	2.78 (1.33, 5.79)	0.0064	2.90 (1.33, 6.31)	0.0072		
	RNP 68	4.72 (1.30, 17.11)	0.0182			8.62 (1.05, 71.00)	0.0452
	RNP A					3.89 (1.07, 14.17)	0.0391
	Chromatin					3.13 (1.09, 8.97)	0.0334
	Sm/RNP					3.27 (1.09, 9.78)	0.0340
Hemolytic anemia	Chromatin					0.10 (0.01, 0.95)	0.0446
Renal disease	dsDNA			2.38 (1.15, 4.94)	0.0197		
Proteinuria	La	0.37 (0.15, 0.91)	0.0293				
	Sex			1.97 (1.04, 3.71)	0.0368		
Cellular casts	dsDNA			3.69 (1.46, 9.32)	0.0058		
	Chromatin			2.90 (1.27, 6.59)	0.0113		
	Sm/RNP			3.65 (1.55, 8.64)	0.0032		
Oral ulcer	Sm/RNP			1.97 (1.04, 3.71)	0.0368		
Photosensitivity	52 kD Ro			0.38 (0.16, 0.92)	0.0329		
	Sex	2.30 (1.00, 5.29)	0.0492				
Malar rash	La	0.43 (0.21, 0.89)	0.0234				
	Sex	3.17 (1.37, 7.33)	0.0069				

in AA patients exhibiting and not exhibiting the specific SLE clinical criteria. The sample size for the HI patients was too small and, thus, the analysis could not be performed. Repeating this analysis utilizing ANA positivity as observed using indirect immunofluorescence found no significant differences in the prevalence of SLE clinical criteria in ANA-positive SLE patients of any ethnicity. Autoantibody frequency and SLE clinical criteria associations were further examined by multivariate analysis.

3.4. Leukopenia Is Associated with Anti-Ribosomal P Antibodies; while Lymphopenia Is Associated with Anti-52 kD Ro and Anti-RNP 68 Antibodies. Multivariate modeling results are shown in Table 3. Conditional logistic regression models were explored for all SLE clinical criteria with at least one significant univariate association. All 10 autoantibodies and sex served as covariates for these models. In the univariate analysis, both leukopenia and lymphopenia were correlated with a few autoantibodies in both EA and AA. However, in the multivariate model, leukopenia was associated with the presence of anti-ribosomal P in both EA and AA with both ORs greater than 3. This increased risk was almost 4-fold in EA. Anti-52 kD Ro and anti-RNP 68 antibodies were enriched in EA patients with lymphopenia; however, only anti-52 kD Ro autoantibodies were associated with lymphopenia in AA. Only one multivariate model with more than a single covariate was identified in HI patients. Anti-chromatin antibodies showed a positive association with lymphopenia; while anti-dsDNA antibodies had a negative association with this criterion.

3.5. Individuals with Anti-Sm/RNP and Anti-dsDNA Antibodies Are Positively Associated with Cellular Casts in AA and Anti-Chromatin Antibodies are Positively Associated with Proteinuria in EA. Significant associations between renal disorder subcriteria (proteinuria and cellular cast) and an array of covariates including autoantibodies and sex were observed in both AA and EA. However, models revealed that covariates differed between AA and EA. In AA patients, development of cellular casts was associated with anti-dsDNA and anti-Sm/RNP antibodies. Sex was the only covariate correlated with proteinuria in the AA patients, in which females were more likely to develop proteinuria. Chromatin autoantibodies were enriched in EA SLE patients with

Table 3: Conditional multivariate adjusted logistic regression models within European-American, African-American, and Hispanic populations using ACR SLE criteria or subcriteria as outcome and individual autoantibodies and sex as covariates.

Criteria/subcriteria	Covariates	European American		African American		Hispanic	
		Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value	Odds ratio (95% CI)	*P* value
Hematological disorder	60 kD Ro	3.31 (1.57, 6.99)	0.0017				
	RNP 68	4.37 (1.10, 3.74)	0.0361				
	Sm/RNP			3.03 (1.21, 7.61)	0.0184		
	La			3.59 (1.07, 12.06)	0.0384		
	RNP A					11.93 (1.53, 93.37)	0.0182
Leukopenia	Ribo P	3.77 (1.22, 11.60)	0.0207	3.16 (1.25, 8.00)	0.0154		
Lymphopenia	RNP 68	5.23 (1.40, 19.54)	0.0139				
	52 kD Ro	2.95 (1.39, 6.29)	0.0050	2.90 (1.33, 6.31)	0.0072		
	dsDNA					0.18 (0.05, 0.69)	0.0124
	Chromatin					6.05 (1.70, 21.54)	0.0055
Hemolytic anemia	Chromatin					0.10 (0.01, 0.95)	0.0446
Renal disease	Chromatin	2.56 (1.32, 4.95)	0.0054				
	La	0.33 (0.13, 0.88)	0.0273				
	Sm	0.28 (0.10, 0.76)	0.0127				
	dsDNA			2.38 (1.11, 5.08)	0.0255		
	Sex			4.40 (1.17, 15.56)	0.0283		
Proteinuria	Chromatin	2.34 (1.23, 4.44)	0.0092				
	La	0.29 (0.11, 0.74)	0.0096				
	Sm	0.36 (0.14, 0.94)	0.0366				
	Sex			4.04 (1.11, 14.74)	0.0346		
Cellular cast	dsDNA			3.26 (1.25, 8.50)	0.0158		
	Sm/RNP			2.91 (1.24, 6.81)	0.0138		
Seizures	Sm/RNP	5.33 (1.15, 24.80)	0.0329				
	RNP A	0.013 (0.02, 0.67)	0.0156				
Oral ulcer	Sm/RNP			1.97 (1.04, 3.71)	0.0368		
Malar rash	La	0.42 (0.20, 0.89)	0.0233				
	Sex	3.23 (1.38, 7.55)	0.0068				
Photosensitivity	52 kD Ro			0.07 (0.01, 0.53)	0.0103		
	La			11.87 (1.37, 103.19)	0.0250		
	Sex	2.30 (1.00, 5.29)	0.0495				

proteinuria. Interestingly, while chromatin antibodies were positively associated with proteinuria, antibodies toward Sm and La were negatively associated.

3.6. Sex and Anti-La Responses Are Associated with Mucocutaneous Criteria, while Anti-Sm/RNP and Anti-RNP Antibodies Are Enriched in European-American SLE Patients with Seizures. Associations between mucocutaneous clinical SLE presentation were assessed in EA and AA SLE patients. Anti-La antibodies were negatively associated with malar rash, while female sex was positively associated with higher odds of developing malar rash. Female sex in EA patients and anti-La antibodies in AA patients were correlated with photosensitivity. Interestingly, a negative association between anti-52 kD Ro antibodies and photosensitivity was observed in AA patients. An association between oral ulcers and anti-Sm/RNP antibodies was also only observed in AA (Table 3). Anti-Sm/RNP antibodies were enriched in EA patients with seizures (OR 5.33, 95% CI 1.15–24.8). No significant autoantibody and ACR criteria/subcriteria associations were observed in HI SLE patients.

4. Discussion

The goal of this study was to use multiplex autoantibody detection technology to determine associations between the presence of specific autoantibodies and classification criteria within a large, ethnically diverse cohort of SLE patients. We screened a panel of 10 autoantibody specificities that are often detected in SLE (dsDNA, chromatin, ribosomal P, 60 kD Ro, 52 kD Ro, La, Sm, Sm/RNP, RNP 68, and RNP A). Our study, as well as those of others, has described the BioPlex 2200 assay as a highly sensitive method for the detection of these autoantibody specificities [27–32]. Understanding associations between specific autoantibodies and SLE criteria as detected by this new methodology which is in widespread clinical use provides key insights into prognostic

relevance for clinical application and may improve screening tests for diagnostic purposes.

Our study participants demonstrate a diverse, representative SLE patient population. While HI patients had a statistically lower age at participation (37.9 ± 12.8) than the other groups (EA 43.2 ± 13.6, AA 40.7 ± 12.4, and other 39.9 ± 12), this is consistent with the earlier age of SLE onset in the overall HI population [33, 34]. The most prevalent criteria in our cohort were ANA, arthritis, immunologic, and hematologic criteria, similar to those observed in previous studies [34–36].

Ethnic differences in autoantibody prevalence and association with ACR SLE classification criteria are observed in our study. We report a detailed association analysis between multiple autoantibodies and hematological disorder in our large Hispanic cohort, which has not been previously reported. We observed a significant difference between AA BioPlex 2200 ANA-positive and ANA-negative SLE patients. Hematological disorder ($P = 0.001$), lymphopenia ($P = 0.030$), and immunological disorder ($P = 0.0068$) were significantly enriched in ANA-positive AA patients. However, when a similar analysis was performed using indirect immunofluorescence, these associations disappeared. This is most likely due to the difference in specificity and sensitivity between the two assays. It is important to note that our EA patient group did have the lowest autoantibody prevalence for all tested specificities. Thus, the use of the BioPlex 2200 ANA may require the use of traditional autoantibody assays to confirm absence of ANA in this population subgroup. In AA patients, multiple autoantibodies associate with hematologic involvement in SLE. The associations between SLE autoantibody specificities and ACR criterion observed in our study confirm those observed in previous work [9, 33–35].

Our initial univariate conditional analysis demonstrates association between multiple autoantibodies. However, our multivariate adjusted conditional logistic regression analysis shows that antibodies to 60 kD Ro and RNP 68 are significantly independently enriched in EA patients with hematological disorders. In AA patients, anti-SM/RNP and anti-La antibodies are correlated with hematological disorder, while anti-RNP A antibodies alone are highly associated with hematological disorder in HI patients. Our results show that antibodies to ribonucleoproteins are highly prevalent in patients with hematological disorder. These results differ than those of Agmon-Levin et al. [2] and To and Petri [37]. Here, Sm/RNP antibodies were underrepresented in SLE patients with hematologic criteria.

In our multivariate analysis, a significant association between anti-52 kD Ro, anti-ribosomal P, anti-RNP antibodies and the hematological ACR criterion is observed in EA and AA patients. Previous studies have identified correlations between anti-Ro and both lymphopenia and leukopenia or with lymphopenia alone and suggested a moderate association between anti-dsDNA and lymphopenia mostly in EA patient cohorts [12, 13]. A significant enrichment of anti-RNP 68 antibodies was observed in EA patients with lymphopenia. HI patients showed unique antibody associations with hematological ACR criterion. Interestingly, while antibodies to dsDNA were inversely associated with lymphopenia, anti-chromatin antibodies were directly associated with lymphopenia in HI patients. Our analysis has not only replicated the association between antibody to Ro and lymphopenia, but also revealed the inverse correlation between anti-dsDNA antibodies and lymphopenia in HI patients. However, differences in hematological criteria, especially luekopenia, may be due to benign ethnic neutropenia [38–41], a well-described characteristic that is not a manifestation of lupus, in some cases.

The association of dsDNA antibody specificity with renal disease has been widely demonstrated [8, 14, 42] and is also confirmed in our study. Interestingly, we have observed different distinct autoantibody associations with renal disease in EA and AA patients. The overall autoantibody associations with renal disease in EA are anti-chromatin and anti-Sm antibodies. Anti-dsDNA antibodies and female sex are more common in patients with renal disease; while anti-La antibodies are underrepresented in SLE patients with renal disease. These autoantibody association differences were maintained when examining renal disease subcriteria (proteinuria and cellular casts). In AA patients, female sex is associated with proteinuria; while anti-dsDNA and anti-Sm/RNP antibodies are correlated with cellular casts. The association between anti-Sm/RNP antibodies and renal criteria has not been previously described. In EA SLE patients, anti-Sm is mildly associated with proteinuria with odds ratio approaching 1.00, further studies are necessary to confirm this effect. No significant association between autoantibodies and renal involvement was observed in HI patients. Thus, our study suggests that anti-chromatin and absence of anti-La antibodies are the main predictors for renal involvement driven by prevalence of proteinuria in EA patients. However, the lack of autoantibody associations with cellular casts in EA SLE patients might be due to low numbers of EA patients being tested for cellular casts. Additionally, our study suggests that anti-dsDNA and anti-Sm/RNP antibodies are the strong correlates for renal disease as measured by cellular casts in AA patients.

Ethnic differences in mucocutaneous manifestations of SLE are observed in our patient cohort. In our study population, EA patients show an increased prevalence of malar rash and photosensitivity; while AA patients exhibit increased frequency for discoid rash which is consistent with previous studies [43, 44]. Our autoantibody results indicate that in EA, malar rash is positively correlated with female sex; anti-La antibodies are negatively associated with malar rash in EA patients, while anti-La and anti-52 kD Ro antibodies are enriched in AA patients with photosensitivity. Several previously reported studies have observed an association between anti-RNP antibodies and photosensitivity [45, 46], which is not seen in our study. However, it is important to note that these two previous studies utilized a primarily Asian cohort [46, 47]. It is possible that this association also exists in our population, but the relatively small number of Asian study participants prevents this observation.

The goal of this study was to examine associations between the prevalence of autoantibodies as detected by a Luminex bead-based assay (BioRad BioPlex 2200) and the occurrence of various clinical criteria in a large, ethnically

diverse SLE patient cohort. The major findings of this paper identify associations between several antibody specificities and hematological disease, more specifically leukopenia and lymphopenia, and identify ethnic differences in autoantibody associations with renal subcriteria. Work is currently underway to better understand the mechanisms underlying these associations, particularly between leukopenia, lymphopenia, and autoantibody production. Autoantibodies play a crucial role in the diagnosis of SLE and a better understanding of the relationships between antibody prevalence and the presentation of other clinical criteria will further strengthen their prognostic implications.

Conflict of Interests

J. B. Harley is a consultant for Bio-Rad. The Bio-Rad BioPlex 2200 ANA screening kits were provided free of charge from Bio-Rad for this study. The remaining authors report no conflict of interests.

Acknowledgments

The authors would like to thank Lupus Family Registry and Repository personnel, participants, and the referring physicians for making this study possible. The authors thank David Wiist, Jr. and Aaron Guthridge for their technical assistance. They also thank Steven Binder, Ph.D. and Tony Prestigiacomo, Ph.D. for their technical expertise with the Bio-Rad BioPlex 2200 instrument and data interpretation. They also thank the OMRF Clinical Immunology Laboratory and more specifically Cathy Velte, Camille Anderson, and Sandy Long. This work was supported by grants from the National Institutes of Health (RR015577, RR015577-10S1, GM103510, AI082714, AR053483 (J. A. James), ARC24717, ARC49084, AR060366, AI094377 (S. K. Nath), N01-AR-6-2277 (K. L. Moser Silvis and J. B. Harley), RR029882 (G. S. Gilkeson and D. L. Kamen), and AR049459 (G. S. Gilkeson and D. L. Kamen)) and the US Department of Veterans Affairs. This work is also supported in part by an OMRF predoctoral fellowship award (B. F. Bruner) and by the Lou Kerr Chair in Biomedical Research (J. A. James). This study was made possible in part by the Kirkland Scholar Award Program at the Hospital for Special Surgery in New York City and is funded exclusively by Rheuminations, Inc., a nonprofit foundation dedicated to supporting research leading to the treatment and cure of lupus. The contents of this work are the sole responsibility of the authors and do not necessarily represent the official views of the NIH or its relevant institutes.

References

[1] Y. Sherer, A. Gorstein, M. J. Fritzler, and Y. Shoenfeld, "Autoantibody explosion in systemic lupus erythematosus: more than 100 different antibodies found in SLE patients," *Seminars in Arthritis and Rheumatism*, vol. 34, no. 2, pp. 501–537, 2004.

[2] N. Agmon-Levin, M. Mosca, M. Petri, and Y. Shoenfeld, "Systemic lupus erythematosus one disease or many?" *Autoimmunity Reviews*, vol. 11, no. 8, pp. 593–595, 2012.

[3] M. Mahler, K. Miyachi, C. Peebles, and M. J. Fritzler, "The clinical significance of autoantibodies to the proliferating cell nuclear antigen (PCNA)," *Autoimmunity Reviews*, vol. 11, no. 10, pp. 771–775, 2012.

[4] L. D. Heinlen, M. T. McClain, L. L. Ritterhouse et al., "60 kD Ro and nRNP A frequently initiate human lupus autoimmunity," *PLoS One*, vol. 5, no. 3, p. e9599, 2010.

[5] L. D. Heinlen, M. T. McClain, J. Merrill et al., "Clinical criteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms," *Arthritis and Rheumatism*, vol. 56, no. 7, pp. 2344–2351, 2007.

[6] M. R. Arbuckle, M. T. McClain, M. V. Rubertone et al., "Development of autoantibodies before the clinical onset of systemic lupus erythematosus," *New England Journal of Medicine*, vol. 349, no. 16, pp. 1526–1533, 2003.

[7] M. R. Arbuckle, J. A. James, G. J. Dennis et al., "Rapid clinical progression to diagnosis among African-American men with systemic lupus erythematosus," *Lupus*, vol. 12, no. 2, pp. 99–106, 2003.

[8] M. R. Arbuckle, J. A. James, K. F. Kohlhase, M. V. Rubertone, G. J. Dennis, and J. B. Harley, "Development of anti-dsDNA autoantibodies prior to clinical diagnosis of systemic lupus erythematosus," *Scandinavian Journal of Immunology*, vol. 54, no. 1-2, pp. 211–219, 2001.

[9] L. D. Heinlen, L. L. Ritterhouse, M. T. McClain et al., "Ribosomal P autoantibodies are present before SLE onset and are directed against non-C-terminal peptides," *Journal of Molecular Medicine*, vol. 88, no. 7, pp. 719–727, 2010.

[10] B. E. Gilliam, A. K. Ombrello, R. W. Burlingame, P. H. Pepmueller, and T. L. Moore, "Measurement of autoantibodies in pediatric-onset systemic lupus erythematosus and their relationship with disease-associated manifestations," *Seminars in Arthritis and Rheumatism*, vol. 41, no. 6, pp. 840–848, 2012.

[11] J. B. Harley, A. L. Sestak, L. G. Willis, S. M. Fu, J. A. Hansen, and M. Reichlin, "A model for disease heterogeneity in systemic lupus erythematosus. Relationships between histocompatibility antigens, autoantibodies, and lymphopenia or renal disease," *Arthritis and Rheumatism*, vol. 32, no. 7, pp. 826–836, 1989.

[12] C. A. Hitchon and C. A. Peschken, "Sm antibodies increase risk of death in systemic lupus erythematosus," *Lupus*, vol. 16, no. 3, pp. 186–194, 2007.

[13] L. M. Vilá, G. S. Alarcón, G. McGwin, H. M. Bastian, B. J. Fessler, and J. D. Reveille, "Systemic lupus erythematosus in a multiethnic US cohort, XXXVII: association of lymphopenia with clinical manifestations, serologic abnormalities, disease activity, and damage accrual," *Arthritis Care and Research*, vol. 55, no. 5, pp. 799–806, 2006.

[14] E. J. Ter Borg, G. Horst, E. J. Hummel, P. C. Limburg, and C. G. M. Kallenberg, "Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long-term, prospective study," *Arthritis and Rheumatism*, vol. 33, no. 5, pp. 634–643, 1990.

[15] M. Reichlin, T. F. Broyles, O. Hubscher et al., "Prevalence of autoantibodies to ribosomal P proteins in juvenile-onset systemic lupus erythematosus compared with the adult disease," *Arthritis and Rheumatism*, vol. 42, no. 1, pp. 69–75, 1999.

[16] S. Malik, G. R. Bruner, C. Williams-Weese et al., "Presence of anti-La autoantibody is associated with a lower risk of nephritis and seizures in lupus patients," *Lupus*, vol. 16, no. 11, pp. 863–866, 2007.

[17] V. D. Pradhan, S. S. Badakere, L. S. Bichile, and M. P. Dolas, "Antibodies to Ro/SS-A and La/SS-B in systemic lupus erythematosus and other autoimmune disorders," *Journal of Association of Physicians of India*, vol. 50, no. 6, pp. 762–765, 2002.

[18] T. Grader-Beck and F. M. Wigley, "Raynaud's phenomenon in mixed connective tissue disease," *Rheumatic Disease Clinics of North America*, vol. 31, no. 3, pp. 465–481, 2005.

[19] G. A. McCarty, J. B. Harley, and M. Reichlin, "A distinctive autoantibody profile in black female patients with lupus nephritis," *Arthritis and Rheumatism*, vol. 36, no. 11, pp. 1560–1565, 1993.

[20] C. E. Tseng, J. P. Buyon, M. Kim et al., "The effect of moderate-dose corticosteroids in preventing severe flares in patients with serologically active, but clinically stable, systemic lupus erythematosus: findings of a prospective, randomized, double-blind, placebo-controlled trial," *Arthritis and Rheumatism*, vol. 54, no. 11, pp. 3623–3632, 2006.

[21] M. T. McClain, M. R. Arbuckle, L. D. Heinlen et al., "The prevalence, onset, and clinical significance of antiphospholipid antibodies prior to diagnosis of systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 50, no. 4, pp. 1226–1232, 2004.

[22] E. M. Tan, A. S. Cohen, and J. F. Fries, "The 1982 revised criteria for the classification of systemic lupus erythrematosus," *Arthritis and Rheumatism*, vol. 25, no. 11, pp. 1271–1277, 1982.

[23] M. C. Hochberg, "Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 40, no. 9, p. 1725, 1997.

[24] A. Rasmussen, S. Sevier, J. A. Kelly et al., "The lupus family registry and repository," *Rheumatology*, vol. 50, no. 1, pp. 47–59, 2011.

[25] M. T. McClain, L. D. Heinlen, G. J. Dennis, J. Roebuck, J. B. Harley, and J. A. James, "Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry," *Nature Medicine*, vol. 11, no. 1, pp. 85–89, 2005.

[26] S. R. Binder, M. C. Genovese, J. T. Merrill, R. I. Morris, and A. L. Metzger, "Computer-assisted pattern recognition of autoantibody results," *Clinical and Diagnostic Laboratory Immunology*, vol. 12, no. 12, pp. 1353–1357, 2005.

[27] B. F. Bruner, J. M. Guthridge, R. Lu et al., "Comparison of autoantibody specificities between traditional and bead-based assays in a large, diverse collection of SLE patients and family members," *Arthritis and Rheumatism*. In press.

[28] N. Bardin, S. Desplat-Jego, L. Daniel, N. Jourde Chiche, and M. Sanmarco, "BioPlex 2200 multiplexed system: simultaneous detection of anti-dsDNA and anti-chromatin antibodies in patients with systemic lupus erythematosus," *Autoimmunity*, vol. 42, no. 1, pp. 63–68, 2009.

[29] J. G. Hanly, L. Su, V. Farewell, and M. J. Fritzler, "Comparison between multiplex assays for autoantibody detection in systemic lupus erythematosus," *Journal of Immunological Methods*, vol. 358, no. 1-2, pp. 75–80, 2010.

[30] J. G. Hanly, K. Thompson, G. McCurdy, L. Fougere, C. Theriault, and K. Wilton, "Measurement of autoantibodies using multiplex methodology in patients with systemic lupus erythematosus," *Journal of Immunological Methods*, vol. 352, no. 1-2, pp. 147–152, 2010.

[31] K. G. Moder, M. H. Wener, M. H. Weisman et al., "Measurement of antinuclear antibodies by multiplex immunoassay: a prospective, multicenter clinical evaluation," *Journal of Rheumatology*, vol. 34, no. 5, pp. 978–986, 2007.

[32] O. Shovman, B. Gilburd, O. Barzilai et al., "Evaluation of the BioPlex 2200 ANA screen. Analysis of 510 healthy subjects: incidence of natural/predictive autoantibodies," *Annals of the New York Academy of Sciences*, vol. 1050, pp. 380–388, 2005.

[33] C. S. Lau, G. Yin, and M. Y. Mok, "Ethnic and geographical differences in systemic lupus erythematosus: an overview," *Lupus*, vol. 15, no. 11, pp. 715–719, 2006.

[34] K. Kumar, S. Chambers, and C. Gordon, "Challenges of ethnicity in SLE," *Best Practice and Research*, vol. 23, no. 4, pp. 549–561, 2009.

[35] A. I. Quintero-Del-Rió, D. Bacino, J. Kelly, T. Aberle, and J. B. Harley, "Familial systemic lupus erythematosus: a comparison of clinical manifestations and antibody presentation in three ethnic groups," *Cellular and Molecular Biology*, vol. 47, no. 7, pp. 1223–1227, 2001.

[36] D. L. Kamen, M. Barron, T. M. Parker et al., "Autoantibody prevalence and lupus characteristics in a unique African American population," *Arthritis and Rheumatism*, vol. 58, no. 5, pp. 1237–1247, 2008.

[37] C. H. To and M. Petri, "Is antibody clustering predictive of clinical subsets and damage in systemic lupus erythematosus?" *Arthritis and Rheumatism*, vol. 52, no. 12, pp. 4003–4010, 2005.

[38] M. A. Nalls, J. G. Wilson, N. J. Patterson et al., "Admixture mapping of white cell count: genetic locus responsible for lower white blood cell count in the Health ABC and Jackson Heart Studies," *American Journal of Human Genetics*, vol. 82, no. 1, pp. 81–87, 2008.

[39] D. Reich, M. A. Nalls, W. H. L. Kao et al., "Reduced neutrophil count in people of African descent is due to a regulatory variant in the Duffy antigen receptor for chemokines gene," *PLoS Genetics*, vol. 5, no. 1, p. e1000360, 2009.

[40] A. P. Reiner, G. Lettre, M. A. Nalls et al., "Genome-Wide association study of white blood cell count in 16,388 african americans: the continental Origins and Genetic Epidemiology network (COGENT)," *PLoS Genetics*, vol. 7, no. 6, p. e1002108, 2011.

[41] T. B. Haddy, S. R. Rana, and O. Castro, "Benign ethnic neutropenia: what is a normal absolute neutrophil count?" *Journal of Laboratory and Clinical Medicine*, vol. 133, no. 1, pp. 15–22, 1999.

[42] P. Alba, L. Bento, M. J. Cuadrado et al., "Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis," *Annals of the Rheumatic Diseases*, vol. 62, no. 6, pp. 556–560, 2003.

[43] G. S. Cooper, C. G. Parks, E. L. Treadwell et al., "Differences by race, sex and age in the clinical and immunologic features of recently diagnosed systemic lupus erythematosus patients in the southeastern United States," *Lupus*, vol. 11, no. 3, pp. 161–167, 2002.

[44] A. L. Sestak, S. K. Nath, J. A. Kelly, G. R. Bruner, J. A. James, and J. B. Harley, "Patients with familial and sporadic onset SLE have similar clinical profiles but vary profoundly by race," *Lupus*, vol. 17, no. 11, pp. 1004–1009, 2008.

[45] W. X. Li, H. F. Pan, L. H. Li et al., "Prevalence and clinical significance of 15 autoantibodies in patients with new-onset systemic lupus erythematosus," *Irish Journal of Medical Science*, vol. 179, no. 4, pp. 623–627, 2010.

[46] X. Tang, Y. Huang, W. Deng, L. Tang, W. Weng, and X. Zhang, "Clinical and serologic correlations and autoantibody clusters

in systemic lupus erythematosus: a retrospective review of 917 patients in South China," *Medicine*, vol. 89, no. 1, pp. 62–67, 2010.

[47] W. X. Li, H. F. Pan, L. H. Li et al., "Prevalence and clinical significance of 15 autoantibodies in patients with new-onset systemic lupus erythematosus," *Irish Journal of Medical Science*, vol. 179, no. 4, pp. 623–627, 2010.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 301274, 6 pages
doi:10.1155/2012/301274

Review Article

Neonatal Lupus Erythematosus

Kam Lun Hon[1] and Alexander K. C. Leung[2]

[1] *Department of Pediatrics, The Chinese University of Hong Kong, Prince of Wales Hospital, 6/F, Clinical Sciences Building, Shatin, Hong Kong*
[2] *Department of Pediatrics, Alberta Children's Hospital, The University of Calgary, Calgary, AB, Canada T2N 1N4*

Correspondence should be addressed to Kam Lun Hon, ehon@cuhk.edu.hk

Received 4 June 2012; Revised 29 July 2012; Accepted 2 August 2012

Academic Editor: Hiroshi Okamoto

Neonatal lupus erythematosus (NLE) refers to a clinical spectrum of cutaneous, cardiac, and systemic abnormalities observed in newborn infants whose mothers have autoantibodies against Ro/SSA and La/SSB. The condition is rare and usually benign and self-limited but sometimes may be associated with serious sequelae. We review the pathophysiology, clinical features, and management of infants with this condition. Neonates with NLE should be managed at a tertiary care center. Multidisciplinary team involvement may also be indicated. In mothers with anti-Ro/SSA and/or anti-La/SSB antibodies and infants with congenital heart block, the risk of recurrence in subsequent offspring is 17–25%. Therefore, careful monitoring of subsequent pregnancies with serial ultrasonography and echocardiography is essential.

1. Introduction

Neonatal lupus erythematosus (NLE) refers to a clinical spectrum of cutaneous, cardiac, and systemic abnormalities observed in newborn infants whose mothers have autoantibodies against Ro/SSA, La/SSB, and, less commonly, U1-ribonucleoprotein (U1-RNP) [1–3]. The condition was first described in 1954 by McCuistion and Schoch who reported a case of transient lupus skin lesion in an infant with an ANA-positive mother [4]. The most common presentation is a nonscarring, nonatrophic skin lesion which resemble subacute cutaneous lupus erythematosus. The infants may have no skin lesions at birth but develop them during the first weeks of life. Cardiac, hematological, hepatobiliary, central nervous, and pulmonary systems may also be involved. NLE is associated with transplacental passage of autoantibodies such as anti-RoSSA and anti-La/SSB [5, 6]. The condition is usually benign and self-limited but sometimes may be associated with serious sequelae.

2. Pathophysiology

A number of studies have suggested that NLE is caused by the transplacental passage of maternal autoantibodies [5, 7]. These autoantibodies may cause damage to the developing tissue and increase the risk of bearing infants with NLE. Approximately 98% of affected infants have maternal transfer of autoantibodies against Ro/SSA, La/SSB, and, less commonly, U1-RNP. However, only 1-2% of mothers with these autoantibodies have neonates with NLE, regardless of whether the mothers are symptomatic or not [8].

The 52-kD Ro/SSA (Ro52) ribonucleoprotein is an antigenic target strongly linked with the autoimmune response in mothers whose children have NLE, congenital heart block, and other conduction abnormalities [9]. Anti-Ro52/SSA autoantibodies antagonize the serotonin-induced L-type calcium channel activation on human fetal atrial cells and trigger an inflammatory response, leading ultimately to fibrosis and scarring of the atrioventricular node, sinus node, and His bundle [9, 10]. This may explain the electrophysiological abnormalities in NLE and the pathogenesis of the cardiac rhythm disturbances, which may lead to diminished cardiac output and the subsequent development of congestive heart failure [9]. In a rat model, Boutjdir et al. [11] demonstrated that IgG containing anti-Ro/SSA and -La/SSB antibodies induces complete AV block in beating hearts and in multicellular preparations, thus implicating a preferential interaction of these autoantibodies with calcium channels and/or

associated regulatory proteins. This is consistent with the observed inhibition of calcium channels that may be a critical factor contributing to the pathogenesis of complete heart block. These conduction defects are caused by anti-Ro/SSA and anti-La/SSB antibodies as well as other autoantibodies against cardiac adrenoceptors and muscarinic acetylcholine receptors [12].

The antibodies associated with heart block and with cutaneous disease are believed to be different; antibodies against the 52/60-kD Ro/SSA and 48-kD La/SSB ribonucleoproteins are associated with heart block, whereas antibodies against the 50-kD La/SSB ribonucleoprotein are associated with cutaneous disease [12, 13].

On the other hand, anti-U1RNP autoantibodies are usually associated with atypical cutaneous lesions without cardiac or systemic abnormalities in a small number of NLE cases and may play a role in the pathogenesis of thrombocytopenia [10]. It has been demonstrated that the anti-U1RNP antibody from patients with connective tissue disease can directly recognize a variety of antigens on the endothelial surface of the pulmonary artery, including the components of U1RNP or other unknown polypeptides. These results suggest that binding to HPAECs of this autoantibody may be one of the triggers of endothelial cell inflammation in various connective tissue diseases [14]. The spectrum of cutaneous disease in U1RNP antibody-positive infants is similar to Ro/SSA antibody-positive infants with NLE. Complete heart block was not a feature of U1RNP antibody-positive NLE. HLA typing studies show a more diverse immunogenetic pattern in U1RNP antibody-positive mothers of infants with NLE compared with Ro/SSA antibody-positive mothers.

It has been shown that the amount of maternal antibodies, rather than their presence, is associated with fetal tissue injury [13]. However, only some neonates exposed to these antibodies develop complications. Therefore, other factors such as titers of maternal antibodies, genetic predisposition, and environmental factors such as viral infection may be involved. Additionally, induction of apoptosis in cultured cardiomyocytes has been demonstrated to result in the expression of Ro/La antigens on the cell surface for recognition by circulating maternal antibodies [15]. It is speculated that *in vivo* such opsonized apoptotic cardiocytes promote an inflammatory response by resident macrophages with damage to surrounding conducting tissue.

In addition to its presence in the skin and heart, the Ro antigen is also found in the liver, bowel, lungs, brain, and blood cells—the tissues that are most often affected by NLE [3]. Ultraviolet radiation and estrogens increase Ro antigen expression on the surface of the keratinocyte [3]. Although ultraviolet radiation can induce or exacerbate the skin lesions, it is not required for their development [10]. Because of limited opportunity for solar exposure in neonates and young infants, photosensitivity is more commonly seen after phototherapy for neonatal hyperbilirubinemia [10].

3. Epidemiology

NLE is a rare acquired autoimmune disease that occurs in 1 of every 20,000 live births in the USA [5]. Elsewhere, epidemiology is usually described in small case series [7, 16]. The presence of certain major histocompatibility complexes such as human leukocyte antigen B8 and human leukocyte antigen DR3 in the mother predisposes the infant to NLE and congenital heart block [17, 18]. Although there is no apparent racial predilection, disparity in outcomes between minorities and whites has been observed [5, 10, 16, 18–20]. Like many autoimmune diseases, reports from the Research Registry for Neonatal Lupus/US indicate that the female-to-male ratio is approximately 2 : 1 with cutaneous NLE, but the gender distribution for cardiac disease is approximately equal [21, 22]. The risk of NLE or congenital heart block developing in a woman who tests positive for Ro/SSA who has never had a child with NLE or congenital heart block is less than 1%. Many seropositive mothers with anti-Ro/SSA and anti-La/SSB antibodies give birth to infants who do not show signs and symptoms of NLE. However, in those who have had an infant with NLE, the risk of cardiac and/or skin disease for a future pregnancy is high. The incidence of congenital heart block is 15–30% in infants with NLE [19]. Heart block usually develops *in utero* between the 18th and 24th weeks of pregnancy. Infants born to mothers with hypothyroidism due to thyroid autoantibodies and anti-Ro/SSA positivity are at nine times higher risk of developing congenital complete heart block than infants born to mothers with only anti-Ro/SSA positivity [23].

Approximately 40–60% of mothers are asymptomatic when the infants are diagnosed to have NLE [8]. The remaining mothers may have SLE, Sjögren syndrome, rheumatoid arthritis, or undifferentiated autoimmune disorder. Mothers with primary Sjögren syndrome or undifferentiated autoimmune syndrome have a greater risk of delivering an infant with congenital complete heart block than those with SLE [12, 24]. There is no association with paternal autoimmune diseases [12].

4. Clinical Manifestations

The most common clinical manifestations of NLE are, in decreasing order of frequency, dermatologic, cardiac, and hepatic abnormalities [1, 5, 10, 16, 25]. Some infants may also have hematologic, neurologic, or splenic abnormalities [5, 7, 10, 16]. One or more systems may be involved. Wisuthsarewong et al. performed a retrospective study to review clinical manifestations on 17 patients (10 girls and 7 boys) with NLE seen at the Department of Pediatrics, Siriraj Hospital from 1993 to 2008 [10]. Cutaneous, cardiac, hepatobiliary, and hematological involvement was found in 70.6%, 64.7%, 52.9%, and 35.3% of infants, respectively.

Cutaneous lesions may be present at birth but often appear within the first few weeks of life [26, 27]. Annular erythematous or polycystic plaques with or without fine scales characterize NLE and appear predominately on the scalp, neck, or face (typically periorbital in distribution), but similar plaques may appear on the trunk or extremities [10, 26]. The dermatitis resembles the rash of subacute cutaneous lupus erythematosus rather than the malar rash of SLE [25].

Periorbital erythema, referred to as "raccoon eye" or "owl eye," is a very common characteristic [3, 10]. At times, the lesions may be urticarial, desquamative, ulcerative, or crusted [28, 29]. Bullous lesions may be seen with a particular predilection for the soles of the feet [25].

In one study, cutaneous involvement was characterized as erythematous patches (91.7%), subacute cutaneous lupus erythematosus lesions (50%), petechiae (41.7%), persistent cutis marmorata (16.7%), and discoidal lesions (8.3%) [10]. In some infants, solar exposure seems to precipitate the eruption [30]. These lesions typically last for weeks or months and then resolve spontaneously consequent to the disappearance of maternal antibodies in the neonatal circulation [26]. Active erythematous lesions after the first year of life should be suspect. Dyspigmentation is frequent but usually resolves spontaneously. Atrophic lesions and, rarely, atrophic scars may develop [10, 27]. Telangiectasia is often prominent and is the sole cutaneous manifestation reported in some patients. The atrophic telangiectatic changes are most evident near the temples and scalp and do not necessarily occur in the same sites as the erythematous lesions [26]. The latter site may occasionally be associated with a permanent alopecia. Telangiectasia, scarring, and atrophic changes are expected to persist.

The cardiac manifestations include conduction abnormalities (first-, second-, and third-degree heart block) and cardiomyopathy [1, 2, 5, 24, 31]. Third-degree heart block, once established, is usually irreversible [26]. Congenital heart block may present as bradycardia noted *in utero* or during physical examination at birth [24]. Conduction disturbances may also present as irregular heartbeat and prolongation of the QT interval [24]. Congenital heart block may be associated with endocardial fibroelastosis and cardiomyopathy [32]. In some cases, myocarditis and pericarditis can develop which may lead to bradycardia. Heart failure is a well-recognized complication during the neonatal period.

The clinical pictures of hepatobiliary involvement may take the forms of elevation of liver enzymes (such as aspartate aminotransferase and alanine aminotransferase) and/or conjugated hyperbilirubinemia occurring a few weeks or months after birth and resolving thereafter. Some infants may have mild hepatomegaly and, less commonly, splenomegaly [25]. The hepatomegaly and splenomegaly are usually transient. Cholestatic hepatitis and hepatic failure may also occur.

Hematologic disturbances (e.g., hemolytic anemia, thrombocytopenia, and neutropenia) may occur in the first 2 weeks of life. Infants with hematological involvement are usually asymptomatic [25]. Autoantibodies, mainly anti-Ro, bind directly to the neutrophil and cause neutropenia. Thrombocytopenia may manifest as petechiae. Hematologic symptoms usually appear at around the second week of life and disappear by the end of the second month. Lymphopenia is a relatively common finding in adults with SLE but is not a characteristic hematologic abnormality of NLE [26].

Other abnormalities such as hydrocephalus and macrocephaly may occur [33]. Aseptic meningitis and myelopathy have rarely been reported [10]. Pneumonitis may manifest as tachypnea and/or tachycardia.

5. Diagnosis and Differential Diagnosis

The diagnosis is usually established based on the clinical features and the demonstration of NLE-associated antibodies in the serum of the mother or the affected infant [5, 10, 16]. NLE can mimic many conditions [5, 10, 16]. Differential diagnosis of NLE includes seborrheic dermatitis, atopic dermatitis, neonatal acne, tinea corporis, psoriasis, granuloma annulare, erythema multiforme, Langerhans cell histiocytosis, congenital rubella, congenital syphilis, Bloom syndrome, and Rothmund-Thomson syndrome [3].

6. Laboratory Investigations

NLE is associated with the anti-Ro/SSA antibody in more than 90% of patients [9]. Occasionally, patients only have anti-La/SSB or anti-U1RNP antibodies. Screening of infants with NLE for the presence of these antibodies is strongly recommended [2]. Many asymptomatic mothers have positive putative antibodies during pregnancy [10]. As such, these mothers of patients suspected of having neonatal lupus erythematosus should be screened for antinuclear, anti-double-stranded DNA, anti-Ro/SSA, anti-La/SSB, and anti-U1-RNP antibodies, irrespective of their symptoms or clinical status [9]. As anti-Ro/SSA antibodies can be detected in one in 200 pregnant women, the risk for an anti-Ro/SSA-positive woman to have an infant with NLE is relatively low [26]. On the other hand, high anti-Ro/SSA levels correlate with the risk of cardiac complications. Serial prenatal ultrasonography/electrocardiography should therefore be performed on pregnant women with high anti-Ro titers ($\geq$50 U/mL) [13]. Prenatal ultrasonography may help identify NLE that affects the heart. Echocardiography may reveal various types of structural deformities in the heart; combined electrocardiography and 24-hour Holter monitoring may reveal various cardiac conduction disorders or different types of heart blocks.

Laboratory investigations may reveal pancytopenia, thrombocytopenia, leukopenia, or elevated transaminase level [34].

Skin biopsy is useful in patients with NLE when the diagnosis is in doubt. Histologic examination shows interface dermatitis, keratinocyte damage, moderate hyperkeratosis, follicular plugging, and vacuolar degeneration in the basal cell layer. Epidermal atrophy may be found [26]. Inflammatory infiltrate may be intense with bulla formation histologically. An immunofluorescent examination reveals a granular deposition of immunoglobulin G (IgG) at the dermoepidermal junction; IgM and C3 deposition may also be evident.

Skin biopsy is not pathognomonic. Various inflammatory and infectious conditions may show similar histological features. In typical cases of NLE and positive autoantibodies, skin biopsy is not mandatory to confirm the diagnosis.

7. Treatment and Followup

Neonates with NLE should be managed at a tertiary care center. Multidisciplinary team involvement may also be

indicated. Patients with NLE with cardiac involvement require regular monitoring to assess cardiac function and the need for a pacemaker. A pacemaker is often necessary for those who are unable to compensate for a slow heart rate. Serial echocardiography to monitor for a prolonged PR interval should also be arranged. If the cardiac involvement is severe, activity may have to be restricted in the young child.

Sunscreens may be useful in the treatment of cutaneous lupus erythematosus, but a neonate is less likely to be exposed to sunlight excessively. Nevertheless, solar exposure should be avoided if possible. Parents should be advised to apply sunscreen well before solar exposure and to use a sunscreen with a high SPF that provides a broad-spectrum (UV-A) coverage which is water resistant. Behavior modification to include solar avoidance should be encouraged. Protective clothing is highly desirable. Strategies aimed at preventing disease before irrevocable scarring ensues are a high priority. Skin lesions of NLE can be treated with mild topical corticosteroids. Antimalarial agents have potential toxicity and a slow onset of action that their use in the treatment of this transient condition is probably not indicated [26]. Laser therapy may be considered for residual telangiectasia. Systemic corticosteroids and immunosuppressive agents are generally not indicated in the treatment of NLE [26]. Children with NLE need continued followup, especially before adolescence and if the mother herself has an autoimmune disease [35]. Although the child may not be at increased risk of developing SLE, the development of some form of autoimmune disease in early childhood may be of concern.

Infants with severe hepatic and hematological involvement may require treatment with systemic corticosteroids, intravenous immunoglobulin, and/or immunosuppressive agents [10].

8. Prognosis

The morbidity and mortality of SLE of childhood depend on the organ systems affected [5, 7]. Children with NLE have an excellent long-term outcome when only skin lesions are present [36]. The cutaneous lesions usually disappear by 6 months of age coincident with the clearance of maternal antibodies from the child's circulation [5, 24, 31]. Involvement of the skin may, rarely, lead to scar formation. Although children with cutaneous disease may be more prone to develop SLE or autoimmunity later in life, this is mainly due to their genetic predisposition, not that they had NLE. Their nonaffected siblings are also at risk for development of SLE or autoimmunity. While the cutaneous lesions of NLE are themselves benign, cutaneous NLE is associated with a 6–10-fold risk for a subsequent child with cardiac NLE [5, 24, 31].

NLE with cardiac involvement is associated with a 20–30% mortality rate in the neonatal period [5, 24, 31]. Mortality is particularly high in cases of congenital heart block with concurrent cardiomyopathy [19, 26]. Death most often results from congestive heart failure caused by congenital heart block. Approximately 57 to 66% of patients with congenital heart block eventually require a pacemaker [5, 24, 31, 36]. Those with pacemakers are at risk of developing dilated cardiomyopathy in their lives [37]. Deaths may also occur later in life as a result of the failure of the pacemaker. However, many children with congenital heart block may be relatively asymptomatic until adolescence, when they begin to exercise. At that time, they may develop syncope and require a pacemaker implantation.

The recurrence rate of congenital heart block is low, about 15%, but this is nearly three times higher than the risk for congenital heart block in a primigravida with the putative antibodies [12]. Prospective clinical trials on use of antenatal fluorinated steroids in women with anti-SSA/Ro and/or anti-SSB/La antibodies and fetuses with heart block identified *in utero* are required before definitive recommendations can be made. A number of anecdotal cases support the use of dexamethasone for treatment of hydrops and possibly incomplete block [12].

Most patients with NLE affecting liver or blood have transient disease that spontaneously resolves within 4–6 months. In some cases, cholestatic hepatitis and liver failure may occur which is associated with a poor prognosis. Anemia, thrombocytopenia, and neutropenia are self-limited. However, if severe thrombocytopenia is present, internal bleeding can lead to a poor prognosis.

9. Future Pregnancies

Although the fetal disease is called neonatal lupus erythematosus, this is considered a misnomer since only about 25% of mothers actually fulfill criteria for the diagnosis of SLE [12]. Furthermore, asymptomatic mothers do not invariably become ill [12]. Mothers of infants with NLE, particularly infants with congenital heart block, have a 2-fold to 3-fold increased risk of having an affected infant in a subsequent pregnancy. On the other hand, the risk for an unselected anti-Ro/SSA-positive woman has been estimated at 1-2% [26]. A prospective controlled study of pregnancy outcome in 100 women with autoimmune diseases and anti-Ro/SSA antibodies showed that the prevalence of congenital heart block in newborns of prospectively followed up women already known to be anti-Ro/SSA positive and with known connective tissue disorders was 2% [24, 38].

In mothers with anti-Ro/SSA and/or anti-La/SSB antibodies and infants with congenital heart block, the risk of recurrence in subsequent offspring is 17–25% [2, 39]. Therefore, carefully monitoring of subsequent pregnancies with serial ultrasonography and echocardiography, particularly at 18–24 weeks' gestation, is essential. Intravenous immunoglobulin merits evaluation as a potential prophylactic approach in mothers who have previously had an affected child [40]. However, two studies failed to demonstrate benefit in outcome from intravenous immunoglobulin [41, 42]. On the other hand, the use of hydroxychloroquine for patients with SLE has been associated with a lower rate of NLE during pregnancy [43].

Shinohara et al. assessed the possibility of preventing cardiac or cutaneous manifestations of NLE or treating the fetus with congenital heart block by administering corticosteroid therapy to the mother [44]. Eighty seven offspring of 40 anti-Ro/SSA-positive mothers, followed up from 1979 to

1996, were evaluated. None of 26 neonates whose mothers received corticosteroid maintenance therapy initiated before 16 weeks' gestation demonstrated congenital heart block, whereas 15 of 61 neonates whose mothers received no corticosteroids during pregnancy or began receiving steroid therapy after 16 weeks' gestation had congenital heart block. Complete congenital heart block, once developed, did not respond to corticosteroid treatment *in utero*. Four infants whose mothers received corticosteroid treatment before 16 weeks' gestation had skin lesions of NLE. The authors concluded that once established, complete congenital heart block was irreversible, and maternal corticosteroid therapy did not effectively prevent cutaneous LE. However, prenatal maintenance therapy with prednisolone or betamethasone given to the mother starting early in pregnancy (before 16 weeks' gestation) might reduce the risk of developing antibody-mediated congenital heart block in the offspring [44].

Mothers with SLE should be treated with drugs that are effective and safe for the fetus [45]. Such an approach may diminish or reduce the prevalence of complete heart block associated with NLE. Tincani et al. recently reported increased occurrence of learning disabilities in children born to mothers with SLE [45]. Corticosteroids and some immunosuppressive drugs can be used in pregnancy to control maternal disease. Some data suggest that prolonged fetal exposure to dexamethasone may impair cerebral development [46]. On the other hand, Tincani et al. followed 6 children (age range, 14–65 months), born to patients treated with dexamethasone because of congenital heart block [45]. These children were found to have a normal intelligence quotient [45]. However, the authors remarked that information about long-term outcome of children exposed to immunosuppressive drugs "*in utero*" are still lacking, and more efforts are needed in this research area.

10. Summary

Neonatal lupus erythematosus (NLE) refers to a clinical spectrum of cutaneous, cardiac, and systemic abnormalities observed in newborn infants whose mothers have autoantibodies against Ro/SSA and La/SSB. The condition may be associated with serious sequelae. Neonates with NLE should be managed at a tertiary care center, and multidisciplinary team involvement may be indicated.

References

[1] S. Garcia and A. C. Campos-de-Carvalho, "Neonatal lupus syndrome: the heart as a target of the immune system," *Anais da Academia Brasileira de Ciencias*, vol. 72, no. 1, pp. 83–89, 2000.

[2] L. K. Hornberger and N. Al Rajaa, "Spectrum of cardiac involvement in neonatal lupus," *Scandinavian Journal of Immunology*, vol. 72, no. 3, pp. 189–197, 2010.

[3] M. F. Perez, M. E. de Torres, M. M. Buján, A. Lanoël, A. B. Cervini, and A. M. Pierini, "Neonatal lupus erythematosus: a report of four cases," *Anais Brasileiros de Dermatologia*, vol. 86, no. 2, pp. 347–351, 2011.

[4] C. H. McCuistion and E. P. Schoch Jr., "Possible discoid lupus erythematosus in newborn infant: report of a case with subsequent development of acute systemic lupus erythematosus in mother," *AMA Archives of Dermatology and Syphilology*, vol. 70, no. 6, pp. 782–785, 1954.

[5] J. P. Buyon and R. M. Clancy, "Neonatal lupus syndromes," *Current Opinion in Rheumatology*, vol. 15, no. 5, pp. 535–541, 2003.

[6] K. Ayed, Y. Gorgi, I. Sfar, and M. Khrouf, "Congenital heart block associated with maternal anti SSA/SSB antibodies: a report of four cases," *Pathologie Biologie*, vol. 52, no. 3, pp. 138–147, 2004.

[7] L. Li, G. F. Dong, F. Z. Han, Y. Cui, Y. Z. Shi, and X. Zhang, "Neonatal lupus erythematosus: a report of 7 cases and review of 87 cases of China," *Zhonghua Er Ke Za Zhi*, vol. 49, pp. 146–150, 2011.

[8] M. Shahian, A. Khosravi, and M. H. Anbardar, "Early cholestasis in neonatal lupus erythematosus," *Annals of Saudi Medicine*, vol. 31, no. 1, pp. 80–82, 2011.

[9] P. Eftekhari, L. Salle, F. Lezoualc'h et al., "Anti-SSA/Ro52 autoantibodies blocking the cardiac 5-HT4 serotoninergic receptor could explain neonatal lupus congenital heart block," *European Journal of Immunology*, vol. 30, pp. 2782–2790, 2000.

[10] W. Wisuthsarewong, J. Soongswang, and R. Chantorn, "Neonatal lupus erythematosus: clinical character, investigation, and outcome," *Pediatric Dermatology*, vol. 28, no. 2, pp. 115–121, 2011.

[11] M. Boutjdir, L. Chen, Z. H. Zhang, C. E. Tseng, N. El-Sherif, and J. P. Buyon, "Serum and immunoglobulin G from the mother of a child with congenital heart block induce conduction abnormalities and inhibit L-type calcium channels in a rat heart model," *Pediatric Research*, vol. 44, no. 1, pp. 11–19, 1998.

[12] J. P. Buyon, "Neonatal lupus: bedside to bench and back," *Scandinavian Journal of Rheumatology*, vol. 25, no. 5, pp. 271–276, 1996.

[13] E. Jaeggi, C. Laskin, R. Hamilton, J. Kingdom, and E. Silverman, "The importance of the level of maternal anti-Ro/SSA antibodies as a prognostic marker of the development of cardiac neonatal lupus erythematosus. A Prospective Study of 186 antibody-exposed fetuses and infants," *Journal of the American College of Cardiology*, vol. 55, no. 24, pp. 2778–2784, 2010.

[14] M. Okawa-Takatsuji, S. Aotsuka, S. Uwatoko et al., "Endothelial cell-binding activity of anti-U1-ribonucleoprotein antibodies in patients with connective tissue diseases," *Clinical and Experimental Immunology*, vol. 126, no. 2, pp. 345–354, 2001.

[15] M. E. Miranda-Carus, A. D. Askanase, R. M. Clancy et al., "Anti-SSA/Ro and anti-SSB/La autoantibodies bind the surface of apoptotic fetal cardiocytes and promote secretion of TNF-α by macrophages," *Journal of Immunology*, vol. 165, no. 9, pp. 5345–5351, 2000.

[16] J. Liu, Y. H. Yang, Y. T. Lin, and B. L. Chiang, "Clinical characteristics of neonatal lupus erythematosus," *Journal of Microbiology, Immunology and Infection*, vol. 34, no. 4, pp. 265–268, 2001.

[17] P. H. Schur, I. Meyer, M. Garovoy, and C. B. Carpenter, "Associations between systemic lupus erythematosus and the major histocompatibility complex: clinical and immunological considerations," *Clinical Immunology and Immunopathology*, vol. 24, no. 2, pp. 263–275, 1982.

[18] S. Miyagawa, K. Shinohara, T. Fujita et al., "Neonatal lupus erythematosus: analysis of HLA class II alleles in mothers and siblings from seven Japanese families," *Journal of the American Academy of Dermatology*, vol. 36, no. 2, part 1, pp. 186–190, 1997.

[19] P. M. Izmirly, A. Saxena, M. Y. Kim et al., "Maternal and fetal factors associated with mortality and morbidity in a multi-racial/ethnic registry of anti-SSA/Ro-associated cardiac neonatal lupus," *Circulation*, vol. 124, pp. 1927–1935, 2011.

[20] S. Miyagawa, "Neonatal lupus erythematosus: a review of the racial differences and similarities in clinical, serological and immunogenetic features of Japanese versus Caucasian patients," *Journal of Dermatology*, vol. 32, no. 7, pp. 514–522, 2005.

[21] J. P. Buyon, R. Hiebert, J. Copel et al., "Autoimmune-associated congenital heart block: demographics, mortality, morbidity and recurrence rates obtained from a national neonatal lupus registry," *Journal of the American College of Cardiology*, vol. 31, no. 7, pp. 1658–1666, 1998.

[22] A. R. Neiman, L. A. Lee, W. L. Weston, and J. P. Buyon, "Cutaneous manifestations of neonatal lupus without heart block: characteristics of mothers and children enrolled in a national registry," *Journal of Pediatrics*, vol. 137, no. 5, pp. 674–680, 2000.

[23] D. Spence, L. Hornberger, R. Hamilton, and E. D. Silverman, "Increased risk of complete congenital heart block in infants born to women with hypothyroidism and anti-Ro and/or anti-La antibodies," *Journal of Rheumatology*, vol. 33, no. 1, pp. 167–170, 2006.

[24] A. Brucato, M. Frassi, F. Franceschini et al., "Risk of congenital complete heart block in newborns of mothers with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis: a prospective study of 100 women," *Arthritis & Rheumatism*, vol. 44, pp. 1832–1835, 2001.

[25] E. Silverman and E. Jaeggi, "Non-cardiac manifestations of neonatal lupus erythematosus," *Scandinavian Journal of Immunology*, vol. 72, no. 3, pp. 223–225, 2010.

[26] L. A. Lee, "Cutaneous lupus in infancy and childhood," *Lupus*, vol. 19, no. 9, pp. 1112–1117, 2010.

[27] C. Lynn Cheng, S. Galbraith, and K. Holland, "Congenital lupus erythematosus presenting at birth with widespread erosions, pancytopenia, and subsequent hepatobiliary disease," *Pediatric Dermatology*, vol. 27, no. 1, pp. 109–111, 2010.

[28] Y. Penate, D. Lujan, J. Rodriguez et al., "Neonatal lupus erythematosus: 4 cases and clinical review," *Actas Dermosifiliogr*, vol. 96, pp. 690–696, 2005.

[29] D. Elish and N. B. Silverberg, "Neonatal lupus erythematosus," *Cutis*, vol. 77, no. 2, pp. 82–86, 2006.

[30] R. Cimaz, M. Biggioggero, L. Catelli, S. Muratori, and S. Cambiaghi, "Ultraviolet light exposure is not a requirement for the development of cutaneous neonatal lupus," *Lupus*, vol. 11, no. 4, pp. 257–260, 2002.

[31] P. M. Izmirly, C. Llanos, L. A. Lee, A. Askanase, M. Y. Kim, and J. P. Buyon, "Cutaneous manifestations of neonatal lupus and risk of subsequent congenital heart block," *Arthritis and Rheumatism*, vol. 62, no. 4, pp. 1153–1157, 2010.

[32] G. Guettrot-Imbert, L. Cohen, L. Fermont et al., "A new presentation of neonatal lupus: 5 Cases of isolated mild endocardial fibroelastosis associated with maternal anti-SSA/Ro and anti-SSB/La antibodies," *Journal of Rheumatology*, vol. 38, no. 2, pp. 378–386, 2011.

[33] C. A. Boros, D. Spence, S. Blaser, and E. D. Silverman, "Hydrocephalus and macrocephaly: new manifestations of neonatal lupus erythematosus.," *Arthritis and Rheumatism*, vol. 57, no. 2, pp. 261–266, 2007.

[34] Q. Yang, X. M. Shao, Y. Cao et al., "Neonatal lupus erythematosus: analysis of 8 cases," *Zhonghua er Ke Za Zhi*, vol. 46, no. 1, pp. 18–21, 2008.

[35] V. Martin, L. A. Lee, A. D. Askanase, M. Katholi, and J. P. Buyon, "Long-term followup of children with neonatal lupus and their unaffected siblings," *Arthritis and Rheumatism*, vol. 46, no. 9, pp. 2377–2383, 2002.

[36] W. Sun, T. M. Yuan, L. H. Chen, and H. M. Yu, "Neonatal lupus erythematosus: three case reports and review of the chinese literature," *Clinical Pediatrics*, vol. 49, no. 7, pp. 627–634, 2010.

[37] M. A. Akin, A. Baykan, S. Sezer, and T. Gunes, "Review of literature for the striking clinic picture seen in two infants of mothers with systemic lupus erythematosus," *Journal of Maternal-Fetal and Neonatal Medicine*, vol. 24, no. 8, pp. 1022–1026, 2011.

[38] A. Brucato, A. Doria, M. Frassi et al., "Pregnancy outcome in 100 women with autoimmune diseases and anti-Ro/SSA antibodies: a prospective controlled study," *Lupus*, vol. 11, no. 11, pp. 716–721, 2002.

[39] C. H. Yang, J. Y. Chen, S. C. Lee, and S. F. Luo, "Successful preventive treatment of congenital heart block during pregnancy in a woman with systemic lupus erythematosus and anti-Sjögren's syndrome A/Ro antibody," *Journal of Microbiology, Immunology and Infection*, vol. 38, no. 5, pp. 365–369, 2005.

[40] J. P. Buyon, R. M. Clancy, and D. M. Friedman, "Cardiac manifestations of neonatal lupus erythematosus: guidelines to management, integrating clues from the bench and bedside," *Nature Clinical Practice Rheumatology*, vol. 5, no. 3, pp. 139–148, 2009.

[41] D. M. Friedman, C. Llanos, P. M. Izmirly et al., "Evaluation of fetuses in a study of intravenous immunoglobulin as preventive therapy for congenital heart block: results of a multicenter, prospective, open-label clinical trial," *Arthritis and Rheumatism*, vol. 62, no. 4, pp. 1138–1146, 2010.

[42] C. N. Pisoni, A. Brucato, A. Ruffatti et al., "Failure of intravenous immunoglobulin to prevent congenital heart block: findings of a multicenter, prospective, observational study," *Arthritis and Rheumatism*, vol. 62, no. 4, pp. 1147–1152, 2010.

[43] P. M. Izmirly, M. Y. Kim, C. Llanos et al., "Evaluation of the risk of anti-SSA/Ro-SSB/La antibody-associated cardiac manifestations of neonatal lupus in fetuses of mothers with systemic lupus erythematosus exposed to hydroxychloroquine," *Annals of the Rheumatic Diseases*, vol. 69, no. 10, pp. 1827–1830, 2010.

[44] K. Shinohara, S. Miyagawa, T. Fujita, T. Aono, and K. I. Kldoguchi, "Neonatal lupus erythematosus: results of maternal corticosteroid therapy," *Obstetrics and Gynecology*, vol. 93, no. 6, pp. 952–957, 1999.

[45] A. Tincani, C. B. Rebaioli, M. Frassi et al., "Pregnancy and autoimmunity: maternal treatment and maternal disease influence on pregnancy outcome," *Autoimmunity Reviews*, vol. 4, no. 7, pp. 423–428, 2005.

[46] O. Baud, L. Foix-L'Helias, M. Kaminski et al., "Antenatal glucocorticoid treatment and cystic periventricular leukomalacia in very premature infants," *The New England Journal of Medicine*, vol. 341, no. 16, pp. 1190–1196, 1999.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 876456, 4 pages
doi:10.1155/2012/876456

Review Article

Vascular Disease in Systemic Lupus Erythematosus

Athina Pyrpasopoulou, Sofia Chatzimichailidou, and Spyros Aslanidis

2nd Propedeutic Department of Internal Medicine, Hippokration General Hospital, Konstantinoupoleos 49, 54643 Thessaloniki, Greece

Correspondence should be addressed to Athina Pyrpasopoulou, a.pyrpasopoulou@doctors.org.uk

Received 29 April 2012; Accepted 3 August 2012

Academic Editor: Tatiana S. Rodriguez-Reyna

Vascular disease, either as a direct complication of the disease or developing as an accompanying comorbidity impairs significantly the quality of life of patients with SLE and represents the most frequent cause of death in established lupus. This paper aims to give an overview of the prevalence of the different forms of vasculopathy that can be encountered in a lupus patient, describe their pathogenesis, and address their impact on disease severity and outcome.

1. Introduction

Vascular disease is frequent in patients with systemic lupus erythematosus and represents the most frequent cause of death in established disease. In this context, vasculopathy can be directly aetiologically implicated in the pathogenesis of the disease, presenting as an acute/subacute manifestation of lupus (e.g., antiphospholipid syndrome, lupus vasculitis). Alternatively, it can develop as an important accompanying comorbidity (steroid-related atherosclerotic disease), or represent the synergistic pathogenetic outcome of augmented atherosclerosis within a proinflammatory environment.

This paper aims to give an overview of the prevalence of the different forms of vasculopathy that can be encountered in a lupus patient, describe their pathogenesis, and address their impact on disease severity and outcome.

2. Epidemiology

Systemic lupus erythematosus (SLE) is a multiorgan autoimmune disease with an increased incidence rate of thrombotic events (9–37%), occurring at a younger age compared to the control population [1].

The high rates of reported cardiovascular comorbidity [2] appear to be largely attributed to the significant predisposition of SLE patients for atherosclerosis and coronary artery disease (CAD) [3]; CAD is 5-6 times more frequent in SLE patients when compared to healthy controls, independent of other coexisting cardiovascular risk factors [4], and represents the majority of vascular events in SLE patients [5]. This excess risk is especially pronounced in women of younger age (35–44yrs; >50-fold) [6, 7].

Additionally, SLE patients are at a 20% higher risk for stroke; 3–15% experience a nonfatal thrombotic stroke [8], its incidence however usually recurrent and associated with a higher mortality rate than matched controls [9].

The rate of thrombotic events is higher in patients with disease of recent onset, when compared to patients with other autoimmune diseases and remains so throughout the course of the disease [10]; in the LUMINA study, which included multiethnic SLE patients of recent diagnosis, age, damage accrual at enrolment, and antiphospholipid antibodies, as well as the use of higher dosages of glucocorticoids were associated with a shorter time interval to thrombotic events [1]. Besides recorded clinical disease, subclinical involvement is significantly increased compared to healthy individuals, reaching 30% in reported studies [11].

Cardiovascular disease is the most frequent cause of death in established disease (>8 yrs after diagnosis; [12]): infection and renal involvement attributing for the majority of cases during the early stages (first year of diagnosis). As mortality rates of SLE patients tend to decrease significantly over time, and patients' life expectancy increases, its prevalence among an aged population becomes more pronounced, and its relevance gains increasing significance [13].

3. Atherosclerosis in Lupus

Atherosclerotic disease has been detected in lupus patients from cohort studies in a significant proportion of the population (≥30%), even when corrected for all other comorbidities [3, 14]. The prevalence was higher compared to corresponding groups, when patients included had previously been treated with atherogenically predisposing treatments, such as steroids, or surprisingly, in younger patients (age < 55 years).

Subclinical disease reached levels of 52% and was confirmed with a variety of techniques, such as carotid and brachial artery Doppler ultrasound, coronary artery angiography, and SPECT dual-isotope myocardial perfusion imaging [15].

Why do SLE patients develop more atherosclerosis? For once, cardiovascular risk factors seem to be more prevalent among SLE patients: hypertension, diabetes mellitus, sedentary lifestyle, hyperlipidemia, hypertriglyceridemia, hyperhomocysteinemia, and even premature menopause. However, as mentioned previously, after correcting for all predisposing cardiovascular risk factors, lupus still qualifies as an independent atherogenic (cardiovascular) risk factor [10, 16].

The SLE-associated proinflammatory cytokine burden and SLE-related treatments such as corticosteroids, that is, at a dose >10 mg/daily promote atherogenesis. These two parameters tend to interconnect, as disease of higher intensity and thus higher proinflammatory burden qualifies for the use of corticosteroids in lupus. Patients without previous history of steroid treatment tend to have more carotid atherosclerotic plaques, possibly due to less efficiently controlled disease activity. However, in lupus, aortic stiffness and increased pulse wave velocity do not always correlate with proven carotid atherosclerosis [17, 18]. As the pathogenesis of the disease unravels and more pro- and anti-inflammatory (i.e., protective) molecules become identified and their role in the course of the disease characterized, associations with increased aortic stiffness (e.g., C-reactive protein, VCAM, and C3) or vasoprotection (TGFb-1) become established [19].

4. Antiphospholipid Syndrome

The clinical antiphospholipid syndrome, an autoimmune syndrome usually developing in the context of systemic lupus erythematosus, is a condition defined as a predisposition for arterial and/or venous thromboses and/or recurrent miscarriages or other obstetric emergencies (premature birth, preeclampsia, etc.) in association with hematologic abnormalities and specific antibodies targeted against phospholipid-binding plasma proteins [20]. Indeed, retrospective studies in patients with clinical thrombotic events revealed a higher prevalence of positivity for antiphospholipid antibodies [21]. Thrombosis within the context of antiphospholipid syndrome may occur even in histologically normal vessels. However, in the majority of aPL-positive patients, seropositivity *per se* does not suffice for the development of clinical events. Thrombotic events seem to occur more readily in lupus patients with coexistent atherosclerosis [22]. Moreover, according to the second-hit hypothesis, a second trigger event—such as cigarette smoking, oral contraceptives, surgical procedures, prolonged immobilization Definition Immobilization refers to the process of holding a joint or bone in place with a splint, cast, or brace. This is done to prevent an injured area from moving while it heals, hypertension, or a genetic prothrombotic state increases the likelihood of an aPL-positive patient developing a vascular event [23–25].

Recently, the presence of microangiopathy, defined as capillary microhemorhages, and diagnosed with the aid of capillaroscopy, has been proposed as an augmentary screening tool for aPL-seropositive patients who are prone to develop clinical thrombotic manifestations [26].

5. Livedoid Vasculopathy: Thrombotic Disease and/or Vasculitis?

Besides overt vessel obstruction, vascular disease in lupus, especially when affecting medium- and small-sized vessels, may contain both vasculopathic and vasculitic pathophysiologic parameters.

Livedoid vasculopathy, a condition which can be observed in patients with systemic lupus erythematosus/antiphospholipid syndrome or specific forms of systemic vasculitis (mainly polyarteritis nodosa and cryoglobulinemia), is associated with chronic ulcerations of the lower extremities and characterized by uneven perfusion [27]. Initial isolated or focal petechiae or purpura progress to form asymmetrical, irregular, painful, and recurrent leg ulcers which heal in the form of atrophic white scars. In lupus in particular, analogous lesions may also be observed in the upper extremities (elbows) and seem to be associated with disease manifestations affecting other systems (e.g., central nervous system) [28].

The pathogenesis of livedoid vasculopathy has not been fully elucidated, or rather, cannot be solely attributed to a particular mechanism, as both hypercoagulable states, as well as autoimmune diseases, appear to associate with and contribute to its development [29].

The typical histological findings show dermal blood vessel occlusion [30] a diagnosis biochemically strengthened by increased prothrombotic markers (prothrombin gene variations, hyperhomocysteinemia, protein C deficiency, increased activity of plasminogen-activator-inhibitor, and activated protein C resistance). The histopathological findings of intravascular fibrin, segmental hyalinization, and endothelial proliferation clearly support the thrombotic parameter of its pathogenesis [31]. The presence of immunoreactants in the vessel wall and circulating immune complexes (such as rheumatoid factor) are in favor of its immunological component; the absence however of fibrinoid necrosis and inflammatory infiltration of the vessel wall differentiate livedoid vasculopathy from true vasculitides. The cutaneous features as well as nervous system manifestations (neuropathies or central nervous system involvement) in patients with livedoid vasculopathy may respond to immunosuppressive treatment.

6. Lupus Vasculitis

Distinction of inflammatory lupus vasculitis from antiphospholipid syndrome, which may present with similar clinical manifestations, is of major significance in terms of clinical management. Inflammatory vascular disease is triggered by the in situ formation, or the deposition, of immune complexes within the vessel wall.

Vasculitis may manifest in as high as 56% of lupus patients throughout their life, in contrast to antiphospholipid syndrome which has a prevalence of 15%. Patients with vasculitis are mainly male and tend to be of younger age [32].

Antibodies against endothelial cells have been identified as a major endothelial cell cytotoxic effector and have been implicated in the pathogenesis of several connective tissue diseases, predominantly vasculitides [33]. More than 80% of systemic lupus erythematosus patients are positive for antiendothelial cell antibodies (AECAs) [34, 35]. Antigens that react with AECAs include heparinlike compounds, DNA and DNA-histone complexes, ribosome proteins, elongation factor 1a, adenyl cyclase-associated protein, profilin II, plasminogen activator inhibitor, fibronectin, and b2-glycoprotein. AECA-endothelial cell interaction attracts monocyte adhesion and induces secretion of chemoattractant proteins and cytokines, thus triggering vasculitis.

Besides AECAs, the presence of antineutrophil cytoplasmic autoantibodies (ANCAs), mainly associated with primary systemic vasculitides, has also been reported in 15-20% of SLE patients [36].

Other forms of SLE-related vasculitis include drug-induced vasculitis [37] and infection-induced vasculitis [38] either through direct compromise of the vascular wall by pathogens, or through antigen-induced autoimmune and inflammatory processes.

7. SLE-Associated Vascular Disease: Burden and Outcome

In conclusion, vascular disease appears to be inherent in the pathogenesis and clinical manifestations of systemic lupus erythematosus; its severity ranging from mild cutaneous disease to severe organ dysfunction, such as renal or central nervous system vasculitis, atherosclerotic cardiovascular or cerebrovascular events, or catastrophic antiphospholipid syndrome.

In lupus, more readily than perhaps in any other systemic autoimmune disease, vascular disease may combine, in the same individual, atherosclerotic, and thrombotic disease with systemic vasculitis *per se*, and autoinflammatory-driven degeneration of the vessel wall.

In a histopathological study of autopsied SLE patients, histological characterization of vessel involvement and size of affected vessels were associated with the cause of death. Fibrinoid degeneration, intimal thickening, thrombosis, and sclerosis were identified and recorded. The principal manifestations of the disease were found to be associated with smaller-sized arteries. However, patients with medium-sized arterial involvement usually presented with more frequent thrombotic events and exhibited higher morbidity rates than the rest of the patients [39].

Lifestyle and pharmaceutical prevention measures, regular screening for subclinical disease, alertness for early clinical signs, accurate differential diagnosis, and targeted treatment may prove organ- or even life-saving especially in younger patients with more aggressive disease [40].

References

[1] P. I. Burgos, G. McGwin Jr., J. D. Reveille, L. M. Vilá, and G. S. Alarcón, "Factors predictive of thrombotic events in LUMINA, a multi-ethnic cohort of SLE patients (LXXII)," *Rheumatology*, vol. 49, no. 9, pp. 1720–1725, 2010.

[2] D. P. M. Symmons and S. E. Gabriel, "Epidemiology of CVD in rheumatic disease, with a focus on RA and SLE," *Nature Reviews Rheumatology*, vol. 7, no. 7, pp. 399–408, 2011.

[3] B. J. Skaqqs, B. H. Hahn, and M. McMahon, "Accelerated atherosclerosis in patients with SLE-mechanisms and management," *Nature Reviews Rheumatology*, vol. 8, no. 4, pp. 214–223, 2012.

[4] I. N. Bruce, "'Not only...but also': factors that contribute to accelerated atherosclerosis and premature coronary heart disease in systemic lupus erythematosus," *Rheumatology*, vol. 44, no. 12, pp. 1492–1502, 2005.

[5] M. B. Urowitz, D. Gladman, D. Ibañez et al., "Atherosclerotic vascular events in a multinational inception cohort of systemic lupus erythematosus," *Arthritis Care and Research*, vol. 62, no. 6, pp. 881–887, 2010.

[6] C. Benqtsson, M. L. Ohman, O. Nived, and S. Rantapää Dahlqvist, "Cadiovascular event in systemic lupus erythematosus in northern Sweden: incidence and predictors in a 7-year follow-up study," *Lupus*, vol. 21, no. 4, pp. 452–459, 2012.

[7] J. M. Kahlenberg and M. J. Kaplan, "The interplay of inflammation and cardiovascular disease in systemic lupus erythematosus," *Arthritis Research & Therapy*, vol. 13, article 203, 2011.

[8] E. Somers, L. S. Magder, and M. Petri, "Antiphospholipid antibodies and incidence of venous thrombosis in a cohort of patients with systemic lupus erythematosus," *Journal of Rheumatology*, vol. 29, no. 12, pp. 2531–2536, 2002.

[9] C. C. Chiu, C. C. Huang, W. L. Chan et al., "Increased risk of ischemic stroke in patients with systemic lupus erythematosus: a nationwide population-based study," *Internal Medicine*, vol. 51, no. 1, pp. 17–21, 2012.

[10] J. Romero-Díaz, F. Vargas-Vóracková, E. Kimura-Hayama, L. F. Cortázar-Benítez, R. Gijón-Mitre, S. Criales et al., "Systemic lupus erythematosus risk factors for coronary artery calcifications," *Rheumatology*, vol. 51, no. 1, pp. 110–119, 2012.

[11] C. Jurcut, S. Caraiola, D. Nitescu et al., "Subclinical vascular disease in patients with systemic lupus erythematosus: the additive deleterious effect of the antiphospholipid syndrome," *Joint Bone Spine*. In press.

[12] M. B. Urowitz, A. A. Bookman, B. E. Koehler, D. A. Gordon, H. A. Smythe, and M. A. Ogryzlo, "The bimodal mortality pattern of systemic lupus erythematosus," *American Journal of Medicine*, vol. 60, no. 2, pp. 221–225, 1976.

[13] G. Chehab, R. Fischer-Betz, and M. Schneider, "Changes in mortality and morbidity in systemic lupus eythematosus," *Zeitschrift für Rheumatologie*, vol. 70, no. 6, pp. 480–485, 2011.

[14] M. McMahon, B. H. Hahn, and B. J. Skaggs, "Systemic lupus erythematosus and cardiovascular disease: prediction and

potential for therapeutic intervention," *Expert Review of Clinical Immunology*, vol. 7, no. 2, pp. 227–241, 2011.

[15] S. A. Croca and A. Rahman, "Imaging assessment of cardiovascular disease in systemic lupus erythematosus," *Clinical and Developmental Immunology*, vol. 2012, Article ID 694143, 7 pages, 2012.

[16] N. S. Wade and A. S. Major, "The problem of accelerated atherosclerosis in systemic lupus erythematosus: insights into a complex co-morbidity," *Thrombosis and Haemostasis*, vol. 106, no. 5, pp. 849–857, 2011.

[17] E. M. Zardi and A. Afeltra, "Endothelial dysfunction and vascular stiffness in systemic lupus erythematosus: are they early markers of subclinical atherosclerosis?" *Autoimmunity Reviews*, vol. 9, no. 10, pp. 684–686, 2010.

[18] C. A. Roldan, J. Joson, C. R. Qualls, J. Sharrar, and W. L. Sibbitt Jr., "Premature aortic stiffness in systemic lupus erythematosus by transesophageal echocardiography," *Lupus*, vol. 19, no. 14, pp. 1599–1605, 2010.

[19] Y. Sherer, H. Zinger, and Y. Shoenfeld, "Atherosclerosis in systemic lupus erythematosus," *Autoimmunity*, vol. 43, no. 1, pp. 98–102, 2010.

[20] J. L. Rodriquez-Garcia, M. L. Bertolaccini, M. J. Quadrado, G. Sanna, O. Ateka-Barrutia, and M. A. Khamashta, "Clinical manifestations of antiphospholipid syndrome (APS) with and without antiphospholipid antibodies (the so-called "seronegativeAPS")," *Annals of the Rheumatic Diseases*, vol. 71, no. 2, pp. 242–244, 2012.

[21] X. Deng and X. Liu, "Reevaluation of predictive value of ACL and anti-β2GP1 antibody for thrombosis in patients with systemic lupus erythematosus: from a perspective of a practical world," *Rheumatology International*. In press.

[22] J. Frostegård, "Systemic lupus erythematosus and cardiovascular disease," *Lupus*, vol. 17, no. 5, pp. 364–367, 2008.

[23] P. L. Meroni, M. O. Borqhi, E. Raschi, and F. Tedesco, "Pathogenesis of antiphospholipd syndrome: understanding the antibodies," *Nature Reviews Rheumatology*, vol. 7, no. 6, pp. 330–339, 2011.

[24] G. R. V. Hughes, "Hughes Syndrome (the antiphospholipid syndrome). Ten clinical lessons," *Autoimmunity Reviews*, vol. 7, no. 3, pp. 262–266, 2008.

[25] A. Ruffatti, T. Del Ross, M. Ciprian et al., "Risk factors for a first thrombotic event in antiphospholipid antibody carriers: a prospective multicentre follow-up study," *Annals of the Rheumatic Diseases*, vol. 70, no. 6, pp. 1083–1086, 2011.

[26] A. Pyrpasopoulou, A. Triantafyllou, P. Anyfanti, S. Douma, and S. Aslanidis, "Capillaroscopy as a screening test for clinical antiphospholipid syndrome," *European Journal of Internal Medicine*, vol. 22, no. 6, pp. e158–e159, 2011.

[27] P. R. Criado, E. A. Rivitti, M. N. Sotto et al., "Livedoid vasculopathy: an intriguing cutaneous disease," *Anais Brasileiros de Dermatologia*, vol. 86, no. 5, pp. 961–977, 2011.

[28] T. Yasue, "Livedoid vasculitis and central nervous system involvement in systemic lupus erythematosus," *Archives of Dermatology*, vol. 122, no. 1, pp. 66–70, 1986.

[29] B. Sopeña, M. T. Pérez-Rodríguez, A. Rivera, J. A. Ortiz-Rey, J. Lamas, and M. C. Freire-Dapena, "Livedoid vasculopathy and recurrent thrombosis in a patient with lupus: seronegative antiphospholipid syndrome?" *Lupus*, vol. 19, no. 11, pp. 1340–1343, 2010.

[30] A. Shimizu, A. Tamura, M. Yamanaka, H. Amano, Y. Nagai, and O. Ishikawa, "Case of livedoid vasculopathy with extensive dermal capillary thrombi," *Journal of Dermatology*, vol. 37, no. 1, pp. 94–97, 2010.

[31] S. Khenifer, L. Thomas, B. Balme, and S. Dalle, "Livedoid vasculopathy: thrombotic or inflammatory disease?" *Clinical and Experimental Dermatology*, vol. 35, no. 7, pp. 693–698, 2010.

[32] P. Cieślik, A. Hrycek, and P. Kłuciński, "Vasculopathy and vasculitis in systemic lupus erythematosus," *Polskie Archiwum Medycyny Wewnetrznej*, vol. 118, no. 1-2, pp. 57–63, 2008.

[33] P. Guilpain and L. Mouthon, "Antiendothelial cells autoantibodies in vasculitis-associated systemic diseases," *Clinical Reviews in Allergy and Immunology*, vol. 35, no. 1-2, pp. 59–65, 2008.

[34] Y. Renaudineau, C. Dugué, M. Dueymes, and P. Youinou, "Antiendothelial cell antibodies in systemic lupus erythematosus," *Autoimmunity Reviews*, vol. 1, no. 6, pp. 365–372, 2002.

[35] S. Praprotnik, M. Blank, P. L. Meroni, B. Rozman, A. Eldor, and Y. Shoenfeld, "Classification of anti-endothelial cell antibodies into antibodies against microvascular and macrovascular endothelial cells," *Arthritis & Rheumatism*, vol. 44, pp. 1484–1494, 2001.

[36] M. Galeazzi, G. Morozzi, G. D. Sebastiani et al., "Anti-neutrophil cytoplasmic antibodies in 566 European patients with systemic lupus erythematosus: prevalence, clinical associations and correlation with other autoantibodies," *Clinical and Experimental Rheumatology*, vol. 16, no. 5, pp. 541–546, 1998.

[37] M. Dobre, J. Wish, and L. Negrea, "Hydralazine-induced ANCA-positive pauci-immune glomerulonephritis: a case report and literature review," *Renal Failure*, vol. 31, no. 8, pp. 745–748, 2009.

[38] T. Avcin, M. Canova, P. Guilpain et al., "Infections, connective tissue diseases and vasculitis," *Clinical and Experimental Rheumatology*, vol. 26, no. 1, supplement 48, pp. S18–S26, 2008.

[39] H. Hashimoto, S. Maekawa, H. Nasu, T. Okada, Y. Shiokawa, and Y. Fukuda, "Systemic vascular lesions and prognosis in systemic lupus eryhtematosus," *Scandinavian Journal of Rheumatology*, vol. 13, no. 1, pp. 45–55, 1984.

[40] P. Soltész, G. Kerekes, H. Dér et al., "Comparative assessment of vascular function in autoimmune rheumatic diseases: considerations of prevention and treatment," *Autoimmunity Reviews*, vol. 10, no. 7, pp. 416–425, 2011.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 725249, 7 pages
doi:10.1155/2012/725249

Review Article

Osteonecrosis in Systemic Lupus Erythematosus: An Early, Frequent, and Not Always Symptomatic Complication

Paola Caramaschi, Domenico Biasi, Ilaria Dal Forno, and Silvano Adami

Rheumatology Unit, Department of Medicine, University of Verona, 37134 Verona, Italy

Correspondence should be addressed to Paola Caramaschi, paola.caramaschi@ospedaleuniverona.it

Received 16 May 2012; Accepted 16 June 2012

Academic Editor: Hiroshi Okamoto

Osteonecrosis may complicate the course of systemic lupus erythematosus and may contemporaneously affect multiple joints. The major risk factor associated with the development of osteonecrosis is the use of glucocorticoid at high doses. Recent studies using serial MRI, which represents the "gold standard" for the early detection of osteonecrosis, yielded some interesting findings about the natural history of this clinical entity. Osteonecrosis in the majority of the cases is asymptomatic and occurs early in the course of the disease. Its later occurrence is associated with lupus flare that requires the increase of corticosteroid dose. The optimal treatment of osteonecrosis is controversial. In case of silent osteonecrosis involving a small area conservative strategy is usually adequate. When lesions are symptomatic surgical treatment as core decompression or free vascularized fibular grafting is required; extracorporeal shockwave treatment may represent an alternative therapeutic approach. When the lesion has a medium-large dimension or involves a weight-bearing area bone collapse is a common complication requiring total joint replacement. Coadministration of bisphosphonate or warfarin with high doses of corticosteroid might be a promising preventive strategy of osteonecrosis.

1. Introduction

Osteonecrosis is a clinical entity characterized by death of bone marrow and trabecular bone as a result of a compromised artery supply. It may follow a trauma resulting in the displacement of a bone fragment that disrupts blood supply causing bone ischemia (posttraumatic osteonecrosis) or may complicate the course of many systemic diseases or conditions (atraumatic osteonecrosis). In a proportion of osteonecrosis a triggering event cannot be identified (idiopathic forms).

Osteonecrosis mainly affects long-bone epiphyses, in particular femoral head and condyles, distal end of the tibia, and humeral head and often shows a multifocal distribution. Systemic conditions known as risk factors for osteonecrosis are corticosteroid treatment, Cushing syndrome, organ transplant, systemic lupus erythematosus (SLE), antiphospholipid antibody syndrome, thrombophilia, alcoholism, dyslipidemia, Caisson disease, sickle cell disease, Gaucher disease, HIV infection, pancreatitis, inflammatory bowel diseases, and pregnancy [1, 2].

Osteonecrosis may be silent or may clinically present with a gradual onset characterized by mild or vague pain that may worsen after ambulation and subsequently progress to severe pain when bone collapse occurs; in other cases osteonecrosis may cause a sudden onset deep joint pain that initially is elicited by movement and later is present also at rest.

Osteonecrosis may be asymptomatic and not progressive when a small fragment of bone tissue is involved. In case of medium-large bone area involvement usually the lesion progresses and eventually the necrotic tissue collapses. The clinical implication is a severe joint damage requiring arthroplasty. Negative prognostic factor is also the involvement of a weight-bearing area, such as the femoral head.

During the recent years magnetic resonance imaging (MRI) has become the "gold standard" technique for the early recognition of osteonecrosis considering the high sensitivity and specificity; it may detect early stage, silent

osteonecrotic lesions, which are not diagnosed by both plain radiography and bone scan. Osteonecrosis is defined by MRI image of low-intensity band (band-like pattern) on T1-weighted images [3] which is attributed to the reaction at the interface between dead and normal bone.

In SLE the prevalence of symptomatic lesions in studies carried when MRI was not available varied from 2.1 to 30% [4–16]. More recent MRI studies allowed the diagnosis of osteonecrotic lesions in higher percentage of the patients. By serial MRI evaluation of hips and knees in 72 SLE patients on high doses of corticosteroid therapy osteonecrosis was observed in 32 subjects (44%), with a multifocal distribution in the majority of the cases. Thus 92 joints had osteonecrosis features, with a slightly predominance of knee involvement [17]. In all cases the bone lesions were asymptomatic at the time of the diagnosis and during the one year followup.

Osteonecrosis often involves more than one skeletal site; a case of SLE with twelve simultaneous osteonecrotic lesions was reported [18]; long-bone epiphyses are usually involved, but flat bones as ilium, sternum, talus, and vertebral bodies may be affected too [10, 19]. When osteonecrosis at one skeletal site is found in a SLE patient, a screening for other concomitant lesions is generally recommended since multifocal involvement is not infrequent.

Symptomatic osteonecrosis does not increase mortality but heavily impacts on quality of life and causes a significant physical disability as measured by the Health Assessment Questionnaire [16].

Progression from clinically occult osteonecrosis detected by MRI to symptomatic lesions is not frequent [10, 20]. A review about the natural history of asymptomatic osteonecrosis of femoral head showed that patients affected by SLE presented the most benign course, while in subjects suffering from sickle cell anemia the rate of progression to symptomatic disease and/or femoral head collapse was the highest [21].

Among a cohort of 500 SLE cases 19 patients had undergone at least one total joint replacement. In most of these cases rheumatoid arthritis was associated to SLE [22].

2. Skeletal Manifestations in Systemic Lupus Erythematosus

SLE is a complex autoimmune disease that may involve any organ and apparatus; it is characterized by great heterogeneity and the clinical picture may vary considerably from patient to patient and in the same subject from the onset to the following course. The involvement of the bone tissue includes osteoporosis and osteonecrosis, both unrelated to the autoimmune pathophysiology of the disease. The etiology of osteoporosis is multifactorial: corticosteroid treatment, limited physical activity and premature ovarian failure favoured also by cyclophosphamide treatment courses may contribute to bone loss [23]; chronic systemic inflammation per se may favour bone loss through increased production of TNF, a cytokine that influences osteoclast maturation and activity [24]. High levels of oxidized LDLs promote the activation of both T cells and the transcription factor peroxisome proliferator-activated receptor (PPAR)γ2 [25], a key regulator of both osteoblast and adipocyte differentiation from mesenchymal stem cells, which reduces the osteoblast precursor number in favour of the adipocyte cell number [26]. SLE patients are invariably encouraged to avoid sunshine exposure and this increases the risk of vitamin D deficiency.

3. Pathogenetic Mechanisms That Lead to Atraumatic Osteonecrosis

Although the pathogenesis of nontraumatic osteonecrosis is not completely understood, it is known that many factors may induce bone ischemia and favour bone necrosis by intraluminal or extraluminal obliteration [1]. Intraluminal occlusion may be due to the release of nitrogen gas bubbles that impinge the blood vessels as occurs in acute decompression syndrome or Caisson disease or may be provoked by fat embolism, which is favoured by dyslipidemia and glucocorticoid treatment or by thrombosis. A thrombophilic status has been associated with osteonecrosis. In a study that evaluated 45 cases of osteonecrosis 82.2% and 46.7% of the patients showed at least one and two risk factors for thrombosis, respectively, in comparison with 30% and 2.5% of the controls [27]. It has also been reported that patients affected by osteonecrosis are more likely to have hypofibrinolytic 4G polymorphism of the plasminogen activator inhibitor-1 gene, methylenetetrahydrofolate reductase gene mutation with higher concentration of homocysteine, low protein S values, and high lipoprotein(a) levels than controls [28]. Sickle vasoocclusion in sickle cell anaemia is another mechanism which may lead to intraluminal obliteration causing both symptomatic and asymptomatic osteonecrosis, which frequently complicates the course of the disease, requiring joint replacement in many cases [29].

Extraluminal obliteration is due to bone marrow pressure elevation, which impairs intraosseous blood flow because of the bone inelasticity. This mechanism, first hypothesized in 1972 [30], may be due to glucocorticoid treatment and alcohol abuse that cause bone marrow adipocyte hypertrophy.

Chronic corticosteroid administration promotes fat conversion of red marrow, as demonstrated by a MRI study of the proximal femur in a cohort of premenopausal women affected by SLE. The magnitude of fat conversion paralleled the daily prednisolone intake and was more evident in patients who had had osteonecrosis. The increase of bone marrow fat content may elevate marrow pressure reducing arterial perfusion [31]. Other mechanisms leading to increase of marrow pressure are bone edema, bleeding or histiocyte proliferation, typical histologic feature of Gaucher disease. All these observations suggest that the so-called atraumatic osteonecrosis represents the final common pathway of many different conditions leading to impaired bone blood supply.

A nationwide epidemiologic survey performed in Japan in 2005 permitted to obtain clinical data from 1502 patients with atraumatic osteonecrosis of the femoral head. Systemic glucocorticoid therapy and alcoholism were the most frequent risk factors, being present in 51% and 31% of the cases, respectively. SLE represents the most

frequent underlying disease in steroid-induced osteonecrosis (31.2% of the cases), followed by nephrotic syndrome (6.3% of the cases); polymyositis/dermatomyositis, thrombocytopenic purpura, bronchial asthma, and inflammatory eye diseases were the underlying illness in about 4% of the patients, whereas rheumatoid arthritis in less than 1%. These proportions clearly do not mirror the epidemiology of the diseases. The most common disorders requiring corticosteroid treatment are bronchial asthma and rheumatoid arthritis, where osteonecrosis is relatively rare. The high incidence of osteonecrosis in SLE, nephrotic syndrome, and other autoimmune diseases might be related to the higher corticosteroid doses used but also to the underlying disease [32].

In a large Japanese survey it was found that patients who receive glucocorticoid are at approximately 20-fold greater risk to develop osteonecrosis in comparison with nonusers [33]. Glucocorticoid administration was also found to be a significant risk factor for the rare atraumatic osteonecrosis of the distal radius and ulna [34].

In a recent large study on the incidence of osteonecrosis among patients requiring corticosteroid treatment it was reported that daily prednisone-equivalent dose > 40 mg/day in comparison with lower doses was associated with an OR of 4.2 to develop osteonecrosis [35]. It was also found that male sex was an additional risk factor for the development of the disease.

Besides the aforementioned effects of corticosteroids on fat content of bone marrow, these drugs may impair bone integrity by inducing apoptosis of osteoblasts and osteocytes [36] and through the downregulation of osteoprotegerin and the upregulation of RANKL impairing the balance between bone formation and bone resorption [37]. Even tough a genetic predisposition has never been reported, the association with some polymorphisms has been found. The multidrug resistance 3435 TT haplotype was found to be protective for the development of osteonecrosis both in SLE patients [38] and in renal transplant recipients [39]. Interestingly this haplotype is associated with a reduced intracellular concentration of corticosteroids.

4. Factors Associated with the Development of Osteonecrosis in Systemic Lupus Erythematosus

The use of corticosteroid therapy is the major risk factor for the development of osteonecrosis in SLE [4, 17, 40–42].

The yearly incidence of osteonecrosis at hips and knees was prospectively investigated by MRI in a large cohort of patients requiring corticosteroid treatment for a variety of underlying diseases. MRI osteonecrosis (most often asymptomatic) developed in 255 (37%) of 687 joints evaluated in 173 SLE cases as compared with 107 (21%) of 512 joints in 129 non-SLE cases. The highest dose of corticosteroid was slightly different between SLE patients and subjects affected by other diseases (56.0 and 51.3 mg/day, resp.). The logistic regression analysis revealed that SLE patients had a higher risk to develop osteonecrosis versus other autoimmune or systemic diseases with an OR of 2.6. These results further confirm that SLE per se is a risk factor for osteonecrosis [35].

Vascular involvement, altered lipid metabolism, haemostatic abnormalities, and thrombophilia due to antiphospholipid antibodies have been considered as additional risk factors for the development of osteonecrosis in SLE. These conditions are generally reflecting a very high disease activity requiring high glucocorticoid dosages. However, contrasting data have emerged from studies focused on the association between osteonecrosis and disease activity. Fialho et al. reported a positive correlation between the development of osteonecrosis and the SLEDAI score (an integrated index of disease activity) ≥ 8 in the year preceding the clinical diagnosis of osteonecrosis [43]. On the contrary other studies did not find a correlation with disease activity [4, 5, 40] or severity [41].

Inconclusive results were yielded by analyzing the association of osteonecrosis with some typical features of SLE like vasculitis [4, 8], Raynaud's phenomenon [4, 6, 9], antiphospholipid antibody positivity [5, 6, 8, 10, 40, 44], and with administration of antimalarial drugs [4, 11]. Many authors reported a higher frequency of Cushingoid body habitus among SLE patients who developed osteonecrosis [8, 45, 46].

Osteonecrosis is relatively uncommon in growing individuals but its incidence rapidly increases in adolescents up to the rate seen in adults [35]. This may be related to the growing proportion of fatty marrow with advancing age. Fatty marrow as compared with red marrow has less collateral flow and is then more vulnerable to ischemic injury whereas children appear to better tolerate ischemia because they have abundant vascularity due to the sinusoidal network in hematopoietic red marrow [47]. A higher blood supply of femur head in pediatric patients after corticosteroid administration was demonstrated by dynamic MRI evaluation [48].

Another study on the prevalence of MRI detected osteonecrosis was carried out in Japan by Nakamura et al. Osteonecrosis MRI features were found in 260 of 676 joints (38%) in 169 SLE patients on treatment with corticosteroids. The authors ranked their cohort according to age: pediatric (<15 years old), adolescent (15–20 years old), and adult (>20 years old) with a prevalence of necrosis of 6%, 49% and 41% of the joints, respectively. None of the patients younger than 14 years developed osteonecrosis. These results indicate that patient age at the time of corticosteroid therapy initiation is an independent risk factor for osteonecrosis in SLE [49]. In a similar study in recipients of kidney transplantation no osteonecrosis cases were observed in children younger than 10 years [50].

The presence of arthritis [4], renal involvement [11], and the prevalence of anti-DNA and of various anti-ENA antibodies [5] could not be identified as risk factors for osteonecrosis. No differences in lipid profile were observed between SLE patients with and without osteonecrosis [11, 43, 44]. In a number of studies the incidence of osteonecrosis was significantly associated with the extensive use of immune suppressants drugs [4, 5, 44].

The aforementioned studies on the prevalence/incidence of osteonecrosis in SLE cannot be easily compared for

their differences regarding important factors, such as age, duration of disease, duration of followup, dose, type, and route of administration of glucocorticoids. The definition of osteonecrosis was also different. It ranged from "clinical osteonecrosis" (the diagnosis having been achieved for the appearance of symptoms), X-ray, and eventually MRI-detected disease. For the latter it is not yet clear to what proportion these patients will develop full clinical picture of the disease, particularly keeping into account that effective treatment able to prevent the worsening of the disease is still lacking. What remains well established in all studies is the crucial role played by corticosteroid therapy in SLE patients.

5. Relationship between Osteonecrosis Development and Type of Corticosteroid Treatment

The relationship between development of osteonecrosis and corticosteroid treatment has been extensively investigated. Important risk factors appear to be the cumulative dose even for treatment cycles [5, 7, 9], the peak daily dose [5, 8, 44], the mean daily dose [5, 46], and the duration of use of high doses [51]. The use of methylprednisolone pulses was reported to be associated with osteonecrosis [38, 45], but this has not been confirmed by others [5, 16].

In all aforementioned studies the diagnosis of osteonecrosis was clinical and supported by traditional X-ray.

The epidemiological scenario has completely changed when the presence of osteonecrosis was identified also in asymptomatic patients by MRI. The use of this technique changed substantially the epidemiology of osteonecrosis and provided also interesting and unexpected data regarding the natural history of osteonecrosis in SLE. For example, in one of these studies it was found that osteonecrosis may occur soon after the beginning of corticosteroid therapy; all lesions were present within five months (on average after 3.1 months), and no new cases were observed afterward! In this particular study the corticosteroid dose was not significantly associated with osteonecrosis [17].

These results are somewhat supported by another interesting study prospectively evaluating by repeated MRI 291 joints in 106 SLE patients without osteonecrosis after the initial corticosteroid treatment. During the long follow-up period (on average 13.6 years) incidental osteonecrosis was observed only in 6 bone segments. In all these cases the osteonecrosis developed after a SLE flare requiring the increase of corticosteroid dosage. No cases of osteonecrotic lesions were found in patients who received a low or medium corticosteroid dose, equivalent to <30 mg/day of prednisone. Thus, it appears that if a SLE patient does not develop osteonecrosis after an initial course of high steroid therapy he will rarely present the complication afterwards unless the dose of corticosteroid is considerably increased as a consequence of a disease flare [52].

A study was focused on the MRI evolution over 10 years in SLE patients who already suffered from a noncollapsed and asymptomatic osteonecrosis at the hips or knees (238 (44%) out of 537 joints). During the followup in about half of the lesions spontaneous reduction of necrotic areas was observed with complete regression in 9% of the cases. Progression of the osteonecrotic lesions occurred in 14% of the cases, invariably associated with an increase of corticosteroid dosage due to SLE recurrence [53].

6. Treatment

The treatment of osteonecrosis in SLE patients is similar to that of osteonecrosis due to other causes. The goal of the treatment of osteonecrotic lesions is to preserve joint integrity by preventing bone collapse.

When osteonecrosis involves less than 10% of the femoral head or less than 1/3 of the weight-bearing portion the outcome is usually favourable and surgical treatment is not required [1]; the conservative treatment includes analgesics and the use of devices to allow nonweight bearing; these measures are usually effective in alleviating pain.

In case of symptomatic lesions surgical treatment is usually required.

Core decompression technique was performed for the first time in 1962 in order to reduce bone marrow pressure and to improve perfusion of ischemic bone [54]. Mont et al. treated by core decompression 31 hips in 18 SLE patients; in 21 cases (68%) a subsequent total joint replacement was required; all these cases were in an advance stage of the disease. The authors concluded that an early detection of the lesion and a prompt treatment are crucial to obtain optimal results [55]. Core decompression is usually ineffective when osteonecrosis involves more than 25% of the femoral head or more than 2/3 of the weight-bearing portion [1]. Core decompression was successfully performed in cases of osteonecrosis of the knee and of the talus in the precollapsed stages [55, 56].

Concentrated autologous bone marrow aspirate transplantation following core decompression was successfully performed in 8 out 9 SLE cases with osteonecrosis of the femur head involving more than 2/3 of the weight-bearing portion; only the patient who showed an advanced stage lesion failed to obtain benefit and later required joint replacement. Non perioperative complications were observed [57].

Another option is free vascularised fibular grafting; this procedure was applied in 80 osteonecrotic lesions of the hip among 50 SLE patients who were followed at least for two years after the procedure, on average for 4.3 years; none of the cases required hip arthroplasty, suggesting that free vascularised fibular grafting may allow to maintain joint function [58].

Transtrochanteric anterior rotational osteotomy demonstrated to be effective in a long-term study, showing a hip survival rate of 73.7% after 25 years from the surgical approach [59].

A 19-year-old woman affected by SLE who developed bilateral femur osteonecrosis that affected more than 2/3 of the weight-bearing area was treated with extracorporeal shockwave under general anesthesia obtaining significant pain relief, hip functional amelioration, and reduction of bone edema [60]. Shockwave treatment may favour

neovascularization through an increased release of angiogenic factors [61] and may enhance bone mass and bone strength as demonstrated in rabbits [62]. After this first favourable experience, 15 SLE patients with 26 hips affected by osteonecrosis were treated with extracorporeal shockwave; hip replacement was necessary in 12% of the cases. The outcome was similar to that observed in a group of non-SLE patients with osteonecrosis [63].

The outcome of hip arthroplasty in SLE-associated osteonecrosis is not different from that observed in other indications; an overall survival probability of 94.6% at 5 years and of 81.8% at 9 years was reported with minimal perioperative morbidity [64]. In 19 SLE patients who underwent 26 hip arthroplasty interventions, two early, nonrecurrent dislocations, and one low-grade prosthetic infection were described [65].

Less favourable results were obtained for knee arthroplasty in SLE patients with osteonecrosis; a good outcome was reported in 11 out of 25 cases. No differences were found when patients were stratified by amount of corticosteroid use, cemented versus cementless fixation, and SLE activity [66].

Alendronate 70 mg per week was tested in a randomized placebo controlled study in 40 patients affected by unilateral or bilateral nontraumatic osteonecrosis of the femoral head with a necrotic area larger than 30%. Only 2 out of 29 femoral heads collapsed in the group treated with alendronate in comparison with 19 out of 25 femoral heads in the control group. Joint replacement was necessary in 1 alendronate-treated patient as compared with 16 cases of the placebo group [67]. A prospective study evaluating the effectiveness of the contemporaneous administration in SLE patients of high corticosteroid dose with bisphosphonate should be planned in order to establish if this class of drugs may prevent the onset or may halt the progression of osteonecrotic lesions.

The preventive strategy for osteonecrosis development in SLE patients requiring high corticosteroid dose to control disease activity has been seldom studied. Sixty newly diagnosed SLE patients treated with ≥ 40 mg of prednisolone daily were alternatively assigned to two options, a warfarin group and a control group; warfarin was given together with the initiation of corticosteroid therapy. Silent and symptomatic osteonecrosis developed in 21% and 4.8% of the hips in the warfarin group, respectively, and in 33% and 14% of the hips in the control group, respectively. Despite the lack of a statistically significant difference, the observed results indicate that larger studies are warranted in order to verify the efficacy of anticoagulation therapy for preventing osteonecrosis at least in SLE patients [68].

According to the actual understanding of the pathophysiology of osteonecrosis, some preventing benefits might be expected from the treatment with lipid lowering agents, antiplatelet drugs, and antiresorptives. However, in a small study in SLE patients with disease flare requiring increased corticosteroid dosage, no beneficial effects were observed with the concomitant administration of statin, antiplatelet, and bisphosphonate [69].

7. Conclusion

Osteonecrosis frequently complicates the course of SLE, usually occurs soon after the initiation of corticosteroid therapy at high doses or, more rarely, after increasing the dosage for SLE flares. The complication may have a significant impact on functional ability and may require in some cases total joint replacement.

The presence of localized asymptomatic forms can be nowadays identified by MRI. By using this technique the incidence of osteonecrosis in SLE patients appears to be considerably higher than hitherto thought.

Recent MRI serial evaluation of hips and knees shed more light on the natural history of osteonecrosis, which does not progress if the dose of corticosteroid is maintained low and possibly thanks to promising preventive strategies, such as warfarin and bisphosphonates. However, at the moment the best documented preventive strategy is a judicious glucocorticoid use which is now possible by adopting steroid-sparing therapies, including the new biological agents.

References

[1] P. Lafforgue, "Pathophysiology and natural history of avascular necrosis of bone," *Joint Bone Spine*, vol. 73, no. 5, pp. 500–507, 2006.

[2] M. Abu-Shakra, D. Buskila, and Y. Shoenfeld, "Osteonecrosis in patients with SLE," *Clinical Reviews in Allergy and Immunology*, vol. 25, no. 1, pp. 13–23, 2003.

[3] N. Sugano, T. Atsumi, K. Ohzono, T. Kubo, T. Hotokebuchi, and K. Takaoka, "The 2001 revised criteria for diagnosis, classification, and staging of idiopathic osteonecrosis of the femoral head," *Journal of Orthopaedic Science*, vol. 7, no. 5, pp. 601–605, 2002.

[4] D. D. Gladman, M. B. Urowitz, V. Chaudhry-Ahluwalia, D. C. Hallet, and R. J. Cook, "Predictive factors for symptomatic osteonecrosis in patients with systemic lupus erythematosus," *Journal of Rheumatology*, vol. 28, no. 4, pp. 761–765, 2001.

[5] C. C. Mok, C. S. Lau, and R. W. S. Wong, "Risk factors for avascular bone necrosis in systemic lupus erythematosus," *British Journal of Rheumatology*, vol. 37, no. 8, pp. 895–900, 1998.

[6] A. Rascu, K. Manger, H. G. Kraetsch, J. R. Kalden, and B. Manger, "Osteonecrosis in systemic lupus erythematosus, steroid-induced or a lupus-dependent manifestation?" *Lupus*, vol. 5, no. 4, pp. 323–327, 1996.

[7] M. Abeles, J. D. Urman, and N. F. Rothfield, "Aseptic necrosis of bone in systemic lupus erythematosus. Relationship to corticosteroid therapy," *Archives of Internal Medicine*, vol. 138, no. 5, pp. 750–754, 1978.

[8] M. A. Mont, C. J. Glueck, I. H. Pacheco, P. Wang, D. S. Hungerford, and M. Petri, "Risk factors for osteonecrosis in systemic lupus erythematosus," *Journal of Rheumatology*, vol. 24, no. 4, pp. 654–662, 1997.

[9] E. S. Weiner and M. Abeles, "Aseptic necrosis and glucocorticosteroids in systemic lupus erythematosus: a reevaluation," *Journal of Rheumatology*, vol. 16, no. 5, pp. 604–608, 1989.

[10] M. Y. Mok, V. T. Farewell, and D. A. Isenberg, "Risk factors for avascular necrosis of bone in patients with systemic lupus erythematosus: is there a role for antiphospholipid antibodies?" *Annals of the Rheumatic Diseases*, vol. 59, no. 6, pp. 462–467, 2000.

[11] P. Uea-Areewongsa, S. Chaiamnuay, P. Narongroeknawin, and P. Asavatanabodee, "Factors associated with osteonecrosis in Thai lupus patients: a case control study," *Journal of Clinical Rheumatology*, vol. 15, no. 7, pp. 345–349, 2009.

[12] J. H. Klippel, L. H. Gerber, L. Pollak, and J. L. Decker, "Avascular necrosis in systemic lupus erythematosus. Silent symmetric osteonecroses," *American Journal of Medicine*, vol. 67, no. 1, pp. 83–87, 1979.

[13] A. A. Kalla, I. D. Learmonth, and P. Klemp, "Early treatment of avascular necrosis in systemic lupus erythematosus," *Annals of the Rheumatic Diseases*, vol. 45, no. 8, pp. 649–652, 1986.

[14] R. A. Asherson, F. Liote, B. Page et al., "Avascular necrosis of bone and antiphospholipid antibodies in systemic lupus erythematosus," *Journal of Rheumatology*, vol. 20, no. 2, pp. 284–288, 1993.

[15] L. Cozen and D. J. Wallace, "Avascular necrosis in systemic lupus erythematosus: clinical associations and a 47-year perspective," *American Journal of Orthopedics*, vol. 27, no. 5, pp. 352–354, 1998.

[16] D. D. Gladman, V. Chaudhry-Ahluwalia, D. Ibañez, E. Bogoch, and M. B. Urowitz, "Outcomes of symptomatic osteonecrosis in 95 patients with systemic lupus erythematosus," *Journal of Rheumatology*, vol. 28, no. 10, pp. 2226–2229, 2001.

[17] K. Oinuma, Y. Harada, Y. Nawata et al., "Osteonecrosis in patients with systemic lupus erythematosus develops very early after starting high dose corticosteroid treatment," *Annals of the Rheumatic Diseases*, vol. 60, no. 12, pp. 1145–1148, 2001.

[18] L. G. Darlington, "Osteonecrosis at multiple sites in a patient with systemic lupus erythematosus," *Annals of the Rheumatic Diseases*, vol. 44, no. 1, pp. 65–66, 1985.

[19] S. N. Oh, W. H. Jee, S. M. Cho et al., "Osteonecrosis in patients with systemic lupus erythematosus: MR imaging and scintigraphic evaluation," *Clinical Imaging*, vol. 28, no. 4, pp. 305–309, 2004.

[20] K. Nagasawa, H. Tsukamoto, Y. Tada et al., "Imaging study on the mode of development and changes in avascular necrosis of the femoral head in systemic lupus erythematosus: long-term observations," *British Journal of Rheumatology*, vol. 33, no. 4, pp. 343–347, 1994.

[21] M. A. Mont, M. G. Zywiel, D. R. Marker, M. S. McGrath, and R. E. Delanois, "The natural history of untreated asymptomatic osteonecrosis of the femoral head: a systematic literature review," *Journal of Bone and Joint Surgery A*, vol. 92, no. 12, pp. 2165–2170, 2010.

[22] A. F. Mourão, M. Amaral, J. Caetano-Lopes, and D. Isenberg, "An analysis of joint replacement in patients with systemic lupus erythematosus," *Lupus*, vol. 18, no. 14, pp. 1298–1302, 2009.

[23] N. E. Lane, "Therapy Insight: osteoporosis and osteonecrosis in systemic lupus erythematosus," *Nature Clinical Practice Rheumatology*, vol. 2, no. 10, pp. 562–569, 2006.

[24] J. Zwerina, S. Hayer, M. Tohidast-Akrad et al., "Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factor-induced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction," *Arthritis and Rheumatism*, vol. 50, no. 1, pp. 277–290, 2004.

[25] S. S. Davies, A. V. Pontsler, G. K. Marathe et al., "Oxidized alkyl phospholipids are specific, high affinity peroxisome proliferator-activated receptor gamma ligands and agonists," *Journal of Biological Chemistry*, vol. 276, no. 19, pp. 16015–16023, 2001.

[26] F. Parhami, Y. Tintut, W. G. Beamer, N. Gharavi, W. Goodman, and L. L. Demer, "Atherogenic high-fat diet reduces bone mineralization in mice," *Journal of Bone and Mineral Research*, vol. 16, no. 1, pp. 182–188, 2001.

[27] L. C. Jones, M. A. Mont, T. B. Le et al., "Procoagulants and osteonecrosis," *Journal of Rheumatology*, vol. 30, no. 4, pp. 783–791, 2003.

[28] C. J. Glueck, R. A. Freiberg, R. N. Fontaine, T. Tracy, and P. Wang, "Hypofibrinolysis, thrombophilia, osteonecrosis," *Clinical Orthopaedics and Related Research*, no. 386, pp. 19–33, 2001.

[29] A. Almeida and I. Roberts, "Bone involvement in sickle cell disease," *British Journal of Haematology*, vol. 129, no. 4, pp. 482–490, 2005.

[30] J. Arlet, P. Ficat, G. Lartigue, and M. A. Tran, "Clinical research on intraosseous pressure in the upper femoral metaphysis and epiphysis in humans. Application to the diagnosis of ischemia and necrosis," *Revue du Rhumatisme et des Maladies Osteo-Articulaires*, vol. 39, no. 11, pp. 717–723, 1972.

[31] B. C. Vande Berg, J. Malghem, F. E. Lecouvet, J.-P. Devogelaer, B. Maldague, and F. A. Houssiau, "Fat conversion of femoral marrow in glucocorticoid-treated patients: a cross-sectional and longitudinal study with magnetic resonance imaging," *Arthritis and Rheumatism*, vol. 42, no. 7, pp. 1405–1411, 1999.

[32] W. Fukushima, M. Fujioka, T. Kubo, A. Tamakoshi, M. Nagai, and Y. Hirota, "Nationwide epidemiologic survey of idiopathic osteonecrosis of the femoral head," *Clinical Orthopaedics and Related Research*, vol. 468, no. 10, pp. 2715–2724, 2010.

[33] M. Sakaguchi, T. Tanaka, W. Fukushima, T. Kubo, and Y. Hirota, "Impact of oral corticosteroid use for idiopathic osteonecrosis of the femoral head: a nationwide multicenter case-control study in Japan," *Journal of Orthopaedic Science*, vol. 15, no. 2, pp. 185–191, 2010.

[34] D. A. Stroh, D. M. Laporte, D. A. Marker, A. J. Johnson, and M. A. Mont, "Atraumatic osteonecrosis of the distal radius and ulna: case series and review," *Journal of Hand Surgery*, vol. 37, no. 1, pp. 134–141, 2012.

[35] T. Shigemura, J. Nakamura, S. Kishida et al., "Incidence of osteonecrosis associated with corticosteroid therapy among different underlying diseases: prospective MRI study," *Rheumatology*, vol. 50, no. 11, pp. 2023–2028, 2011.

[36] R. S. Weinstein, "Glucocorticoids, osteocytes, and skeletal fragility: the role of bone vascularity," *Bone*, vol. 46, no. 3, pp. 564–570, 2010.

[37] M. García-Carrasco, C. Mendoza-Pinto, R. O. Escárcega et al., "Osteoporosis in patients with systemic lupus erythematosus," *Israel Medical Association Journal*, vol. 11, no. 8, pp. 486–491, 2009.

[38] X. Y. Yang and D. H. Xu, "MDR1(ABCB1) gene polymorphisms associated with steroid-induced osteonecrosis of femoral head in systemic lupus erythematosus," *Pharmazie*, vol. 62, no. 12, pp. 930–932, 2007.

[39] T. Asano, K. A. Takahashi, M. Fujioka et al., "ABCB1 C3435T and G2677T/A polymorphism decreased the risk for steroid-induced osteonecrosis of the femoral head after kidney transplantation," *Pharmacogenetics*, vol. 13, no. 11, pp. 675–682, 2003.

[40] F. A. Houssiau, A. N'Zeusseu Toukap, G. Depresseux et al., "Magnetic resonance imaging-detected avascular osteonecrosis in systemic lupus erythematosus: lack of correlation with antiphospholipid antibodies," *British Journal of Rheumatology*, vol. 37, no. 4, pp. 448–453, 1998.

[41] R. Prasad, D. Ibanez, D. Gladman, and M. B. Urowitz, "The role of non-corticosteroid related factors in osteonecrosis (ON) in systemic lupus erythematosus: a nested case-control

study of inception patients," *Lupus*, vol. 16, no. 3, pp. 157–162, 2007.

[42] A. Zonana-Nacach, S. G. Barr, L. S. Magder et al., "Damage in systemic lupus erythematosus and its association with corticosteroids," *Arthritis and Rheumatism*, vol. 43, no. 8, pp. 1801–1808, 2000.

[43] S. C. M. S. Fialho, E. Bonfá, L. F. Vitule et al., "Disease activity as a major risk factor for osteonecrosis in early systemic lupus erythematosus," *Lupus*, vol. 16, no. 4, pp. 239–244, 2007.

[44] J. Calvo-Alén, G. McGwin, S. Toloza et al., "Systemic lupus erythematosus in a multiethnic US cohort (LUMINA): XXIV. Cytotoxic treatment is an additional risk factor for the development of symptomatic osteonecrosis in lupus patients: results of a nested matched case-control study," *Annals of the Rheumatic Diseases*, vol. 65, no. 6, pp. 785–790, 2006.

[45] L. Massardo, S. Jacobelli, M. Leissner, M. González, L. Villarroel, and S. Rivero, "High-dose intravenous methylprednisolone therapy associated with osteonecrosis in patients with systemic lupus erythematosus," *Lupus*, vol. 1, no. 6, pp. 401–405, 1992.

[46] T. M. Zizic, C. Marcoux, and D. S. Hungerford, "Corticosteroid therapy associated with ischemic necrosis of bone in systemic lupus erythematosus," *American Journal of Medicine*, vol. 79, no. 5, pp. 596–604, 1985.

[47] D. G. Mitchell, M. E. Steinberg, M. K. Dalinka, V. M. Rao, M. Fallon, and H. Y. Kressel, "Magnetic resonance imaging of the ischemic hip. Alterations within the osteonecrotic, viable, and reactive zones," *Clinical Orthopaedics and Related Research*, no. 244, pp. 60–77, 1989.

[48] J. Nakamura, Y. Miura, S. Ikenoue et al., "Blood supply of proximal femur during steroid therapy in SLE patients using dynamic MRI," in *Proceedings of the 77th Annual Meeting of the American Academy of Orthopaedic Surgeons*, New Orleans, La, USA, March 2010.

[49] J. Nakamura, T. Saisu, K. Yamashita, C. Suzuki, M. Kamegaya, and K. Takahashi, "Age at time of corticosteroid administration is a risk factor for osteonecrosis in pediatric patients with systemic lupus erythematosus: a prospective magnetic resonance imaging study," *Arthritis and Rheumatism*, vol. 62, no. 2, pp. 609–615, 2010.

[50] P. Stern and H. G. Watts, "Osteonecrosis after renal transplantation in children," *Journal of Bone and Joint Surgery A*, vol. 61, no. 6, pp. 851–856, 1979.

[51] S. Migliaresi, U. Picillo, L. Ambrosone et al., "Avascular osteonecrosis in patients with SLE: relation to corticosteroid therapy and anticardiolipin antibodies," *Lupus*, vol. 3, no. 1, pp. 37–41, 1994.

[52] J. Nakamura, S. Ohtori, M. Sakamoto, A. Chuma, I. Abe, and K. Shimizu, "Development of new osteonecrosis in systemic lupus erythematosus patients in association with long-term corticosteroid therapy after disease recurrence," *Clinical and Experimental Rheumatology*, vol. 28, no. 1, pp. 13–18, 2010.

[53] J. Nakamura, Y. Harada, K. Oinuma, S. Iida, S. Kishida, and K. Takahashi, "Spontaneous repair of asymptomatic osteonecrosis associated with corticosteroid therapy in systemic lupus erythematosus: 10-year minimum follow-up with MRI," *Lupus*, vol. 19, no. 11, pp. 1307–1314, 2010.

[54] R. P. Ficat, "Treatment of avascular necrosis of the femoral head," *The Hip*, pp. 279–295, 1983.

[55] M. A. Mont, A. C. Fairbank, M. Petri, and D. S. Hungerford, "Osteonecrosis in patients with systemic lupus erythematosus develops very early after starting high dose corticosteroid treatment," *Clinical Orthopaedics and Related Research*, vol. 334, pp. 91–97, 1997.

[56] R. E. Delanois, M. A. Mont, T. R. Yoon, M. Mizell, and D. S. Hungerford, "Atraumatic osteonecrosis of the talus," *Journal of Bone and Joint Surgery A*, vol. 80, no. 4, pp. 529–536, 1998.

[57] T. Yoshioka, H. Mishima, H. Akaogi, S. Sakai, M. Li, and N. Ochiai, "Concentrated autologous bone marrow aspirate transplantation treatment for corticosteroid-induced osteonecrosis of the femoral head in systemic lupus erythematosus," *International Orthopaedics*, vol. 35, no. 6, pp. 823–829, 2010.

[58] Y. Sun, C. Q. Zhang, S. B. Chen, J. G. Sheng, D. X. Jin, and B. F. Zeng, "Treatment of femoral head osteonecrosis in patients with systemic lupus erythematosus by free vascularised fibular grafting," *Lupus*, vol. 18, no. 12, pp. 1061–1065, 2009.

[59] G. Motomura, T. Yamamoto, K. Suenaga et al., "Long-term outcome of transtrochanteric anterior rotational osteotomy for osteonecrosis of the femoral head in patients with systemic lupus erythematosus," *Lupus*, vol. 19, no. 7, pp. 860–865, 2010.

[60] P. C. Lin, C. J. Wang, K. D. Yang, F. S. Wang, J. Y. Ko, and C. C. Huang, "Extracorporeal shockwave treatment of osteonecrosis of the femoral head in systemic lupus erythematosis," *Journal of Arthroplasty*, vol. 21, no. 6, pp. 911–915, 2006.

[61] C. J. Wang, F. S. Wang, K. D. Yang et al., "Shock wave therapy induces neovascularization at the tendon-bone junction. A study in rabbits," *Journal of Orthopaedic Research*, vol. 21, no. 6, pp. 984–989, 2003.

[62] C. J. Wang, K. D. Yang, F. S. Wang, C. C. Hsu, and H. H. Chen, "Shock wave treatment shows dose-dependent enhancement of bone mass and bone strength after fracture of the femur," *Bone*, vol. 34, no. 1, pp. 225–230, 2004.

[63] C. J. Wang, J. Y. Ko, Y. S. Chan et al., "Extracorporeal shockwave for hip necrosis in systemic lupus erythematosus," *Lupus*, vol. 18, no. 12, pp. 1082–1086, 2009.

[64] M. H. Huo, E. A. Salvati, M. G. Browne, P. M. Pellicci, T. P. Sculco, and N. A. Johanson, "Primary total hip arthroplasty in systemic lupus erythematosus," *Journal of Arthroplasty*, vol. 7, no. 1, pp. 51–56, 1992.

[65] P. Zangger, D. D. Gladman, M. B. Urowitz, and E. R. Bogoch, "Outcome of total hip replacement for avascular necrosis in systemic lupus erythematosus," *Journal of Rheumatology*, vol. 27, no. 4, pp. 919–923, 2000.

[66] M. A. Mont, T. H. Myers, K. A. Krackow, and D. S. Hungerford, "Total knee arthroplasty for corticosteroid associated avascular necrosis of the knee," *Clinical Orthopaedics and Related Research*, no. 338, pp. 124–130, 1997.

[67] K. A. Lai, W. J. Shen, C. Y. Yang, C. J. Shao, J. T. Hsu, and R. M. Lin, "The use of alendronate to prevent early collapse of the femoral head in patients with nontraumatic osteonecrosis: a randomized clinical study," *Journal of Bone and Joint Surgery A*, vol. 87, no. 10, pp. 2155–2159, 2005.

[68] K. Nagasawa, Y. Tada, S. Koarada et al., "Prevention of steroid-induced osteonecrosis of femoral head in systemic lupus erythematosus by anti-coagulant," *Lupus*, vol. 15, no. 6, pp. 354–357, 2006.

[69] F. Sekiya, K. Yamaji, K. Yang, H. Tsuda, and Y. Takasaki, "Investigation of occurrence of osteonecrosis of the femoral head after increasing corticosteroids in patients with recurring systemic lupus erythematosus," *Rheumatology International*, vol. 30, no. 12, pp. 1587–1593, 2010.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 207190, 9 pages
doi:10.1155/2012/207190

Review Article

The Role of Hyaluronan and CD44 in the Pathogenesis of Lupus Nephritis

Susan Yung and Tak Mao Chan

Department of Medicine, University of Hong Kong, Room 302 New Clinical Building, Queen Mary Hospital, Pokfulam, Hong Kong

Correspondence should be addressed to Susan Yung, ssyyung@hku.hk and Tak Mao Chan, dtmchan@hku.hk

Received 7 May 2012; Accepted 11 June 2012

Academic Editor: Hiroshi Okamoto

Systemic lupus erythematosus (SLE) is a prototype autoimmune disease that affects multiorgan systems. Lupus nephritis is one of the most severe manifestations of SLE whereby immune-mediated inflammation can lead to permanent damage within the glomerular, tubulo-interstitial, and vascular compartments of the kidney, resulting in acute or chronic renal failure. The mechanisms that regulate host inflammatory responses and tissue injury are incompletely understood. Accumulating evidence suggests that hyaluronan and its interaction with its cell surface receptor CD44 plays an important role in mediating pathogenic mechanisms in SLE. This paper discusses the putative mechanisms through which hyaluronan and CD44 contribute to the pathogenesis of SLE, with particular emphasis on lupus nephritis.

1. Introduction

Systemic lupus erythematosus (SLE) is a severe autoimmune disease characterized by a breakdown of immune tolerance and production of autoantibodies. Although the etiology of SLE remains to be fully elucidated, accumulating evidence suggests that genetic, environmental, infectious, and hormonal factors may predispose individuals to the development of SLE [1–3]. This disease predominantly affects females of Afro-American, Hispanic, and Asian descent and can be mild or life threatening depending on the organs involved.

Renal involvement occurs in up to 60% of SLE patients and is a strong predictor of morbidity and mortality [4]. Onset of lupus nephritis is initiated by the deposition of anti-double stranded (ds) DNA antibodies in the kidney parenchyma, which results in complement activation, infiltration of immune cells, and induction of inflammatory and fibrotic processes in the kidney. If these tissue-damaging processes are not sufficiently controlled, destruction of the normal kidney parenchyma and its replacement by fibrous tissue will ensue, which will lead to endstage renal failure [4]. The exact mechanisms through which anti-dsDNA antibodies are deposited in the kidney to mediate kidney injury remains to be fully defined but current knowledge suggests that they can bind directly to mesangial cells through annexin II or α-actinin [5–7] or indirectly to components of the glomerular basement membrane through nucleosomes [8, 9].

The extracellular matrix (ECM) was previously considered to function solely as a structural support that maintained the architecture of tissues and organs, but there is now compelling evidence to show that ECM components also play critical roles during inflammatory processes. Their accumulation and subsequent degradation is a cardinal feature of autoimmune diseases. Hyaluronan (HA) is a major component of the ECM that can directly regulate inflammatory processes through its interaction with CD44, its cell surface receptor [10, 11]. Depending on its molecular weight HA may possess either anti-inflammatory or pro-inflammatory properties. We have demonstrated that serum HA levels in patients with lupus nephritis correlate with disease activity, and that intrarenal HA expression is also increased in lupus nephritis, induced in part by anti-dsDNA antibodies [12]. This paper will discuss the putative roles of HA and CD44 in SLE, with particular emphasis on their roles in mediating inflammatory processes during lupus nephritis.

2. Hyaluronan and CD44

2.1. Synthesis of HA. HA is a nonsulfated, negatively charged glycosaminoglycan that is composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine [13]. Unlike other glycosaminoglycans, HA is not attached to a protein core and is synthesized on the inner surface of the plasma membrane [13]. HA is synthesized by HA synthases (HAS) and currently three mammalian HAS have been identified, namely, HAS I, HAS II, and HAS III, which utilize UDP-α-N-acetyl-D-glucosamine and UDP-α-glucuronate as substrates for the synthesis of HA [14]. Under physiologic conditions, HA is synthesized as a macromolecule with a MW of 10^5–10^7 Da depending on the tissue type [15]. Following its synthesis, HA is directed to the cell surface where it interacts with CD44, or is assembled into pericellular or extracellular matrices [16]. Studies have demonstrated that all three HAS isozymes can contribute to the synthesis of high MW (HMW) HA, but HAS I and HAS III may also produce low MW (LMW) HA depending on the condition of the microenvironment [14].

2.2. Functions of HA under Physiological and Pathological Conditions. Despite its simple chemical structure, HA remains one of the most complex and multifaceted components of the ECM that contributes to diverse biological functions such as the structural stability of basement membranes, maintenance of water balance, plasma protein distribution, sequestration of free radicals, and regulation of cell proliferation, migration, and phenotype [17]. Native HA possesses anti-inflammatory, anti-angiogenic, and immunosuppressive properties [18]. They also provide a protective glycocalyx around endothelial, epithelial, and mesothelial cells that protect these cells from injury, apoptosis, and leukocyte adhesion [19–21]. HA undergoes constant turnover during the daily maintenance of basement membranes. It is degraded into small, nonbiologically active fragments which is rapidly removed through the liver.

The turnover and remodeling of the ECM is a dynamic process that occurs during normal development and tissue repair, and replenishment of ECM components is critical in order to preserve the structural and functional integrity of tissues. These processes become aberrant in pathological conditions associated with chronic inflammation where accumulation of ECM constituents is often observed, which perturbs tissue structure resulting in organ dysfunction. HA accumulates at sites of injury during chronic renal inflammation, where they form long cable-like structures that act as an adhesive matrix for the binding of leukocytes and macrophages. Mesangial cells and proximal tubular epithelial cells have been shown to contribute to the synthesis of these cable-like structures [22, 23]. In line with its anti-inflammatory properties, it has been suggested that binding of leukocytes to HA cables prevents them from interacting with adhesion molecules, thus limiting inflammatory processes in the glomerulus and tubulo-interstitium. Furthermore, it is also conceivable that the HA cable may serve as a temporary scaffold that prevents the loss of ECM components during extreme tissue remodeling [24]. Macrophages have been shown to regulate the clearance of the provisional HA matrix, and this process is essential before a permanent matrix can be synthesized.

Unlike other glycosaminoglycans where modifications in their sulfation pattern, deacetylation and epimerization define their biological roles, the functional role in HA is dictated by its molecular weight and its interaction with its binding proteins, the latter termed the hyaladherins. HA undergoes depolymerization either through oxidative stress or enzymatic cleavage by various hyaluronidases during tissue injury and inflammatory processes [10, 11, 25]. LMW HA have biological properties that are distinct from their parent molecule and have been shown to promote inflammatory and angiogenic processes through increased cell proliferation, activation of signaling transduction pathways and induction of chemokine and cytokine secretion in macrophages, dendritic cells, mesothelial cells, mesangial cells, epithelial cells, and chondrocytes [10, 26–32]. The clearance of HA fragments is therefore imperative for the resolution of tissue injury. The removal of LMW HA from sites of injury is dependent on their interaction with CD44 since targeted deletion of CD44 in mice with bleomycin-induced lung injury resulted in the accumulation of HA fragments, unremitting inflammation, and perpetual tissue damage, a finding that was not observed in wild-type mice [33]. The distinct biological roles of HMW and LMW HA thus far identified are summarized (Table 1). An in-depth review of the interaction of HA with hyaladherins and mechanisms of degradation is outside the scope of this paper [10, 11, 13, 16].

2.3. CD44. CD44 is a transmembrane glycoprotein with a wide tissue distribution and is found on leukocytes, and epithelial, endothelial, and smooth muscle-like cells. The human CD44 gene is located on the short arm of chromosome 11 and consists of 20 exons of which 10 are variant exons (v1–v10) that can undergo alternative splicing to generate multiple CD44 isoforms [34]. The genomic structure of CD44 is shown in Figure 1. Post-translational modifications of the CD44 molecule such as N- and O-glycosylation, and the attachment of heparan sulfate and/or chondroitin sulfate glycosaminoglycan chains may further increase the number of CD44 isoforms. Such post-translational modifications are tissue specific and bestow upon the CD44 molecule an ability to sequester growth factors and cytokines, thereby allowing greater accrual of its variability and functions [35–37]. It has been hypothesized that over one hundred CD44 isoforms can be generated, although to date only 26 have been identified. The predominant form of CD44 expressed in normal tissues does not contain any spliced exons and is designated hematopoetic or standard CD44 (CD44H or CD44s resp.). It can undergo post-translational modifications and has a molecular weight of 80–100 kDa.

CD44 can interact with various cell surface and extracellular ligands but its principal ligand is HA [38, 39]. It is noteworthy that binding of HA to CD44 is not constitutive but is activation dependent [40]. In this respect, quiescent leukocytes express inactive forms of CD44 that do not bind to HA and must be activated before it can interact with HA.

Table 1: Functions of native and depolymerized hyaluronan.

Native hyaluronan	Hyaluronan fragments
Contributes to tissue integrity and maintenance of epithelial cell phenotype	Induces chemokine and cytokine secretion in infiltrating, renal tubular epithelial and endothelial cells
Contributes to water balance and regulation of tissue hydration	Induces phosphorylation of signaling pathways, for example, MAPK
Contributes to transportation and distribution of plasma proteins	Induces cell proliferation and migration in chondrocytes, endothelial cells and fibroblasts
Protects against tissue damage by scavenging free radicals	Activates NFκB
Anti-inflammatory-can inhibit activation of inflammatory cells	Induces nitric oxide synthase
Protects against apoptosis	Suppresses cell death and apoptosis in cell culture
Anti-angiogenic	Promotes angiogenesis
Immunosuppressive-prevents ligand binding to surface receptors	Increases matrix protein synthesis, for example, collagen type I
Inhibits phagocytosis	Increases transcription of matrix metalloproteinases

Hyaluronan fragments: range from 4 to 40 saccharide units.

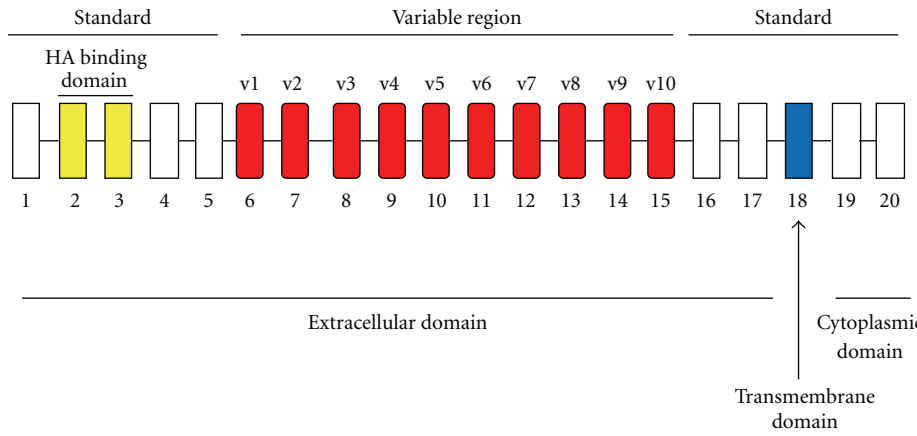


Figure 1: Genomic structure of CD44. The gene encoding for human CD44 consists of 20 exons. The standard form of CD44 contains exons 1–5, 16–18, and 20. Variants forms of CD44 comprise the standard form of CD44 and the insertion of various combinations of variant exons (v1–v10). Exon 19 is normally absent in most CD44 transcripts and its inclusion results in a shorter variant form of CD44.

Recognition of HA by CD44 is dependent on the degree of post-translational modifications, its phosphorylation status, sulfation pattern and ability to form multivalent aggregates on the cell surface [41–44]. Binding of HA to CD44 is a relatively weak interaction in comparison to other cell receptor-ligand interactions such as those that involve integrins or cadherins, but in some instances weak interactions are an advantage particularly when leukocytes require to be in close proximity in order to exchange chemical signals prior to their activation and maturation [45, 46]. The interaction of CD44 with HA has been shown to enhance various cellular functions such as cell proliferation and migration, and activation of PKC, PI3K and MAPK-signaling pathways which have all been shown to induce inflammatory processes in autoimmune diseases including lupus nephritis [47, 48].

CD44 plays an important role in many physiological and pathological processes that include cell-cell and cell-matrix interactions, cell migration, lymphocyte activation and extravasation, and presentation of growth factors, cytokines and chemokines to their cognate receptors. Increased synthesis of CD44 and/or generation of new isoforms is often associated with pathological conditions and CD44 expression can be altered by pro-inflammatory cytokines and chemokines such as TNF-α, IL-1β, IL-8 and RANTES in both lymphoid and non-lymphoid cells. There is increasing evidence to suggest that CD44 plays a pivotal role in autoimmune diseases and its expression is increased in synovial cells in patients with rheumatoid arthritis, which correlates with synovial inflammation [49]. The administration of antibodies against CD44 can significantly reduce inflammatory processes in murine models of collagen- or proteoglycan-induced arthritis and experimental autoimmune encephalomyelitis [50–52]. CD44-HA interactions in normal murine B cells have been shown to induce cell activation, proliferation and differentiation [53]. Readers are referred to reviews by Taylor and Gallo [17] and Jiang et al. [11], which discuss the role of CD44 and HA as immune regulators during pathological disorders.

3. Hyaluronan and CD44 in the Pathogenesis of SLE

Alterations in the distribution pattern of HA and CD44 have been shown to play an important role in the development of SLE. Elevated serum HA levels have been observed in patients and mice with active lupus nephritis, and murine anti-dsDNA antibodies have been shown to cross-react with HA [12, 54–56]. In the next section, we will discuss the contributing role of HA and CD44 in SLE with particular emphasis of their roles in the progression of lupus nephritis.

3.1. HA, CD44, and Immune Cells. An important step in the initiation and propagation of lupus nephritis is the recruitment of immune cells, namely T cells, B cells, macrophages, and dendritic cells, to sites of injury including the kidney [57–62]. Polyclonal B-cell activation precedes the development of clinical nephritis [63], thereby highlighting the crucial role of leukocytes in the pathogenesis of disease. The mechanism of local immune regulation and leukocyte-mediated kidney injury is not well delineated and is a topic of much interest. HA can induce chemokine and cytokine secretion in both lymphoid and nonlymphoid cells and therefore assumes an important role in the activation, recruitment, and retention of lymphocytes at sites of injury [26, 64–66]. We have demonstrated that in patients with active lupus nephritis subpopulations of glomerular lymphocytic infiltrates possess cell surface HA, a finding that is not observed in healthy individuals [12]. Although the mechanism through which HA regulates the activities of immune cells in the kidney during lupus nephritis remains to be defined, studies have shown that through its interaction with CD44, HA can induce murine B-cell activation, T cell, and macrophage effector functions and dendritic cell maturation [53, 67, 68]. Siegelman et al. demonstrated that CD44-HA interactions contributes to leukocyte rolling [69], a process that is essential for their extravasation to sites of injury. These researchers further observed that a subpopulation of circulating peripheral blood T cells strongly expressed CD44-dependent adhesion in SLE patients and their existence correlated with disease activity [70]. T cells that possess increased expression of CD44 have an enhanced capacity to infiltrate the kidney and induce inflammation [71], and this is dependent on the colocalization of CD44 with F-actin and phosphorylated ezrin, radixin, and moesin (ERM) at their polar caps, resulting in their polarization and conversion from freely circulating lymphocytes to those that can adhere to the endothelium and migrate into injured tissues, a process mediated through Rho-associated, coiled coil containing protein kinase (ROCK) activation (Figure 2) [71]. Genetic deletion of CD44 or inhibition of CD44 expression using a peptide based on the CDR1 sequence of a human anti-DNA antibody inhibited lymphoproliferation in lupus-prone mice and non-autoimmune mice immunized with a monoclonal anti-DNA antibody, respectively [72, 73], thereby highlighting the importance of CD44 in the pathogenesis of SLE. Crispín et al. [74] demonstrated that CD44v3 and CD44v6 expression are increased on $CD4^+$ and $CD8^+$ T cells isolated from patients with SLE, which correlated with disease activity, whereas CD44v6 on T cells was associated with lupus nephritis and positivity for anti-dsDNA antibodies [74].

Apoptosis and the phagocytic clearance of apoptotic cells from sites of injury are tightly regulated processes that are essential for the maintenance of tissue structure and function. The recognition and removal of apoptotic bodies is mediated by macrophages. Studies have demonstrated that CD44 on the surface of macrophages plays an important role in the clearance of apoptotic bodies and this process is dependent on the prior activation of intracellular pathways such as tyrosine phosphorylation of p561ck and interaction with cytoskeletal proteins [75]. Defective clearance of apoptotic cells is a cardinal feature of SLE that results in persistent inflammation and autoimmunity, since chromatin fragments and cellular components that escape from nondigested apoptotic cells can serve as immunogens that will further exacerbate disease pathogenesis [75, 76]. Studies have demonstrated that the expression of variant CD44 isoforms is induced in activated macrophages that are present at sites of inflammation and this may alter the repertoire of CD44 ligands [77]. Furthermore, studies have demonstrated that CD44 expression is reduced on monocytes/macrophages in SLE patients, which inversely correlate with the percentage of apoptotic neutrophils [78]. Therefore, a reduction in CD44 expression together with a change in CD44 isoform on monocytes/macrophages will impair their ability to recognize and remove apoptotic cells from sites of injury. Although the mechanism through which CD44 expression is altered in SLE patients remains to be investigated, it is possible that changes in cytokine expression in the microenvironment may contribute.

Increased expression of interferon-inducible genes is a prominent feature in SLE. Recent analysis of the interferon pathway showed an association between CD44 and SLE [79]. In a recent study, CD44 has also been linked to thrombocytopenia in SLE patients [80–82].

3.2. HA, CD44, and Resident Renal Cells. In the normal kidney, HA is found solely in the medullary and papillary interstitium of the kidney where it contributes to the mechanical stability of tubules and blood vessels, and also in the concentration of urine, whilst the expression of CD44 is restricted to passenger leukocytes and resident macrophages [83–85]. Accumulation of HA in the renal cortex is observed in patients and mice with active lupus nephritis and in autoimmune crescentic glomerulonephritis [12, 86]. *In vitro* studies have demonstrated that mesangial cells, proximal tubular epithelial cells and interstitial fibroblasts are able to synthesize HA and it is likely that these cells all contribute to the synthesis of HA in renal diseases [12, 87–91]. We and others have demonstrated that HA and CD44 expression is increased in the glomerular and tubulo-interstitial compartments of the kidneys, with predominant expression of HA and CD44 in the periglomerular area and in atrophic tubules of patients and mice with active lupus nephritis [12, 54, 85, 92, 93]. The accumulation of HA in the kidney was shown to correlate with the infiltration of lymphocytes in the tubulo-interstitium and tissue damage [92]. *In vitro* studies have shown that proinflammatory mediators involved in

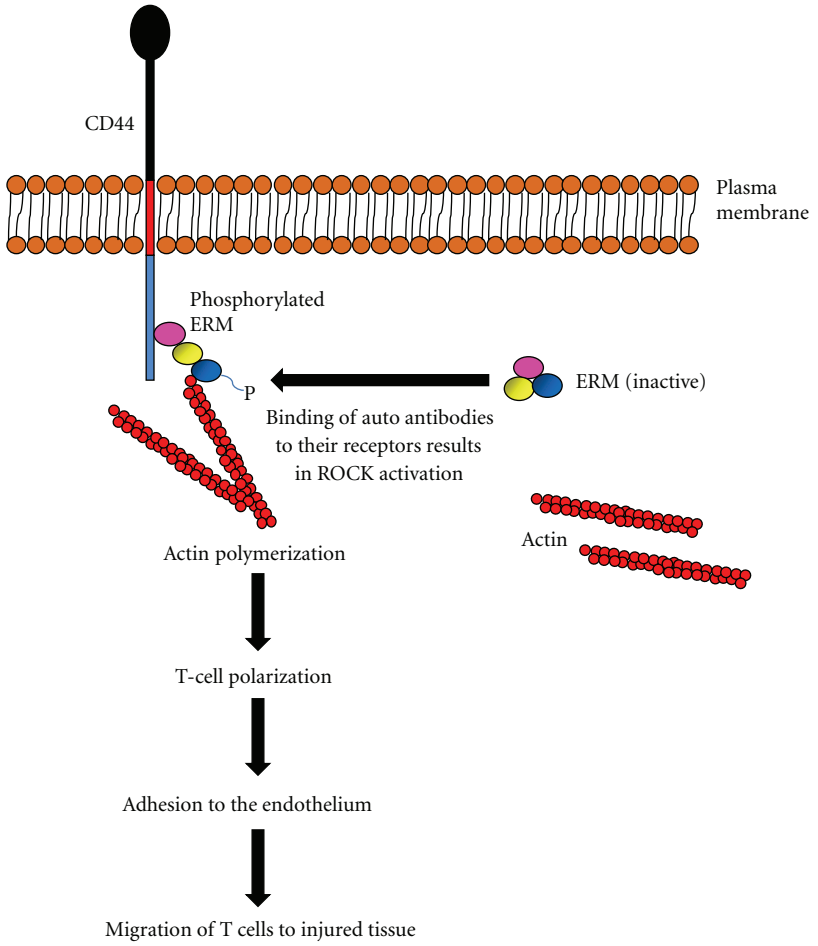


Figure 2: Schematic diagram showing the effect of ERM activation on T-cell function in SLE patients. Autoantibodies such as anti-CD3/T-cell receptor (TCR) antibodies bind to CD3/TCR complex in circulating T cells and induce ROCK activation, which in turn mediates ERM phosphorylation. Once activated, ERM directly interacts with CD44 and F actin resulting in their colocalization at the polar caps of T cells, leading to actin polymerization, T-cell polarization, adhesion to the endothelium and subsequent chemotactic migration to sites of injury in the kidney.

the pathogenesis of lupus nephritis such as TNF-α and IFN-γ can increase HA synthesis in proximal tubular epithelial cells [92], and therefore may contribute to increased synthesis of HA in lupus patients.

We have previously demonstrated that human polyclonal anti-dsDNA antibodies can induce IL-1β, IL-6, and TNF-α in cultured human mesangial cells and proximal tubular epithelial cells [12, 94]. We further demonstrated that anti-dsDNA antibodies can induce HA synthesis in human mesangial cells and proximal tubular epithelial cells, with the production of both HMW and LMW HA, and this induction was dependent on increased synthesis of HAS II mRNA, and IL-1β and IL-6 secretion [12, 95]. Our observation that increased circulating HA levels in patients with lupus nephritis correlated with anti-dsDNA antibodies substantiates the likelihood that anti-dsDNA antibodies contribute to increased HA synthesis during pathogenesis of disease [12]. Considering that LMW HA possesses pro-inflammatory properties, that anti-dsDNA antibodies can induce LMW HA in resident renal cells may represent a pathogenic mechanism through which anti-dsDNA antibodies induce inflammatory processes in the kidney parenchyma during lupus nephritis.

Exogenous LMW, but not HMW HA, has been shown to induce *de novo* synthesis of MCP-1 mRNA and protein secretion in proximal tubular epithelial cells, and this induction was dependent on the interaction of HA with CD44 [64]. Intrarenal MCP-1 expression is increased in both the glomerular and tubulo-interstitial compartments of the kidney during lupus nephritis and precedes leukocyte infiltration, proteinuria, and renal damage [96]. The importance of MCP-1 in the pathogenesis of lupus nephritis is underscored by studies by Tesch et al. [97], which demonstrated that lupus-prone mice rendered genetically deficient in MCP-1 showed less severe renal histology and proteinuria [97]. Studies have also demonstrated that exogenous LMW HA can induce ICAM-1 and VCAM-1 in murine cortical tubular epithelial cells, suggesting that HA may play a role in the adhesion of leukocytes to resident renal cells [31]. We have demonstrated that inhibition of HA synthesis in NZBWF1/J mice is associated with an improvement

in clinical parameters of disease and decreased intrarenal expression of IL-6 and TNF-α [54].

4. Conclusion

Despite its simple structure, HA is a multifaceted macromolecule that, depending on its molecular weight, is involved in tissue homeostasis and pathological processes. Through its interaction with CD44, HA regulates leukocyte infiltration, secretion of inflammatory mediators, and clearance of apoptotic cells processes that dictate the severity of lupus nephritis. Although studies have demonstrated that the interaction of HA with toll-like receptors can modulate inflammatory processes in animal models of bleomycin-induced lung injury, there is currently no data on the interaction of HA and toll-like receptors in the pathogenesis of lupus nephritis. Further research into the interaction of HA with other binding proteins will provide us with a better understanding of their roles in the pathophysiology of lupus nephritis and whether targeting HA or CD44 may serve as a novel therapeutic strategy.

Acknowledgments

This study was supported by the Hong Kong Research Grants Council General Research Fund (HKU 7812/08M), UGC Matching Grant Schemes (Phases IV and V), and the Estate of the late Mr. Chan Wing Hei. S. Yung is partially supported by the Wai Hung Charitable Foundation Limited and the Endowment Fund established for the Yu Chiu Kwong Professorship in Medicine awarded to T. M. Chan.

References

[1] Y. Deng and B. P. Tsao, "Genetic susceptibility to systemic lupus erythematosus in the genomic era," *Nature Reviews Rheumatology*, vol. 6, no. 12, pp. 683–692, 2010.

[2] D. A. González, B. B. Díaz, M. D. C. Rodríguez Pérez, A. G. Hernández, B. N. D. Chico, and A. C. de León, "Sex hormones and autoimmunity," *Immunology Letters*, vol. 133, no. 1, pp. 6–13, 2010.

[3] P. Sarzi-Puttini, F. Atzeni, L. Iaccarino, and A. Doria, "Environment and systemic lupus erythematosus: an overview," *Autoimmunity*, vol. 38, no. 7, pp. 465–472, 2005.

[4] J. S. Cameron, "Lupus nephritis," *Journal of the American Society of Nephrology*, vol. 10, no. 2, pp. 413–424, 1999.

[5] S. Yung, K. F. Cheung, Q. Zhang, and T. M. Chan, "Anti-dsDNA antibodies bind to mesangial annexin II in lupus nephritis," *Journal of the American Society of Nephrology*, vol. 21, no. 11, pp. 1912–1927, 2010.

[6] G. Mostoslavsky, R. Fischel, N. Yachimovich et al., "Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: a case for tissue injury by molecular mimicry," *European Journal of Immunology*, vol. 31, no. 4, pp. 1221–1227, 2001.

[7] B. Deocharan, X. Qing, J. Lichauco, and C. Putterman, "α-Actinin is a cross-reactive renal target for pathogenic anti-DNA aptibodies," *Journal of Immunology*, vol. 168, no. 6, pp. 3072–3078, 2002.

[8] M. Kalaaji, K. A. Fenton, E. S. Mortensen et al., "Glomerular apoptotic nucleosomes are central target structures for nephritogenic antibodies in human SLE nephritis," *Kidney International*, vol. 71, no. 7, pp. 664–672, 2007.

[9] M. Kalaaji, E. Mortensen, L. Jørgensen, R. Olsen, and O. P. Rekvig, "Nephritogenic lupus antibodies recognize glomerular basement membrane-associated chromatin fragments released from apoptotic intraglomerular cells," *American Journal of Pathology*, vol. 168, no. 6, pp. 1779–1792, 2006.

[10] D. Jiang, J. Liang, and P. W. Noble, "Hyaluronan in tissue injury and repair," *Annual Review of Cell and Developmental Biology*, vol. 23, pp. 435–461, 2007.

[11] D. Jiang, J. Liang, and P. W. Noble, "Hyaluronan as an immune regulator in human diseases," *Physiological Reviews*, vol. 91, no. 1, pp. 221–264, 2011.

[12] S. Yung, R. C. W. Tsang, J. K. H. Leung, and T. M. Chan, "Increased mesangial cell hyaluronan expression in lupus nephritis is mediated by anti-DNA antibody-induced IL-1β," *Kidney International*, vol. 69, no. 2, pp. 272–280, 2006.

[13] T. C. Laurent and J. R. E. Fraser, "Hyaluronan," *The FASEB Journal*, vol. 6, no. 7, pp. 2397–2404, 1992.

[14] N. Itano, T. Sawai, M. Yoshida et al., "Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties," *The Journal of Biological Chemistry*, vol. 274, no. 35, pp. 25085–25092, 1999.

[15] J. Y. Lee and A. P. Spicer, "Hyaluronan: a multifunctional, megaDalton, stealth molecule," *Current Opinion in Cell Biology*, vol. 12, no. 5, pp. 581–586, 2000.

[16] B. P. Toole, "Hyaluronan: from extracellular glue to pericellular cue," *Nature Reviews Cancer*, vol. 4, no. 7, pp. 528–539, 2004.

[17] K. R. Taylor and R. L. Gallo, "Glycosaminoglycans and their proteoglycans: host-associated molecular patterns for initiation and modulation of inflammation," *The FASEB Journal*, vol. 20, no. 1, pp. 9–22, 2006.

[18] R. Stern, A. A. Asari, and K. N. Sugahara, "Hyaluronan fragments: an information-rich system," *European Journal of Cell Biology*, vol. 85, no. 8, pp. 699–715, 2006.

[19] S. Yung, G. J. Thomas, and M. Davies, "Induction of hyaluronan metabolism after mechanical injury of human peritoneal mesothelial cells in vitro," *Kidney International*, vol. 58, no. 5, pp. 1953–1962, 2000.

[20] S. Yung and T. M. Chan, "Pathophysiology of the peritoneal membrane during peritoneal dialysis: the role of hyaluronan," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 180594, 11 pages, 2011.

[21] N. Nagy, T. Freudenberger, A. Melchior-Becker et al., "Inhibition of hyaluronan synthesis accelerates murine atherosclerosis: novel insights into the role of hyaluronan synthesis," *Circulation*, vol. 122, no. 22, pp. 2313–2322, 2010.

[22] A. Wang and V. C. Hascall, "Hyaluronan structures synthesized by rat mesangial cells in response to hyperglycemia induce monocyte adhesion," *The Journal of Biological Chemistry*, vol. 279, no. 11, pp. 10279–10285, 2004.

[23] W. Selbi, C. A. de la Motte, V. C. Hascall, A. J. Day, T. Bowen, and A. O. Phillips, "Characterization of hyaluronan cable structure and function in renal proximal tubular epithelial cells," *Kidney International*, vol. 70, no. 7, pp. 1287–1295, 2006.

[24] A. J. Day and C. A. de la Motte, "Hyaluronan cross-linking: a protective mechanism in inflammation?" *Trends in Immunology*, vol. 26, no. 12, pp. 637–643, 2005.

[25] P. W. Noble, "Hyaluronan and its catabolic products in tissue injury and repair," *Matrix Biology*, vol. 21, no. 1, pp. 25–29, 2002.

[26] C. M. McKee, M. B. Penno, M. Cowman et al., "Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages: the role of HA size and CD44," *Journal of Clinical Investigation*, vol. 98, no. 10, pp. 2403–2413, 1996.

[27] C. M. McKee, C. J. Lowenstein, M. R. Horton et al., "Hyaluronan fragments induce nitric-oxide synthase in murine macrophages through a nuclear factor κB-dependent mechanism," *The Journal of Biological Chemistry*, vol. 272, no. 12, pp. 8013–8018, 1997.

[28] B. Haslinger, S. Mandl-Weber, A. Sellmayer, and T. Sitter, "Hyaluronan fragments induce the synthesis of MCP-1 and IL-8 in cultured human peritoneal mesothelial cells," *Cell and Tissue Research*, vol. 305, no. 1, pp. 79–86, 2001.

[29] C. Fieber, P. Baumann, R. Vallon et al., "Hyaluronan-oligosaccharide-induced transcription of metalloproteases," *Journal of Cell Science*, vol. 117, no. 2, pp. 359–367, 2004.

[30] N. Guo, X. Li, M. M. Mann, M. L. Funderburgh, Y. Du, and J. L. Funderburgh, "Hyaluronan synthesis mediates the fibrotic response of keratocytes to transforming growth factor *β*," *The Journal of Biological Chemistry*, vol. 285, no. 42, pp. 32012–32019, 2010.

[31] B. Oertli, B. Beck-Schimmer, X. Fan, and R. P. Wüthrich, "Mechanisms of hyaluronan-induced up-regulation of ICAM-1 and VCAM-1 expression by murine kidney tubular epithelial cells: hyaluronan triggers cell adhesion molecule expression through a mechanism involving activation of nuclear factor-κB and activating protein-1," *Journal of Immunology*, vol. 161, no. 7, pp. 3431–3437, 1998.

[32] S. Ohno, H. J. Im, C. B. Knudson, and W. Knudson, "Hyaluronan oligosaccharides induce matrix metalloproteinase 13 via transcriptional activation of NFκB and p38 MAP kinase in articular chondrocytes," *The Journal of Biological Chemistry*, vol. 281, no. 26, pp. 17952–17960, 2006.

[33] P. Teder, R. W. Vandivier, D. Jiang et al., "Resolution of lung inflammation by CD44," *Science*, vol. 296, no. 5565, pp. 155–158, 2002.

[34] L. A. Goldstein and E. C. Butcher, "Identification of mRNA that encodes an alternative form of H-CAM(CD44) in lymphoid and nonlymphoid tissues," *Immunogenetics*, vol. 32, no. 6, pp. 389–397, 1990.

[35] J. Lesley, R. Hyman, and P. W. Kincade, "CD44 and its interaction with extracellular matrix," *Advances in Immunology*, vol. 54, pp. 271–335, 1993.

[36] B. Greenfield, W. C. Wang, H. Marquardt et al., "Characterization of the heparan sulfate and chondroitin sulfate assembly sites in CD44," *The Journal of Biological Chemistry*, vol. 274, no. 4, pp. 2511–2517, 1999.

[37] M. Jones, L. Tussey, N. Athanasou, and D. G. Jackson, "Heparan sulfate proteoglycan isoforms of the CD44 hyaluronan receptor induced in human inflammatory macrophages can function as paracrine regulators of fibroblast growth factor action," *The Journal of Biological Chemistry*, vol. 275, no. 11, pp. 7964–7974, 2000.

[38] A. Aruffo, I. Stamenkovic, M. Melnick, C. B. Underhill, and B. Seed, "CD44 is the principal cell surface receptor for hyaluronate," *Cell*, vol. 61, no. 7, pp. 1303–1313, 1990.

[39] J. Lesley, V. C. Hascall, M. Tammi, and R. Hyman, "Hyaluronan binding by cell surface CD44," *The Journal of Biological Chemistry*, vol. 275, no. 35, pp. 26967–26975, 2000.

[40] J. Lesley and R. Hyman, "CD44 can be activated to function as an hyaluronic acid receptor in normal murine T cells," *European Journal of Immunology*, vol. 22, no. 10, pp. 2719–2723, 1992.

[41] K. S. Hathcock, H. Hirano, S. Murakami, and R. J. Hodes, "CD44 expression on activated B cells: differential capacity for CD44- dependent binding to hyaluronic acid," *Journal of Immunology*, vol. 151, no. 12, pp. 6712–6722, 1993.

[42] J. Lesley, N. English, A. Perschl, J. Gregoroff, and R. Hyman, "Variant cell lines selected for alterations in the function of the hyaluronan receptor CD44 show differences in glycosylation," *Journal of Experimental Medicine*, vol. 182, no. 2, pp. 431–437, 1995.

[43] J. Lesley, N. Howes, A. Perschl, and R. Hyman, "Hyaluronan binding function of CD44 is transiently activated on T cells during an in vivo immune response," *Journal of Experimental Medicine*, vol. 180, no. 1, pp. 383–387, 1994.

[44] S. Katoh, Z. Zheng, K. Oritani, T. Shimozato, and P. W. Kincade, "Glycosylation of CD44 negatively regulates its recognition of hyaluronan," *Journal of Experimental Medicine*, vol. 182, no. 2, pp. 419–429, 1995.

[45] C. Underhill, "CD44: the hyaluronan receptor," *Journal of Cell Science*, vol. 103, no. 2, pp. 293–298, 1992.

[46] S. M. Denning, P. T. Le, K. H. Singer, and B. F. Haynes, "Antibodies against the CD44 p80, lymphocyte homing receptor molecule augment human peripheral blood T cell activation," *Journal of Immunology*, vol. 144, no. 1, pp. 7–15, 1990.

[47] D. F. Barber, A. Bartolomé, C. Hernandez et al., "PI3K*γ* inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus," *Nature Medicine*, vol. 11, no. 9, pp. 933–935, 2005.

[48] S. Yung, Q. Zhang, C. Z. Zhang, K. W. Chan, L. Lui, and T. M. Chan, "Anti-DNA antibody induction of protein kinase C phosphorylation and fibronectin synthesis in human and murine lupus and the effect of mycophenolic acid," *Arthritis and Rheumatism*, vol. 60, no. 7, pp. 2071–2082, 2009.

[49] B. F. Haynes, L. P. Hale, K. L. Patton, M. E. Martin, and R. M. McCallum, "Measurement of an adhesion molecule as an indicator of inflammatory disease activity: up-regulation of the receptor for hyaluronate (CD44) in rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 34, no. 11, pp. 1434–1443, 1991.

[50] K. Mikecz, F. R. Brennan, J. H. Kim, and T. T. Glant, "Anti-CD44 treatment abrogates tissue oedema and leukocyte infiltration in murine arthritis," *Nature Medicine*, vol. 1, no. 6, pp. 558–563, 1995.

[51] S. Nedvetzki, M. Walmsley, E. Alpert, R. O. Williams, M. Feldmann, and D. Naor, "CD44 involvement in experimental collagen-induced arthritis (CIA)," *Journal of Autoimmunity*, vol. 13, no. 1, pp. 39–47, 1999.

[52] S. Brocke, C. Piercy, L. Steinman, I. L. Weissman, and T. Veromaa, "Antibodies to CD44 and integrin *α*4, but not L-selectin, prevent central nervous system inflammation and experimental encephalomyelitis by blocking secondary leukocyte recruitment," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 12, pp. 6896–6901, 1999.

[53] A. Rafi, M. Nagarkatti, and P. S. Nagarkatti, "Hyaluronate-CD44 interactions can induce murine B-cell activation," *Blood*, vol. 89, no. 8, pp. 2901–2908, 1997.

[54] T. M. Chan, W. W. Tse, M. Chau, and S. Yung, "Suppression of hyaluronan synthesis with 4-methylumbelliferone in NZB/W F1 mice is associated with reduced renal inflammation and renal function improvement," *Journal of the American Society of Nephrology*, vol. 22, article 134A, 2011.

[55] C. Hansen, E. Otto, K. Kuhlemann, G. Förster, and G. J. Kahaly, "Glycosaminoglycans in autoimmunity," *Clinical and*

Experimental Rheumatology, vol. 14, supplement 15, pp. S59–S67, 1996.

[56] P. Faaber, J. Capel, G. Rijke, G. Vierwinden, L. B. van de Putte, and R. A. Koene, "Cross-reactivity of anti-DNA antibodies with proteoglycans," *Clinical and Experimental Immunology*, vol. 55, no. 3, pp. 502–508, 1984.

[57] D. M. Klinman, R. A. Eisenberg, and A. D. Steinberg, "Development of the autoimmune B cell repertoire in MRL-lpr/lpr mice," *Journal of Immunology*, vol. 144, no. 2, pp. 506–511, 1990.

[58] K. Moreth, R. Brodbeck, A. Babelova et al., "The proteoglycan biglycan regulates expression of the B cell chemoattractant CXCL13 and aggravates murine lupus nephritis," *Journal of Clinical Investigation*, vol. 120, no. 12, pp. 4251–4272, 2010.

[59] T. K. Means, E. Latz, F. Hayashi, M. R. Murali, D. T. Golenbock, and A. D. Luster, "Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9," *Journal of Clinical Investigation*, vol. 115, no. 2, pp. 407–417, 2005.

[60] H. Bagavant, U. S. Deshmukh, H. Wang, T. Ly, and M. F. Shu, "Role for nephritogenic T cells in lupus glomerulonephritis: progression to renal failure is accompanied by T cell activation and expansion in regional lymph nodes," *Journal of Immunology*, vol. 177, no. 11, pp. 8258–8265, 2006.

[61] A. Chang, S. G. Henderson, D. Brandt et al., "In situ B Cell-mediated immune responses and tubulointerstitial inflammation in human lupus nephritis," *Journal of Immunology*, vol. 186, no. 3, pp. 1849–1860, 2011.

[62] L. Schiffer, R. Bethunaickan, M. Ramanujam et al., "Activated renal macrophages are markers of disease onset and disease remission in lupus nephritis," *Journal of Immunology*, vol. 180, no. 3, pp. 1938–1947, 2008.

[63] D. M. Klinman, "Polyclonal B cell activation in lupus-prone mice precedes and predicts the development of autoimmune disease," *Journal of Clinical Investigation*, vol. 86, no. 4, pp. 1249–1254, 1990.

[64] B. Beck-Schimmer, B. Oertli, T. Pasch, and R. P. Wüthrich, "Hyaluronan induces monocyte chemoattractant protein-1 expression in renal tubular epithelial cells," *Journal of the American Society of Nephrology*, vol. 9, no. 12, pp. 2283–2290, 1998.

[65] J. Hodge-Dufour, P. W. Noble, M. R. Norton et al., "Induction of IL-12 and chemokines by hyaluronan requires adhesion-dependent priming of resident but not elicited macrophages," *Journal of Immunology*, vol. 159, no. 5, pp. 2492–2500, 1997.

[66] M. R. Horton, C. M. McKee, C. Bao et al., "Hyaluronan fragments synergize with interferon-γ to induce the C-X-C chemokines mig and interferon-inducible protein-10 in mouse macrophages," *The Journal of Biological Chemistry*, vol. 273, no. 52, pp. 35088–35094, 1998.

[67] R. Galandrini, N. Albi, G. Tripodi et al., "Antibodies to CD44 trigger effector functions of human T cell clones," *Journal of Immunology*, vol. 150, no. 10, pp. 4225–4235, 1993.

[68] C. Termeer, F. Benedix, J. Sleeman et al., "Oligosaccharides of hyaluronan activate dendritic cells via toll-like receptor 4," *Journal of Experimental Medicine*, vol. 195, no. 1, pp. 99–111, 2002.

[69] M. H. Siegelman, H. C. DeGrendele, and P. Estess, "Activation and interaction of CD44 and hyaluronan in immunological systems," *Journal of Leukocyte Biology*, vol. 66, no. 2, pp. 315–321, 1999.

[70] P. Estess, H. C. DeGrendele, V. Pascual, and M. H. Siegelman, "Functional activation of lymphocyte CD44 in peripheral blood is a marker of autoimmune disease activity," *Journal of Clinical Investigation*, vol. 102, no. 6, pp. 1173–1182, 1998.

[71] Y. Li, T. Harada, Y. T. Juang et al., "Phosphorylated ERM is responsible for increased T cell polarization, adhesion, and migration in patients with systemic lupus erythematosus," *Journal of Immunology*, vol. 178, no. 3, pp. 1938–1947, 2007.

[72] G. F. Weber, "The absence of CD44 ameliorates Faslpr/lpr disease," *Autoimmunity*, vol. 37, no. 1, pp. 1–8, 2004.

[73] U. Sela, N. Mauermann, R. Hershkoviz et al., "The inhibition of autoreactive T cell functions by a peptide based on the CDR1 of an anti-DNA autoantibody is via TGF-β-mediated suppression of LFA-1 and CD44 expression and function," *Journal of Immunology*, vol. 175, no. 11, pp. 7255–7263, 2005.

[74] J. C. Crispín, B. T. Keenan, M. D. Finnell et al., "Expression of CD44 variant isoforms CD44v3 and CD44v6 is increased on T cells from patients with systemic lupus erythematosus and is correlated with disease activity," *Arthritis and Rheumatism*, vol. 62, no. 5, pp. 1431–1437, 2010.

[75] J. Savill, I. Dransfield, C. Gregory, and C. Haslett, "A blast from the past: clearance of apoptotic cells regulates immune responses," *Nature Reviews Immunology*, vol. 2, no. 12, pp. 965–975, 2002.

[76] M. Herrmann, R. E. Voll, O. M. Zoller, M. Hagenhofer, B. B. Ponner, and J. R. Kalden, "Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 41, no. 7, pp. 1241–1250, 1998.

[77] M. C. Levesque and B. F. Haynes, "In vitro culture of human peripheral blood monocytes induces hyaluronan binding and up-regulates monocyte variant CD44 isoform expression," *Journal of Immunology*, vol. 156, no. 4, pp. 1557–1565, 1996.

[78] A. P. Cairns, A. D. Crockard, J. R. McConnell, P. A. Courtney, and A. L. Bell, "Reduced expression of CD44 on monocytes and neutrophils in systemic lupus erythematosus: relations with apoptotic neutrophils and disease activity," *Annals of the Rheumatic Diseases*, vol. 60, no. 10, pp. 950–955, 2001.

[79] P. S. Ramos, A. H. Williams, J. T. Ziegler et al., "Genetic analyses of interferon pathway-related genes reveal multiple new loci associated with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 63, no. 7, pp. 2049–2057, 2011.

[80] K. M. Kaufman, J. Rankin, I. T. Harley, J. A. Kelly, J. B. Harley, and R. H. Scofield, "A genetic marker within the CD44 gene confirms linkage at 11p13 in African-American families with lupus stratified by thrombocytopenia, but genetic association with CD44 is not present," *Genes and Immunity*, vol. 3, 1, pp. S86–S88, 2002.

[81] R. H. Scofield, G. R. Bruner, J. A. Kelly et al., "Thrombocytopenia identifies a severe familial phenotype of systemic lupus erythematosus and reveals genetic linkages at 1q22 and 11p13," *Blood*, vol. 101, no. 3, pp. 992–997, 2003.

[82] C. J. Lessard, I. Adrianto, J. A. Kelly et al., "Identification of a systemic lupus erythematosus susceptibility locus at 11p13 between PDHX and CD44 in a multiethnic study," *American Journal of Human Genetics*, vol. 88, no. 1, pp. 83–91, 2011.

[83] P. Hansell, V. Goransson, C. Odlind, B. Gerdin, and R. Hallgren, "Hyaluronan content in the kidney in different states of body hydration," *Kidney International*, vol. 58, no. 5, pp. 2061–2068, 2000.

[84] P. Roy-Chaudhury, T. F. Khong, J. H. Williams et al., "CD44 in glomerulonephritis: expression in human renal biopsies, the Thy 1.1 model, and by cultured mesangial cells," *Kidney International*, vol. 50, no. 1, pp. 272–281, 1996.

[85] P. S. Benz, X. Fan, and R. P. Wüthrich, "Enhanced tubular epithelial CD44 expression in MRL-lpr lupus nephritis," *Kidney International*, vol. 50, no. 1, pp. 156–163, 1996.

[86] Z. Jun, P. A. Hill, H. Y. Lan et al., "CD44 and hyaluronan expression in the development of experimental crescentic glomerulonephritis," *Clinical and Experimental Immunology*, vol. 108, no. 1, pp. 69–77, 1997.

[87] J. Ren, V. C. Hascall, and A. Wang, "Cyclin D3 mediates synthesis of a hyaluronan matrix that is adhesive for monocytes in mesangial cells stimulated to divide in hyperglycemic medium," *The Journal of Biological Chemistry*, vol. 284, no. 24, pp. 16621–16632, 2009.

[88] S. Jones and A. O. Phillips, "Regulation of renal proximal tubular epithelial cell hyaluronan generation: implications for diabetic nephropathy," *Kidney International*, vol. 59, no. 5, pp. 1739–1749, 2001.

[89] D. R. Michael, A. O. Phillips, A. Krupa et al., "The human hyaluronan synthase 2 (HAS2) gene and its natural antisense RNA exhibit coordinated expression in the renal proximal tubular epithelial cell," *The Journal of Biological Chemistry*, vol. 286, no. 22, pp. 19523–19532, 2011.

[90] E. Pedagogos, T. D. Hewitson, K. M. Nicholls, and G. J. Becker, "Hyaluronan and rat renal fibroblasts: in vitro studies," *Nephron*, vol. 88, no. 4, pp. 347–353, 2001.

[91] M. Takeda, T. Babazono, K. Nitta, and Y. Iwamoto, "High glucose stimulates hyaluronan production by renal interstitial fibroblasts through the protein kinase C and transforming growth factor-β cascade," *Metabolism*, vol. 50, no. 7, pp. 789–794, 2001.

[92] E. Feusi, L. Sun, A. Sibalic, B. Beck-Schimmer, B. Oertli, and R. P. Wüthrich, "Enhanced hyaluronan synthesis in the MRL-Fas(lpr) kidney: role of cytokines," *Nephron*, vol. 83, no. 1, pp. 66–73, 1999.

[93] L. Daniel, H. Sichez, R. Giorgi et al., "Tubular lesions and tubular cell adhesion molecules for the prognosis of lupus nephritis," *Kidney International*, vol. 60, no. 6, pp. 2215–2221, 2001.

[94] S. Yung, R. C. W. Tsang, Y. Sun, J. K. H. Leung, and T. M. Chan, "Effect of human anti-DNA antibodies on proximal renal tubular epithelial cell cytokine expression: implications on tubulointerstitial inflammation in lupus nephritis," *Journal of the American Society of Nephrology*, vol. 16, no. 11, pp. 3281–3294, 2005.

[95] T. M. Chan, R. C. W. Tsang, K. W. Chan, and S. Yung, "Anti-DNA antibodies stimulate hyaluronan synthesis in proximal tubular epithelial cells through the induction of IL-6 and IL-1β," *Journal of the American Society of Nephrology*, vol. 17, article 352A, 2006.

[96] G. Pérez de Lema, H. Maier, E. Nieto et al., "Chemokine expression precedes inflammatory cell infiltration and chemokine receptor and cytokine expression during the initiation of murine lupus nephritis," *Journal of the American Society of Nephrology*, vol. 12, no. 7, pp. 1369–1382, 2001.

[97] G. H. Tesch, S. Maifert, A. Schwarting, B. J. Rollins, and V. R. Kelley, "Monocyte chemoattractant protein 1-dependent leukocytic infiltrates are responsible for autoimmune disease in MRL-Fas(lpr) mice," *Journal of Experimental Medicine*, vol. 190, no. 12, pp. 1813–1824, 1999.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 834291, 15 pages
doi:10.1155/2012/834291

Review Article

Cutaneous Manifestations of Systemic Lupus Erythematosus

Luís Uva, Diana Miguel, Catarina Pinheiro, João Pedro Freitas, Manuel Marques Gomes, and Paulo Filipe

Faculdade de Medicina de Lisboa, Clinica Universitária de Dermatologia, Avenido Prof. Egas Moniz, 1649-035 Lisboa, Portugal

Correspondence should be addressed to Paulo Filipe, pfilipe@fm.ul.pt

Received 4 May 2012; Accepted 4 June 2012

Academic Editor: Hiroshi Okamoto

Systemic lupus erythematosus (SLE) is a multiorgan autoimmune disease of unknown etiology with many clinical manifestations. The skin is one of the target organs most variably affected by the disease. The American College of Rheumatology (ACR) established 11 criteria as a classificatory instrument to operationalise the definition of SLE in clinical trials. They were not intended to be used to diagnose individuals and do not do well in that capacity. Cutaneous lesions account for four of these 11 revised criteria of SLE. Skin lesions in patients with lupus may be specific or nonspecific. This paper covers the SLE-specific cutaneous changes: malar rash, discoid rash, photosensitivity, and oral mucosal lesions as well as SLE nonspecific skin manifestations, their pathophysiology, and management. A deeper thorough understanding of the cutaneous manifestations of SLE is essential for diagnosis, prognosis, and efficient management. Thus, dermatologists should cooperate with other specialties to provide optimal care of SLE patient.

1. Introduction

The nosographic concept of lupus erythematosus (LE) includes 3 major subtypes: chronic cutaneous LE, subacute cutaneous LE, and systemic or acute cutaneous LE. Besides these 3 subtypes, other less frequent clinical varieties may occur [1].

Systemic lupus erythematosus (SLE) is a multiorgan autoimmune disease of unknown etiology that can have many clinical manifestations (Table 1). The skin is involved in up to 85% of systemic lupus erythematosus (SLE) cases and may be the only organ involved in cutaneous lupus erythematosus (CLE).

The diagnosis of the cutaneous manifestations of LE is based on clinical, histopathology, and immunohistology of skin lesions. In addition, serum autoantibodies are considered immunologic markers for distinct clinical types of the illness. The Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) is used as a clinical tool that standardizes the way disease activity is described and provides guidelines for identifying a clinical change. This clinical tool quantifies disease activity and damage in cutaneous lupus erythematosus. The activity score is based on the erythema, scale, mucous membrane lesions, and nonscarring alopecia. A recent study gives us a foundation for the practical use of the CLASI in clinical trials as a tool to measure disease severity and responsiveness to therapy [2].

In 1982, the diagnosis criteria for SLE were published by the American College of Rheumatology (ACR) which were revised in 1997 and are currently used in clinical practice [3]. Undoubtedly useful, mainly for differential diagnosis between systemic LE and other rheumatologic diseases, such criteria are commonly inadequate for some LE subsets. Concerning cutaneous manifestations, the ACR criteria include malar rash, discoid rash, photosensitivity, and oral ulcers. It must be pointed out that the immunologic study does not include the immunohistology of the skin (lupus band test).

2. Malar Rash

The first criterion of the ACR is malar rash (sensitivity 57%; specificity 96%), which is characterized by an erythematous rash over the cheeks and nasal bridge (Figure 1). Malar rash is a fixed erythema that typically spares the nasolabial folds. It is a butterfly-shaped or vespertilio rash that can be flat or raised over the cheeks and bridge of the nose. It lasts from days to weeks and is occasionally painful or pruritic.

Table 1: Cutaneous manifestations of SLE.

(1) Malar rash
(2) Discoid LE (DLE)
(a) Localized DLE
(b) Generalized DLE
(3) Photosensitivity
(4) Mucosal DLE
(a) Oral DLE
(b) Conjunctival DLE
(c) Nasal DLE
(d) Genital DLE
(5) Subacute cutaneous lupus erythematosus
(6) Alopecia
(7) Lupus panniculitis/lupus profundus
(8) Lichenoid DLE (LE/lichen planus overlap)
(9) Small vessel cutaneous leukocytoclastic vasculitis secondary to LE
(a) Dependent palpable purpura
(b) Urticarial vasculitis
(10) Secondary atrophie blanche
(11) Periungual telangiectasias
(12) Livedo reticularis
(13) Raynaud's phenomenon
(14) Bullous lesions (BSLE)

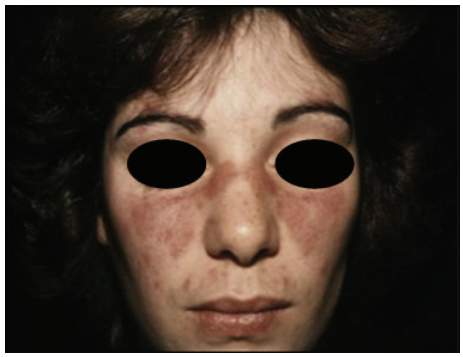


Figure 1: Malar rash.

3. Photosensitivity

The second criterion is photosensitivity (sensitivity 43%; specificity 96%). Exposure to ultraviolet light causes skin rash or other symptoms of SLE flareups. A macular or a diffuse erythematous rash occurs in sun-exposed areas, as the face, arms, or hands and that generally persists for more than 1 day. Sometimes erythematous papules or macules on the dorsal aspects of the hands classically sparing the knuckles are observed (Figure 2).

4. Discoid Rash

The third feature may be discoid rash (sensitivity 18%; specificity 59%). Discoid lupus erythematosus (DLE), a chronic dermatological disease, is the most common form of

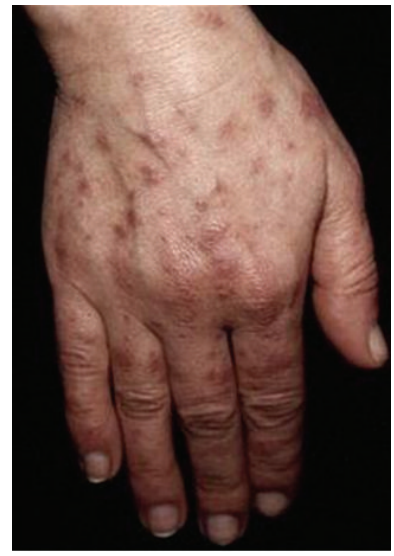


Figure 2: Photosensitive lesions.

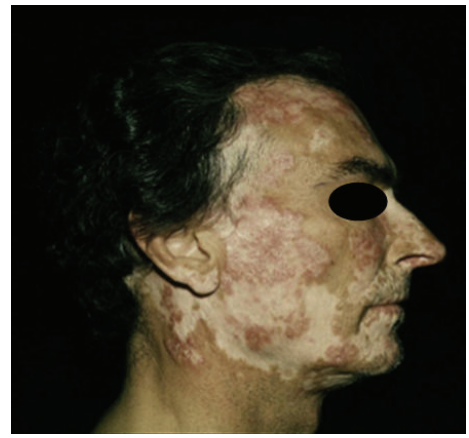


Figure 3: Discoid rash.

chronic CLE. Lesions may be part of systemic lupus or may represent discoid lupus without organ involvement, which is a separate diagnostic entity.

Lesions are disc-shaped, erythematous plaques of varying size, and contain areas of follicular hyperkeratoses, which are painful if lifted manually. Disease progression can result in pigmentary changes, permanent, depressed scarring, atrophy, and alopecia (Figure 3). Lesions spread centrifugally and may merge. Although most patients manifest lesional confinement to the head and neck area, a variant termed generalized/disseminated DLE is recognized, for which the minimum criterion is the presence of DLE lesions above and below the neck. Mucosal surfaces may be affected by lesions that appear identical to DLE of the skin or by lesions that may simulate lichen planus. Palms and soles can be also involved, but this occurs in less than 2% of patients [4].

DLE lesions may become hypertrophic or verrucous. This subset is manifested by wart-like lesions, more often on the extensor arms. Hypertrophic lesions of LE must be differentiated from warts, keratoacanthomas, or squamous

cell carcinoma. These lesions are more difficult to treat [5]. Lupus panniculitis is a form of chronic CLE that may be accompanied by typical DLE lesions or may occur in patients with SLE [6].

Discoid lupus erythematosus occurs most frequently in women (female/male ratio of 3 : 1) who are 40 to 50 years old [7, 8].

Occurrence of lesions, which may be disfiguring on visible sun-exposed areas, is emotionally devastating and can be added to the psychological burden of the disease. DLE has been reported to have a dramatic negative impact on the patient's quality of life, leading to physical and psychological disability [9, 10].

DLE may occur in patients with SLE, and some patients (<5%) with DLE progress to SLE [11].

Patients with DLE rarely fulfill 4 or more of the criteria used to classify SLE. Serologic abnormalities are uncommon. Serious systemic disease is rare, but when it occurs, patients may develop life-altering sequelae. Malignant degeneration of chronic lesions of lupus erythematosus (LE) is possible, although rare, leading to nonmelanoma skin cancer. Dark-skinned individuals may be more prone to skin cancer because of the lack of pigmentation within the chronic lesion, combined with chronic inflammation and continued sun damage.

Patients may complain of mild pruritus or occasional pain within lesions, but most patients are asymptomatic. Patients with widespread involvement often have hematologic and serologic abnormalities, are more likely to develop SLE, and are more difficult to treat.

5. Pathophysiology of Malar Rash, Photosensitivity, and Discoid Rash

The impact of UV irradiation on initial triggering, and on perpetuation of the various cutaneous manifestations of LE, suggests that abnormal photoreactivity is one important factor in LE.

Photosensitivity shows a strong association with the manifestation of all CLE subtypes, and the abnormal reactivity to ultraviolet (UV) light is an important factor in the pathogenesis of both cutaneous and systemic disease. A potentially crucial role in the initiation of the autoimmune reaction cascade has been attributed to UV-induced keratinocyte apoptosis [12]. Interestingly, a significantly higher number of apoptotic nuclei in the epidermis has been described in primary and UV-induced skin lesions of CLE patients compared with normal donors [13]. This is in analogy with the evidence that impair clearance of apoptotic cells may trigger the immune response in patients with autoimmune disorders. Apoptotic cells accumulate in the germinal centers of lymph nodes from patients with SLE, which might be due to impaired phagocytic activity or caused by the absence of tangible body macrophages [14], indicating that apoptotic cells accumulate, and, subsequently, enter late stages of apoptotic cell death including secondary necrosis.

The chromatin nonhistone DNA binding protein high mobility group box one (HMGB1), released during cell activation and death, may also be involved in the inflammatory clearance of apoptotic cells, which justifies the release of HMGB1, detected in the serum of SLE patients as well as an increased expression of HMGB1, was demonstrated in skin lesions of lupus patients. HMGB1 makes easier interaction and uptake (followed by inflammation) by macrophages and dendritic cells through receptor for advanced glycation end-products and Toll-like receptors 2, 4, and 9 due the connection with nucleosomes and DNA released from apoptotic cells.

Apoptosis or clearance of apoptotic cells has been reported as an important pathophysiological characteristic in autoimmune diseases such as systemic lupus erythematosus, therefore targeting HMGB1 might have an important role on the inflammation control [15].

Nitric oxide (NO), an important regulator of apoptosis, has been implicated in the course of various autoimmune diseases. Interestingly, NO has been shown to protect against UVA-induced apoptosis by increasing Bcl-2 expression and inhibiting UVA-induced upregulation of Bax protein in endothelial cells [16].

In addition, an antiapoptotic role for NO in keratinocytes was suggested after UVB irradiation. Furthermore, UV exposure has also been shown to modulate local production of NO by the constitutively expressed nitric oxide synthase (iNOS). It has also been reported that iNOS is expressed in human skin in the first 2 days after exposure to UVA and UVB [17]. In contrast, in CLE patients, an iNOS-specific signal appeared only 72 h after UV exposure and persisted in the evolving skin lesions up to 1 month, evidencing a delayed and prolonged expression of iNOS in the LE skin. It has further been studied that NO production is increased in patients with SLE, possibly due to the upregulated iNOS expression in activated endothelial cells and keratinocytes [18].

Ultraviolet irradiation leads to release of interleukin-10 (IL-10) by keratinocytes, which may be related with increased autoantibody production and apoptotic damage in skin lesions of LE patients [19]. An interferon-alpha (IFN-α) or "type I IFN signature" has been found in patients with SLE. Lesional skin from LE patients has shown a high number of plasmacytoid dendritic cells (pDCs) which are the primary cellular source of IFN-α in LE skin lesions [20]. Interferon-inducible protein-10 (IP-10 or CXCL10), a monokine induced by gamma interferon (MIG or CXCL9) and interferon-a/p-inducible Mx 78 kDa protein (MxA), is downstream surrogate marker for IFN-α expression [21]. IP-10 and MIG recruit CXCR-3 expressing T cells into skin and are abundantly expressed in patients with LE [19].

CJun N-terminal kinase (JNK) is activated by UV radiation [22, 23]. Even low of UVB radiation such as 1 mJ/cm^2 are capable of inducing JNK activation and the apoptosis of keratinocytes [24]. The expression of iNOS seems also to be dependent upon JNK activity [25].

Naturally occurring $CD4^+CD25^+$ regulatory T cells (T_{reg}) have emerged as another important factor in self-tolerance and mechanisms in autoimmune diseases [26]. A decreased number of peripheral T_{reg} were found in SLE patients compared with normal healthy donors and a significant

correlation could be detected between the number of $CD4^+CD25^+$ T cells and disease activity [27]. As suggested by Miyara et al., sensitivity of T_{reg} to CD95L-mediated apoptosis could explain the loss of $CD4^+CD25^+$ T cells in patients with active SLE [28].

Recently, a superfamily of small chemotactic proteins has been shown to regulate lymphocyte trafficking of some inflammatory conditions, and it has been demonstrated that UV exposure induces the expression of T cell attracting chemokines [29]. Furthermore, it was shown that CXCR3 ligands, CXCL9, CXCL10, and CXCL11, are overexpressed in patients with CLE. Additionally, it has been reported that the CCR4 ligand TARC/CCL17 is increased in skin and in the serum of patients with CLE [30].

The pathophysiology of cutaneous LE is not clarified, and to find a solution to this problem, appropriate animal models can be helpful to study autoimmune diseases, although no animal model perfectly mimics a human disease.

MRL/lpr mouse is a good model for the spontaneous development of skin lesions similar to those seen in human, but also other models such as transgenic, knockout mice, TCR$\alpha-/-$ mice treated with fluorouracil and ultraviolet B light, may contribute to ongoing research, which will shed more light on the pathophysiological relevance of the different cellular and molecular factors *in vivo*, leading to a more complete understanding of the complex events and in SLE [31].

6. Treatment of Malar Rash, Photosensitivity, and Discoid Rash

Therapy begins with the use of sun-protective measures, including sunscreens, protective clothing, and behavior alteration. Ultraviolet A and B (UVA and UVB) radiations have been implicated in the initiation and exacerbation of skin lesions. As a result, current standard of care includes minimizing sun exposure, and the use of broad spectrum sunscreens. Despite sunscreens are widely used to photoprotect patients with photosensitive lupus erythematosus, standardized controlled studies that can prove their efficacy for this indication have been lacking.

The regular use of sunscreens is beneficial to LE patients because it prevents the UV radiation-induced skin lesions. Effective protection, however, might vary considerably between different sunscreens. A recent study demonstrated that a highly protective sunscreen is able to block the development of UV-induced skin lesions in all patients with the disease. This study confirms that the use of a broad-spectrum (UVB and UVA) sunscreen can effectively protect photosensitive patients with CLE from developing skin lesions.

Topical and intralesional corticosteroids are used for limited disease; however, long-term use may lead to significant side effects, especially on the face. Topical tacrolimus and pimecrolimus have also been shown to be effective on facial lesions in DLE in some patients. In hyperkeratotic lesions, topical retinoids have been reported to be helpful. Recently, topical imiquimod was reported to be effective. When skin lesions are not controlled with topical agents or intralesional corticosteroids, systemic therapy may be indicated.

Antimalarials are the gold standard systemic agents used for DLE. The Food and Drug Administration approved only hydroxychloroquine for the treatment of DLE, but other antimalarials have been used. Most existing regimens have been based on limited clinical experience and empirical data. Recent data says that hydroxychloroquine use is possibly associated with a delay in the development of integument damage and disease activity was associated with a shorter time to integument damage. African Americans have a higher probability of developing integument damage than Caucasians and Hispanics [32].

In 1993, cigarette smoking was suggested to interfere with antimalarial efficacy in treating patients with cutaneous lupus erythematosus (CLE). There are some data which says that cigarette smoking may interfere in a direct manner with the effectiveness of hydroxychloroquine and chloroquine in CLE. It has been hypothesized that the resistance of CLE to antimalarials can be explained by a modification of its metabolism, usually by the induction of cytochrome P450 (in which antimalarial agents are partly metabolized) by the constituents of cigarette smoke. The nonadherence to treatment by smokers could be one of the reasons about how cigarette smoking interferes with CLE treatment. Evidence-based data with long-term followup is required to understand the diminished antimalarial response. Taking into account that smoking negatively affects a number cutaneous conditions, dermatologists are active participants in smoking prevention and cessation [33]. A recent study has shown that there was no significant relationship between cigarette smoking and hydroxychloroquine concentrations, and this is a a strong argument against a direct effect of smoking on hydroxychloroquine metabolism [34].

Up to 30% of DLE subjects are not responsive to the available drugs, and even for those who are responders, long-term use may be precluded by toxicity (e.g., retinal toxicity of antimalarials). As a consequence, no more than 8 months of treatment is recommended. Various studies have shown benefits of thalidomide, with high response rates, even in disease refractory to antimalarials. Adverse events, such as neuropathy, have advised us to not use thalidomide as a first-line agent. Other medications that have been used empirically in subjects that were nonresponders to antimalarial therapy include oral retinoids, clofazimine, dapsone, azathioprine, methotrexate, mycophenolate mofetil, and other cytokine-blocking medications. These medications also have dose-limiting toxicities, including GI side effects, hepatotoxicity, neuropathy, malignancy, and bone marrow suppression [35].

7. Oral Ulcers

The fourth criterion of ACR is oral ulcers (including oral or nasopharyngeal ulcers) [36].

Lupus should be considered in all patients who experience painless or painful oral (or less frequently nasal or vaginal) ulcers. Palatal ulcers are most specific for SLE (Figure 4).

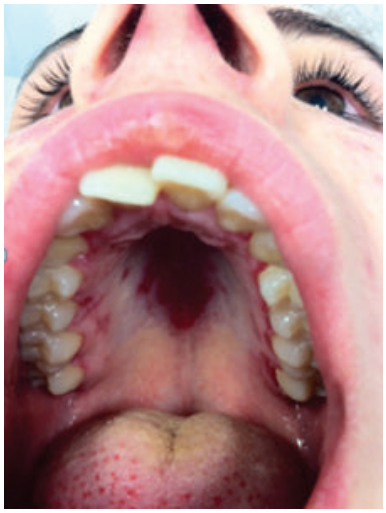


Figure 4: Palatal ulcers.

The prevalence of oral lesions is reported to be 7–52% of patients with SLE [3, 36–48]. Some studies have shown that up to 57% of mucosal lesions were painful whilst other earlier observations stated that up to 82% of oral ulcers observed were painless. This disparity may be due to differences in the type of lesion, whereas erythematous lesions are typically painless, discoid lesions are more often painful. Due to a significant proportion of asymptomatic oral lesions, a careful examination of the oral cavity in all lupus patients must be performed. The relationship between mucosal lesions and systemic disease activity is also nonconsensual. One study specified an association of oral ulceration with clinical systemic activity, although this did not correlate with significant changes in titers of serum complement (C3) or anti-DNA antibodies [38]. It was suggested that patients with oral ulcers have a higher mortality than those without oral ulcers [40], although this has not been confirmed by further studies. It was shown that the overall prevalence of oral lesions was not related to disease activity. However, discoid lesions and ulceration have mainly been seen in patients with active disease.

The buccal mucosa, hard palate, and vermilion border are the locations most frequently involved by lesions [36], which can be three types (discoid lesions, erythematosus lesions, and ulcers) and may coexist [38], leading to oedema and petechiae [36]. Discoid lesions appear as central areas of erythema with white spots surrounded by radiating white striae and telangiectasia at the periphery [38]. Erythematous lesions are often accompanied by oedema and petechial reddening on the hard palate, although they are usually found incidentally as flat macular areas with poorly defined borders [36]. Ulcers tend to occur in crops and are shallow. They are usually 1-2 cm in diameter and in about one-third of patients may extend into the pharynx [38].

No evidence-based recommendations exist for the treatment of oral lupus. A recent large international survey found that mucocutaneous lesions are treated most frequently with antimalarials, steroids and azathioprine are reserved for more severe cases. Thalidomide and cyclosporin are more often used as second-line agents in Europe, whilst North American centers tend to prefer methotrexate [48]. Antileprosy drugs such as dapsone and clofazime have been shown to be beneficial [49, 50].

Preventive dental care is an important issue. Patients have a tendency to consume a diet that promotes dental decay because of impaired taste. The use of chlorhexidine mouthwashes will help to contain periodontal disease and infection. Local treatment of mucous membrane ulcers with hydrogen peroxide gargle, buttermilk gargle, or steroid-impregnated gel may be beneficial. Intralesional injection of corticosteroids may be an option [51]. Suspected infections should be treated with antiviral, antifungal, or antibacterial agents after a swab has been taken for culture and microbial sensitivities.

8. Alopecia

Alopecia is an often less specific cutaneous feature of SLE, occurring in about 45 percent of people with lupus at some time during the course of the disease. It often affects the temporal regions or creates a patchy pattern of hair loss. Most frequently, the hair loss occurs at the onset of the illness and may be one of the first symptoms of the disease. When the disease is under control, the hair should grow back. Sometimes there is a rash in the scalp, usually subacute or chronic discoid that interferes with the hair follicle. In this situation, the patient is left with a permanent area of cicatricial alopecia.

The bulge area involvement of the follicles by the inflammation that characterized chronic CLE supports the possibility that damage to the stem cells, which reside on the bulge region, may be one triggering factor to permanent follicles loss. Therefore, the pathogenesis of scarring process in CLE may be explained based on follicular stem cells. Cytokeratin 15 (CK15), a marker of stem cells, has been used to show the bulge region involvement in the scarring process in primary cicatricial alopecia and DLE [52]. Drugs used to treat lupus, such as prednisone and immunosuppressive therapies, also may be responsible of reversible hair loss.

9. Subacute Cutaneous Lupus Erythematosus

Subacute cutaneous lupus erythematosus (SCLE) is a photosensitive, nonscarring, nonindurated form of lupus erythematosus. SCLE lesions are related with immunogenetic background that includes the production of Ro/SS-A autoantibodies. Patients who have SCLE skin lesions represent a distinctive subset of LE that has a good prognosis with respect to life-threatening systemic manifestations of LE.

SCLE skin lesions often initiate as a papular eruption or a small plaque with a slight scaling and may simulate polymorphous light eruption (Figure 5). The enlargement and fusion of these lesions can form either plaques with scaling, in the papulosquamous variant, which may simulate psoriasis or lichen planus, or annular and/or polycyclic

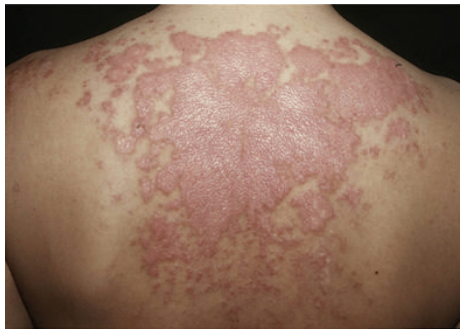


Figure 5: Subacute cutaneous lupus erythematosus.

lesions, in the annular variant, that may mimic erythema annulare centrifugum.

In addition to papulosquamous variant and annular variant there are unusual variants of SCLE, as tumid lupus erythematosus (TLE) characterized by a cutaneous deeper involvement where little or no scaling is seen. Subacute classification of TLE is controversial, and some authorities have proposed that this variant is better classified as chronic cutaneous lupus erythematosus.

Rowell syndrome, a variant including erythema multiforme-like lesions in association with DLE and chilblains may exist, but it is not sure that this is a distinct entity.

Sun exposure can induce an exacerbation of the disease, and some patients report worsening each spring and summer. Most patients with SCLE are asymptomatic but mild pruritus could happen in some of those, especially when the lesions occur on the lower extremities.

The etiopathogenesis of SCLE skin lesions is thought to result from different stages such as decline of tolerance/induction of autoimmunity (ultraviolet light, photosensitizing drugs/chemicals, cigarette smoking, infection, psychological stress); susceptibility genetic patrimony (HLA 8.1 ancestral haplotype (C2, C4 deficiency, TNF-α-308A polymorphism), C1q deficiency); increasing/maturation of autoimmune responses (high levels of autoantibodies (Ro/SS-A), immune complexes, autoreactive T cells); tissue injury/complaint induction resulting from various autoimmune effector mechanisms (e.g., direct T-cell-mediated cytotoxicity, antibody-dependent cell-mediated cytotoxicity) [53].

The aim of treatment in subacute cutaneous lupus erythematosus (SCLE) is to improve the patient's appearance and prevent the development of additional lesions.

Besides sun-protective measures, therapy includes corticosteroids (topical, intralesional) and antimalarials. Treatment with single-agent or combination with aminoquinoline antimalarial will suffice for 75% of SCLE patients. In particular clinical cases, the remaining 25%, have been treated with other pharmacologic forms, as antiinflammatory or systemic immunosuppressive-immunomodulatory therapies, which includes auranofin, dapsone, thalidomide, retinoids, interferon, and immunosuppressive agents [53–56].

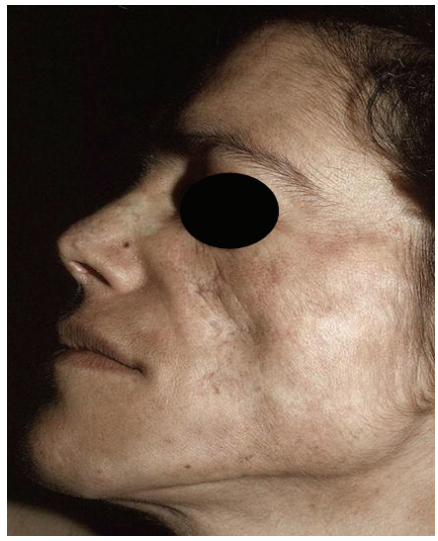


Figure 6: Lupus profundus.

10. Lupus Profundus or Lupus Erythematosus Panniculitis

Lupus profundus (LP) is a form of cutaneous lupus erythematosus, which may be the unique manifestation or appear before or after the clinical onset of SLE. Lupus profundus consists of deep brawny indurations or subcutaneous nodules occur under normal or, less often, involved skin; the overlying skin may be erythematous, atrophic, ulcerated, and, on healing, may leave a depressed scar (Figure 6). The most common sites of involvement are proximal extremities, particularly the lateral aspects of the arms and shoulders, thighs, buttocks, trunk, breast, face, and scalp. It can be associated with DLE or SLE. The frequency of occurrence of LP in SLE has been reported to be 2%. The etiology is uncertain. Cytokines and circulating immune complexes may enhance inflammation and hypodermal necrosis observed in LP. Histologically, lymphocytic lobular panniculitis and a characteristic hyaline sclerosis of the adipose tissue are defined.

The most common type of treatment is nonsteroidal anti-inflammatory drugs, or NSAIDS. As an option, antimalarial, drugs, adrenal corticosteroids, and immunosuppressive drugs can be used for treatment as well as chemotherapy drugs for the most severe cases.

11. Lichen Planus in LE

LE and lichen planus are usually seen as individual entities. Their overlap comprises patients who have clinical, histological, and/or immunopathological characteristics of both diseases simultaneously. The clinical presentation is a pruritic papular eruption characterized by its violaceous color polygonal shape and, sometimes, fine scale. It is most commonly found on the flexor surfaces of the upper extremities, on the genitalia, and on the mucous membranes. Pruritus is common in lichen planus but varies in severity depending

on the type of lesion and the extent of involvement. Hypertrophic lesions are extremely pruritic while oral lesions may be asymptomatic or have a burning sensation, or they may even be painful if erosions are present. Large, annular, hypertrophic lesions and mucous membrane involvement are more likely to become chronic.

Pathophysiologically, lichen planus is thought to be an immunologically mediated disorder.

It has been suggested that CD8+ cytotoxic T cells recognize an unknown antigen associated with the major histocompatibility complex (MHC) class I on lesional keratinocytes and lyse them [57]. T cells and keratinocytes express interferon-γ (IFN-γ) and interleukin-6 (IL-6) [58], and T cells also express lymphocyte function-associated antigen-1 (LFA-1).

Mononuclear cells infiltrating the skin, the majority of which are CD8+, as well as basal keratinocytes, express tumour necrosis factor-α (TNF-α) and TNF-R1 [59]. Activated T cells secreting IFN-γ induce keratinocyte expression of human leukocyte antigen (HLA)-DR [60], and the presence of epidermotropic T cells correlates with that of HLA-DR-expressing keratinocytes and Langerhans' cells.

The role of chemokines in the pathophysiology of lichenoid tissue reactions regards of recruitment and local activation of cytotoxic Th1 cells and plasmacytoid dendritic cells. Infiltrating CD8+ T cells, as well as keratinocytes, express a variety of different chemokines [61–63]. RANTES (regulated upon activation, normal T cell expressed and secreted) secreted by T cells may trigger mast cell degranulation with consequent release of TNF-α, which in turn up-regulates lesional T cell RANTES secretion; such mechanisms may contribute to chronicity of T-cell infiltration and clinical disease [57].

The first-line treatments of cutaneous lichen planus are topical steroids and a second choice would be systemic steroids for symptom control, which leads to a faster resolution. Oral acitretin has been shown to be effective [64]. Many other treatments are used, including mycophenolate mofetil, which efficacy is uncertain, and sulfasalazine in patients with generalized lichen planus [65].

Other cutaneous manifestations related, but not specific, to SLE, include the following:

(i) Raynaud's phenomenon;

(ii) cutaneous vasculitis;

(iii) periungual telangiectasias;

(iv) urticarial vasculitis;

(v) livedo reticularis;

(vi) atrophie blanche;

(vii) bullous lesions.

The nonspecific skin lesions, mainly found in active phase of SLE, are characteristic of cutaneous lupus but can also be included on the clinical picture of another disease and it is not possible to establish a histopathological distinction between them [66].

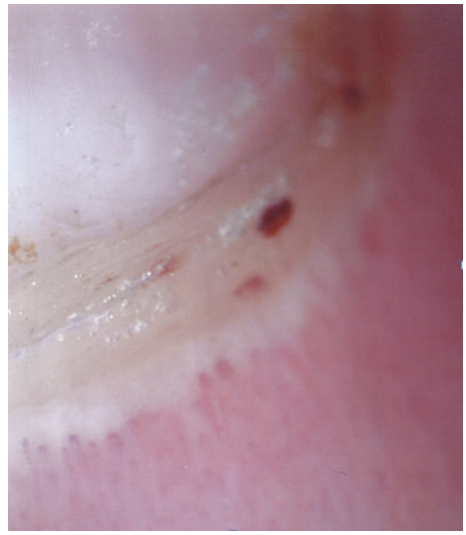


Figure 7: Capillaroscopy.

12. Raynaud's Syndrome

The Raynaud's syndrome (RS) is an exaggerated vascular response to cold temperature or emotional stress, secondary to identified diseases. This vascular lability is manifested clinically by sharply demarcated color changes in the skin of the digits. Abnormal vasoconstriction of digital arteries and cutaneous arterioles, due to local vascular responses, is thought to be the basis of this disorder [67]. These defective events are reversible, contrary to irreversible causes of ischemia such as vasculitis or thrombosis. It most commonly affects the digits of the fingers but may affect the toes, nose, ears, or even the tongue. Raynaud's phenomenon may be observed with blue, white, and red color change at the distal digital tips. Capillaroscopy can be performed with an ophthalmoscope to search for dilated capillary nailfold loops, giant capillaries, and microhemorrhages (Figure 7).

Management of Raynaud's syndrome involves protecting the fingers and the toes from cold, trauma, and infection.

Unfortunately, patients with autoimmune disorders such as SLE and associated Raynaud's phenomenon do not usually respond well to therapy.

Pharmacologic therapy includes calcium channel blockers, prostacyclin analogues, and pentoxifylline. Key areas of ongoing research include a topical nitroglycerin and a Rho-kinase inhibitor (vasodilator) [68].

13. Cutaneous Vasculitis

Cutaneous vasculitis is presented in a multivariety of morphological lesions such as punctuate lesions, palpable purpura, urticaria, ulcers, papules, erythematosus plaques or macules, and erythema with necrosis that may be self-limiting or relapsing (Figure 8). Cutaneous lesions may be the sole manifestation of the vasculitis or may be part of a systemic involvement.

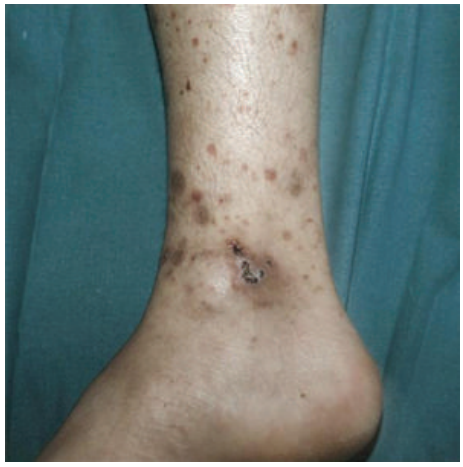


Figure 8: Vasculitis.

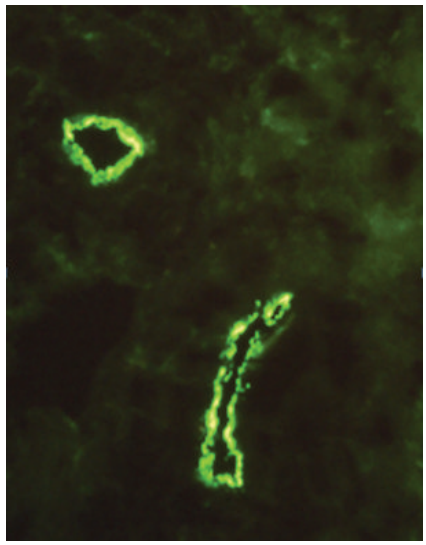


Figure 9: Immune reactant deposits in vessel.

The most common form of vasculitis seen in LE is a small vessel vasculitis, mediated via circulating immune complexes or by the direct effects of antibodies to cell surface components. Immune complexes are formed in the microvasculature, leading to complement activation and inflammation. Antibody-antigen complexes deposit on the basement membranes of skin. In active SLE, this process has been confirmed by demonstration of complexes of nuclear antigens such as DNA, immunoglobulins, and complement proteins in the skin.

Occasionally, deposition of immune reactants in dermal vessels can be observed, corresponding to vascular involvement (Figure 9). The relative percentages of the different immunoglobulins vary according to authors.

SLE vasculitis is frequently treated with antimalarials, but its discontinuation may result in an SLE flare even in remitted patients. A combination of drugs, plasmapheresis, and intravenous immunoglobulin, along with high-dose steroids and cytotoxicagents, are employed in the treatment of severe SLE vasculitis. Recent data suggests that patients with SLE vasculitis may benefit from a number of autoimmune disease therapies such as switching cytokine responses from Th1 to Th2, and the manipulation of toll-like receptors, chemokines, and FcR receptors. Specific B-cell therapies (e.g., anti-Blys, B-cell depletion) may also emerge as potential treatments for SLE vasculitis.

Responsiveness of cutaneous lupus to rituximab is complex. Discoid lesions do not respond. Acute non-discoid LE and vasculitis in patients with active systemic disease initially improved along with other manifestations. However, some patients switched to a disseminated discoid pattern following B-cell repopulation. This may be explained by the expansion of a T-cell population during B-cell depletion that becomes activated during repopulation. Alternatively transient and incomplete B-cell depletion may alter the pathological B-cell repertoire. The role of B-cells may vary between different patterns of skin disease in SLE and rituximab may not be the most appropriate therapy for all patients. Careful monitoring of the skin is needed when using rituximab in SLE [69].

The last studies about therapy are focused on B-cell targets, T-cell downregulation and costimulatory blockade, cytokine inhibition, and the modulation of complement. Several biological agents have been developed, although with several disappointments in trials [70]. Belimumab has been the only one to be approved in March 2011 by the US Food and Drug Administration (FDA) for the treatment of adult patients with active autoantibody-positive systemic lupus erythematosus who are receiving standard therapy (corticosteroids, antimalarials, immunosuppressives, and NSAIDs) [71]. Other biological therapies proposed for SLE treatment, but not all approved, are, as B-cell targets, Rituximab and Belimumab showing reduction in disease activity; as T-cell targets, Efalizumab (reduces cutaneous SLE manifestations) and Sirolimus (for refractory SLE); as cytokine inhibitors, Infliximab (for lupus nephritis) [70].

14. Urticarial Vasculitis

Urticarial vasculitis is an eruption of erythematous wheals that clinically resemble urticarial but histologically shows changes of leukocytoclastic vasculitis. Inversely to urticaria it is usually painful or nonpruritic and typically persists for more than 24 hours. It usually resolves with hyperpigmentation or purpura.

Urticarial vasculitis is a type III hypersensitivity reaction in which antigen-antibody complexes are deposited in the vascular lumina. This reaction activates complement and induces neutrophils chemotaxis. Once activated, neutrophils release proteolytic enzymes, such as collagenase and elastase, damaging the vascular wall. Eosinophils may be involved in the early stages of the vasculitic lesions. Patients with hypocomplementemic urticarial vasculitis are more likely to show autoantibodies to C1q and vascular endothelial cells.

Patients with urticarial vasculitis can be subdivided into two groups, those with normal complement levels and those coursing with hypocomplementemia [72, 73]. The last one

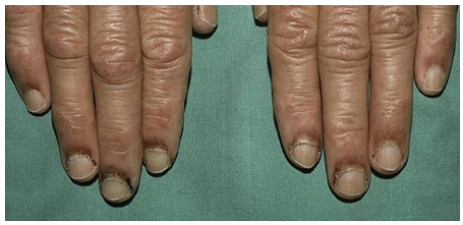


Figure 10: Periungual telangiectasia.

is more likely to exhibit systemic manifestations, including constitutional symptoms (fever, malaise, and fatigue), arthralgia, arthritis, serositis, glomerulonephritis, interstitial nephritis, and Raynaud's phenomenon. Angioedema-like lesions are present in 40% of patients, frequently involving the lips, tongue, periorbital tissue and hands [73]. Some patients may present conjunctivitis and episcleritis [74].

Antibodies against C1q are diagnostic markers for hypocomplementary urticarial vasculitis [72–74]. Anti-C1q antibodies were detected in 30% of patients with SLE and 80% of SLE patients with glomerulonephritis [75]. Intravenous methylprednisolone and cyclophosphamide or high-dose oral corticosteroids, colchicine, dapsone, hydroxychloroquine, and low-dose methotrexate have been reported to be effective treatments [76].

15. Periungual Telangiectasias

Dilated capillaries of the nailfolds have been found in LE patients (Figure 10). It is better detected by capillaroscopy. Nailfold telangiectasias in SLE patients were associated with anti-U1RNP antibodies [77]. In addition, telangiectasias and erythema of the nailfold were found in 76% of patients who hadboth DLE and SLE, but none in patients with DLE in the absence of SLE, suggesting that this is a rather sensitive indicator for systemic disease activity [78]. Dilated capillary loops dropout are the hallmarks of "scleroderma-pattern" capillaroscopic changes, however, when seen in SLE patients, this pattern of nailfold appears to correlate strongly with Raynaud's phenomenon.

16. Livedo Reticularis

Livedo reticularis is a common cutaneous reaction consisting of a mottled reticulated vascular pattern that appears like a lace-like purplish violaceous discoloration frequently on the lower extremities (Figure 11) [79]. The discoloration is caused by swelling of the medium veins in skin which makes them more visible. It can be caused by any condition that makes venules swell. The condition may be normal or may be related to severe underlying pathology. It may be aggravated by exposure to cold.

The diagnosis in a patient with livedo reticularis requires a search for associated subcutaneous nodules, retiform purpura, necrosis, and secondary ulceration.

A detailed history can provide valuable information concerning associated diseases such as LES.

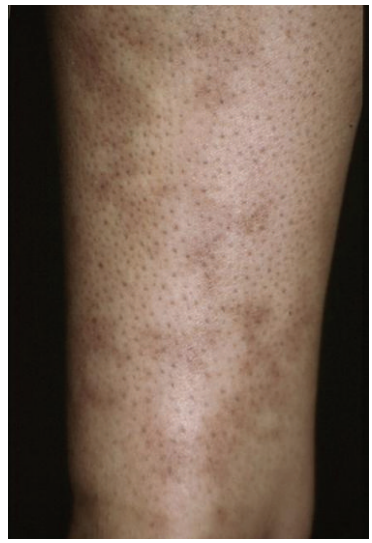


Figure 11: Livedo reticularis.

Treatment options should be carefully assessed and individualized to each case. Livedo reticularis associated with systemic vasculitis should be treated with corticosteroids and immunosuppressants; doses and combinations will vary according to the clinical condition of the patient and the extent of organ involvement. Serious organ dysfunction requires the use of corticosteroids and cyclophosphamide pulse therapy, and combining low doses of corticosteroids with methotrexate or azathioprine is a good option for maintenance treatment [80].

17. Atrophie Blanche

Atrophie blanche is a particular type of scar arising on the lower leg that occurs after a skin injury when the blood supply is poor. One can classify atrophie blanche into primary and secondary types. In the latter such as LES [81].

The clinical presentation is painful petechial, purpuric papules, or hemorrhagic bullae. This last one become, necrotic and forms ulcers, which in turn become atrophic angular scars with hyperpigmentation of the surrounding skin usually on the lower extremities [82, 83].

Atrophie blanche can be the result of circulating immune complexes that are deposited into vessel wall resulting in activation of complement fractions, chemoattraction of neutrophils and fibrin deposition. The release of lysosomal enzymes and reactive oxygen species subsequently lead to secondary vascular damage and inflammatory tissue destruction [84]. Atrophie blanche can also be induced by coagulopathy and this is supported by the fact that fibrins deposit within vessels is the earliest pathogenic change [84, 85].

Systemic corticosteroids, which may be used for recalcitrant cutaneous vasculitis, are ineffective in atrophie blanche. As a matter of fact, prolonged use of corticosteroids in atrophie blanche may result in significant adverse events such as osteoporosis, Cushing's syndrome, hypertension, and

glucose intolerance [81]. The most popular regimens include low dose aspirin and dipyridamole which are generally well tolerated and have minimal side-effects [86]. Alternatively pentoxifylline may be used as a rheologic drug consequently improving blood flow [87]. Minidose heparin (SC heparin 5,000 U 12 hourly) has also been reported to be effective in some cases of Atrophie Blanche [88].

18. Bullous Systemic Lupus Erythematosus

Bullous systemic lupus erythematosus (BSLE) is an autoantibody-mediated subepidermal blistering disease that occurs in patients with SLE. Blisters and vesicules may arise on erythematous or normal skin and are nonscarring. Lesions occur on sun-exposed or flexural skin. Blistering often parallels flares of SLE involving other organ systems, in particular the kidney. Camisa and Sharma proposed criteria for this distinct subset of vesiculobullous skin lesions occurring in patients with SLE [89]: a diagnosis of SLE based on American College of Rheumatology criteria [90]; vesicles and bullae arising upon but not limited to sun-exposed skin [91]; histopathology compatible with dermatitis herpetiformis [92]; negative indirect immunofluorescence (IDIF) for circulating basement membrane zone antibodies [93]; direct immunofluorescence (DIF) positive for IgG and/or IgM and often IgA at the basement membrane zone. Others have suggested this classification to be revised because of the heterogeneity of clinical and immunohistological presentation [94]. BSLE can be defined as an acquired subepidermal blistering disease in a patient with SLE, in which immune reactants are present at the basement membrane zone on direct, or indirect, immunofluorescence. Direct immunofluorescence microscopy demonstrates immunoglobulin G (with or without immunoglobulin A and immunoglobulin M) deposits at the basement membrane zone (BMZ). Evidence of antibodies to type VII collagen via DIF or IDIF on salt-split skin, immunoblotting, immunoprecipitation, ELISA, or immunoelectron microscopy can be demonstrated.

In patients with BSLE, antibodies directed at the BMZ likely mediate the blistering phenotype by directly interfering with adhesive connections at the dermoepidermal junction and through induction of complement-dependent inflammation that leads to tissue injury and dermoepidermal separation. Proteolytic damage caused by recruited neutrophils contributes to the latter process.

In type 1 BSLE (which accounts for most cases), antibodies against type VII collagen may weaken or block anchoring fibril-mediated connections between the lamina densa of the basement membrane and the papillary dermis. In both EBA and BSLE, antigenic epitopes reside within the NC1 and NC2 domains of type VII collagen, which are localized to the lamina densa and the underlying dermis, respectively. Antibodies recognizing bullous pemphigoid antigen 1, laminin-5, and laminin-6 have also been described in patients with BSLE.

Certain individuals may have a genetic predisposition to develop autoimmunity to BMZ antigens and to SLE. For example, BSLE and SLE are associated with an increased prevalence of the HLA class II DR2 haplotype. The antigen-presenting protein encoded by the DR2-associated DRB1*1501 allele (found in BSLE patients) has been postulated to be involved in presenting type VII collagen epitopes to T lymphocytes.

BSLE occurs in the setting of SLE; thus, ANA test results generally are positive. Anti-dsDNA, anti-Sm, anti-Ro/SS-A, anti-La/SS-B, and anticardiolipin antibodies may also be detected. Other laboratory abnormalities related to SLE can include low levels of complement (i.e., C3, C4, CH50), anemia, leukopenia, thrombocytopenia, proteinuria or cellular casts upon urinalysis, and an elevated erythrocyte sedimentation rate.

All 5 criteria are used to classify type 1 BSLE, whereas only the first 4 criteria are used for type 2 (undetermined location of antigen or dermal antigen other than type VII collagen) and type 3 (epidermal antigen) BSLE [94].

Dapsone is the initial treatment of choice for BSLE. The response is usually dramatic, with cessation of new blister formation within 1-2 days and rapid healing of existing lesions. Low doses (25–50 mg/day) are often effective, although a higher dosage is sometimes required. Rapid recurrences may occur upon withdrawal of dapsone, with prompt remission after reinstitution of therapy [95]. However, discontinuance of dapsone therapy is usually possible within a year. Prednisone may be effective in patients intolerant to dapsone, have a poor response to dapsone, or require treatment of concurrent systemic manifestations of SLE. Combination therapy with prednisone and dapsone can also be beneficial. Methotrexate, azathioprine, and mycophenolate mofetil represent additional therapeutic options.

Not all blistering eruptions that occur in patients with lupus erythematosus (LE) represent BSLE. Such patients may present with a severe form of acute or subacute cutaneous LE (SCLE) that resembles erythema multiforme (Rowell syndrome) or toxic epidermal necrolysis (TEN).

The eruptions can develop rapidly or evolve over several weeks. In toxic epidermal necrolysis-like acute cutaneous LE, photodistributed diffuse or patchy erythema evolves (usually rapidly) into flaccid bullae (positive Nikolsky sign, unlike BSLE) and widespread sheet-like full-thickness epidermal detachment [94].

The term acute syndrome of apoptotic pan-epidermolysis (ASAP) has been proposed for the TEN-like cutaneous injury pattern that can occur in settings of LE, acute graft versus host disease, pseudoporphyria, and the classic drug-hypersensitivity syndrome. Fas-Fas ligand interactions have been implicated in the massive keratinocyte apoptosis that characterizes ASAP. TEN-like cutaneous LE must be differentiated from drug-induced TEN occurring in a patient with LE. Patients with TEN-like acute cutaneous LE often have significant systemic disease activity (e.g., lupus nephritis, or cerebritis). Extensive eruptions of TEN-like LE require prompt institution of therapy with intravenous immunoglobulin and/or systemic corticosteroids. Less fulminant manifestations of erythema multiforme—like LE can be treated with antimalarials, corticosteroids

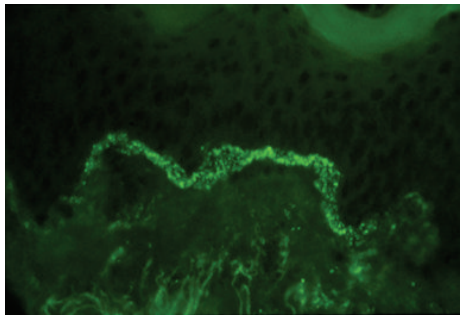


Figure 12: Lupus band test.

(topical or systemic) and other agents in the therapeutic armamentarium for LE [96].

19. Lupus Band Test

The deposition of immunoglobulin and/or complements at the dermoepidermal junction is a histological feature of LE. Examination of tissue may be done on lesional skin or on nonlesional skin. Nonlesional skin biopsies may be performed on sun-exposed or nonexposed areas. Testing of nonlesional, nonexposed skin is termed the lupus band test.

By immunohistology, approximately 70% of patients with various subtypes of LE show a positive lupus band test when skin biopsies are performed in normal appearing skin. The normal appearing skin of patients carrying the diagnosis of chronic cutaneous LE, are almost always negative for lupus band test; however, when performed in the cutaneous lesions, lupus band test is positive in about 80% of patients. The fluorescent pattern of dermoepidermal skin deposits of complement or immunoglobulins are either in granules, either in thick band (Figure 12). In some SLE cases, *in situ* ANA deposits were observed. This pattern was first detected by Gilliam in patients with Sharp mixed connective-tissue disease but latter was shown to be not exclusive of the Sharp syndrome, but is also detected in some SLE. It corresponds to an *in situ* epidermis nuclear deposition of the circulating Anti-SSA of those patients.

20. Discussion

The American College of Rheumatology (ACR) established 11 criteria in 1982, which were revised in 1997 as a classificatory instrument to operationalise the definition of SLE in clinical trials. They were not intended to be used to diagnose individuals and do not do well in that capacity.

The term "photosensitivity" defined as a rash resulting of an unusual reaction to sunlight by patient history or physician observation is poorly defined, although it is listed as one of the ACR criteria for the classification of SLE. This is an extremely broad definition that can be fulfilled by a variety of other conditions, such as polymorphous light eruption, photoallergic contact dermatitis, and dermatomyositis. In addition, a high disagreement between patient history of photosensitivity and a decreased minimal erythema dose was documented [97]. Concluding that the use of photosensitivity as a classification criterion for SLE remains questionable, at least when it is assessed by patient or physician history according to the ACR criteria. Moreover, the "malar rash," a further ACR criterion used for the classification of SLE, is often indistinguishable from photosensitivity and, therefore, the two criteria are not completely independent [98].

Up to 73% of patients with systemic LE report photosensitivity, although this correlates poorly with results of phototesting using standardized protocols. Repeated single-patient observations indicate that sunlight may precipitate systemic disease *de novo* or aggravate existing disease. Variation in disease activity related to sun exposure using objective variables has not been shown in large cohort studies; however, two recent studies show that although cutaneous manifestations are more common in the summer months, systemic disease activity is increased in the 3–6 months following maximal potential sun exposure. These observations suggest that summer UV light exposure may lead to flares, after a latency period of several months.

In addition, only 50% of patients with CLE are aware of an adverse effect of sunlight on their disease and, therefore, a negative history of photosensitivity does not necessarily exclude any effect of sun exposure on their disease.

Phototesting with a standardized protocol for UVA and UVB irradiation is an optimal way to evaluate photosensitivity in patients with CLE, confirming that abnormal reactivity to sunlight is an important factor in the pathogenesis of the disease.

Standardized photoprovocation tests with artificial UVA and UVB irradiation are an alternative way to evaluate photosensitivity in patients with CLE demonstrating some differences regarding the various subtypes. However, UV exposure by artificial light sources can trigger systemic organ manifestations [99], therefore, photoprovocation tests should not be performed in all patients with SLE. In the past years, phototesting has been crucial in further characterizing the highly photosensitive subtype intermittent systemic lupus erythematous (ICLE) and has also been shown to be very helpful for the education of patients on photoprotection measures [19]. Therefore, consequent protection against UV light as well as other physical and mechanical injuries are of significant value for the course and prognosis of this disease.

The frequently seen erythematous papules on the dorsal aspects of the hands, typically sparing the knuckles, are completely opposed to the one observed in dermatomyositis.

A revision of ACR criteria is desired, in order to include other dermatologic signs and symptoms, like hand papules sparing the Knuckles. Recently, new classification criteria have been validated. The Systemic Lupus International Collaborating Clinics (SLICC) is an international group, which dedicates their work to the clinical research of SLE. A large set of patient scenarios rated by experts was experimented successfully for the new SLICC classification criteria. They require the presence, at least, of one clinical criterion (nonscarring alopecia is included) and one immunologic criterion for a classification of SLE. Despite that, the diagnostic of nephritis compatible with lupus by biopsy (in the presence of SLE antibodies) is enough for classification.

SLICC classification criteria have more sensitivity, but not specificity, than the revised ACR criteria. ACR and SLICC have, statistically, a similar performance. Patients without antibodies or low complement, the hallmark of SLE, cannot be classified as having SLE. The SLICC classification criteria are an alternative for SLE clinical care and research [100].

The lupus band test as an immunologic test for Lupus patients can also be a helpful instrument for differential diagnosis between DLE and SLE.

References

[1] M. C. Hochberg, "Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 40, no. 9, article 1725, 1997.

[2] R. Klein, S. Moghadam-Kia, J. LoMonico et al., "Development of the CLASI as a tool to measure disease severity and responsiveness to therapy in cutaneous lupus erythematosus," *Archives of Dermatology*, vol. 147, no. 2, pp. 203–208, 2011.

[3] E. M. Tan, A. S. Cohen, and J. F. Fries, "The 1982 revised criteria for the classification of systemic lupus erythrematosus," *Arthritis and Rheumatism*, vol. 25, no. 11, pp. 1271–1277, 1982.

[4] L. C. Parish, R. J. Kennedy, and J. Hurley, "Palmar lesions in lupus erythematosus," *Archives of Dermatology*, vol. 96, no. 3, pp. 273–276, 1967.

[5] C. R. Spann, J. P. Callen, J. B. Klein, and K. B. Kulick, "Clinical, serologic and immunogenetic studies in patients with chronic cutaneous (discoid) lupus erythematosus who have verrucous and/or hypertrophic skin lesions," *Journal of Rheumatology*, vol. 15, no. 2, pp. 256–261, 1988.

[6] P. B. Martens, K. G. Moder, and I. Ahmed, "Lupus panniculitis: clinical perspectives from a case series," *Journal of Rheumatology*, vol. 26, no. 1, pp. 68–72, 1999.

[7] A. Kuhn, M. Sticherling, and G. Bonsmann, "Clinical manifestations of cutaneous lupus erythematosus," *JDDG*, vol. 5, no. 12, pp. 1124–1137, 2007.

[8] S. Panjwani, "Early diagnosis and treatment of discoid lupus erythematosus," *JABFM*, vol. 22, no. 2, pp. 206–213, 2009.

[9] J. Albrecht and V. P. Werth, "Development of the CLASI as an outcome instrument for cutaneous lupus erythematosus," *Dermatologic Therapy*, vol. 20, no. 2, pp. 93–101, 2007.

[10] A. Y. Finlay, "Quality of life measurement in dermatology: a practical guide," *British Journal of Dermatology*, vol. 136, no. 3, pp. 305–314, 1997.

[11] S. D. Prystowsky and J. N. Gilliam, "Discoid lupus erythematosus as part of a larger disease spectrum. Correlation of clinical features with laboratory findings in lupus erythematosus," *Archives of Dermatology*, vol. 111, no. 11, pp. 1448–1452, 1975.

[12] A. Kuhn, M. Herrmann, S. Kleber et al., "Accumulation of apoptotic cells in the epidermis of patients with cutaneous lupus erythematosus after ultraviolet irradiation," *Arthritis and Rheumatism*, vol. 54, no. 3, pp. 939–950, 2006.

[13] I. Baumann, W. Kolowos, R. E. Voll et al., "Impaired uptake of apoptotic cells into tingible body macrophages in germinal centers of patients with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 46, no. 1, pp. 191–201, 2002.

[14] U. S. Gaipl, A. Kuhn, A. Sheriff et al., "Clearance of apoptotic cells in human SLE," *Current Directions in Autoimmunity*, vol. 9, pp. 173–187, 2006.

[15] D. A. Abdulahad, J. Westra, P. C. Limburg, C. G. M. Kallenberg, and M. Bijl, "HMGB1 in systemic lupus Erythematosus: its role in cutaneous lesions development," *Autoimmunity Reviews*, vol. 9, no. 10, pp. 661–665, 2010.

[16] C. V. Suschek, V. Krischel, D. Bruch-Gerharz et al., "Nitric oxide fully protects against UVA-induced apoptosis in tight correlation with Bcl-2 up-regulation," *The Journal of Biological Chemistry*, vol. 274, no. 10, pp. 6130–6137, 1999.

[17] A. Kuhn, K. Fehsel, P. Lehmann, J. Krutmann, T. Ruzicka, and V. Kolb-Bachofen, "Aberrant timing in epidermal expression of inducible nitric oxide synthase after UV irradiation in cutaneous lupus erythematosus," *Journal of Investigative Dermatology*, vol. 111, no. 1, pp. 149–153, 1998.

[18] H. M. Belmont, D. Levartovsky, A. Goel et al., "Increased nitric oxide production accompanied by the up-regulation of inducible nitric oxide synthase in vascular endothelium from patients with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 40, no. 10, pp. 1810–1816, 1997.

[19] A. Kuhn, P. H. Krammer, and V. Kolb-Bachofen, "Pathophysiology of cutaneous lupus erythematosus—novel aspects," *Rheumatology*, vol. 45, supplement 3, pp. iii14–iii16, 2006.

[20] J. Wenzel and T. Tüting, "Identification of type I interferon-associated inflammation in the pathogenesis of cutaneous lupus erythematosus opens up options for novel therapeutic approaches," *Experimental Dermatology*, vol. 16, no. 5, pp. 454–463, 2007.

[21] J. Wenzel, M. Uerlich, E. Wörrenkämper, S. Freutel, T. Bieber, and T. Tüting, "Scarring skin lesions of discoid lupus erythematosus are characterized by high numbers of skin-homing cytotoxic lymphocytes associated with strong expression of the type I interferon-induced protein MxA," *British Journal of Dermatology*, vol. 153, no. 5, pp. 1011–1015, 2005.

[22] T. Uehara, X. X. Peng, B. Bennett et al., "c-Jun N-terminal kinase mediates hepatic injury after rat liver transplantation," *Transplantation*, vol. 78, no. 3, pp. 324–332, 2004.

[23] T. Uehara, B. Bennett, S. T. Sakata et al., "JNK mediates hepatic ischemia reperfusion injury," *Journal of Hepatology*, vol. 42, no. 6, pp. 850–859, 2005.

[24] R. Caricchio, L. McPhie, and P. L. Cohen, "Ultraviolet B radiation-induced cell death: critical role of ultraviolet dose in inflammation and lupus autoantigen redistribution," *Journal of Immunology*, vol. 171, no. 11, pp. 5778–5786, 2003.

[25] R. Korhonen, K. Linker, A. Pautz, U. Förstermann, E. Moilanen, and H. Kleinert, "Post-transcriptional regulation of human inducible nitric-oxide synthase expression by the Jun N-terminal kinase," *Molecular Pharmacology*, vol. 71, no. 5, pp. 1427–1434, 2007.

[26] S. Sakaguchi, "Naturally arising Foxp3-expressing $CD25^+$ $CD4^+$ regulatory T cells in immunological tolerance to self and non-self," *Nature Immunology*, vol. 6, no. 4, pp. 345–352, 2005.

[27] J. C. Crispin, A. Martínez, and J. Alcocer-Varela, "Quantification of regulatory T cells in patients with systemic lupus erythematosus," *Journal of Autoimmunity*, vol. 21, no. 3, pp. 273–276, 2003.

[28] M. Miyara, Z. Amoura, C. Parizot et al., "Global natural regulatory T cell depletion in active systemic lupus erythematosus," *Journal of Immunology*, vol. 175, no. 12, pp. 8392–8400, 2005.

[29] S. Meller, F. Winterberg, M. Gilliet et al., "Ultraviolet radiation-induced injury, chemokines, and leukocyte recruitment:

an amplification cycle triggering cutaneous lupus erythematosus," *Arthritis and Rheumatism*, vol. 52, no. 5, pp. 1504–1516, 2005.

[30] J. Wenzel, S. Henze, E. Wörenkämper et al., "Role of the chemokine receptor CCR4 and its ligand thymus- and activation-regulated chemokine/CCL17 for lymphocyte recruitment in cutaneous lupus erythematosus," *Journal of Investigative Dermatology*, vol. 124, no. 6, pp. 1241–1248, 2005.

[31] F. Furukawa and T. Yoshimasu, "Animal models of spontaneous and drug-induced cutaneous lupus erythematosus," *Autoimmunity Reviews*, vol. 4, no. 6, pp. 345–350, 2005.

[32] G. J. Pons-Estel, G. S. Alarcón, L. A. González et al., "Possible protective effect of hydroxychloroquine on delaying the occurrence of integument damage in lupus: LXXI, data from a multiethnic cohort," *Arthritis Care and Research*, vol. 62, no. 3, pp. 393–400, 2010.

[33] N. Ezra and J. Jorizzo, "Hydroxychloroquine and smoking in patients with cutaneous lupus erythematosus," *Clinical and Experimental Dermatology*, vol. 37, no. 4, pp. 327–334, 2012.

[34] J. Dutz and V. P. Werth, "Cigarette smoking and response to antimalarials in cutaneous lupus erythematosus patients: evolution of a dogma," *Journal of Investigative Dermatology*, vol. 131, no. 10, pp. 1968–1970, 2011.

[35] J. P. Callen, "Update on the management of cutaneous lupus erythematosus," *British Journal of Dermatology*, vol. 151, no. 4, pp. 731–736, 2004.

[36] R. Jonsson, G. Heyden, N. G. Westberg, and G. Nyberg, "Oral mucosal lesions in systemic lupus erythematosus—a clinical, histopathological and immunopathological study," *Journal of Rheumatology*, vol. 11, no. 1, pp. 38–42, 1984.

[37] J. D. Urman, M. B. Lowenstein, M. Abeles, and A. Weinstein, "Oral mucosal ulceration in systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 21, no. 1, pp. 58–61, 1978.

[38] M. Schiodt, "Oral discoid lupus erythematosus. III. A histopathologic study of sixty-six patients," *Oral Surgery Oral Medicine and Oral Pathology*, vol. 57, no. 3, pp. 281–293, 1984.

[39] E. L. Dubois and D. L. Tuffanelli, "Clinical manifestations of systemic lupus erythematosus. Computer analysis of 520 cases," *JAMA*, vol. 190, pp. 104–111, 1964.

[40] J. F. Fries and H. R. Holman, "Systemic lupus erythematosus: a clinical analysis," *Major Problems in Internal Medicine*, vol. 6, 1975.

[41] A. M. Harvey, L. E. Shulman, P. A. Tumulty, C. L. Conley, and E. H. Schoenrich, "Systemic lupus erythematosus: review of the literature and clinical analysis of 138 cases," *Medicine*, vol. 33, no. 4, pp. 291–437, 1954.

[42] D. Estes and C. L. Christian, "The natural history of systemic lupus erythematosus by prospective analysis," *Medicine*, vol. 50, no. 2, pp. 85–95, 1971.

[43] Z. Al Rawi, H. Al Shaarbaf, E. Al Raheem, and S. J. Khalifa, "Clinical features of early cases of systemic lupus erythematosus in Iraqi patients," *British Journal of Rheumatology*, vol. 22, no. 3, pp. 165–171, 1983.

[44] E. B. Tsianos, A. G. Tzioufas, and M. D. Kita, "Serum isoamylases in patients with autoimmune rheumatic diseases," *Clinical and Experimental Rheumatology*, vol. 2, no. 3, pp. 235–238, 1984.

[45] M. C. Hochberg, R. E. Boyd, and J. M. Ahearn, "Systemic lupus erythematosus: a review of clinico-laboratory features and immunogenetic markers in 150 patients with emphasis on demographic subsets," *Medicine*, vol. 64, no. 5, pp. 285–295, 1985.

[46] M. Pistiner, D. J. Wallace, S. Nessim, A. L. Metzger, and J. R. Klinenberg, "Lupus erythematosus in the 1980s: a survey of 570 patients," *Seminars in Arthritis and Rheumatism*, vol. 21, no. 1, pp. 55–64, 1991.

[47] C. Vitali, W. Bencivelli, D. A. Isenberg et al., "Disease activity in systemic lupus erythematosus: report of the Consensus Study Group of the European Workshop for Rheumatology Research. II. Identification of the variables indicative of disease activity and their use in the development of an acticity score," *Clinical and Experimental Rheumatology*, vol. 10, no. 5, pp. 541–547, 1992.

[48] C. Vitali, A. Doria, A. Tincani et al., "International survey on the management of patients with SLE. I. General data on the participating centers and the results of a questionnaire regarding mucocutaneous involvement," *Clinical and Experimental Rheumatology*, vol. 14, supplement 16, pp. S17–S22, 1996.

[49] J. T. Jakes, E. L. Dubois, and F. P. Quismorio, "Antileprosy drugs and lupus erythematosus," *Annals of Internal Medicine*, vol. 97, no. 5, p. 788, 1982.

[50] R. P. Hall, T. J. Lawley, H. R. Smith, and S. I. Katz, "Bullous eruption of systemic lupus erythematosus. Dramatic response to dapsone therapy," *Annals of Internal Medicine*, vol. 97, no. 2, pp. 165–170, 1982.

[51] A. E. Soto-Rojas, A. R. Villa, J. Sifuentes-Osornio, D. Alarcón-Segovia, and A. Kraus, "Oral manifestations in patients with Sjogren's syndrome," *Journal of Rheumatology*, vol. 25, no. 5, pp. 906–910, 1998.

[52] K. Al-Refu and M. Goodfield, "Hair follicle stem cells in the pathogenesis of the scarring process in cutaneous lupus erythematosus," *Autoimmunity Reviews*, vol. 8, no. 6, pp. 474–477, 2009.

[53] R. D. Sontheimer, "Subacute cutaneous lupus erythematosus: 25-Year evolution of a prototypic subset (subphenotype) of lupus erythematosus defined by characteristic cutaneous, pathological, immunological, and genetic findings," *Autoimmunity Reviews*, vol. 4, no. 5, pp. 253–263, 2005.

[54] T. S. Housman, J. L. Jorizzo, M. A. McCarty, S. E. Grummer, A. B. Fleischer, and P. G. Sutej, "Low-dose thalidomide therapy for refractory cutaneous lesions of lupus erythematosus," *Archives of Dermatology*, vol. 139, no. 1, pp. 50–54, 2003.

[55] M. Sticherling, G. Bonsmann, and A. Kuhn, "Diagnostic approach and treatment of cutaneous lupus erythematosus," *JDDG*, vol. 6, no. 1, pp. 48–60, 2008.

[56] N. Usmani and M. Goodfield, "Efalizumab in the treatment of discoid lupus erythematosus," *Archives of Dermatology*, vol. 143, no. 7, pp. 873–877, 2007.

[57] T. B. S. Burns, N. Cox, and C. Griffiths, *Lichen Planus and Lichenoid Disorders. Rook?s Textbook of Dermatology*, Wiley-Blackwel, Oxford, UK, 8th edition, 2010.

[58] A. Fayyazi, S. Schweyer, A. Soruri et al., "T lymphocytes and altered keratinocytes express interferon-γ and interleukin 6 in lichen planus," *Archives of Dermatological Research*, vol. 291, no. 9, pp. 485–490, 1999.

[59] A. Khan, C. S. Farah, N. W. Savage, L. J. Walsh, D. J. Harbrow, and P. B. Sugerman, "Th1 cytokines in oral lichen planus," *Journal of Oral Pathology and Medicine*, vol. 32, no. 2, pp. 77–83, 2003.

[60] J. N. W. N. Barker, H. A. Navsaria, I. M. Leigh, and D. M. MacDonald, "Gamma-interferon induced human keratinocyte HLA-DR synthesis: the role of dermal activated T lymphocytes," *British Journal of Dermatology*, vol. 119, no. 5, pp. 567–572, 1988.

[61] Z. Z. Zhao, P. B. Sugerman, L. J. Walsh, and N. W. Savage, "Expression of RANTES and CCR1 in oral lichen planus and association with mast cell migration," *Journal of Oral Pathology and Medicine*, vol. 31, no. 3, pp. 158–162, 2002.

[62] W. Iijima, H. Ohtani, T. Nakayama et al., "Infiltrating $CD8^+$ T cells in oral lichen planus predominantly express CCR5 and CXCR3 and carry respective chemokine ligands RANTES/CCL5 and IP-10/CXCL10 in their cytolytic granules: a potential self-recruiting mechanism," *American Journal of Pathology*, vol. 163, no. 1, pp. 261–268, 2003.

[63] M. C. Little, C. E. M. Griffiths, R. E. B. Watson, M. N. Pemberton, and M. H. Thornhill, "Oral mucosal keratinocytes express RANTES and ICAM-1, but not interleukin-8, in oral lichen planus and oral lichenoid reactions induced by amalgam fillings," *Clinical and Experimental Dermatology*, vol. 28, no. 1, pp. 64–69, 2003.

[64] B. Cribier, C. Frances, and O. Chosidow, "Treatment of lichen planus: an evidence-based medicine analysis of efficacy," *Archives of Dermatology*, vol. 134, no. 12, pp. 1521–1530, 1998.

[65] M. Omidian, A. Ayoobi, M. Mapar, A. Feily, and B. Cheraghian, "Efficacy of sulfasalazine in the treatment of generalized lichen planus: randomized double-blinded clinical trial on 52 patients," *Journal of the European Academy of Dermatology and Venereology*, vol. 24, no. 9, pp. 1051–1054, 2010.

[66] V. P. Werth, "Clinical manifestations of cutaneous lupus erythematosus," *Autoimmunity Reviews*, vol. 4, no. 5, pp. 296–302, 2005.

[67] F. M. Wigley, "Raynaud's phenomenon," *The New England Journal of Medicine*, vol. 347, no. 13, pp. 1001–1008, 2002.

[68] B. Goundry, L. Bell, M. Langtree, and A. Moorthy, "Diagnosis and management of Raynaud's phenomenon," *BMJ*, vol. 344, article e289, 2012.

[69] E. M. Vital, S. Dass, M. H. Buch et al., "Re-treatment of rheumatoid arthritis patients who were initial nonresponders to rituximab: comment on the article by Thurlings et al," *Arthritis and Rheumatism*, vol. 60, no. 6, p. 1867, 2009.

[70] M. Postal, L. T. Costallat, and S. Appenzeller, "Biological therapy in systemic lupus erythematosus," *International Journal of Rheumatology*, vol. 2012, Article ID 578641, 9 pages, 2012.

[71] E. G. Boyce and B. E. Fusco, "Belimumab: review of use in systemic lupus erythematosus," *Clinical Therapeutics*, vol. 34, no. 5, pp. 1006–1022, 2012.

[72] J. J. Wisnieski, "Urticarial vasculitis," *Current Opinion in Rheumatology*, vol. 12, no. 1, pp. 24–31, 2000.

[73] J. Venzor, W. L. Lee, and D. P. Huston, "Urticarial vasculitis," *Clinical Reviews in Allergy and Immunology*, vol. 23, no. 2, pp. 201–216, 2002.

[74] J. J. Wisnieski, A. N. Baer, J. Christensen et al., "Hypocomplementemic urticarial vasculitis syndrome: clinical and serologic findings in 18 patients," *Medicine*, vol. 74, no. 1, pp. 24–41, 1995.

[75] C. E. H. Siegert, M. D. Kazatchkine, A. Sjöholm, R. Würzner, M. Loos, and M. R. Daha, "Autoantibodies against C1q: view on clinical relevance and pathogenic roles," *Clinical and Experimental Immunology*, vol. 116, no. 1, pp. 4–8, 1999.

[76] M. Y. Her, J. Y. Song, and D. Y. Kim, "Hypocomplementemic urticarial vasculitis in systemic lupus erythematosus," *Journal of Korean Medical Science*, vol. 24, no. 1, pp. 184–186, 2009.

[77] R. N. V. Furtado, M. L. C. Pucinelli, V. V. Cristo, L. E. C. Andrade, and E. I. Sato, "Scleroderma-like nailfold capillaroscopic abnormalities are associated with anti-U1-RNP antibodies and Raynaud's phenomenon in SLE patients," *Lupus*, vol. 11, no. 1, pp. 35–41, 2002.

[78] C. Cardinali, M. Caproni, E. Bernacchi, L. Amato, and P. Fabbri, "The spectrum of cutaneous manifestations in lupus erythematosus—the Italian experience," *Lupus*, vol. 9, no. 6, pp. 417–423, 2000.

[79] R. P. B. Rapini, L. Jean, Jorizzo, and L. Joseph, *Dermatology*, vol. 2, Mosby, St. Louis, Mo, USA, 2007.

[80] C. Herrero, A. Guilabert, and J. M. Mascaró-Galy, "Diagnosis and treatment of livedo reticularis on the legs," *Actas Dermo-Sifiliograficas*, vol. 99, no. 8, pp. 598–607, 2008.

[81] C. Y. K. Kwok and W. K. Cheong, "Atrophie blanche—a special form of vasculopathy," *Singapore Medical Journal*, vol. 38, no. 3, pp. 123–124, 1997.

[82] L. M. Milstone, I. M. Braverman, P. Lucky, and P. Fleckman, "Classification and therapy of atrophie blanche," *Archives of Dermatology*, vol. 119, no. 12, pp. 963–969, 1983.

[83] M. Elisaf, S. Nikou-Stefanaki, A. A. Drosos, and H. M. Moutsopoulos, "Atrophie blanche. Clinical diagnosis and treatment," *Annales de Medecine Interne*, vol. 142, no. 6, pp. 415–418, 1991.

[84] C. S. McCalmont, T. H. McCalmont, J. L. Jorizzo, W. L. White, B. Leshin, and H. Rothberger, "Livedo vasculitis: vasculitis or thrombotic vasculopathy?" *Clinical and Experimental Dermatology*, vol. 17, no. 1, pp. 4–8, 1992.

[85] J. K. Shornick, B. K. Nicholes, P. R. Bergstresser, and J. N. Gilliam, "Idiopathic atrophie blanche," *Journal of the American Academy of Dermatology*, vol. 8, no. 6, pp. 792–798, 1983.

[86] C. R. Drucker and W. C. Duncan, "Antiplatelet therapy in atrophie blanche and livedo vasculitis," *Journal of the American Academy of Dermatology*, vol. 7, no. 3, pp. 359–363, 1982.

[87] G. C. Sauer, "Pentoxifylline (Trental) therapy for the vasculitis of atrophie blanche," *Archives of Dermatology*, vol. 122, no. 4, pp. 380–381, 1986.

[88] R. L. Jetton and G. S. Lazarus, "Minidose heparin therapy for vasculitis of atrophie blanche," *Journal of the American Academy of Dermatology*, vol. 8, no. 1, pp. 23–26, 1983.

[89] E. A. I. M. Freedberg, K. Wolff et al., *Katz SI. Dermatitis Herpetiformis. Fitzpatrick's Dermatology in General Medicine*, McGraw-Hill, Health Professions Division, New York, 5th edition, 1999.

[90] A. Dupuy, M. Viguier, C. Bédane et al., "Treatment of refractory pemphigus vulgaris with rituximab (anti-CD20 monoclonal antibody)," *Archives of Dermatology*, vol. 140, no. 1, pp. 91–96, 2004.

[91] A. R. Ahmed, Z. Spigelman, L. A. Cavacini, and M. R. Posner, "Treatment of pemphigus vulgaris with rituximab and intravenous immune globulin," *The New England Journal of Medicine*, vol. 355, no. 17, pp. 1772–1779, 2006.

[92] F. Wojnarowska, R. A. Marsden, B. Bhogal, and M. M. Black, "Chronic bullous disease of childhood, childhood cicatricial pemphigoid, and linear IgA disease of adults: a comparative study demonstrating clinical and immunopathologic overlap," *Journal of the American Academy of Dermatology*, vol. 19, no. 5, part 1, pp. 792–805, 1988.

[93] F. E. R. Wojnarovska and S. Burge, "Bullous eruptions," in *Textbook of Dermatology*, R. H. Champion, Ed., pp. 1880–1883, Blackwell, Oxford, UK, 6th edition, 1998.

[94] P. Patrício, C. Ferreira, M. M. Gomes, and P. Filipe, "Autoimmune bullous dermatoses: a review," *Annals of the New York Academy of Sciences*, vol. 1173, pp. 203–210, 2009.

[95] A. M. Alahlafi, P. Wordsworth, and F. Wojnarowska, "The lupus band: do the autoantibodies target collagen VII?" *British Journal of Dermatology*, vol. 150, no. 3, pp. 504–510, 2004.

[96] A. M. Alahlafi, P. Wordsworth, L. Lakasing, D. Davies, and F. Wojnarowska, "The basement membrane zone in patients with systemic lupus erythematosus: immunofluorescence studies in the skin, kidney and amniochorion," *Lupus*, vol. 13, no. 8, pp. 594–600, 2004.

[97] A. Doria, C. Biasinutto, A. Ghirardello et al., "Photosensitivity in systemic lupus erythematosus: laboratory testing of ARA/ACR definition," *Lupus*, vol. 5, no. 4, pp. 263–268, 1996.

[98] J. Albrecht, J. A. Berlin, I. M. Braverman et al., "Dermatology position paper on the revision of the 1982 ACR criteria for systemic lupus erythematosus," *Lupus*, vol. 13, no. 11, pp. 839–849, 2004.

[99] A. Kuhn, M. Sonntag, D. Richter-Hintz et al., "Phototesting in lupus erythematosus: a 15-year experience," *Journal of the American Academy of Dermatology*, vol. 45, no. 1, pp. 86–95, 2001.

[100] M. Petri, A. M. Orbai, G. S. Alarcón et al., "Derivation and validation of systemic lupus international collaborating clinics classification criteria for systemic lupus erythematosus," *Arthritis and Rheumatism*, 2012. In press.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 265823, 5 pages
doi:10.1155/2012/265823

Research Article

Identification of TNIP1 Polymorphisms by High Resolution Melting Analysis with Unlabelled Probe: Association with Systemic Lupus Erythematosus

Jie Zhang,[1,2,3] Yuewen Chen,[1,3,4] Yong Shao,[1,2,3] Qi Wu,[1,3] Ming Guan,[5] Wei Zhang,[1,3] Jun Wan,[3,6] and Bo Yu[2]

[1] *Shenzhen Key Lab for Translational Medicine of Dermatology, Shenzhen PKU-HKUST Medical Center, No. 1120 Lian-Hua Road, Futian District, Shenzhen, Guangdong 518036, China*
[2] *Department of Dermatology, Shenzhen Hospital, Peking University, Shenzhen, Guangdong 518036, China*
[3] *Biomedical Research Institute, Shenzhen PKU-HKUST Medical Center, No. 1120 Lianhua Road, Futian District, Shenzhen, Guangdong 518036, China*
[4] *JNU-HKUST Joint Laboratory, Jinan University, Guangdong 510632, China*
[5] *Department of Clinical Laboratory, Shanghai Worldwide Medical Center, Huashan 200040, China*
[6] *Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong*

Correspondence should be addressed to Jun Wan, wanj@ust.hk and Bo Yu, yubomd@hotmail.com

Received 6 March 2012; Accepted 31 May 2012

Academic Editor: Hiroshi Okamoto

Background. TNFα-induced protein 3 (TNFAIP3) interacting with protein 1 (TNIP1) acts as a negative regulator of NF-κB and plays an important role in maintaining the homeostasis of immune system. A recent genome-wide association study (GWAS) showed that the polymorphism of TNIP1 was associated with the disease risk of SLE in Caucasian. In this study, we investigated whether the association of TNIP1 with SLE was replicated in Chinese population. *Methods*. The association of TNIP1 SNP rs7708392 (G/C) was determined by high resolution melting (HRM) analysis with unlabeled probe in 285 SLE patients and 336 healthy controls. *Results*. A new SNP rs79937737 located on 5 bp upstream of rs7708392 was discovered during the HRM analysis. No association of rs7708392 or rs79937737 with the disease risk of SLE was found. Furthermore, rs7708392 and rs79937737 were in weak linkage disequilibrium (LD). Hypotypes analysis of the two SNPs also showed no association with SLE in Chinese population. *Conclusions*. High resolution melting analysis with unlabeled probes proves to be a powerful and efficient genotyping method for identifying and screening SNPs. No association of rs7708392 or rs79937737 with the disease risk of SLE was observed in Chinese population.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease influenced by both genetic and environmental factors [1, 2]. Recently, the genetic background of this complex disease was robustly revealed by a series of genome-wide association studies (GWAS) [3]. Several SLE susceptive genes including MHC, BLK, ITGAM, STAT4, IRF5, BANK1, and ETS1 were identified although the function of these candidate genes in the pathological development of SLE was still largely unknown [4–10]. Among these candidate genes, the polymorphisms on TNFα induced protein 3 (TNFAIP3 or A20) interacting protein 1 (TNIP1) have been found to associate with the disease risk of several autoimmune diseases including psoriasis and SLE [8, 9, 11–15]. TNIP1, also known as A20 binding inhibitors of NF-κB (ABIN1), can interact with TNFAIP3 and IκB kinase γ/NF-κB essential modulator (IKKγ/NEMO) and acts as a negative regulator of NF-κB signal pathway [16]. Additionally, TNIP1 was also involved in inhibiting the processing of the p105, a precursor of NF-κB [17].

It has been reported that SNP rs7708392 (C/G) on 5q33.1 that resides within an intron of TNIP1 is associated with the disease risk of SLE in the Caucasian population [9]. However,

whether this association is also presented in Chinese Han population remains unknown. In this study, we examined the polymorphism of SNP rs7708392 (C/G) in 285 patients and 336 normal controls in the Chinese population using high-resolution melting analysis with unlabelled probe.

2. Methods

2.1. Study Populations. A total of 285 patients (26 males and 259 females; median age 29 years, range 12–55) who fulfilled the American College of Rheumatology criteria for SLE [18] and 336 ethnically matched healthy controls (28 males and 308 females; median age 28 years, range 17–46) were recruited from Shenzhen Hospital, Peking University. The control group had neither family history nor symptoms related to SLE. The study was approved by the institutional review board of the Shenzhen Hospital and written informed consent was taken from all patients.

2.2. Genotyping. Genomic DNA was isolated from peripheral blood cells by using Innogent genomic DNA extraction kit (Innogent, China) according to the manufactory instructions. Genotyping was assayed by high resolution melting with unlabeled probe as previously described [19]. Briefly, asymmetric PCR reaction was performed in a volume of 20 μL containing 20 ng of genomic DNA, 1 $\times$ PCR buffer (Takara, Japan), 200 μM dNTPs, 0.5 U rTaq DNA polymerase (Takara, Japan), 0.05 μM forward primer, 0.5 μM excess reverse primer, and 0.5 μM C3-blocked probe. The PCR reactions were performed in a S1000 Thermal Cycler (Bio-Rad, USA). The conditions included an initial denaturation at 94°C for 2 min, followed by 50 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 20 s, and a final extension at 72°C for 5 minutes. The 10 μL of PCR products were supplied with 1 μL LCGreen Plus Dye (Idaho Technology, USA) and transferred to a capillary tube on the HR-1 (Idaho Technology, USA). The samples were first denatured at 95°C for 30 s and rapidly cooled to 40°C for 30 s, then melted from 55°C to 90°C with a 0.3°C/s ramp rate. Melting curves were analyzed with LightScanner software (Idaho Technology, USA). All the primers and probes were designed by Lighscaner probe design software (Idaho Technology, USA). The sequences of the primers used in PCR were as follows: forward 5′-TGG TCA ATT CTC CCA ACC GA-3′, reverse 5′-ACT TCA AGG TCA GAC CCT AAA-3′ and three unlabeled C3 block probes used during the genotyping were listed as follows: probe 5′-GCT GAT TCC AGT TAT TGT GAC TAG TCT ACT-3′, probe-1 5′-CGA GGA GAG GCT GAT TCC AAT TAT T-3′, and probe-2 5′-TTA TTG TGA CTA GTC TAC TAA GTT CCA GA-3′. The position of the primers and probe on the genomic DNA sequence are presented in Figure 2(a), respectively.

2.3. Statistical Analysis. The SNP was analyzed for an association with the disease by means of comparison of the minor allele frequency (MAF) in patients and controls as well as the constancy of Hardy-Weinberg equilibrium using chi-square test or Fisher's exact test. The magnitude of association was expressed as odds ratio (OR) with a 95% confidence interval (CI). Linkage disequilibrium (LD) and haplotype analysis were carried out by SHEsis software [20]. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Discovery of a New SNP Located on 5 bp Upstream of SNP rs7708392. High resolution melting analysis with unlabeled probes is a newly developed method for SNP detection with low cost and high efficiency [21, 22]. Single mutation in the genomic DNA sequence could cause mispairing within the unlabeled probe region, which produces a shift of melting peaks. Typically, three kinds of melting curves representing three genotypes (wildtype, heterozygote, and mutant) could be well distinguished. Then, this method was employed to detect the genotypes of SNP rs7708392. Interestingly, more than three melting curves were observed in the melting curves during genotyping (Figure 1), implying that a new unknown SNP might also exist on the probe region. The following DNA sequencing results revealed that a new mutation (G/A) locating on 5 bp upstream of SNP rs7708392 (G/C) was found (Figure 1(d)). This new SNP was submitted to NCBI and assigned an accession number rs79937737 (ss244236678).

To discriminate these two SNPs independently, we redesigned two new probes which could specifically match to each SNP, respectively (Figure 2(a)). As presented in Figure 2, both probe 1 and probe 2 could clearly distinguish three genotypes. Then, these probes were used for genotyping all the SLE and normal samples.

3.2. No Association of rs7708392 or rs79937737 with the Disease Risk of SLE. Table 1 shows genotype and allele frequencies of SNPs rs7708392 and rs79937737 in SLE patients and healthy controls. Genotype frequencies were in Hardy-Weinberg's equilibrium in the patients and controls. Neither genotype nor allele frequencies of rs7708392 or rs79937737 showed statistically significant differences between SLE patients and controls. Haplotype analysis showed that SNPs rs7708392 and rs79937737 were in weak linkage disequilibrium (LD) ($r^2 = 0.020$). Furthermore, all the haplotypes generated from these two SNPs showed no significant association with the disease risk of SLE (Table 2).

4. Discussion

In comparison with other traditional SNP screening methods, high melting curve analysis (HRMA) is a powerful and cost-effective method for SNP screening [21, 22]. However, it is occasionally difficult to discriminate the wildtype and homomutant since the melting temperature shifts between these genotypes are almost undetectable (less than 0.4°C). By using a ~30 bp C3-blocked probe to target the SNP of interest, the melting temperature shift could be amplified to 3~4°C which can be much feasible to detect. Moreover, some undiscovered SNPs locating on the probe region could

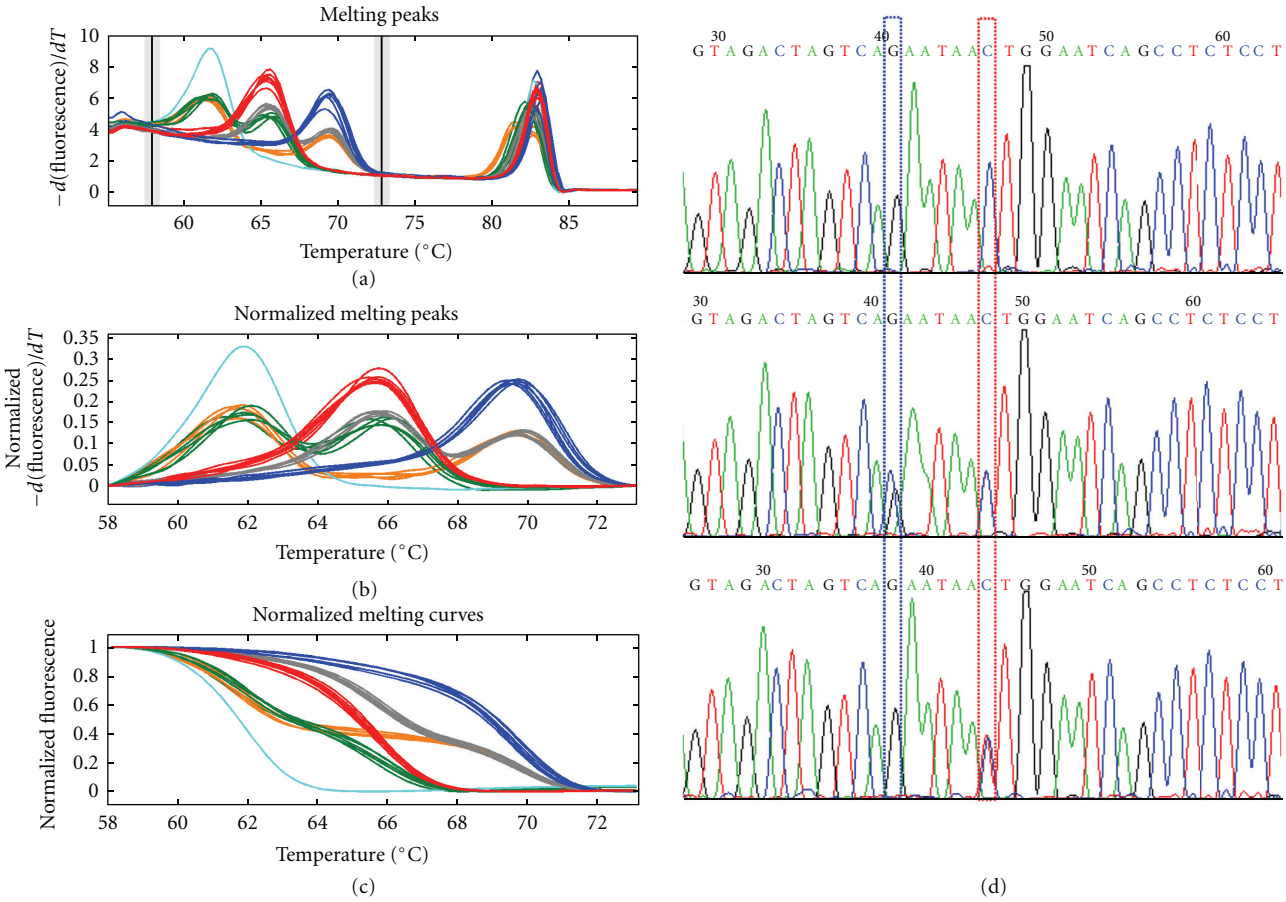


Figure 1: SNP genotyping by HRM with unlabeled probe. (a) Derivative melting curves of unlabeled probe and amplicon for genotyping of SNP rs7708392. (b) Normalized difference curves of unlabeled probe region. (c) Normalized melting curves of unlabeled probe region. Unlike the classical SNP genotyping (wildtype, heterozygote, and homozygote), six types of curves were observed implying a new SNP also presented in probe region. (d) DNA sequencing result of SNP rs7708392. The sequencing was performed by using reverse primer of the PCR amplicon. The blue box indicates the SNP rs7708392 harbors G/C mutation by reverse sequencing. The red box shows that the polymorphism on the new SNP is C/T by reverse sequencing.

Table 1: Genotype and allele frequencies of TNIP1 SNPs in SLE cases and controls*.

SNP, population	Number of subjects	Genotype frequency, *n* (%)			*P* value	Allele frequency, *n* (%)		*P* value	OR (95% CI)
		Major homozygote	Heterozygote	Minor homozygote		Major allele	Minor allele		
m75rs7708392									
Genotype or allele	CC	CG	GG	C	G				
Cases	283	179 (63.3)	91 (32.2)	13 (4.6)	0.915	449 (79.3)	117 (20.7)	0.697	1.056 (0.803 ~ 1.389)
Controls	336	207 (61.6)	113 (33.6)	16 (4.8)		527 (78.4)	145 (21.6)		
m75rs79937737									
Genotype or allele	GG	AG	AA	G	A				
Cases	283	239 (84.4)	43 (15.2)	1 (0.4)	0.500	521 (92.0)	45 (8.0)	0.523	1.148 (0.751 ~ 1.757)
Controls	336	289 (86.0)	47 (14)	0 (0)		625 (93.0)	47 (7.0)		

*SNP: single-nucleotide polymorphism; SLE: systemic lupus erythematosus; OR: odds ratios; 95% CI: 95% confidence interval.

also be observed during the genotyping, which would be helpful for identifying new SNPs. In our case, an unknown SNP existing in the probe region gave rise to a completely new pattern of melting curves. Six types of melting curves were shown up, implying that at least two SNPs were located in the probe region. Even though it was still difficult to deduce which genotype was represented by each melting curve, respectively, the potential advantage of the HRMA with unlabeled probe in identifying new SNPs was well appreciated in our work.

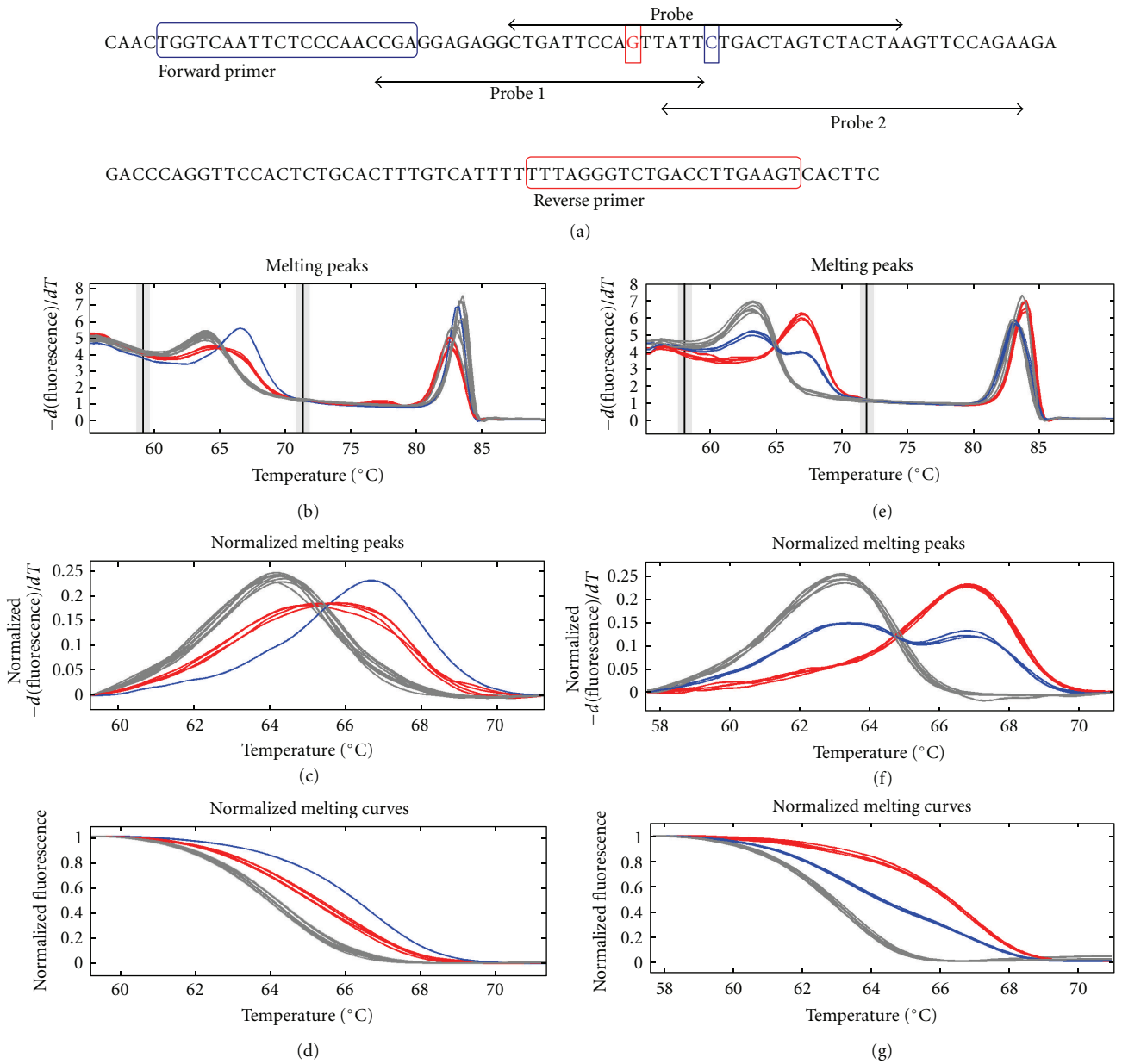


Figure 2: SNP genotyping by HRM with specific probes targeting each SNP, respectively. (a) The location of probes and PCR primers. Probe 1 targets the new-discovered SNP. Probe 2 targets rs7708392. The probe containing both SNPs is also shown (b)–(g). The derivative melting curve, normalized difference curve, and normalized melting curve for each genotyping assay by using probe 1 and probe 2 are shown as indicated.

Table 2: Haplotype analysis of TNIP1 SNPs in SLE cases and controls*.

Haploptype	Cases, *n* (%)	Controls, *n* (%)	*P* value	OR (95% CI)
AC	44.26 (7.8)	46.84 (7)	0.568	1.132 (0.739 ~ 1.735)
AG	0.74 (0.1)	0.16 (0)	0.590	5.357 (0.272 ~ 105.552)
GC	404.74 (71.5)	480.16 (71.5)	0.983	1.003 (0.783 ~ 1.285)
GG	116.26 (20.5)	144.84 (21.6)	0.664	0.941 (0.715 ~ 1.238)

*SNP: single-nucleotide polymorphism; SLE: systemic lupus erythematosus; OR: odds ratios; 95% CI: 95% confidence interval.

As a negative regulator of NF-κB signal pathway, TNIP1 might play an important role in NF-κB associated innate and adaptive immune response. Until recently, the potential role of TNIP1 during the disease development of SLE has been appreciated since the polymorphism of TNIP1 is associated with the disease risks of SLE in Caucasian population [9]. A recent work reported that rs7708392 was associate with SLE in Japanese population by using a fluorescence probe based TaqMan SNP genotyping assay [15]. In the present work, we found that SNP rs7708392 (G/C) was not relevant to the disease risk of SLE in Chinese population. This discrepancy might cause by the ethnic divergence. Intriguingly, we identified a new SNP (rs79937737) locating on just 5 bp upstream of rs7708392. This new SNP showed low mutation frequency since almost no homo-mutant was observed in our samples. However, these two SNPs were in weak linkage disequilibrium ($r^2 = 0.02$). This nonlinkage disequilibrium in such a short distance on the genome indicates that rs79937737 might be a newly developed polymorphism during the evolution of genome. Whether rs79937737 also exists in other populations needs to be carefully investigated in the future.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

The study was supported by the Research Grants of Shenzhen Science and Technology Project. The authors thank Shenzhen Biomedical Research Support Platform for the technical help. J. Zhang, Y. Chen, J. Wan, and B. Yu contribute equally to this paper.

References

[1] C. J. Edwards and C. Cooper, "Early environmental exposure and the development of lupus," *Lupus*, vol. 15, no. 11, pp. 814–819, 2006.

[2] S. R. Block, J. B. Winfield, and M. D. Lockshin, "Studies of twins with systemic lupus erythematosus. A review of the literature and presentation of 12 additional sets," *American Journal of Medicine*, vol. 59, no. 4, pp. 533–552, 1975.

[3] R. R. Graham, G. Hom, W. Ortmann, and T. W. Behrens, "Review of recent genome-wide association scans in lupus," *Journal of Internal Medicine*, vol. 265, no. 6, pp. 680–688, 2009.

[4] J. B. Harley, M. E. Alarcón-Riquelme, L. A. Criswell et al., "Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci," *Nature Genetics*, vol. 40, no. 2, pp. 204–210, 2008.

[5] G. Hom, R. R. Graham, B. Modrek et al., "Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX," *The New England Journal of Medicine*, vol. 358, no. 9, pp. 900–909, 2008.

[6] R. R. Graham, C. Cotsapas, L. Davies et al., "Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus," *Nature Genetics*, vol. 40, no. 9, pp. 1059–1061, 2008.

[7] S. V. Kozyrev, A. K. Abelson, J. Wojcik et al., "Functional variants in the B-cell gene BANK1 are associated with systemic lupus erythematosus," *Nature Genetics*, vol. 40, no. 2, pp. 211–216, 2008.

[8] J. W. Han, H. F. Zheng, Y. Cui et al., "Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus," *Nature Genetics*, vol. 41, no. 11, pp. 1234–1237, 2009.

[9] V. Gateva, J. K. Sandling, G. Hom et al., "A large-scale replication study identifies TNIP1, PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus," *Nature Genetics*, vol. 41, no. 11, pp. 1228–1233, 2009.

[10] W. Yang, N. Shen, D. Q. Ye et al., "Genome-wide association study in asian populations identifies variants in ETS1 and WDFY4 associated with systemic lupus erythematosus," *PLoS Genetics*, vol. 6, no. 2, Article ID e1000841, 2010.

[11] C. F. He, Y. S. Liu, Y. L. Cheng et al., "TNIP1, SLC15A4, ETS1, RasGRP3 and IKZF1 are associated with clinical features of systemic lupus erythematosus in a Chinese Han population," *Lupus*, vol. 19, no. 10, pp. 1181–1186, 2010.

[12] R. P. Nair, K. C. Duffin, C. Helms et al., "Genome-wide scan reveals association of psoriasis with IL-23 and NF-κB pathways," *Nature Genetics*, vol. 41, no. 2, pp. 199–204, 2009.

[13] J. T. Elder, "Genome-wide association scan yields new insights into the immunopathogenesis of psoriasis," *Genes and Immunity*, vol. 10, no. 3, pp. 201–209, 2009.

[14] Y. Li and A. B. Begovich, "Unraveling the genetics of complex diseases: susceptibility genes for rheumatoid arthritis and psoriasis," *Seminars in Immunology*, vol. 21, no. 6, pp. 318–327, 2009.

[15] A. Kawasaki, S. Ito, H. Furukawa et al., "Association of TNFAIP3 interacting protein 1, TNIP1 with systemic lupus erythematosus in a Japanese population: a case-control association study," *Arthritis Research & Therapy*, vol. 12, no. 5, p. R174, 2010.

[16] L. Verstrepen, I. Carpentier, K. Verhelst, and R. Beyaert, "ABINs: a20 binding inhibitors of NF-κB and apoptosis signaling," *Biochemical Pharmacology*, vol. 78, no. 2, pp. 105–114, 2009.

[17] S. Cohen, A. Ciechanover, Y. Kravtsova-Ivantsiv, D. Lapid, and S. Lahav-Baratz, "ABIN-1 negatively regulates NF-κB by inhibiting processing of the p105 precursor," *Biochemical and Biophysical Research Communications*, vol. 389, no. 2, pp. 205–210, 2009.

[18] M. C. Hochberg, "Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 40, no. 9, p. 1725, 1997.

[19] J. Montgomery, C. T. Wittwer, R. Palais, and L. Zhou, "Simultaneous mutation scanning and genotyping by high-resolution DNA melting analysis," *Nature Protocols*, vol. 2, no. 1, pp. 59–66, 2007.

[20] Y. Y. Shi and L. He, "SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci," *Cell Research*, vol. 15, no. 2, pp. 97–98, 2005.

[21] C. T. Wittwer, "High-resolution DNA melting analysis: advancements and limitations," *Human Mutation*, vol. 30, no. 6, pp. 857–859, 2009.

[22] R. H. A. M. Vossen, E. Aten, A. Roos, and J. T. Den Dunnen, "High-resolution melting analysis (HRMA)—more than just sequence variant screening," *Human Mutation*, vol. 30, no. 6, pp. 860–866, 2009.

Hindawi Publishing Corporation
Autoimmune Diseases
Volume 2012, Article ID 290898, 9 pages
doi:10.1155/2012/290898

Review Article

Ocular Manifestations of Systemic Lupus Erythematosus: A Review of the Literature

Neal V. Palejwala, Harpreet S. Walia, and Steven Yeh

Section of Vitreoretinal Disease and Surgery, Department of Ophthalmology, Emory Eye Center, Emory University School of Medicine, Atlanta, 30322 GA, USA

Correspondence should be addressed to Steven Yeh, steven.yeh@emory.edu

Received 5 May 2012; Accepted 31 May 2012

Academic Editor: Hiroshi Okamoto

About one-third of patients suffering from systemic lupus erythematosus have ocular manifestations. The most common manifestation is keratoconjunctivitis sicca. The most vision threatening are retinal vasculitis and optic neuritis/neuropathy. Prompt diagnosis and treatment of eye disease is paramount as they are often associated with high levels of systemic inflammation and end-organ damage. Initial management with high-dose oral or IV corticosteroids is often necessary. Multiple "steroid-sparing" treatment options exist with the most recently studied being biologic agents.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic, autoimmune, connective tissue disorder affecting multiple organ systems often with a relapsing and remitting clinical course. Prevalence, clinical manifestations, and morbidity vary significantly between the developing and industrialized worlds. While SLE is more common in people of African and Asian descent, thrombotic complications are more common in Caucasian patients [1]. The highest prevalence has been reported in Italy, Spain, Martinique, and the UK Afro-Caribbean population [2]. The median age of onset is between the late teens and early 40s with a 9 times higher incidence in women compared to men. Ocular manifestations—occurring in up to one third of patients—can be associated with significant morbidity and also a marker for overall systemic disease activity.

2. Genetic Considerations

Concordance rates for SLE among monozygotic and dizygotic twins are 25% and 2%, respectively, suggesting a significant genetic contribution [3]. Major histocompatibility complex genes, such as HLA-A1, B8, and DR3 [4], as well as alleles that cause deficiency in complement components—C1q, C2, and C4 [5]—have all been linked to lupus.

3. Mechanism of Disease

SLE is a complex disease process demonstrating dysregulation of the immune system at multiple levels. Autoantibodies against double-stranded DNA were first isolated from kidney specimens in patients with lupus nephritis in 1967 [6]. Other autoantibodies that have been implicated in disease include anti-Ro, La, Sm, nucleosome, NMDA receptor, phospholipid, and α-actinin. Two major theories exist on how these autoantibodies cause tissue damage. The first model suggests that anti-double-stranded DNA antibodies bind to circulating nucleosomes to form immune complexes that then get deposited in end-organ capillary beds such as the renal glomerulus and activate immune/inflammatory responses [7]. The second hypothesizes that these autoantibodies cross-react with normal renal proteins causing tissue destruction [8]. The source of autoantigens that trigger the formation of the aforementioned antibodies is thought to arise from apoptotic cells. Normally, early complement factors, such as C1q, bind cellular debris from apoptotic cells, which facilitate phagocytosis by macrophages. Deficiency of

such complement factors is an independent risk factor for the development of SLE [5].

Mass production of autoantibodies relies on multiple factor, which have each independently been targeted as potential immunotherapy in the treatment of lupus. Important steps include T-cell activation via antigen binding to the T-cell receptor and proper costimulation; T-cell activation of B cells; production of cytokines such as TNF-α, INF-γ, IL-10, and B-lymphocyte stimulator.

Medications, hormonal influences, and other factors such as sunlight have all been implicated in disease exacerbation. Drug-induced lupus, most commonly due to procainamide, hydralazine, and quinidine, usually presents with disease involving the skin and joints with renal and CNS manifestations being much more rare [9]. Hormonal replacement therapy has been associated with an increased risk of mild-to-moderate flares [10].

4. Diagnostic Criteria

According to the 1982 revised criteria for systemic lupus erythematosus, a diagnosis of SLE can be made by the serial or simultaneous presentation of at least 4 of the following 11 criteria: malar rash, discoid rash, photosensitivity, oral ulcers, nonerosive arthritis, serositis, renal dysfunction, neurological derangements (i.e., seizures or psychosis), hematologic disorder (i.e., anemia, leukopenia, thrombocytopenia), immunologic disorder (i.e., anti-DNA antibody, anti-Sm antibody, and false positive VDRL testing), and presence of antinuclear antibodies.

5. Ocular Manifestations

SLE can affect the periorbita, ocular adnexa, eye, and optic nerve. The most common association is keratoconjunctivitis sicca, while the most visually devastating sequelae occur secondary to optic nerve involvement and retinal vaso-occlusion.

5.1. Orbit. Orbital involvement is a rare manifestation of SLE. Vasculitis, myositis, and panniculitis have all been described. Signs and symptoms include proptosis, enophthalmos, orbital pain, blurred vision, chemosis, and restriction of extraocular movements.

Orbital vasculitis leads to nonperfusion of the globe and extraocular muscles. This has been shown to cause irreversible vision loss secondary to ischemic injury to the optic nerve as well as elevated intraocular pressure from neovascular glaucoma [11].

Orbital myositis is often initially misdiagnosed as bacterial orbital cellulitis, as it usually presents with significant pain, proptosis, periorbital swelling, and globe restriction. CT and orbital ultrasound are both valuable in demonstrating enlargement of one or multiple extraocular muscles. Creatinine kinase, aldolase, and myoglobin levels are markedly elevated. Inflammation and symptoms typically respond to steroids [12, 13].

Subcutaneous inflammation secondary to SLE was first described by Kaposi in 1883, and the term "lupus erythematosus panniculitis" was coined in 1940 [14]. It is most commonly encountered in the setting of discoid lupus erythematosus. Clinical findings include tender deep subcutaneous nodules usually involving the proximal extremities, trunk, face, and scalp [15]. Orbital involvement is very rare and has only been reported in a handful of paper. Histopathology shows perivascular lymphocytic infiltration [16]. Response to steroids can be quite dramatic in most cases [16–18]; however, few cases have shown a more virulent course with significant enophthalmos secondary to fat atrophy [19] and even melting of orbital structures [20].

5.2. Periorbita. Periorbital edema is an uncommon manifestation of systemic and discoid lupus erythematosus with an overall incidence of 4.8% [21]. It is most common in patients of African decent [22]. Violaceous swelling with overlying eczematous changes without any skin necrosis is seen. Some cases can resemble chronic blepharitis [23]. Etiologies include nephrosis, increased vascular permeability, dermal mucin deposits, and angioedema secondary to C1 deficiency. Treatment options include observation [23], topical/intradermal/systemic corticosteroids [24], and antimalarials [23].

5.3. Eyelids. Typical lesions of discoid lupus erythematosus are slightly raised, scaly, and atrophic. Most commonly, they occur on the head, face, neck, and other sun-exposed areas. Rarely does it affect the eyelids. Histopathologic study shows a hyperkeratotic epithelium with liquefactive degeneration of the basal layer and a dense perivascular/periappendageal lymphocytic infiltration [25, 26]. Diagnosis in most cases is delayed because lesions are often mistaken for blepharitis and eczema. Patients most commonly present with chronic erythema, blepharoconjunctivitis with inflammation of the meibomian glands. Long-term complications include madarosis, lid scarring, and cicatricial ectropion/entropion [26, 27].

5.4. Ocular Surface. The most common ocular manifestation of SLE is keratoconjunctivitis sicca with the majority of patients endorsing at least one dry eye symptom [28]. Dryness can occur from multiple etiologies. Most patients with SLE develop a secondary Sjogren's syndrome. In their review of 283 SLE patients, Manoussakis et al. [29] identified 9.2% who had developed Sjogren's syndrome (SS). The SLE-SS group had a higher frequency of Raynaud's phenomenon, anti-Ro antibody, anti-La antibody, and rheumatoid factor and a lower frequency of renal involvement, lymphadenopathy, and thrombocytopenia. These patients tend to undergo a more benign course with a significantly reduced mortality and need for immunosuppression [30]. The hallmark of disease is a decreased production of the aqueous layer of the tear film.

An abundance of proinflammatory markers such as IL-17 [31, 32] can be found in the tear film of SLE patients. These are some of the same markers that are found in

cicatrizing inflammatory conditions such as Steven Johnsons syndrome. Clinical findings can include symblepharon formation, forniceal foreshortening, and exposure keratopathy. Histopathological findings include loss of goblet cells, keratinization of the conjunctival epithelium, monocellular infiltration, and granuloma formation in the substantia propria [32]. Immunopathology shows immune complex deposition within the epithelial basement membrane with an increased number of CD4+ and CD8+ T cells, B cells, and macrophages [32, 33].

5.5. Episclera/Sclera. Episcleritis is generally a benign inflammation of the episclera. Typically occurring in young women, symptoms include a dull ache, red eye, and tearing. Decreased visual acuity and severe pain are uncommon. Systemic associations are extremely rare in adults, and a systemic workup is not necessary. Incidence in adult patients with SLE has been reported at 2.4% [34]. However, in children, episcleritis is much more rare but systemic associates are much more common. Read et al. [35] found 6 of 9 patients in their series on pediatric episcleritis to have systemic connective tissue disease. Treatment options include observation or topical/systemic nonsteroidal anti-inflammatory drugs.

Scleritis is a more painful and potentially a vision-threatening condition that warrants prompt treatment. Anterior scleritis can be nodular or diffuse and presents with a red, painful eye that is tender to touch. The injected deep episcleral vessels give a violaceous due to the sclera, which is best appreciated in natural light (Figure 1). Posterior scleritis on the other hand may not be associated with a red eye because it affects sclera posterior to the equator of the globe. Presenting symptoms are pain, blurred vision, limited eye movements, and proptosis. Blurred vision is most commonly caused by exudative retinal detachment, macular distortion due to a large scleral mass, and cystoid macular edema.

5.6. Cornea. Corneal epitheliopathy, scarring, ulceration, and filamentary keratitis can all occur secondary to keratoconjunctivitis sicca. More rare corneal complications include peripheral ulcerative keratitis [36], which can be a marker of active systemic vasculitis, interstitial keratitis, and keratoendothelitis [37]. Spectral microscopy has been used to show dysfunctional appearing corneal endothelial cells in both patients with corneal edema and asymptomatic patients [38].

Corneal biomechanical properties differ in SLE. Yazici et al. [39] used Reichert ocular response analyzer measurements to show that corneal hysteresis and corneal resistance factor were both lower in SLE patients which can lead to an underestimated IOP and development of keratoconus [40].

5.7. Retina. Lupus retinopathy is one of the most common vision-threatening complications of systemic lupus erythematosus with an incidence of up to 29% in patients with active systemic disease. A strong correlation exists between presence of retinopathy and CNS disease [41]. The most common pattern of retinopathy is microangiopathy

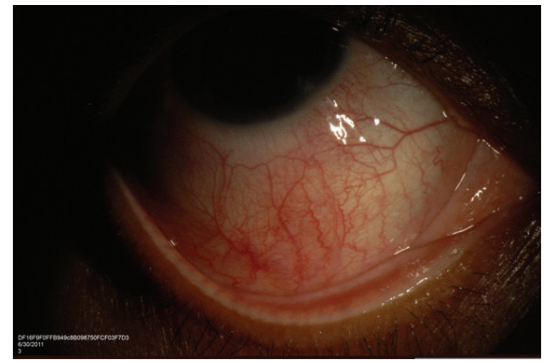


Figure 1: Slit-lamp photo demonstrating diffuse anterior scleritis in a patient with SLE.

similar to diabetic and hypertensive retinopathy. The earliest findings are small intraretinal hemorrhages and cotton wool spots [42]. Pathogenesis is attributed to deposition of immune complexes in the vessel wall and microemboli. Histopathology shows immunoglobulin and complement deposits, perivascular monocellular infiltrate, and rarely fibrinoid necrosis [43, 44]. Studies using fluorescein angiography describe hyperpermeability of arterioles and venules as well as capillary nonperfusion [45]. Although it is poor prognostic factor for survival, visual outcomes in this group are usually very good [46].

Retinal vasculitis, a subset of retinal vasculopathy featuring inflammation of the retinal arterioles or venules, tends to have poorer visual outcomes and present in an acute onset fashion. A large percentage of these patients have concomitant antiphospholipid antibodies including anticardiolipin and lupus anticoagulant. In one study, 77% of patients with SLE and retinal involvement had positive antiphospholipid antibody titers, whereas only 29% of SLE patients without retinal disease had positive titers [47]. Histopathologic specimens show fibrinoid change with thrombus formation without a true arteritis [48]. CNS vascular disease demonstrates similar pathology, thus providing a link between CNS vasculitis and severe lupus vasculopathy [49]. In 1984, Hall et al. [50] first reported the link between severe lupus retinal vasculopathy and presence of antiphospholipid antibodies. Since that time, multiple cases have been demonstrating severe vision loss secondary to central retinal artery/vein occlusions, vitreous hemorrhage, retinal ischemia, and neovascularization [47–53]. While the milder form of retinal vasculopathy is mediated by immune-complex deposition and inflammation, the more severe vaso-occlusive disease stems from fibrinoid degeneration/necrosis without significant inflammation.

Immunosuppression has been successful in improving the appearance of the retinopathy; however, visual recovery has only been reported in few cases. The permanent loss of visual acuity is likely due to retinal ischemia. Addition of anticoagulation to immunosuppression helps to stabilize retinal disease and prevent further vascular events [48]. Other therapies that have been reported for severe disease include plasmapheresis [54] and plasma exchange

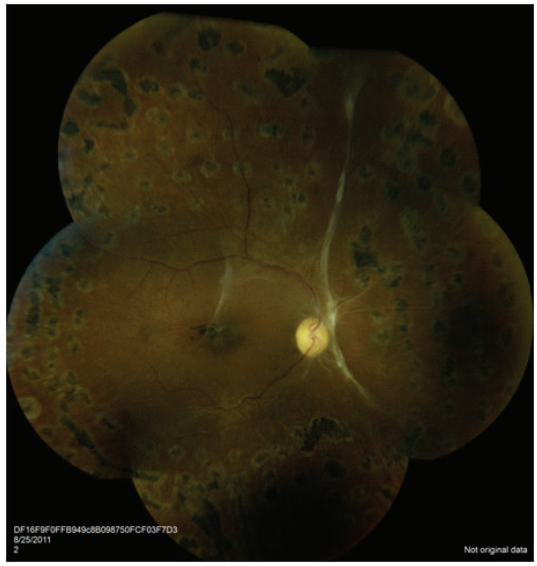
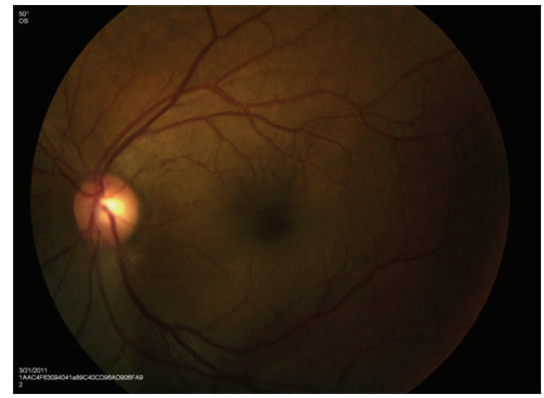


Figure 2: Fundus photograph demonstrating severe retinal vasculitis. Significant ischemia is present which is highlighted by the attenuated and sclerotic vasculature. Panretinal photocoagulation was required to treat ischemic and neovascular complications.

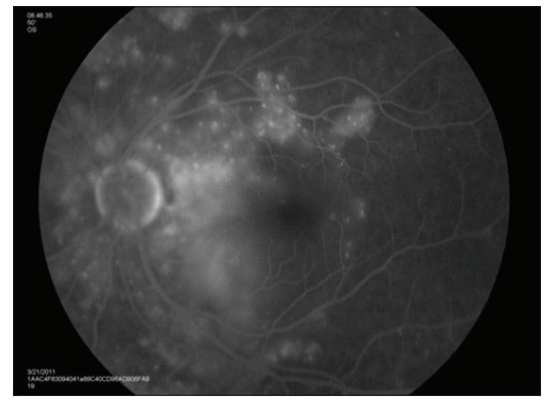
[55]. Panretinal photocoagulation, intravitreal antivascular endothelial growth factor agents, and vitrectomy may also be considered for the treatment of complications of ocular ischemia (Figure 2).

5.8. Choroid. Lupus choroidopathy with exudative retinal detachments is a rare ocular manifestation with fewer than 40 patients reported in the literature (Figure 3). It is generally seen in patients who have highly active disease including CNS vasculitis and nephropathy as well as uncontrolled blood pressure. Clinical diagnostic ophthalmic imaging is paramount for the diagnosis of choroidal and retinal pathologies. Specifically indocyanine green is extremely valuable for evaluating choroidal vascular and tissue inflammation, while fluorescein angiography is helpful in identifying optic nerve inflammation, retinal vascular disease, retinal ischemia, and macular edema. Baglio et al. [56] used indocyanine green angiography (ICG) to demonstrate that subtle changes in the choroidal circulation can be seen in patients with SLE-associated nephropathy, while similar findings are not seen in SLE patients without renal involvement. The pathogenesis is thought to be multifactorial; uncontrolled hypertension [57], immune complex deposition in the choriocapillaris [58], and antiretinal pigment epithelium antibodies [59] have all been implicated as contributing factors.

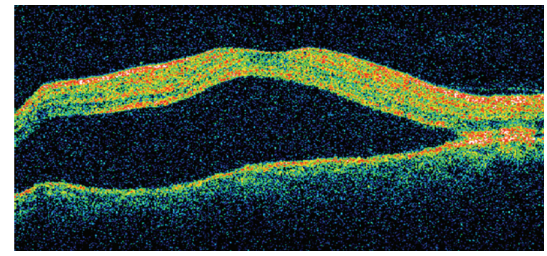
Recently, ICG imaging has been used to visualize the choroidal circulation in lupus choroidopathy. Studies have shown focal, transient early-phase hypofluorescence followed by late-phase diffuse hyperfluorescence, distortion of the large choroidal vessels, and also focal clusters of choroidal hyperfluorescence in the intermediate phase. Transient early hypofluorescence and late hyperfluorescence



(a)



(b)



(c)

Figure 3: (a) Macular serous retinal detachment in a patient with lupus choroidopathy. (b) Multiple areas of hyperfluorescence seen on fluorescein angiography caused by increased vascular permeability of the choroidal circulation. (c) Large accumulation of subretinal fluid is seen on optical coherence tomography.

are likely secondary to choroidal vascular perfusion delay with subsequent leakage due to an increase in vascular permeability, which are also observed in other vascular and inflammatory diseases. Unique findings include focal areas of hyperfluorescence in the intermediate frames, which may represent ICG staining of immune complexes [60].

Although it is a marker of high disease activity, lupus choroidopathy has been shown to be responsive to corticosteroids and other forms of immunosuppression. Given its associations with CNS and renal disease, the presence of

choroidopathy is likely an indication for aggressive, long-term immunosuppression.

5.9. Optic Nerve/Central Nervous System. Optic nerve disease is a rare manifestation of SLE and consists of optic neuritis and ischemic optic neuropathy [61]. Presenting visual acuity in SLE-associated optic neuritis is poor with most patients seeing worse than 20/200 [62]. In the Optic Neuritis Treatment Trial (ONTT), only 35.9% had a similar vision [63]. Visual recovery is variable in most patients and can range anywhere from full recovery to count fingers vision. In a study by Lin et al. [62] only 50% of patients recovered to better than 20/25, while 37.5% maintained a visual acuity worse than 20/200. In ONTT, 87% of patients recovered to better than 20/25 at 5 years of followup [63]. The increased severity of disease in SLE-associated optic neuritis compared to idiopathic optic neuritis stems from differences in pathogenesis. SLE-optic neuritis is not due to a primary inflammatory demyelinating process but rather an ischemic process that can cause subsequent demyelination and axonal necrosis. The degree of axonal loss correlates to visual outcome [64]. Luckily, the optic neuritis responds dramatically to corticosteroid treatment [65]. Early diagnosis and prompt treatment with high-dose corticosteroids is associated with better visual outcomes [62].

The neuromyelitis optica spectrum disorders (NMOSDs) are characterized by a combination of optic neuritis and transverse myelitis. Few cases have been reported in the literature of the presentation of NMOSD in SLE [66–68]. A recent paper by Jarius et al. [69] demonstrates a high association of aquaporin-4 antibodies in patients with connective tissue disease and symptoms suggestive of NMOSD. The antibodies cause tissue destruction by complement activation. Aquaporin-4 antibody positivity has important clinical implications as it is associated with a relapsing course of myelitis and optic neuritis and can lead to blindness and immobility quickly if not treated [70]

Optic neuropathy in SLE is caused by an ischemic process that affects the small vessels supplying both the optic nerve head and retrobulbar nerve. It usually presents as an acute loss of vision with an altitudinal visual field defect with or without optic disc edema. The disease is most commonly bilateral except in patients with circulating antiphospholipid antibodies. In this subset, a focal thrombotic event in the ciliary vasculature is thought to occur as opposed to a generalized vasculitis [71]. Standard treatment for lupus optic neuropathy includes intravenous high-dose corticosteroids followed by an extended oral taper [72]. Other studies have shown success with other immunosuppressive agents such as cyclophosphamide, cyclosporine, methotrexate, and azathioprine [73, 74].

Eye movement abnormalities are common in SLE and have been reported in up to 29% of patients [75]. Ischemic microvascular disease of the brainstem is usually the etiology. Sixth nerve palsies are the most common cause of disconjugate gaze abnormalities [75], while internuclear ophthalmoplegia is the most common cause of conjugate gaze abnormalities [76–78].

Retrochiasmal involvement can cause visual hallucinations, visual field defects, nystagmus, and cortical blindness. Few cases of idiopathic intracranial hypertension have been reported. 60% of cases reported in the literature are associated with antiphospholipid antibodies [79].

6. Therapeutic Considerations

Treatment options for SLE range from nonsteroidal anti-inflammatory drugs, corticosteroids, antimalarials, immunomodulatory, and biologic agents. Significant ocular involvement—orbital inflammation, scleritis, retinal vasculitis, choroiditis, and optic neuritis—warrants systemic therapy. The goal of treatment is to suppress immune activity, specifically decreasing the level of autoantibodies.

Corticosteroids are the mainstay and most effective short-term therapy for SLE [80]. They inhibit both the innate and adaptive immune response by preventing proliferation and inducing apoptosis of T cells, B cells, and macrophages as well as reducing levels of cytokines and prostaglandins [81, 82]. Generally, by the time patients present with ocular manifestation, they have a high level of systemic inflammation. Previous papers have shown that a high correlation exists between CNS vasculitis and retinal vasculitis [49] as well as nephritis and choroiditis [56]. Nguyen et al. recently described a series of four of 28 patients with choroidopathy who died from lupus-related complications [83]. Early and aggressive treatment is needed for this group to prevent increased morbidity and mortality. Thus, it is of extreme importance that patients presenting with severe ocular manifestations be treated with high-dose oral or even IV steroids early on in the disease course. Periocular steroid injections may have a role in unilateral/asymmetric disease; however, they should be used cautiously and avoided in patients with scleritis.

Steroid-sparing immunosuppressive agents are used in a large amount of patients secondary to treatment failure or harmful side effects of corticosteroids. Antimalarials such as chloroquine and more commonly hydroxychloroquine are often used. These medications are highly efficacious in curtailing future flares with fewer side effects compared to other immunomodulatory drugs such as alkylating agents. However, ocular effects of these drugs are well known. Irreversible vision loss secondary to a drug-induced maculopathy has been well documented in the literature. Factors associated with high risk of developing maculopathy include greater than 5–7 years of therapy, greater than a cumulative dose of 1000 g of hydroxychloroquine, impairment of liver or kidney function, obesity, age greater than 65, and preexisting retinopathy. The American Academy of Ophthalmology recommends a baseline-dilated eye exam on all patients starting hydroxychloroquine followed by annual exams starting at 5 years after initiating therapy. A Humphrey 10–2 automated visual field test along with multi-focal electroretinogram, spectral domain optical coherence tomography, or fundus autofluorescence should be performed at each of these visits. Discontinuation of the drug should be recommended at the earliest sign of toxicity [84]. Unfortunately, cases of

progression of retinopathy despite cessation of therapy have been reported [85].

Methotrexate, azathioprine, mycophenolate mofetil, cyclosporine A, cyclophosphamide, and chlorambucil have all been employed with varying degrees of success.

In the past few years, newer drugs, categorized as biological agents, have emerged targeting specific molecules involved in B- and T-cell activation. One of the first to be utilized in SLE was rituximab, a chimeric murine/human anti-CD20 antibody. Multiple studies have shown clinical improvement in refractory patients [86, 87]. Rituximab has also shown efficacy in treating noninfectious forms of ocular inflammation including that secondary to SLE [88].

7. Conclusions

In summary, a myriad of ocular manifestations of systemic lupus erythematosus have been described, and in some patients, these findings may be a presenting sign of systemic disease. Moreover, their presence can be a sign or a marker of disease activity. In the cases of choroidopathy and retinopathy, ophthalmic findings can be a poor prognostic systemic risk factor with the potential for both ophthalmic and systemic morbidity. For this reason, treatment typically involves a considered assessment of both the systemic and ophthalmic findings in determining the proper therapy and duration of treatment. Close communication between the consultant ophthalmologist and treating rheumatologist is critical in the effective management of these complex clinical situations.

Conflict of Interests

This manuscript has not been previously submitted to any other journal. The authors have no financial conflict of interests.

Funding

This research was funded in part by an unrestricted grant from Research to Prevent Blindness (Emory Eye Center, Emory University School of Medicine).

References

[1] M. Tikly and S. V. Navarra, "Lupus in the developing world—is it any different?" *Best Practice and Research*, vol. 22, no. 4, pp. 643–655, 2008.

[2] D. P. D'Cruz, M. A. Khamashta, and G. R. Hughes, "Systemic lupus erythematosus," *The Lancet*, vol. 369, no. 9561, pp. 587–596, 2007.

[3] K. E. Sullivan, "Genetics of systemic lupus erythematosus: clinical implications," *Rheumatic Disease Clinics of North America*, vol. 26, no. 2, pp. 229–256, 2000.

[4] M. J. Walport, C. M. Black, and J. R. Batchelor, "The immunogenetics of SLE," *Clinics in Rheumatic Diseases*, vol. 8, no. 1, pp. 3–21, 1982.

[5] M. J. Walport, "Complement and systemic lupus erythematosus," *Arthritis Research*, vol. 4, supplement 3, pp. S279–S293, 2002.

[6] D. Koffler, P. H. Schur, and H. G. Kunkel, "Immunological studies concerning the nephritis of systemic lupus erythematosus," *Journal of Experimental Medicine*, vol. 126, no. 4, pp. 607–624, 1967.

[7] J. H. M. Berden, R. Licht, M. C. J. Van Bruggen, and W. J. M. Tax, "Role of nucleosomes for induction and glomerular binding of autoantibodies in lupus nephritis," *Current Opinion in Nephrology and Hypertension*, vol. 8, no. 3, pp. 299–306, 1999.

[8] J. L. Michaud, L. I. Lemieux, M. Dubé, B. C. Vanderhyden, S. J. Robertson, and C. R. J. Kennedy, "Focal and segmental glomerulosclerosis in mice with podocyte-specific expression of mutant α-actinin-4," *Journal of the American Society of Nephrology*, vol. 14, no. 5, pp. 1200–1211, 2003.

[9] R. Rubin, "Drug induced lupus," in *Dubois' Lupus Erythematosus*, B. H. Wallace and D. J. Hahn, Eds., pp. 885–916, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 6th edition, 2002.

[10] J. P. Buyon, M. A. Petri, M. Y. Kim et al., "The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial," *Annals of Internal Medicine*, vol. 142, no. 12 I, 2005.

[11] P. Stavrou, P. I. Murray, K. Batta, and C. Gordon, "Acute ocular ischaemia and orbital inflammation associated with systemic lupus erythematosus," *British Journal of Ophthalmology*, vol. 86, no. 4, pp. 474–475, 2002.

[12] B. S. Grimson and K. B. Simons, "Orbital inflammation, myositis, and systemic lupus erythematosus," *Archives of Ophthalmology*, vol. 101, no. 5, pp. 736–738, 1983.

[13] S. Serop, R. N. G. Vianna, M. Claeys, and J. J. De Laey, "Orbital myositis secondary to systemic lupus erythematosus," *Acta Ophthalmologica*, vol. 72, no. 4, pp. 520–523, 1994.

[14] H. L. Arnold Jr, "Lupus erythematosus profundus, commentary and report of four more cases," *Archives of Dermatology*, vol. 73, no. 1, pp. 15–33, 1956.

[15] A. García-Díez and J. Fraga, "Lupus erythematosus panniculitis," *Dermatologic Clinics*, vol. 26, no. 4, pp. 453–463, 2008.

[16] D. R. Jordan, H. McDonald, B. Olberg, D. McKim, and R. McKendry, "Orbital panniculitis as the initial manifestation of systemic lupus erythematosus," *Ophthalmic Plastic and Reconstructive Surgery*, vol. 9, no. 1, pp. 71–75, 1993.

[17] R. E. Feinfield, R. J. Hesse, and S. A. Rosenberg, "Orbital inflammatory disease associated with systemic lupus erythematosus," *Southern Medical Journal*, vol. 84, no. 1, pp. 98–99, 1991.

[18] S. Siebert and U. Srinivasan, "Proptosis can be the presenting feature of systemic lupus erythematosus," *Annals of the Rheumatic Diseases*, vol. 63, no. 8, pp. 908–909, 2004.

[19] B. P. Arthurs, M. K. Khalil, F. Chagnon, S. K. Lindley, D. P. Anderson, and M. Burnier, "Orbital infarction and melting in a patient with systemic lupus erythematosus," *Ophthalmology*, vol. 106, no. 12, pp. 2387–2390, 1999.

[20] T. Y. Kao, M. K. Yoon, T. J. McCulley, B. S. Ruben, and T. N. Hwang, "Acquired enophthalmos in lupus erythematosus profundus," *Journal of Neuro-Ophthalmology*, vol. 30, no. 1, pp. 64–66, 2010.

[21] E. L. Tuffanelli and D. L. Dubois, "Cutaneous manifestations of systemic lupus erythematsus," *Archives of Dermatology*, vol. 90, pp. 377–386, 1964.

[22] J. A. Gómez-Puerta, S. Levy, M. A. Khamashta, and G. R. V. Hughes, "Periorbital oedema in systemic lupus erythematosus," *Lupus*, vol. 12, no. 11, pp. 866–869, 2003.

[23] T. Gupta, M. Beaconsfield, G. E. Rose, and D. H. Verity, "Discoid lupus erythematosus of the periorbita: clinical dilemmas, diagnostic delays," *Eye*, vol. 26, no. 4, pp. 609–612, 2012.

[24] V. Gunasekera, H. Jayaram, S. Kashani, N. M. G. Toma, and J. M. Olver, "Refractory discoid lupus erythematosis of the eyelid successfully treated with intra-lesional triamcinolone," *Eye*, vol. 22, no. 9, pp. 1205–1206, 2008.

[25] P. B. Donzis, M. S. Insler, D. M. Buntin, and L. E. Gately, "Discoid lupus erythematosus involving the eyelids," *American Journal of Ophthalmology*, vol. 98, no. 1, pp. 32–36, 1984.

[26] C. Huey, F. A. Jakobiec, T. Iwamoto, R. Kennedy, E. R. Farmer, and R. Green, "Discoid lupus erythematosus of the eyelids," *Ophthalmology*, vol. 90, no. 12, pp. 1389–1398, 1983.

[27] N. Acharya, R. Pineda, H. S. Uy, and C. S. Foster, "Discoid lupus erythematosus masquerading as chronic blepharoconjunctivitis," *Ophthalmology*, vol. 112, no. 5, pp. e19–e23, 2005.

[28] J. L. Jensen, H. O. Bergem, I. M. Gilboe, G. Husby, and T. Axéll, "Oral and ocular sicca symptoms and findings are prevalent in systemic lupus erythematosus," *Journal of Oral Pathology and Medicine*, vol. 28, no. 7, pp. 317–322, 1999.

[29] M. N. Manoussakis, C. Georgopoulou, E. Zintzaras et al., "Sjögren's syndrome associated with systemic lupus erythematosus: clinical and laboratory profiles and comparison with primary sjögren's syndrome," *Arthritis and Rheumatism*, vol. 50, no. 3, pp. 882–891, 2004.

[30] D. Xu, X. Tian, W. Zhang, X. Zhang, B. Liu, and F. Zhang, "Sjögren's syndrome-onset lupus patients have distinctive clinical manifestations and benign prognosis: a case-control study," *Lupus*, vol. 19, no. 2, pp. 197–200, 2010.

[31] J. Y. Oh, M. K. Kim, H. J. Choi et al., "Investingating the relationship between serum interluekin-17 levels and systemic immune-mediated diseases in patients with dry eye syndrome," *Korean J Ophthalmol*, vol. 25, no. 2, pp. 73–76, 2011.

[32] P. Frith, S. M. Burge, P. R. Millard, and F. Wojnarowska, "External ocular finidngs in lupus erythematosus: a clinical and immunopathological study," *British Journal of Ophthalmology*, vol. 74, no. 3, pp. 163–167, 1990.

[33] A. Heiligenhaus, J. E. Dutt, and C. Stephen Foster, "Histology and immunopathology of systemic lupus erythematosus affecting the conjunctiva," *Eye*, vol. 10, no. 4, pp. 425–432, 1996.

[34] R. Sitaula, D. N. Shah, and D. Singh, "The spectrum of ocular involvement in systemic lupus erythematosus in a teriary eye care center in Nepal," *Ocular Immunology and Inflammation*, vol. 19, no. 6, pp. 422–425, 2011.

[35] R. W. Read, A. H. Weiss, and D. D. Sherry, "Episcleritis in childhood," *Ophthalmology*, vol. 106, no. 12, pp. 2377–2379, 1999.

[36] E. M. Messmer and C. S. Foster, "Vasculitic peripheral ulcerative keratitis," *Survey of Ophthalmology*, vol. 43, no. 5, pp. 379–396, 1999.

[37] J. H. Varga and T. C. Wolf, "Bilateral transient keratoendotheliitis associated with systemic lupus erythematosus," *Annals of Ophthalmology*, vol. 25, no. 6, pp. 222–223, 1993.

[38] P. Ruusuvaara and K. Setala, "Keratoendotheliitis fugax hereditaria. A clinical and specular microscopic study of a family with dominant inflammatory corneal disease," *Acta Ophthalmologica*, vol. 65, no. 2, pp. 159–169, 1987.

[39] A. T. Yazici, N. Kara Yuksel K, H. Altinkaynak, O. Baz, E. Bozkurt, and A. Demirok, "The biomechanical properties of the cornea in patients with systemic lupus erythematosus," *Eye*, vol. 25, no. 8, pp. 1005–1009, 2011.

[40] A. Y. Nemet, S. Vinker, I. Bahar, and I. Kaiserman, "The association of keratoconus with immune disorders," *Cornea*, vol. 29, no. 11, pp. 1261–1264, 2010.

[41] F. J. Stafford-Brady, M. B. Urowitz, D. D. Gladman, and M. Easterbrook, "Lupus retinopathy. Patterns, associations, and prognosis," *Arthritis and Rheumatism*, vol. 31, no. 9, pp. 1105–1110, 1988.

[42] O. Ushiyama, K. Ushiyama, S. Koarada et al., "Retinal disease in patients with systemic lupus erythematosus," *Annals of the Rheumatic Diseases*, vol. 59, no. 9, pp. 705–708, 2000.

[43] J. S. Aiello, "Ocular findings in lupus erythematosus," *American Journal of Ophthalmology*, vol. 35, no. 6, pp. 837–843, 1952.

[44] A. J. Aronson, N. G. Ordonez, K. R. Diddie, and J. T. Ernest, "Immune-complex deposition in the eye in systemic lupus erythematosus," *Archives of Internal Medicine*, vol. 139, no. 11, pp. 1312–1313, 1979.

[45] J. G. Lanham, T. Barrie, E. M. Kohner, and G. R. V. Hughes, "SLE retinopathy: evaluation by fluorescein angiography," *Annals of the Rheumatic Diseases*, vol. 41, no. 5, pp. 473–478, 1982.

[46] F. J. Stafford-Brady, M. B. Urowitz, D. D. Gladman, and M. Easterbrook, "Lupus retinopathy. Patterns, associations, and prognosis," *Arthritis and Rheumatism*, vol. 31, no. 9, pp. 1105–1110, 1988.

[47] A. Montehermoso, R. Cervera, J. Font et al., "Association of antiphospholipid antibodies with retinal vascular disease in systemic lupus erythematosus," *Seminars in Arthritis and Rheumatism*, vol. 28, no. 5, pp. 326–332, 1999.

[48] A. Au and J. O'Day, "Review of severe vaso-occlusive retinopathy in systemic lupus erythematosus and the antiphospholipid syndrome: associations, visual outcomes, complications and treatment," *Clinical and Experimental Ophthalmology*, vol. 32, no. 1, pp. 87–100, 2004.

[49] D. A. Jabs, S. L. Fine, M. C. Hochberg, S. A. Newman, G. G. Heiner, and M. B. Stevens, "Severe retinal vaso-occlusive disease in systemic lupus erythematosus," *Archives of Ophthalmology*, vol. 104, no. 4, pp. 558–563, 1986.

[50] S. Hall, H. Buettner, and H. S. Luthra, "Occlusive retinal vascular disease in systemic lupus erythematosus," *Journal of Rheumatology*, vol. 11, no. 6, pp. 846–850, 1984.

[51] W. K. Jeon and S. Lee, "Aggravated capillary non-perfusion after intravitreal bevacizumab for macular edema secondary to systempic lupus erythematosus and anti-phospholipid syndrome," *Lupus*, vol. 21, no. 3, pp. 335–337, 2012.

[52] I. Leibovitch, M. Goldstein, A. Loewenstein, and A. Barak, "Combined central retinal artery and vein occlusion in a patient with systemic lupus erythematosus," *Rheumatology*, vol. 40, no. 10, pp. 1195–1196, 2001.

[53] A. H. Durukan, Y. Akar, Z. Bayraktar, A. Dinc, and O. F. Sahin, "Combined retinal artery and vein occlusion in a patient with asystemic lupus erythematosus and anitphospholipid syndrome," *Canadian Journal of Ophthalmology*, vol. 40, no. 1, pp. 87–89, 2005.

[54] T. G. Papadaki, I. P. Zacharopoulos, G. Papaliodis, B. Iaccheri, T. Fiore, and C. S. Foster, "Plasmapheresis for lupus retinal vasculitis," *Archives of Ophthalmology*, vol. 124, no. 11, pp. 1654–1656, 2006.

[55] E. Damato, M. Chilov, R. Lee, A. Singh, S. Harper, and A. Dick, "Plasma exchange and rituximab in the management of acute retinal vaso-occlusive disease in systemic lupus erythematosus," *Ocular Immunology and Inflammation*, vol. 19, no. 5, pp. 379–381, 2011.

[56] V. Baglio, M. Gharbiya, C. Balacco-Gabrieli et al., "Choroidopathy in patients with systemic lupus erythematosus with or without nephropathy," *Journal of Nephrology*, vol. 24, no. 4, pp. 522–529, 2011.

[57] D. Hannouche, J. F. Korobelnik, I. Cochereau et al., "Systemic lupus erythematosus with choroidopathy and serous retinal detachment," *International Ophthalmology*, vol. 19, no. 2, pp. 125–127, 1995.

[58] M. M. Schwartz and J. L. Roberts, "Membranous and vascular choroidopathy: two patterns of immune deposits in systemic lupus erythematosus," *Clinical Immunology and Immunopathology*, vol. 29, no. 3, pp. 369–380, 1983.

[59] T. Matsuo, T. Nakayama, T. Koyama, and N. Matsuo, "Multifocal pigment epithelial damages with serous retinal detachment in systemic lupus erythematosus," *Ophthalmologica*, vol. 195, no. 2, pp. 97–102, 1987.

[60] M. Gharbiya, F. Bozzoni-Pantaleoni, F. Augello, and C. Balacco-Gabrieli, "Indocyanine green angiographic findings in systemic lupus erythematosus choroidopathy," *American Journal of Ophthalmology*, vol. 134, no. 2, pp. 286–290, 2002.

[61] E. J. Feinglass, F. C. Arnett, C. A. Dorsch, T. M. Zizic, and M. B. Stevens, "Neuropyschiatric manifestations of systemic lupus erythematosus: diagnosis, clinical spectrum, and relations to other features of the disease," *Medicine*, vol. 55, no. 4, pp. 323–329, 1976.

[62] Y. C. Lin, A. G. Wang, and M. Y. Yen, "Systemic lupus erythematosus-associated optic neuritis: clinical experience and literature review," *Acta Ophthalmologica*, vol. 87, no. 2, pp. 204–210, 2009.

[63] Optic Neuritis Study Group, "The clinical profile of acute optic neuritis: experience of the optic neuritis treatment trial," *Archives of Ophthalmology*, vol. 109, no. 12, pp. 1673–1678, 1991.

[64] C. H. Smith et al., "Optic neuritis," in *Walsh and Hoyt's Clinical Neuro-Ophthalmology*, N. R. Miller and N. J. Newman, Eds., vol. 1, pp. 293–347, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 6th edition, 2005.

[65] R. M. Siatkowski, I. U. Scott, A. M. Verm et al., "Optic neuropathy and chiasmopathy in the diagnosis of systemic lupus erythematosus," *Journal of Neuro-Ophthalmology*, vol. 21, no. 3, pp. 193–198, 2001.

[66] P. Sergio, B. Mariana, O. Alberto et al., "Association of neuromyelitis optic (NMO) with autoimmune disorders: report of two cases and review of the literature," *Clinical Rheumatology*, vol. 29, no. 11, pp. 1335–1338, 2010.

[67] J. G. Kenik, K. Krohn, R. B. Kelly, M. Bierman, M. D. Hammeke, and J. A. Hurley, "Transverse myelitis and optic neuritis in systemic lupus erythematosus: a case report with magnetic resonance imaging findings," *Arthritis and Rhumatisim*, vol. 30, no. 8, pp. 947–950, 1987.

[68] J. Birnbaum and D. Kerr, "Optic neuritis and recurrent myelitis in a woman with systemic lupus erythematosus," *Nature Clinical Practice Rheumatology*, vol. 4, no. 7, pp. 381–386, 2008.

[69] S. Jarius, C. Jacobi, J. de Seze et al., "Frequency and syndrome specificity of antibodies to aquaporin-4 in neurological patients with rheumatic disorders," *Multiple Sclerosis Journal*, vol. 17, no. 9, pp. 1067–1073, 2011.

[70] B. G. Weinshenker, D. M. Wingerchuk, S. Vukusic et al., "Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis," *Annals of Neurology*, vol. 59, no. 3, pp. 566–569, 2006.

[71] D. Giorgi and C. Balacco Gabrieli, "Optic neuropathy in systemic lupus erythematosus and antiphospholipid syndrome (APS): clinical features, pathogenesis, review of the literature and proposed ophthalmological criteria for APS diagnosis," *Clinical Rheumatology*, vol. 18, no. 2, pp. 124–131, 1999.

[72] M. Frigui, F. Frikha, D. Sellemi, F. Chouayakh, J. Feki, and Z. Bahloul, "Optic neuropathy as a presenting feature of systemic lupus erythematosus: two case reports and literature review," *Lupus*, vol. 20, no. 11, pp. 1214–1218, 2011.

[73] T. D. Myers, J. R. Smith, M. S. Wertheim, R. A. Egan, W. T. Shults, and J. T. Rosenbaum, "Use of corticosteroid sparing systemic immunosuppression for treatment of corticosteroid dependent optic neuritis not associated with demyelinating disease," *British Journal of Ophthalmology*, vol. 88, no. 5, pp. 673–680, 2004.

[74] J. T. Rosenbaum, J. Simpson, and C. M. Neuwelt, "Successful treatment of optic neuropathy in association with systemic lupus erythematosus using intravenous cyclophosphamide," *British Journal of Ophthalmology*, vol. 81, no. 2, pp. 130–132, 1997.

[75] J. R. Keane, "Eye movement abnormalities in systemic lupus erythematosus," *Archives of Neurology*, vol. 52, no. 12, pp. 1145–1149, 1995.

[76] M. Galindo, J. L. Pablos, and J. J. Gomez-Reino, "Internuclear ophthalmoplegia in systemic lupus erythematosus," *Seminars in Arthritis and Rheumatism*, vol. 28, no. 3, pp. 179–186, 1998.

[77] M. S. Cogen, L. B. Kline, and E. R. Duvall, "Bilateral internuclear ophthalmoplegia in systemic lupus erythematosus," *Journal of Clinical Neuro-Ophthalmology*, vol. 7, no. 2, pp. 69–73, 1987.

[78] M. W. Meyer and J. H. Wild, "Letter: unilateral internuclear ophthalmoplegia in systemic lupus erythematosus," *Archives of Neurology*, vol. 32, no. 7, p. 486, 1975.

[79] R. Barahona-Hernando, J. J. Rios-Blanco, I. Mendez-Meson et al., "Idiopathic intracranial hypertension and systemic lupus erythematosus: a case report and review of the literature," *Lupus*, vol. 18, no. 12, pp. 1121–1123, 2009.

[80] G. C. Lo and M. S. Tsokos, "Treatment of systemic lupus erythematosus: new advances in targeted therapy," *Annals of the New York Academy of Sciences*, vol. 1247, pp. 138–152, 2012.

[81] J. S. Goodwin, D. Atluru, S. Sierakowski, and E. A. Lianos, "Mechanism of action of glucocorticosteroids. Inhibition of T cell proliferation and interleukin 2 production by hydrocortisone is reversed by leukotriene B4," *Journal of Clinical Investigation*, vol. 77, no. 4, pp. 1244–1250, 1986.

[82] R. Newton, "Molecular mechanisms of glucocorticoid action: what is important?" *Thorax*, vol. 55, no. 7, pp. 603–613, 2000.

[83] Q. D. Nguyen, H. S. Uy, E. K. Akpek, S. L. Harper, D. N. Zacks, and C. S. Foster, "Choroidopathy of systemic lupus erythematosus," *Lupus*, vol. 9, no. 4, pp. 288–298, 2000.

[84] M. F. Marmor, U. Kellner, T. Y. Y. Lai, J. S. Lyons, and W. F. Mieler, "Revised recommendations on screening for chloroquine and hydroxychloroquine retinopathy," *Ophthalmology*, vol. 118, no. 2, pp. 415–422, 2011.

[85] M. Michaelides, N. B. Stover, P. J. Francis, and R. G. Weleber, "Retinal toxicity associated with hydroxychloroquine and chloroquine: risk factors, screening, and progression despite cessation of therapy," *Archives of Ophthalmology*, vol. 129, no. 1, pp. 30–39, 2011.

[86] L. F. Pinto, C. J. Velaásquez, C. Prieto, L. Mestra, E. Forero, and J. D. Marquez, "Rituximab induces a rapid and sustained remission in Colombian patients with severe and refractory systemic lupus erythematosus," *Lupus*, vol. 20, no. 11, pp. 1219–1226, 2011.

[87] B. Terrier, Z. Amoura, P. Ravaud et al., "Safety and efficacy of rituximab in systemic lupus erythematosus: results from 136 patients from the French autoimmunity and rituximab registry," *Arthritis and Rheumatism*, vol. 62, no. 8, pp. 2458–2466, 2010.

[88] E. Miserocchi, I. Pontikaki, G. Modorati, M. Gattinara, P. L. Meroni, and V. Gerloni, "Anti-CD 20 monoclonal antibody (rituximab) treatment for inflammatory ocular diseases," *Autoimmunity Reviews*, vol. 11, no. 1, pp. 35–39, 2011.