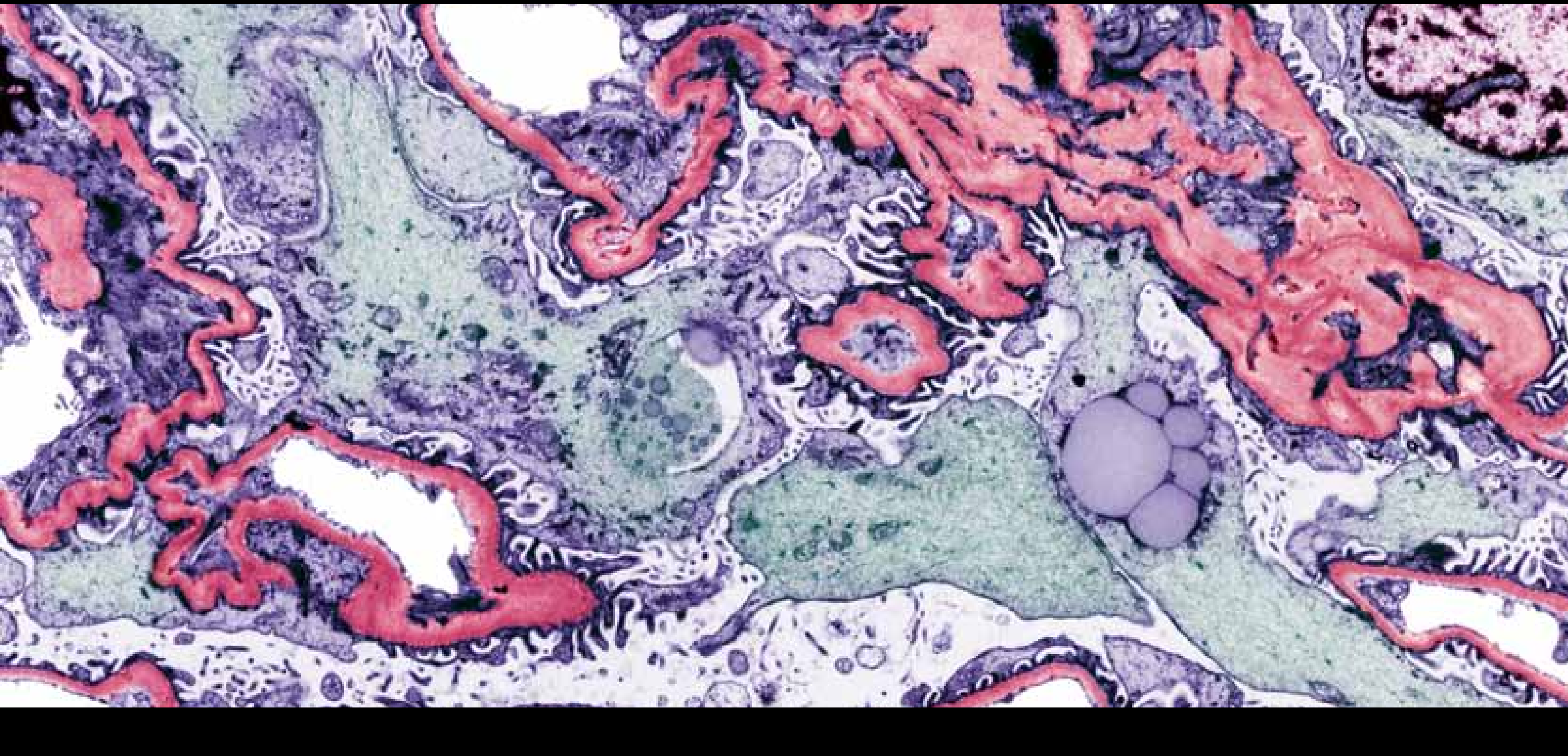
Autoimmune Diseases

Systemic Lupus Erythematosus 2014

Guest Editors: Juan-Manuel Anaya, Yehuda Shoenfeld, and Ricard Cervera



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Editorial

Systemic Lupus Erythematosus 2014

Juan-Manuel Anaya,[1,2] Yehuda Shoenfeld,[3] and Ricard Cervera[4]

[1] *Center for Autoimmune Diseases Research (CREA), School of Medicine and Health Sciences, Universidad del Rosario, 11001000 Bogota, Colombia*
[2] *Méderi Hospital Universitario Mayor, 11001000 Bogota, Colombia*
[3] *Zabludowitcz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Sackler Faculty of Medicine, Tel Aviv University, 52621 Tel-Hashomer, Israel*
[4] *Department of Autoimmune Diseases, Hospital Clinic, Calle Villarroel 170, Barcelona, Catalonia, Spain*

Correspondence should be addressed to Juan-Manuel Anaya; juan.anaya@urosario.edu.co

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Systemic lupus erythematosus (SLE, lupus) is the prototype of systemic autoimmune disease (AD). Immune system activation in SLE is characterized by exaggerated B-cell and T-cell responses and loss of immune tolerance against self-antigens. Production and defective elimination of antibodies, circulation and tissue deposition of immune complexes, and complement and cytokine activation contribute to clinical manifestations that range from fatigue and joint pain to severe, life-threatening organ damage [1].

In this special issue, nine papers were selected covering important topics of the disease from B lymphocytes (e.g., autoantibodies and B-cell depletion therapy), immunosenescence, and genetics to disease complications such as cardiovascular disease (CVD), preeclampsia, fatigue, and depression.

B lymphocytes are the effectors of humoral immunity, providing defense from pathogens through different functions including antibody production. In the context of ADs, B lymphocytes play an essential role by not only producing autoantibodies but also functioning as antigen presenting cells and as a source of cytokines as pointed out by G. J. Tobón and colleagues, who reviewed the functions of B lymphocytes in autoimmunity and ADs with a special focus on their abnormalities in SLE.

We recognize today that disease manifestations are determined by the diversity of autoantibodies appearing in SLE [2, 3]. This explains the different clinical presentations within individuals with SLE. In this issue, E. Cozzani and colleagues reviewed the most important autoantibodies in SLE and their correlation between immunopathological features and clinical aspects. Recommendations for determining antinuclear antibodies, anti-double stranded DNA antibodies, specific antibodies, and validation of methods have been published elsewhere [4].

Given that autoantibody production is the hallmark of SLE, it is not surprising that B-cell depletion therapy is a promising therapeutic option in the management of SLE. Rituximab (RTX), a chimeric anti-CD20 monoclonal antibody, has been used off-license in the management of severe refractory SLE since 2002. In this special issue, F. Bonilla-Abadía and colleagues report the results of a retrospective and descriptive observational study of patients with SLE refractory to conventional treatment who were treated with RTX as remission induction therapy and maintenance. They observed a significant reduction in the conventional immunosuppressive drug dose and the number of relapses of disease suggesting that RTX could be effective and safe in patients with SLE refractory to conventional therapy.

An important matter about SLE for 2014 will be the progress and even release of results of the ongoing trials with the new biological therapies including epratuzumab, a humanized anti-CD22 monoclonal antibody, and subcutaneous belimumab, a human monoclonal antibody that inhibits B-lymphocyte stimulator, as well as the investigator-initiated trials with RTX (i.e., RITUXILUP and Ring) [5].

Senescence is a normal biological process that occurs in all organisms and involves a decline in cell functions. In the context of the immune system, this phenomenon is known as immunosenescence and refers to the immune function deregulation. A complete review about this topic is also presented in this issue.

ADs are observed in genetically susceptible individuals in whom their clinical expression is modified by permissive and protective environments occurring over time [6]. A plethora of new susceptibility genetic variants for ADs has emerged. As per the case of SLE, more than 100 loci have been replicated by several independent studies that modify the risk to acquire the disease. In this issue, J. E. Molineros and colleagues from Oklahoma Medical Research Foundation report a replication study in which 22 recently identified SLE susceptibility genes were strongly associated with Malaysians.

In order to better understand the genetic basis of SLE that might be due to natural selection, P. S. Ramos and colleagues report an original study showing positive selection at several SLE-associated loci. Their results "provide corroborating evidence in support of recent positive selection as one mechanism underlying the elevated population frequency of SLE risk loci and should stimulate future research that integrates signals of natural selection to help identify functional SLE risk alleles."

Concerning environmental factors involved in SLE induction, we are surprised each time by novel and modern factors. One of the last ones belongs to the ASIA (Autoimmune Syndrome Induced by Adjuvants). In this sense, human papillomavirus quadrivalent (types 6, 11, 16, and 18) vaccine, recombinant (Gardasil), which became almost mandatory in many countries, has aluminum as adjuvant. It is not surprising that 6 cases of SLE following Gardasil vaccination were reported recently [7]. Further studies on this topic are expected to come to light in 2014.

CVD is a major concern in patients with SLE, whose disease expression varies depending on several factors including ancestry. J. Amaya-Amaya and colleagues report a high rate of CVD in Latin American patients with SLE and encourage preventive population strategies aimed to facilitate the suppression of cigarette smoking and coffee consumption to the tight control of dyslipidemia and other modifiable risk factors for such complication.

One of the obstetric complications of SLE is preeclampsia. A. Schramm and M. E. B. Clowse, after providing an overview of the pathogenesis of preeclampsia, preeclampsia in lupus pregnancies, and previous trials for prevention of preeclampsia with aspirin treatment, recommend low-dose aspirin administration for all lupus patients starting prior to 16 weeks of gestation. Further clinical trials are needed to confirm this recommendation.

Patients with SLE report higher levels of cognitive difficulties, depression, pain, and fatigue. R. Fonseca and colleagues report a case-control study in which significant lower scores in quality-of-life dimensions related to physical impairment were found in patients with SLE as compared with controls. Authors suggest that unexplained fatigue in SLE may signify an early sign of immune activation flare-up.

We hope readers of Autoimmune Diseases will enjoy this special issue and be encouraged to translate this new knowledge into practice.

Juan-Manuel Anaya
Yehuda Shoenfeld
Ricard Cervera

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Review Article

Aspirin for Prevention of Preeclampsia in Lupus Pregnancy

Amelie M. Schramm[1] **and Megan E. B. Clowse**[2]

[1] *Department for Internal Medicine 3 and Institute for Clinical Immunology, Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany*
[2] *Department of Medicine, Duke University Medical Center, P.O. Box 3535, Trent Drive, Durham, NC 27710, USA*

Correspondence should be addressed to Megan E. B. Clowse; megan.clowse@duke.edu

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Preeclampsia, the onset of hypertension and proteinuria during pregnancy, is a common medical disorder with high maternal and fetal mortality and morbidity. The underlying pathology remains poorly understood and includes inflammation, endothelial dysfunction, and an unbalanced thromboxane A_2/prostacyclin ratio. For women with systemic lupus erythematosus (SLE), particularly those with preexisting renal disease or with active lupus, the risk of developing preeclampsia is up to 14% higher than it is among healthy individuals. The mechanism is still unknown and the data for preventing preeclampsia in lupus pregnancies are rare. Modulating the impaired thromboxane A_2/prostacyclin ratio by administration of low-dose aspirin appears to be the current best option for the prevention of preeclampsia. After providing an overview of the pathogenesis of preeclampsia, preeclampsia in lupus pregnancies, and previous trials for prevention of preeclampsia with aspirin treatment, we recommend low-dose aspirin administration for all lupus patients starting prior to 16 weeks of gestation. Patients with SLE and antiphospholipid syndrome should receive treatment with heparin and low-dose aspirin during pregnancy.

1. Introduction

Preeclampsia is defined by an increase in blood pressure (>140/90) and proteinuria (>300 mg/24 hr) in the latter half of pregnancy. While modern obstetrical management has made it less dangerous than before, it is still associated with an increased rate of maternal and fetal mortality and is an important cause for preterm birth [1]. It is difficult to identify pregnancies at particularly high risk for preeclampsia before it presents clinically; however, there are several known risk factors. These include first birth, a first birth with a new father, prior hypertension or renal disease, diabetes, and prior preeclampsia. Both systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) put pregnancies at high risk for this complication. Uterine Doppler studies can identify pregnancies with particularly high uterine artery pressures, which may be an indicator of early placental changes that lead to preeclampsia [1]. The goal of this paper is to provide insight into the mechanisms through which SLE and APS contribute to preeclampsia and the potential role that low-dose aspirin may play in mitigating this risk.

2. Pathogenesis

2.1. Pathophysiology of Preeclampsia. As a common medical disorder during pregnancy, preeclampsia causes hypertension (systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg), proteinuria (>300 mg/24 h), and in rare cases additional symptoms like hyperreflexia, seizures (eclampsia), acute renal failure, pulmonary complications, the triad of hemolysis, elevated liver enzymes, and low platelets (HELLP syndrome).

The disease typically starts after 20 weeks of gestation and is an important factor for maternal and fetal mortality and morbidity. In the United States, around 5–8% of pregnant women are affected [2]. The only definitive treatment is delivery of the baby. While preeclampsia's underlying pathology remains poorly understood, the onset of this condition

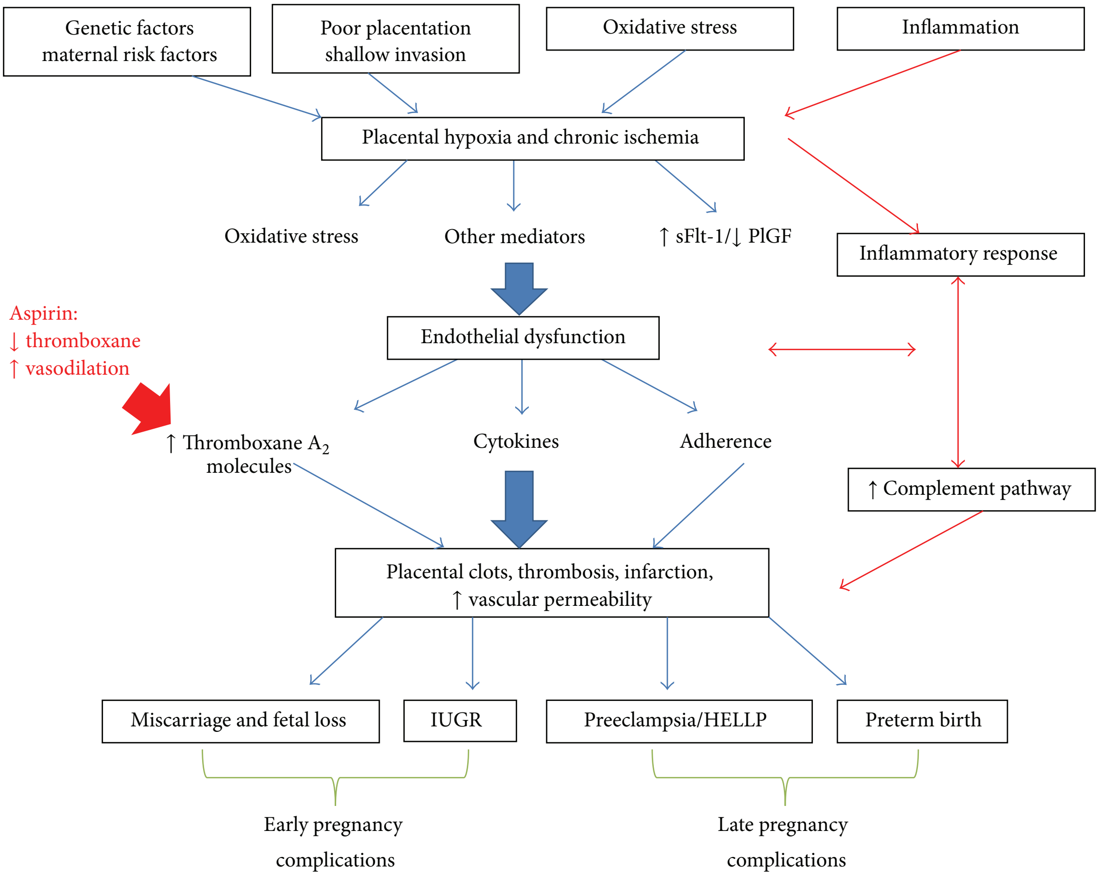
Genetic factors maternal risk factors
Poor placentation shallow invasion
Oxidative stress
Inflammation
Placental hypoxia and chronic ischemia
Oxidative stress
Other mediators
↑ sFlt-1/↓ PlGF
Inflammatory response
Aspirin:
↓ thromboxane
↑ vasodilation
Endothelial dysfunction
↑ Thromboxane A2 molecules
Cytokines
Adherence
↑ Complement pathway
Placental clots, thrombosis, infarction, ↑ vascular permeability
Miscarriage and fetal loss
IUGR
Preeclampsia/HELLP
Preterm birth
Early pregnancy complications
Late pregnancy complications

Figure 1: Mechanisms of preeclampsia.

involves shallow trophoblast invasion with poor placentation, inflammation, dysregulation of angiogenic factors, and ischemia, all of which lead to the central mechanism of endothelial dysfunction. Endothelial dysfunction causes, among other things, activation of platelets, a rise in thromboxane levels, and an ensuing clotting cascade (Figure 1).

2.1.1. Poor Placental Vascular Remodeling. Implantation and placental development happen in the first trimester of pregnancy. Fetal cytotrophoblasts invade the maternal spiral arteries and convert these high-resistance, muscular arteries to high-capacitance, elastic vessels. Insufficient spiral artery transformation is strongly associated with the pathology of severe preeclampsia. In preeclamptic placentas, shallow trophoblast invasion prevents the necessary vascular remodeling, which leads to decreased perfusion, hypoxia, and chronic placental ischemia [3].

2.1.2. Imbalance of Angiogenic Factors. Chronic ischemia caused by poor vascularization is associated with the placental production of angiogenic factors like vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and soluble fms-like tyrosine kinase-1 (sFlt-1). VEGF encourages the growth of blood vessels, supports proper function of endothelial cells, and stimulates NO production in vascular walls [4, 5]. SFlt-1 is a naturally occurring VEGF antagonist which binds free VEGF and occupies the VEGF receptor [6]. Experiments and animal models suggest that VEGF and sFlt-1 may play a role in the pathogenesis of preeclampsia [7]. During healthy pregnancies, levels of placental growth factor (PlGF) increase in the first and second trimesters and fall in the third trimester. The level of antiangiogenic factor sFlt-1 usually remains steady during the first part of pregnancy and rises during the last trimester. In blood samples of patients with preeclampsia, however, lower levels of PlGF throughout gestation and increased levels of sFlt-1 levels at 26 and 29 weeks of gestation are detectable [8–10]. An elevated sFlt-1/PlGF ratio during the second trimester, but not the first trimester, can help detect preeclampsia even before the presentation of clinical symptoms [11–13]. Increased levels of sFlt-1 also can be found in lupus patients with preeclampsia [14].

2.1.3. Inflammation. Systemic maternal inflammation is also involved in the pathogenesis of preeclampsia. Levels of circulating proinflammatory mediators like IL-6, IL-8, TNF-α, and monocyte chemoattractant protein 1 (MCP-1) are markedly higher in preeclamptic pregnancies than in healthy pregnancies [19]. Noninfectious leukocyte infiltration of the villi (fetal) and the decidua (maternal interface) was found in preeclamptic placentas, along with an uncontrolled, increased activation of the complement system, with elevated levels of complement-activation factor Bb. Activation of the complement system is commonly seen as an important mechanism linking inflammation and coagulation [20]. Inflammation may therefore be a trigger for the development of preeclampsia in patients with SLE [21].

2.1.4. Thromboxane. Poor placental perfusion leads to activation of platelets and the clotting cascade, resulting in an imbalance among vasoactive prostaglandins. The ratio between the prostaglandins thromboxane and prostacyclin modulates vascular blood flow, with thromboxane A_2 acting as a vasoconstrictor and promoting platelet aggregation, while prostacyclin acts as a vasodilator and inhibits aggregation. Increased thromboxane and reduced prostacyclin levels are associated with infarction and thrombotic vasculopathy, which are well-known features in preeclamptic placentas [22].

The constitutive enzyme cyclooxygenase 1 produces thromboxane A_2 in platelets and primarily prostacyclin in endothelial cells. Aspirin, a common irreversible inhibitor of cyclooxygenases, acts particularly in platelets. The impact of aspirin use in preeclampsia prevention is shown by data which demonstrate an aspirin-induced decrease of thromboxane concentration and mediation of the unbalanced thromboxane A_2/prostacyclin ratio [23] (Figure 2). Aspirin thus improves placental blood flow and minimizes risk of placental thrombosis, which serves as the rationale for administering prophylactic low-dose aspirin for prevention of preeclampsia.

2.2. Pregnancy in Patients with Systemic Lupus Erythematosus. Systemic lupus erythematosus (SLE) is a pervasive autoimmune disease which can affect nearly every organ and tissue in the body with an extremely broad variability in severity. Women with SLE are often diagnosed in their childbearing years [24]. As SLE usually has no influence on female fertility, pregnancies are common among these women [25].

Maternal and fetal risk for serious medical and pregnancy complications is significantly higher for women with SLE than for healthy women. National analysis showed a 20-fold higher risk for maternal mortality among lupus patients, who are also at increased risk for preterm labor (OR 2.4), Cesarean section (OR 1.7), and fetal growth restriction (OR 2.6) [26]. Compared to healthy individuals, women with SLE, especially those with preexisting renal disease or with active SLE before and during pregnancy, have a higher risk for developing preeclampsia. Up to 30% of all lupus pregnancies are complicated by preeclampsia [27, 28].

While active lupus is the main predictor for pregnancy complications, other identified risk factors are strongly associated with preeclampsia, including preexisting hypertension, antiphospholipid syndrome (APS), obesity, positive anti-double-stranded DNA antibodies (dsDNA) or anti-ribonucleoprotein (RNP) antibodies, low complement [2], and thrombocytopenia at onset of pregnancy [29]. Thrombocytopenia that occurs in lupus pregnancy before 15 weeks of gestation is usually due to SLE activity (platelet-specific antibodies) or APS. After 25 weeks, low platelet counts are more commonly caused by preeclampsia/HELLP syndrome [27].

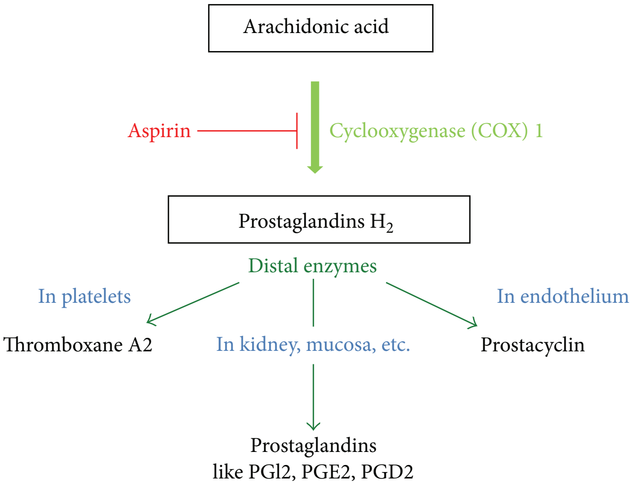

Figure 2: Interaction of aspirin with COX 1.

Differentiating between lupus activity and pregnancy-related complications presents a major challenge in the management of SLE pregnancies. In both SLE and preeclampsia, for example, women develop worsening proteinuria with hypertension and edema. Yet it remains essential to accurately determine whether symptoms are due to lupus or to preeclampsia, given the different treatments required for preeclampsia (delivery) and SLE (immunosuppression) [2]. Decreased levels of complement and active urine sediment could suggest lupus nephritis, whereas elevated serum uric acid and low urine calcium are more typical for preeclampsia. Additionally, the presence of concomitant lupus symptoms—like arthritis, serositis, skin lesions, or rising levels of dsDNA-antibodies—could point to SLE activity.

While women who experience increased SLE activity during pregnancy are the most at risk, even those with quiescent lupus are more likely to develop preeclampsia than healthy women. This suggests that SLE and preeclampsia may share a common underlying pathology.

2.2.1. Endothelial Dysfunction. Chronic autoimmune diseases with systemic inflammation lead to an increased risk for cardiovascular disease (CVD), with atherosclerosis and vascular alterations through changes in vascular adhesion molecules, increased transendothelial permeability, impaired antithrombotic properties, and reduced production of vasodilators and thrombomodulin expression. Thrombomodulin limits aggregation of platelets and activation of the complement pathway. The presence of proinflammatory cytokines, however, lowers thrombomodulin and increases levels of the procoagulant tissue factor [30].

Impaired endothelial repair is also linked to endothelial dysfunction in SLE. While patients with SLE have similar numbers of endothelial progenitor cells compared to healthy people, these cells exhibit impaired migratory and adhesive properties [31]. We suggest that the endothelial dysfunction inherent in SLE may contribute to the risk of preeclampsia in this population.

Table 1: Treatment of anti-phospholipid antibodies in pregnancy.

Clinical presentation	Suggested treatment
Patients with aPL and no history of thrombosis and no series of fetal loss or early delivery due to preeclampsia or placental insufficiency	Addition of low-dose aspirin throughout pregnancy
Patients with APS and no history of thrombosis but with previous historyof stillbirth, recurrent fetal loss, or other APS-associated pregnancy complications	Heparin or LMWH (usual prophylactic dose) during pregnancy and 6 weeks postpartum Low-dose aspirin throughout pregnancy
Patients with APS and prior history of thrombosis or embolism	Heparin or LMWH (usual therapeutic dose) during pregnancy and 6 weeks postpartum followed by optional conversion on warfarin Low-dose aspirin throughout pregnancy

2.2.2. Inflammation. During pregnancy, T-cells play an important role in modulating the maternal immune system as it adapts to a semiallogeneic fetus [32]. Fewer regulatory T-cells (Treg) and increased T helper-17 cell (Th17) activity have been found in women with preeclampsia. Similar changes are common in active SLE. One study of SLE pregnancies, for example, documented particularly low levels of Treg cells in the context of preeclampsia [33–36]. We suggest that the immune dysregulation seen in SLE may contribute to the risk of preeclampsia. Reduced systemic inflammation and normalized T-cell activity during lupus quiescence may lead to a reduction of pregnancy complications in the absence of lupus activity.

2.3. Antiphospholipid Syndrome. Antiphospholipid syndrome (APS) is a prothrombotic disorder which can cause thrombosis, embolism, or stroke and is highly associated with pregnancy complications like miscarriage and preeclampsia. The antibodies of APS are circulating anti-phospholipid antibodies (aPL), which are characterized as anti-cardiolipin antibodies (aCL), lupus-anticoagulant (LAC), and β2-glycoprotein antibodies (anti-β2-GPI). Patients are diagnosed by the combination of detectable antibodies and clinical findings (including thrombosis, embolism, and stroke) or obstetrical failures (including three sequential early pregnancy losses, a second- or third-trimester loss, or severe early preeclampsia).

2.3.1. APS and Pregnancy. The risk for preeclampsia is more than ninefold higher in APS patients than in healthy women [37]. The pregnancy complications of APS appear to result from the interaction of prothrombotic factors, inflammation, and trophoblast pathologies. Phospholipid-binding proteins—annexin V, protein C, prothrombin, or anti-β2-GPI—are antigen targets for aPL. Whereas most human cells translocate β2-glycoprotein on their surface only during apoptosis or pathological conditions, trophoblasts continually present β2-GPI on their cell membranes. This could explain β2-GPI placental tropism and placenta-related pregnancy complications in women with APS [38].

Clots, thrombosis, and placental infarction due to the presence of antibodies and the following platelet activation are common in APS placentas. While aPL does not react with resting endothelial cells, Chen et al. found that a triggering event such as phagocytosis of necrotic trophoblastic debris allows aPL to influence endothelial cells for a prolonged period. After a trigger event, aPL can maintain activation of endothelial cells even without the further presence of necrotic trophoblastic debris. Chronic activation of the endothelium leads to an imbalance between thromboxane A_2 and prostacyclin [39]. Correction of this imbalance by low-dose aspirin may explain the benefit of aspirin administration in APS pregnancy.

Pregnant women with anti-phospholipid antibodies are at risk for catastrophic APS (CAPS), which presents with rapid evolution of thrombosis, often microthrombi, in 3 or more organs. CAPS is deadly: up to half of women who develop this in pregnancy die, as do half of the infants. The role that aspirin might play in the prevention or treatment of CAPS is unclear, but aggressive anticoagulation, plasmapheresis, and immunosuppression are all suggested therapies. CAPS may present concurrently with preeclampsia and/or HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome [40].

Treatment guidelines suggest combining therapies of low-dose aspirin and heparin for all APS patients during pregnancy (Table 1). Depending on the patient's history of thrombosis, miscarriage, or other pregnancy complications, the medication may be extended to anticoagulation with full therapeutic dose [41, 42].

Studies have documented a decrease in pregnancy loss among patients with APS who receive heparin treatment, but heparin does not appear to be as effective in preventing late pregnancy complications such as preeclampsia [42]. In addition to its anticoagulant properties, heparin can interrupt the interaction between aPL and β2-GPI, inhibit the complement pathway, and provide supplemental anti-inflammatory effects, but it does not influence trophoblast migration or placentation. This may explain heparin's lack of significant benefit for prevention of late pregnancy complications like preeclampsia, despite its effective prevention of early pregnancy problems in APS patients [38]. Aspirin, on the other hand, may play a role in preventing late pregnancy complications.

Table 2: Data from meta-analyses of aspirin for preeclampsia prevention.

Meta-analysis (first author)	Onset of treatment	Inclusion criteria	Intervention	Methods	Results
Duley et al. Cochrane Review 2007 [15]	Before and after 20 weeks of gestation	*High risk criteria*: Previous severe preeclampsia Chronic hypertension Renal disease Autoimmune disease Diabetes	Antiplatelet agent (low dose aspirin or dipyridamole) versus placebo or no antiplatelet agent	59 trials (37,560 women) with low, moderate, and high risk groups treated with or without antiplatelet agents *Outcome*: preeclampsia Secondary outcome: preterm birth and neonatal outcome	17% risk reduction with use of antiplatelet agents (RR 0.83, 95% CI 0.77, 0.89)
Trivedi 2011 [16]	7–32 weeks of gestation	*High risk criteria*: Previous severe preeclampsia Essential hypertension Underlying vascular disorder Gestational diabetes mellitus Maternal age > 40 Positive Doppler ultrasonography	Low-dose aspirin 40–160 mg versus placebo	19 trials with low risk group (16,550 women) and high risk group (11,687 women) for developement of preeclampsia Each group treated with low-dose aspirin or placebo *Outcome*: preeclampsia Secondary outcome: preterm delivery (<37 week) and IUGR	*High risk group*: preeclampsia incidence: 10.7% low-dose aspirin group, 12.5% placebo group → risk reduction of preeclampsia with low-dose aspirin: 21% (RR 0.79, 95% CI 0.65, 0.97) 16% reduction in risk for preterm delivery (RR 0.84, 95% CI 0.71, 0.99) *Low risk group*: preeclampsia incidence: 4.3% low-dose aspirin group, 4.4% placebo group → no significant risk reduction of preeclampsia wtih low-dose aspirin (RR 0.86, 95% CI 0.64, 1.17) 2% reduction in risk for preterm delivery (RR 0.98, 95% CI 0.90, 1.07)
Roberge et al. 2012 [17]	Before 16 weeks of gestation	*Risk factors*: Chronic hypertension Previous severe preeclampsia Abnormal uterine doppler Obesity First pregnancy Sjögren Syndrome	Low dose asprin 50–150 mg versus placebo	5 trials with 556 women at risk of preeclampsia treated with low dose aspirin or placebo *Outcome*: preterm and term preeclampsia	Risk reduction of preterm preeclampsia with low dose aspirin: 89% (RR 0.11, 95% CI 0.04, 0.33) No effects of low dose aspirin on term preeclampsia (RR 0.98, 95% CI 0.42, 2.33)
Villa et al. 2013 [18]	At/before 16 weeks of gestation	*Risk factors*: Abnormal uterine artery doppler flow velocimetry	Low dose aspirin 50–150 mg versus placebo/no treatment	346 women treated with aspirin or placebo *Outcome*: preeclampsia Secondary outcome: preterm (<37 week), term or severe preeclampsia	Low dose aspirin group: significant reduced risk of preeclampsia (RR 0.6, 95% CI 0.37–0.83) and severe preeclampsia (RR 0.3, 95% CI 0.11–0.69)

3. Clinical Trials of Low-Dose Aspirin Administration for Prevention of Preeclampsia

Until recently, preeclampsia has been resistant to preventive treatment. Low-dose aspirin, however, has shown beneficial effects in a wide range of clinical trials for prevention of placenta-associated pregnancy complications. While the definition of high risk for preeclampsia has not been entirely consistent between studies, patients identified as high risk typically have a history of preeclampsia or fetal growth restriction, abnormal uterine artery Doppler, essential hypertension, obesity, and/or diabetes mellitus. A few studies also included women with underlying vascular disorders or autoimmune diseases, but the number of patients studied with rheumatologic disease is very small.

Two meta-analyses demonstrated beneficial effects of aspirin for prevention of preeclampsia: among women at high risk for this complication, antiplatelet treatment reduced the risk by 17–21% (Table 2) [15, 16]. In Trivedi's analysis, the incidence of preeclampsia in the high-risk group was 10.7% with low-dose aspirin administration and 12.5% with placebo administration (RR 0.79, $P = 0.02$). The low-risk group, however, showed no significant differences in preeclampsia incidence with aspirin (4.3%) and with placebo (4.4%, RR 0.86, $P = 0.35$). These meta-analyses included fairly heterogeneous studies, with low-dose aspirin initiation ranging from 7 to 32 weeks of gestation.

More robust findings emerged from two meta-analyses that were restricted to studies in which low-dose aspirin was started prior to 16 weeks of gestation. One analysis found that while low-dose aspirin introduced <16 weeks of gestation decreased the risk for severe preeclampsia, perinatal death, and fetal growth restriction, low-dose aspirin initiation after 16 weeks of gestation did not provide this protective effect [17]. Villa et al. demonstrated significantly reduced risk for preeclampsia (RR 0.6, CI 0.27–0.83) and severe preeclampsia (RR 0.3, CI 0.11–0.69) with low-dose aspirin administration in women with abnormal uterine artery flow [18]. Roberge et al. found that low-dose aspirin administration resulted in an 89% risk reduction for preterm preeclampsia but did not decrease risk for term preeclampsia. Based on this data, it appears that early initiation of low-dose aspirin is important and that it may be most effective in preventing preterm and severe preeclampsia [17].

The range of aspirin dosages with positive effects appears to be quite flexible. Across studies, the administered low-dose aspirin dose was between 40 and 160 mg/day, yet there was no difference in potency [16]. Previous trials also investigated the maternal and neonatal outcomes of pregnancies exposed to low-dose aspirin, and treatment appears to be safe for both mother and newborn [15, 43]. Compared to women in the control groups, for example, pregnant women treated with low-dose aspirin experienced no significant difference in risk of maternal or neonatal bleeding [16]. Case-controlled data also showed no increased risk of congenital abnormalities [44]. And, unlike high-dose NSAIDs, low-dose aspirin does not appear to increase the risk for ductus arteriosus closure *in utero* [45]. Analysis for preconceptional low-dose aspirin administration for prevention of preeclampsia after *in vitro* fertilization (IVF) found no significant reduction of hypertensive pregnancy complications compared to placebo group [22].

4. Conclusion: Impact of Anticoagulation for Prevention of Preeclampsia in Lupus Patients

The task of lowering the risk for preeclampsia in women with SLE is challenging. Maintaining SLE quiescence may reduce this risk by minimizing the impact of chronic inflammation, but endothelial dysfunction due to systemic lupus is not currently amenable to treatment. Aspirin, however, can interfere with the subsequent pathological process of a vasoconstrictive, procoagulant, and platelet-activating state and may prevent preeclampsia by modulating the thromboxane A_2/prostacyclin ratio to optimize placental blood flow and prevent placental thrombosis.

While aspirin will not eliminate all cases of preeclampsia, it is currently the best and safest available drug for influencing the pathogenesis and clinical presentation of preeclampsia. Although there is no trial evidence for the use of low-dose aspirin to prevent preeclampsia in SLE, aspirin could lower the risk for lupus patients to an extent comparable to the risk reduction demonstrated in trials assessing low-dose aspirin treatment for other high-risk groups. Therefore, we would expect aspirin treatment to offer a risk reduction of up to 20% for preeclampsia development in lupus patients, suggesting the possibility of lowering preeclampsia incidence from 15% in all lupus pregnancies to around 12% in lupus patients with low-dose aspirin treatment.

4.1. Preeclampsia Prophylaxis in Lupus Pregnancies. Based on the pathogenic role of thromboxane in placental perfusion and the higher incidence of preeclampsia in SLE pregnancy, we recommend low-dose aspirin administration for all pregnant women with SLE, with therapy being initiated prior to 16 weeks of gestation and continuing throughout pregnancy. Women with SLE and APS should continue aspirin treatment as a preeclampsia prophylaxis and add heparin or LMWH. For pregnant women without a history of thrombosis, lower prophylactic dosing of heparin or LMWH is appropriate, but for pregnant women with a history of thrombosis, heparin administration should be increased to a full antithrombotic dose.

A future clinical trial of the use of aspirin as preeclampsia prevention in SLE should be performed to generate more exact recommendations.

Conflict of Interests

Megan E. B. Clowse serves as a consultant for UCB but on issues separate from those discussed in this paper. Schramm has no conflict of interests.

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Research Article

Evaluation of SLE Susceptibility Genes in Malaysians

Julio E. Molineros,[1] Kek Heng Chua,[2] Celi Sun,[1] Lay Hoong Lian,[3] Prasenjeet Motghare,[1] Xana Kim-Howard,[1] and Swapan K. Nath[1]

[1] *Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, 1025 NE 13th Street, Oklahoma City, OK 73104, USA*
[2] *Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[3] *Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

Correspondence should be addressed to Swapan K. Nath; swapan-nath@omrf.org

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Systemic Lupus Erythematosus (SLE) is a clinically heterogeneous autoimmune disease with strong genetic and environmental components. Our objective was to replicate 25 recently identified SLE susceptibility genes in two distinct populations (Chinese (CH) and Malays (MA)) from Malaysia. We genotyped 347 SLE cases and 356 controls (CH and MA) using the ImmunoChip array and performed an admixture corrected case-control association analysis. Associated genes were grouped into five immune-related pathways. While CH were largely homogenous, MA had three ancestry components (average 82.3% Asian, 14.5% European, and 3.2% African). Ancestry proportions were significantly different between cases and controls in MA. We identified 22 genes with at least one associated SNP ($P < 0.05$). The strongest signal was at HLA-DRA ($P_{\text{Meta}} = 9.96 \times 10^{-9}$; $P_{\text{CH}} = 6.57 \times 10^{-8}$, $P_{\text{MA}} = 6.73 \times 10^{-3}$); the strongest non-HLA signal occurred at *STAT4* ($P_{\text{Meta}} = 1.67 \times 10^{-7}$; $P_{\text{CH}} = 2.88 \times 10^{-6}$, $P_{\text{MA}} = 2.99 \times 10^{-3}$). Most of these genes were associated with B- and T-cell function and signaling pathways. Our exploratory study using high-density fine-mapping suggests that most of the established SLE genes are also associated in the major ethnicities of Malaysia. However, these novel SNPs showed stronger association in these Asian populations than with the SNPs reported in previous studies.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a heterogeneous autoimmune disease, in terms of both clinical presentation and incidence and severity across ethnically diverse populations. Asians are among those with a greater risk of SLE and have more severe disease presentations such as lupus nephritis [1]. SLE has strong and complex genetic components. While several genomewide association studies (GWAS) have been reported for European SLE populations, few Asian GWAS have been performed [2–4]. Among European identified SLE loci were HLA loci *HLA-DRA* [5] and *ATG5* [5], immune signal transduction loci *BANK1* [6], *BLK* [5], *LYN* [5], TLR, and IFN pathway related loci *IFIH1* [7], *STAT4* [8], *TNFAIP3* [9], *IRF7* [5], *IRF8* [7], as well as *NCF2* [7], *IL10* [10], *PHRF1* [5], *CD44* [11], *ICAM1_ICAM4* [7], *TYK2* [7], and *UBE2L3* [5]. Loci identified through Asian GWAS include *ETS1* [12], *SLC15A4* [12], *IKZF1* [12], *RASGRP3* [12], *TNFSF4* [12], and *TNIP1* [10].

Malaysia has a population of around 28 million with three major ethnic groups (Malays (60.3%), Chinese (22.9%), and Indians (7.1%)). SLE patients and controls from Malaysia offer a unique opportunity to explore the effect of different ancestral backgrounds [13] on SLE genetic architecture. We explored association of SLE-associated loci identified through GWAS in two majority populations, Chinese and Malays. Given that these cohorts may be admixed, we expect that ancestry proportion may influence SLE association. Although previous studies [13–18] reported genetic associations with some candidate genes in Malaysians. To our knowledge, this is the first study which assessed SLE susceptibility genes using large scale targeted fine-mapping on Malaysian populations.

Our objective was to replicate and fine-map genetic association in 25 previously reported SLE susceptibility loci and to assess population structure and individuals admixture in the two ethnically distinct Malaysian cohorts.

2. Materials and Methods

2.1. Subjects and Genotyping. We genotyped 347 cases and 356 controls from the two major Malaysian ethnic groups (Malays (MA) and Chinese (CH)) using the Illumina custom designed ImmunoChip array [19] as part of a separate ongoing genetic association project. The ImmunoChip is a dense fine-mapping genotype array that contains ~196,000 SNPs from 184 genes associated with at least one of 12 autoimmune diseases, including SLE. Genotyping was conducted through the Genotyping Core Facility of the Oklahoma Medical Research Foundation (OMRF), Oklahoma City, USA. Subjects were recruited in compliance with the Internal Review Boards of OMRF and the University of Malaya Medical Centre. All SLE cases fulfilled the ACR criteria for SLE classification [20, 21]. Controls were matched by ethnicity and gender. Our CH cohort included 288 cases and 292 controls (187 males and 393 females); MA included 59 cases and 64 controls (48 males and 75 females) (Supplementary Table 1 in Supplementary Material available online at http://dx.doi.org/10.1155/2014/305436).

2.2. Quality Control. Individuals were removed from analysis if they were genetically related to other study subjects ($r > 0.25$), as estimated through the relatedness coefficient implemented in GCTA, or if they were outliers (mean ± 2 standard deviations) determined by principal component analysis. SNPs were excluded according to the following criteria: poor genotyping clustering, missing genotype rate greater than 90%, Hardy-Weinberg disequilibrium $P < 0.001$ in controls, or minor allele frequency below 0.5% (Supplementary Figure 1). SNP positions were aligned with HG19. The analysis set contained 6,991 SNPs from 25 previously reported genes genotyped on 580 CH and 123 MA unrelated individuals.

2.3. Population Structure. In order to estimate population structure of our cohorts, we selected 14,134 SNPs with very low intermarker linkage disequilibrium (LD, $r^2 < 0.2$). This SNP set was enriched by variants with pairwise allele frequency difference >20%. We merged our cohorts with individuals from the 1000 Genomes Project (103 CEU, 100 CHB + 100 JPT, and 101 YRI). We estimated the first ten principal components using GCTA [22], as well as the mean and standard deviation for the first three principal components within each cohort (Figure 1). The same dataset was used to estimate individual admixture proportions in ADMIXTURE [23]. We estimated models of admixture using 1 to 7 ancestry components and determined the optimal admixture model by minimizing the cross-validation error using the Bayesian information criterion and the Akaike information criterion. Mean ancestry between cases and controls was compared with a two-tailed t-test.

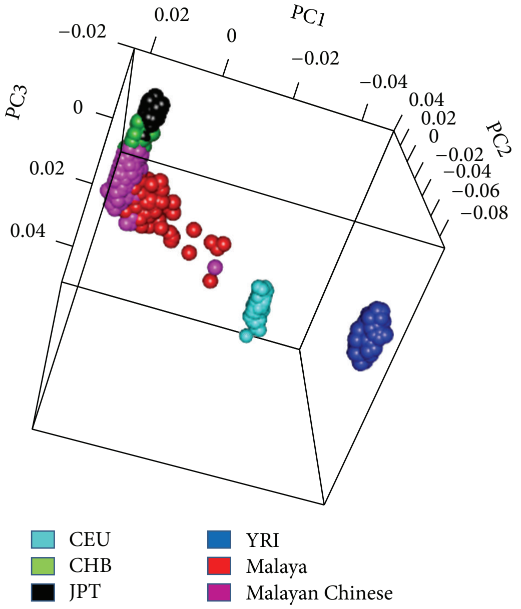

Figure 1: Principal components analysis of Chinese and Malay cohorts. Notably the Malaysian Chinese were a more homogeneous population than the Malays (CEU: North Europeans from CEPH; CHB: Beijing Chinese; JPT: Japanese; YRI: Yorubans from Nigeria).

2.4. Association Analysis. We performed individual SNP case-control association analysis using a chi-square statistic in PLINK [24]. Given the sample size of our cohorts and that this is a replication study, association was considered significant if $P < 0.05$ (alpha = 0.05). We guarded against type 1 error by performing permutation tests (100,000 permutations). Possible influence of admixture was corrected using a logistic regression model in PLINK [24] with the Asian ancestry proportion as a covariate. We used meta-analysis (Fisher's combined P value, four degrees of freedom) to combine association P values from both cohorts. For SNPs which were not significant in either cohort or when odds ratios were not in the same direction, no P_{Meta} was calculated. All associated SNPs passed the permutation test (results not shown).

The best SNP was selected for each region starting with the most significant combined P. We performed epistasis analysis using PLINK [24] and GAIA [25] in order to identify possible gene-gene interactions. We performed a conditional analysis using a logistic regression model (PLINK) for all significant SNPs from *STAT4* and *HLA-DRA* regions. We used the strongest associated SNP from each loci as the initial conditioned SNP to identify additional independent variants.

In order to check for additional sources of stratification, we used mixed models as implemented on EMMAX [26] (Supplementary Table 2).

RegulomeDB [27] and HaploReg [28] were used to identify functional elements overlapping with the selected SNPs.

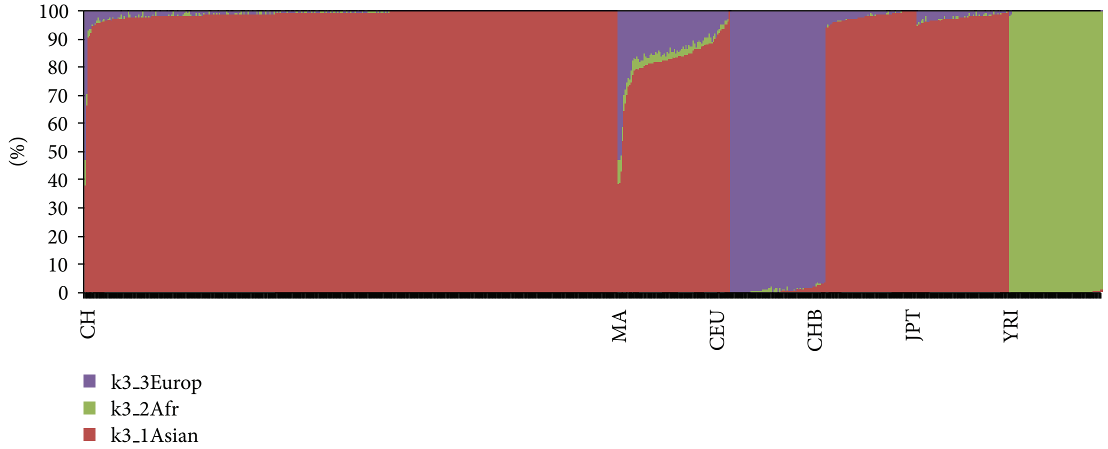

FIGURE 2: Admixture proportions of Chinese and Malays. Malaysian Chinese had less evidence of admixture from Europeans and Africans than Malaysian Malays (CH: Malaysian Chinese; MA: Malaysian Malays; CEU: North Europeans from CEPH; CHB: Beijing Chinese; JPT: Japanese; YRI: Yorubans from Nigeria).

2.5. Pathway Analysis. We chose to study five main pathways which contained the majority of the 25 target genes. All of these pathways are reported to be involved in SLE pathogenesis [29]. In order to determine if there were overrepresented pathways in these two cohorts we performed a gene set enrichment analysis (GSEA) weighted by the strength of the meta-analysis association using i-GSEA4GWAS [30]. The objective of this mode of GSEA was to identify the possible biological mechanisms that involve associated loci, and to identify candidate causal SNPs that affect the normal function in these pathways. Since we used a small set of loci we looked for pathways that contained at least two reported genes.

2.6. Power Analysis. We estimated the required sample size for detection of additional association signals in our cohorts using the method developed by Hoggart et al. [31] for alpha = 0.05. This method takes into account the effect of admixture on the probability of identifying an associated variant in a mixed population. The parameters included populations with similar characteristics as CH and MA (admixture proportions of 10% for CH and 20% for MA) and a power of detection of 80%.

3. Results

3.1. Population Structure. Based on the proportions of the 1000 Genomes Project populations we estimated optimal population structure for Asian, African, and European ancestries. CH were very homogenous compared to the MA. As expected, mean Asian ancestral proportion was the highest in both populations ($\mathrm{ASN}_{\mathrm{CH}} = 99.0 \pm 3.2$; $\mathrm{ASN}_{\mathrm{MA}} = 82.3 \pm 10.6$), followed by European ancestry ($\mathrm{EUR}_{\mathrm{CH}} = 0.7 \pm 2.7$; $\mathrm{EUR}_{\mathrm{MA}} = 14.5 \pm 9.4$) and then African ancestry ($\mathrm{AFR}_{\mathrm{CH}} = 0.3 \pm 0.7$; $\mathrm{AFR}_{\mathrm{MA}} = 3.2 \pm 1.6$) (Figure 2). There was a significant mean ancestry difference between cases and controls in MA (case/control: 84.5/80.3: $P_{\mathrm{ASN}} = 0.02$; 3/3.3: 12.5/16.4: $P_{\mathrm{AFR}} = 0.27$; $P_{\mathrm{EUR}} = 0.02$) but not in CH.

3.2. Association Analysis. We identified associated SNPs in 20 previously reported genes in either cohort. However, not all associated SNPs were significant in both cohorts. In CH published non-HLA loci SNPs showed significant association with SLE (Supplementary Table 3), including *ETS1* (rs1128334, $P_{\mathrm{CH}} = 2.4 \times 10^{-3}$), *IRF8* (rs2280381, $P_{\mathrm{CH}} = 1.38 \times 10^{-2}$), *TNFAIP3* (rs5029939, $P_{\mathrm{CH}} = 1.62 \times 10^{-2}$), *STAT4* (rs3821236, $P_{\mathrm{CH}} = 1.86 \times 10^{-2}$), and *RASGRP3* (rs13385731, $P_{\mathrm{CH}} = 3.63 \times 10^{-2}$). In MA, *IKZF1* (rs4917014, $P_{\mathrm{MA}} = 1.06 \times 10^{-2}$), *RASGRP3* (rs13385731, $P_{\mathrm{MA}} = 2.14 \times 10^{-2}$), *KIAA1542* (rs4963128, $P_{\mathrm{MA}} = 2.25 \times 10^{-2}$), *TNIP1* (rs10036748, $P_{\mathrm{MA}} = 2.55 \times 10^{-2}$), and *IL21R* (rs3093301, $P_{\mathrm{MA}} = 3.28 \times 10^{-2}$) were significantly associated with SLE. For the HLA locus, we replicated association for rs9271366 (*HLA-DRB1_HLA-DQA1* $P_{\mathrm{Meta}} = 1.33 \times 10^{-6}$, $P_{\mathrm{CH}} = 2.62 \times 10^{-6}$; $P_{\mathrm{MA}} = 2.92 \times 10^{-2}$), consistent with the previous report on Malays and Chinese [13].

For all genes SNPs with the strongest association in this study differed from those previously reported. We identified 22 previously reported genes with at least one associated variant; the most significantly associated SNP for each gene was based on Fisher's combined *P* value. The strongest association was in the HLA region in the vicinity of *HLA-DRA* (rs6911777, $P_{\mathrm{Meta}} = 9.96 \times 10^{-9}$, $P_{\mathrm{CH}} = 6.58 \times 10^{-8}$, and $P_{\mathrm{MA}} = 6.73 \times 10^{-3}$). The strongest non-HLA association was observed at *STAT4* (rs7568275, $P_{\mathrm{Meta}} = 1.68 \times 10^{-7}$, $P_{\mathrm{CH}} = 2.88 \times 10^{-6}$, and $P_{\mathrm{MA}} = 2.99 \times 10^{-3}$). Also Asian identified *TNFSF4* (rs10798269, $P_{\mathrm{Meta}} = 5.98 \times 10^{-3}$, $P_{\mathrm{CH}} = 3.87 \times 10^{-2}$, and $P_{\mathrm{MA}} = 1.88 \times 10^{-2}$) and *SLC15A4* (rs6486738, $P_{\mathrm{Meta}} = 4.88 \times 10^{-2}$, $P_{\mathrm{CH}} = 0.122$, and $P_{\mathrm{MA}} = 6.9 \times 10^{-2}$) were replicated. We identified variants in LD with published variants *RASGRP3* (rs13425999, $r^2 = 0.95$, $P_{\mathrm{Meta}} = 6.82 \times 10^{-3}$, $P_{\mathrm{CH}} = 3.30 \times 10^{-2}$, and $P_{\mathrm{MA}} = 2.79 \times 10^{-2}$), *TNIP1* (rs3792782,

Table 1: SLE susceptibility genes in Malay (MA) and Chinese (CH).

Gene	Cytogenetic band	SNP	Base position	A1/A2	Chinese				Malay				Meta-analysis *P* value*
					F_A	F_U	*P* value	OR 95% CI	F_A	F_U	*P* value	OR 95% CI	
TNFSF4-LOC730070	1q25	rs10798269	173,309,713	A/G	0.342	0.401	3.87×10^{-2}	0.78 (0.61–0.99)	0.314	0.460	1.88×10^{-2}	0.54 (0.32–0.9)	5.98×10^{-3}
NCF2	1q25	rs13306575	183,532,437	T/C	0.080	0.043	8.11×10^{-3}	1.95 (1.18–3.22)	0.119	0.070	1.93×10^{-1}	1.78 (0.74–4.28)	1.17×10^{-2}
MAPKAPK2-IL10	1q31-32	rs2232360	207,040,659	A/G	0.328	0.351	3.98×10^{-1}	0.9 (0.71–1.15)	0.576	0.397	5.06×10^{-3}	2.07 (1.24–3.45)	—
RASGRP3	2p25.1-24.1	rs13425999	33,702,203	T/C	0.129	0.174	3.03×10^{-2}	0.7 (0.51–0.97)	0.102	0.203	2.79×10^{-2}	0.44 (0.21–0.93)	6.82×10^{-3}
IFIH1	2q24	rs13023380	163,154,363	A/G	0.010	0.002	5.56×10^{-2}	6.14 (0.74–51.13)	0.025	0.103	1.42×10^{-2}	0.23 (0.06–0.82)	—
STAT4	2q32.2-32.3	rs7568275	191,966,452	G/C	0.453	0.317	2.88×10^{-6}	1.78 (1.4–2.27)	0.422	0.236	2.99×10^{-3}	2.36 (1.33–4.19)	1.68×10^{-7}
BANK1	4q24	rs17031870	102,940,788	G/A	0.092	0.058	2.89×10^{-2}	1.64 (1.05–2.56)	0.237	0.143	5.95×10^{-2}	1.87 (0.97–3.59)	1.26×10^{-2}
TNIP1	5q32-33.1	rs3792782	150,456,677	C/T	0.214	0.247	1.81×10^{-1}	0.83 (0.63–1.09)	0.305	0.453	1.70×10^{-2}	0.53 (0.31–0.89)	2.09×10^{-2}
HLA-DRA	6p21.3	rs6911777	32,409,996	C/T	0.264	0.137	6.57×10^{-8}	2.26 (1.67–3.05)	0.161	0.055	6.73×10^{-3}	3.32 (1.34–8.21)	9.96×10^{-9}
PRDM1-ATG5	6q21	rs9398065	106,546,034	C/G	0.075	0.039	9.52×10^{-3}	1.97 (1.17–3.31)	0.119	0.039	1.95×10^{-2}	3.31 (1.15–9.5)	1.78×10^{-3}
TNFAIP3	6q23	rs5029928	138,189,942	T/C	0.083	0.046	1.02×10^{-2}	1.88 (1.15–3.05)	0.059	0.031	2.87×10^{-1}	1.96 (0.56–6.86)	2.00×10^{-2}
C7orf721-IKZF1	7p13-11.1	rs11185603	50,306,810	G/C	0.236	0.282	7.61×10^{-2}	0.79 (0.61–1.03)	0.144	0.302	3.25×10^{-3}	0.39 (0.21–0.74)	2.30×10^{-3}
BLK	8p23-22	rs11782375	11,294,934	C/T	0.277	0.380	1.88×10^{-4}	0.62 (0.49–0.8)	0.297	0.375	1.94×10^{-1}	0.7 (0.41–1.2)	4.09×10^{-4}
LYN	8q13	rs7828258	56,867,945	T/C	0.223	0.242	4.38×10^{-1}	0.9 (0.68–1.18)	0.136	0.281	5.18×10^{-3}	0.4 (0.21–0.77)	1.61×10^{-2}
KIAA1542-PHRF1	11p15.5	rs4963128	589,564	T/C	0.083	0.070	4.01×10^{-1}	1.2 (0.78–1.86)	0.059	0.156	1.51×10^{-2}	0.34 (0.14–0.84)	—
IRF7	11p15.5	rs7943546	612,148	C/T	0.024	0.033	3.99×10^{-1}	0.74 (0.37–1.49)	0.025	0.055	2.46×10^{-1}	0.45 (0.11–1.79)	3.26×10^{-1}
PDHX-CD44	11p13	rs12362140	35,142,019	A/C	0.007	0.027	7.46×10^{-3}	0.25 (0.08–0.75)	0.085	0.148	1.22×10^{-1}	0.53 (0.24–1.2)	7.27×10^{-3}
ETS1	11q23.3	rs76404385	128,333,055	T/C	0.202	0.138	3.41×10^{-3}	1.59 (1.16–2.17)	0.178	0.063	5.02×10^{-3}	3.25 (1.38–7.65)	2.05×10^{-4}
SLC15A4	12q24.32	rs6486738	129,432,715	G/C	0.295	0.337	1.22×10^{-1}	0.82 (0.64–1.05)	0.203	0.305	6.90×10^{-2}	0.58 (0.32–1.05)	4.88×10^{-2}
IL21R	16p11	rs8060368	27,412,414	T/C	0.012	0.039	3.59×10^{-3}	0.3 (0.13–0.71)	0.170	0.109	1.72×10^{-1}	1.66 (0.8–3.46)	—
ITGAM	16p11.2	rs12444713	31,378,235	A/G	0.284	0.369	2.48×10^{-3}	0.68 (0.53–0.87)	0.5	0.52	7.10×10^{-1}	1.1 (0.67–1.82)	—
ORF8-LOC100131952	16q24.1	rs34912238	86,001,903	T/C	0.039	0.077	6.25×10^{-3}	0.49 (0.29–0.82)	0.076	0.031	1.15×10^{-1}	2.56 (0.77–8.55)	—
ICAM1-ICAM4	19p13.2	rs5498	10,395,683	G/A	0.250	0.267	5.06×10^{-1}	0.91 (0.7–1.19)	0.314	0.242	2.11×10^{-1}	1.43 (0.82–2.51)	—
TYK2	19p13.2	rs12975591	10,627,814	G/A	0.415	0.454	1.84×10^{-1}	0.85 (0.67–1.08)	0.232	0.121	2.62×10^{-2}	2.19 (1.09–4.41)	—
UBE2L3	22q11.21	rs2236642	21,989,621	T/C	0.089	0.127	3.61×10^{-2}	0.67 (0.46–0.98)	0.144	0.172	5.51×10^{-1}	0.81 (0.41–1.62)	9.77×10^{-2}

*When ORs from CH and MA are in different directions, we did not perform the meta-analysis.

— indicated SNPs where no meta-analysis was performed.

OR: odds ratio. Minor allele frequencies are given for the minor allele A1. F_A: minor allele frequency for cases; F_U: minor allele frequency for controls.

$r^2 = 0.74$, $P_{\text{Meta}} = 2.09 \times 10^{-2}$, $P_{\text{CH}} = 0.181$, and $P_{\text{MA}} = 1.7 \times 10^{-2}$), *C7orf72-IKZF1* (rs11185603, $r^2 = 1$, $P_{\text{Meta}} = 2.3 \times 10^{-3}$, $P_{\text{CH}} = 7.61 \times 10^{-2}$, and $P_{\text{MA}} = 3.25 \times 10^{-3}$). Even though these variants have a stronger association signal, they can be explained by their published counterparts.

We also identified a variant in *ETS1* (rs76404385, $P_{\text{Meta}} = 2.05 \times 10^{-4}$, $P_{\text{CH}} = 3.41 \times 10^{-3}$, and $P_{\text{MA}} = 5.02 \times 10^{-3}$) that was completely independent of published variant (rs1128334 $r^2 = 0$). European GWAS identified loci *IL10* (rs2232360, $P_{\text{CH}} = 0.398$, and $P_{\text{MA}} = 5.06 \times 10^{-3}$), *BANK1* (rs17031870, $P_{\text{Meta}} = 1.26 \times 10^{-2}$, $P_{\text{CH}} = 2.89 \times 10^{-2}$, and $P_{\text{MA}} = 5.95 \times 10^{-2}$), *PRDM1-ATG5* (rs9398065, $P_{\text{Meta}} = 1.78 \times 10^{-3}$, $P_{\text{CH}} = 9.52 \times 10^{-3}$, and $P_{\text{MA}} = 1.95 \times 10^{-2}$), *BLK-FAM167A* (rs11782375, $P_{\text{Meta}} = 4.09 \times 10^{-4}$, $P_{\text{CH}} = 1.88 \times 10^{-4}$, and $P_{\text{MA}} = 0.194$), *LYN* (rs7828258, $P_{\text{Meta}} = 1.61 \times 10^{-2}$, $P_{\text{CH}} = 4.38 \times 10^{-1}$, and $P_{\text{MA}} = 5.18 \times 10^{-3}$), *PDHX-CD44* (rs12362140, $P_{\text{Meta}} = 7.27 \times 10^{-3}$, $P_{\text{CH}} = 7.46 \times 10^{-3}$, and $P_{\text{MA}} = 0.122$), *ITGAM* (rs12444713, $P_{\text{CH}} = 2.48 \times 10^{-3}$, and $P_{\text{MA}} = 0.71$), *NCF2* (rs13306575, $P_{\text{Meta}} = 1.17 \times 10^{-2}$, $P_{\text{CH}} = 8.11 \times 10^{-3}$, and $P_{\text{MA}} = 0.193$), *IFIH1* (rs13023380, $P_{\text{CH}} = 5.56 \times 10^{-2}$, and $P_{\text{MA}} = 1.42 \times 10^{-2}$), *TNFAIP3* (rs5029928, $P_{\text{Meta}} = 2 \times 10^{-2}$, $P_{\text{CH}} = 1.02 \times 10^{-2}$, and $P_{\text{MA}} = 0.287$), *PHRF1* (rs4963128, $P_{\text{CH}} = 0.4$, and $P_{\text{MA}} = 1.51 \times 10^{-2}$), *IL21R* (rs8060368, $P_{\text{CH}} = 3.59 \times 10^{-3}$, $P_{\text{MA}} = 0.172$), *IRF8* (rs34912238, $P_{\text{CH}} = 6.25 \times 10^{-3}$, and $P_{\text{MA}} = 0.115$), and *ICAM1-ICAM4-TYK2* region (rs12975591, $P_{\text{CH}} = 0.184$, and $P_{\text{MA}} = 3.06 \times 10^{-2}$) also had a strong combined association with SLE (Table 1).

Notably, the scales of the odds ratio for rs7568275 (*STAT4*: $\text{OR}_{\text{CH}} = 1.78$, $\text{OR}_{\text{MA}} = 2.36$), rs9398065 (*PRDM1-ATG5*: $\text{OR}_{\text{CH}} = 1.97$, $\text{OR}_{\text{MA}} = 3.31$), rs5029928 (*TNFAIP3*: $\text{OR}_{\text{CH}} = 1.88$, $\text{OR}_{\text{MA}} = 1.96$), and rs76404385 (*ETS1*: $\text{OR}_{\text{CH}} = 1.59$, $\text{OR}_{\text{MA}} = 3.25$) were very close to *HLA-DRA* levels of OR (rs6911777 $\text{OR}_{\text{CH}} = 2.26$, $\text{OR}_{\text{MA}} = 3.32$).

Among the aforementioned 22 SNPs, we identified that rs11782375 (*FAM167A_BLK*) overlaps with an eQTL that potentially affects gene expression [32]. Additionally, rs13425999 (*RASGRP3*), rs5029928 (*TNFAIP3*), and rs11185603 (*IKZF1*) were identified as likely to affect binding by RegulomeDB [27]. These three SNPs contained enhancer and promoter histone marks in multiple cell types (in particular lymphoblastoid cell type GM12787) and also colocated with DNAse binding sites.

We used conditional analysis to identify multiple independent SNPs for each gene. In particular, *STAT4* had rs6740131 ($P_{\text{CH}} = 2.89 \times 10^{-4}$, $P_{\text{CH}} = 5.76 \times 10^{-3}$ after conditioning) as an additional independent SNP in CH. In the case of HLA, there were two additional independent SNPs in CH (rs2239806, $P_{\text{CH}} = 9.3 \times 10^{-5}$, and $P_{\text{CH}} = 2.47 \times 10^{-6}$ after conditioning and rs532098, $P_{\text{CH}} = 7.44 \times 10^{-5}$, and $P_{\text{CH}} = 7.05 \times 10^{-5}$ after conditioning).

3.3. Pathway Related Loci. We identified five important pathways involved in SLE pathogenesis which contained at least one of the 25 genes examined in our study. Both B- and T-cell function and signaling pathways had the greatest number of associated variants (Table 2). Neutrophil/monocyte function and signaling had four significantly associated SNPs, whereas TLR and type I IFN signaling pathways each included five genes with significantly associated SNPs. NFκB signaling also contained SNPs significantly associated with SLE. We did not observe any significantly associated SNPs in DNA degradation apoptosis and clearance of cellular debris pathways.

The only significantly enriched pathway was hsa04514 [cell adhesion molecules (CAMs)]. We derived four causal SNPs that potentially explain enrichment of this pathway, where rs2071554 (nonsynonymous, coding (deleterious) in *HLA-DOB*) and rs1129740 (nonsynonymous, coding in *HLA-DQA1*) are candidate causal SNPs through their RECEPTOR_ACTIVITY/TRANSMEMBRANE_RECEPTOR_ACTIVITY; rs8084 (essential splice site and intronic in *HLA-DQB*) and rs7192 (nonsynonymous, coding in *HLA-DRA*) were candidate causal SNPs through TRANSMEMBRANE_RECEPTOR_ACTIVITY.

3.4. Gene-Gene Interactions. We did not identify any gene-gene interaction between significant SNPs in either cohort.

3.5. Admixture Correction. We determined potential effects of admixture on associated variants within these pathways by adjusting case-control association analysis with admixture proportions. After admixture correction, only two MA SNPs were no longer significantly associated ($P > 0.05$). All CH SNPs passed the association threshold ($P < 0.05$).

4. Discussion

In this fine-mapping study we examined two understudied Malaysian populations to replicate previously known SLE genetic associations and to localize the most associated SNP within known SLE genes. Since SLE heterogeneity may be amplified in admixed populations, we adjusted association for admixture (Asian and European). We also categorized associated variants by pathways involvement and identified particular pathways with accumulation of reported associated variants in our Malaysian populations.

We found no effect of admixture on CH, which was not surprising since they are considered a homogenous population. In fact, the ancestry proportions of European and African were very small, and the minor allele frequencies for the top 25 genes were remarkably similar (Figure 3). Correlation between allele frequencies of CH versus CHB ($P = 0.94$) was higher than MA versus CHB ($P = 0.65$) further supporting our conclusion of the similarity between CH and CHB.

We replicated SLE association in *RASGP3* [12], *STAT4* [8], *TNIP1* [10], *IKZF1* [7], *IL21R* [33], *ETS1* [12], and *IRF8* [7]. It is not surprising that we did not identify more previously reported loci since the majority of loci were identified from studies of European and European American populations. Given the differences in LD structure between European and Asian populations, we identified new SNPs associated with SLE which could be either causal or in LD with the true causal SNPs within gene.

Associated variants were framed within their possible functional roles in immune-related pathways. The most

Table 2: Replicated genes and five immune-related pathways. Cells marked with an X represent presence of SNPs associated with SLE in those genes in either cohort. *Marks genes present in the pathway.

Gene	B-cell function and signaling	Neutrophil and monocyte function and signaling	NFκB signaling	T-cell function and signaling	TLR and type I IFN signaling
ATG5			*		
BANK1	X*				
BLK	X*				
CD44				X*	
ETS1	X*			X*	
HLA-DR2	X*			X*	
HLA-DR3	X*			X*	
ICAMs		X*			
IFIH1					X*
IKZF1	X*			X*	
IL10	X*	X*		X*	
IL21	X*			X*	
IRF7					*
IRF8	X*	X*			X*
ITGAM		X*			
LYN	X*				
NCF2	X*				
PHRF1					X*
PRDM1	X*			X*	X*
RASGRP3	X*				
SLC15A4			X*		
STAT4				X*	X*
TNFAIP3			X*		
TNFSF4				X*	
TNIP1			X*		
TYK2				*	
UBE2L3			*		
Total	13	4	3	10	5

important SLE-associated pathways in these populations were related to the B- and T-cell function and signaling pathways. We also introduced a causality model for gene set enrichment based on HLA SNPs in the cell adhesion molecules pathway (hsa04514). We identified four SNPs with potential functional effects through an eQTL and histone marks.

Although these results are encouraging, our study is limited due to the small sample size of our cohorts. Given the admixture proportions of these cohorts, we have estimated that we would require at least 1000 cases and controls to identify novel genomewide significant variants ($P < 5 \times 10^{-8}$) with moderate effects (OR > 1.5) for MA and almost double that for CH. Future large scale admixture mapping with the MA will be especially useful to identify novel SLE susceptibility genes. On the other hand, the CH population can be useful for straightforward association mapping for identifying novel genes or localizing the most likely causal variants.

In conclusion, our high-density fine-mapping on SLE targeted genes is one of the first such undertakings in Malaysian populations. Based on our rigorous analysis, we were able to replicate European and Asian SLE-associated loci in both Malaysian Malays and Malaysian Chinese and were able to identify additional variants that might serve as better tag SNPs for causal variants within these cohorts.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

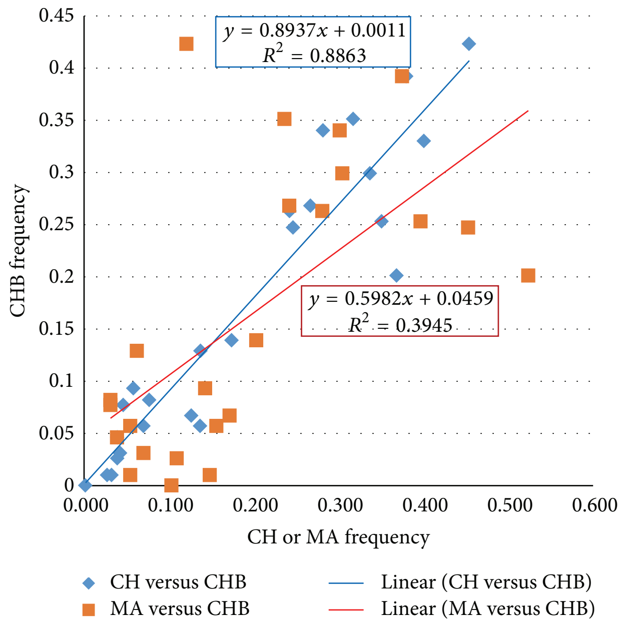

FIGURE 3: Comparison of allele frequencies of the top 25 SNPs from CH versus HapMap Chinese from Beijing (CHB) and MA versus CHB.

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Review Article

Serology of Lupus Erythematosus: Correlation between Immunopathological Features and Clinical Aspects

Emanuele Cozzani, Massimo Drosera, Giulia Gasparini, and Aurora Parodi

Di.S.Sal, Section of Dermatology, IRCCS Azienda Ospedaliera, Universitaria San Martino-IST, 16132 Genoa, Italy

Correspondence should be addressed to Emanuele Cozzani; emanuele.cozzani@unige.it

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the aberrant production of a broad and heterogenous group of autoantibodies. Even though the presence of autoantibodies in SLE has been known, for more than 60 years, still nowadays a great effort is being made to understand the pathogenetic, diagnostic, and prognostic meaning of such autoantibodies. Antibodies to ds-DNA are useful for the diagnosis of SLE, to monitor the disease activity, and correlate with renal and central nervous involvements. Anti-Sm antibodies are highly specific for SLE. Anti-nucleosome antibodies are an excellent marker for SLE and good predictors of flares in quiescent lupus. Anti-histone antibodies characterize drug-induced lupus, while anti-SSA/Ro and anti-SSB/La antibodies are associated with neonatal lupus erythematosus and photosensitivity. Anti-ribosomal P antibodies play a role in neuropsychiatric lupus, but their association with clinical manifestations is still unclear. Anti-phospholipid antibodies are associated with the anti-phospholipid syndrome, cerebral vascular disease, and neuropsychiatric lupus. Anti-C1q antibodies amplify glomerular injury, and the elevation of their titers may predict renal flares. Anti-RNP antibodies are a marker of Sharp's syndrome but can be found in SLE as well. Anti-PCNA antibodies are present in 5–10% of SLE patients especially those with arthritis and hypocomplementemia.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of autoreactive B and T cells, responsible for the aberrant production of a broad and heterogeneous group of autoantibodies (Table 1). Indeed, in 2004 Sherer et al. reported that one hundred sixteen autoantibodies have been described in SLE patients [1]. In SLE, especially in its systemic form (SLE), autoantibodies directed to nuclear (ANAs), cytoplasmtic, and cellular membrane antigens are considered the serological hallmark. ANAs consist of various types of autoantibodies characterized by different antigen specificities. These nuclear antigens include single strand (ss) and double strand (ds) DNA (deoxyribonucleic acid), histone proteins, nucleosome (histone-DNA complex), centromere proteins, and extractable nuclear antigens (ENA) (Smith antigen (Sm), Ro, La, ribonucleoprotein (RNP), etc.). ANAs are present in about 95% of SLE patients with an active disease. In patients with prevalent cutaneous lesions, ANAs have been found positive in 75% of cases.

Therefore, considering the very wide spectrum of discovered autoantibodies, the aim of the present paper is to highlight the most promising and significant ones from both immunopathologic and clinical perspectives.

The presence of autoantibodies in SLE was envisaged when lupus phenomenon was described by Hargraves et al. in 1948 [2] and then proven when it was understood that it was due to neutrophil phagocytosis of cell nuclei opsonised by autoantibodies. In 1957, antibodies to DNA were identified [3] and in 1966 Tan and Kunkel found autoantibodies directed to antigens different from DNA and described the anti-Sm antibodies [4].

Even though the presence of autoantibodies in SLE has been known for more than 60 years, still nowadays a great effort is being made to understand the pathogenetic, diagnostic, and prognostic meaning of such autoantibodies. In particular, studies have focused on ANAs, anti-C1q antibodies, and anti-phospholipid antibodies.

Demonstrating the pathogenic role of autoantibodies is an arduous task; nevertheless recent data from murine, and

Table 1: Correlation between antibodies reactivity lupus subtypes and diagnostic utility.

Antibody	Target	Diagnostic utility	Associated lupus subtypes (prevalence)	Other associated diseases	References
ANAs	The cell nucleus	High sensitivity, but specificity is low	SLE (98%) LN (100%) MCTD Drug-induced lupus Discoid lupus (35%)	Hepatic diseases (autoimmune hepatitis A), malignancies, chronic infections, thyroid diseases, elderly people, SS, SSc, PM, DM, juvenile chronic arthritis, Felty's syndrome, relapsing polychondritis, and rheumatoid arthritis	[12, 21, 22]
Anti-dsDNA	Double strand DNA	High sensitivity and specificity for SLE. Correlate with disease activity	SLE (70–98%) LN (70%) NPSLE (44, 4–81, 6%)	RA, HIV and parvovirus B19 infections, myeloma, and type 1 autoimmune hepatitis	[12, 21, 23–26]
Anti-Sm	Sm[1]	Low sensitivity, but high specificity for SLE	SLE (20–40%) LN (14%) MCTD (8%)	EBV infections	[12, 21, 27, 28]
Anti-nucleosome	Nuclesome[2]	High sensitivity and specificity for SLE. Correlate with disease activity	SLE (61–85%) LN (60–90%) MTCD (41%)	RA, SSc, and SS	[14, 21, 29, 30]
Anti-histone	Histone	Low IgM Higher IgG	SLE (70%) LN (37%) Anti-H2A and Anti-H2b specific for SLE induced by drugs (96–100%)	Rheumatoid arthritis, SSc, PBC[3], Alzheimer's disease, dementia, and infections	[12, 21, 31, 32]
Anti-SSA/Ro	SSA/Ro (proteins 60/52 kD)[4]	High prognostic value for NLE in pregnant women	SLE (30%) LN (31%) NLE (especially CHB) (90%) SCLE (70–80%) Discoid Lupus (5–20%)	SSc, IIM[5], PBC, RA, and SS	[12, 21, 33–36]
Anti-SSB/La	SSB/La[6]	Moderate	SLE (10%) LN (14%) Protective for LN SCLE (30%) NLE (90%)	SS	[12, 21, 35, 36]
Anti-ribosomal P	Ribosomes[7]	Moderate	SLE (13–40%) LN (6%) NPSLE (especially psychosis and depression) (21%)	Hepatic diseases, malignancies, and RA	[12, 21, 37, 38]

Table 1: Continued.

Antibody	Target	Diagnostic utility	Associated lupus subtypes (prevalence)	Other associated diseases	References
Anti-phospholipid	Phospholipids[8] (of cardiolipin, LACs are the most important ones)	High if Anti-PL syndrome is suspected	SLE (30–40%) LN (20–80%) Anti-PL syndrome: venous thrombosis, arterial thrombosis, recurrent pregnancy loss, thrombocytopenia and haemolytic anaemia, livedo reticularis, and skin ulcers.	Other autoimmune diseases, infections, malignancies, and drug-induced disorders, rheumatoid arthritis	[12, 21, 39]
Anti-C1q	C1q[9]	Low but useful to monitor evolution of LE nephritis	SLE (17–46%) LN (40–100%) CLE (44%)	Hypocomplementemic urticarial vasculitis syndrome, rheumatoid arthritis, and renal disease	[21, 40, 41]
Anti-RNP	RNP[10]	Unclear	SLE (20–30%) MCTD (100%)	Sharp's syndrome scleroderma, polymyositis, rheumatoid arthritis, SSc, Sjögren's syndrome	[12, 42, 43]
Anti-PCNA	PCNA[11]	Low	SLE (5–10%)	Chronic hepatitis B and C	[44, 45]

[1] In biology, Sm proteins are a family of RNA-binding proteins found in virtually every cellular organism.

[2] A nucleosome is the basic unit of DNA packaging in eukaryotes, consisting of a segment of DNA wound in sequence around eight histone protein cores.

[3] Primary biliary cirrhosis.

[4] Ro60 is a ribonuclear protein containing small uridine-rich nucleic acids known. Protein 60 KD is located into the nucleus and nucleolus, while protein 52 is located into the cytoplasm.

[5] Idiopathic inflammatory myopathies.

[6] SSB/La particle is a 48–50 kDa nuclear phosphoprotein composed of 2 distinct regions of 28 and 23 kDa.

[7] P0, P1, and P2 of 38, 19, and 17 kDa, respectively, of the 60S subunit.

[8] Anionic phospholipis including cardiolipin (CL), LA, phophatidylserine (PS), phsphatidylinositol (PI), and phosphatidic acid (PA).

[9] C1q is a cationic glycoprotein of 410–450 kDa which binds to the Fc portions of immunoglobulins in immune complexes to initiate complement activation via the classical pathway.

[10] 3 ribonucleoproteins: of 70 kDa (U1), 33 kDa (protein A), and 22 kDa (protein C), respectively.

[11] Anti-proliferating cell nuclear antigen.

human models have clarified the key role of autoantibodies in severe organ involvements, such as nephritis and neuropsychiatric dysfunctions [5]. Common autoantibody-mediated mechanisms of damage in SLE include immune complex-mediate damage, cell surface binding and cytotoxicity, reactivity with autoantigens expressed on apoptotic or activated cell surface, penetration into living cells, and binding to cross-reactive extracellular molecules [6].

Beyond elucidating the mechanisms behind the disease, understanding the pathogenetic role of autoantibodies, might have therapeutic implications. Indeed, in a recent article Diamond et al., after discovering the antigenic specificity of a subset of anti-DNA antibodies, hypothesized a potential therapeutic strategy, using peptides to block the antigen-binding site of the pathogenetic antibody [7].

Pisetsky gives another extremely interesting perspective, based on different sources [8–10], on the role of ANAs in autoimmune diseases, hypothesizing a protective role of such antibodies [11]. ANAs would prevent the disease by inhibiting the immunological activity of nuclear antigens, promoting their clearance in a nonphlogistic way or blocking the formation of immune complexes. Indeed, in SLE anti-SSA/Ro and anti-SSB/La antibodies seem to exert a protective role from lupus nephritis [12]. This hypothesis requires further investigations but could translate into other interesting findings in SLE as well.

However, the biggest effort was made to understand the clinical implications of antibodies found in the sera of patients affected by SLE. Indeed, the diagnostic and prognostic values of such antibodies are well known and no less than two of the American College of Rheumatology (ACR) criteria for SLE [13] regard immunological abnormalities:

"10. Immunologic disorder:

1. Anti-DNA: antibody to native DNA in abnormal titer or
2. Anti-Sm: presence of antibody to Sm nuclear antigen or
3. Positive finding of antiphospholipid antibodies on:
    - An abnormal serum level of IgG or IgM anticardiolipin antibodies,
    - A positive test result for lupus anticoagulant using a standard method,
    - A false-positive test result for at least 6 months confirmed by treponema pallidum immobilization or fluorescent treponemal antibody absorption test

11. Positive antinuclear antibody: An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs"

Many authors have recently questioned the validity of these criteria, for example, Bizzaro et al. demonstrated through a study of meta analysis that the anti-nucleosome antibodies (AnuA) test is superior for diagnostics than the test for anti-dsDNA antibodies [14]. Furthermore Doria et al. underline that the test for anti-ribosomal P protein antibodies has a sensitivity and specificity for the classification of SLE similar to that of anti-Sm antibodies and that it could possibly substitute anti-Sm antibodies in the ACR criteria [15, 16].

Furthermore, anti-ribosomal P protein antibodies correlate with the activity of the disease and are associated with neuropsychiatric manifestations of SLE, while anti-Sm antibodies are frequently static over the disease course and it is difficult to link them with clinical manifestations. Nevertheless, the Systemic Lupus International Collaborating Clinics (SLICC) group recently revised and validated the ACR SLE classification criteria, maintaining and further emphasizing the same immunological criteria [17]. Indeed, according to the SLICC rules, patients must satisfy at least 4 criteria, including at least one immunologic criterion, or the patient must have biopsy-proven lupus nephritis in the presence of antinuclear antibodies or anti-double stranded DNA antibodies.

ANAs can be useful to identify particular subsets of LE: Anti-dsDNA is associated with renal involvement, anti-Ro/SSA antibodies with photosensitive rash especially subacute lupus erythematosus (SCLE) as well as with serositis and haematological manifestations, anti-P ribosomal protein with neuropsychiatric disorders, and anti-RNP with arthritis, Raynaud's, and puffy fingers. In this regard, another interesting point of view is given by Shivastava and Khanna [18], who propose the cluster theory: according to which distinct autoantibody clustering correlates to particular clinical syndromes. Cluster 1 (anti-Sm and anti-RNP) is characterized by the lowest incidence of proteinuria, anaemia, lymphopenia, and thrombocythemia. Cluster 2 (anti-dsDNA, anti-Ro and anti-La) is associated with a higher rate of nephritic syndrome and leukopenia. Cluster 3 (anti-ds-DNA, LAC and aCL) is expectedly associated with thrombotic events [19]. Moreover, Ching et al. [20] studied the serological profiles of SLE patients, finding that most of them segregated into one of two distinct clusters defined by autoantibodies against Sm/anti-RNP or Ro/La autoantigens. The Sm/RNP cluster was associated with a higher prevalence of serositis in comparison to the Ro/La cluster.

2. Techniques

ANAs can be detected by various assays: indirect immunofluorescence (IIF) using cultured cells as substrates, enzyme-linked immunosorbent assay (ELISA), and farr radioimmunoassay (RIA).

IIF and ELISA are most popular in routine work. ELISA is more sensitive but less specific while IIF is sensitive, reproducible, and easy to perform. ELISA is preferable when the exact titration of ANAs is needed in the follow-up of SLE.

Lately, multiplexed ELISA assays have been used for ANAs titration and these new sophisticated techniques are able to detect simultaneously multiple autoantibodies from a single sample. Until now, various studies report overall agreement between the detection of lupus autoantibodies by conventional ELISA and by multiplexed ELISA assays [46–48].

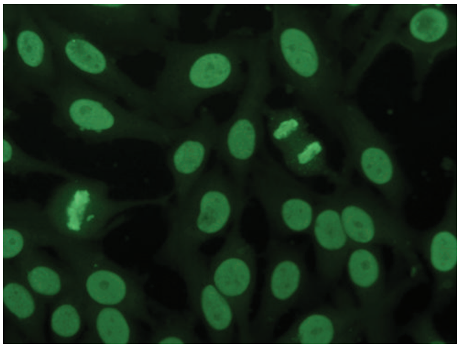

Figure 1: IIF on Hep2 cells: homogeneous pattern. Dilution 1 : 40.

Monolayer of cultured cells, particularly HEp2 (a human laryngeal carcinoma cell line), is now considered the gold standard for IIF. In cultured cells used for IIF, the antigens are in the native location and form, undenatured or minimally denatured, and the nuclei and nucleoli are clearly visible in dividing cells.

About 40 different fluoroscopic patterns have been described in IIF, related to different antibody specificities. The most common are homogeneous, peripheral or ring, speckled, nucleolar, pleomorphic speckled, nuclear dots, and nuclear membrane. Generally, the homogenous pattern is linked to SLE (Figure 1). ANA pattern has some correlation with clinical subsets, such as a shrunken peripheral pattern with renal disease, a fine particulate pattern in SCLE, and a homogeneous pattern with anti-histone antibodies [49]. However, the homogenous pattern can be found in many other autoimmune diseases and, in contrast, various ANA patterns may coexist in the same disease. For these reasons, more specific tests, such as the anti-dsDNA test or anti-ENA test are necessary for a precise diagnosis, according to the well-known “cascade testing” as suggested by guidelines [50]. Indeed, it must be kept in mind that ANAs may be found not only in autoimmune diseases, but also hepatic diseases, malignancies, chronic infections, thyroid diseases, and even in individuals with no medical condition, particularly elderly people [51, 52].

Ippolito et al. [53] report the results of current serologic tests for SLE are generally consistent with the historical ones. However, probably due to their better sensitivity, current serological tests yield a certain percentage of additional positives. Further, due to a lower sensitivity in the past tests for C3 and C4 detected more frequently the depletion of these factors.

3. Anti-DNA Antibodies

Anti-DNA antibodies constitute a subgroup of antinuclear antibodies that bind to either single-stranded or double-stranded DNA [54]. Both subtypes of DNA-binding antibodies may be found in SLE. Nonetheless, while some authors highlighted a possible role of anti-ssDNA antibodies in the diagnosis and follow-up of SLE, especially when anti-dsDNA antibodies were negative [55, 56], others doubted the specificity and utility of this test [57–60]. Instead, because of their high specificity, anti-dsDNA antibodies are universally used as a diagnostic criterion for SLE (70–98% of patients are positive for such antibodies) [12] and for monitoring the clinical course of the patient [61] (every 6 weeks, for example), especially in the presence of an immunosuppressive treatment that reduces their production. IIF on *Crithidia luciliae* (Figure 1), RIA, and ELISA is the most commonly used assays to detect anti-dsDNA antibodies. IIF-based *Crithidia* assay is probably the most specific technique, but ELISA is the most practical and clinically relevant method. In IIF anti-dsDNA antibodies correlate with a shrunken peripheral ANA pattern [49]. It is generally accepted that anti-dsDNA antibodies, in particular of the IgG isotype, have an important pathogenetic role in SLE. A clear-cut relationship exists, for example, between anti-dsDNA antibodies (R4A antibody) [7] and disease activity in nephritis [62]. Anti-DNA-DNA immune complexes can deposit in the mesangial matrix and their subsequent complement activation leads to inflammation and mesangial nephritis. Moreover, anti-dsDNA antibodies also contribute to the end-stage lupus nephritis by directly binding exposed chromatine fragments in glomerular basement membrane [5]. On the other hand, IgM-class anti-dsDNA antibodies seem to have a protective role for nephropathy [63, 64]. Furthermore De Giorgio et al. demonstrated that a subset of anti-DNA antibodies cross-reacts with N-methyl-D-aspartate receptors (NMDAR), and through an excitotoxic mechanism, could induce neuronal apoptosis. Anti-NMDAR antibodies are present in 40% of lupus patients and some reports have supported the correlation between such antibodies and the presence of neuropsychiatric lupus [23, 65, 66], while others have not [67]. More recently, Franchin et al. have demonstrated that anti-NMDAR antibodies also bind C1q; therefore, they hypothesized that this subset of anti-DNA antibodies contributes in lupus pathogenesis through direct targeting of C1q on glomeruli and also through removal of soluble C1q thereby limiting the ability of C1q to suppressor of immune activation [68].

4. Anti-Sm Antibodies

Sm antigen consists of at least 4 proteins: B (28 kDa), B1 (29 kDa), D (19 kDa), and E (13 kDa). Anti-Sm antibodies are a highly specific marker for SLE and Anti-Sm reactivity is not described in other diseases. Their sensitivity is however low. In fact, anti-Sm antibodies are detectable only in 20% of SLE white patients, but 30–40% in black and Asian people. Clinical correlations of these autoantibodies remain unclear [12] and generally show persistent expression over time [11]. In some studies anti-Sm titers were found to fluctuate with disease activity and treatment [69], but it is unclear whether serial monitoring predicts relapse [70].

5. Anti-Nucleosome Antibodies

The antigen consists of pairs of 4 core histones: H2A, H2B, H3, and H4, forming the histone octamer around which 200 pairs of basis of DNA are wound twice, with H1 bound

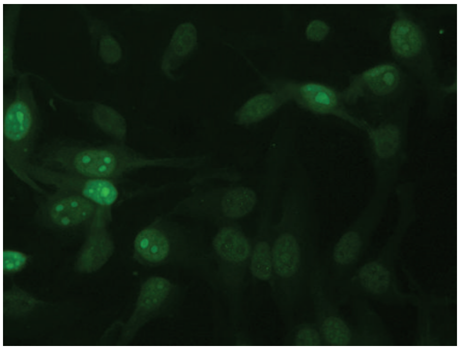

Figure 2: IIF on HEp2 cells: speckeld and nuclear and nucleolar staining (anti-SSA/Ro antibodies). Dilution 1 : 40.

on the outside. Anti-nucleosomes antibodies (ANuA) react exclusively to nucleosomes and not to individual histones or native non-protein-complexed DNA [71].

Although anti-nucleosome antibodies can be seen in IIF as homogeneous pattern (Figure 2), only ELISA detects them.

They represent the first serological marker of SLE described and, at present, nucleosomes are considered a major autoantigen in SLE in which they are positive in about 85% of patients and probably play an important pathogenetic role [29]. There is major evidence that nucleosome antibodies play an important role in the pathogenesis of SLE, being the first ones to appear in murine lupus models before the onset of any other autoantibodies, which are only later produced by B cells, stimulated by nucleosome-specific T cells through epitope spreading [72]. In glomerulonephritis, nucleosomes facilitate binding of autoantibodies to glomerular basement membranes with an increased permeability and inflammatory response [5, 73].

According to Bizzaro's meta-analysis ANuA test appears to have an adequate level of diagnostic accuracy for SLE, with equal specificity, but higher sensitivity, positive likelihood ratio, and diagnostic odds ratio than anti-dsDNA antibodies test [14]. Indeed, they could be one of the most sensitive markers in the diagnosis of SLE, especially in anti-ds-DNA-negative patients [74]. Furthermore, there is a strong correlation between the level of anti-nucleosome antibodies and lupus disease severity [23, 75, 76]. ANuAs are probably better to predict flares in quiescent lupus [77].

6. Anti-Histone Antibodies

The target antigens are 5 major classes of histones (H1, H2A, H2B, H3, and H4), which organize and constrain the topology of DNA.

ELISA is the only reliable method for detection of anti-histone antibodies. It is important to use IgG-specific antibodies and not IgM that are not specifically related to the disease. Using IIF on standard substrates anti-histone antibodies produces a homogeneous, chromosome-positive staining of the nucleus.

These autoantibodies are characteristic of particular subset of SLE. In fact, anti-H2A-H2b antibodies are a sensitive test in drug-induced SLE. About 96% of patients with SLE induced by procainamide [31] and 100% of patients with SLE induced by penicillamine, isoniazid [32], and methyldopa have anti-histone antibodies. Nonetheless, they are also present in idiopathic SLE (70% of patients with SLE [12]), in rheumatoid arthritis, Felty's syndrome, Sjögren's syndrome (SS), systemic sclerosis (SSc) [78], primary biliary cirrhosis, infectious diseases (including HIV infection), and even neurological disorders such as Alzheimer's disease and dementia. In our experience, anti-histone antibodies are found in 10% of SLE patients and in 40% of SSc patients [79]. However, because of their low specificity these anti-histone antibodies albeit more prevalent, are not pathognomonic of drug-induce SLE [80]. This apparent paradox might be explained by the fact that the metabolites of offending drugs probably have the capacity to disrupt nonspecifically central immune tolerance to chromatin [81]. From a pathogenetic point of view, the histone-anti-histone antibody system might play a role in the perpetuation of murine lupus nephritis [82] and recently Sui et al. demonstrate in their study a strong association between simultaneous positivity to anti-DNA, anti-nucleosome, and anti-histone antibodies and renal disease activities, especially in proliferative glomerulonephritis [83].

7. Anti-SSA/Ro Antibodies

SSA/Ro antigen is a ribonucleoprotein containing small uridine-rich nucleic acids known as hY1, hY3, hY4, and hY5 (hY is the abbreviation of human cytoplasmic). SSA/Ro antigen consists of at least of 4 proteins: 45, 52, 54, and 60 kDa, respectively, with the best known of them being the 52 and 60 kDa proteins [84].

The most sensitive and specific method for detection of anti-SSA/Ro antibodies is ELISA. Using tumoral cell lines transfected with SSA/Ro antigen (HEp 2000) as a substrate, IIF is useful too, showing a typical speckled nuclear and nucleolar staining (Figure 2).

Anti-SSA/Ro antibodies might have a pathogenetic role in the initiation of tissue damage especially in photosensitive SLE, for ultraviolet radiation has been shown to induce *de novo* synthesis and the expression on the cell surface of SSA/Ro polypeptides in keratinocytes [85, 86].

Since the 1980s, it was known that anti-SSA/Ro and anti-SSB/La antibodies can cross the maternal placenta and determine neonatal lupus erythematosus (NLE). Indeed, anti-SSA/Ro as well as also anti-SSB/La antibodies bind to fetal heart conduction tissue and inhibit cardiac repolarization [87], determining isolated complete atrioventricular block (CHB). Other frequently observed manifestations of NLE are cutaneous rash, haematological disorders (thrombocytopenia, anemia, and leukopenia), and liver dysfunction [88], all of which tend to resolve within the time of clearance of maternal antibodies from the infant's circulation.

In a recent paper, it was reported that newborns from mothers with high to moderate titers of anti-SSA/Ro antibodies are more likely to develop cardiac manifestations of NLE, independently from the anti-SSB/La titers, while infants with prenatal exposure to high titers of anti-SSB/La antibodies were most likely to present non-cardiac manifestations [89].

Anti-SSA/Ro antibodies can be detected in 70–100% of patients with SS, in 30–70% of patients in particular in SCLE and NLE (70–80%) and with a lower frequency also in discoid LE (5–20%). Antibodies to the 52 kDa subunit are more specific for SS while antibodies to the 60 kDa subunit are more frequent in SLE patients. Anti-Ro and Anti-La antibodies are found earlier than other SLE-related autoantibodies and are present on average 6.6 years before the the diagnosis of SLE [33]. A close association between anti-SSA/Ro antibodies and late onset of SLE (average age of 50) was suggested [34]. Anti-SSA/Ro antibodies correlate with photosensitivity, SCLE, cutaneous vasculitis (palpable purpura), and haematological disorders (anemia, leukopenia, and thrombocytopenia) [35, 90–92].

There are discordant data regarding the association between anti-SSA/Ro titers and the disease activity, but it seems that anti-SSA/Ro antibody levels tend to decline when patients are treated with cytotoxic drugs [93–97].

Recently, greater attention is being paid toward distinguishing the two subtypes of anti-SSA/Ro: anti-SSA/Ro60 and anti-Ro52/TRIM21. A recent retrospective study conducted by Menendez et al. supports their routine distinction in clinical practice, since the two subtypes show different associations with different clinical subtypes of SLE. Indeed, anti-SSA/Ro60 are more frequently reported in association with SLE and CLE. Nevertheless, the pattern with both anti-SSA/Ro60 and anti-Ro52/TRIM21 is more frequent in SCLE and anti-Ro52/TRIM21 is more strongly associated with CHB [98]. In particular, the antibodies that seem to be strictly linked to CHB are directed against peptide aa 200–239 of subunit 52 kDa of Ro/SSA antigen [99].

8. Anti-SSB/La Antibodies

The SSB/La particle is a 48–50 kDa nuclear phosphoprotein composed of 2 distinct regions of 28 and 23 kDa [100]. The larger domain contains a RNA binding site that binds RNA polymerase III transcripts. Although anti-SSB/La antibodies were originally detected by immunodiffusion and counterimmunoelectrophoresis, they are now commonly detected by ELISA and immunoblotting.

Even though there is no direct evidence of a pathogenetic role of anti-SSB/La antibodies in SS and SLE, their presence in maternal blood is strongly associated with NLE and congenital heart block. In fact, both SSB/La and SSA/Ro antibodies bind to the surface of the fibres of the heart suggesting that the maternal anti-SSB/La and anti-SSA/Ro antibodies bind to the surface of cardiac muscle cells and damage them. Anti-SSB/La antibodies are the serological marker of SS [101]: if detected by ELISA, anti-SSB/La antibodies are present in 90% of patients with primary SS and 50% with secondary SS. In SLE, anti-SSB/La antibodies are instead present only in about 10% of patients with lower prevalence of renal disease. About 30% of patients with SCLE have anti-SSB/La antibodies.

9. Anti-Ribosomal P Antibodies

Ribosomes are complex macromolecular structures incorporating both protein and ribonucleic acid (RNA) elements. Mammalian ribosomes are formed by the 60S and 40S subunit. The 40S subunit is a ribonucleoprotein complex containing a single 18S species of RNA and 33 different basic proteins. The 60S subunit incorporates 3 distinct species of RNA, 46 different basic proteins, and 3 phosphoproteins named P0, P1, and P2 of 38, 19 and 17 kDa, respectively, that are the most important antigen targets of anti-ribosomal antibodies [102].

The specificity of autoantibodies directed against ribosomal components is evaluated by immunoblotting, but their presence is already suggested in IIF by a cytoplasmatic pattern. In the routine work, however, they are usually detected by ELISA. In comparative studies immunoblotting and ELISA seem to give the same diagnostic accuracy [103]. More recently, the international multicentre evaluation of the clinical accuracy of a new ELISA based on recombinant P polypeptides demonstrated that a combination of all three P proteins resembling the native heterocomplex P0 $(P1/P2)_2$ as antigen gives the best accuracy [104].

Anti-ribosomal P antibodies seem to have an intriguing pathogenetic potential that needs further investigations. Indeed, anti-ribosomal P antibodies may exert different cellular effects by binding to the surface of T cells, monocytes, and endothelial cells [21].

They are able to penetrate into living cells by binding a cell-surface 38 kDa protein, which is the corresponding surface version of P0 ribosomal protein. In this way, they can cause cellular dysfunction and tissue damage by inhibiting protein synthesis, inducing apoptosis or proinflammatory cytokine production [105]. More recently, two independent groups elucidated the neuropathogenic potential of anti-ribosamal P antibodies [106, 107]. Moreover, Caponi et al. demonstrated that anti-ribosomal P antibodies in some cases can cross react with cardiolipin, ssDNA, dsDNA, and also nucleosomes. Such data indicate a partial overlapping of anti-ribosmal P antibodies with the other autoantibody populations detected frequently in SLE. For this reason anti-ribosomal P might have a similar pathogenetic role, for instance, in NPSLE [108].

The autoimmune response to ribosomal components is quite specific for SLE. Anti-ribosomal P antibodies occur in 13–20% of Caucasian SLE patients and in more than 40% of Asian patients [37].

Since the first prospective study in 1987 by Bonfa et al. [52] reporting a strong association between anti-ribosomal P antibodies and lupus psychosis, many other studies tried to confirm the utility of such antibodies in predicting NPSLE. However, the results were contrasting [38, 109]. Anyhow, many studies report associations with psychosis and especially depression.

10. Anti-Phospholipid Antibodies

The study of anti-phospholipid antibodies (aPL) antibodies began in 1906 when Wasserman introduced his serological test for syphilis [110]. In 1941, the active component was found to be a phospholipid, which was called cardiolipin [111]. After the 1950s, it became clear that people with positive Wasserman-test did not necessarily have syphilis but that they may have instead an autoimmune disorder, including

SLE [112]. The term *lupus anticoagulant* (LAC) first used in 1972 should be abandoned because LA can be found in patients without SLE and it is associated with thrombosis and not with bleeding [113].

Anti-PL antibodies recognize a number of anionic negatively charged phospholipids, including cardiolipin (CL), LAC, phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol, and phosphatidic acid (PA). Neutrally charged autoantigen targets include phosphatidyl ethanolamine, phosphatidyl choline, platelet activating factor and sphingomyelin. These antibodies are usually detected with radioimmunoassay and ELISA. CL remains the most commonly used antigen for detecting anti-PL antibodies with ELISA. It is now clear, however, that the optimal binding of anti-PL antibodies depends on cofactors; the best known of them is termed Beta2-Glycoprotein I (Beta2GP1), that is, a 50 kDa B2 globulin involved in the regulation of blood coagulation [114]. ELISA testing for Beta2GP1 is also available [12].

As mentioned before, anti-PL antibodies are not confined to SLE patients but can be found in other autoimmune diseases, infections, malignant, and drug-induced disorders as well as in some apparently healthy individuals. In addition, anti-PL antibodies are positive in 30–40% of SLE patients, but only 1/3 of them develop clinical features of anti-PL syndrome, namely, venous thrombosis, arterial thrombosis, recurrent pregnancy loss, thrombocytopenia and haemolytic anaemia, *livedo reticularis,* and skin ulcers [39]. Furthermore, aPL antibodies are involved in cerebral vascular disease and are also implied in the pathogenesis of focal damage in NPSLE. In particular, anti-beta2GPI antibodies are the most thrombogenic and may exert a pathogenetic potential either as a strong procoagulant factor in the cerebral circulation or by directly interacting with neuronal tissue [5].

11. Anti-C1q Antibodies

C1q is a cationic glycoprotein of 410–450 kDa, which binds to the Fc portions of immunoglobulins in immune complexes to initiate complement activation via the classical pathway [115]. C1q is produced by macrophages, monocytes, dendritic cells, fibroblasts, and epithelial cells and acts like a binding molecule between debris from cellular apoptosis (apoptotic blebs) and macrophages. Therefore, anti-C1q antibody development seems to be related to a deficiency in apoptotic cell clearance, as suggested by the fact that such antibodies from SLE patients specifically bind to C1q on apoptotic cells [116].

Anti-C1q antibodies are commonly detected by ELISA. From a pathogenic point of view anti-C1q antibodies probably amplify glomerular injury but only when C1q has already been brought to the site by other types of glomerular-reactive autoantibodies [117]. Furthermore, Hegazy et al. recently reported in their study a strong correlation between anti-C1q antibodies and cutaneous lupus and hypothesised a potential pathogenetic role in such context [40].

They are found in SLE with a prevalence ranging from 17% to 46%, especially in patients with nephritis [41]. Moroni suggests that the elevation of their titers may predict renal flares even better than anti-dsDNA antibody levels [118]. Elevated titres of anti-C1q antibodies are usually associated with the proliferative forms of lupus nephritis and with subendothelial deposits of immune complexes. They are therefore a useful marker for assessing both disease activity and progression of the renal disease [118]. Anti-C1q antibodies can be found also in other autoimmune diseases such as hypocomplementemic urticarial vasculitis syndrome, rheumatoid arthritis, Felty's syndrome, rheumatoid vasculitis, Sjögren's syndrome, membranoproliferative glomerulonephritis (MPGN), and IgA nephropathy [119, 120].

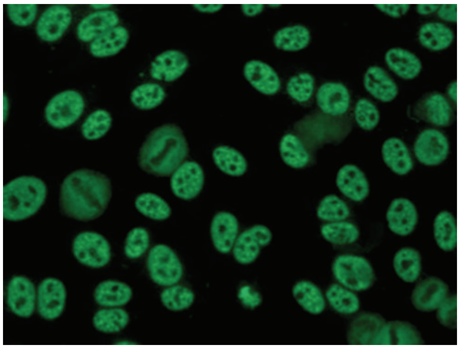

Figure 3: IIF on Hep2 cells: speckled pattern (anti-RNP antibodies). Dilution 1 : 40.

12. Anti-RNP Antibodies

Anti-RNP antibodies are directed to at least 3 proteins of 70 kDa (U1), 33 kDa (protein A), and 22 kDa (protein C), respectively. In IIF anti-RNP antibodies produce a fine speckled staining (Figure 3). Anti-U1small nuclear (sn) RNP antibodies are considered pathognomonic for Sharp's syndrome (mixed connective tissue disease or MCTD), but they can be found in 20–30% of patients with SLE as well [42]. Their presence is associated with HLA DR4 and their prevalence is higher in African American patients [12]. Other diseases in which anti-U1snRNP activity is described include rheumatoid arthritis, polymyositis, SSc, and Sjögren's syndrome (SS). Data from recent experimental studies promote the hypothesis that U1snRNP antibodies participate in both innate and adaptive immune responses, implicating them in the pathogenesis of connective tissue disease [121]. According to some authors anti-RNP antibodies are more prevalent in patients with Raynaud's phenomenon and are associated with milder renal involvement [122]. Although, ultimately anti-U1 RNP antibodies do not reflect the disease activity and their utility in monitoring the latter remains unclear.

13. Anti-Proliferating Cell Nuclear Antigen (PCNA) Antibodies

Anti-PCNA antibodies can be detected by using IIF on cultured cells in which they show a characteristic nuclear speckled pattern of varying intensity (Figure 4). ELISA kits are also available. PCNA is an auxiliary protein for DNA polymerase delta. PCNA expression increases proportionally to DNA synthesis and/or cell growth, beginning in late G1, increasing in S, and decreasing in G2 cellular phases.

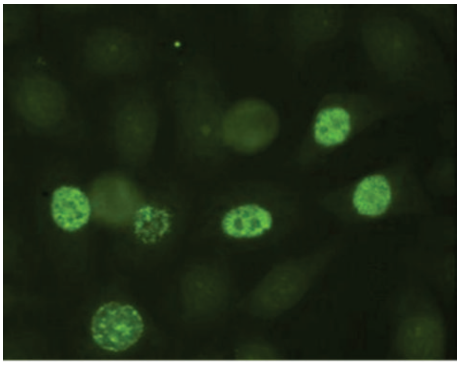

Figure 4: IIF on Hep2 cells: speckled pattern of varying intensity (anti-PCNA antibodies). Diluition 1 : 40.

Anti-PCNA antibodies are present in 5–10% of SLE patients especially those with arthritis and hypocomplementemia [44]. After treatment with steroids or cytotoxic drugs, anti-PCNA antibodies become undetectable.

14. Serology of SLE in Overlap Syndromes

SLE can be associated with other autoimmune diseases such as Sjögren's syndrome (SS), systemic sclerosis (SSc), rheumatoid arthritis (RA), dermatomyositis (DM)/polymyositis (PM), and determining overlap syndromes (OSs). OSs share clinical and immunological features of two or more distinct autoimmune diseases and might also have their own peculiar features. From a serological point of view OSs can be associated with a specific antibody profile (MCTD and SLE/SS) or not associated with a specific antibody profile (rhupus syndrome, SLE/SSc). MCTD has mixed features of SLE, SSc, DM/PM, and RA, in which anti-U1snRNP antibodies are the specific antibodies of the disease (see above). Anti-Ro/SS-A, anti-ssDNA, anti-Sm, anti-dsDNA [123], and anti-PL antibodies [124] have also been detected; nevertheless, they are not specific of MCTD. Recently, autoantibodies to angiotensin-converting enzyme 2 (ACE2) [125] were also reported in MCTD. SLE/SS patients have a higher frequency of SS-related immunological markers, such as rheumatoid factor (RF), polyclonal hypergammaglobulinemia, anti-Ro/SSA, and anti-La/SSB, while SLE-related antibodies are less frequent [126]. Anti-La/SSB antibodies are considered the serological markers of this OS. Most authors define rhupus syndrome as a condition characterized by signs and symptoms of both SLE and RA [127, 128]. In patients affected by such OS no specific antibody is identifiable and specific autoantibodies for SLE (anti-dsDNA and anti-Sm) and RA (anti-citrullinated peptides ACPA) coexist [126]. SLE/SSc overlap is a rare condition, in which a specific serological marker has not been identified yet, but a high incidence of anti-dsDNA and anti-Scl70 antibodies has been reported [126].

15. Conclusions

The comprehension of pathogenetic mechanisms is the starting point for the development of new and better laboratory tests, with various clinical implications. For example, the discovery of the cross-reactivity of certain types of anti-dsDNA antibodies with the NMDA receptor helped to comprehend the pathogenesis of NPSLE, but the detection of such antibodies in patients' sera could also be a potential predictive marker of the risk of developing NP disorders in SLE. Furthermore, distinguishing between the two different subtypes of anti-SSA/Ro antibodies might have interesting clinical implications. A better knowledge of the specificities of the antibodies might be a useful tool to subclassify patients with lupus and to predict which clinical manifestations they might develop. Detecting simultaneously a battery of various antibodies with multiplexed ELISA could be helpful for this purpose.

For the diagnosis of lupus certainly ds-DNA antibodies are an excellent biomarker, but we believe that perhaps ANuAs might be a better one, in accordance with Bizzaro's meta analysis, and considering that from a pathogenetic point of view these autoantibodies are the first ones to appear.

The role played by autoantibodies in the pathogenesis of lupus is yet to be revealed in many respects and the strive to find new and more valid biomarkers for a better management of the disease is constant, being lupus such a complex disease. Therefore, we believe there is still room for improvement as far as lupus serology is concerned.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Silent Burdens in Disease: Fatigue and Depression in SLE

R. Fonseca,[1] M. Bernardes,[2] G. Terroso,[2] M. de Sousa,[3] and M. Figueiredo-Braga[1]

[1] *Department of Clinical Neurosciences and Mental Health, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal*
[2] *Rheumatology Department, São João Hospital, 4200-319 Porto, Portugal*
[3] *IBMC/GABBA, University of Porto, 4150-180 Porto, Portugal*

Correspondence should be addressed to M. Figueiredo-Braga; mmfb@med.up.pt

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At a time when health is being recognized as more than just avoiding death, age and comorbidity are becoming increasingly important aspects of chronic disease. Systemic Lupus Erythematous (SLE) is probably one of the best paradigms of modern chronic disease, sitting at the crossroads of numerous somatic health problems, immune activation, depression, pain, and fatigue. One hundred forty-eight female participants were enrolled in the present study: 50 diagnosed with SLE, 45 with major depressive disorder (MDD), and 53 age-matched controls. Statistically significant lower scores in quality-of-life dimensions related to physical impairment were found in SLE. Patients with MDD presented significant levels of pain, reduced physical summary component (PSC), and general health scores different from healthy controls. Fatigue was reported in 90% of women with SLE and 77.8% of the MDD patients in contrast with 39.6% in the control group. Significant correlations were seen among fatigue severity, age, and educational level in SLE. From our own previous work and more recent work on the association of immune activation and depression, unexplained fatigue in SLE may signify an early sign of immune activation flare-up. The search for cytokine markers should perhaps be extended to fatigue in SLE.

1. Introduction

The more recent analysis of the Global Burden of Disease has identified mental disorders and musculoskeletal diseases as some of the major contributors to Years Lived in Disability (YLD) [1]. One consequence of this study that has particularly interested us has led to the question of how such global findings can be reflected in the care of the individual patient and the understanding of the complexity of comorbidity in chronic disease. Although fatigue, anxiety, and depression crosstalk with the clinical presentation and progression of Systemic Lupus Erythematosus (SLE), clinicians generally pay more attention to the somatic health problems posed by illness [2]. This can be explained in part by present-day medical education's emphasis on the biological, genomic, and statistically significant dimensions of disease.

Studies of SLE patients have shown, however, the impact of individual elements such as anxiety and depression on the course of disease as experienced by individual patients and how, collectively, such elements have an impact on health care costs [3].

SLE is also a particularly good example of how progress in the dissection of genes and molecules involved in autoimmunity continues to be disappointingly reflected in helping the individual patient [4]. In addition, SLE as a chronic inflammatory autoimmune disease with multisystem involvement, secondary to the production of autoantibodies and to the production of Type 1 interferon by innate immune cells [5, 6], represents a singularly revealing model of the crossroads that individual clinicians or clinical teams must face to deal adequately with an individual patient.

Presently, clinical evaluation of disease relies mostly upon objective criteria, contemplating clinical features whose significance may nevertheless escape the patient. Particular symptoms such as pain and fatigue can, on the contrary, be experienced and reported exclusively by the patient, escaping regular clinical assessment. We would like to identify such individual burdens as silent burdens of disease.

Table 1: Sociodemographic characterization.

	Total $n = 148$	SLE (1) $n = 50$	Depression (2) $n = 45$	Controls (3) $n = 53$	P	Post hoc analysis
Age (years)[a]	44.5 (11.6)	44.1 (10.1)	41.7 (13.7)	47.2 (11.8)	0.108[c]	
Education (years)[a]	10.7 (4.8)	9.1 (3.9)	13.0 (4.2)	10.9 (5.4)	0.001[c]	2 > 1, 3
Marital status[b]						
Single	26 (17.6)	8 (16.0)	10 (22.2)	8 (15.1)		
Married	101 (68.2)	38 (76.0)	25 (55.6)	38 (71.7)		
Divorced	15 (10.1)	2 (4.0)	9 (20.0)	4 (7.5)	0.254[d]	
Widow	4 (2.7)	1 (2.0)	1 (2.2)	2 (3.6)		
Common-law marriage	2 (1.4)	1 (2.0)	0 (0.0)	1 (1.9)		
Employment status[b]						
Active	86 (58.1)	15 (30.0)	27 (60.0)	44 (83.0)	0.000[d]	
Nonactive	62 (41.9)	35 (70.0)	18 (40.0)	9 (17.0)		

[a]Mean (standard deviation), [b]n (%), [c]ANOVA, [d]Chi-square test.

Eventually, the disrupted immunological tolerance seen in SLE patients results in immune complex deposition that ends in permanent damage, most frequently affecting the musculoskeletal, cutaneous, renal, central nervous, and gastrointestinal systems [7]. The diversity of the resulting clinical manifestations led to the establishment of consensual, valuable, sensitive, and specific diagnostic criteria: the American College of Rheumatology (ACR) Criteria for Classification of Systemic Lupus Erythematosus. Although not formally contemplated in these criteria, fatigue is the most common symptom in SLE, affecting 67% to 90% of the patients, even in mild disease presentations [8–10]. Patients often describe their fatigue as debilitating, causing a severe impact on personal, family, and social functioning [10]. Resulting from a complex interplay of biological, behavioral, and psychological factors, fatigue appears to have a privileged association with depressive symptoms, independently of genetic background [11]. Indeed, fatigue cannot be seen as a purely physical sign, as it is also a common symptom in depressive disorder [12].

Anxiety and depression are highly prevalent among patients with lupus, thought to represent central nervous system involvement [13] or immune dysfunction manifestation [14, 15], or denoting the emotional burden of the disease [16, 17]. Recently, a link has been established among neurotransmitter dysfunction, immune activation (lymphocyte abnormalities and cytokine expression), and major depression [18, 19]. In this paper, we consider fatigue and depression as examples of silent burdens of disease, in a cross-sectional study of three groups of participants: patients with SLE, patients with major depressive disorder, and age- and sex-matched controls.

The principal objective of the work was twofold: to highlight the importance of single patient derived symptoms over the course of the disease and to stress the importance of SLE as a modern model chronic disease as important as cancer, if not more so. This importance is derived from the multiple effects resulting from the challenge of crosstalk between the immunological system and other systems, in the absence of infection.

2. Study Participants and Methods

One hundred forty-eight female participants were enrolled in the present study, 50 of whom were patients diagnosed with SLE, recruited from the rheumatology outpatient clinic of the São João Hospital, EPE. All SLE patients fulfilled the ACR diagnostic criteria. Only adult patients (>18 years old) without diagnosed psychiatric comorbidities were selected. All patients attended routine visits at the hospital and completed regular clinic and laboratorial assessments.

Patients with lupus were compared to one group of 53 healthy age-matched subjects and a group of 45 patients with major depressive disorder (MDD), followed by one of the authors (mmfb) in her private psychiatric clinic. Psychiatric patients were diagnosed by a psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) [20]. The study was submitted and approved by the Ethical Committee of the São João Hospital (EPE). Participants received oral and written information about the study's goals, methods, expected benefits, and discomfort, after which they gave their oral and written informed consent. The confidentiality and privacy of the collected data were guaranteed during the data collection and analysis stages according to the Declaration of Helsinki.

The sociodemographic characterization of the populations studied is shown in Table 1.

3. Data Collection

Following a cross-sectional approach, psychosocial data were collected between September 2012 and June 2013. A first contact established the willingness of the patients to participate. After a first verbal consent was obtained, written informed consent was mailed and retrieved from all participants before the study began. Recruited patients and controls were subsequently interviewed by phone by a trained interviewer (RF). The literature corroborates phone interviews as valid and precise tools for psychological data collection [21–25].

Participants' sociodemographic data included age, educational level, employment status (active/nonactive), and marital status. Laboratorial and SLE standardized clinical evaluation were obtained exclusively in the SLE patients through the clinical records.

4. Instruments

4.1. Psychosocial Evaluation. Psychological variables were obtained through a battery test: the Fatigue Severity Scale (FSS), the Hospital Anxiety and Depression Scale (HADS), the Pittsburgh Sleep Quality Index (PSQI), and the Short Form-36 Health Survey (SF-36v2).

Self-reported fatigue was evaluated through the short version of the FSS [26]. This unidimensional nine-item Likert scale was designed to assess fatigue in chronic medical and rheumatologic conditions and is recommended as the instrument of choice for research purposes in studies involving patients diagnosed with SLE [27]. The Portuguese version of the FSS used in the present work has been validated for SLE patients [28].

The FSS demonstrates good internal consistency (Cronbach's α = 0.89 in SLE patients), test-retest reliability (0.84), and construct and discriminative validity and is sensitive to change. Each question is scored from 1 to 7, and a final score is obtained from the mean of all scored items. Higher scores reveal increasing severity of fatigue. Presence of clinical levels of fatigue was defined by a FSS score >3, as proposed by Krupp and collaborators [26]. The use of FSS assessment in an SLE population by telephone interview has been established [29].

The Hospital Anxiety and Depression Scale (HADS) [30] is used to assess depressive and anxiety symptoms. This questionnaire contains 14 items, scored from 0 to 3, to achieve a total of 0 to 21. It is divided into two sets of seven questions aiming to detect, respectively, depressive and anxiety states. Scores exceeding 8 points indicate possible mood disorder, and 10 points delimit pathological situations. This instrument does not contain items focused in physical indicators of psychological distress or somatic complaints, which improves its sensitivity to anxiety and depression in physically ill individuals. Factor analysis confirmed the bidimensionality of the scale and the correlation between the anxiety and depression subscales. Both subscales showed suitable internal consistency (Mean Cronbach's alpha = 0.83 for HADS-A and 0.82 for HADS-D) [31]. Telephone-administered mode has been described for the HADS, maintaining similar psychometric properties [32].

Participants were also screened for sleep quality through the Pittsburgh Sleep Quality Index (PSQI). A weighted global score of 0 to 21, reflecting a four-week time interval, is yielded from the seven components subjectively evaluated: sleep latency, sleep disturbances, sleep duration, sleep quality, sleep efficiency, use of sleep medications, and daytime dysfunction. This instrument has good psychometric properties, with high homogeneity, reliability (Cronbach's alpha = 0.83), and validity [33, 34]. Poor sleepers are identified by a PSQI score >5 [33]. When used in a telephone interview, the PSQI presented an adequate internal consistency and proved to be a reliable mode for sleep quality assessment [35, 36].

The Short-Form 36 Health Survey Version 2.0 (SF-36v2) was elected to assess health-related quality of life (HRQoL) [37]. This scale comprises 36 self-rated questions, reproducing eight domains: physical functioning (PF), role limitations due to physical problems (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE), and mental health (MH). These domains can be individually evaluated or summarized in the physical (PSC) and mental (MSC) summary components. A gradual score ranging from 0 to 100 in each area reflects enhanced quality of life. The SF-36 can be either self-administered or administered by a trained interviewer, either in person or by telephone [38, 39]. The SF-36-v2 is the most widely used questionnaire to assess quality-of-life-related outcomes in SLE patients, providing the additional possibility of comparing the obtained results with those of other healthy or patient populations [40].The Cronbach's alpha for studies enrolling SLE patients was recently computed from the available literature (Cronbach's α = 0.71–0.95) [41].

4.2. Clinical SLE Indexes. Disease activity was estimated with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [42]. This physician-rated instrument reports 16 clinical and eight laboratorial descriptors appraised during the previous 10 or 30 days. The SLEDAI score retrieved during the last (most recent) patient visit was used. Patients were grouped into four categories, according to SLEDAI total score, representing increasing activity of the disease: no activity (0), mild activity (1–5), moderate activity (6–10), high activity (11–19), and very high activity (20) [43]. A score of 6 or more was considered as clinically active disease.

The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index [44] was used to compile irreversible impairment in all SLE patients. This index reflects accumulated damage continuously present in a six-month period and targets 12 distinct organs/systems.

4.3. Laboratory Evaluation. Laboratorial evaluation included in the patient's clinical records was used to determine immune activation, inflammatory status, antibody profile, and vitamin D quantifications. Immune activation was determined by autoantibodies detection: antidouble stranded DNA, nucleosome, Smith, Sjogren Syndrome A, Sjogren Syndrome B, histones, cardiolipin (IgG and IgM isotypes), β2-glycoprotein I (IgG and IgM isotypes), antiribonucleoproteins, and Ribosomal P substance. The C-Reactive Protein (CRP), inactive C1, C1q, C3c, C4, and CH50 complement fractions and Erythrocyte Sedimentation Rate (ESR) determinations were also considered. Blood and serological measurements were performed according to standardized methods in the hospital's laboratory, and standardized cutoff levels were established. Only two laboratory tests correlate with fatigue: CH50 and anti-ds-DNA determinations (data not shown).

Table 2: Fatigue assessment.

	Total $n = 148$	SLE (1) $n = 50$	Depression (2) $n = 45$	Controls (3) $n = 53$	P	Post hoc analysis
FSS global score[a,b]	4.3 (1.7) 1.0–7.0	5.2 (1.3) 1.6–7.0	4.5 (1.4) 1.7–7.0	3.2 (1.8) 1.0–10.4	0.000[d]	2, 1 > 3
Fatigue severity level[c]						
Nonclinical	36 (24.3)	5 (10.0)	6 (13.3)	25 (47.2)		
Clinical	101 (68.2)	45 (90.0)	35 (77.8)	21 (39.6)	0.000[e]	
Missing	11 (7.4)		4 (8.9)	7 (13.2)		

[a]Mean (standard deviation); [b]minimum–maximum; [c]n (%); [d]ANOVA; [e]Chi-square test.

Table 3: Correlations (Pearson) between fatigue and psychosocial and anthropometric characteristics, quality of life, and sleep quality.

	SLE (1) $n = 50$	Depression (2) $n = 45$	Controls (3) $n = 53$
Age (years)	0.489**	−0.061	0.243
Education (years)	−0.476**	−0.043	−0.294
HADS			
Anxiety	0.542**	0.202	0.397**
Depression	0.576**	0.402*	0.410**
SF-36			
PSC	−0.717**	−0.351*	−0.466**
Physical functioning	−0.648**	−0.103	−0.318*
Role limitations due to physical health problems	−0.699**	−0.393*	−0.302*
Social functioning	−0.354*	−0.389*	−0.226
Mental health	−0.464**	−0.416**	−0.435**
Role limitations due to emotional problems	−0.315*	−0.249	−0.274
Vitality	−0.677**	−0.323*	−0.660**
Bodily pain	−0.563**	−0.450**	−0.287
General health	−0.617**	−0.418**	−0.580**
PSQI global score	0.401**	0.334*	0.425**
BMI (kg/m^2)	0.135	−0.82	−0.301*

*$P < 0.05$, **$P < 0.01$; PSC: Physical summary component; BMI: body mass index.

4.4. Statistical Analysis. Statistical analysis was performed using the Statistical Package for Social Science 18.0 (SPSS).

A descriptive analysis of the obtained results was performed, and the data were expressed as frequencies (%), minimums, maximums, means, and standard deviations.

Analysis of variance (ANOVA) was used to investigate differences between groups, and Pearson Chi-square test was conducted for categorical variables. Post-hoc Tukey test was performed to provide a stratified comparison between groups, when necessary. For correlation analysis, the Spearman's coefficient was computed. Confidence intervals of 95% and a significance level of 0.05 were adopted.

5. Results

5.1. Fatigue Assessment: Significant Associations with Age, Psychological Suffering, and Educational Level (Tables 1, 2, and 3). The sociodemographic characterization of the three groups showed that all participants presented similar age, with a mean value of 44.5 years and similar marital status. Regarding employment status and education, however, lower scores were observed in the SLE group, with statistical significance when compared to depressed and control women (Table 1).

Fatigue was not an exclusive burden of SLE. It was reported in 90% of women with SLE and 77.8% of the female MDD patients, in contrast with 39.6% in the control group.

The global score of FSS revealed similar fatigue severity in SLE and MDD patients (5.2 ± 1.3 and 4.5 ± 1.4, resp.), which is significantly higher than the scores found in the control group (3.2 ± 1.8, $P = 0.0001$) (Table 2).

The result of the search for significant correlations between fatigue and markers of psychological suffering is detailed as a correlational analysis in Table 3.

Significant correlations were detected between fatigue severity, age, and education exclusively in patients with SLE. Higher fatigue was associated with older age, possibly reflecting the cumulative physical impairment of disease and aging.

Table 4: Anthropometric characterization, health-related behaviors, and sleep quality.

	Total $n = 148$	SLE (1) $n = 50$	Depression (2) $n = 45$	Controls (3) $n = 53$	P	Post hoc analysis
BMI (kg/m^2)[a]	24.9 (5.5)	24.8 (4.1)	25.7 (7.8)	24.3 (4.0)	0.466[c]	
BMI categories[b]						
Underweight	7 (4.7)	1 (2.0)	3 (6.7)	3 (5.7)	0.701[d]	
Normal range	85 (57.4)	31 (62.0)	24 (53.3)	30 (56.6)		
Preobesity	32 (21.6)	13 (26.0)	8 (17.8)	11 (20.8)		
Obesity	18 (12.2)	5 (10.0)	8 (17.8)	5 (9.4)		
Missing	6 (4.1)	0 (0.0)	2 (4.4)	4 (7.5)		
Smoking habits[b]						
Smokers	34 (23.0)	10 (20.0)	18 (40.0)	6 (11.3)	0.003[d]	
Nonsmokers	114 (77.0)	40 (80.0)	27 (60.0)	47 (88.7)		
Alcohol consumption[b]						
Yes	13 (8.8)	3 (6.0)	2 (4.4)	8 (15.1)	0.124[d]	
No	135 (91.2)	47 (94.0)	43 (95.6)	45 (84.9)		
Physical activity[b]						
Yes	63 (42.6)	18 (36.0)	18 (40.0)	27 (50.9)	0.283[d]	
No	85 (57.4)	32 (64.0)	27 (60.0)	26 (49.1)		
Sleep						
PSQI global score[a]	10.3 (3.8)	10.9 (4.0)	11.3 (3.7)	9.0 (3.4)	0.007[c]	2, 1 > 3
Quality of sleep[b]						
Good sleepers	16 (10.8)	3 (6.0)	3 (6.7)	10 (18.9)	0.140[d]	
Poor sleepers	126 (85.1)	46 (92.0)	39 (86.7)	41 (77.4)		
Missing	6 (4.1)	1 (2.0)	3 (6.7)	2 (3.8)		

[a]Mean (standard deviation), [b]n (%), [c]ANOVA, [d]Chi-square test; BMI: body mass index.

Lower education was seen to be related to higher fatigue scores.

Significant correlations between anxiety and fatigue were detected in SLE and control population ($r = 0.542$ and $r = 0.397$, resp.).

Mental health scores, vitality, and depressive symptoms presented significant associations with fatigue severity, regardless of participant group (Table 3).

In the SLE group of women, education correlated significantly with anxiety (HADS-A: $r = 0.313$, $P = 0.027$), depressive symptoms (HADS D $r = 0.452$, $P = 0.001$), and bodily pain ($r = 0.527$, $P < 0.005$).

5.2. Weight, Sleep, and Physical Activity (Table 4). In order to clarify classically reported associations among fatigue, excessive weight, sleep abnormalities, and physical activity, participants were subjected to anthropometric, sleep quality, and health-related behavior evaluations.

In our study, disturbed sleep quality affected the three groups, although higher global PSQI scores ($P = 0.007$), representing poorer sleep quality, were detected in SLE and MDD patients.

The presence of obesity and preobesity was equally distributed across the studied sample, and BMI scores were similar in all the groups. Evaluation of health-related behaviors revealed that physical activity and alcohol consumption were similar in the three groups. Smoking habits, on the contrary, were more prevalent in psychiatric patients (Table 4).

5.3. Fatigue and Quality-of-Life Dimensions: Physical Summary Components, Pain, and General Health (Table 5). Fatigue has been extensively associated with poorer health-related quality of life in SLE patients. Accordingly, we detected statistically significant lower scores in quality-of-life dimensions related to physical impairment in SLE patients; results in physical summary components, bodily pain and general health were statistically significantly different in the SLE group when compared to the MDD group and control subjects. MDD patients, however, presented significant levels of pain, reduced PSC, and general health scores different from healthy controls.

Other dimensions expressed in mental summary components (social functioning, mental health, and role limitations due to emotional problems), while showing significant impairment in SLE patients, presented lower scores in the MDD group when compared to the control population (Table 5).

5.4. Fatigue, Anxiety, and Depression (Table 6). Loss of energy or exhaustion can characterize fatigue and also be regarded as a depressive symptom. In the present study, measures of depression were obtained using HADS depression subscale in the three groups of participants, and they revealed

Table 5: Health-related quality of life.

	Total $n = 148$	SLE (1) $n = 50$	Depression (2) $n = 45$	Controls (3) $n = 53$	P	Post hoc analysis
SF-36						
Physical functioning[a]	71.8 (26.7)	55.8 (30.5)	75.9 (22.2)	84.3 (16.7)	0.000[b]	1 < 2, 3
Role limitations due to physical health problems[a]	62.5 (30.8)	44.5 (32.6)	59.8 (23.5)	81.3 (22.4)	0.000[b]	1 < 2 < 3
Social functioning[a]	63.0 (28.6)	61.5 (31.0)	46.6 (22.5)	78.1 (22.5)	0.000[b]	2 < 1 < 3
Mental health[a]	56.8 (22.1)	55.8 (20.2)	43.0 (17.5)	69.3 (20.2)	0.000[b]	2 < 1 < 3
Role limitations due to emotional problems[a]	70.1 (27.9)	68.7 (29.0)	52.4 (22.9)	86.5 (20.5)	0.000[b]	2 < 1 < 3
Vitality[a]	45.6 (22.9)	34.9 (23.1)	39.4 (16.8)	70.0 (18.8)	0.000[b]	1, 2 < 3
Bodily pain[a]	60.7 (31.6)	43.7 (34.6)	64.5 (26.1)	73.7 (25.5)	0.000[b]	1 < 2, 3
General health[a]	49.8 (23.7)	31.3 (18.5)	56.2 (21.2)	61.9 (19.2)	0.000[b]	1 < 2, 3
PSC[a]	0.0 (1.0)	−0.8 (0.9)	0.4 (0.7)	0.5 (0.6)	0.000[b]	1 < 2, 3
MSC[a]	0.0 (1.0)	0.1 (0.9)	−0.8 (0.8)	0.6 (0.8)	0.000[b]	2 < 3 < 1

[a] Mean (standard deviation); [b] ANOVA; PSC: physical summary component; MSC: mental summary component.

Table 6: Depression and anxiety symptoms.

	Total $n = 148$	SLE (1) $n = 50$	Depression (2) $n = 45$	Controls (3) $n = 53$	P	Post hoc analysis
HADS-D[a]	6.4 (4.5)	6.7 (5.0)	8.4 (4.0)	4.5 (3.5)	0.000[b]	2, 1 > 3
HADS-A[a]	8.8 (4.6)	8.5 (4.8)	11.4 (3.7)	6.8 (4.1)	0.000[b]	2 > 1, 3

[a] Mean (standard deviation); [b] ANOVA.

Table 7: Correlation (Pearson) between fatigue and disease-related markers in SLE patients.

	FSS
SLEDAI	−100
SLICC	0.043

the occurrence of similar depressive symptoms in SLE patients and MDD patients (6.7 ± 5.0 and 8.4 ± 4.0, resp.), which are significantly higher than in the normal population. Anxiety presented higher scores in MDD patients (11.4±3.7), which are statistically significantly different from the SLE and control groups.

5.5. Lack of Correlations between Clinical SLE Evaluation and Severity of Fatigue (Table 7). Clinical indexes used to assess disease activity and damage in SLE patients did not present significant associations with fatigue severity, failing to translate subjective patients' complaints into clinical standardized evaluation.

6. Discussion

The spectacular progress in our understanding of the molecular and genetic basis of disease is transforming clinical practice and the nature of patient-physician interaction. Indeed, distracted by the panoply of biological markers at his/her disposal, the clinician may miss subjective symptoms of relevance to the development of disease. Fatigue, anxiety, and depression fall in the category of subjective symptoms that may escape the attention of clinicians *and* patients. Because of their impact in the course of a chronic disease such as SLE, such symptoms will become of increasing clinical and social value as the concept of health itself changes. As emphasized by others, "health is about more than avoiding death" [1].

As shown by the results of the present study, some correlations of fatigue, for example, with sleep quality were common to all groups examined, namely, SLE, MDD, and the control group. A correlation of fatigue with anxiety was also seen within the control group.

The presence of obesity and BMI scores was similar in all groups just as alcohol consumption and physical activity. Smoking habits were more prevalent in the psychiatric group.

We would like to highlight two observations made exclusively in SLE: the correlation with age and education and the correlation with depression (not exclusively seen in SLE).

6.1. Fatigue and Depression. In a recent analysis of fatigue in monozygotic and dizygotic twins, chronic fatigue and psychological distress were strongly associated without evidence of genetic covariation, implying, according to the authors, that the "association is environmental" [11]. In an earlier large analysis of a World Health Organization longitudinal study of fatigue and depression, Skapinakis et al. concluded that unexplained fatigue and depression might act as independent factors of each other [45].

However, Palagini et al., in a review based on the search of SLE and depression as key-words in several major databases, concluded that to date, the relationship between depression and SLE disease activity appears controversial, stressing the need for identification of SLE-specific biomarkers of depression. Methodological limitations are present in the available literature, and the standardization of methodologies should be considered a high priority in SLE research [46].

In the present cross-sectional study, we confirmed the existence of a link between fatigue and depression in the patients studied. From our own previous work [14] and more recent work on the association of immune activation and depression, unexplained fatigue may signify an early alert signal of a flare-up of immune activation [18, 19], and the search for cytokine markers in SLE [47] should perhaps be extended to fatigue in SLE.

6.2. Fatigue and Education Level. Comparison of educational levels can be done only between the SLE and control groups. The link between education level and fatigue strengthens the point made by others about the importance of the role of health professionals, including nurses, in explaining the disease to patients [48]. Patients with lower education levels challenge clinicians and other health professionals' ability to gather and share important information. Reduced educational achievement may affect a patient's ability to understand, and, in addition, to seek appropriate clarification of doubts regarding the disease, the treatment, or its expected outcomes [49]. Lack of knowledge can comprehensibly add more anxiety and suffering to the difficulty of living with a disease with uncertain evolution and unpredictable flares.

7. Conclusion

Giving the attention that fatigue and depression may deserve as silent burdens of disease in SLE, we may be preventing a deleterious progression of a disease and, thus, diminishing the costs recently estimated in Sweden, where a total of 339 patients with the mean age of 55 years were analyzed. The mean Health Related Quality of Life (HRQoL) measured through the five-item EQ-5D instrument was 0.64, and total costs were estimated at €22,594 (direct costs €7,818; indirect costs €14,776). Disease activity, fatigue, and corticosteroid doses had a statistically significant impact on costs and HRQoL. This study demonstrates that Swedish patients with SLE have low HRQoL and incur high societal costs that are both associated with and most likely driven by disease activity, fatigue, and corticosteroid use [3].

The objective measure of costs has, like molecular and genetic progress, become a carefully "listened to" burden of health care and chronic disease.

We wish to conclude by returning to our starting reference to the Global Burden of Disease study, referencing one of their interpretations: *"Prevalences of the most common causes of YLDs such as mental and behavioural disorders and musculoskeletal disorders have not decreased. Health systems will need to address the needs of the rising numbers of individuals with a range of disorders that largely cause disability but not mortality"*—such as SLE [1].

The health system will work well proportionally to the attention and time clinicians can give to one patient. We hope this study of SLE as a model of all modern chronic diseases, with all its limitations as a cross-sectional study and its small numbers, will nevertheless help clinicians and patients to become aware of the importance of the weight of their silent burden in the global burden of modern disease, where *"health is about more than avoiding death"* [1].

Identifying silent burdens in SLE is essential at all times to the care and follow-up of disease progression.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Review Article

Genes Associated with SLE Are Targets of Recent Positive Selection

Paula S. Ramos,[1] Stephanie R. Shaftman,[2] Ralph C. Ward,[2] and Carl D. Langefeld[3]

[1] *Department of Medicine, Medical University of South Carolina, Charleston, SC 29425, USA*
[2] *Department of Public Health Sciences, Medical University of South Carolina, Charleston, SC 29425, USA*
[3] *Department of Public Health Sciences, Wake Forest School of Medicine and Center for Public Health Genomics, Winston-Salem, NC 27157, USA*

Correspondence should be addressed to Paula S. Ramos; ramosp@musc.edu

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The reasons for the ethnic disparities in the prevalence of systemic lupus erythematosus (SLE) and the relative high frequency of SLE risk alleles in the population are not fully understood. Population genetic factors such as natural selection alter allele frequencies over generations and may help explain the persistence of such common risk variants in the population and the differential risk of SLE. In order to better understand the genetic basis of SLE that might be due to natural selection, a total of 74 genomic regions with compelling evidence for association with SLE were tested for evidence of recent positive selection in the HapMap and HGDP populations, using population differentiation, allele frequency, and haplotype-based tests. Consistent signs of positive selection across different studies and statistical methods were observed at several SLE-associated loci, including *PTPN22*, *TNFSF4*, *TET3-DGUOK*, *TNIP1*, *UHRF1BP1*, *BLK*, and *ITGAM* genes. This study is the first to evaluate and report that several SLE-associated regions show signs of positive natural selection. These results provide corroborating evidence in support of recent positive selection as one mechanism underlying the elevated population frequency of SLE risk loci and supports future research that integrates signals of natural selection to help identify functional SLE risk alleles.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease whose prevalence, incidence, and disease severity are known to vary among ethnic groups. Increased prevalence has been reported among African-Americans, Asians, Hispanics, and Native Americans (reviewed elsewhere [1, 2]). The reasons for the ethnic disparities remain elusive. According to the "hygiene hypothesis" first proposed by Strachan two decades ago [3], the increased disease prevalence of autoimmune and allergic diseases in industrialized countries may be due to modern society's limited pathogen exposure. The Hygiene Hypothesis posits that humans have adapted to infectious exposures that were the norm in the past and that exposure was protective against autoimmune disease. Over many generations environmental pressure may have favored alleles that allow humans to respond to immune system challenges differently but resulted in an increased risk of autoimmune diseases. This could be a mechanism explaining the number of SLE risk alleles that are common in the population.

Human genome variation at the population level is shaped by four evolutionary processes: mutation, migration, random genetic drift, and natural selection. *Natural selection* is the process by which a trait, in the context of the organism's environment, becomes either more or less common in a population as a function of the effect of the inherited trait on the differential reproductive success. This ability to survive and reproduce and contribute to the gene pool of the next generation is known as *fitness*. Natural selection drives *adaptation*, the evolutionary process whereby over generations the members of a population become better suited to survive and reproduce in that environment. While *negative selection* decreases the prevalence of traits that diminish individuals' fitness, *positive selection* increases the prevalence of adaptive traits. Left untreated, SLE would have a reproductive fitness cost, defined as the ability to raise offspring that successfully reproduce. Thus, some evolutionary process must sustain the relative high frequency of SLE risk alleles seen in current populations around the world. We hypothesize that since the human genome is shaped by adaptation to environmental pressures at the population level, one plausible reason for the higher frequency of disease-risk alleles may be the direct effect of population-specific positive natural selection.

There is compelling evidence that natural selection is acting on a significant fraction of all genes (~3%) [4–7] and as much as 10% of the human genome [8]. Multiple studies have identified genes involved in immune-related functions to be under selection [8–10], including the *HLA* [11–14] (associated with all autoimmune diseases), *BTLA* [10] (associated with rheumatoid arthritis), *ITPR3* [10] (SLE, type 1 diabetes, Grave's disease), *PTPN22* [10] (rheumatoid arthritis, Crohn's disease, type 1 diabetes, vitiligo), *ITGAX* [10] (SLE), and *BLK* [10] (SLE, rheumatoid arthritis, Kawasaki disease). Finally, we have recently provided evidence that variants within the *APOL1* gene known to be under selective pressure in some African populations predispose to end-stage kidney disease in SLE [15]. Given the increasing evidence of selection at loci associated with human autoimmune diseases, identification of alleles under selection may provide further insight into SLE susceptibility and help understand the natural history of SLE predisposition.

2. Methods

A list of genetic regions with compelling evidence of association with SLE was compiled from the literature. This list includes results that met genome-wide significance in any genome-wide association study (GWAS) or transethnic study of SLE and common or rare variants that are considered established SLE-predisposing loci from candidate gene and other studies. The list of regions was based on the literature as of August 2013 and comprises 89 genes in 74 genomic regions.

This list was built upon all the SLE-associated regions described in recent reviews [16–19], which include common and rare variants from candidate gene studies with compelling evidence of association with SLE. We included all reported risk variants for SLE using data from the National Human Genome Research Institute's Catalog of Published GWAS (http://www.genome.gov/gwastudies) accessed on August 30th, 2013 [20]. Finally, we searched PubMed (http://www.ncbi.nlm.nih.gov/pubmed) for all large-scale transethnic or multiracial studies in SLE and catalogued all variants with a reported meta-analysis P value $< 5 \times 10^{-7}$. The references for these more recent studies are included in Table 1. Given the paucity of studies conducted in some minority populations, and in order to avoid differential bias due to the number of reported associations in different ethnic groups, we chose to include all variation regardless of the population(s) where they were reported and ignore the information about the population(s) where they have been reported to date.

Assuming no other influencing factors, the advantageous alleles at a locus under positive selective pressure will tend

Table 1: Genetic regions with compelling evidence for association with SLE.

Gene(s) region	Chr	Pos (Mb)
C1q [21]	1	22.96
IL12RB2 [22]	1	67.55
PTPN22 [22–25]	1	114.16
FCGR2A, FCGR3A [26, 27]	1	159.74
TNFSF4 [22, 28–33]	1	171.42
NMNAT2 [22, 24, 32]	1	181.48
NCF2 [22]	1	181.79
APOBEC4 [28]	1	181.88
CFH [34]	1	194.89
CFHR1, CFHR4 [34]	1	196.79
CRP [35]	1	199.72
IL10 [22]	1	205.01
LYST [22]	1	233.89
RASGRP3 [28, 32]	2	33.51
TET3, DGUOK [28]	2	74.21
IFIH1 [22, 36]	2	162.83
STAT4 [22–24, 32, 37–41]	2	191.60
PDCD1 [42]	2	242.44
SCN10A [28]	3	38.71
TREX1 [43]	3	48.48
DNASE1L3 [44]	3	58.15
PXK [22–24]	3	58.29
TMEM39A [45]	3	120.63
CD80 [28]	3	120.73
AFF1 [46]	4	88.15
BANK1 [23, 28, 47]	4	102.93
LEF1 [46]	4	109.19
IL21 [48]	4	123.75
PPP2CA [49]	5	133.53
TNIP1 [22, 28, 32]	5	150.39
PTTG1 [22, 32]	5	159.78
C4 [50]	6	32.09
HLA-DRB1 [24, 39, 51–53]	6	32.59
ITPR3 [54]	6	33.70
UHRF1BP1 [22, 28]	6	34.87
BACH2 [28]	6	90.69
ATG5, PRDM1 [22–24, 28]	6	106.53
TNFAIP3 [22, 28, 32, 38, 55]	6	138.23
ICA1 [22, 24]	7	8.12
JAZF1 [22]	7	27.84
IKZF1 [28, 32]	7	50.31
IRF5, TNPO3 [22, 24, 28, 39, 40, 56, 57]	7	128.37
XKR6 [24]	8	10.79
BLK [22–24, 28, 39, 40]	8	11.39
LYN [24]	8	56.95

Table 1: Continued.

Gene(s) region	Chr	Pos (Mb)
ARMC3 [22]	10	23.26
LRRC18, WDFY4 [28, 32, 33]	10	49.89
ARID5B, RTKN2 [28]	10	63.94
SLC29A3 [28]	10	72.75
PHRF1, IRF7 [22–24]	11	0.58
CD44, PDHX [28, 58]	11	34.94
DDX6 [22, 32]	11	118.13
ETS1 [28, 32, 33]	11	127.83
CREBL2, GPR19, CDKN1B [28]	12	12.66
DRAM1 [28]	12	102.27
SLC15A4 [28, 32]	12	127.84
ELF1 [28]	13	40.40
C2 [59]	14	20.75
CSK [60]	15	72.86
DNASE1 [61]	16	3.64
CLEC16A [28]	16	11.04
PRKCB [62]	16	23.75
SEZ6L2 [28]	16	29.79
ITGAM, ITGAX [23, 24, 28, 63]	16	31.18
IRF8 [22, 45]	16	84.49
IKZF3, ZPBP2 [45]	17	37.91
CD226 [22, 57, 64]	18	65.68
TYK2 [22, 57]	19	10.32
ICAM1, ICAM4, ICAM5 [65]	19	10.40
ACP5 [66]	19	11.55
DDA1 [28]	19	17.28
UBE2L3 [22–24, 28]	22	20.25
SCUBE1 [24]	22	41.93
IRAK1, MECP2 [22, 23, 67, 68]	X	152.93

The reference list for each gene region does not intent to be exhaustive; instead, only the first and/or strongest associations reported to date are mentioned. A comprehensive list of all the studies that report each region have been recently reviewed elsewhere [16–18]. Chr: chromosome; Pos: position (in Mega basepairs) according to Human Genome Build hg18.

to stochastically increase in prevalence over generations. This can lead to allele frequency differences between populations, which can be detected using statistics that compare the genetic variability within and between populations [69]. It can also lead to the haplotype carrying the advantageous allele to remain longer than genetic distance predicts around alleles of equal frequency, which can be measured using haplotype-based statistics [7]. The evidence of selection in each SLE-associated region was analyzed using both population differentiation, allele frequency spectrum, and haplotype-based statistics in the HapMap II and HGDP populations as implemented in the Haplotter (http://haplotter.uchicago.edu/) [7] and the Human Genome Diversity Project (HGDP) Selection Browsers (http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP/) [70], respectively.

Haplotter displays the results of a scan for positive selection in the human genome using the International HapMap Project data (http://haplotter.uchicago.edu/) [7]. These data consist of ~800,000 polymorphic SNPs in three distinct population samples of unrelated individuals: 89 Japanese and Han Chinese individuals from Tokyo and Beijing, respectively, denoted as East Asian (ASN), 60 individuals of northern and western European origin (CEU), and 60 Yoruba (YRI) from Ibadan, Nigeria. It shows results on the autosomes only. Results from several selection statistics are displayed, including (1) the fixation index (F_{ST}), (2) the Tajima's D, and (3) the integrated haplotype score (iHS). In situations where selection is restricted to certain populations or geographical locations, the allele frequencies at the locus that is undergoing selection may vary significantly between different populations. The fixation index F_{ST} provides a metric of the magnitude of global allele frequency differentiation between populations at a locus [69, 71]. F_{ST} is directly related to the variance in allele frequency among populations and, conversely, to the degree of resemblance among individuals within populations. If F_{ST} is small, it means that the allele frequencies within each population are similar; if it is large, it means that the allele frequencies are different [72]. The Tajima's D is based on the frequencies of the polymorphisms segregating in a locus [73]. As described [7], positive selection results in an excess of high frequency derived alleles compared to neutral expectations when the selected allele has swept to high frequencies. Positive selection also results in an excess of low frequency polymorphisms, especially when the selected allele is close to fixation or right after fixation. This skewing of SNP frequencies in different directions can be detected by Tajima's D, which is based on the frequencies of SNPs segregating in the region of interest [73]. Signals of selective sweeps will result in high negative D. The integrated haplotype score (iHS) uses the lengths of the haplotypes surrounding each core SNP to identify SNPs for which alleles have rapidly risen in frequency [7, 74]. It is based on linkage disequilibrium (LD) surrounding a positively selected allele compared with background, providing evidence of recent positive selection at a locus [7]. An iHS score > 2.0 reflects the fact that haplotypes on the ancestral background are longer compared with those on the derived allelic background.

For these analyses, genome-wide SNP data from Phase II of the HapMap Project were used to investigate if the regions associated with SLE showed evidence of selection in the CEU, YRI, and ASN populations using these three metrics (iHS, Tajima's D, and F_{ST}). Regions of 1 Mb around each of the 74 regions in Table 1 were queried, and, when higher than 2, the maximum value on the Y-axis ($-\log(Q)$) in this 1 Mb interval was recorded. As described by Voight et al. [7], the $-\log(Q)$ value represents the negative log of the rank of the observed statistic for a given SNP divided by the total number of SNPs. The statistic that is ranked is obtained independently for each of the three statistics separately for each population. For D, the estimated value of D was used for ranking. For iHS, for each SNP, 25 SNPs on either side of the SNP are scanned for $|\mathrm{iHS}| > 2$. The proportion of SNPs in this 51 SNP window with $|\mathrm{iHS}| > 2$ is computed. For F_{ST}, the statistic to be ranked is obtained in a similar manner as that for iHS except for each population comparison, the thresholds for defining a significant F_{ST} is based on the top 5% cutoff for each population comparison. The different thresholds used for F_{ST} were CEU-YRI: 0.2976, CEU-ASN: 0.2055, and YRI-ASN: 0.3374. Haplotter also displays the F_{ST} value of the SNPs in the top 1% within each population comparison, which were also recorded, if any such SNPs were present in the 1 Mb interval. In addition to these, Haplotter shows an empirical P value estimated for each gene and for each population, as detailed by Voight et al. [7]. When this P value showed significant evidence for selection, the value was recorded.

The HGDP Selection Browser displays results from a series of genome-wide scans for natural selection using single nucleotide polymorphism (SNP) genotype data from the Human Genome Diversity-CEPH Panel (HGDP), a dataset containing 938 individuals from 53 populations typed on the Illumina 650Y platform (http://hgdp.uchicago.edu/cgi-bin/gbrowse/HGDP/) [70]. Summary statistics regarding haplotype structure and population differentiation on this data can be queried in the browser. These include the iHS, the F_{ST}, and the cross-population extended haplotype homozygosity test (XP-EHH) [74]. While the iHS detects partial selective sweeps of moderate frequency (~50%–80%), the XP-EHH detects selected alleles that have risen to near fixation in one population (above 80% frequency) [7, 74]. As described by Pickrell et al. [70], the F_{ST} was calculated on the level of population groupings identified by Rosenberg et al. [75]; that is, if a SNP has high F_{ST}, most of the variance in allele frequencies is captured by the seven labels identified in that paper. In the browser, plotted is the $-\log_{10}$ of the empirical P value for each SNP—the higher this plotted $-\log_{10} P$ value, the more extreme (high) the F_{ST} value is compared the rest of the genotyped SNPs. The iHS was calculated as in Voight et al. [7] and smoothed across windows. Plotted is the $-\log_{10}$ of the P value for a window centered at the SNP; high values again indicate potential signals of positive selection. The test statistic was the fraction of SNPs with $|\text{iHS}| > 2$. The XP-EHH was calculated as in Sabeti et al.'s work [74]. The test statistic was the maximum XP-EHH. Again, the plotted measure is a measure of how extreme a SNP is with regard to the rest of the genome, and high values indicate outliers potentially due to the action of natural selection. The iHS and XP-EHH have been calculated in each individual population, as well as in the following groupings: Bantu-speaking populations, Europeans, Middle Easterners, Central Asians, East Asians, Americans, and Oceanians.

Regions of 1 Mb around each of the 74 regions in Table 1 were queried, and the maximum value on the Y-axis ($-\log(P)$) in this 1 Mb interval was recorded.

3. Results

To test whether SLE susceptibility loci show evidence of positive selection, a list of 74 genetic regions with compelling evidence of association with SLE was compiled (Table 1). In order to test whether SLE-associated loci show evidence for recent positive selection, 1 Mb regions around each of the 74 regions were queried. Regions where the maximum $-\log(Q) > 3$ (for Haplotter) or $-\log(P) > 3$ (for HGDP) for the F_{ST}, D, iHS, or XP-EHH were considered as showing evidence for recent positive selection (Tables 2 and 3). In addition, regions that in the HapMap populations had SNPs with F_{ST} values in the top 1% within each population comparison, or whose empirical P value estimated for each gene and for each population showed significant evidence for selection (P value < 0.001) were also considered to show evidence for selection. Of the 74 regions associated with SLE, 19 showed evidence of selection in a HapMap population (Table 2), and 16 exhibited a signal of selection in a HGDP population (Table 3). Many of these loci also had corroborating evidence using different metrics.

In the HapMap data multiple regions displayed evidence of population differentiation, as indicated by the F_{ST}, which was the highest in the *PTPN22*, *TET3-DGUOK*, *ITPR3*, *ITGAM*, and *CD226* regions. Several SNPs with very high F_{ST} (in the top 1% within each population comparison) were identified in these and other regions, especially *XKR6-BLK* ($F_{ST} = 0.92$ in YRI versus ASN), *TET3-DGUOK* ($F_{ST} = 0.85$ in YRI versus ASN, and $F_{ST} = 0.80$ in YRI versus CEU), *CD226* ($F_{ST} = 0.80$ in CEU versus YRI), *LRRC18-WDFY4* ($F_{ST} = 0.80$ in YRI versus ASN), *IFIH1* ($F_{ST} = 0.78$ in CEU versus YRI), *PTPN22* ($F_{ST} = 0.75$ in YRI versus ASN), and *ITGAM* ($F_{ST} = 0.75$ in YRI versus ASN). The highest allele frequency differences, as indicated by the D statistic, were detected in the *PTPN22*, *IFIH1*, *ITPR3*, and *XKR6-BLK* regions. The *ITPR3* region also had a high iHS. This and *BLK* are the regions that displayed the most consistently strong evidence for selection according to all three metrics. The *ITPR3* gene lies at 6p21, adjacent to the centromeric end of the extended MHC region, after the class II flanking region. *XKR6* and *BLK* lie on the same chromosomal inversion at 8p23.1. *PTPN22*, *ITPR3*, and *CD226* exhibited the strongest evidence for selection according to the frequency-based statistics. Finally, several regions included genes whose empirical P value showed significant evidence for selection. These genes included *XKR6* ($P = 0.004$ in ASN) and *UHRF1BP1* ($P = 0.006$ in CEU). Other genes were significant in several regions, such as the *TET3-DGUOK* region (*DUSP11* and *STAMBP* with $P = 0.005$ and $P = 0.007$, resp., in CEU). The *PTPN22*, *ITGAX* (near *ITGAM*), *ITPR3*, and *BLK* regions were recently reported to be under selection (in YRI, YRI, YRI, and ASN, resp.) in a candidate gene study by Grossman et al. [10], who used full-genome sequence variation from the 1000 Genomes Project and the composite of multiple signals (CMS) test.

Since the regions in Table 2 showed evidence of selection in the HapMap samples, the evidence centered at the specific SNP associated with SLE were tested (Supplementary Table 1 in the Supplementary Material available online at http://dx.doi.org/10.1155/2014/203435). Specifically, Haplotter displays the iHS and F_{ST} for common SNPs. Of the queried SLE-associated SNPs, the highest evidence of population differentiation was shown by rs9937837 in *ITGAM* ($F_{ST} = 0.81$ in YRI versus ASN). Evidence for association according to the iHS test was observed in *CFHR1-CFHR4* (rs16840639, iHS $= -2.63$ in YRI), *NMNAT2* (rs2022013, iHS $= 2.50$ in ASN), *APOBEC4* (rs10911390, iHS $= -2.36$ in ASN), *CFH*

Table 2: Regions with evidence for selection on the HapMap populations.

Gene region	Chr	Mb	iHS		D			F_{ST}		Empirical P value	
			Max − log(Q)	Pop	Max − log(Q)	Pop	Max − log(Q)	Value	Pop	Min P value	Pop
PTPN22	1	114.158	—	—	3.6	YRI	3.2	0.75	YRI versus ASN	—	—
TNFSF4	1	171.419	2.5	ASN	2.3	ASN	2.7	0.60	YRI versus ASN	0.005	ASN
NMNAT2	1	181.484	2.5	ASN	2.4	CEU	—	—	—	0.004	ASN
NCF2	1	181.791	2.5	ASN	—	—	—	0.65	CEU versus YRI	0.004	ASN
APOBEC4	1	181.882	2.5	ASN	—	—	—	0.65	CEU versus YRI	0.004	ASN
CFH	1	194.888	—	—	—	—	3.0	0.60	YRI versus ASN	—	—
CFHR1, CFHR4	1	196.789	2.0	YRI	—	—	3.0	0.60	YRI versus ASN	—	—
TET3, DGUOK	2	74.212	2.7	CEU	2.6	ASN	3.2	0.85	YRI versus ASN	0.001	CEU
IFIH1	2	162.832	—	—	3.8	CEU	2.2	0.78	CEU versus YRI	—	—
TREX1	3	48.481	2.4	ASN	2.1	ASN	—	—	—	0.002	ASN
TNIP1	5	150.390	—	—	3.0	CEU	—	0.65	CEU versus YRI	—	—
ITPR3	6	33.697	3.4	YRI	3.3	YRI	3.3	0.60	YRI versus ASN	—	—
UHRF1BP1	6	34.868	2.5	CEU	2.4	YRI	—	0.50	—	0.004	CEU
XKR6	8	10.791	2.7	ASN	3.3	ASN	2.6	0.92	YRI versus ASN	0.003	ASN
BLK	8	11.389	2.7	ASN	3.2	ASN	2.6	0.92	YRI versus ASN	0.005	ASN
ARMC3	10	23.257	—	—	2.5	CEU	2.5	0.65	YRI versus ASN	—	—
LRRC18, WDFY4	10	49.893	—	—	2.0	ASN	2.5	0.80	YRI versus ASN	—	—
ITGAM	16	31.179	—	—	—	—	3.4	0.75	YRI versus ASN	—	—
CD226	18	65.681	—	—	3.1	CEU	3.7	0.80	CEU versus YRI	—	—

Regions were considered to show evidence for selection if the maximum $-\log(Q) > 3$ for either the F_{ST}, D, or iHS, or it had SNPs with F_{ST} values in the top 1% within each population comparison, or the empirical P value estimated for the SLE-associated gene and for each population showed significant evidence for selection (P value < 0.01). Cells that did not meet these thresholds or whose $-\log(Q) > 2$ are marked with (—). The table shows the highest $-\log(Q)$ value and respective population for the iHS, D, and F_{ST}, the F_{ST} statistic (value) for SNPs in the top 1% and the population comparison, and the minimum empirical P value in each region. Q is the rank of the observed statistic for a given SNP divided by the total number of SNPs. The statistic that is ranked is obtained independently for each of the three statistics separately for each population. For iHS, for each SNP, 25 SNPs on either side of the SNP are scanned for $|\text{iHS}| > 2$. The proportion of SNPs in this 51 SNP window with $|\text{iHS}| > 2$ is computed. For D, the estimated value of D was used for ranking. For F_{ST}, the statistic to be ranked is obtained in a similar manner as that for iHS except for each population comparison, the thresholds for defining a significant F_{ST} is based on the top 5% cutoff for each population comparison. See Methods for details. Chr: chromosome, Mb: mega basepairs, Max: maximum, Min: minimum, Pop: population, ASN: East Asian, CEU: European, YRI: African.

Table 3: Regions with evidence for selection in the HGDP populations.

Gene region	Chr	Mb	F_{ST}	iHS		XP-EHH	
			Max − log(P)	Max − log(P)	Pop	Max − log(P)	Pop
PTPN22	1	114.158	2.5	3	Afr	3.5	Afr
TNFSF4	1	171.419	4.5	2.5	EAsia	3.5	EAsia
CRP	1	199.719	3.5	—	—	2.5	Afr, Eur
IL10	1	205.008	4	2	MEast, EAsia	2.5	SAsia EAsia
TET3, DGUOK	2	74.212	2.5	2	SAsia	3.5	MEast, SAsia
TNIP1	5	150.390	3.5	1.5	MEast	3	Amer
PTTG1	5	159.781	—	3.5	Afr	2.8	MEast, Afr
UHRF1BP1	6	34.868	—	3	Amer	3.5	Amer
IKZF1	7	50.315	3.5	3	EAsia	2.5	EAsia
BLK	8	11.389	4	3	SAsia, MEast, Afr	4	EAsia
ARMC3	10	23.257	2.5	2.5	MEast	3.5	MEast
SLC15A4	12	127.844	3.5	—	—	2.5	Afr, Eur
CLEC16A	16	11.038	2	4	Amer	4	Amer
ITGAM	16	31.179	2.5	2	EAsia	3.5	EAsia
IRF8	16	84.490	2.5	2	SAsia	4	SAsia
SCUBE1	22	41.929	2.5	2	Oceania	3	Oceania

Regions were considered to show evidence for selection if the maximum $-\log_{10}(P) > 3$ for either the F_{ST}, iHS, or XP-EHH. The table shows the highest $-\log_{10}$(empirical P value) and respective population for the F_{ST}, iHS, and XP-EHH in each region. Regions whose $-\log_{10}(P) < 2$ are marked with (—). See Methods for details. Chr: chromosome, Mb: mega basepairs, Max: maximum, Pop: population. Populations: Bantu-speaking Africans (Afr), Europeans (Eur), Middle Easterners (MEast), Eastern Asians (EAsia), South Asians (SAsia), Americans (Amer), and Oceanians (Oceania).

Table 4: Summary of regions with evidence for selection on both the HapMap and HGDP populations.

Gene region			HapMap				HGDP	
	iHS	D	F_{ST}		Min empirical P value	F_{ST}	iHS	XP-EHH
		Max − log(Q)	Max − log(Q)	Value		Max − log(P)	Max − log(P)	Max − log(P)
PTPN22		3.6	3.2	0.75			3.0	3.5
TNFSF4				0.6	0.005	4.5		
TET3, DGUOK			3.2	0.85	0.001			3.5
TNIP1		3.0		0.65		3.5		3.0
UHRF1BP1	|2.28|*				0.004		3.0	3.5
BLK				0.92	0.005	4.0	3.0	4.0
ITGAM			3.4	0.75				3.5

Please refer to footnotes on Tables 2 and 3 for details. *iHS = −2.28 for rs11755393.

(rs6677604, iHS = −2.30 in YRI), *UHRF1BP1* (rs11755393, iHS = −2.28 in CEU), and *CD226* (rs727088, iHS = 2.14 in CEU). The evidence for selection at the *UHRF1BP1* variant was recently reported in a study of candidate inflammatory-disease SNPs using the same statistic and HapMap II data [76].

In the HGDP data, the highest XP-EHH was detected in the *BLK*, *CLEC16A*, and *IRF8* regions and the maximum iHS in the *CLEC16A* and *PTTG1* regions. The *CLEC16A*, *BLK*, *PTPN22*, and *UHRF1BP1* regions showed strong evidence for selection under the haplotype-based statistics. *TNFSF4*, *IL10*, and *BLK* were the regions showing the highest degree of population differentiation. The *TNFSF4* and *BLK* regions showed the strongest most consistent evidence of selection according to all three metrics. Using the same HapMap II data, Raj and colleagues [76] previously reported SNPs with a significant signal of selection in *CLEC16A* (rs12708716, iHS = 2.29 in CEU) and *UHRF1BP1* (rs11755393, iHS = −2.28 in CEU). As mentioned, the *BLK* and *ITGAX-ITGAM* regions were recently reported to be under selection (in ASN and YRI, resp.) in a candidate genes study using the 1000 Genomes Project samples [10]. For the genes in Table 2, an inspection of the worldwide distribution of allele frequencies for the SNPs associated with SLE (Supplementary Table 2) revealed interesting patterns for SNPs in *BLK*, *ITGAM*, and *CLEC16A* (Figure 1).

Comparing the results of the tests for selection in the HapMap and the HGDP samples shows that there are seven genetic regions captured by at least one test in both datasets (Table 4). The common regions captured by the majority of tests were that of the *PTPN22*, *UHRF1BP1*, and *BLK* genes. While the region of the *TNIP1* gene was captured in both the HapMap and HGDP populations by the frequency spectrum and population differentiation statistics (D and F_{ST}), the region of the *UHRF1BP1* gene was captured by the haplotype-based statistics. The evidence for selection in these seven genetic regions (Table 4) is strengthened by the fact that they show consistent evidence across different studies and analytic methods.

4. Discussion

The diversity exhibited in the human genome is a result of stochastic population genetics processes such as mutation, migration, drift, and selection. SLE disproportionately affects women of child bearing age and without treatment would tend to put affected individuals at a reproductive disadvantage; here, reproductive disadvantage not only includes conception but the ability to raise offspring that successfully reproduce. Thus, strong alternative forces or changing selective pressure must exist that permits the relative high frequency of these risk alleles seen in current populations around the world. Infectious diseases and pathogenic exposures have been postulated to be important factors resulting in strong selective pressure and might provide such alternative pressures. This study investigated whether SLE susceptibility loci show signs of recent positive selection by comparing these regions to the background distribution of genetic variation.

Two important studies have computed several genome-wide tests for selection in two main reference populations, the HapMap and the HGDP populations [7, 70], and implemented the results in genetic browsers. These browsers were queried to assess whether SLE-associated genetic regions have shown evidence for selection in the HapMap and HGDP populations.

This study reports several SLE-associated loci that show evidence for selection in the HapMap populations, and several SLE-associated loci that show evidence for selection in the HGDP populations. Seven genetic regions showed evidence for selection on both the HapMap and HGDP populations. These include the regions of the *PTPN22*, *TNFSF4*, *TET3-DGUOK*, *TNIP1*, *UHRF1BP1*, *BLK*, and *ITGAM* genes. In addition to the regions that are concordant, the different results obtained with the different metrics and datasets are expected, mostly due to the different coverage of the SNP arrays used, local adaptation in different ethnic groups, and the different test statistics which are likely recovering selective events from different time periods and for different stages of the selective sweep [77].

Several of these genes have been previously reported to show patterns of genetic variation that are consistent with evidence for recent positive selection. For example, in their search for inflammatory-disease SNPs that localize to regions of the genome where patterns of genetic variation are consistent with that expected under a model of recent positive selection, Raj and colleagues [76] also reported SNPs

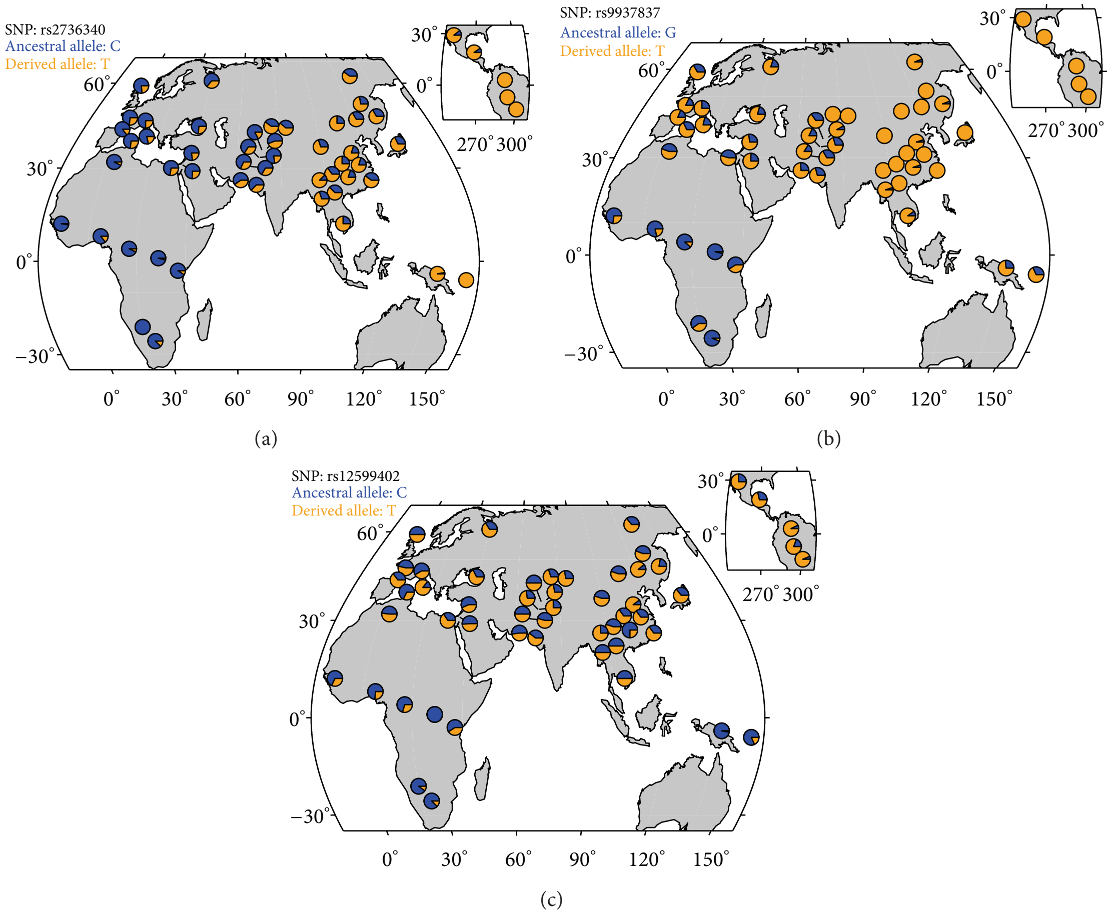

Figure 1: Worldwide distribution of allele frequencies for SLE-associated SNPs rs2736340 in *BLK* (a), rs9937837 in *ITGAM* (b), and rs12599402 in *CLEC16A* (c).

in *CLEC16A* and *UHRF1BP1* that exhibit a significant signal of selection using the iHS test. Furthermore, they show that the SLE susceptibility allele in *UHRF1BP1* is associated with decreased *UHRF1BP1* RNA expression in different cell subsets, suggesting that the SLE risk allele is under recent selection and has a regulatory effect [76]. Furthermore, *UHRF1BP1* has been shown to be significantly differentially expressed in dendritic cells after Mycobacterium tuberculosis (MTB) infection [78]. Using full-genome sequence variation from the 1000 Genomes Project and the composite of multiple signals (CMS) test, Grossman et al. [10] reported the *PTPN22*, *ITGAX* (near *ITGAM*), *ITPR3*, and *BLK* regions to show evidence for recent positive selection.

Several of the immune genes that have been identified in regions under selection are under the selective pressure of known pathogens, such as the Duffy blood group atypical chemokine receptor (*DARC*) gene to *Plasmodium vivax* malaria [79], ras homolog family member A (*RHOA*), and OTU domain ubiquitin aldehyde binding 1 (*OTUB1*) genes to *Yersinia pestis* (plague) [80], or the tyrosylprotein sulfotransferase 1 (*TPST1*) gene to *HIV* [81]. Several genetic regions associated with susceptibility to different autoimmune diseases show evidence of selection that has been attributed to host-pathogen coevolution, including the multiple major histocompatibility complex (MHC) [82–84] and the celiac risk locus *SH2B3* as a protective factor against bacterial infection [85]. Karlsson et al. [86] have recently reported that cholera has exerted strong selective pressure on proinflammatory pathways, and Jostins et al. [87] reported considerable overlap between susceptibility loci for inflammatory bowel disease and mycobacterial infection. Variants in the *IFIH1* gene, whose protein is a cytoplasmic helicase that recognizes RNA of picornaviruses and mediates induction of interferon response to viral RNA, have been shown to affect *IFIH1* function and host antiviral response [88]. In the context of SLE predisposing loci, Clatworthy et al. [89] have shown that *FCGR2B* is important in controlling the immune response to *Plasmodium falciparum*, the parasite responsible for the most severe form of malaria, and suggests that the higher frequency of human *FCGR2B* polymorphisms predisposing to SLE in Asians and Africans may be maintained because these variants reduce susceptibility to malaria. The complement component (3b/4b) receptor 1 (*CR1*) gene has been shown to be a *P. falciparum* resistance gene [90] used by the parasite for host invasion. Machado et al. [91] have suggested that helminth infection has driven positive selection of *FCGR*s variation. Finally, Grossman et al. [10] implicated *Salmonella typhimurium* and other exposures that directionally drive selection of the toll-like receptor 5 (*TLR5*) gene [92]. Given that infectious organisms are strong agents of natural selection, it is plausible that alleles selected for protection against infection predispose to autoimmune diseases.

It is important to acknowledge the challenges and limitations inherent to the study of traits with complex genetic architectures and/or a less clear influence on survival and reproduction, such as SLE. As Castiblanco and colleagues [93] recently articulated, the differences in allele and genotype frequencies of diverse human populations depend upon their evolutionary and epidemiological history, including environmental exposures, which might explain why some risk alleles to autoimmunity may be protective factors to infectious diseases and vice versa in a given population (e.g., *PTPN22* [94, 95] and TNF [96]). Immune and infectious agents have been recognized as among the strongest selective pressures for natural populations, as shown by the identification of candidate adaptive alleles that functionally contribute to biological variation in contemporary populations. However, clarifying the relationship between the functional alleles and reproductive fitness in the environment in which they rose to a high frequency in the ancestors of the study population can rarely be attained. In complex diseases such as SLE, despite the established associations to specific regions or polymorphisms, the true causal variants still remain largely unknown. The emerging availability of genome-wide functional data allows the integration of an unprecedented amount of biological information to help identify potential functional variants and characterize their biological impact. Recent examples demonstrate how the integration of signatures of positive selection with phenotypic association studies and/or with regulatory data can improve the identification of functional loci [10, 97–99]. Also, the complex genetic architecture of SLE, resulting from the effects of many alleles of small effects, suggests that adaptation is likely to have occurred by simultaneous selection on variants at many loci. In this scenario, the response to selection is due to small frequency shifts of many alleles. However, most methods to detect selection rely on rapid fixation of strongly selected alleles. The development of novel analytical approaches to detect more subtle signatures of selection will improve the identification of selection signatures in complex diseases like SLE. Clearly, much remains to be done until the functional adaptive SLE risk loci are identified, the phenotypic consequences of these risk alleles elucidated, and the relationship between the functional alleles and reproductive fitness clarified. Recent progresses will provide the necessary tools to accelerate the discovery of these functional adaptive variants that increase the risk of SLE, which will improve knowledge about the etiology and deepen our understanding of the natural history of SLE. Further research regarding exploration of the interplay between infection, type of exposure, additional environmental factors, and autoimmunity will result in the discovery of multiple factors underpinning perhaps newly identified physiopathology mechanisms of SLE and autoimmune diseases [93].

In summary, this study has systematically queried the HapMap and HGDP populations for evidence for selection at SLE susceptibility regions and provides a comprehensive catalog of regions with both evidence for recent positive selection and association with SLE. These results provide support for recent positive selection influencing genetic variation associated with SLE, suggesting that population-specific selective pressures may be one of the factors behind the high frequency of SLE risk alleles in the population and differential disease risk. Finally, these results support future analyses aimed at identifying the specific selective pressures and characterizing the functional mechanisms of adaptation and disease predisposition.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Rituximab for Remission Induction and Maintenance in Refractory Systemic Lupus Erythematosus

Fabio Bonilla-Abadía,[1] Nicolás Coronel Restrepo,[2] Gabriel J. Tobón,[1] Andrés F. Echeverri,[1] Evelyn Muñoz-Buitrón,[3] Andres Mauricio Castro,[3] Mercedes Andrade Bejarano,[4] and Carlos A. Cañas[1]

[1] *Rheumatology Unit, Fundación Valle del Lili, ICESI University, Carrera 98 18-49, Cali, Colombia*
[2] *Internal Medicine Unit, Fundación Valle del Lili, CES University, Cali, Colombia*
[3] *Clinical Research Unit, Fundación Valle del Lili, Cali, Colombia*
[4] *School of Statistics, Universidad del Valle, Cali, Colombia*

Correspondence should be addressed to Fabio Bonilla-Abadía; fbac1982@hotmail.com

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Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease with high morbidity if untreated. Sometimes, despite aggressive treatments, the disease remains active with cumulative organic damage. We conducted a retrospective and descriptive observational study of patients with SLE refractory to conventional treatment who were treated with rituximab (RTX) as remission induction therapy and maintenance. There was a significant reduction in the conventional immunosuppressive drug dose and the number of relapses of disease. RTX appeared to be effective and safe for the induction and maintenance of remission in patient with SLE refractory to conventional treatment.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease with no permanent cure or high morbidity if left untreated [1]. Sometimes, despite aggressive treatments with high dose of glucocorticoids and immunosuppressive drugs, the disease remains active with cumulative organic damage [2]. The disease severity appears to be higher in Hispanics compared to Caucasians. Thus, the overall survival rates vary by race and ethnic background with a 5-year survival rate of approximately 95% among whites, 90% among blacks, and 87% among Hispanics [3]. The management of patients with SLE depends on the type of organ involvement and severity of the disease. When manifestations are severe, with threatening life conditions, the treatment is based on high-dose steroids, plasmapheresis, intravenous gamma globulin, and various types of immunosuppressants such as cyclophosphamide, azathioprine, or mofetil mycophenolate [4]. Some patients remain with active disease despite full immunosuppressive drugs. Two monoclonal antibodies targeting B cells have been used successfully in refractory SLE: belimumab directed against B-cell activating factor (BAFF) [5] and Rituximab which is a genetically engineered chimeric anti-CD20 monoclonal antibody. CD20 is a B-cell surface antigen that is expressed only on pre-B and mature B cells. It is not present on stem cells and is lost before differentiation of B cells into plasma cells. Therefore, rituximab causes a selective transient depletion of the CD20+ B-cell subpopulation [6]. Rituximab (RTX) is currently approved for the management of B-cell lymphomas, rheumatoid arthritis (RA) and systemic vasculitis ANCA (antineutrophil cytoplasmic antibodies) positive [7, 8], and it is used as a single dose for SLE crisis with varying results [9–11]. There are few reports in the literature about routine and chronic use of RTX as a maintenance drug therapy in SLE [12]. Being a chronic and incurable disease with high rates of relapse, we decide to treat indefinitely a group of patients that previously had favorable response to single doses. In this study, we evaluate the efficacy and safety of RTX both as a rescue and maintenance agent in a group of patients with refractory SLE.

2. Patients and Methods

The data was taken from the electronic medical records of patients at a fourth-level center (Fundación Valle del Lili) in Cali, Colombia, throughout twelve years (from August/2001 to April/2013). Patients were eligible for the study if they had provided authorization for review of their medical records, were older than 18 years old at the time of the study, had a diagnosis of SLE based on the criteria of the American College of Rheumatology [13], and had refractory SLE to conventional treatments: hydroxychloroquine, high dose steroids, cyclophosphamide, azathioprine, and mofetil mycophenolate, which despite receiving recommended doses and validated protocols in the adequate time persisted with active disease (defined as a SLEDAI score higher than 4) [14]. Patients with active infections or cancer were excluded from the study. Medical records were reviewed and demographic and clinical data, including the number of RTX cycles, frequency and severity of relapses, glucocorticoids doses, and type and doses of conventional immune-suppressor agents, were collected. Assess of lupus activity was done with SLEDAI score [15]. Patients with refractory SLE were treated with RTX both as rescue medication for lupus flare and for maintenance (dose of 1 gr at day 0 and 1 gr at day 15 with retreatment every 9 months). Administration of acetaminophen 1 g, diphenhydramine 50 mg, and prednisone 50 mg prior to each RTX infusion was done. RTX was given as additional agent to the treatment received. We did not use the CD20+ cell counts as an indicator of the moment to retreat these patients.

2.1. Statistical Analysis. An exploratory analysis of the data was made using percentages for categorical variables and medians (interquartile range (IQR)) for continuous variables. The Wilcoxon nonparametric test was done for comparisons. Data analysis was done using STATA 1.2. software. P values less than 0.05 were considered significant for all statistical tests. The study was approved by the ethics committee of Fundación Valle del Lili research center.

3. Results

Out of 350 patients of the initial cohort, eighteen patients with refractory and active SLE were included in the analysis. Seventeen out of eighteen patients were women and all patients were Hispanic. The median age was 28.5 years (range: 22–36). At the time of inclusion, median SLEDAI score was 12.5 (range: 8–18). Median initial doses were as follow: prednisone, 25 mg/day (range: 15–20); mofetil mycophenolate, 2.0 gr/day; azathioprine, 100 mg/day (2 mg/kg/day); hydroxychloroquine, 200 mgr/day; and endovenous cyclophosphamide, 750 mgr each month. The mean follow-up was 37.5 months (range: 18–63) with a mean average of 5 RTX cycles (range: 3–8). Baseline clinical characteristics, treatment history, and refractory organ system involvement in these 18 patients at the time of their first RTX course are described in Table 1. At the end of follow-up, SLEDAI score was 0 in fourteen patients, 2 in three patients, and 4 in one patient ($P = 0.0002$)—(Table 2 and Figure 1). Median prednisone dose at the end of follow-up was 3.75 mg/day (range: 2.5–5) ($P = 0.0002$). In addition, mofetil mycophenolate and azathioprine were both discontinued in all patients ($P = 0.0071$ and $P = 0.052$, resp., compared to baseline), and hydroxychloroquine median dose decreased to 75 mgr/day. The median relapses rate at the beginning of RTX treatment was 3 per year (IQR 3–5), decreasing to 0 (IQR 0-1) at the end of follow-up.

Table 1: Baseline characteristics of the 18 systemic lupus erythematosus patients and their main refractory and active involvement.

Characteristics	Values ($n = 18$)
Age, years*	28.5 (22–36)
Female, Gender	17 (94.4)
Prednisone dose*	25 (15–50)
Use of Azathioprine	5 (27)
Use of Cyclophosphamide	6 (33)
Use of Mycophenolate	9 (50)
Use of Hydroxychloroquine	11 (61)
Relapses/year*	3 (3–5)
Renal criteria**	11 (61.1)
Hematological criteria***	13 (72.2)
Cardiopulmonary criteria	8 (44.4)
Musculoskeletal criteria	14 (77.8)
Low C3	13 (72.2)
Low C4	10 (55.6)
Anti-DNA positive	9 (50)

All the data correspond to n (%) with the exemption of those marked with *.
*Median IQR (interquartile range).
**Proteinuria (higher than 0.5 g/day), casts (erythrocytes, hemoglobin, granular, tubular, or mixed), hematuria, and pyuria.
***Leukopenia (less than 4.000 cells/mm^3), thrombocytopenia (less than 100.000 cells/mm^3), and hemolytic anemia.

Because the use of RTX in rescue dose in our patients with acute disease was favorable in all but one, we decided to retreat them every nine months.

Three patients presented an adverse reaction to RTX at the first course of treatment consistent with cytokine release syndrome [16]. However, the application of the drug was not suspended in these cases, and a desensitization protocol was successfully performed in the hospital.

4. Discussion

Here, we present a case series that evaluated and demonstrated safety and efficacy of RTX therapy in induction and maintenance for the treatment of SLE.

Two randomized clinical trials have been published trying to prove the clinical effectiveness of RTX in SLE

Table 2: SLEDAI Score in the initial and at the end of the last cycle of RTX.

(a)

Patient	SLEDAI score	
	Beginning	End
1	40	0
2	8	0
3	14	0
4	18	2
5	14	0
6	8	2
7	18	0
8	8	0
9	7	0
10	11	0
11	8	0
12	14	0
13	22	2
14	14	0
15	6	0
16	22	4
17	11	0
18	9	0

(b)

	Beginning	End	*P* value
Score	12.5 (8–18)	0 (0-0)	$P = 0.0002$

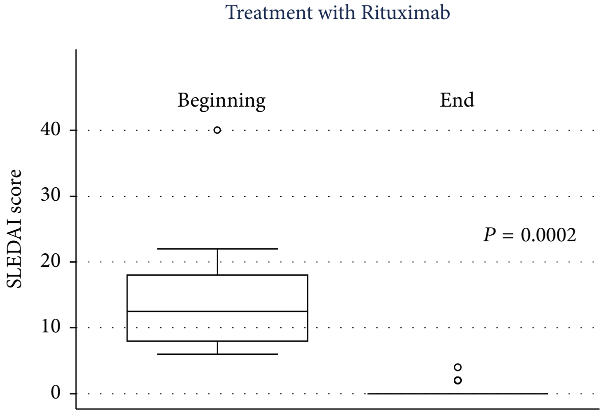

Figure 1: Box plot diagram of the SLEDAI score at the beginning and at the end of the last cycle of RTX.

with mixed results. The EXPLORER study [9], a case-control study conducted in 257 patients with extrarenal SLE, showed no statistical significance in reduction in disease activity between RTX and conventional immunosuppressive therapy. The LUNAR study [10] randomized 144 patients to receive either RTX or placebo, under a mofetil-mycophenolate-based immunosuppression and steroids, showing a significant improvement in the levels of C3, C4, and anti-DNA but no differences in renal response rates at week 52 of treatment. Pinto et al. [11] conducted a prospective study of a cohort of 42 patients with refractory SLE in Colombia, adding RTX as a rescue therapy in one initial dose, with 36% of the patients showing complete remission and an overall significant reduction in steroid use. The preferred scheme used for ablation of B cells with RTX was an initial dose of 1 g and then 1 g in two weeks. Subsequent doses were not indicated. The excellent clinical response in Colombian patients may be explained by racial grounds, which has been shown in the present study and in other recent publications included Latin American population [17–19]. All patients including in our series were mestizos. More studies are needed to confirm these findings.

Our scheme was done based on the protocols used in RA patients and as now it is beginning to be recommended in refractory granulomatosis with polyangiitis [20]. All patients showed to the end of the study remission criteria. This follow-up study showed that depletion of B lymphocytes with repeated RTX is effective and safe in patients with SLE. A decrease in the number of relapses by disease activity was also evident. Relapses were prevented with RTX retreatment and conventional immunosuppressive doses were decreased gradually. Adverse events related to the infusion were few and there was no contraindication for retreatment with RTX. All patients in this cohort reached remission of the disease.

One of the most interesting findings and strengths of this study is that only few reports have shown the effectiveness of RTX retreatment in SLE patients, as used in a routine way in rheumatoid arthritis patients and not only at the moment of refractory involvement. Several shortcomings are presented in our study. First of all, this is a retrospective series, and no randomized assessment was done. In addition, only 18 patients were evaluated and no B-cell count was done to define the adequate moment to retreatment.

In conclusion, RTX seems to be effective and safe for the induction and maintenance of remission in Colombian mestizo patients with SLE refractory to conventional immunosuppressive therapy. Our results provide important information for the design of future studies in order to confirm the results obtained here.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Cardiovascular Disease in Latin American Patients with Systemic Lupus Erythematosus: A Cross-Sectional Study and a Systematic Review

Jenny Amaya-Amaya, Juan Camilo Sarmiento-Monroy,
Julián Caro-Moreno, Nicolás Molano-González, Rubén D. Mantilla,
Adriana Rojas-Villarraga, and Juan-Manuel Anaya

Center for Autoimmune Diseases Research (CREA), School of Medicine and Health Sciences, Universidad del Rosario,
Carrera 24 No. 63C-69, 111221 Bogotá, Colombia

Correspondence should be addressed to Jenny Amaya-Amaya; jecamaya@gmail.com

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Objective. This study was performed to determine the prevalence of and associated risk factors for cardiovascular disease (CVD) in Latin American (LA) patients with systemic lupus erythematosus (SLE). *Methods*. First, a cross-sectional analytical study was conducted in 310 Colombian patients with SLE in whom CVD was assessed. Associated factors were examined by multivariate regression analyses. Second, a systematic review of the literature on CVD in SLE in LA was performed. *Results*. There were 133 (36.5%) Colombian SLE patients with CVD. Dyslipidemia, smoking, coffee consumption, and pleural effusion were positively associated with CVD. An independent effect of coffee consumption and cigarette on CVD was found regardless of gender and duration of disease. In the systematic review, 60 articles fulfilling the eligibility criteria were included. A wide range of CVD prevalence was found (4%–79.5%). Several studies reported ancestry, genetic factors, and polyautoimmunity as novel risk factors for such a condition. *Conclusions*. A high rate of CVD is observed in LA patients with SLE. Awareness of the observed risk factors should encourage preventive population strategies for CVD in patients with SLE aimed at facilitating the suppression of cigarette smoking and coffee consumption as well as at the tight control of dyslipidemia and other modifiable risk factors.

1. Introduction

Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease (AD), characterized by the production of numerous pathogenic autoantibodies [1]. Diverse heritable, hormonal, and environmental factors and immune-system aberrations contribute to the clinical expression of the disease [2]. The heterogeneous nature of SLE explains the broad spectrum of clinical manifestations (i.e., subphenotypes). SLE affects predominantly women (female-to-male ratio 9 : 1) of child-bearing age [3]. The annual incidence and prevalence range from 1.4 to 11 cases per 100,000 population, and from 7.4 to 159.4 cases per 100,000 population, respectively [4] depending on a variety of factors, including age, gender, and ancestry. African, Hispanic, and Asian ancestry significantly influence both the risk of developing the disorder and outcome [4]. A bimodal mortality was described by Urowitz et al. [5] characterized by an early peak in the first 3 years after diagnosis due to active disease, infections and glomerulonephritis, and a second peak, 4–20 years after SLE diagnosis, in which cardiovascular disease (CVD) is the main feature and cause of death. Although overall mortality for patients with SLE has improved over the past 30 years, mortality due to CVD has remained almost the same [6].

CVD is the leading cause of mortality, responsible for about 30% of deaths worldwide. Globally, 80% of total CVD deaths occur in developing countries [7]. In addition, there is strong epidemiologic evidence that CVD risk among SLE patients compared to the general population is at least doubled [8]. Noteworthy, the excessive cardiovascular (CV) events observed in SLE individuals are not fully explained by classic risk factors. Several SLE-specific factors, including disease activity and duration, and possibly specific manifestations and therapies, further increase CV risk [8]. In fact, SLE *per se* seems to be an independent risk factor for the development of accelerated atherosclerosis [9].

SLE is not uncommon in Latin America (LA), the geographical area defined by Mexico, Central America, South America, and the islands of the Caribbean, a rapidly growing region with almost 600 million inhabitants [10]. Latin Americans are considered a highly admixed population due to a mixed ethnicity (so called *mestizos*) that is mainly derived from a European and Amerindian inheritance [11]. The increased prevalence of chronic diseases in LA has been attributed to diverse causes including the ageing of the population and lifestyle factors such as smoking, physical inactivity, and excess alcohol intake [12]. Despite important advances in recent decades, LA remains one of the world's most unequal regions [13]. Enormous cultural differences in health perceptions in LA exist, correlating with individuals' economic and health conditions [12]. So far, some studies regarding SLE have documented differences in health status, disease prevalence, treatment outcomes, and healthcare use among different ethnic groups, suggesting that minorities influence SLE health disparities [14–19]. Thus, this study was performed to determine the prevalence and associated risk factors for CVD in Latin American patients with SLE.

2. Material and Methods

2.1. Study Population. First, a cross-sectional analytical study was conducted in 310 Colombian patients with SLE in whom CVD was assessed. The subjects were seen at the Center for Autoimmune Diseases Research (CREA) in Bogota, Colombia. All of them fulfilled the 1997 update American College of Rheumatology classification criteria for SLE [20]. This study was done in compliance with Act 008430/1993 by Ministry of Health of the Republic of Colombia, which classified it as a minimal-risk research. The institutional review board of the Universidad del Rosario approved the study design.

Information on patient socio-demographic and cumulative clinical and laboratory data, as well as household description, were obtained by interview, standardized report form, physical examination and chart review. All data were collected in an electronic and secure database. Socio-demographic variables included age at SLE onset, disease duration, educational and socioeconomic status, current occupation, smoking habits, coffee consumption, expositional factors and physical activity. Age at onset of the disease was defined as the first subjective experience of the symptom(s) and/or sign(s) described in any of the items of the classification criteria [21]. Duration of disease was considered as the difference between age at onset and the date of first participation in the study. Educational level was recorded as the number of years of education and was divided into two groups (more or less than 9 years) of education based on the "General Law of Education" in Colombia [22, 23]. Socioeconomic status was categorized on the basis of national legislation and was divided into low (1 and 2) and high (3–6) status. Smoking habits was assessed as ever; 1–6, 6–15, and >15 packages/year; or quitter cigarette consumption. Coffee intake was asked as yes or not, and measured in cups per day (1-2, 2–4, >4). Several expositional factors were also questioned, including the use of silicone implants, hair dyes, pesticides and organic solvents [24].

2.2. Clinical Variables. Clinical and laboratory variables were registered as present or absent at any time during the course of the disease. Clinical features of the disease were included taking into account the revised American College of Rheumatology criteria [20] and others manifestations as follows: polyautoimmunity (coexistence of an additional AD in the same individual on the basis of international criteria) [25, 26], multiple autoimmune syndrome (presence of more than two AD in the same patient) [26–28]; familial autoimmunity, and familial autoimmune disease were also registered as the presence of any other AD and SLE in first degree relatives (FDR) respectively [27–29]. Regarding pharmacological treatment, current or past use of azathioprine, mycophenolate mofetil, cyclophosphamide, methotrexate, antimalarials (i.e., clhoroquine, hydroxychloroquine), glucocorticoids (i.e., prednisolone, metilprednisolone, and deflazacort), and biological therapy (i.e., Rituximab) were recorded.

2.3. Cardiovascular Assessment. Five subphenotypes were defined and assessed: first, hypertension, defined as having a blood pressure ≥140/90 mm Hg or using any antihypertensive medication [30]: systolic and diastolic blood pressures were measured twice with at least a 15-minute interval between and the averages were recorded. Second, history of stroke, third, coronary event (i.e., unstable angina, myocardial infarction (MI)), fourth, thrombotic event (other than coronary disease and carotid involvement, requiring anticoagulant treatment), fifth, carotid disease (doppler criteria or intima-media thickness ≥0.9).

2.4. Laboratories Measurements. Relevant laboratory variables associated with SLE were recorded. Antinuclear antibodies, antidouble strand DNA antibodies, precipitating antibodies to extractable nuclear antigens (Sm, U1-RNP, Ro/SS-A, La/SS-B), anticardiolipin IgG and IgM, antibeta 2-glycoprotein 1 IgG and IgM antibodies, and lupus anticoagulant were extracted from the patient's clinical record. Other autoantibodies including rheumatoid factor, anticyclic citrullinated peptide, -thyroperoxidase enzyme, -thyroglobulin, -Scl 70, -centromere, -mitochondrial, and -smooth muscle antibodies were also recorded. Inflammatory biomarkers, including erythrocyte sedimentation rate (ESR), and serum high sensitive C-reactive protein (CRP) levels, as well as white blood cell and platelet count, hemoglobin levels, mean corpuscular volume, coombs test, complement (i.e., C3 and C4 levels), TSH, tetraiodothyronine T4, venereal disease research laboratory, and creatinine were extracted from patient's clinical record. Likewise, serum levels of total cholesterol (TC), triglycerides (TGL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and glycemia were determined by blood tests provided by every patient.

2.5. Assessment of Traditional Risk Factors for CVD. Patients were assessed for traditional CVD risk factors including current age (≥45 and ≥55 years for men and women, resp.) [31–34]. Type 2 diabetes mellitus (T2DM) was defined as having a fasting plasma glucose level ≥7 mmol/L (126 mg/dL), or taking any antidiabetic agents at the time of assessment [35]. A diagnosis of dyslipidemia was given if the patient had (a) hypercholesterolemia, defined as taking lipid-lowering medication as a surrogate, or having a fasting plasma TC ≥200 mg/dL (b) HDL ≤40 mg/dL, (c) hypertriglyceridemia (TGL ≥150 mg/dL), or (d) elevated LDL (≥100 mg/dL) [31, 36]. Current hemoglobin ≤12 g/dL established a diagnosis of anemia and current levels of creatinine (abnormal defined as ≥1.2 mg/dL) were evaluated as well. History of premature CAD in FDR was also assessed [37]. Patients and their past medical records were evaluated for the current or past use of aspirin or hormone replacement therapy.

2.6. Statistical Analysis. First, univariate analyses were done. Categorical variables were analyzed by frequencies. Kolmogorov-Smirnov normality test was done to evaluate normality for quantitative continuous variables. Parametric data are expressed as the mean and standard deviation, and nonparametric data are described as the median and interquartile range. Second, associations between traditional and nontraditional CVD risk factors were assessed by bivariate analyses. The presence of CVD was assessed through chi square tests or Fisher's exact tests when the variable was dichotomous. Parametric values were analyzed by t student test. Nonparametric values were analyzed by Mann-Whitney U test. Clinical variables with a $P \leq 0.25$ were included in a multivariable model. Finally, a multivariate binomial logistic regression model having CVD as the dependent variable was fit. As independent factors, the model included the traditional and nontraditional associations that were statistically significant in bivariate analyses and those variables that were biologically plausible. Moreover, other logistic regression models were made, including interactions between independent factors. In both circumstances, the models were adjusted by gender and duration of the disease. Adequacies of logistic models were assessed using the Hosmer-Lemeshow goodness-of-fit test. The Nagelkerke R^2 (i.e., pseudo-R^2) was used to estimate the percentage of variance explained by the models. Adjusted odds ratios (AOR) were calculated with 95% confidence intervals (CI). The Wald statistic test was used to evaluate the significance of individual logistic regression coefficients for each independent variable. Statistical analyses were done by the Statistical Package for the Social Sciences (SPSS, v.20, Chicago, IL, USA).

2.7. Systematic Review of Literature. The Preferred reporting items for Systematic Reviews and Meta-analyses guidelines were followed [38] to systematically search in the following databases: PubMed, EMBASE, Scopus, SciELO, and Virtual Health Library—which includes BIREME, LILACS and many others LA sources—about CVD and SLE in LA population. Three reviewers did the search and extraction data independently (AAJC, SMJC, and CMJ) using predefined eligibility criteria, from inception up to February 2013.

The search was done in PubMed, using the following Medical Subject Headings (MeSH terms) groups: "Lupus Erythematosus, Systemic," "Cardiovascular Diseases;" "Latin America," "Ethnic Groups," "Brazil," "Mexico," "Colombia," "Chile," "Cuba," "Panama," "Venezuela," "Bolivia," "Peru," "Argentina," "Uruguay," "Paraguay," "Ecuador," "Nicaragua," "Surinam," "French Guiana," "Guatemala," "Honduras," "Belize," "Costa Rica," "El Salvador," "Puerto Rico," "Dominican Republic," and "Haiti." Each one of them was cross-referenced with the following MeSH terms/keywords: "risk factors," "traditional risk factors," "classic risk factors," "nontraditional risk factors," "novel risk factors," "hypertension," "metabolic syndrome,“ "obesity," "smoking," "tobacco," "dyslipidemia," "advanced age," "menopausal status," "family history of CVD," "hyperhomocysteinemia," "sedentary lifestyle," "renal impairment," "male gender," "type 2 diabetes mellitus," "insulin resistance," "hormone replacement therapy," "coffee," "ancestry," "polymorphism, genetic," "poliautoimmunity," "autoantibodies," "antibodies, antiphospholipid," "lupus coagulation inhibitor," "circulation anticoagulants," "antibodies, anticardiolipins," "beta 2-glycoprotein I," "endothelial cells antibodies," "systemic inflammation," "c-reactive protein," "blood sedimentation," "tumor necrosis factor," "cytokines," "immune complex," "disease activity," "SLEDAI," "organ damage," "SDI," "duration of illness," "immune cells aberrations," "glucocorticoids," "steroids," "DMARD," "antirheumatic agents," "methotrexate," "biological therapy," "rituximab," "anti-Inflammatory agents, non-steroidal," "azathioprine," "vasculopathy," "lupus nephritis," "premature menopause," "endogenous dyslipidemia," "sociodemographic factors," "hypovitaminosis D," "vitamin D deficiency," "low vitamin D," "osteoporosis," "biomarkers," "antiphospholipid syndrome," "thyroiditis, autoimmune," "Graves Disease," "Hashimoto Disease," "scleroderma," "Sjögren's syndrome," "rheumatoid arthritis," "hypertension," "ischemic heart disease," "coronary artery disease," "acute coronary syndrome," "congestive heart failure," "myocardial infarction," "stroke," "angina," "thrombosis," "deep vein thromboses," "pulmonary embolism," "periphery arterial disease," and "atherosclerosis." Each term was cross-referenced for the greatest number of results. No limits regarding language, period of publication, or publication type were used.

The same terms were used for searching in EMBASE and Scopus databases. Each MeSH term and keyword was translated into DeCS (Health Sciences Descriptors) in order to explore sources of information in Spanish, Portuguese, and English through SciELO and Virtual Health Library databases.

2.8. Study Selection, Data Extraction, and Quality Assessment. Reviewers screened all titles and abstracts and applied the eligibility criteria in order to identify studies that were appropriate for inclusion [39]. A study was included if (a) the abstract was available, (b) it contained original data, (c) it used accepted classification criteria for SLE, (d) it measured CV risk factors (traditional and/or nontraditional), (e) it examined as a clinical endpoints: hypertension, ischemic heart disease (IHD), coronary artery disease (CAD), acute coronary syndrome (ACS), MI, angina, congestive heart failure (CHF), stroke, thrombosis, peripheral arterial disease (PAD), and subclinical atherosclerosis, and (d) it includes LA population. In order to complete the systematic review, several authors were contacted by E-mail for full text and those references from the articles that seemed to be relevant to the review were hand-searched.

Articles were excluded from the analysis if they dealt with juvenile SLE or were done on animal models (i.e., murine models) instead of SLE patients. Studies were also excluded if they were reviews or case reports, or if they discussed topics not related to CVD, and/or were not done on LA population. Blinded reviewers (AAJC, SMJC, and MJC) organized selected articles on the basis of publication source, country, author, year, type of study, sample size, traditional and nontraditional risk factors, cardiovascular outcomes evaluated, and main results. Only novel risk factors with statistical significance were included. For details, see Supplementary Table S1 in the supplementary material available online at http://dx.doi.org/10.1155/2013/794383. Each record was classified based on the quality score of the studies that was assigned by applying the levels established by the Oxford Centre for Evidence-based Medicine 2011 in order to evaluate the risk of bias [40]. The search results were compared and disagreements were resolved by consensus.

3. Results

3.1. Description of the Study Population. Out of a total of 310 patients, 91.3% (283/310) were women. The median (interquartile range) of age and duration of the disease was 37 (22) and 5 (9) years, respectively. CVD was observed in 36.5% (113/310). The most frequent condition was hypertension (25.2%) (Tables 1 and 2).

3.2. Factors Associated with CVD. Current age, ever smoking, coffee consumption, polyautoimmunity (i.e., antiphospholipid syndrome), dyslipidemia, use of cytotoxic drugs, serositis, renal involvement (i.e., nephrotic syndrome), and thrombocytopenia were all risk factors significantly associated with CVD. Instead, ethnicity and leukopenia were factors negatively associated with CVD (Table 3).

3.3. Adjusted Effects of Risk Factors for CVD. Dyslipidemia, pleural effusion, polyautoimmunity, and renal compromise were variables significantly associated with CVD, regardless of gender and duration of the disease by logistic regression analysis (Table 4). The association between CVD and smoking habit remained statistically significant after establishing interaction with coffee consumption (Table 5).

3.4. Systematic Literature Review. There were 21,161 articles identified in PubMed, EMBASE, and Scopus databases search. Additional records identified through other sources included 814 articles (SciELO and Virtual Health Library). Therefore, the database searches provided a total of 21,975 publications. Of these, 19,729 were identified as duplicates. A total of 2,246 full text articles were assessed for eligibility. Only 115 articles were included for methodological analysis.

TABLE 1: Demographic and clinical characteristics of 310 patients with SLE.

Characteristic	Median (IQR)
Age (y)	37 (22)
Duration of disease (y)	5.0 (9.0)
Characteristic	**Mean (SD)**
Age at SLE onset (y)	38 (15.4)
Age of diagnosis (y)	39.8 (15.8)
Sociodemographic characteristic	**% (*n*/*N*)**
Female	91.3 (283/310)
High education level	85.6 (255/298)
High socioeconomic status	79.5 (236/297)
Mixed occupation	23.2 (71/306)
Housewife	21.2 (65/306)
Exposure to hair dyes	40.6 (125/308)
Clinical manifestation	**% (*n*/*N*)**
Cutaneous compromise	88.1 (273/310)
Arthropathy	87.7 (272/310)
Neurological involvement	10 (31/310)
Hematological criteria	34.5 (107/310)
Immunological criteria	78.1 (242/310)
Raynaud's phenomenon	39.4 (122/310)
Vasculitis	18.4 (57/310)
Alopecia	47.7 (148/310)
Livedo reticularis	19 (59/310)
Pleural effusion	23.5 (73/310)
Pulmonary hypertension	6.5 (20/310)
Pulmonary embolism	2.9 (9/310)
Pericarditis	14.5 (45/310)
Lupus nephritis	46.5 (144/310)
Nephritic syndrome	5.5 (17/310)
Nephrotic syndrome	16.8 (58/310)
Histological pattern	
Normal	8.5 (7/82)
Mesangial glomerulonephritis	15.9 (13/82)
Focal segmental glomerulonephritis	13.4 (11/82)
Proliferative glomerulonephritis	41.5 (34/82)
Membranous glomerulonephritis	11 (9/82)
Autoimmune disease(s)	**% (*n*/*N*)**
Polyautoimmunity	26.1 (81/310)
MAS	6.1 (19/310)
Familial autoimmunity in FDR	30.3 (94/310)
RA	3.9 (12/310)
APS	8.7 (27/310)
SS	8.7 (27/310)
AITD	6.8 (21/310)

Table 1: Continued.

Characteristic	Median (IQR)
Comorbidities	% (*n*/*N*)
Fibromyalgia	9.7 (30/309)
Depression	21.4 (66/309)
Epilepsy	3.9 (12/309)
Peptic ulcer disease	38.5 (119/309)
Anemia	8.1 (25/309)
Osteoporosis	5.8 (18/309)
Malaria	2.3 (7/303)
Hepatitis A	7.1 (22/309)
Miscarriage	20.2 (57/282)
Drugs	% (*n*/*N*)
Azathioprine	33.9 (105/310)
Antimalarial	80.6 (250/310)
Mycophenolate Mofetil	15.8 (49/310)
Steroid	78.4 (243/310)
Rituximab	7.4 (23/310)
Cytotoxic agents	12.3 (38/310)
Biological treatment	8.4 (26/310)
Methotrexate	34.8 (108/310)
Laboratories findings	% (*n*/*N*)
Anemia	25.9 (76/293)
Leukopenia	38 (114/300)
Lymphopenia	78.7 (85/202)
Thrombocytopenia	7.8 (23/296)
C-reactive protein (+)	35.4 (57/161)
Erythrocyte sedimentation rate (+)	46.2 (104/225)
VDRL (+)	24.5 (26/204)
Abnormal serum creatinine	9.7 (26/268)
Abnormal creatinine clearance	56.6 (94/166)
24 hours proteinuria (+)	37.7 (80/212)
Hematuria	57.4 (58/101)
Pyuria	36.1 (56/155)
Antinuclear antibodies (+)	98.6 (287/291)
Lupus anticoagulant (+)	51 (52/102)
Anti-dsDNA antibodies (+)	54.6 (147/269)
Low complement 3 (C3)	60.8 (160/263)
Low complement 4 (C4)	31.4 (83/264)
aCL IgG (+)	33.8 (79/234)
aCL IgM (+)	32.4 (73/225)
Beta 2-glycoprotein antibodies IgG (+)	29.4 (10/34)
Beta 2-glycoprotein antibodies IgM (+)	28.6 (6/21)
Anti-Ro antibodies (+)	48.6 (122/251)
Anti-La antibodies (+)	26.2 (64/244)
Anti-Sm antibodies (+)	36 (89/247)
Anti-RNP antibodies (+)	46 (109/237)
Rheumatoid factor (+)	34.6 (27/78)
Citrullinated peptide antibodies (+)	34.3 (12/35)
Thyroid stimulating hormone (+)	51.5 (53/207)
Thyroid peroxidase antibodies (+)	38.6 (17/44)
Thyroglobulin antibodies (+)	20 (6/30)

CVD: cardiovascular disease; SLE: systemic lupus erythematosus; IQR: interquartile range; SD: standard deviation; Y: years; MAS: multiple autoimmune syndrome; FDR: first-degree relatives; RA: rheumatoid arthritis; APS: antiphospholipid syndrome; SS: Sjögren's syndrome; AITD: autoimmune thyroid disease; VDRL: venereal disease research laboratory; aCL: anticardiolipin antibodies.
Arthropathy was defined as the presence of at least one of the following: arthritis, arthralgia, hands edema, or Jaccoud's arthropathy.
Cutaneous compromise was defined as the presence of at least one of the following: photosensitivity, oral ulcers, malar rash, discoid lupus, subacute lupus, and urticaria.
Neurological involvement were defined as the presence of at least one of the following: seizures, psychosis, and peripheral nerve compromise.
Hematological criteria were defined as the presence of at least one of the following: hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia.
Immunological criteria were defined as the presence of at least one of the following: anti-dsDNA, anti-Sm, anticardiolipin IgG or IgM, lupus anticoagulant, false-positive test VDRL, or fluorescent treponemal antibody absorption test.

Finally, 60 articles that had interpretable data and fulfilled the eligibility criteria were included. In 3 papers, data extraction was made from abstract [41–43]. For details, see Supplementary Table S1. There were 29 from Brazil [42, 44–71], 14 from Mexico [43, 72–84], 6 from United States of America [16–18, 85–87], 5 from Argentina [14, 41, 88–90], 4 from Puerto Rico [91–94], and 1 from Colombia [95] and Chile [96]. Five studies correspond to SLE LUMINA (LUpus in MInorities: nature versus nurture) multiethnic cohort [16–18, 85, 86]. The LUMINA cohort is comprised of patients of Hispanic (from Texas and Puerto Rico), African-American and Caucasian background. Two studies correspond to GLADEL'S (Grupo Latino Americano De Estudio de Lupus) longitudinal inception cohort [14, 97]. Thirty studies were cross-sectional, 15 were case-controls, 11 were cohort, 2 nested case-control and 2 were an inception cohort. The flowchart for systematic literature review and articles included in the analysis are shown in Figure 1.

3.5. Prevalence, Risk Factors, and Subphenotypes of CVD. Out of total of 60 articles that fulfilled eligibility criteria, 46 had interpretable data regarding CVD frequency, which corresponds to a prevalence range of 4%–79.5%. Several *classic* CV risk factors such as metabolic syndrome (MetS), obesity, dyslipidemia, hypertension, T2DM, sedentary lifestyle, male gender, smoking, advanced age, hyperhomocysteinemia, renal impairment, family history of CVD, and menopausal status were described. Several studies reported *nontraditional* risk factors such as ancestry, certain single-nucleotide polymorphisms, SLE *per se*, polyautoimmunity, autoantibodies (i.e., antiphospholipid), markers of systemic inflammation

Table 2: Cardiovascular disease characteristics of 310 patients with SLE.

	% (n/N)
Clinical manifestation	
Cardiovascular disease	36.5 (113/310)
Hypertension	25.2 (78/310)
Stroke	16.8 (52/310)
Coronary disease	2.6 (8/310)
Thrombotic event	1.6 (5/310)
Carotid disease	0.6 (2/310)
Risk factors	
Type 2 diabetes mellitus	1.9 (6/309)
Dyslipidemia	18.1 (56/309)
Ever smoking	39.21 (120/306)
1 to 6 packages/year	4.2 (13/306)
6 to 15 packages/year	2.6 (8/306)
More than 15 packages/year	2.3 (7/306)
Quitter	30.1 (92/306)
Coffee consumption	61.5 (187/304)
1 to 2 cups/day	30.9 (94/304)
2 to 4 cups/day	22.7 (69/304)
More than 4 cups/day	7.9 (24/304)
Never	38.5 (117/304)
Laboratories findings	
Abnormal triglycerides	34.3 (12/35)
Abnormal total cholesterol	44.4 (16/22)
Abnormal high-density cholesterol	58.8 (20/34)
Abnormal low-density cholesterol	33.3 (8/24)
Abnormal glycemic	25.7 (9/35)

(i.e., CRP), SLE disease activity, SLE duration, organ damage, immune cells aberrations, medication (i.e., glucocorticoids), vasculopathy, lupus nephritis, endogenous dyslipidemia, bone mineral density, education level, and monthly income. A broad spectrum of CV subphenotypes including hypertension, IHD, CAD, ACS, MI, angina, CHF, stroke, thrombosis, peripheral arterial disease, subclinical atherosclerosis, and mortality due to CVD were described in LA individuals with SLE. For details, see Figure 2 and Supplementary Table S1.

4. Discussion

The analysis of Colombian patients with SLE discloses a high prevalence of CVD (36.5%). In contrast, prospective North Americans cohort studies showed a prevalence and annual incidence of CVD between 6 and 10%, and 1.5%, respectively [9, 98, 99]. Meanwhile, a case control study on the British General Practice Research Database showed higher risk of CVD in patients with SLE than rheumatoid arthritis [100]. Furthermore, in Italian population, Doria et al. [101] demonstrated rates of 10–40% for subclinical atherosclerosis. This inconsistency may reflect methodological obstacles such as differences in the definition of CVD outcome. Since CVD begins by endothelial dysfunction, hypertension was considered as the first subphenotype to be assessed.

4.1. Epidemiology of Atherosclerosis among Patients with SLE. Carotid plaque is prevalent in 21% of SLE patients under age of 35 and in up to 100% of those over age 65 [102]. The most striking example of raised risk of MI comes from the University of Pittsburgh SLE cohort, in which women with SLE aged 35–44 were >50 times more likely to experience MI than women without SLE from the Framingham Offspring study (RR 52.4; 95% CI 21.6–98.5) [8, 98].

Independent predictive risk factors for CV events had been assessed in five large prospective cohorts of patients with SLE, including Baltimore (1992, $n = 229$) [103], Pittsburg (1997, $n = 498$) [98], LUMINA (2004, $n = 546$) [17], Toronto (2007, $n = 561$) [104] and Systemic Lupus International Collaborating Clinics-Registry for Atherosclerosis (2010, $n = 637$) [105]. These cohorts found association of diverse classic risk factors (i.e., older age at diagnosis, smoking, hypercholesterolemia, male gender, and hypertension), as well as novel risk factors (i.e., longer duration of SLE and glucocorticoid use, antiphospholipid antibodies, and neuropsychiatric lupus), with CVD in SLE patients [105, 106]. In a recent meta-analysis, Schoenfeld et al. [8] showed that epidemiologic data strongly support that SLE patients are at elevated relative risk of CVD. The risks of MI, CHF, CVA, and CVD mortality are all increased among SLE patients compared to general population risks. The variability regarding the relative importance of risk factors for CVD among SLE patients in past epidemiologic studies is likely due in part to different design methods and different patient and comparison groups.

4.2. CVD in Hispanics with SLE. CVD has been assessed in LUMINA multiethnic cohort and GLADEL's longitudinal inception cohort, which demonstrated differences in sociodemographic, clinical (i.e., subphenotypes), immunologic, and therapeutic characteristics, in SLE patients with CV events [14–19, 85, 86, 93]. The present study adds further evidence about the high frequency of CVD in patients with SLE, their traditional risk factors (i.e., dyslipidemia, and smoking), and highlights coffee consumption as a factor for such a complication. Through the systematic review several factors and outcomes related to CVD were also identified (Table 6).

4.3. Cigarette Smoking and Coffee Consumption Independently Influence the Risk of Developing CVD. Several studies have assessed smoking as an independent risk factor for CV atherosclerotic disease [17, 107–111]. Gustafsson et al. [110] found that smoking may be the main traditional risk factor promoting increased CV risk in 208 SLE patients (RR 3.4, 95% CI 1.3–9.2). Previously, the same group found that smoking was predictive of MI, stroke, peripheral vascular disease (PVD) or CV mortality among the same patient population [109]. Toloza et al. [17] prospectively followed SLE patients over a median follow-up of 73.8 months and compared those who had a CVD event to those who did not as part of the LUMINA study. Current cigarette use was significantly associated with a 3.7-times increased risk of

Table 3: Characteristics associated with CVD in 310 patients with SLE.

Characteristic	Cardiovascular disease 113/310 % (n/N)	Noncardiovascular disease 197/310 % (n/N)	OR (95% CI)	P
Sociodemographic characteristic				
Age (y)	Median (IQR): 40 (23)	Median (IQR): 36 (21)		0.059
Age at SLE onset (y)	Median (IQR): 30 (19)	Median (IQR): 26 (18)		0.175
Age of diagnosis (y)	Median (IQR): 34 (22)	Median (IQR): 28 (18)		0.041
Ever smoking	50.9 (57/112)	37.1 (73/194)	1.75 (1.09–2.61)	0.019
Coffee	70.5 (79/112)	55.2 (108/192)	1.94 (1.19–3.18)	0.009
Hair dye	46 (52/113)	37.4 (73/195)	1.42 (0.89–2.29)	0.139
Pesticides	3.5 (4/113)	1 (2/197)	3.56 (0.64–19.75)	0.122
Autoimmune disease(s)				
MAS	3.5 (4/113)	7.1 (14/197)	0.48 (0.15–1.49)	0.196
Familial autoimmunity in FDR	19.5 (22/113)	25.4 (50/197)	0.71 (0.40–1.25)	0.235
RA	0.9 (1/113)	5.6 (11/197)	0.15 (0.01–1.19)	0.062
APS	16.8 (19/113)	4.1 (8/197)	4.77 (2.01–11.31)	0.0001
AITD	4.4 (5/113)	9.1 (16/197)	0.52 (0.18–1.47)	0.213
Comorbidities				
Type 2 diabetes mellitus	3.5 (4/113)	1 (2/197)	3.56 (0.64–19.75)	0.196
Dyslipidemia	28.3 (32/113)	12.2 (24/197)	2.83 (1.56–5.11)	0.0001
Fibromyalgia	14.2 (16/113)	7.1 (14/197)	2.14 (1.00–4.57)	0.045
Treatment				
Antimalarials	77 (87/113)	82.7 (163/197)	0.69 (0.39–1.23)	0.217
Mycophenolate Mofetil	19.5 (22/113)	13.7 (27/197)	1.52 (0.82–2.82)	0.181
Cytotoxics agents	17.7 (20/113)	9.1 (16/197)	2.13 (1.07–4.23)	0.027
Clinical variable				
Discoid lupus	5.3 (6/113)	9.1 (16/197)	0.55 (0.21–1.44)	0.225
Alopecia	41.5 (47/113)	51.3 (101/197)	0.67 (0.42–1.08)	0.101
Subacute	6.2 (7/113)	3 (6/197)	2.10 (0.68–6.41)	0.239
Urticaria	19.5 (22)	10.2 (20/197)	2.14 (1.11–4.12)	0.021
Vasculitis	15 (17/113)	20.3 (40/197)	0.89 (0.37–1.29)	0.250
Neurological involvement	13.3 (15/113)	8.1 (16/197)	1.73 (0.82–3.65)	0.146
Headache	28.2 (33/113)	18.9 (37/197)	1.79 (1.03–3.06)	0.035
Psychosis	7.1 (8/113)	3.8 (7/197)	2.08 (0.72–5.86)	0.164
Serositis	37.2 (42/113)	23.9 (47/197)	1.88 (1.14–3.12)	0.013
Pleural effusion	31.9 (36/113)	18.9 (37/197)	2.02 (1.18–3.44)	0.009
Hands edema	32.7 (37/113)	15.9 (33/197)	2.41 (1.40–4.16)	0.0001
Renal involvement	56.6 (84/113)	40.9 (80/197)	1.91 (1.19–3.05)	0.006
Nephrotic	27.4 (31/113)	10.7 (21/197)	3.16 (1.71–5.84)	0.0001
Pulmonary haemorrhage	3.5 (4/113)	0.5 (1/197)	7.19 (0.79–65.16)	0.061
Laboratory findings				
Thrombocytopenia	11.9 (13/109)	5.3 (10/187)	2.39 (1.01–5.67)	0.041
Leukopenia	30.6 (34/111)	42.3 (80/189)	0.60 (0.36–0.98)	0.044
Lymphopenia	71.6 (78/109)	78.7 (148/188)	0.68 (0.39–1.17)	0.163
Abnormal creatinine	17 (17/100)	5.4 (8/169)	3.61 (1.54–8.47)	0.002
Abnormal creatinine clearance	64.4 (38/59)	52.3 (56/107)	1.64 (0.85–3.17)	0.133
Proteinuria (+)	50 (41/82)	30 (39/130)	2.33 (1.31–4.13)	0.003
aCLIgG (+)	40.7 (35/88)	29.7 (44/149)	1.62 (0.93–2.82)	0.087
Lupus anticoagulant (+)	58.5 (24/41)	45.9 (29/61)	1.66 (0.74–3.70)	0.211

CVD: cardiovascular disease; SLE: systemic lupus erythematosus; OR: odds ratio; CI: confidence interval; IQR: interquartile range; SD: standard deviation; Y: years; MAS: multiple autoimmune syndrome; FDR: first-degree relatives; RA: rheumatoid arthritis; APS: antiphospholipid syndrome; AITD: autoimmune thyroid disease; aCL: anticardiolipin antibodies.

Table 4: Factors associated with CVD in patients with SLE*.

Characteristic	β	AOR	95% CI	P
Dyslipidemia	0.971	2.64	1.32–5.28	0.005
Pleural effusion	0.751	2.12	1.17–3.84	0.013
Ever smoking	0.602	1.83	1.07–3.10	0.025
Coffee consumption	0.559	1.75	1.01–3.04	0.043
Renal involvement	0.476	1.61	0.94–3.84	0.081

CVD: cardiovascular disease; SLE: systemic lupus erythematosus; β: β coefficient; AOR: adjusted odds ratio; 95% CI: 95% confidence interval.
*The model was adjusted by gender and duration of the disease.

Table 5: Factors associated with CVD in patients with SLE including interaction between smoking and coffee consumption*.

Characteristic	β	AOR	95% CI	P
APS	1.55	4.71	1.81–12.2	0.001
Dyslipidemia	1.07	2.92	1.54–5.55	0.001
Pleural effusion	0.78	2.19	1.20–3.98	0.011
Smoking and coffee	0.60	1.82	1.05–3.13	0.03

CVD: cardiovascular disease; SLE: systemic lupus erythematosus; β: β coefficient; AOR: adjusted odds ratio; CI: confidence interval; APS: antiphospholipid syndrome.
*Adjusted by gender and duration of the disease including interaction between smoking and coffee consumption. P-values persisted significant despite the evaluation of the four possible combinations (i.e., smoking, coffee, smoking and coffee, none) through the adjustment of the multivariate model.

having a CVD event. In the PROFILE population, another multicenter, multiethnic study population, Bertoli et al. [111] found that smoking acted as an independent risk factor associated with a 2-fold decrease in time to a CV event among 1,333 SLE patients over a 6.4-year follow-up period.

Several studies have evaluated the association between coffee consumption and CVD in the general population with controversial results. Two Dutch studies [112, 113] found no association between coffee intake, high blood pressure, and CVD. Despite the classification of coffee consumption differed among studies, some results suggest that habitual coffee consumption is associated with increased risk of hypertension [114]. In the same way, Klag et al. [115] demonstrated over many years of followup that coffee drinking is associated with small increases in blood pressure but appears to play a small role in the development of hypertension. When they compared with nondrinkers at baseline, coffee drinkers had a greater incidence of hypertension during follow-up (18.8% versus. 28.3%; $P = 0.03$). Relative risk (95% confidence interval) of hypertension associated with drinking 5 or more cups a day was 1.35 (0.87–2.08) for baseline intake and 1.60 (1.06–2.40) for intake over followup. Other effects attributed to coffee drinking are the increase in systemic vascular resistance, increased serum cholesterol levels, arterial stiffness, plasma rennin activity, epinephrine and norepinephrine, driving an unfavorable effect on endothelial function in healthy population [116]. On the other hand an Australia study [117] detected a negative association between coffee, hypertension, and MetS. Likewise, an increase in flow-mediated dilation and a decrease in CRP levels related to coffee drinking have been observed regardless of CAD [118, 119].

In order to isolate the interaction of smoking and coffee consumption, two regression models were made in which both the independent effect of coffee and smoking consumption on CVD as well as their interaction remains significant, demonstrating synergism between them (i.e., multiplicative effect). Otherwise, coffee consumption has not been evaluated systematically in SLE patients with CVD. However, since there is not a universal accepted tool for assessing coffee consumption, a bias concerning this variable is not precluded. Furthermore, membership bias could also exist because coffee consumption in Colombia is a well-defined tradition.

4.4. Traditional Risk Factors for CVD in SLE. Diverse lupus cohorts had shown the influence of advanced age, dyslipidemia, obesity, hypertension, and hyperhomocysteinemia, as classical risk factors for CVD [109, 120, 121]. Younger patients with SLE have the greatest relative risk compared to their healthy counterparts, but the absolute risk of CVD among SLE patients increases with advancing age [8]. de Souza et al. [58] observed that young Brazilian patients with SLE presented higher prevalence of carotid plaque than controls.

López-Jaramillo et al. [122] showed that the concentration of proinflammatory cytokines is higher in the LA population than in developed countries, suggesting a higher susceptibility to develop systemic low-degree inflammation at a given level of abdominal obesity, which contributes to the burden of CVD in this population. The inflammatory milieu of SLE leads to deregulation of lipid metabolism pathways, which contribute to the increased risk of atherosclerotic disease among these patients [123, 124]. Five large cohort studies have shown hypercholesterolemia as a risk factor for CVD in SLE patients [98, 103, 125–127]. Our results confirm the role of dyslipidemia as an independent risk factor for CVD in LA patients with SLE [50, 52, 53, 58, 60, 62–64, 66, 80, 81, 83, 84, 89]. For more details see Table 6.

4.5. Nontraditional Risk Factors for CVD in SLE. It is well known that while traditional CVD risk factors are undoubtedly important in increasing the CVD risk among SLE patients, these do not fully account for the elevated risk of CVD in this population [9]. Thereby, SLE-associated factors play an important role in the premature atherosclerosis process characteristic of these patients [128, 129]. Evidence strongly suggests that atherosclerotic plaque is largely driven

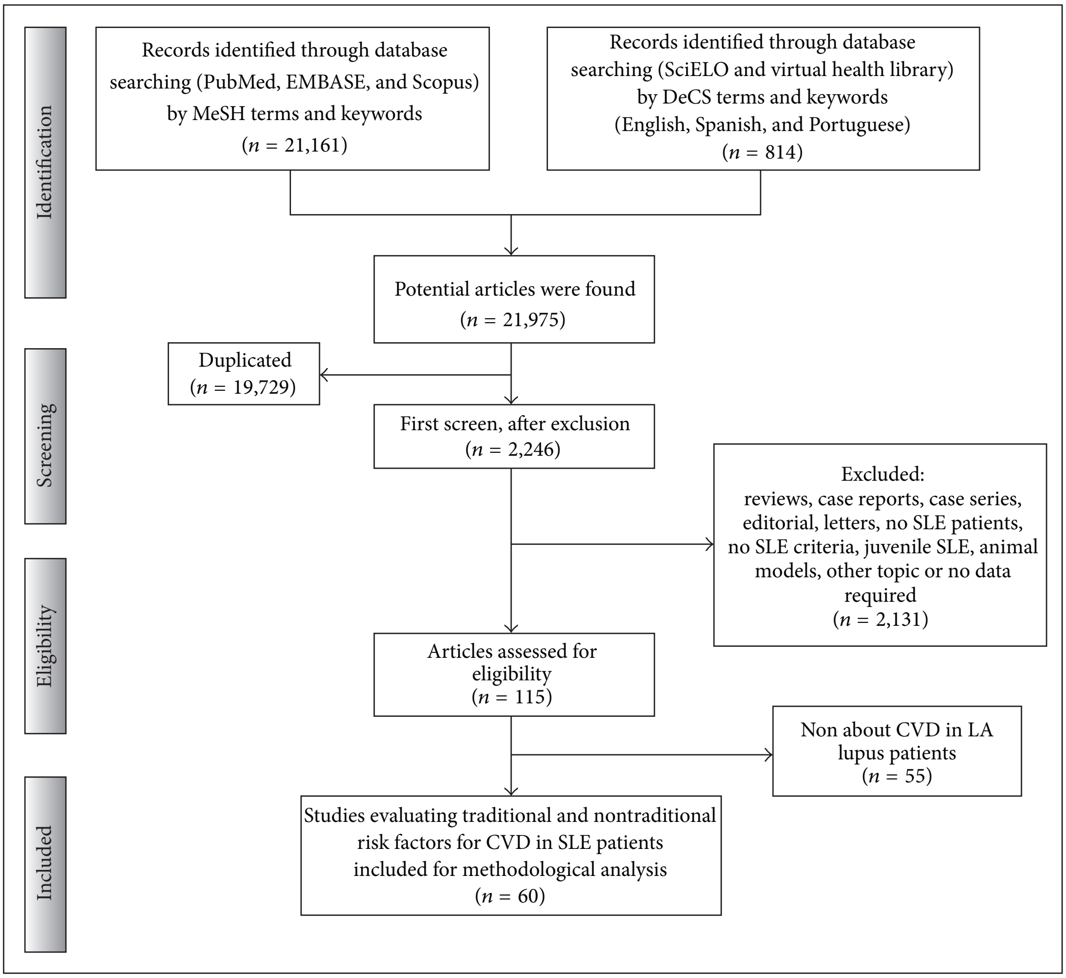
Identification
Screening
Eligibility
Included
Records identified through database searching (PubMed, EMBASE, and Scopus) by MeSH terms and keywords (n = 21,161)
Records identified through database searching (SciELO and virtual health library) by DeCS terms and keywords (English, Spanish, and Portuguese) (n = 814)
Potential articles were found (n = 21,975)
Duplicated (n = 19,729)
First screen, after exclusion (n = 2,246)
Excluded: reviews, case reports, case series, editorial, letters, no SLE patients, no SLE criteria, juvenile SLE, animal models, other topic or no data required (n = 2,131)
Articles assessed for eligibility (n = 115)
Non about CVD in LA lupus patients (n = 55)
Studies evaluating traditional and nontraditional risk factors for CVD in SLE patients included for methodological analysis (n = 60)

Figure 1: Flowchart of the systematic literature review. SLE: systemic lupus erythematosus, CVD: cardiovascular disease, LA: Latin America.

by inflammation and an active immunological response [130–132] and points to SLE itself as an independent risk factor for premature CAD [133, 134]. Nontraditional risk factors for CVD in ADs may be classified into genetics, AD-related, and miscellaneous [135, 136].

Family and twin studies have repeatedly supported a role for heredity in CAD, particularly in young individuals. Several genetic markers have been proposed as predisposing factors for CVD in SLE patients [87, 137, 138]. The SLE-associated risk factors represent a broad spectrum of conditions related to the autoimmune nature of the disease. All of these pathways may eventually converge into a shared proatherogenic phenotype [139]. Table 6 summarizes the state of the art of CVD risk factors in LA patients with SLE.

4.6. Assessment and Management of CV Risk in SLE Patients. Physicians often face the question of how to personalize treatment and prevention of CV events. Framingham risk score (FRS) is widely used to stratify asymptomatic patients into different CV risk categories in order to target the intensity of primary medical intervention. This score is strongly influenced by age and therefore has limited usefulness in young patients with SLE [140]. Therefore, evaluation of only traditional CAD risk factors in lupus patients may result in the underestimation of their future overall CAD risk. The contribution of inflammatory biomarkers should be also considered to gain a complete picture of the CAD risk in patients with underlying conditions that increase inflammation such as SLE [141]. The Reynolds Risk score incorporates CRP concentration in the risk model and reclassifies approximately 50% of women in the 10-year FRS 5–20% risk category into different risk categories [142]. Another proposed approach corresponds to Systematic Coronary Risk Evaluation that is recommended by the EULAR experts and is widely used for CV assessment in patients with rheumatoid arthritis and other forms of inflammatory arthritis [143]. Neither of these charts includes the broad variety of risk factors that are disease-specific and could potentially explain the increased burden due to CV events. Therefore, CV risk in SLE patients is consistently underestimated with these scales.

Recently, Petri and Magder [144] proposed a data-driven risk equation of CV risk in SLE, based on data collected in a longitudinal cohort that can better estimate 10-year CV risk than the Framingham equation. In this model an integer score is given to each variable including age over 40, male gender, systolic blood pressure over 140, TC over 160,

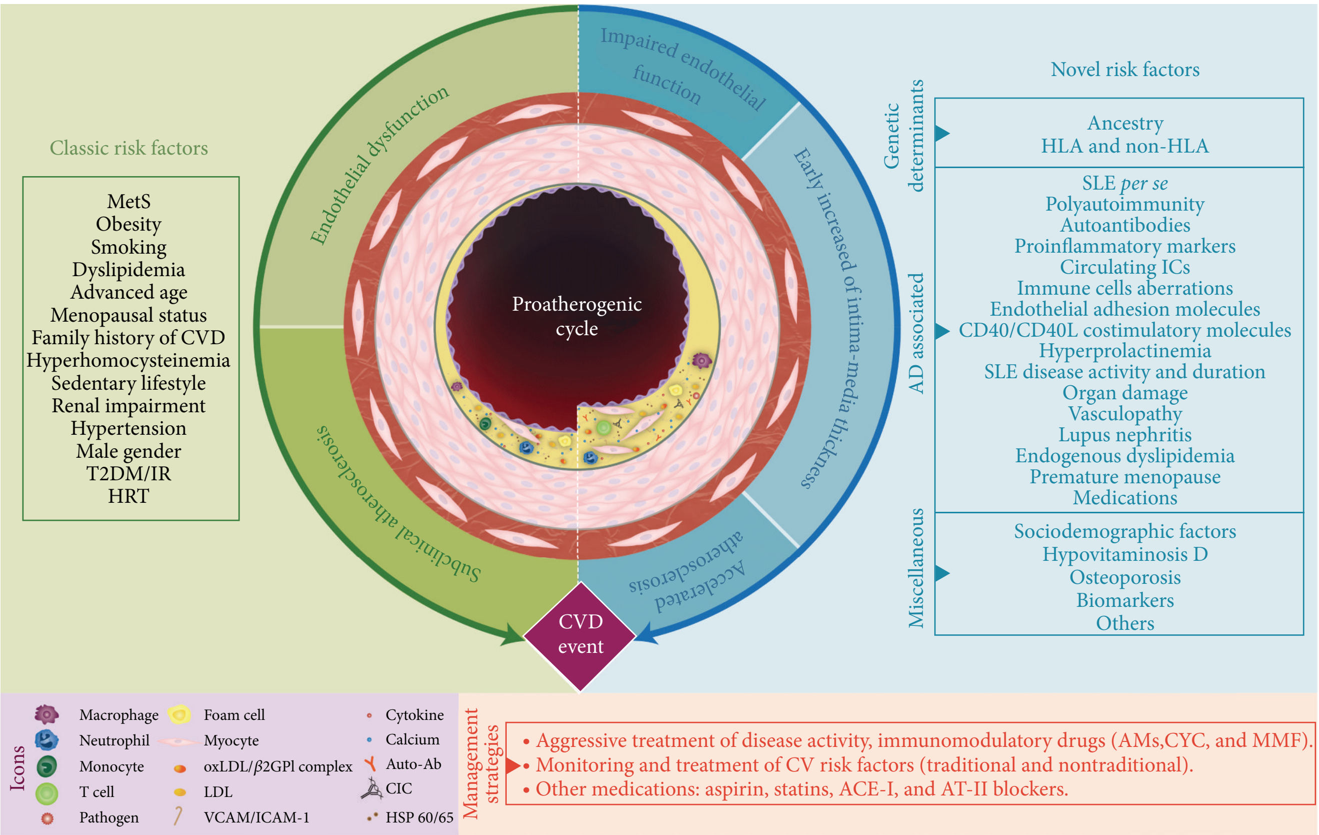
Classic risk factors
MetS
Obesity
Smoking
Dyslipidemia
Advanced age
Menopausal status
Family history of CVD
Hyperhomocysteinemia
Sedentary lifestyle
Renal impairment
Hypertension
Male gender
T2DM/IR
HRT
Endothelial dysfunction
Subclinical atherosclerosis
Impaired endothelial function
Early increased of intima-media thickness
Accelerated atherosclerosis
Proatherogenic cycle
CVD event
Novel risk factors
Genetic determinants
Ancestry
HLA and non-HLA
AD associated
SLE *per se*
Polyautoimmunity
Autoantibodies
Proinflammatory markers
Circulating ICs
Immune cells aberrations
Endothelial adhesion molecules
CD40/CD40L costimulatory molecules
Hyperprolactinemia
SLE disease activity and duration
Organ damage
Vasculopathy
Lupus nephritis
Endogenous dyslipidemia
Premature menopause
Medications
Miscellaneous
Sociodemographic factors
Hypovitaminosis D
Osteoporosis
Biomarkers
Others
Icons
Macrophage
Neutrophil
Monocyte
T cell
Pathogen
Foam cell
Myocyte
oxLDL/β2GPl complex
LDL
VCAM/ICAM-1
Cytokine
Calcium
Auto-Ab
CIC
HSP 60/65
Management strategies
• Aggressive treatment of disease activity, immunomodulatory drugs (AMs,CYC, and MMF).
• Monitoring and treatment of CV risk factors (traditional and nontraditional).
• Other medications: aspirin, statins, ACE-I, and AT-II blockers.

FIGURE 2: Traditional and autoimmune-related mechanisms of cardiovascular disease in systemic lupus erythematosus. A complex interaction between traditional and disease-specific traits leads to premature atherosclerotic process. Several risk factors (left) have been described since The Framingham Heart Study, known as classic risk factors, which over time conduce to endothelial dysfunction, subclinical atherosclerosis, and CV event manifest. In the autoimmune setting (right), a broad spectrum of novel risk factors contribute to development of premature vascular damage. This damage is represented by impaired endothelial function and early increased of Intima-Media Thickness which are surrogates of the accelerated atherosclerosis process, which is perpetuated by a chronic proinflammatory milieu. The cornerstone of management of CV risk include an aggressive treatment of disease activity, the continuous monitoring and treatment of modifiable CV risk factors, as well as the use of other medications in order to diminish de CV burden. CVD: cardiovascular disease, SLE: systemic lupus erythematosus, MetS: metabolic syndrome, T2DM: type 2 diabetes mellitus, IR: insulin resistance, HRT: hormone replacement therapy, CIC: Circulating Immune complex, oxLDL/B2GPI complex: oxidized-low density lipoprotein/β2 glycoprotein I, HDL: high density lipoprotein, Auto-Ab: auto-antibodies, AMs: antimalarials, CYC: cyclophosphamide, AZA: azathioprine, MMF: mycophenolate mofetil, ACE-I: angiotensin-converting enzyme inhibitors, AT-II blockers: angiotensin II receptor blockers.

smoking, T2DM, mean SLE disease activity index (SLEDAI), history of lupus anticoagulant, and low mean C3. Using this model, together in the absence of SLE-specific risk factors, the estimated risk is higher than what would be projected based on the FRS. Nevertheless, this model is not exempt: limitations on, for example, HDL and LDL are not available at all routine visits. Further validation of this model is warranted.

SLE entails a CVD equivalent to T2DM; thus, lower lipid goals, aspirin use, and an aggressive monitoring are required [145, 146]. Studies aimed to evaluate whether or not traditional treatment regimens prevent or slow atherosclerosis in SLE patients have been published [147]. The recent randomized controlled Lupus Atherosclerosis Prevention Study suggested that atorvastatin do not slow progression of subclinical atherosclerosis [148].

There are several new mechanisms of action described for antimalarials, many of them with beneficial effects in the management of CV risk in patients with SLE [149, 150]. There is evidence that antimalarials reduce serum cholesterol and LDL levels, elevate HDL cholesterol, and when taken concomitantly with steroids can reduce serum cholesterol [151]. Penn et al. [152] suggested that hydroxychloroquine use is associated with a lower fasting glucose and a decrease in the homeostasis model assessment-insulin resistance (HOMA-IR) index. Rekedal et al. [153] showed that hydroxychloroquine initiation was associated with a significantly greater reduction in HbA1c as compared to methotrexate initiation among diabetic patients with rheumatic diseases. In addition, beneficial effects of hydroxycloroquine on thrombosis formation have also been described. Multiple retrospective cohort studies have shown a reduced incidence of thrombotic events and improved overall survival in patients with SLE treated with antimalarials [154–157].

The presence of low vitamin D levels has been associated with disrupting self-tolerance. Therefore, it is tempting to speculate that vitamin D might prove useful as a preventive

Table 6: Traditional and nontraditional risk factors associated with cardiovascular disease and systemic lupus erythematosus in Latin America.

Risk factor associated with CVD	Comments	Reference(s)
	Traditional	
Hypertension	Hypertension influences the risk of death by CVD in SLE patients.	[64, 89]
	Hypertension acts as CVD subphenotype as well as a risk factor.	[45, 49, 71, 88]
	Patients with SLE were at increased risk of thrombosis when it is associated with hypertension.	[17, 80, 84]
	Compared with patients without atherosclerotic plaque, those with plaque had higher prevalence of hypertension.	[54, 63, 66, 73]
	Lupus patients with abnormal myocardial scintigraphic findings and hypertension, as a risk factor for CAD, had a higher risk of abnormal findings on coronary angiography.	[52, 53]
	Patients with lupus had higher hypertension prevalence than controls with noninflammatory disorders.	[14, 68, 90]
T2DM	T2DM influence on abnormal myocardial perfusion in asymptomatic patients with SLE.	[53]
	Alterations in glycemic profile were associated with traditional risk factors for CHD and lupus characteristics, including CVD, damage index, and renal involvement.	[17, 68, 81]
	Patients with SLE and T2DM were at increased risk of thrombosis. This risk remains elevated throughout the course of the disease.	[16, 80]
	T2DM is an independent risk factor for atherosclerotic plaque and CAC.	[63, 71, 84]
Dyslipidemia	The main risk factor for death in SLE was heart involvement, which was influenced by dyslipidemia.	[50, 89]
	High levels of TGL were associated with myocardial perfusion abnormalities and endothelial dysfunction	[52, 53, 83]
	There was high prevalence of dyslipidemia as risk factor for thrombotic events.	[60, 62, 80]
	Alterations in lipid profile was a risk factor for premature CAC in young women with SLE.	[66, 84]
	CAD was more prevalent in dyslipidemic women with SLE than controls.	[64, 81]
	Compared with patients without atherosclerotic plaque, those with plaque had high level of TGL and LDL.	[58, 63]
Male gender	Male gender was a risk factor for developing severe organ damage (CVD) and mortality in SLE patients.	[16, 17, 53, 60]
	Males with SLE were at increased risk of thrombosis and CAC. This risk remains elevated throughout the course of the disease.	[80, 84, 85]
	Patients had more peripheral vascular and gonadal involvement compared with published data from non-Hispanic SLE populations.	[76]
MetS	SLE patients had a high prevalence of MetS that directly contributes to increasing inflammatory status and oxidative stress.	[69]
	MetS was associated with traditional risk factors for CHD and lupus characteristics, including CVD, damage Index, and renal involvement.	[68, 81]
	Presence of MetS was related to CVD in SLE patients.	[90, 94]
Obesity	Patients with SLE who had excess weight present distinct clinical-laboratory findings, sociodemographic characteristics, and treatment options when compared to normal weight patients.	[17, 71, 81]
	Excess weight is associated with some traditional risk factors for CVD and SLE poor prognosis.	[58, 65, 68]
	Increase weight influence on abnormal myocardial perfusion in asymptomatic patients with SLE.	[53, 64]
	SLE patients with high BMI have increased QT interval parameters when compared to controls. This prolongation may lead to an increased CV risk.	[55]
	Major values in BMI were related with the presence of CAD and carotid plaque.	[58, 63, 64]

Table 6: Continued.

Risk factor associated with CVD	Comments	Reference(s)
Smoking	Smoking is an important determinant in the occurrence of thrombotic (central and/or peripheral, arterial and/or venous) events in SLE patients.	[18, 69, 81]
	Smoking was an independent risk factor for atherosclerotic plaque and thrombosis.	[63, 68, 80]
	Smoking habit influence on abnormal myocardial perfusion in asymptomatic patients with SLE.	[53]
	Smoking was a risk factor for premature CAC in young women with SLE.	[66, 84]
	CAD was more prevalent in women with SLE.	[64, 85, 86]
Advance age	Several traditional risk factors, including age, appear to be important contributors to atherosclerotic CV damage.	[16, 71]
	The presence of CVD has been associated with older age.	[16, 59]
	Age was directly related with atherosclerotic plaque formation.	[63]
Menopausal status	High percentage of SLE patients with abnormal angiographic findings was in postmenopausal status.	[52]
	There is high prevalence of premature menopausal status as a risk factor for CVD.	[60]
	Postmenopausal status was a risk factor for premature CAC in young women with SLE.	[66, 68, 84]
	Postmenopausal women had a higher prevalence of subclinical AT and abnormal myocardial perfusion in asymptomatic patients with SLE.	[53, 63]
Family history of CVD	Familial history of CVD was an independent risk factor for atherosclerotic process.	[17, 63, 68]
	Family history of CVD was a risk factor for premature CAC in young women with SLE.	[66, 84]
	Family history of CVD influence on abnormal myocardial perfusion in asymptomatic patients with SLE.	[53]
HRT	HRT use was not associated with the occurrence of vascular arterial events in the LUMINA patients. HRT use in women with SLE should be individualized, but data suggest its use may be safe if aPL antibodies are not present or vascular arterial events have not previously occurred.	[17]
Hyperhomocysteinemia	Hyperhomocysteinemia was a risk factor for CAC in SLE patients.	[84]
	The presence of polyautoimmunity and hyperhomocysteinemia was risk factors for thrombotic events.	[41]
	Nontraditional	
Genetic determinants		
Ancestry	There are several differences regarding clinical (including CVD), prognostic, socioeconomic, educational, and access to medical care features in GLADEL cohort according to ancestry (White, Mestizo, and African-LA).	[14]
Non-HLA	An SNP in FGG rs2066865 demonstrated association with arterial thrombosis risk in Hispanic Americans patients with SLE.	[87]
	The CRP GT20 variant is more likely to occur in African-American and Hispanic SLE patients than in Caucasian ones, and SLE patients carrying the GT20 allele are more likely to develop vascular arterial events (LUMINA multiethnic cohort).	[86]
SLE-associated		
Poliautoimmunity	The presence of APS was the major independent contributor to the development of severe organ damage in Brazilian patients with SLE.	[54]
	APS and its characteristic antibodies may contribute to the development of thrombotic events in Brazilian and Mexican lupus patients.	[57, 78]
	APS had high impact in CVD and survival in Brazilian lupus patients.	[42]
	Polyautoimmunity (APS) may suggest concerted pathogenic actions with other autoantibodies in the development of thrombotic events in Mexican patients with SLE.	[78]

Table 6: Continued.

Risk factor associated with CVD	Comments	Reference(s)
SLE *per se*	SLE diagnosis was significantly associated with carotid plaque formation and development of CV event in Brazilian patients with SLE.	[58]
	High percentage of patients with abnormal angiographic findings had higher ACR criteria number for SLE Brazilian patients with SLE.	[52]
Autoantibodies	One of the independent predictors of vascular events in a multiethnic US cohort (LUMINA) was the presence of any aPL antibody.	[17]
	anti-β2GPI antibodies were strongly associated with thrombosis in patients with Mexicans with SLE. The decrease of anti-β2GPI levels at the time of thrombosis may indicate a pathogenic role.	[77]
	The higher frequency of aPT found in Mexican patients with SLE with thrombosis may suggest concerted pathogenic actions with other autoantibodies in the development of thrombotic events.	[78]
	Patients with aCL antibodies seem to be at an increased risk for arterial and venous thrombotic events in Puerto Ricans and Chilean patients with SLE.	[92, 96]
	There was correlation between lupus anticoagulant and thrombotic events in Brazilian lupus patients.	[50]
	aCL antibodies were associated with thrombotic events, mainly in high titers in Chilean SLE patients.	[96]
	aCL antibodies were significantly associated with CV events and showed an association with echocardiographic abnormalities in Brazilian patients with SLE.	[51]
	Mexican patients had more peripheral vascular compared with published data from non-Hispanic SLE populations.	[76]
Immune cells aberrations	Complement fixing activity of aCL antibodies seems to be relevant in thrombotic venous events in Brazilian patients with SLE.	[57]
Inflammatory markers	Increased ESR was independently associated with MetS in Puerto Ricans lupic patients.	[94]
	One of the independent predictors of vascular events in a multiethnic US cohort (LUMINA) was elevated serum levels of CRP.	[16, 17]
Endogenous dyslipidemia	HDL distribution and composition (−HDL2b, +HDL3b, and +HDL3c) were abnormal in noncomplicated Mexican SLE patients.	[79]
	Low HDL levels and increased TGL levels were associated with atherosclerosis by cIMT measurement in Colombian lupic patients.	[95]
	SLE patients have a lipid profile abnormality in Brazilian patients with SLE. This pattern of dyslipoproteinemia may increase the risk of developing CAD.	[47]
Disease activity	Disease activity (SLAM) is an important determinant in the occurrence of thrombotic (central and/or peripheral, arterial, and/or venous) events in the LUMINA cohort.	[18]
	SLEDAI scores were positively correlated with BMI and WC in Brazilian population with SLE.	[69]
	Higher disease activity was independently associated with MetS and thrombosis in Puerto Ricans and Mexican SLE patients.	[80, 94]
	Higher score of SLICC was associated with atherosclerotic plaque in Brazilian SLE patients.	[58]
	High scores in diseases activity index (SLEDAI and SLICC) were associated with myocardial perfusion abnormalities in Brazilian SLE patients.	[52]
	Brazilian SLE patients have a lipid profile abnormality which is aggravated by disease activity and may reside in a defect of VLDL metabolism.	[47]
	Disease activity was predictor of CAC in Mexican SLE patients.	[84]
	Higher disease activity was independently associated with MetS in Puerto Ricans patients with SLE.	[94]

Table 6: Continued.

Risk factor associated with CVD	Comments	Reference(s)
Organ damage	Baseline and accrued damage increase mortality risk (including due to CVD) in Brazilian patients with SLE.	[61]
	Mexican patients had more peripheral vascular involvement (measured by SDI), compared with published data from non-Hispanic SLE populations.	[76]
	In Brazilian SLE patients, MetS was associated with both traditional risk factors for CHD and lupus characteristics including damage index.	[68]
	There was a correlation between IMT and revised damage index (SLICC) in Brazilian SLE patients.	[58]
	Atherosclerotic CV damage in SLE is multifactorial, and disease-related factors (including CRP levels and SDI at baseline) appear to be important contributors to such an occurrence (LUMINA multiethnic cohort).	[16]
Long duration	Longer duration of SLE was associated with atherosclerotic plaque and CV events in Brazilian population.	[58, 59]
	A correlation between IMT and duration of the disease was found in Brazilian patients with SLE.	[63]
	Disease duration was independent predictor for premature CAC in young Brazilian women with SLE.	[66]
Medications	PDN > 10 mg/day was independently associated with MetS in Puerto Ricans SLE patients.	[94]
	In Brazilian SLE patients, there was a correlation between IMT and the duration of PDN use.	[63]
	IHD was observed in two types of Mexican SLE patients: those with long-term steroid therapy and those with frank episodes of vasculitis.	[73]
Vasculopathy	Current vasculitis was associated with abnormal myocardial scintigraphy in Brazilian patients with SLE.	[53]
	Puerto Ricans patients with SLE and RP seem to be at increased risk for arterial and venous thrombotic events.	[92]
	IHD was observed in two types of Mexican SLE patients: those with long-term steroid therapy and those with frank episodes of vasculitis.	[73]
Renal involvement	In Brazilian SLE patients, MetS was associated with traditional risk factors for CHD and lupus characteristics, including damage index and renal involvement (nephritic syndrome).	[68]
Miscellaneous		
BMD	Decreased BMD was an independent predictor for premature CAC in Brazilian young women with SLE.	[66]
Sociodemographic factors	A low education and monthly income were associated with MetS in Mexican patients with SLE and RA.	[81]

aCL: anticardiolipins antibodies; ACR: American College of Rheumatology; anti-β2GPI: anti-beta2 glycoprotein 1 antibodies; aPT: antiprothrombin antibodies; aPL: antiphospholipid antibodies; APS: antiphospholipid syndrome; AT: atherosclerosis; BMD: bone mineral density; BMI: body mass index; CAC: coronary artery calcification; CAD: coronary artery disease; cIMT: carotid Intimal Medial Thickness; CHD: coronary heart disease; CRP: C-reactive protein; CV: cardiovascular; CVD: cardiovascular disease; ESR: erythrocyte sedimentation rate; GLADEL: Grupo Latino Americano De Estudio de Lupus; HDL: high-density lipoprotein cholesterol; HRT: hormone replacement therapy; IHD: ischemic heart disease; IMT: intimal media thickness; LA: Latin America; LDL: low-density lipoprotein cholesterol; LUMINA: LUpus in MInorities: NAture versus nurture cohort; MetS: metabolic syndrome; PDN: prednisolone; RP: Raynaud's phenomenon; T2DM: type 2 diabetes mellitus; TGL: triglycerides; SLAM: Systemic Lupus Activity Measure; SLE: systemic lupus erythematosus; SLEDAI: systemic lupus erythematosus disease activity index; SLICC: Systemic Lupus International Collaborating Clinics score; SDI: SLICC damage index; SNP: single-nucleotide polymorphism; VLDL: very low-density lipoprotein cholesterol; WC: waist circumference.

agent by reducing the risk of developing an autoimmune response [158]. In addition, vitamin D has been found to have antithrombotic properties improving the risk of CVD. Vitamin D deficiency has been linked to the development of atherosclerosis. As a corollary, the use of vitamin D may be useful to improve microvascular endothelial function [158].

5. Limitations of the Study

As a cross-sectional study, it is placed at level 4 of evidence according to Oxford Evidence Based Medicine [40]. Although the study sample size is not negligible, it would have been more valuable to have had an appropirate followup to establish valid associations between CVD, novel risk factors, and SLE. This, in turn, could have improved both internal and external validities.

The diversity of parameters defining CVD in SLE precluded homogenization of analysis and assessment in the systematic review. Often, one or two CVD subphenotypes were assessed as primary outcomes but many did not consider general CVD compromise. Therefore, it was not possible to perform a meta-analysis and to establish true measures of association such as odds ratios. Finally, we acknowledge that publication bias may exist.

6. Final Remarks and Conclusions

CVD is a major cause of morbidity and mortality in SLE patients. SLE and CVD share common pathophysiology mechanisms (i.e., systemic and chronic inflammation) with secondary accelerated atherosclerosis. Since traditional risk factors do not completely explain the high rates of CVD in patients with SLE, novel risk factors related to autoimmunity are now recognized. In the current study classical risk factors such as dyslipidemia, coffee consumption, and smoking habit are highlighted, and SLE-related factors (i.e., SLE-antiphospholipid syndrome polyautoimmunity, pleural effusion, and renal involvement) are confirmed. In addition, several factors associated with CVD in LA patients with SLE were reviewed. Altogether, our results should encourage preventive population strategies for CVD in patients with SLE [106, 159], aimed at facilitating the suppression of cigarette smoking and coffee consumption as well as to the tight control of dyslipidemia and other modifiable risk factors.

Conflict of Interests

The authors have indicated that they have no conflict of interests regarding the content of this paper.

Author's Contribution

Jenny Amaya-Amaya and Juan Camilo Sarmiento-Monroy contributed equally to this work.

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Review Article

Immunosenescence, Aging, and Systemic Lupus Erythematous

Gladis Montoya-Ortiz

Center for Autoimmune Diseases Research (CREA), School of Medicine and Health Sciences, Universidad del Rosario, Carrera 24 No. 63C-69, Bogota, Colombia

Correspondence should be addressed to Gladis Montoya-Ortiz; gladis.montoya@gmail.com

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Senescence is a normal biological process that occurs in all organisms and involves a decline in cell functions. This process is caused by molecular regulatory machinery alterations, and it is closely related to telomere erosion in chromosomes. In the context of the immune system, this phenomenon is known as immunosenescence and refers to the immune function deregulation. Therefore, functions of several cells involved in the innate and adaptive immune responses are severely compromised with age progression (e.g., changes in lymphocyte subsets, decreased proliferative responses, chronic inflammatory states, etc.). These alterations make elderly individuals prone to not only infectious diseases but also to malignancy and autoimmunity. This review will explore the molecular aspects of processes related to cell aging, their importance in the context of the immune system, and their participation in elderly SLE patients.

1. Introduction

Aging can be defined as the progressive decay of tissue functions which eventually results in organ dysfunction and death. This decline may be the result of the loss of postmitotic cell function or the lack of replacement of such cells due to a decreased stem cell ability to maintain cell division and replication [1]. If the organism suffers damage and it is irreparable, the senescence or aging process will take place by limiting the cells' proliferative potential. Some control mechanisms include differential gene expression which may be detrimental [2]. However, there is a renewal mechanism that ensures damaged cell replacement. This singular mechanism corresponds to a set of proliferating precursor cells that provide a source of cell replacement within the tissues. The immune system provides an interesting case of replacement: cells that die by apoptosis are replaced by new ones, a process which is essential for immune system longevity and for adequate functionality. This review will describe main molecular mechanisms implicated in immunosenescence and their relationship with autoimmune disease, particularly related to systemic lupus erythematous (SLE).

2. Aging Molecular Mechanisms

One of the most striking features of cell aging is its close relationship with telomere length [3]. There is an inverse relationship between telomere length and cellular aging; for example, very short telomeres force their cells to enter senescence. Human telomeres contain guanine-rich repetitive sequences (i.e., TTAGGG) which are gradually lost in each mitotic division. This occurs by the fact that the DNA polymerase is unable to replicate linear chromosomes in a process known as telomere erosion (Figure 1) [4–6]. This process functions as a mitotic clock for which the length of the telomeres represents the number of cell divisions sustained by the cells [7].

There is also a significant variability with respect to the speed and quality of aging between and within populations [8]. This heterogeneity results from interaction between genetic, environmental, and stochastic factors. In this regard, several epigenetic alterations have been associated with aging and diseases caused by aging (e.g., DNA methylation state, histone modification, miRNA, etc.) [9]. Several studies about DNA methylation have shown loss of methylcytosines with age, especially in CpG islands within Alu repetitive sequences

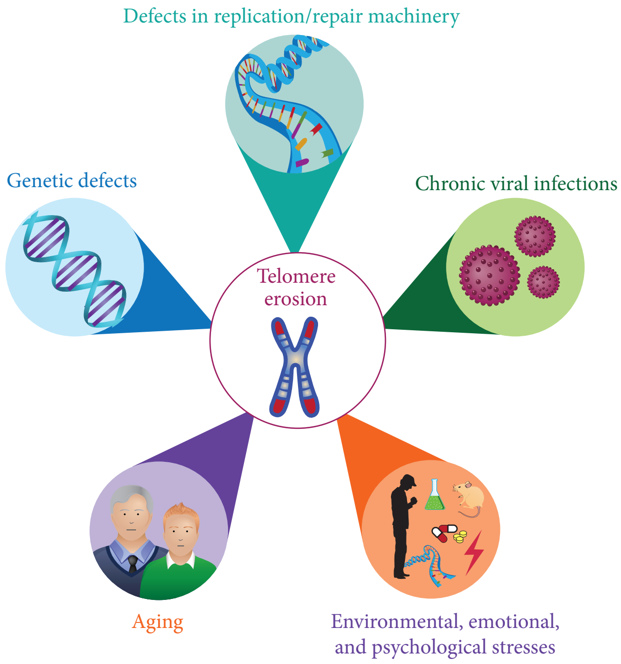
Defects in replication/repair machinery
Genetic defects
Chronic viral infections
Telomere erosion
Aging
Environmental, emotional, and psychological stresses

Figure 1: Factors related to telomere erosion. The mechanisms contributing to the loss of telomere length include genetic defects, chronic viral infections, defects in repair machinery, aging, and stress.

and endogenous retroviral sequences. On top of this, a study of monozygotic twins showed that, for other young people, they retain similar methylation profiles while other twins who were between 50 and 60 years old had different methylation profiles and an H3 and H4 differential acetylation state [10].

Another mechanism related to epigenetic changes in aging involves chromatin remodeling. This includes H3K9, H3K27, and H4K20 trimethylation, decreased H3K9 acetylation, and increased H3S10 phosphorylation [11]. A decrease in H3K27 methylation together with an augmentation in H3S10 phosphorylation supports the idea of a change in the heterochromatin and euchromatin dynamics in aging cells. In addition, there are several chromatin remodeling-related proteins that suffer alterations during aging. These include the histone deacetylases (HDACs), the sirtuin 1 (SIRT1) protein, and the histone methyltransferases [12].

Finally, several studies in both murine and humans have shown that miRNAs may influence aging and longevity. Recently, multiple miRNAs related to aging have been described including lin-4, miR-1, miR-145, miR-140, miR-34a, and miR-449th, and some of them modulate cell senescence critical molecules such as class I HDAC, SIRT1, p21, p53, and pRb. Another important miRNA related to TCR signaling (miR181a) has serious implications in elderly people and autoimmunity (this topic will be discussed later). Recently, Liu et al. summarized the miRNAs involved in cell senescence [13].

3. Immunosenescence

One characteristic of elderly people is their inability to respond properly to vaccines and infections. This condition could be the result of their low immune system efficiency [14] and occurs because of thymic involution in which the thymus loses its ability to produce and replace naïve T cells on the periphery. As a result, thymic dysfunction produces a decrease in cell-mediated response to foreign antigens, self-tolerance, and naïve T-cell population. In turn, it could increase the autoproliferation of T cells and eventually the induction of premature T-cell maturation which would also conduce to tolerance reduction [15]. These alterations lead to not only modifications in lymphocyte subsets but also to functional changes in cell population subsets. For instance, longitudinal studies have demonstrated an association between immunosenescence and an increase in cytomegalovirus (CMV) anergic CD8 T cells [16].

One of the main characteristics of the immune system is the constant renewal of its cells. At the same time, this renewal is highly dependent on the efficiency of telomere maintenance. Immune system cells are derived from hematopoietic progenitor cells that come from myeloid and lymphoid lineages. These cells are constantly dividing and differentiating throughout their lifespan and that leads to changes in their telomere length. Note that a high rate of telomere loss in the first years of life has been observed, perhaps because of their high rate of mitotic division. However, this telomere loss is not a linear process over time since, in older people, it is possible to find significant telomere erosion. In fact, several studies have shown a decline in the length of telomeres with aging [17]. A lot has been written regarding the relationship between aging, thymic degeneration, and changes in the bone marrow cells [18–20]. However, we will focus only on immune senescence with an emphasis on circulating cell populations.

There are reports about age-related changes in peripheral blood cell populations: increase in monocytes, decreased lymphocytes, decrease in naïve cells, and increase in memory cells (Table 1) [21]. Curiously, memory T cells ($CD4^+$-$CD45RO^+$ and $CD8^+$-$CD45RO^+$) increase with age and are preferentially located in tissue, whereas there is a similar proportion of $CD45RA^+$ and $CD45RO^+$ subsets in peripheral blood [22]. Unlike somatic cells, lymphocytes have a robust capability to proliferate given their clonal expansion and present an overexpression in the telomerase. This process ensures no significant telomere shortening during each division. Interestingly, T cells possess several special features regarding their phenotype and their telomere length. T-cell memory cells have shorter telomeres than naïve T cells, and $CD28^+$ T cells have longer telomeres than $CD28^-$ T cells.

Immune aging or immunosenescence not only affects adaptive response but also has implications in the innate response (Table 1) [23]. It has been found that older individuals who exhibit a breakdown of their innate immune barriers such as epithelial skin barriers, lungs, and gastrointestinal tract could be vulnerable to a pathogen attack. Among the cell types involved in innate response, there are neutrophils, macrophages, and natural killer (NK) cells, which also suffer

Table 1: Age-associated changes in immune cell populations and functions.

Cell type	Characteristics	References
	Innate immunity	
Neutrophils	↓ Phagocytic chemotaxis capability ↓ Superoxide anion production ↓ Ability to respond to soluble factors (GM-CSF) and bacteria (LPS and fMLP) ↓ Molecule recruitment into lipid raft, apoptosis, and signal transduction	[23, 70, 87, 140]
Dendritic cells	↓ Cell number, antigen presentation, TLR-mediated signaling, IFN I/III production, chemotaxis, and endocytosis ↓ Ability to stimulate lymphocytes in the ill elderly ↑ Function in the healthy elderly	[23, 102, 141]
Macrophages	↓ Phagocytic activity and chemotaxis ↑ Synthesis of proinflammatory cytokines (IL-6, IL-8, TNF-α, and IL-1β) ↓ Apoptosis, superoxide production, and signal transduction ↓ TLR expression and function ↑ PGE2 production ↓ MCH class II production	[23, 87, 142]
NK cells	↑ CD56dimCD57$^+$ population ↓ Function of cytotoxicity ↓ Secretion of IFN-γ induced by Interleukin 2 (IL-2) ↓ HLA-DR, IFN-α, CD57, and CD95 ↓ Cell proliferation ↑ Production of IL-1, IL-4, IL-6, IL-8, and TNF-α	[72, 143, 144]
	Adaptive immunity	
Cellular response		
Thymus	Involution from age of 9 months, thymic remnant after 50 years	[20, 145]
T Cells	Variable number (↓ proliferation to PHA, varying age, and health status)—HLA B8/DR3 associated with high proliferative responses ↑ Proportion of memory cells (CD45RO$^+$), especially tissue CD8$^+$ ↓ Proportion of naïve cells (CD45RA$^+$) ↓ Proliferative capacity ↓ Synthesis of IL-2 receptor and IL-2 in memory cells ↓ CD28$^+$ ↑ CD28$^-$ T cells—mainly CD8$^+$ CD28$^-$ (characterized by oligoclonal expansion, shortening of telomeres, potentially decreased proliferation, resistance to apoptosis, and increased production of TNF-α and IL-6) ↓ CD4 T lymphocytes Change from Th1 response to Th2 response with ↓ cell-mediated responses directed against intracellular bacteria (Th1 function) and relative preservation of humoral response (Th2 function) ↓ Treg population (CD4$^+$ CD25$^+$) that plays a role in the manifestations of autoimmunity Impaired immunological synapse formation and signaling pathways (calcium response and phosphorylations) ↓ CD4/CD8 rate	[14, 145–147]
Humoral response		
B Cells	↓ Pre-B lymphocytes with peripheral B lymphocyte count unchanged ↑ CD5$^+$ B cells (CD19$^+$ CD5$^+$ clones B) that produce low affinity antibodies without cooperation of T cell ↓ Naïve B cells Accumulation of memory B cells with ↓ diversity and affinity of antibodies Reaching primary humoral response (dependent T cell cooperation). Conserved secondary humoral response	[14, 33, 50, 53, 145, 148]

Table 1: Continued.

Cell type	Characteristics	References
Immunoglobulins	↑ Serum levels of IgA and IgG (IgG1, IgG2, and IgG4). Monoclonal immunoglobulin production by $CD19^+$ $CD5^+$ clones. Secretion of non-organ-specific self-antibodies (rheumatoid factor, antinuclear antibodies, antiphospholipid antithyroglobulines, and parietal cells).	[51, 113]
Interleukins	↓ IL-2 production because of the following: ↓ cooperation of T cells with antibody producer B cells, ↑ production of IL-4, IL-6, IL-8, IL-10, and TNF-α, ↓ production of IL-1 and IFN-γ.	[14, 106, 145]

functional alterations through aging (Table 1). Immunosenescence also affects the response to immunization. There are several reports indicating low response to infectious agents in elderly individuals [24, 25]. Latent proinflammatory status in old subjects is due to involution of thymus with subsequent alteration of function and balance of naïve, effector, and memory cells. This status combined with the presence of common and cumulative viral infections in the elderly (such as cytomegalovirus and Epstein-Barr virus) produces overall responses, loss of ability to control infectious diseases, and decreased response to vaccinations. The cytokine environmental balance (i.e., decline in the INFγ : IL-10 ratio) in challenge condition (i.e., influenza or other viral infections) could decline the $CD8^+$ cytotoxic ability, thus conducing to high IL-10 response to virus challenge. Therefore, vaccines that arouse inflammatory cytokines would be expected to enhance protection in elderly subjects.

3.1. Adaptive Immune Response

3.1.1. T Cells. T lymphocytes suffer alterations due to aging. Most of the observed changes are attributed to alterations during the initial activation step of the T-cell receptor (TCR). There is evidence of alteration in the downstream signaling of the TCR in the case of elderly people. This includes a decrease in intracellular free calcium, deficiencies in protein kinase C translocation, low Lck, ZAP70 activation, NFAT impairment, NF-κB translocation, low ras-mitogen activated protein kinase (MAPK) pathways, and a decrease in proteasome activity, [26]. These alterations have been demonstrated in both naïve and memory T cells [27].

TCR has the function to discriminate between self-antigens and respond to foreign peptides. This is caused by its activation threshold level, and therefore, the loss of TCR sensitivity is closely related to aging. As mentioned before, there is an important microRNA—named miR-181a—implicated in this phenomenon which controls the expression of several phosphatases related to the negative regulation of proximal CD4 TCR signaling events. Indeed, in a murine model, miR181a overexpression lowers TCR activation threshold and restores TCR ability to respond to autoantigens [28]. Note that miR-181a expression declines throughout life and shows a significant loss after the age of 70 [29].

Another interesting point is that there are reports indicating changes in gene expression of surface molecules on T cells. Changes in surface molecules may have a negative impact on T-cell activation by increasing phosphatase expression such as DUSP family. Furthermore, a study evaluated the effect of aging on surface molecule gene expression and found that IL-6R, CD8, CD27, and CD28 are downregulated while ILT2 (CD85j), KLRG, KIR, CD44, CD96, Klrf1, and CD94 are upregulated [30, 31]. Some of these molecules (ILT, KLRG, and KIR) function as negative regulators of TCR activation and proliferation (at least in murine models) or as specific molecules of particular T cells such as cytomegalovirus (CMV) peptide-specific T cells (KIR and ILT2). They also appear to be related to T-cell exhaustion although the relationship between aging, senescent, and exhausted T-cell gene expressions seems to be different (Figure 2). An excellent review of this topic was done by Cavanagh et al. [32].

In addition to surface molecule gene expression alteration, there are other processes related to TCR signaling alteration and aging. For instance, it is well known that a cell-intrinsic environment (ROS species, DNA damage) and a cell-extrinsic environment (cytokines) both modulate TCR responses, and they are also altered in the elderly. Moreover, studies have shown that increased oxidative stress produces displacement of LAT from the cell membrane, thus inhibiting TCR signaling. Along with this, activated CD4 T cells from aging humans express increased levels of metallothoineins, which are an important redox system [34]. Additionally, there are alterations in molecules that participate in nuclear and cytoplasmic signaling pathways such as DNA repair kinases (ATM, ATR, and DNA-PKCs), which are activated not only by DNA double-strand breaks (DSB) but also by telomere attrition (as we will see later). Both of them are related to activation of DNA repair kinases (ATM, ATR and DNA-PKcs) [35].

Host environment and specific cytokine profiles have enormous implications in the T-cell signaling threshold. Some cytokines such as IL-7, IL-21, and IL15 have been studied in this regard. These cytokines signal through PI3K, STAT3, and STAT5 [36] and participate in ERK pathway activation. Note that IL-7 and IL-15 have profound implications in lymphopenia development in RA animal models. This has been shown through the fact that when animals are primed with IL-7 or IL-15, T-cell response to autoantigens is enabled. In addition, another cytokine implicated in signaling alteration during aging is the IL-6. This cytokine has implications in the JAK-STAT pathway through activation of

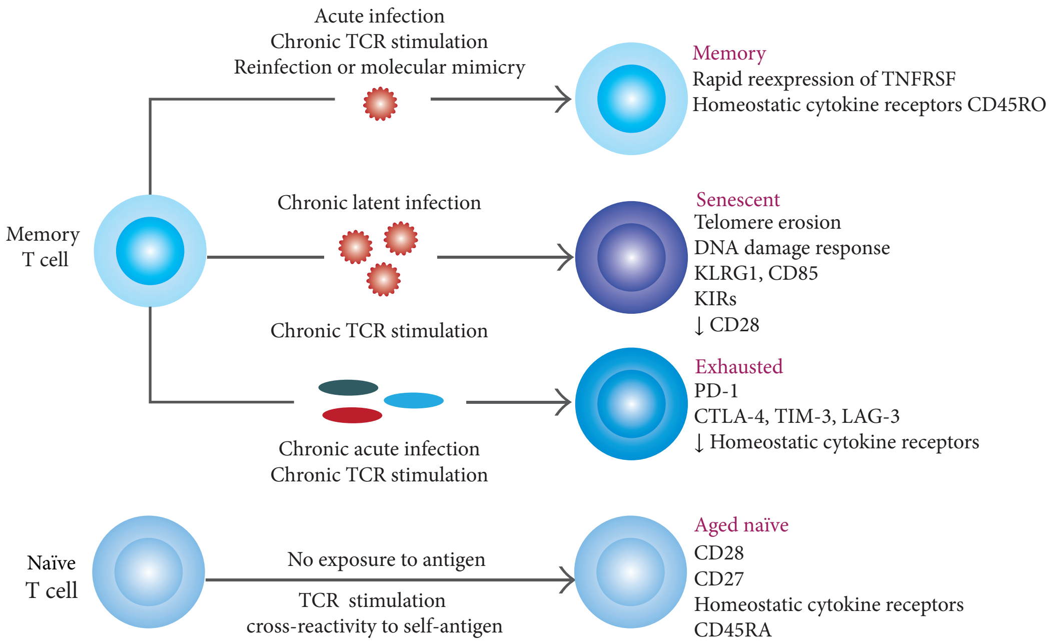
Acute infection
Chronic TCR stimulation
Reinfection or molecular mimicry
Memory
Rapid reexpression of TNFRSF
Homeostatic cytokine receptors CD45RO
Memory
T cell
Chronic latent infection
Chronic TCR stimulation
Senescent
Telomere erosion
DNA damage response
KLRG1, CD85
KIRs
↓ CD28
Exhausted
PD-1
CTLA-4, TIM-3, LAG-3
↓ Homeostatic cytokine receptors
Chronic acute infection
Chronic TCR stimulation
Naïve
T cell
No exposure to antigen
TCR stimulation
cross-reactivity to self-antigen
Aged naïve
CD28
CD27
Homeostatic cytokine receptors
CD45RA

Figure 2: Changes in the T-cell pool and individual cells during aging. A Proportion of T subsets depends on individual infection antecedents and the environment. Memory T-cell subset can change to senescence by a chronic latent infection and chronic TCR stimulation. Meanwhile, exhausted T cells are produced by the same type of stimulation and the chronic acute infection. Aged naïve cells are generated from naïve cells stimulated by self-antigen exposure (adapted from [32]).

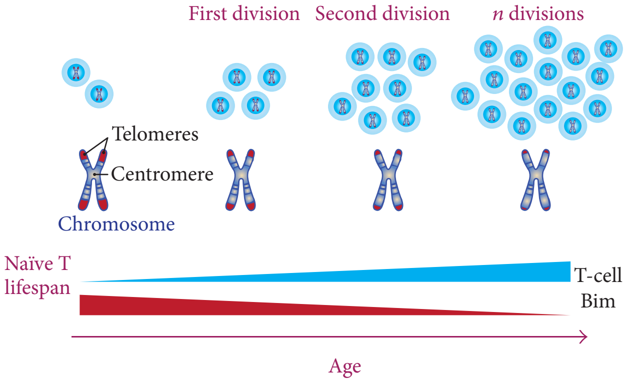
First division
Second division
n divisions
Telomeres
Centromere
Chromosome
Naïve T
lifespan
T-cell
Bim
Age

Figure 3: Schematic representation of T-cell divisions and their implication in telomere erosion and aging. Constantly dividing cells are accompanied by a decrease in their telomere length, which is related to aging phenotype: decreased Bim expression, increased naïve lifespan, and important functional changes.

SOCS3 transcription. Note that STAT molecules are highly phosphorylated in elderly humans, a phenomenon that also occurs through type I interferon T-cell activation [35].

As was discussed previously, telomere attrition is very frequent in elderly people and it is also known that T-cell replication is important for maintaining lymphocyte function. This suggests that T-cells employ the best mechanisms for telomere maintenance during clonal expansion. Indeed, there is evidence that telomerase activity is highly regulated during T lymphocyte development and differentiation [37]. The resting CD4$^+$ and CD8$^+$ and naïve T cells recorded no telomerase activity on the periphery. However, the telomerase is activated by lymphocyte stimulation. The level of telomerase activity decreases during successive stimulations of the lymphocyte (Figure 3) [38]. The rate of telomere shortening seems to be different among CD4$^+$ and CD8$^+$ cells, and it has been estimated to be 33 bp/year for CD4$^+$ T cells and 26 bp/year for CD8$^+$ T cells. One of the most outstanding features of aged CD4$^+$ naïve T cells is their inability to produce significant levels of IL-2 after stimulation of their T-cell receptor (TCR). This inability subsequently leads to poor Th1/Th2 polarization. However, these cells may retain their ability to suffer Th17 differentiation [39, 40], which, in turn, could favor an inflammatory and autoimmune phenotype development. On the other hand, the number of CD4$^+$CD25$^+$FOXP3$^+$ regulatory T cells (Treg) increases (2.4-fold), and they retain and gain functions during aging. Nonetheless, their ability to produce IL-10 is low. They may also contribute to Th17 bias (production of high levels of IL-17, IL-21, and IL-22) and show a decrease in antitumor responses too [40]. Meanwhile, aging CD8$^+$ lymphocytes show a reduction in the diversity of the TCR repertoire, low antitumor response, and marked clonal expansion development but without the ability to replicate after stimulation [26]. Note that Th2 inflammatory cytokines favor antibody production by B cells, and this condition could explain autoantibodies in the aged population.

One of the most important traits of immune aging is the loss of the CD28 surface marker. CD28 is one of the molecules expressed in T cells that provide costimulatory signals that are required for T-cell activation, T-cell proliferation, cytokine production, and T-cell survival promotion. Loss of CD28

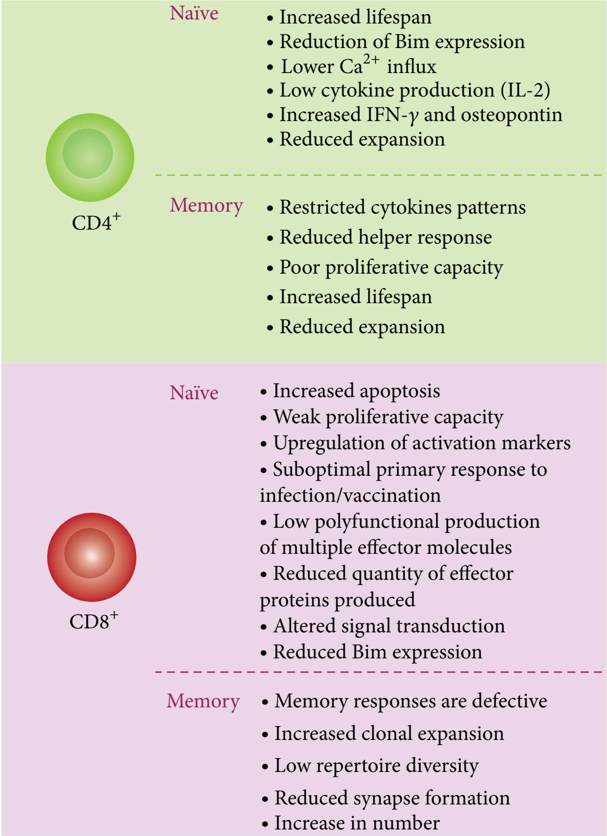
CD4$^+$
Naïve
• Increased lifespan
• Reduction of Bim expression
• Lower Ca^{2+} influx
• Low cytokine production (IL-2)
• Increased IFN-γ and osteopontin
• Reduced expansion
Memory
• Restricted cytokines patterns
• Reduced helper response
• Poor proliferative capacity
• Increased lifespan
• Reduced expansion
CD8$^+$
Naïve
• Increased apoptosis
• Weak proliferative capacity
• Upregulation of activation markers
• Suboptimal primary response to infection/vaccination
• Low polyfunctional production of multiple effector molecules
• Reduced quantity of effector proteins produced
• Altered signal transduction
• Reduced Bim expression
Memory
• Memory responses are defective
• Increased clonal expansion
• Low repertoire diversity
• Reduced synapse formation
• Increase in number

Figure 4: Aged-related functional changes in T-cell subsets. Alterations are produced in both memory and naïve subsets. These alterations depend on T-cell microenvironmental history, exposures to stressor agents, and stochastic events. There are differences in changes between CD4$^+$ and CD8$^+$ concerning aging, but in both cases, there is reduction of naïve subtype, increase in lifespan, and defective immune response.

expression is a phenotypic change associated with senescence in T lymphocytes, and it has been associated with functional alterations such as enhanced cytotoxicity, suppressive functions, and resistance of CD4$^+$ T cells to apoptosis. Loss of CD28 expression is characterized by telomere shortening and reduced proliferative ability, both *ex vivo* and *in vitro* [41, 42]. At birth, virtually all T cells express CD28, but with age, the marker decreases about 40 to 50% for CD8$^+$ T cells and 85 to 90% for CD4$^+$. This reduction in the markers is attributed to repeated antigenic stimulation in peripheral blood [43]. However, when the CD28 is lost, cells suffer reprograming and, as a consequence, they express new receptors such as KIR, CD70, and perforin. Moreover, phenotypic CD28$^-$ T-cell characteristics include interferon gamma (IFN-γ) production, potent cytotoxic capability, and CD158, CD158b, CD158J, DAP12, CD94, and CD244 receptor expression (similar to NK cell characteristics). These receptors give them the potential to interact with accessory cells such as mesenchymal cells, which include the fibroblasts of inflamed joints [44]. Furthermore, in elderly individuals with chronic viral infections and autoimmune diseases (e.g., multiple sclerosis (MS), rheumatoid arthritis (RA), and Wegener's disease), an increase in the frequency of CD28$^-$ T cells has been detected [43]. It has been suggested that autoantigens can lead to clonal expansion of these cells. Thus, there are reports [45, 46] indicating how they can, for instance, show reactivity to myelin basic protein (MBP). The presence of CD4$^+$ CD28$^-$ T cells in both elderly individuals and patients with autoimmune diseases (ADs) has supported the concept that ADs are closely related to the cell aging process. In this regard, the loss of CD28 molecule could favor CTLA-4 interaction with their ligands (CD80 and CD86), which are implicated in autoimmune phenotype too.

The main differential and functional alterations of CD4$^+$ and CD8$^+$ T-cell subsets are summarized in Figure 4.

3.1.2. B Cells. It is known that with aging, there is a decrease in not only the frequency and absolute number of pro-B cells but also in their ability to differentiate into pre-B (between 60 and 90%). Nevertheless, in healthy individuals, mature peripheral B-cell numbers do not change with aging; instead the relationship between naïve and memory cells is altered; that is, there is an increase in long-lived memory cells (homeostatic expansion of antigen-experienced or activated B cells) and a decrease in naïve cells [47]. This condition seems to depend on different factors other than genetic ones. A study comparing old individuals with healthy centenarian offspring could determine that centenarian offspring have more IgD$^+$ CD27$^-$ naïve B cells than older people. Nevertheless, the double negative memory cells (IgD$^-$ CD27$^-$ B cells) are only found in healthy elderly individuals, and there are no differences between groups [48]. Recently, studies have reported a novel peripheral B-cell subset in the elderly named aging-associated B-cell (ABC) subset (CD19$^+$CD11b$^+$CD11c$^+$) [49]. *In vitro*, the ABC subset responds only to innate stimuli producing secretion of autoantibodies and cytokines, and this subset also has the ability to potentiate Th17 polarization, thus relating it to an autoimmune phenotype.

Another important fact is the presence of alterations in the repertoire of the B-cell receptor (BCR), which exhibits a decreased affinity and diversity to the antibody response with aging [33]. Indeed, elderly patients have impaired B-cell proliferation and activation, possibly as a result of defects in their threshold of activation [26]. Also, there is a loss of precision in distinguishing self- from non-self-antigens due to the oligoclonal expansion of the B lymphocyte subpopulation with a high proportion of antigen-experienced cells [50]. This subpopulation expresses CD5 on their surface, thus giving them the ability to produce low affinity antibodies independently of T cells. In the context of autoantibody generation, this is important for triggering an autoimmune response.

Moreover, the germinal centers (GC) from elderly people are small and have few cells producing IgM. In these individuals, levels of immunoglobulins, especially IgA and IgG, are increased [51]. Furthermore, it has been shown that IgG$^+$/IgA$^+$ B-cell subsets (both CD27$^+$ and CD27$^-$) express Ig mutated genes in their variable regions and high levels of CD80 and CD86 on their surface, thus exhibiting a similar B-cell memory phenotype [52]. According to the reports,

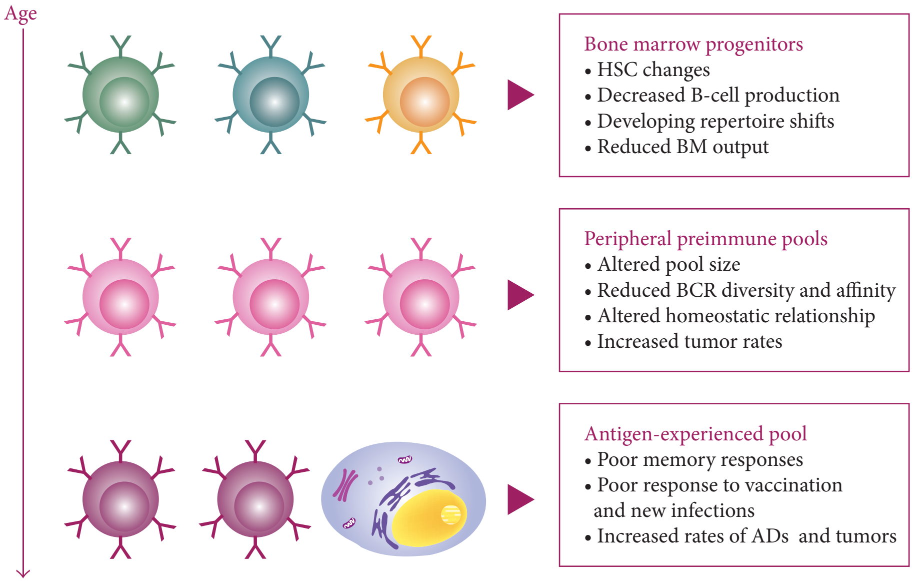
Age
Bone marrow progenitors
• HSC changes
• Decreased B-cell production
• Developing repertoire shifts
• Reduced BM output
Peripheral preimmune pools
• Altered pool size
• Reduced BCR diversity and affinity
• Altered homeostatic relationship
• Increased tumor rates
Antigen-experienced pool
• Poor memory responses
• Poor response to vaccination and new infections
• Increased rates of ADs and tumors

Figure 5: Age-related changes in the generation and function of B cells. There is a reduced output of B cells in the bone marrow, which induces accumulations in the periphery of antigen-experienced subsets with poor immune response and low diversity. HSC: hematopoietic stem cell; BCR: B-cell receptor; BM: bone marrow (adapted from [33]).

this subtype of cells declines with aging [51]. Finally, aging B cells have been observed to produce antibodies with low avidity because of their somatic hypermutation deterioration which leads to a gradual decline in the humoral response. Nevertheless, repertory changes are not synchronous with aging, and decreased diversity has been related to poor health status [53].

An interesting study [54] done to evaluate naïve (CD19$^+$CD27$^-$) and memory (CD19$^+$CD27$^+$) switch B-cell subsets in elderly individuals showed a decrease in total B cells, and, although the quantity of naïve cells increased in percentage, they decreased or remained constant in number. Moreover, another striking result is that the B-cell memory (CD27$^+$) increased in percentage but not significantly. In contrast, memory cells producing IgM subtype decreased in number but not in percentage. Finally, the memory switch cells decreased both in number and percentage with aging. An interesting point about memory B cells is that they have a hyporesponsive state to antigen-induced activation with less clonal expansion or less ability to differentiate into antibody secreting cells [55]. This condition may be caused by the decreasing number of antibody high affinity B cells that elderly people have.

Taken together, these results indicate that there is an accumulation of antigen-experienced B subsets in aging individuals. In these cases, overall B-cell numbers are unchanged, but they vary in their functional abilities (Figure 5).

3.2. Innate Immune Response

3.2.1. Dendritic Cells. Dendritic cells (DCs) are important because they function as a checkpoint between immunity and tolerance. DCs from aging individuals display a basal level of activation, increased secretion of proinflammatory cytokines such as TNF-α and IL-6 [56], and high levels of NF-κB activation. However, they do not exhibit upregulation of CD86 and CD80 molecules on their surface, which suggests that they are partially activated. Another important characteristic is that they are more reactive to self-antigens compared with their young counterparts and display an impaired clearance of apoptotic cells and antigens [57]. This could produce a higher presentation of self-antigens and, consequently, an activation of autoreactive lymphocytes. An interesting point is that these partially activated DCs have a greater ability to stimulate T cells, thus indicating that their ability to induce tolerance to self-antigens is affected.

Some explanations have been given regarding partially activated DCs: (1) an increased age-associated level of proinflammatory mediators and (2) age-associated modifications in autoantigens, which increase their immunogenicity [58].

Note that the functions of myeloid DCs (mDCs) such as IL-12 production, chemotaxis, and their ability to activate naïve CD4 T cells via antigen presentation appear to be altered in elderly individuals [57]. This inability is due to decreased PI3K activation [59], which leads to activation of NF-κB, as it was previously mentioned. This subtype of DCs also shows decreased capability in their antigen processing and increased expression of CD86.

Plasmacytoid DCs (pDCs) from elderly people, in turn, have reduced IFN I and IFN III production after stimulation via Toll-like receptor (TLR) [60]. Additionally, they have an impaired ability for antigen presentation to CD4 and CD8 T cells.

3.2.2. Neutrophils. Neutrophils are the first immune cells that are recruited to the site of infection or to the tissue damage [61]. Besides, there is a debate about whether the numbers of neutrophils change with age, but a variety of studies suggest that there are no number changes. However, there are reports indicating several functional defects in neutrophils from the elderly [62, 63]. The main function decreased in neutrophils is the chemotaxis, followed by the phagocytic activity. Both of them affect the time needed for the neutrophils to reach the infection site and their ability to control the infections, respectively [62, 64, 65]. These two alterations are closely related to increased infections in elderly subjects.

Low phagocytic activity has been associated with reduced surface expression of the Fcγ receptor CD16 [64]. Signaling function of other receptors involved in activation such as fMLp, TLR, retinoic-acid-inducible-gene-1-protein- (RIG-1-) like helicases (RLRs), nucleotide binding domain and leucine-rich-repeat-containing proteins (NLRs), and C3b has been reported to be significantly altered. This alteration is due to changes in signaling molecules but not in the number of their receptors [66, 67]. Some downstream signaling events include phosphoinositide-3 kinase (PI-3 K), MAP kinase, Calcium, protein kinase B, and SHP-1 and Jak-STAT pathways [68]. Interestingly, these alterations are produced by changes in membrane composition including lipid rafts distribution and their structure [69, 70].

3.2.3. NK and NKT Cells. NK cells participate in the innate immune defense against intracellular pathogens and tumor cells, and they mediate MHC-independent cytotoxicity. There are several studies indicating a remodeling of these cells in elderly individuals. The percentage and absolute number of NK cells are increased in healthy aging, and they are characterized by the increment of CD57 expression and expansion of CD56dim NK cells (mature and highly differentiated cells) [71, 72]. Other important features of these cells from aging subjects are decreased proliferative response to cytokines, altered expression of some NK receptors such as natural cytotoxicity receptors (NCRs) [73], CD226 [74], and KLRG-1 [75], and increased expression of HLA-specific killer immunoglobulin-like receptor [73].

At functional level, cytotoxic and proliferation ability and cytokines/chemokines (such as INFγ, RANTES, MIP1a, and IL8) production of NK cells are reduced [76].

NKT cells are important in the clearance of bacterial and viral infections as well as in regulation of immune tolerance and autoimmunity [77]. NKT cells are characterized by expression of a TCR encoded by Vα14/Vβ8.2 gene segments.

The effects of aging on NKT cell number and function have been little studied. In general, nowadays, it is accepted that the absolute number of NKTs within the lymphoid organ increases [78]. In addition, there are reports that show a decrease of proliferative ability and low number of CD1d-restricted NKT cells in the peripheral blood [79]. Studies of inhibition of NKT cell activation demonstrated age-associated decay of proliferative response and retarded type hypersensitivity responses [80]; in addition, results showed that NKT cells contribute to increments of IL-4 and IL-10 production and decreased IFN-γ in aging subjects [81].

3.2.4. Monocyte/Macrophages. Other essential components of innate immune response are macrophages and monocytes. Monocytes respond to inflammation by their differentiation into macrophages and dendritic cells. Studies have demonstrated that CD56$^+$ monocytes subpopulation (high producers of TNF-α via TLRs 2 and 4) is increased with age while their counterpart (CD56$^-$) is decreased [82]. The increment of CD56$^+$ monocytes is paradoxical with the alteration in macrophage TLR function. A study revealed a decrease in IL-6 and TNF-α via TLR1/2 in the elderly, and this fact was related to the decrease of TLR1 on monocyte surface [83]. These results are contradictory with another report which indicates substantial increase of the same cytokines [21]. Thus, this issue required further confirmation, which can be accomplished by the study of phenotype subpopulations (according to the expression levels of the receptors and proteins). In this regard, age-associated changes in TLRs expression on monocytes have been performed [84]. A particular study showed that old patients infected with West Nile virus have a persistent TLR3 expression on macrophages' surface while young patients have reduced expression of this receptor [85]. This feature may produce a higher inflammatory response with the subsequent increased morbidity of elderly subjects.

Furthermore, there are reports about age-related upregulation of CD80 molecule on monocytes after TLR activation [86] which is associated with production of a protective response to influenza vaccination.

Another important age-associated feature of both monocytes and macrophages is that several of their receptors become altered, thus producing cells dysfunction. This produces that clearance of free radical production and phagocytosis are reduced in monocyte/macrophages in the elderly [87]. Also, these alterations may lead to deregulation in clearing of apoptotic cells by macrophages, thus precipitating the exacerbation of inflammatory-aging condition.

4. Infection and Immunosenescence

To produce an adequate response to large numbers of pathogens throughout life, there are homeostatic mechanisms guaranteeing competent memory and a naïve cell pool for prolonging the survival of memory cells. However, under the conditions of advanced age, these mechanisms are seriously affected. As we have seen, during aging, many changes occur in the immune system, which means that immunosenescence becomes a factor contributing significantly to a higher risk and severity of infections. The most important diseases in the elderly are urinary tract infections, influenza and pneumonia, chronic viral infection reactivation (herpes virus and varicella-zoster virus), as well as bacterial (tuberculosis), fungal (candidiasis), or parasitic infections, and, more rarely, opportunistic infections such as *Clostridium* and *Staphylococcus* [88, 89]. Although the immune response to antigens may be preserved in elderly individuals, their ability to be immunized against new antigens is reduced. This may be the result of an increase in the proportion of memory cells and progressive decrease in naïve cells from the thymus [90].

While it is true that aging is associated with the emergence of infectious diseases, it is also true that these infectious events will promote aging. It is well known that viral infections (particularly the herpes virus family) are strong stressors which alter the lymphocyte phenotype and functionality, altered cytokine profile, resistance to apoptosis, and shortened telomeres [91]. These features are similar to those found in the elderly; thus it is possible that viral infections could represent an important extrinsic factor for aging by the repeated antigen stimulation characteristic of persistent latent infections [92]. Furthermore, it has been suggested that latent herpes virus infections are primarily responsible for *in vivo* generation of senescent $CD8^+$ T cells, perhaps due to constant and prolonged virus-specific T-cell proliferation [93]. Additionally, the Epstein-Barr virus (EBV) latent infection has also been associated with telomere shortening in antigen-specific $CD8^-$ T cells because EBV antigens cause a decrease in telomerase activity associated with T-cell proliferation [94]. In contrast (and related to telomere erosion and its relationship with CD28 molecule expression), the majority of T cells in a study done by Vescocini were $CD28^+$ unlike what was found for CMV, which were mainly $CD28^-$ [95]. During human immunodeficiency virus (HIV) infection, in turn, it has been reported that early presence of $CD8^+$ $CD28^-$ T cells is a predictive characteristic of rapid disease progression [96].

These data indicate that chronic infections during aging produce significant changes in the $CD8^+$ cell subset. Additionally, this shows that expansion of $CD28^-$ T cells is age dependent, and they have a positive correlation with proinflammatory cytokines. At the same time, these cytokines are heavily involved in the pathogenesis of immunological disorders which could favor the emergence of different pathologies including ADs.

5. Autoimmune Disease

Currently, it is clear that changes occurring in the immune system during aging affect the onset of ADs. This is due to the fact that aging is related to increased reactivity to self-antigens and loss of tolerance. The overall tendency supports this hypothesis because elderly people experience general systemic inflammation and, at the same time, they aggravate degenerative diseases [97], which, in turn, increase the risk of developing ADs [98, 99]. Proinflammatory cytokines on general systemic inflammation (produced by viral infection) lead to a state called inflammaging, which corresponds to the loss of equilibrium between adequate inflammatory response and efficient anti-inflammatory control in the elderly condition. Later, in normal aging, this control fails to fully neutralize the inflammatory processes.

In addition to this, it is important to remember (as we have seen previously) the epigenetic changes occurring in elderly people and how these may affect important genes involved in autoimmune disorder development [100]. In this regard, there are reports in which some genes associated with ADs are hypermethylated but others are hypomethylated. For instance, FoxP3, a hypermethylated gene, is a member of the forkhead transcription regulator family [101] and is associated with the development of multiple ADs. In contrast, the gene coding for the CD11a chain of lymphocyte function-associated antigen 1 (LFA-1)—a protein which is associated with certain ADs—is hypomethylated with age and thus overexpressed in aging cells [100]. Furthermore, there are other reports on elderly subjects indicating DNA hypomethylation states which could lead to an increase in the immunogenicity [58]

Another important aspect of aging that is closely related to autoimmunity in general and ADs in particular is the increase in inflammatory cytokines and chemokines such as TNF-α, C-reactive protein, IL-8, MCP1, and RANTES [102–104]. There is a substantial amount of evidence of age-associated alterations in the T-cell cytokine profile which could contribute to development of ADs. Studies have shown that there is a change from Th1 to Th2 molecules (mainly IL-4 and IL-6) in the cytokine profile as age advances [105]. IL-6 is a potent proinflammatory cytokine closely related to disability in patients with RA; therefore, IL-6 represents a therapeutic target for this disease [106]. In addition, there are reports of an imbalance between Th17 and Treg cells. A considerable number of IL-17-secreting naïve $CD4^+$ T helper cells have been detected in the elderly in contrast to reduced IL-17-secreting memory $CD4^+$ T helper cells [107].

Some ADs are very frequent in younger patients and are not limited to elderly people although the occurrence or presence of autoantibodies is greater at advanced age [108–111]. Autoantibody production such as rheumatoid factor, as well as antinuclear, antiphospholipid, and antithyroglobulin antibodies, is present during aging [109, 112]. Autoantibody production has been attributed to altered T- and B-cell functions [113], especially to the decrease in antibody affinity maturation. This evidence supports the idea that autoantibody levels may be closely related to the clinical characteristics of the elderly and to patients with ADs.

One of the important causes of dysfunctional immune responses is telomere abnormalities which may lead to autoimmunity. This observation is significant since numerous studies have shown an association between mean telomere length in peripheral blood mononuclear cells (PBMCs) and different diseases [91]. This evidence suggests an increase in $CD8^+$ $CD28^-$ T-cell proportions in several pathologies such as in the case of some ADs.

Moreover, there are reports of telomere length alteration in patients with ADs such as RA [114, 115], scleroderma (SSc) [116], systemic lupus erythematosus (SLE) [117], polyangiitis with granulomatosis [118], psoriasis, and atopic dermatitis [119], suggesting an excessive cell replication with their corresponding telomere erosion. These findings have been interpreted as evidence of T-cell accelerated proliferation in the autoimmune process.

At present, it is believed that there are differences among telomere abnormalities and various ADs. Some of these differences could be explained by the genetic background of the individuals studied. For example, a study performed in patients with SSc and their family members reported short telomeres [116]. The idea of a genetic predisposition to telomere shortening is also suggested in patients with RA, who exhibit telomere erosion in not only memory cells but also in naïve cells. Moreover, this evidence shows acceleration in telomere erosion occurring at the precursor cell level [120].

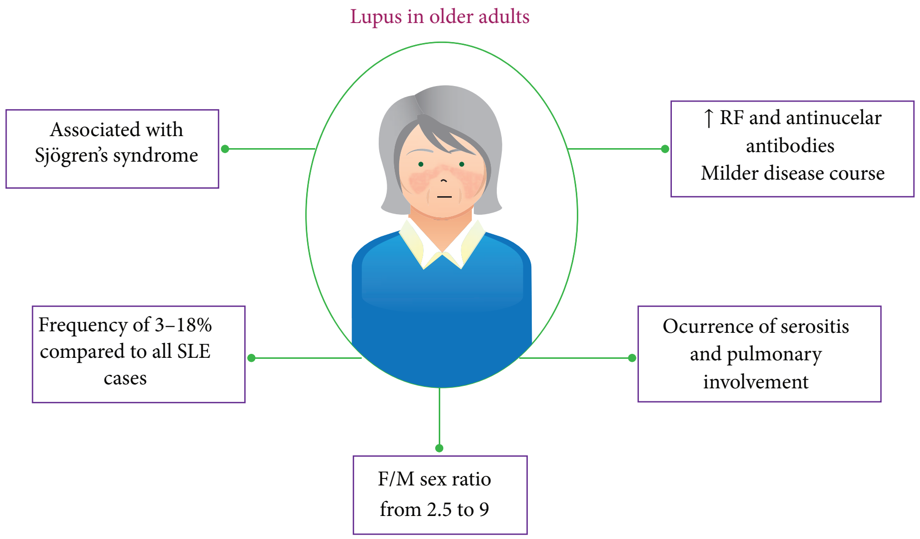
Lupus in older adults
Associated with Sjögren's syndrome
↑ RF and antinucelar antibodies
Milder disease course
Frequency of 3–18% compared to all SLE cases
Ocurrence of serositis and pulmonary involvement
F/M sex ratio from 2.5 to 9

Figure 6: General characteristics of late-onset SLE. There are different manifestations of older SLE patients compared to young patients. RF: rheumatoid factor; F/M: female/male.

Another striking fact is that the genetic predisposition to short telomeres is strongly related to HLA-DR4 haplotype which is shared by RA and T1D in some individuals [121, 122].

5.1. Late-Onset Systemic Lupus Erythematous. Although SLE is considered a disease of the reproductive stage of women, there is evidence that it occurs between 3 and 18% in individuals older than 50 years [123]. Despite that there are aged SLE patients, their clinical manifestations, response to treatment, prognosis, and course of the disease are different in these individuals (Figure 6). For example, clinical manifestations such as malar rash, renal disease, arthritis, and photosensitivity are less frequent in them, while serositis, cytopenias, and pulmonary involvement are more frequent [124–127]. In addition, it has been shown that female/male (F/M) sex ratio declines with age. Studies report F/M ratio from 2.5 to 9 in elderly individuals compared to from 9.1 to 14.4 in young people [126, 128].

A striking feature of these patients is the differential diagnosis due to the SLE overlapping with other diseases. Late-onset rheumatoid arthritis, endocarditis, tuberculosis, neoplasia, polymyalgia rheumatica, temporal arteritis, and Sjögren's Syndrome (SS) had been described is these patients [124, 127]. In the particular case of SS, elderly patients with SLE and without SS have low frequency of compromising renal disease, lymphadenopathy, and thrombocytopenia and high frequency of Raynaud's phenomenon [129].

Besides changes in clinical and serological profiles, serological profiles of aged SLE patients also exhibit alterations [124]. Compared to younger individuals, elderly patients with SLE have high frequency of rheumatoid factor (33% versus 20%) and antinuclear antibodies but low frequency of antiribonucleoprotein (anti-RNP, 10% versus 21%) and anti-Sm (9% versus 17%). There are reports that indicate contradictory results [130], but these differences can be explained by different reasons: ethnicity, sample size, methodology, and so forth.

An important fact is that the severity of the disease appears to decrease with age. It has been reported that late-onset SLE patients have milder disease course which is reflected in a small number of relapses per patient. Additionally, it was found that the prevalence of lupus nephritis and nephrotic syndrome also is lower in elderly patients.

Currently, it is not clear if there is a relationship between telomere loss and SLE. Some studies have shown an increased telomere erosion in SLE patients [100, 101, 131, 132], while others report normal telomere length when compared with healthy controls [102, 103]. Nevertheless, it is clear that there is a reduction in telomerase activity in naïve $CD4^+$ T cells and an increased activity in B cells [101, 103]. In this regard, a recent study showed a differential expression of shelterin complex molecules in patients with lupus [104], but, unfortunately, it was not done cell specific.

According to the report of [132], shorter telomeres are associated with Ro antibodies while longer ones with steroid therapy and increased body mass index. However this study also showed that short telomeres are not related to disease activity or immune cell turnover, but they could be good predictors of premature aging.

Related to this topic, previous studies have indicated that bone marrow mesenchymal stem cells (BMSCs) from SLE patients exhibit not only increased apoptosis and senescence but also impaired capacity of differentiation, immune modulation, proliferation, and secretion of cytokines. Apoptosis and senescence in BMSCs from SLE patients appear, to be due to increased favorable conditions for these processes. There were described increased levels of p16INK4A, Bax, caspase 8, Fas and tumor necrosis factor-a receptor 1, and the respective ligands of the two last. There is also a decreased expression of Bcl-2, CDK4, CDK6, and p-Rb [133, 134].

Programmed cell death (PCD) is an essential mechanism of homeostasis and development and it is very important as an immune response regulator. Hence appropriate clearance of apoptotic cells in the immune system is necessary for regulating inflammation and maintaining self-tolerance. Impaired clearance of apoptotic cells in patients with SLE is considered an important process in the etiology of lupus [135]. This phenomenon is exacerbated in older age, and its deficiencies may account for the development of autoimmunity by the loss of tolerance in lymphoid tissues. This process is mediated by phagocytes and, as it was mentioned before, in elderly subjects; phagocytic functions of macrophages are altered. This functional deregulation may generate danger signals, followed by concomitant exposure of autoantigens and the subsequent autoimmune reaction.

Another mechanism that is related to development of lupus is the molecular mimicry in which B and T cells are activated as a result of an infection. This mechanism makes cells able to recognize self-molecules that are similar to infectious agent molecules, thus originating an autoimmune response [136–139]. In SLE, this response is associated with high levels of anti-Sm autoantibodies due to similar molecular sequence of pathogenic molecules of CMV and EBV, which develop the initial immune response. Although anti-Sm autoantibodies are less frequent in the elderly than in younger individuals, it may be possible that EBV infection—exacerbated with age—can contribute not only to inflammaging development but also to induction, by molecular mimicry, of an immune response towards itself. Further studies in this topic could be interesting.

Abnormalities in TCR signaling which have been documented in SLE patients are similar to those in RA patients. This may also be related to the elderly; for instance, TCR zeta chain expression is defective in these patients [105].

6. Concluding Remarks

Aging is a natural physiological process that could eventually conduce to increases in some pathological conditions. A lot of changes are detected in the immune system of elderly individuals which could contribute to the occurrence of complications such as infection, autoimmunity, and autoimmune disorders. In this regard, it is important to remember that elderly patients with SLE have different clinical and serological manifestations and poorer prognosis comparing with young patients (less insidious onset disease, more occurrences of severe manifestations, and higher frequency of comorbid conditions). Thus it is necessary to implement a proper immunological recognition of these patients in order to produce adequate therapeutic management which must be different because the treatment itself may cause long-term damage.

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Review Article

B Lymphocytes: Development, Tolerance, and Their Role in Autoimmunity—Focus on Systemic Lupus Erythematosus

Gabriel J. Tobón, Jorge H. Izquierdo, and Carlos A. Cañas

Department of Internal Medicine, Division of Rheumatology, Fundación Valle del Lili, ICESI University School of Medicine, Cra 98 No. 18-49, Cali, Colombia

Correspondence should be addressed to Gabriel J. Tobón; gtobon1@yahoo.com

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B lymphocytes are the effectors of humoral immunity, providing defense against pathogens through different functions including antibody production. B cells constitute approximately 15% of peripheral blood leukocytes and arise from hemopoietic stem cells in the bone marrow. It is here that their antigen receptors (surface immunoglobulin) are assembled. In the context of autoimmune diseases defined by B and/or T cell autoreactive that upon activation lead to chronic tissue inflammation and often irreversible structural and functional damage, B lymphocytes play an essential role by not only producing autoantibodies but also functioning as antigen-presenting cells (APC) and as a source of cytokines. In this paper, we describe B lymphocyte functions in autoimmunity and autoimmune diseases with a special focus on their abnormalities in systemic lupus erythematosus.

1. Introduction

Systemic lupus erythematosus (SLE) is the prototype of the systemic autoimmune diseases characterized by multiorgan involvement. This systemic compromise is mediated by a global loss of self-tolerance. The loss of tolerance is a consequence of genetic factors, in the context of specific environmental triggers, with the subsequent development of an altered immune response. Both innate and acquired immune mechanisms are implicated in the disease pathogenesis. Recently, special attention has been focused on the B cell abnormalities. In this paper, we will describe the B cell development, tolerance mechanism, and their implications in autoimmune diseases, with emphasis on SLE.

2. B Cell Development and the B Cell Receptor Formation

Different populations of B cells result in preimmune pools where each cell in these quiescent populations expresses a B cell antigen receptor (BCR) with a unique specificity. When the BCRs come in contact with their specific antigen, several intracellular signals are generated leading to activation, differentiation, and formation of plasma cells and memory B cells. This last subset of B cells maintains protective antibody levels and mediates the response to subsequent antigen challenges. As the mechanisms leading to maturing and antibody production are complex, the alterations of some of these populations or critical steps have been associated with immunodeficiency and autoimmune diseases. Table 1 summarizes the most important features of each of the subpopulations (lineages) of B lymphocytes [1].

2.1. B Cell Development. This process begins from stem cells present in the bone marrow (BM) which, depending on the different stimuli received, will generate B lymphocytes. They are derived from the early lymphoid progenitor, which passes to the common lymphoid progenitor. This produces, first of all, the natural killer (NK) cells and dendritic cells and, secondly, the common lymphoid-2 progenitor (LCA-2) that is responsible for the B cell lineage, which is considered the first stage of immature B lymphocytes. Development of the B cell lineage depends on BM stromal cells that produce mainly interleukin (IL)-7 but also the Fms-like tyrosine kinase 3 (Flt3-L) and on the action of several transcription factors such as PU.1, IKAROS (IKAROS family zinc finger 1), E2A, EBF (early B cell factor 1), PAX5 (paired box gene 5), and IRF8 (interferon regulatory factor 8) [2–5]. In the BM, B cells pass

Table 1: Characteristics of primary B cell subsets and their progenitors.

Differentiation	Subset	Surface phenotype
Progenitor subsets (bone marrow)	Pro-B	B220loCD43+
		AA4.1+
	Pre-B	B220loCD43−, AA4.1+preBcR+
	Immature (23−)	B220lo, sIgM+, sIgD−, CD23−
	Immature (23+)	CD19+, B220+, sIgM+, sIgD−, CD23+
Transitional subsets (spleen)	T1	IgMhiCD23−, B220intAA4.1+
	T2	IgMhiCD23+, B220+AA4.1+
	T3	IgMloCD23+, B220+AA4.1+
Mature primary subsets	Follicular zone	IgMloCD23+, B220hiAA4.1−
	Marginal zone	CD19+IgMhiIgDlo CD23+ CD21+
	B1	CD43+ CD23− CD5+
T-independent responses	Early antibody-forming cells/short-lived plasma cells	B220loCD19+slg+iclghi
T-dependent responses	Early antibody-forming cells/short-lived plasma cells	B220loCD19+slg+iclghi
	Germinal center	B220+CD19+GL7+
	Long-lived plasma cells	B220loslg−iclg+
	Memory	B220+slg+IgD−
Natural antibodies	Peritoneal B1a and B1b	CD43+ CD23− CD5+

through several distinct developmental stages. During this, they acquire their antigen specificity, follow a program of differential surface antigen expression and sequential heavy and light chain gene rearrangement, forming the BCR (initially IgM), that determines the cell maturation stage. Reaching the immature stage, B cells exit the BM and complete their development to the mature or naïve stage, which is signaled by the appearance of IgD in addition to IgM on the cell surface. This development sequence occurs in the absence of any contact with exogenous antigen, a stage known as antigen-independent B cell development [2–5].

2.2. B Cell Receptor Development. Immunoglobulin molecules are composed of 2 identical 50 kd heavy chains and 2 identical 25 kd light chains [6]. The genes encoding immunoglobulins are assembled from segments in a manner that is entirely analogous to the process of T cell receptor genes. The light and heavy chain loci are each composed of a series of V (variable) gene elements, followed by several D (diversity) segments (for the heavy chain gene only), some J (joining) segments, and C (constant region) exons. Heavy chains (H) are assembled from 4 segments (VH, D, JH, and CH). Light chains (L) are assembled from 3 segments (VL, JL, and CL) (Figure 1). The genes for 9 different heavy chain types (IgM, IgD, IgG1–4, IgA1-2, and IgE) are located on chromosome 14 and those for 2 light chain types (κ or λ) are on chromosome 2 and 22, respectively. The variable portions (V) of the H and L chains are in juxtaposition, and this creates the antigen-binding portion of the immunoglobulin molecule. These V regions contain 3 highly variable subregions, or hypervariable sequences, which produce the antigen-binding domain of the molecule. The amino-terminal portions of the chains vary in amino acid sequence from one antibody molecule to another. The carboxyl terminal portions are constant in each subclass of antibody. The H chain constant regions form the Fc domain of the molecule and are responsible for most of the effector functions of the immunoglobulin molecule.

The development process of different subsets of B cells has been extensively reviewed elsewhere [4–7] and summarized in Figure 1. Once a functional IgM and IgD are synthesized, the pre-B cell evolves into an immature B cell. The fully mature BCR includes additional transmembrane proteins designated as Igα and Igβ that activate intracellular signals after receptor binding to antigen [8, 9]. At that point, the mature B cell passes to peripheral lymphoid tissues (Figure 2).

2.3. B Cell Classification according to Their Ontogenic State. As soon as B cells have productively rearranged their immunoglobulin genes, pro-B cells proceed to the pre-B cell stage. On their arrival in the spleen, immature B cells give rise to type-1 (BT1), type-2 (BT2), and possibly type-3 transitional B cells [11]. As transitional B cells, they are pushed into migrating from the BM to secondary lymphoid organs (SLO). Although T1 cells undergo apoptosis in response to BCR engagement, they require signaling via the B cell activating factor belonging to the tumor necrosis factor (TNF) family receptor (BAFF-R, TNFRSF13) to mature to the T2 stage [12]. T2 cells are only present in the spleen and reside in the follicles, whereas T1 cells are found in the red pulp and outer periarterial lymphatic sheath (PALS) [13].

There, they continue maturing and are further selected by antigens. As BT1, they present as CD20+CD5+CD10+/−CD21+/−CD23+/−IgM+IgD+/− and CD38+, but once they have evolved to type 2 (BT2), they become CD20+CD5+/−CD21++CD23+/−IgM++IgD++ and CD38+/−. T2 B cells differentiate into either circulating lymphocytes that get organized as germinal centers (GCs), or noncirculating lymphocytes that populate the marginal zone (MZ). Progression of T2 B cells towards MZ or GCs may be determined by the quality of BCR-evoked signals and the subsequent expression of the Notch proteins [14].

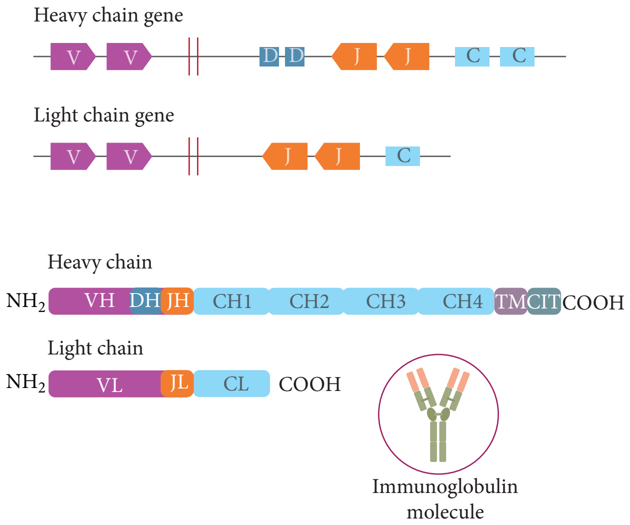

Figure 1: Schematic representation of the components of the H and L chains of immunoglobulins. The light and heavy chain loci are each made up of a series of V (variable) gene elements, followed by several D (diversity) segments (for the heavy chain gene only), some J (joining) segments, and C (constant region) exons. Heavy chains (H) are assembled from 4 segments (VH, D, JH, and CH); light chains (L) are assembled from 3 segments (VL, JL, and CL). The development of the BCR begins when the recombinase enzyme complex catalyzes the fusion of one DH region gene to a JH region gene with the deletion of the intermediate DNA sequences. Next, the recombinase joins one VH region gene to the rearranged DHJH gene. The enzyme terminal deoxynucleotidyl transferase (TdT) is expressed, adding random nucleotides to the sites of VHDHJH joining and enhancing the diversity of amino acid sequences. The rearranged VHDHJH element forms the most 5′ exon of the H chain gene and is followed downstream by exons encoding the constant (C) region (initially μ chain), that pairs with an L chain and produces IgM. When the VHDHJH element is followed downstream by exons encoding the C region for the δ chain, it produces IgD. These events occur as a result of alternative RNA splicing. Finally, if the rearrangement of VH, DH, and JH elements yields an H chain transcript and encodes a functional H chain protein, this heavy chain is synthesized and pairs in with 2 proteins (called λ5 and VpreB), which act as a surrogate light chain, and results in the expression of a pre-BCR. Once a functional heavy chain is produced, the cell downregulates the TdT gene and initiates an L chain rearrangement. It begins first with a κ element and, if this rearrangement is unsuccessful, continues with a λ element. A Vκ element rearranges to a Jκ element and produces a light chain, which, if it is functional, pairs with the H chain to make an immunoglobulin protein.

Alternatively, MZ B cells with mutated immunoglobulin genes, but without activation-induced cytidine deaminase (AICDA), may have passed a germinal center (GC) response [15]. Finally, the expression of sphingosine 1-phosphate receptor 1 on the B cells may overcome the recruiting activity of the B cell-attracting chemokine (BCA)-1 to the GCs [16], and thereby retain B cells within the MZ [17] (Figure 3). The main CD molecules expressed by B cells are summarized in Table 2.

Table 2: Cell surface CD molecules that are preferentially expressed by B cells.

Name	Cellular reactivity	Structure
CD19	Pan-B cell, FDCs?	Ig superfamily
CD20	Mature B cells	MS4A family
CD21	Mature B cells, FDCs	Complement receptor family
CD22	Mature B cells	Ig superfamily
CD23	Activated B cells, FDCs, others	C-type lectin
CD24	Pan-B cell, granulocytes, epithelial cells	GPI anchored
CD40	B cells, epithelial cells, FDCs, others	TNF receptor
CD72	Pan-B cell	C-type lectin
CD79a,b	Surface Ig$^+$ B cells	Ig superfamily

FDCs: follicular dendritic cells; Ig: immunoglobulin.

2.4. Migration of B Cell into the Germinal Centers.

Organization of the B cell follicles and surrounding T cell zones is achieved by the secretion of chemokines by distinct stromal cell subsets. Of these subsets, follicular dendritic cells (FDCs) are essential to retain immune complexes and produce B-lymphocyte chemoattractants (BLC/CXCL13). FDC maintenance requires continual membrane expression of lymphotoxin $\alpha 1\beta 2$ (LT$\alpha 1\beta 2$) trimer as well as TNF secretion by B cells and LTβR and TNF-R1 expression on FDCs [18]. The MZ demarcates the perimeter of the white pulp of the spleen and contains a subset of B cells that likely arises from the transitional B cell compartment [19]. MZ B cells are strategically located to respond to blood-borne antigens and can rapidly differentiate into antibody-producing cells in the red pulp. Upon an encounter with antigens, follicular B cells migrate to the border regions of the PALS/cortex to present bound peptide and costimulate T cells. Reciprocal B cell activation is mediated by engagement of CD40 and provision of cytokine support. CD40-dependent B cell activation is required to undergo proliferative expansion and differentiation in the GC, where somatic hypermutation and enhanced immunoglobulin class switch recombination (CSR) occur. The architecture of the GC is divided into distinct regions: rapidly dividing B cells or centroblasts in the "dark zone" of the GC give rise to centrocytes which occupy the "light zone." The light zone is thought to be the site of B cell selection by FDC-bound antigens that are processed and presented by B cells to primed T cells of the follicular helper CD4+ (Tfh) subtype.

B cell maturation in the GC is accompanied by somatic hypermutation of antibody variable region (V) genes, which provides the molecular basis for the production of B cells bearing high-affinity antigen receptors. These B cells are thought to have a competitive advantage when antigen becomes limiting and GC structures present atrophy. B cells unable to bind antigen or receive sufficient T cell help die *in situ* by apoptosis and are cleared by macrophages, whereas antigen-selected B cells that leave the GC become memory B cells or plasmablasts by a process that is not fully understood. Long-lived plasma cells are actively retained

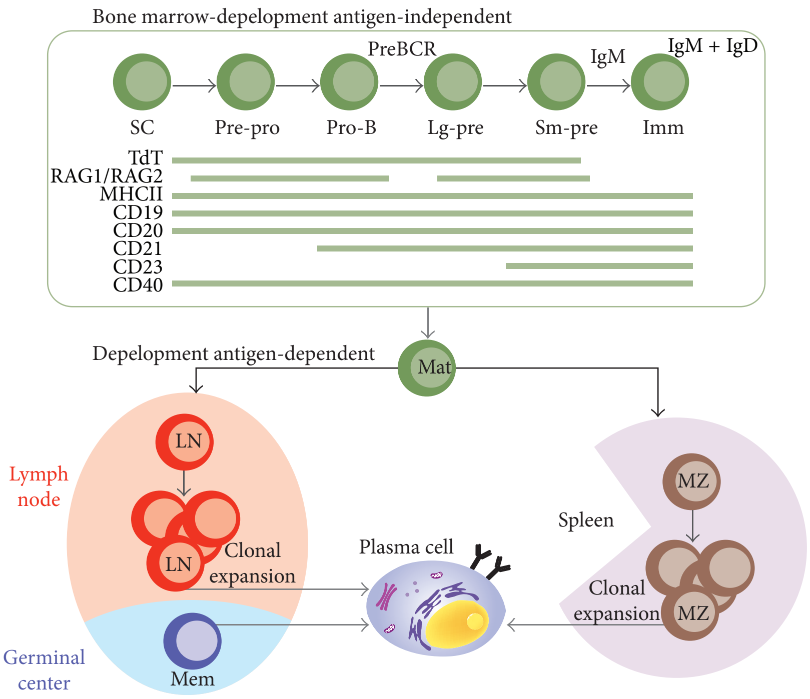
Bone marrow-depelopment antigen-independent
PreBCR
IgM
IgM + IgD
SC
Pre-pro
Pro-B
Lg-pre
Sm-pre
Imm
TdT
RAG1/RAG2
MHCII
CD19
CD20
CD21
CD23
CD40
Depelopment antigen-dependent
Mat
Lymph node
LN
LN
Clonal expansion
Germinal center
Mem
Plasma cell
Spleen
MZ
Clonal expansion
MZ

Figure 2: B cell receptor development and differentiation.

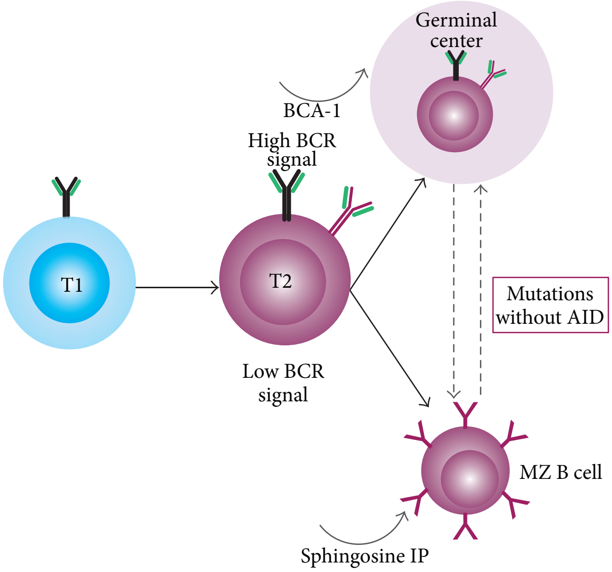
Germinal center
BCA-1
High BCR signal
T1
T2
Low BCR signal
Mutations without AID
MZ B cell
Sphingosine IP

Figure 3: B cell classification based on their ontogenic state. From the transitional type 1 (T1) and T2 B cells, two options depend on the B cell receptor (BCR) evoked signal and the downstream Notch 2 proteins: germinal center (GC) B cells driven by the B cell-attracting (BCA)-1 chemokine (or CXCL13) and MZ B cells with mutations but without activation-induced cytidine deaminase (AID). (Modified from [10]).

in the BM responding to stromal derived factor/CXCL12 as well as survival factors such as IL-6, B cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL). The trafficking of B cells in the lymphoid organs and target tissues is a regulation mechanism of B cell activation and differentiation [20–22].

B cells can act as an antigen delivery system that transports blood-borne antigens into the FDC network region of the spleen [17]. This regulates the GC formation where high affinity antibody-forming B cell differentiation occurs. These migratory responses are extremely dynamic and involve ongoing shuttling of the B cells between the different anatomic sites and the GCs. Chemotactic responses play a key role in orchestrating the cell-cell interactions in the GCs. This process involves ongoing shuttling of the antigen-carrying B cells between the MZ and the GCs. In animal models of autoimmunity, the migration of MZ precursor B cells is promoted by high levels of interferon (IFN)-α produced by plasmacytoid dendritic cells (pDC) in the marginal sinus that antagonize the activity of the S1P1 chemokine receptor. In contrast, within the GCs, IL-17A upregulates the expression of regulators of G protein signaling (RGS) in B cells to desensitize the G protein-coupled receptor (GPCR) signaling pathway of CXCL12 and CXCL13 chemokines [23–25]. This provides a prolonged stable interaction of B and T cells in the GC that induces high levels of AICDA and, as a result, enables the development of pathogenic autoantibody-producing B cells (Figure 4).

2.5. Mature B Cells. Peripheral B cell maturation, homeostasis, and antigen-dependent differentiation are complex processes occurring in distinct anatomic locations. As B cells egress from the BM, further maturation into follicular or MZ B cells is dependent upon the effects of the cytokine BAFF. B cell compartmentalization and cell-cell interactions in the SLO require expression of membrane-bound LTα/β trimers

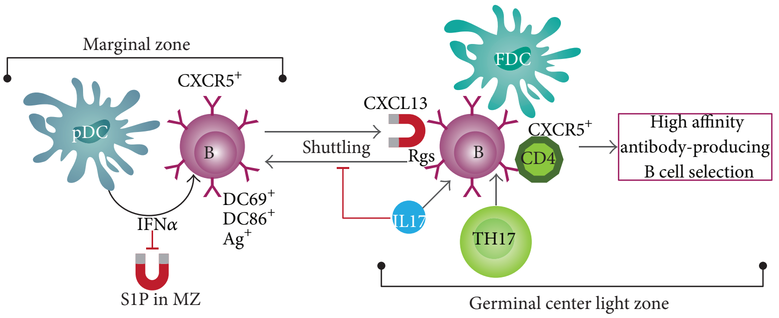
Marginal zone
CXCR5$^+$
pDC
B
DC69$^+$
DC86$^+$
Ag$^+$
IFNα
S1P in MZ
Shuttling
CXCL13
Rgs
FDC
B
CXCR5$^+$
CD4
High affinity antibody-producing B cell selection
IL17
TH17
Germinal center light zone

FIGURE 4: Chemotactic responses play a key role in orchestrating the cell-cell interactions in the germinal centers.

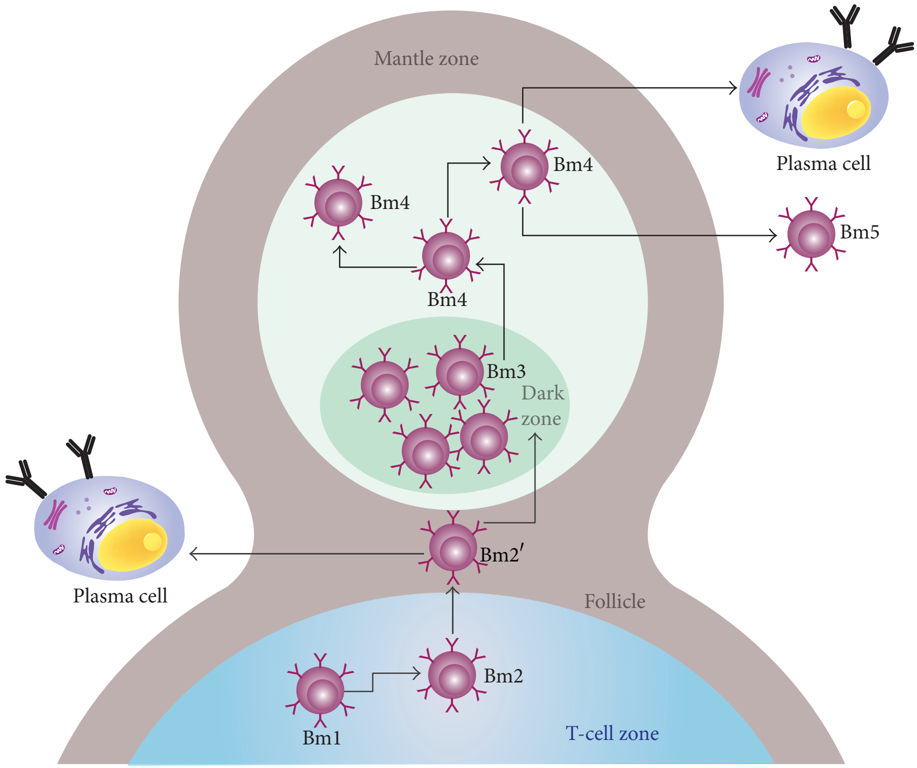
Mantle zone
Plasma cell
Bm4
Bm4
Bm5
Bm4
Bm3
Dark zone
Bm2′
Plasma cell
Follicle
Bm2
Bm1
T-cell zone

FIGURE 5: Germinal center (GC) changing a primary lymphoid follicle (LF) into a secondary LF. The GC is surrounded by the mantle zone, which is comprised of the light and dark zone, and populated by mature B (Bm) cells evolving from Bm1 in the T cell area through plasma cells that come back to the bone marrow.

and TNF, whereas T cell-dependent B cell differentiation requires engagement of CD40 (TNFRSF5) by CD40L on activated CD4+ T cells. CD30 (TNFRSF8) is expressed on activated B cells and has been found to be required for efficient memory B cell generation. CD27 is also implicated in B cell memory.

The development stages of GC B cells are based on the relative expression of IgD and CD38 on mature B (Bm) lymphocytes [26] from naïve cells leaving the BM (Bm1) to memory B cells activated and differentiated by their specific antigen (Bm5). The development starts with CD38−IgD+ naïve Bm1 that progresses into CD38+IgD+ antigen activated Bm2, of which some become CD38++IgD+Bm2′ GC founder cells. These differentiate into CD38++IgD−Bm3 centroblasts and Bm4 centrocytes (Figure 5). Two types of B cells arise from GC reactions: CD38+IgD− early memory B cells that mature locally into CD38−IgD−Bm5 memory B cells and CD38++IgD− plasmablasts, which were first described by Odendahl et al. [27]. The latter return to the BM where they differentiate into long-lived plasma cells. A few cells of each subset escape into the circulation from GCs.

2.6. B Cell Distribution Abnormalities in Systemic Lupus Erythematosus. Several studies show differences of certain peripheral B cell subsets in SLE patients compared to healthy controls. Populations such as transitional B cells (CD24++CD38++), prenaïve and naïve B cells are expanded in the peripheral blood of patients [28], indicating a population shift within the preimmune B cell compartment toward the more immature B cells. Whether these abnormalities reflect an intrinsic B cell defect or are secondary to inflammation or immune deregulation is unclear, but the excess of some cytokines such as BAFF may explain part of these differences. In peripheral blood of healthy controls transitional B cells account for only 2 to 3% of all B cells [29, 30]. In contrast, SLE patients have an increased frequency of approximately 6-7%. This high proportion does not correlate with disease activity and titres of autoantibodies. Due to the lymphopenia seen in SLE patients, the absolute number of transitional B cells is not different to that of controls. The most important check point in SLE seems to be at the transitional stage. High number of self-reactive mature naïve B cells which subsequently originate autoantibody producing plasma cells. This is the most reported characteristic of the abnormal B cell homeostasis in SLE characterized by the expansion of peripheral CD27++ plasmablasts [31], which also correlates with disease activity and the titre of autoantibodies [32]. On the other hand, the frequency of CD19+CD27+ memory B cells seems to be unaffected in SLE patients with active and inactive disease, although the total number of memory B cells is decreased in SLE patients compared to healthy controls [27].

2.7. B Cell Derived Cytokines. IL-7 is important in B cell functioning. This cytokine plays several important roles during B cell development including aiding in the specification and commitment of cells to the B lineage, the proliferation and survival of B cell progenitors, and maturation during the pro-B to pre-B cell transition [33]. Regulation and modulation of IL-7 receptor (IL-7R) signaling is critical during B lymphopoiesis because excessive or deficient IL-7R signaling leads to abnormal or inhibited B cell development [34]. IL-7 works together with *E2A*, *EBF*, *Pax-5*, and other transcription factors to regulate B cell commitment while it also works to regulate immunoglobulin rearrangement by modulating *FoxO* protein activation and *Rag* enhancer activity. Suppressors of cytokine signaling (SOCS) proteins are inhibitors of cytokine activation and, in B cells, function to fine-tune IL-7R signaling. This ensures that appropriate IL-7 signals are transmitted to allow for efficient B cell commitment and development [35].

Recent discoveries have unveiled new insights into B cell derived cytokines, including IFN-γ and IL-4 that modulate the response [36]. They are likely to serve as effectors of some B cell functions. Given the kinetics of B cell generation and the cytokine profile of B lymphocytes, T helper (Th) 1 phenotype may be imprinted by B effector (Be) 1 cells through the expression of IL-2 and IFN-γ by B cells. This is sustained by an IFN-γ/IFN-γ receptor autocrine loop. Conversely, Th2 cells induced naïve B cell polarization into Be2, which produces IL-4 and IL-6 in the absence of GATA-3. In fact, the Th1/Th2 cytokine balance changes with the progress of the immunopathological lesions on autoimmune diseases such as SLE and primary Sjögren's syndrome [37]. Distinct populations of serum cytokines have also been found to differentiate autoimmune disease patients from controls and one patient from another depending on the presence or absence of different organ involvement [38]. B cell produced cytokines may be classified as proinflammatory (IL-1, IL-6, TNF-α, and LT-α), immunosuppressive cytokines (TGF-α and IL-10), or as hematopoietic growth factors (granulocyte/monocytes-colony stimulating factor and IL-17).

2.8. B Cell Transcription Factors. B cell development depends on several transcription factors. One of the most important transcription factors is *Pax5*. *Pax5* restricts the developmental potential of lymphoid progenitors to the B cell pathway by repressing B-lineage-inappropriate genes while it simultaneously promotes B cell development by activating B-lymphoid-specific genes. Therefore, *Pax5* controls gene transcription by recruiting chromatin-remodeling, histone modifying, and basal transcription factor complexes to their target genes [39]. Moreover, *Pax5* contributes to the diversity of the antibody repertoire by controlling VH-DJH recombination. It does this by inducing contraction of the immunoglobulin heavy-chain locus in pro-B cells, which is likely mediated by PAIR elements in the 50 region of the VH gene cluster. Importantly all mature B cell types depend on *Pax5* for their differentiation and function. *Pax5* thus controls the identity of B lymphocytes throughout B cell development. Consequently, conditional loss of *Pax5* allows mature B cells from peripheral lymphoid organs to develop into functional T cells in the thymus via differentiation to uncommitted progenitors in the BM. *Pax5* has also been implicated in some diseases including human B cell malignancies.

3. B Cell Tolerance Mechanisms and Their Role in Autoimmunity

3.1. B cell Tolerance. This mechanism is essential for maintaining nonresponsiveness to thymus-independent self-antigens such as lipids and polysaccharides. B cell tolerance is also important in preventing the development of antibody responses to protein antigens. Both central and peripheral mechanisms are implicated in B cell tolerance. In the central tolerance, the immature B lymphocytes that recognize self-antigens in the BM with high affinity are deleted or activate mechanisms to change their specificity by receptor editing. This fate is defined by the strength of BCR signaling: a strong BCR signal by binding with high affinity to an autoantigen will lead to deletion or receptor editing (see below) while an intermediate binding affinity will permit B cells to survive and continue to the periphery [40].

If a mature B cell recognizes autoantigens in peripheral tissues without specific helper T cell response, this cell may be functionally inactivated by anergy mechanisms or die by apoptosis. The AICDA is required for B cell tolerance in humans. This enzyme is required for CSR and somatic hypermutation. Patients with AICDA deficit develop primary immunodeficiencies and autoimmune complications. Single B cells from AICDA-deficient patients show an abnormal immunoglobulin (Ig) repertoire and high frequencies of autoreactive antibodies [41].

3.2. B Cell Receptor Editing. When the B cell differentiation is ongoing, its receptor presents a phenomenon known as receptor editing, which is the process of antibody gene rearrangement to have a functional BCR and inhibit further rearrangement (allelic exclusion). The receptor editing is a major mechanism of central tolerance in B cells. If a T lymphocyte produces a self-reactive receptor, different mechanisms are initiated to induce the apoptosis of this self-reactive cell (negative regulation). However, B cells have a second chance at escaping this negative regulation by "editing" the specificities of their receptors with additional antibody gene rearrangements. Immature B cells in the BM that encounter multivalent self-antigens revert to pre-B stage and continue to rearrange κ and, if necessary, λ light chain genes and generate newly generated B cells that have a novel light chain that is no longer self-reactive. In this case, immature B cells with novel light chains that are no longer part of a self-reactive BCR migrate to the periphery as BT1 cells where they mature into newly generated IgM and IgD expressing recirculating BT2 cells and, then, into mature recirculating B cells. Furthermore, edited B cells are not simply endowed for life with a single, invariant antigen receptor, because an edited B cell whose initial *Ig* gene is not inactivated during the editing process may exhibit two specificities [42].

The BCR editing process initiates with the allelic exclusion. This is the phenomenon in which B cells usually express a single kind of antibody H chain and L chain, and it is typically enforced at the genetic level with only one allele being productively rearranged. A series of epigenetic mechanisms, including replication timing, DNA methylation, histone modification, nucleosome positioning, and heterochromatization, appear to control H and L chain locus accessibility and which allele is first rearranged [43]. These mechanisms regulate accessibility to recombination machinery and activate feedback inhibition of the rearrangement between H chain and L chains. Once the H chain protein is completed, L chain rearrangements initiate. This process is regulated by isotypic exclusion, a phenomenon in which B cells usually express a single L chain isotype (either κ or λ, not both) and is explained by two properties of L chain rearrangement: first, the κ or λ rearrange at different times during B cell development, and second, the B cells which express λ often have both κ alleles deleted. Based on the analysis of cell lines in mouse and human, it was clear that κ chain nearly always rearranges before λ chain [44, 45].

Another process identified is the secondary rearrangement of H and L chains. In heavy chain, the mechanism is mediated by DH-JH rearrangement, DH-DH fusion, and VH replacement, all of which contribute to the elongation of the third complementarity determining region (CDR3) and promote autoreactivity. During DH-JH rearrangement, a DH gene upstream of the existing DH-JH rearrangement recombines with a JH gene downstream of the DH-JH rearrangement and replaces it by a leapfrogging deletion rearrangement. In a DH-DH fusion, the recombination process links a $5'$ DH segment to a preceding DH-JH rearrangement rather than to a $3'$ JH gene. DH-DH fusion occurs more frequently in murine lupus than in nonautoimmune strains of mice [46, 47]. Finally, during VH replacement, the conventional 23 recombination signal sequence (RSS) of an upstream murine VH undergoes RAG-dependent deletional rearrangement with the cryptic RSS of an existing downstream VH gene which is part of an existing VDJ rearrangement on the same allele. This rearrangement results in replacement of all but the very $3'$ end of the previously rearranged VH with a new VH. Secondary rearrangement, which would consist of either deletion or inversion of the chromosomal DNA between the recombining gene segments, can also occur at the κ locus. These rearrangements are apparently part of an important physiological process underlying failed allelic exclusion and might occur to edit the specificity of a self-reactive BCR (Figure 6).

3.3. Control of Receptor Editing. Receptor editing has a genetic control and has been studied in several models. Pre-B cells expressing IκB show evidence of receptor editing which is consistent with a role for *NFκB* [48]. *PLCγ2* is present in higher quantities in immature B cells, showing increased phosphorylation in response to BCR crosslinking and probably induces the expression of *Rag2* in these cells. However, other data show downregulation of rag induced by PLCγ2 and thus terminate receptor editing. Immature B cells can be induced to edit by BCR crosslinking while transitional B cells cannot. This may be due to an altered signaling pathway through *PLCγ2* [49, 50].

The mechanisms that suppress editing and their potential role in autoimmune diseases are under research.

3.4. B Cell and Autoimmunity. Classically, the immune mechanisms implicated in the development of autoimmune diseases have been categorized into two broad sets of diseases: one set in which the pathological process is driven by T cells and the other in which the humoral B response mediates the disorder by producing autoantibodies that are able to bind tissue self-antigens or by forming immune complexes. In recent years, with the new knowledge about the immune response, this approach—dividing autoimmune diseases into T cell and B cell mediated diseases—has dramatically changed. It is now recognized that T lymphocytes facilitate adaptive immune B responses, and B cells play a reciprocal role during CD4 T cell activation in autoimmune diseases.

For instance, most disease-related autoantibodies are IgGs that are somatically mutated, and this suggests that helper T cells drive the autoimmune B cell response [51]. In addition, B cells have been shown to be important mediators of some autoimmune diseases. These are classically described as T cell mediated and include rheumatoid arthritis (RA), multiple sclerosis (MS), and type 1 diabetes mellitus (T1D). In diseases in which specific autoimmune T cell clones drive the process of inflammation, autoantibody synthesis may represent a marker for the expansion of autoantigen specific B cells that capture and present autoantigen peptides to T cells. As mentioned before, the central tolerance mechanisms are crucial in preventing B cell mediated autoimmune diseases. For instance, the strong BCR signal from binding with high affinity to an autoantigen will lead to deletion or receptor editing of the high affinity. This concept has been demonstrated in several autoimmune animal models, including a double-transgenic mouse model carrying not

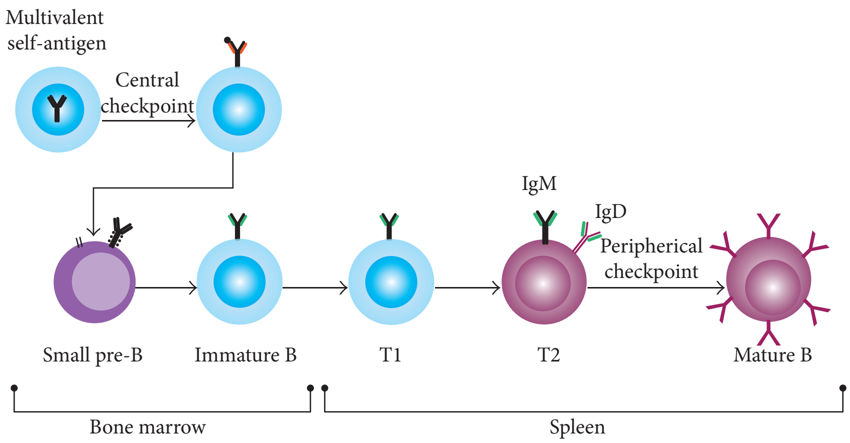

Figure 6: Receptor editing as a major mechanism of central tolerance in B cells. Receptor editing is a major mechanism of central tolerance in B cells. Immature B cells in the bone marrow that encounter multivalent self-antigens revert to the small pre-B stage, continue to rearrange k and, if necessary, l light chain genes, and generate newly B cells that have a novel light chain that is no longer self-reactive. Immature B cells with novel light chains that are no longer part of a self-reactive B cell receptor then migrate to the periphery as T1 B cells where they mature into newly generated IgM and IgD expressing recirculating T2 B cells and, then, into mature recirculating B cells.

only the heavy chain against the myelin oligodendrocyte glycoprotein (MOG) autoantigen but also the light chain. The authors demonstrated that B cells expressing solely the MOG-specific Ig H chain differentiate without tolerance. On the other hand, double-transgenic B cells expressing transgenic Ig H and L chains are subjected to receptor editing [52, 53].

If the signaling potential of the BCR is affected, for example, by overexpression of CD19 or *Ptpn22* polymorphisms (described in several autoimmune diseases), the self-reactive B cells will not be deleted and may reach the periphery [54, 55]. These mechanisms lead to the increase of self-reactive B cells in the periphery and, as a consequence, the possibility of developing autoimmune diseases. Thus, leaky central tolerance increases the risk for subsequent development of autoimmune disease, but additional factors (genetic, hormonal, environmental, etc.) control this progression from autoimmunity to autoimmune disease.

The role of Toll-like receptors (TLR) in B cell and autoimmunity has also been explored. In a study to determine the stimuli contributing to the development into MZ B cells (involved in autoimmunity), TLR9 stimulation by CpG of transitional B cells induces proliferation and specific maturation into B cells with phenotypic markers of MZ B cells. Also the terminal differentiation into antibody-secreting cell was triggered, leading to autoantibodies synthesis. On the other hand, mature B cells do not differentiate into MZ following TLR9 stimulation. These results suggest that transitional B cells are specifically sensitive to TLR9 stimulation to induce autoreactive B cells [56].

3.5. B Cell Functions in Autoimmunity. B cells do not simply produce autoantibodies. In fact, B lymphocytes are uniquely endowed to drive autoimmunity as APC because they can bind native self-proteins through their BCR, process them, and present them to T lymphocytes. To demonstrate the antigen-presenting effect of B cells in autoimmunity, several models and observations have been used. For example, in the murine experimental allergic encephalomyelitis (EAE), B lymphocytes are dispensable when disease is induced by MOG peptides but absolutely required for disease to develop if mice are immunized with MOG protein [57]. In MOG-specific TCR and BCR double-transgenic mice, self-reactive B cells cause severe EAE by presenting endogenous MOG protein to self-reactive T cells rather than by autoantibody production [58, 59]. In addition to this observation in EAE (a classical described T cell disease), B cell depletion by rituximab strongly reduced disease severity, affecting the delayed type hypersensitivity and reducing T cell proliferation and IL-17 production [60]. The IL-6 seems important to mediate these effects as indicated by the findings that rituximab effects are not observed in IL-6 KO mice with EAE.

Another example to show that B cells functions in autoimmunity are not only producing autoantibodies is the transgenic mIgM.MRL-FASlpr mouse. In this model, whose B lymphocytes cannot secrete antibodies but can present antigen, lupus develops spontaneously and T cell activation is comparable to MRL/lpr controls [61]. Likewise, nonobese diabetic (NOD) mice with a mutant IgM heavy chain that cannot be secreted demonstrate that increased insulitis and spontaneous diabetes may occur in the absence of antibody production but require antigen presentation by B cells [62].

The ability of B cells to bind autoantigens through their BCR allows them to act as potent APCs at very low protein concentrations. In the MOG-specific TCR and BCR double-transgenic mice, antigen specific B cells process and present MOG protein to T cells at concentrations that are 100-fold lower than B cells with other BCR specificities. Other functions of B cells are cytokine and chemokine synthesis and ectopic lymphoid neogenesis in autoimmune diseases.

3.6. Amplification of the Autoimmune Response by Epitope Spreading. B cells bind to a specific epitope in antigens via their BCR. After the initial recognition, protein and even protein complexes can be internalized and processed for antigen presentation. The protein may, however, contain several other epitopes besides the epitope originally recognized by the BCR, which can fit in the binding grooves of the MHCII molecules in the B cell. As a consequence, the B cells can present not only the original epitope but also other epitopes of the same protein or protein complex to T lymphocytes and thereby trigger different T cell specificities [63]. This phenomenon, known as epitope spreading, allows autoantigens that were not the initial targets of autoreactive lymphocytes at the onset of autoimmunity to become antigens at later stages [64]. This phenomenon is described in almost all immune diseases and is frequently associated with disease progression [64]. Epitope spreading may trigger the clinically manifested autoimmune disease. As a representative example, the SJL/J mice immunized with protelipid (PLP) proteins develop T cell responses specific to different epitopes in the molecule. These distinct T cell responses contribute to the relapse phases of the EAE and can initiate disease upon secondary adoptive transfer to naïve animals [65]. Epitope spreading also occurs in the NOD mouse model of spontaneous diabetes. In this model, T cell responses and antibodies to type 1 diabetes (T1D), autoantigens, GAD65 and GAD67 isoforms of GAD are observed in mice at 4 weeks of age. At 6 weeks of age, T and B lymphocyte responses for other β cell antigens—peripherin, carboxypeptidase H, and Hsp60—are also detected. By 8 weeks of age, responses to all former antigens are enhanced. The initial GAD specific reactivity in this model coincides with the onset of insulitis whereas the progression of insulitis to β cell destruction with age correlates to the epitope spreading of B and T cells [66]. Temporal progression of autoreactivity to autoimmune disease by epitope spreading also occurs in human autoimmune diseases. In childhood T1D diabetes, insulin autoantibodies (IAA) are the first autoantibodies detected. IAA-positive children that sequentially develop antibodies to other β cell antigens such as GAD and protein tyrosine phosphatase-like proteins IA-2 usually progress to T1D. In contrast, children that remain positive for only IAAs rarely develop the disease [67]. In RA, several reports have shown that the number of antibody specificities increases over time. Like T1D patients, healthy individuals with a broad anticitrullinated peptide antibody (ACPA) profile have a higher risk of developing arthritis [64, 68]. This phenomenon is also observed in SLE patients. In this case, the number of positive antibodies in serums of patients also increases over time until the onset of clinical symptoms as demonstrated in the classic article about autoimmune diseases prediction by Arbuckle et al. [69].

3.7. The Effects of the Cytokine BAFF in B Cell Tolerance and SLE Development. The cytokine BAFF (for B cell activating factor belonging to the TNF family) has emerged since 1999 [70] as one of the critical factors controlling B cell maturation, tolerance, and malignancy. BAFF plays a key role in B cell differentiation, survival, and activation [70]. BAFF, also known as B lymphocyte stimulator (BLyS), is a cytokine that prevents apoptosis of autoreactive B cells [21]. The BAFF family consists of two ligands, a proliferation-inducing ligand (APRIL) and BAFF; and three membrane receptors, BCMA (B cell maturation antigen), TACI (transmembrane activator, calcium modulator, and cyclophylin ligand interactor), and BAFF-R (also known as BR3). The interactions between ligands and receptors vary: thus, BAFF interacts chiefly with BR3 but can interact with all three receptors, whereas APRIL can interact with TACI and BCMA, but not with BR3 [71]. BAFF enhances B cell survival, drives B cell maturation especially at the early transitional stages, and discontinues humoral tolerance by rescuing autoreactive B cells from apoptosis [72]. Figure 7 shows the different receptors for BAFF and APRIL.

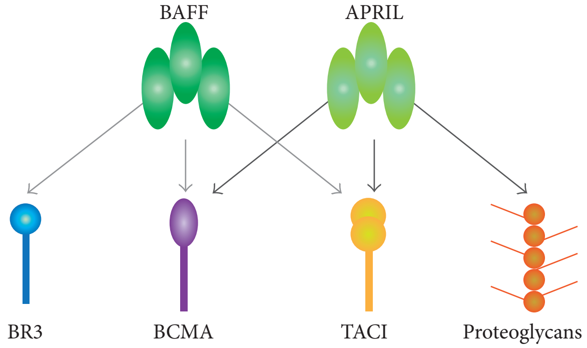

Figure 7: BAFF and APRIL receptors. BAFF binds chiefly to BAFF-R (BR3) but also to BCMA and TACI. APRIL, in turn, interacts with TACI and BCMA, but not with BR3. In addition, APRIL binds to proteoglycans expressed in membranes of lymphoid and nonlymphoid cells.

3.8. Double-Transgenic Mice Expressing Both HEL and Anti-HEL B Cell Receptor. As mentioned before, to avoid the generation of pathogenic autoantibodies, self-reactive lymphocytes have to be deleted or anergized at successive immune checkpoints during B cell development and maturation. Because immunoglobulin gene rearrangement is a random mechanism, 50–75% of the newly generated B cells in the BM have a self-reactive BCR. However, the development of autoimmune disease is rare, affecting up to 5% of the population. Consequently, effective mechanisms exist for preventing immune activation of self-reactive lymphocytes. BAFF is known for its role in the survival of mature B cells. Based on its receptor expression profile, BAFF has no effect on B cell tolerance in the BM but does act at the periphery (Figure 8). BAFF certainly plays a major role in B cell tolerance after the BT1 immature B cell stage. Whether or not BAFF can influence self-reactive BT1 cell elimination is unclear. However, BAFF is certainly needed for the survival of BT2 cells and downstream B cell subsets. BT2 cells, which express high levels of BAFF-R, are indeed dependent on BAFF because of their propensity for apoptosis [73], and B cell ontogenesis is stopped at the T1 stage when BAFF or BAFF-R are lacking [74]. One of the most informative systems for studying B cell tolerance is the double-transgenic (Tg) mouse model which expresses the anti-hen-egg lysozyme (HEL) BCR and HEL simultaneously. When HEL is expressed as a cell surface molecule, self-reactive B cells are deleted or undergo additional *ig* gene rearrangements by the receptor editing mechanisms. When HEL is expressed as a soluble

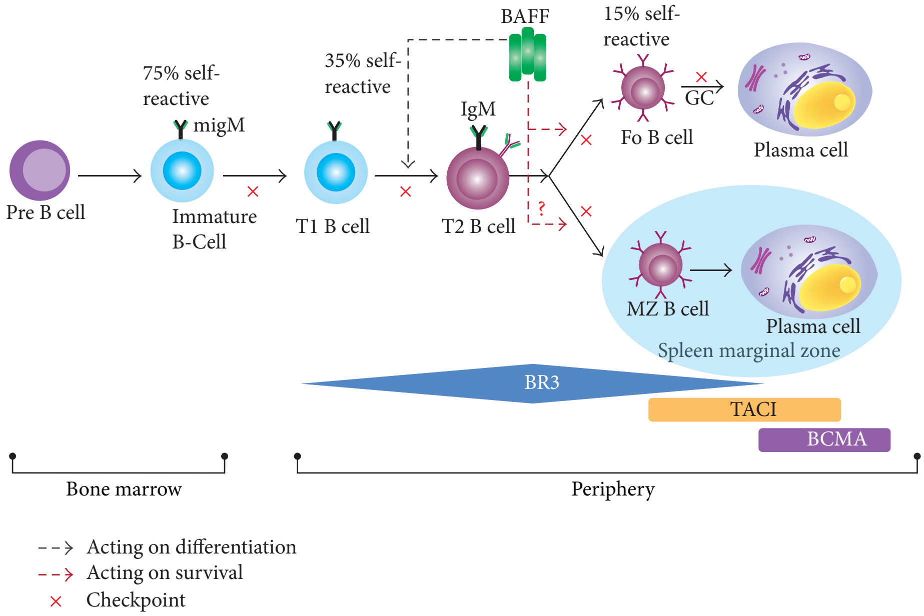

Figure 8: BAFF receptor cell surface expression and self-tolerance during B cell ontogenesis. Data indicate the proportion of self-reactive B cells at specific B cell stages before or after checkpoints as determined in the anti-HEL/HEL transgenic models. Fo: follicular; GC: germinal center; Imm: immature; MZ: marginal zone; Pre: precursor; T1 or 2: transitional type 1 or 2.

protein (sHEL), self-reactive B cells can migrate into the periphery where their fate depends on their ability to compete with non-self-reactive B cells. Without competition, self-reactive BT2 cells persist in an anergic state. In the presence of competition, self-reactive BT2 cells need the cytokine BAFF to sustain their survival and maturation. Because BAFF levels are limited under normal conditions, these self-reactive B cells undergo apoptosis. Thus, if double Tg mice for sHEL/anti-HEL are treated with antagonist for BAFF, survival of sHEL self-reactive B cells is dramatically decreased [75]. In contrast, when BAFF is overexpressed, sHEL self-reactive BT2 cells survive and colonize follicles and MZ in the spleen [76]. Of note, when anti-HEL B cells compete with normal B cells in the animal, excessive expression of BAFF no longer prevents the escape of self-reactive B cells. In this scenario, self-reactive cells are eliminated at a much earlier maturation stage (T1), a stage when B cells express little BAFF-R and as such are unable to sense excessive BAFF production that can only efficiently rescue BT2 cells.

3.9. BAFF-Transgenic Mice. BAFF-Tg mice constitute an effective model for autoimmunity. Overproduction of BAFF in these mice leads to B cell proliferation, auto-antibody production, and, ultimately, development of kidney failure similar to SLE-associated symptoms. Moreover, aging BAFF-Tg mice also present a primary Sjögren's syndrome-like disease, in which they demonstrate inflammation and destruction of salivary glands (SGs) [72]. In addition to the attendant polyclonal hypergammaglobulinemia, BAFF-Tg mice develop elevated titers of multiple autoantibodies, including antinuclear antibodies, anti-double-stranded DNA, rheumatoid factors, circulating immune complexes, and immunoglobulin deposits in kidneys. Some B cell subsets such as BT2 cells, follicular (Fo) B cells, and MZ B cells rise. Moreover, without stimulation, a high number of GCs are found in the spleen and the lymph nodes. Finally, lymphocytes infiltrating the SG are essentially MZ-like B cells. Note that BAFF-Tg mice develop the same pSS manifestations when T cells are removed [77], but in this instance, BAFF exacerbates Toll-like receptor activation of B cells. An alternative model for the development of SS apart from T cells has since been proposed [78].

3.10. CD5 in Its Implications in Autoimmunity. The CD5 is a transmembrane glycoprotein expressed in T lymphocytes and, at lower levels, in the subset of B cells known as B1 cell. The initial interest on CD5+ expressing B cells pointed on the role of these cells in autoimmune diseases, based on the ability of these cells to produce natural polyreactive antibodies, which recognize autoantigens with low affinity [79, 80]. The hypothesis in autoimmune diseases was that these natural antibodies with low affinity to autoantigens may improve this affinity and become in high affinity pathogenic autoantibodies. However, the B1 cells expressing CD5 have phenotypic features similar to transitional anergic murine B lymphocytes. In fact, these cells may produce IL-10 upon activation through the CD40 coreceptor [81, 82]. The regulatory potential of CD5 has been demonstrated by transfections of CD5 in Jok-1 B cell line [83]. In this experiment, the expression of CD5 induces IL-10 production through activating NFAT2 and STAT3. Thus CD5-expressing B cells may present contradictory roles in B lymphocytes function. An elegant study showing how CD5 expression is regulated in B lymphocytes and how it modulates the B cell response has been published. This study analyzed the molecular structure of the human CD5 gene, showing that two different promoters exist (E1A and E1B) [84]. The E1B Interestingly, the CD5-E1A was expressed on the membranes of both T and B lymphocytes, while the CD5-E1B was transcribed into a truncated CD5 isoform in B lymphocytes, not able to express on the membranes. CD5-E1B expression downregulates the level of membrane expression of the conventional CD5-C1A. As a consequence, high levels of CD5-E1B could reduce the inhibitory effects of CD5 on BCR mediated signaling and lead to increased antibody production. Thus, in B cells expressing high or normal levels of membrane CD5, the molecule acts to downregulate BCR mediated signaling. On the other hand, B cells expressing high levels of CD5-E1B, induced probably by external stimuli, would more likely to be activated. To support these results, in B lymphocytes from SLE patients, the levels of CD5-E1B are higher, indicating a more activating B cell [85]. These high levels of CD5-E1B traduces in reduced expression of membrane CD5. In this model, high levels of IL-6 on B cells from SLE patients abrogate the ability to induce the DNA methyl transferase (DNMT1) and then to methylate DNA, affecting the transcription of CD5-E1A, favoring the truncated form E1B. This altered signaling could promote the activation and expansion of autoreactive B cells in SLE patients. Interestingly, in mature B cells from SLE patients, a default in the regulation of *Rag* is present and leads to upregulation of this enzyme and the emergence of autoantibodies [86, 87]. CD5 and IL-6 contribute to this upregulation, indicating the roles of these molecules in SLE pathogenesis.

In murine models CD5 is involved in anergy [88]. This hypothesis has been elegantly demonstrated breeding the HEL transgenic model for B cell anergy onto the CD5 null background. This experiment resulted in a spontaneous loss of B cell tolerance *in vivo*. The study showed high levels of anti-HEL IgM antibodies and enhanced proliferative responses *in vitro* with elevated intracellular calcium levels.

3.11. CD22 in Its Implications in Autoimmunity. Another important B cell molecule which has an effect on autoimmunity development is the CD22. B cell responses are initiated by antigen binding to the BCR and are modified by a broad repertoire of activating and inhibitory transmembrane coreceptors expressed on the B cell surface [89, 90]. In this context, the multifunctional BCR co-receptor, CD22, is interesting since it plays a critical role in establishing and modulating the antigen receptor signaling thresholds for B cell activation [91]. CD22, as part of the BCR complex, can modulate the intensity, quality, and duration of homeostatic and BCR-induced signals in an inhibitory or stimulatory capacity through ligand-dependent and -independent mechanisms [92, 93]. Based on substantial mouse model data, it appears that the predominant effect of CD22 is inhibitory [94]. CD22 is a 135 kDa B lymphocyte restricted type-I transmembrane sialoglycoprotein of the immunoglobulin superfamily [95]. It appears intracellularly during the late pro-B cell stage of ontogeny but shifts to the plasma membrane with B cell maturation. CD22 is expressed at low levels on immature B cells and at higher levels on mature IgM+, IgD+ B cells. However, it is absent on differentiated plasma cells. It is strongly expressed in follicular, mantle, and marginal zone B cells but is weakly present in germinal B cells [96]. As previously mentioned, for the immune system to function effectively, it is essential to mount an appropriate humoral response against potential pathogens while avoiding autoimmunity and reactivity to self-antigens [97]. Understanding the function of CD22 may, therefore, suggest methods for modulating humoral immunity and aid in discovering treatments for autoimmunity [98].

To regulate B lymphocyte functions and migration, the interaction of CD22 with $\alpha 2,6$-linked sialic acid ligands is important. This binding is necessary for its negative regulatory functions [99]. Cell lines expressing CD22 without sialic acidbinding activity are hyperresponsive to BCR stimulation [99].

Recent studies in mouse models have suggested a role for defects and loss of functionality in CD22 in the pathogenesis of autoimmune disease, including SLE. B cells obtained from CD22-deficient mice have been shown to be hyperresponsive to receptor signaling and demonstrate increased Ca2+ fluxes on BCR ligation, which increased serum titers of IgG anti-dsDNA autoantibodies. These antibodies were of multiclonal origin, were somatically mutated, and had high affinity [100].

Epratuzumab is a novel humanized antihuman CD22 IgG1 monoclonal antibody that binds to the extracellular domain of CD22 and induces modest but significant intracellular phosphorylation. Epratuzumab reduces total blood B cells by about 35–40% and has preferential effects on naïve and transitional B cells [101, 102]. Epratuzumab treatment has been used with moderate clinical success in SLE and primary Sjögren's syndrome [103].

3.12. A New Concept in Autoimmunity: Regulatory B Cells. A functional B cell subset, called regulatory B cells, has recently emerged as an important factor for maintaining immune tolerance. This subtype restrains the excessive inflammatory response that occurs during the development of autoimmune diseases. The main regulatory B cell function is mediated by the IL-10 production that inhibits proinflammatory cytokines and supports regulatory T cell differentiation. The regulatory B cells were named in 2002 [104], after the demonstration that IL-10 producing B cells can suppress inflammatory responses in experimental autoimmune encephalomyelitis, collagen-induced arthritis, and autoimmune colitis [105, 106].

In the murine models, regulatory B cells have also been shown to directly inhibit T cell proliferation through cell-cell contact. This may even lead to anergy or apoptosis of T cells [107, 108] and the modulation of the inflammatory response. In this regard, CD40 engagement on B cells appears to be a requisite for the induction of functional B regulatory cells in mice. Stimulation of CD40 brings about the development of B cells with suppressive properties. Furthermore, signaling in the absence of CD40 makes B cells unable to regulate inflammatory response [105, 109].

The murine phenotypic nature of B regulatory cells is still a matter for debate. Two distinct IL-10 producing B cell subpopulations associated with regulatory functions have been identified. One has been recognized as transitional marginal zone precursor B cells expressing a high level of CD21, CD23, CD24, IgM, and CD1d, designed as transitional type 2 (T2)-like cells [110–112]. The second—described as $\mathrm{CD1d}^{\mathrm{hi}}$, CD5+, and $\mathrm{CD19}^{\mathrm{hi}}$ B cells—has been called “B10” cells since IL-10 is the main cytokine produced by these cells [81]. Recent studies have suggested that human B cells can also regulate inflammatory responses [113]. These cells have been studied primarily in autoimmune diseases, including SLE and multiple sclerosis, for which functional as well as numerical defects of these cells have been described [112, 114–116]. A recent publication on patients with SLE described a population of regulatory CD19+CD24++CD38++ B cells [112] as a phenotype reminiscent of preimmune B cells. This subset is able to secrete IL-10 and thus is able to suppress Th1 and Th2 functions after activation. These cells, though present in numbers similar to controls, lack regulatory capacity in SLE patients.

In addition to the described results, another study on human regulatory B cells showed that regulation of T cell proliferation was defective in SLE patients but not in other autoimmune diseases [117]. This paper studied the regulation of T cell responses induced by B cells following CD40 cognate interaction. CD40-induced regulatory B cells partially inhibited T cell proliferation without any soluble factor. In contrast, modulation of Th1 differentiation resulted from CD80- and CD86-dependent interactions and IL-10 production. The suppressive effects were mediated by CD19++IgD+CD38++CD24++CD5++ and appeared to be indirect through the induction of regulatory T cells (CD4+CD25+FoxP3+). The mentioned defect of B cell regulatory effect was found only in SLE patients, indicating that the restoration of efficient B cell regulatory activity could be an innovative and alternative therapy in SLE.

In another interesting paper from the same group, the effects of human B cells with a regulatory potential on dendritic cells have been studied. In an *in vitro* model of cocultures, human activated B cells (CD19+IgDlowCD38+CD24lowCD27−) showed a potential to restrain the development of monocytes into immature dendritic cells and their differentiation into mature dendritic cell, decreased the HLA-DR, CD80, and CD86 expression and the production of IL-12p70 required for antigen presentation and Th1 differentiation [118, 119]. Even more interesting, mature dendritic cells from patients with SLE displayed insensitivity to the regulation of IL-12 induced by B cells. Thus, inefficient B cell regulation may alter the balance between an effector inflammatory response and tolerance induction.

Knowledge about these cells is increasing rapidly, but much remains to be understood regarding the biology of B regulatory cells in murine models and humans. The increasing knowledge may allow the development of targeted therapies in order to increase the B cell regulatory function in autoimmune diseases.

Table 3: Potential targets in B lymphocytes and the therapeutic molecule for the treatment of systemic lupus erythematosus.

Direct B lymphocyte targeting
CD-20 antigen
(i) Rituximab (chimeric monoclonal antibody): EXPLORER and LUNAR studies did not meet primary endpoints.
(ii) Ocrelizumab (humanized monoclonal antibody): phase II prematurely stopped due to infections.
(iii) Ofatumumab (human monoclonal antibody): no studies in SLE.
(iv) Veltuzumab (humanized monoclonal antibody): no studies in SLE.
(v) TRU-015 (engineered protein).
CD-22 antigen
(i) Epratuzumab (humanized monoclonal antibody anti-CD22): phase III study ongoing.
Indirect B lymphocyte targeting
BAFF
(i) Belimumab (LimphoStat B: fully human monoclonal antibody anti-BAFF): FDA approved based on BLISS 52/BLISS 76 phase III studies.
BAFF receptors
(i) Anti-BR3.
(ii) Atacicept (fusion IgG with the extracellular domain of TACI receptor): study in progress.
(iii) Briobacept/BR3-Fc (fusion IgG with the extracellular domain of BAFF receptor—BR3).

BAFF: B lymphocyte Activator Factor belonging to the TNF family; TACI: transmembrane activator and calcium modulator and cyclophylin ligand interactor.

3.13. B Cell Targeted Therapies in SLE. Several B cell molecules can be targeted to treat autoimmune diseases (Table 3). The most widely studied target for achieving B cell depletion in autoimmune disease is the CD20 antigen (human B cell-restricted differentiation antigen), a hydrophobic transmembrane protein with a molecular weight of approximately 35 kDa found on pre-B and mature B cells [120, 121] as well as in over 90% of the B cells in NHL [122]. Pilot studies of an anti-CD20 antibody (rituximab) in SLE were promising [123, 124]. A review of off-label use also suggested significant clinical and serological response [125]. However, two randomized trials showed no superiority of rituximab over standard therapy and did not reach primary or secondary end points [126, 127]. Despite these overall discouraging results, both studies have significant design shortcomings that limit their applicability. A study with another anti-CD20 antibody, ocrelizumab, was stopped prematurely due to an increase of serious infections. As mentioned above, CD22 inhibition with epratuzumab may be an alternative for B cell inhibition in SLE. A phase III study is now undergoing [128].

Another therapeutic approach is the inhibition of BAFF effects on B cell. This inhibition can be done by anti-BAFF or anti-BR3 monoclonal Abs, as well as BR3 or TACI decoy fusion proteins. Selective BAFF blockers prevent BAFF from interacting with its receptors, leaving APRIL available to interact with TACI and BCMA. Drugs in this class include anti-BAFF Ab (Belimumab or LymphoStat B) and a fusion protein consisting of human Ig Fc and of the extracellular BR3 domain (Briobacept, for BAFF-R-Ig). Nonselective BAFF blockers abolish the interactions of both BAFF and APRIL with all their receptors. To date, there is a single drug in this class which is human Ig Fc fused to the extracellular TACI domain (Atacicept, TACI-Ig). Differences in the distribution of the forms of BAFF could denote the potential of patients to respond or to resist to BAFF antagonist therapy. Treatment of B cells with TACI agonist Ab inhibits proliferation *in vitro,* and activation of a chimeric receptor containing TACI intracellular domain induces apoptosis. These results demonstrate also the critical requirement for TACI in regulating B cell homeostasis. The therapeutic effects of anti-BAFF therapy with Belimumab have been demonstrated in patients with SLE, based on two large randomized controlled trials, BLISS 52 and BLISS 76 [129].

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