# Autoimmunity Diseases of the Skin

Guest Editors: Jozélio Freire De Carvalho, Paulo Ricardo Criado, Valéria Aoki, and Yehuda Shoenfeld



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## *Editorial* **Autoimmunity Diseases of the Skin**

#### Jozélio Freire Carvalho,<sup>1</sup> Paulo Ricardo Criado,<sup>2</sup> Valéria Aoki,<sup>2</sup> and Yehuda Shoenfeld<sup>3</sup>

 <sup>1</sup> Rheumatology Division, Aliança Medical Center, Rua das Violetas, 42, AP. 502, 41810-080 Salvador, BA, Brazil
<sup>2</sup> Dermatology Division, Clinic Hospital from Sao Paulo University School of Medicine, 01239-040 Sao Paulo, SP, Brazil

<sup>3</sup> Zabludowitcz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Sackler Faculty of Medicine, Tel Aviv University, 52621 Tel-Hashomer, Israel

Correspondence should be addressed to Jozélio Freire Carvalho; jotafc@gmail.com

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The tegumentary system becomes a scenario for immune responses. The knowledge of these conditions has led to induction of complementary animal models, better knowledge of the pathophysiology, and new tools [1] for diagnosis and therapy of these autoimmune skin disorders.

In this special issue from Autoimmune Diseases we have invited several papers to address the dermatological issue.

One paper of this issue analyzes epidemiological, clinical, and histopathological features of 56 patients with psoriasis in Africa. Severity, HIV associations, and diverse clinical aspects are discussed in this interesting paper. Another paper reviews the genetic aspect of psoriasis and also implications of the pharmacogenomics in predicting responses to therapeutical agents. An interesting recent review on psoriasis immunomodulation and treatment may be obtained at [2, 3]. One of the papers evaluated retrospectively in a large cohort of 155 patients with pemphigus vulgaris the incidence of infections. Interestingly, 94 cases of infection were detected and described. Pemphigus vulgaris was also studied in one paper with the aim to determine the involvement of the anal area in newly diagnosed pemphigus vulgaris patients.

Another paper of this issue reviewed p38 mitogen activated protein kinase (p38 MAPK) in the pathogenesis of pemphigus. P38 MAPK signaling plays a major role in the modulation of immune-mediated inflammatory responses and therefore has been linked with diverse autoimmune diseases.

One paper reviewed vascular alterations in patients with morphea, since it has been proposed that endothelial cell damage may represent the initial and pivotal step in the development of soft tissue changes in morphea.

Another paper of this collection has evaluated the role of interleukin-8 in patients with dermatitis herpetiformis associated with gluten-sensitive enteropathy. It brings a new methodology for treating arthritis. The rational consists that small bowel as a mucosal immune system, responding to gluten ingestion with high levels of interleukin-8, and that the mucosal immune response was associated with the development of the skin lesions in dermatitis herpetiformis. It was previously demonstrated in the Caucasian and it was for the first time shown in the present study in Japanese patients.

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## Research Article

## Severe Skin Forms of Psoriasis in Black Africans: Epidemiological, Clinical, and Histological Aspects Related to 56 Cases

## Komenan Kassi,<sup>1</sup> Oussou Armel Mienwoley,<sup>2,3</sup> Mohamed Kouyate,<sup>4</sup> Sylvanus Koui,<sup>4</sup> and Kouame A. Kouassi<sup>1</sup>

<sup>1</sup> Department of Dermatology and Infectiology, Training and Research Unit of Medical Sciences,

Félix Houphouët Boigny University (FHBU) of Abidjan-Cocody, Abidjan 21 BP 5151, Cote d'Ivoire

<sup>2</sup> Training and Research Unit of Medical Sciences, University of Bouaké, Bouaké 01 BP V18, Cote d'Ivoire

<sup>3</sup> Mother Maria Elisa Andreoli Health Center, Cocody Riviera Palmeraie, Cidex 3, Abijan-Riviera BP 51, Cote d'Ivoire

<sup>4</sup> Department of Histopathology, University Hospital of Treichville, Abidjan 01 BP V3, Cote d'Ivoire

Correspondence should be addressed to Komenan Kassi; siskakomlo@yahoo.fr

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*Bacground.* Psoriasis is an erythematosquamous dermatosis of chronic development. In sub-Saharan Africa, few studies have been focused on complicated forms of psoriasis. *Objective.* The aim is to describe epidemiological, clinical, and histological features of severe skin forms of psoriasis in Cote d'Ivoire. *Material and Methods.* The study was both cross-sectional and descriptive, that focused on patient admitted to the dermatology unit for complicated psoriasis, from January 1st, 1986, to December 31th, 2007. *Results.* Fifty-six patients admitted to hospital for severe skin forms of psoriasis were recorded and included in our study over 7.503 patients hospitalized during the study period. They represented 0.75% of cases. The average age was  $39.6 \pm 3.3$  years. There were 49 male (87.5%) and 7 female patients (12.5%) with a sex ratio of 7. At socioprofessional level, 48 patients (87.5%) were from category 1. Patients' history was dominated by the psoriasis vulgaris. Physical and general signs were dominated by itching (58.9%). The three severe skin forms were observed with predominant erythrodermic psoriasis (60.7%). Fifteen patients (34.9%) were HIV positive. *Conclusion.* Severe skin forms of psoriasis are rare in our setting. But in the quarter of HIV-positive patients, they are dominated by the erythrodermic psoriasis.

#### 1. Introduction

Psoriasis is an erythematosquamous dermatosis of chronic development. It is ubiquitous and seems common in the West where prevalence rate ranges from 2 to 3% in the population at large [1, 2]. The benign forms are the most numerous, around 90%, and raise an aesthetic issue. The severe forms which are life-threatening or threaten the functional prognosis account for about 10% and require an admission to hospital. Psoriasis diagnosis is clinically easy in typical forms. Yet a histological confirmation is required after a biopsy of the skin lesion. Histological images are characteristic with parakeratotic hyperkeratosis and Munro-Sabouraud's microabscesses [2]. In sub-Saharan Africa few studies have

been focused on complicated forms of psoriasis [3]. The objective of this study is to describe the epidemiological, clinical, and histological aspects of psoriasis complicated forms in patients admitted to the Dermatology Unit of Treichville University Hospital.

#### 2. Material and Methods

The study was cross-sectional and descriptive. It focused on all the records of patients admitted to the Dermatology Unit of Treichville University Hospital for complicated forms of psoriasis in the period covering January 1, 1986, to December 31, 2007. In the study records of patients of all sexes and ages suffering from erythrodermic psoriasis, universal psoriasis, or pustular psoriasis confirmed by histology were included. The histological examination has been carried out in the anatomopathology labs of Treichville University Hospital on a sample of the skin lesion biopsy. The sample was stored in 10% formalin. Paraffin inclusion was performed before examining under an optical microscope.

Epidemiological, clinical, and histological data along with HIV status have been recorded in a prescribed survey form.

The socioprofessional status has been subdivided into three categories.

- (i) Category I: patients with monthly pay lower than the index-linked guaranteed minimum wage in Côte d'Ivoire (35,000 FCFA, i.e. 53,8 Euros).
- (ii) Category II: medium ranking civil servants of Ivorian public civil service with an average wage of 137 Euros [3].
- (iii) Category III: patients with more than 300 Euros as monthly wage.

Data analysis has been performed with the software Epi info 6.04 and has consisted in calculating the rates.

#### 3. Results

We selected 56 records of patients suffering from complicated forms of psoriasis out of a total of 7503 hospitalization records during the 22-year study period representing 2.5 cases per year. Cumulative incidence of complicated forms of psoriasis was 0.7%. There were 49 male (87.5%) and 7 female patients (12.5%) which equals a sex ratio of 7. Average age was  $39.6 \pm 3.3$ years with extremes of 4 and 77 years. There were 3 children (5.3%) and 53 adults (94.7%). From the adults, 38 patients (67%) were between 30 and 50 years old. At socioeconomic level, patients earning a monthly income less than 53.8 Euros accounted for 85.7% of cases. Our patients' history was dominated by psoriasis vulgaris in 21 cases (37.5%) followed by medication use in 18 cases (32.1%), tobacco in 14 cases (25%), alcohol in 14 cases (25%), combination of tobacco and alcohol in 8 cases (14.3%), stress in 3 cases (5.3%), and other variables in 7 cases (12.5%). Benzathine-penicillin and non steroidal topical remedies were the most used medecine in respectivily 22% of cases. Three severe forms have been observed. There were erythrodermic psoriasis in 34 cases (60.7%), universal psoriasis in 21 cases (37.5%), and generalized pustular psoriasis in 1 case (1.8%). Physical and general signs were dominated by itching in 33 cases (58.9%) (Table 2). Ungual impairments were observed in 33 patients (58.9%) and were dominated by "thimble-like" aspect in 13 cases (39.4%) followed bysubungual hyperkeratosis and pachyonychia in 4 cases (12.1%), onycholysis in 2 cases (6.1%), and others in 9 cases (27.3%). Patients were screened for HIV infection in 43 cases (76.8%). Fifteen patients (34.9%) were HIV positive with 9 cases of erythrodermic psoriasis and 6 cases of universal psoriasis. Histology involved 11 patients (19.6%). At the epidermic level, hyperkeratosis

TABLE 1: Histological epidermal changes in 11 severe psoriasis cases.

Enidermal changes	Number	Percentage
Epidermai changes	<i>(n)</i>	(n/11)
Hyperkeratosis	11	100.0
Parakeratosis	8	72.7
Parakeratosis and orthokeratosis	3	27.3
Microabscesses	8	72.7
Agranulosis	11	100.0
Thinning of the dermal papilla roof	10	90.9
Interpapillary bud changes		
Acanthosis	10	90.9
Elongation	8	72.7
Club aspect	7	63.6
Exocytosis	8	72.7
Polynuclear and mononuclear neutrophils	5 7	63.6
Mononuclear	1	9.1
Spongiosis	4	36.4
Minimal	3	27.3
Moderate	1	9.1

TABLE 2: Histological dermal changes in 11 severe psoriasis cases.

Dermal changes	Number ( <i>n</i> )	Percentage ( <i>n</i> /11)
Hyperpapillomatosis	9	81.8
Turgidity of dermal papilla	4	36.4
Presence of dilated capillaries	7	63.6
Superficial dermal infiltrate	11	100.0
Lymphocytes	11	100.0
Polynuclear	10	90.9
Neutrophil	8	72.7
Eosinophil	1	9.1
Neutrophil and eosinophil	1	9.1
Histiocytes	10	90.9
Plasmacytes	8	72.7

and agranulosis were observed in all the slides. Munro-Sabouraud abscesses were objectified in 8 slides (72.7%) (Table 1). Histological changes of the dermis were dominated by a lymphocytic infiltrate objectified in all the slides and a hyperpapillomatosis in 9 cases (81.8%) (Table 2).

#### 4. Discussion

The severe psoriasis is rarely seen in the Dermatology Unit of Treichville University Hospital. This rareness has been reported by Kundakci et al. [4] in the Turkish in 2002 with an incidence of 0.07%. Our data is relatively low compared to those raised by Jalal et al. [5] in 2005 in Morocco and Lapeyre et al. [6] in 2007 in France with, respectively, 13 and 4.5 cases per year. This observation should confirm this disease rareness in blacks and particularly in West Africans [3, 7, 8]. Our study has pointed out a clear male predominance with 7 as sex ratio. This male predominance has been reported by many authors [5, 9, 10]. This marked difference observed in our study may be due to the delay in caring for the initially benign skin disorders in male patients for they show little concern for their physical look. Adults accounted for 94.7% and children 5.3%. They were adults of average age (39.6  $\pm$  3.3 years old). This observation is in line with many authors' data [4, 5, 11]. Yet, Fortune et al. [12] in 2010 in Saudi Arabia have observed the severe psoriasis in adults of 22 to 26 years old. Kundakci et al. [4] reported the rareness of the disease in children ( $\leq$ 10 years old; 5.7%).

In our study, the severe psoriasis observed in adults may provide a proof of a late start of the (type II) disease in relation to non pustular clinical forms [1]. More than fourfifths of patients suffering from severe psoriasis were from category I. Public health facilities are visited by poor income patients in most cases due to the social rates charged whereas middle and high income patients would favor private health facilities. Moreover, we should not systematically reject the psychological impact of the bad living condition of category I patients on psoriasis beginning and/or worsening. According to Griffiths and Richards [11], psoriasis is a complex disease combining biological, psychological, and social contributors. Patients have different medical histories. Tobacco intoxication accounted for 25%. Alcohol consumption along with tobacco-alcohol combination accounted for 14.3% of cases. Dereure and Guilhou [8] mentioned tobacco and alcohol to be the psoriasis exogenous risks. Jalal et al. [5] found 8.9% of tobacco intoxication in patients suffering from severe psoriasis. In 2007, in Spain, Huerta et al. [13] reported that tobacco was an independent psoriasis risk factor. In 2008, Kirby et al. [10] observed link between psoriasis severity and weekly alcohol consumption. More than one-third of the patients suffering from severe psoriasis in our study have already been diagnosed with psoriasis vulgaris. The switch from psoriasis vulgaris to severe psoriasis is well known. Jalal et al. [5] in their series have reported a history of psoriasis vulgaris in 89.4% of cases. This precession of the severe psoriasis by the psoriasis vulgaris is caused by some contributors including drugs. Many authors have reported drug involvement in worsening or leading to psoriasis outbreak [2, 5, 6, 13]. Bérard et al. [2] have observed that mechanisms with which environment contributors such as drugs worsen or lead to outbreak are well known. Jalal et al. [5] reported an incidence of 12.5% of patients suffering from severe psoriasis triggered by the use of drug. Lapeyre et al. [6] observed that the erythrodermic psoriasis may be sparked by the introduction of new drugs. Huerta et al. [13] stated that antibiotics' use might cause an existing psoriasis to exacerbate.

The stress may be another contributor likely to lead to psoriasis outbreak or worsen an existing one according to some authors [2, 9, 11, 14]. In our study, 5.3% of patients have pointed out stress presence. However, Huerta et al. [13] stated that there was no connection between psoriasis occurrence risk and stress histories. The three severe skin forms of psoriasis have been observed in our study and are the erythrodermic psoriasis (60.7%), the universal psoriasis (37.5%), and pustular psoriasis (1.8%). Jalal et al. [5] and Lapeyre et al. [6] have reported the three severe skin forms of the psoriasis in their series at the following respective rates: erythrodermic psoriasis (54.4% and 14.2%), universal psoriasis (19.4% and 67.8%), and pustular psoriasis (19.4% and 18.0%). Kundakci et al. [4] reported the pustular psoriasis only with 17 cases. Fatani et al. [9] observed the erythrodermic psoriasis (57.9%) and the pustular psoriasis (42.1%). Our study pointed out rareness of the pustular psoriasis while this clinical form seems to be more common in the Maghreb, in Europe, and in the Middle East. This observation may suggest that there is a difference in the genetic factors associated with the onset of psoriasis between Caucasian and black African patients [8]. Severe skin forms of the psoriasis were accompanied with physical and general signs. Our study provided that there were itching (58.7%), oedema of lower limbs (28.6%), and hyperthermia (26.8%). Fatani et al. [9] have reported itching in 43% of cases. Globe et al. [15] have shown that itching was the most important sign and the most severe in the course of psoriasis. For patients included in this study, itching would cause an important deterioration of the quality of life. Jalal et al. [5] have found hyperthermia in 3% of patients. These physical and general signs are mostly observed in erythrodermic forms of psoriasis. The important impairment of the skin barrier may be the cause of biochemical disorders, thermoregulation changes, and hydroelectrolytic and protein disorders. More than half of our patients (58.9%) had ungual impairments dominated by "thimble-like aspect" (39.4%). Kundakci et al. [4] reported the ungual impairments in 16.4% of cases with "thimble-like aspect" dominating (79.6%). Mak et al. [14] asserted that almost half of the patients suffering from psoriasis had ungual impairment dominated by the "thimble-like aspect." For some authors, HIV infection is a contributing factor to the occurrence of severe and extensive forms of psoriasis [8, 14, 16, 17]. According to most of these authors, there was no change in the incidence of psoriasis patients associated with HIV in comparison to the general population. But, in our series, 15 patients in 43 detected were HIV positive, that is, 34.9% of cases. The prevalence of patients suffering from severe psoriasis associated with HIV seemed high, in 28.8% of cases, yet the study does not allow checking whether severe psoriasis erupted before or after the HIV infection. This limit does not help to assess involvement of HIV infection in occurrence of severe forms of psoriasis. This prevalence observed in our study was lower than in the study conducted in eastern Africa where it was 41.6% of cases (in a population of 61 HIV-positive patients diagnosed for complicated psoriasis) [18]. However, it was higher than in Caucasians. In fact, a study in 2000 on HIV-positive patients showed a prevalence of 2.5% of cases compared to the general population in San Francisco [19]. Another study in Berlin on 700 patients infected by HIV reported a prevalence of 5% of cases, 3 times higher compared to the general population [16]. This high rate of HIV associated with severe forms of psoriasis in our setting could be explained by the high rate of HIV/AIDS incidence and prevalence in sub-Saharan Africa (the most infected region worldwide), in particular in Côte d'Ivoire.

#### 5. Conclusion

Although they are scarce, severe forms of psoriasis are a concern to practitioners for being more often life-threatening

because of the biological disorders and infectious complications they involve. Incidence of these severe cases of psoriasis in HIV-positive patients requires systematic HIV testing.

#### **Conflict of Interests**

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

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## **Review** Article

# Updates on Morphea: Role of Vascular Injury and Advances in Treatment

#### Julio C. Sartori-Valinotti,<sup>1</sup> Megha M. Tollefson,<sup>1</sup> and Ann M. Reed<sup>2</sup>

<sup>1</sup> The Department of Dermatology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA <sup>2</sup> The Department of Pediatrics, Mayo Clinic College of Medicine, Rochester, MN 55905, USA

Correspondence should be addressed to Ann M. Reed; reed.ann18@mayo.edu

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Morphea and systemic sclerosis are fibrosing disorders of the skin that share common inflammatory and immunologic pathways that are responsible for the vascular changes, increased collagen production, and extracellular matrix proliferation seen in both conditions. Recent advances in molecular biology techniques have furthered our knowledge of the potential underlying pathogenic mechanisms and offer new and provocative areas of research for novel diagnostic and therapeutic interventions. This review focuses on the role of vascular injury in the development of morphea, the use of ultrasonography as a diagnostic modality, and well-established and newly proposed treatments.

#### 1. Introduction

Morphea is an inflammatory, fibrosing skin disorder that leads to sclerosis of the dermis and subcutaneous tissue but in some cases may also extend to the fascia, muscle, and underlying bone. Clinically, morphea has an asymmetric distribution and is usually confined to one body area; hence it is also referred to as localized scleroderma. Systemic sclerosis (SSc), however, in addition to symmetric skin changes is characterized by internal organ involvement, sclerodactyly, presence of Raynaud's phenomenon, and nailfold capillary abnormalities. Despite these differences, both entities share common inflammatory and immunologic pathways that are ultimately responsible for the vascular changes, increased collagen production, and extracellular matrix proliferation seen in both conditions. Although the etiology and precise mechanisms that trigger the cascade of molecular events that culminate in skin fibrosis are not fully understood, advances in molecular biology techniques have furthered our knowledge of the potential culprits and offer new and provocative areas of research for novel diagnostic and therapeutic interventions. This brief review will focus on the role of vascular injury in the development of morphea with emphasis on recent basic research data as well as use of ultrasonography as a diagnostic method. Lastly, well-established and newly proposed treatments will be discussed.

#### 2. Clinical Features

Several classification systems have been developed in attempt to grasp the breath of the various forms of presentation of morphea [1, 2]. They are largely based on clinical findings and include, with minor differences, at least four major variants: plaque-type, linear, generalized and a miscellaneous group of morphologically distinct phenotypes.

*Plaque-Type Morphea (Morphea en Plaque or Circumscribed).* It is the most common subtype overall and the most common variant in adults. Most often located on the trunk, it begins as an erythematous-to-violaceous, edematous plaque of several centimeters that extends peripherally over a period of 3 to 5 years before it reaches a plateau phase. This is followed by an involution phase that leaves behind atrophic skin (Figure 1).

Linear Morphea (Including Morphea en Coup de Sabre (Figure 2) and Progressive Hemifacial Atrophy or Parry-Romberg Syndrome). Most common in children and adolescents, it presents as a linear induration on the scalp,



FIGURE 1: Plaque-type morphea.



FIGURE 2: Morphea en coup de sabre.

forehead, trunk, or extremities (Figures 3 and 4), sometimes with involvement of the eye (in the case of facial lesions), underlying fascia, muscle, and bone. The latter may lead to limb atrophy and joint immobilization. Patients with Parry-Romberg syndrome and *en coup de sabre* morphea may also have seizures, headaches, and abnormal intracranial findings on magnetic resonance imaging (MRI) [3]. Linear morphea affecting the mouth has also been described (Figure 5). Antinuclear antibodies, ssDNA, and antihistones antibodies are usually positive.

*Generalized Morphea.* It is defined by the presence of four or more plaque-type lesions affecting two or more body sites or by the insidious onset of a slowly progressing plaquetype morphea on the trunk with eventual involvement of the entire trunk leading to progressive dyspnea due to mechanical restriction of chest cage expansion. Similar to linear morphea, patients in this subgroup have positive serology for antinuclear antibodies, ssDNA, and antihistones antibodies and are more likely to have constitutional symptoms.

*Miscellaneous Group.* Encompasses a variety of phenotypically different lesions including nodular, mixed (combination of two or more variants), guttate, bullous morphea and atrophoderma of Pasini and Pierini.

Irrespective of the clinical subgroup, morphea can be distinguished from SSc by the absence of internal organ involvement, Raynaud's phenomenon and nailfold capillary changes. On the other hand, SSc can be further subdivided into limited SSc (ISSc) and diffuse SSc (dSSC) on the basis of the extent and distribution pattern of skin disease. Sclerodermatous skin changes distal to the elbows or knees are referred to



FIGURE 3: Linear morphea affecting the leg.



FIGURE 4: Linear morphea of the upper extremity.

as ISSC whereas skin thickening proximal to these anatomic landmarks are characteristic of dSSC. A subset of patients with ISSc and calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias comprised the so-called CREST syndrome.

#### 3. Pathogenesis

Vascular Injury as the Crucial Event. It has been proposed that endothelial cell damage may represent the initial and pivotal step in the development of soft tissue changes in morphea and systemic sclerosis. For example, using whole-field digital microscopy and transmission electron microscopy, Frech and coworkers demonstrated that 20 patients with SSc had increased skin interstitial edema, fibrosis, basal lamina lamellation, and endothelial swelling compared to normal controls, irrespective of disease duration, or appreciable clinical features [4]. This is consistent with the clinical findings of Raynaud's phenomenon and nail fold capillary changes seen in the early stages of the disease prior to the development of frank fibrosis. Infection, hypoxia, trauma, radiation, reactive oxygen species, and antiendothelial cell autoantibodies contribute to vascular injury and subsequent recruitment and activation of T and B lymphocytes and mononuclear cells, secretion of proinflammatory mediators and growth factors, endothelial cell apoptosis, and fibroblast activation which in



FIGURE 5: Linear morphea of the mouth. Note subtle changes of the inner upper lip and gingival mucosa around the maxillary right central incisor.

turn leads to vascular and tissue remodeling and fibrosis [5–9].

Under physiologic and pathologic conditions, disruption of the capillary network results in decreased blood flow and tissue ischemia. The ability to withstand hypoxia varies by tissue type and is tightly regulated by hypoxia inducible factors. One of the adaptive responses to diminished tissue oxygen delivery is the formation of new vessels via either angiogenesis and/or vasculogenesis. The former refers to the formation of new vessels from preexisting vessels whereas vasculogenesis represents de novo vessel formation from bone marrow derived endothelial precursor cells (EPC). Cumulative evidence suggests that both processes are defective in SSc despite strong proangiogenic stimuli [10].

Viruses may trigger vascular damage via neointimal proliferation and apoptosis likely through overproduction of profibrotic cytokines including TGF-beta, PDGF-alpha, and PDGF-beta [11]. Cytomegalovirus (CMV) RNA transcripts have been found in the endothelium of patients with sclerodermoid changes [12]. Similarly, parvovirus B19-infected endothelial cells, fibroblasts, and perivascular inflammatory cells of SSc patients have increased expression of TNF-alpha [13] which has been shown to participate in regulation of fibroblast function and endothelial activation [14]. A role for viral infection is further supported by the observation that molecular mimicry between human CMV late protein UL94 and NAG-2, a surface molecule present on endothelial cells and dermal fibroblasts, is responsible for cross-reactivity of human anti-CMV antibodies against the latter and may contribute to chronic sclerodermoid graft versus host disease (GVHD) [15, 16].

Endothelial cell apoptosis is a key feature of SSc and arguably the earliest event [17]. IL-6 and the Fas-pathway have been implicated in endothelial cell apoptosis [18, 19] via mechanisms dependent on the presence of neutrophils and antibody-induced cell-mediated toxicity, respectively. Circulating angiogenic cells are also prone to and undergo apoptosis in SSc through phagocytosis of microparticles and stimulation of acid sphingomyelinase activity [20]. Plasma samples of SSc patients have significantly higher levels of microparticles [21]. They are small, membrane-bound vesicles with altered surface lipids that participate in intercellular signaling [22]. Conversely, dermal fibroblasts are resistant to Fas-mediated apoptosis, perhaps due to deficiency in acid sphingomyelinase, and increased levels of anti-apoptotic proteins cFLIPs and cIAP, partially explaining their survival and contribution to increased extracellular matrix deposition in SSc [23, 24].

Antiendothelial cell autoantibodies (AECAs) likely promote vascular injury, endothelial cell apoptosis, generation of reactive oxygen species, and expression of adhesion molecules on endothelial cells in patients with SSc. They are a heterogeneous group of antibodies against endothelial cellspecific proteins and are present in 22-86% of patients with SSc [25]. Upon interaction with these antibodies, endothelial cells augment the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin resulting in increased leucocyte adhesion. Moreover, they stimulate platelet-derived growth factor (PDGF) pathway and oxidative stress [26]. Beyond their pathogenic role in dermal fibrosis, AECAs have also been linked to complications of SSs, including pulmonary fibrosis and hypertension, and apoptosis of bone marrow EPC [27, 28].

Microarray analyses of EPC from patients with SSc reveal a differential protein expression profile under both basal and hypoxic conditions, with differentiation towards a proinflammatory state. Furthermore, immunohistochemistry of SSc skin samples shows downregulation of TNFS10, TNFAIP3, and HOX-A9 and overexpression of PTGS-2 [29]. Most recently, genomic DNA analysis of eight pairs of monozygotic twins with SSc identified sites that were preferentially hypermethylated or hypomethylated on the X chromosome and corresponded to target genes governing, among other cellular pathways, apoptosis (MTM1), inflammation (ARAF), and oxidative stress (ENOX2) [30]. These latter findings provide some insight into the molecular alterations behind the higher prevalence of morphea and SSc in women.

Vascular abnormalities seem not to be limited to the skin. Patients with SSc have reduced bone marrow vascularity, in spite of normal cell morphology, as measured by microvessel density. Notoriously, peripheral blood mononuclear cells (PMBC) from individuals with SSc release greater amounts of VEGF [31], and its expression is much higher in SSc patients [32, 33] thus suggesting a diminished responsiveness to angiogenic stimuli. Impaired production of TNF-like weak inducer of apoptosis (TWEAK), a newly characterized cytokine, by PMBC may be accountable for aberrant angiogenesis and tissue remodeling in SSc [34]. Lastly, fibroblasts and autoimmunity are also important pathogenic players in morphea and SSc, but in-depth discussion of these topics is beyond the scope of this review.

#### 4. Evaluation

Based on the increasing evidence that vascular changes dominate the early stages of disease development, it is not surprising that there is emerging research looking into ways to rapidly and accurately recognize them. Ultrasonography has gained particular attention as a noninvasive, harmless, and inexpensive diagnostic tool.

Morphea and SSc are characterized by three distinct phases of skin disease: active or edematous; inactive, sclerotic, or fibrosis; and atrophic lesions. Early recognition of the active phase may have both therapeutic and prognostic implications. In a study of 104 morphea lesions, ultrasonography was not inferior to dermatopathologic examination in evaluating active disease. Indeed, when compared to histology, increased cutaneous (dermal or subcutaneous) blood flow and hyperechogenicity of the subcutaneous tissue had both 100% specificity and 100% sensitivity [35]. In keeping with these observations, using fourteen MHz ultrasonography of 16 morphea lesions, hyperechogenicity correlated with the presence of moderate or severe sclerosis on histology. More importantly, ultrasonographic findings were more reliable than clinical-based scores such as the Modified Rodnan Skin Score (mRSS) [36] and have acceptable and reproducible inter- and intraobserver reliability. The inactive and atrophic phases of the disease also exhibit unique sonographic features [37, 38].

Ultrasound can additionally be used to determine the severity of musculoskeletal involvement [39] and endothelial function [40] in SSc and in sclerodermoid GVHD [41]. It has also been shown to be useful in monitoring the response to treatment. For instance, in pediatric patients, the hyperemia and increased echogenicity of active lesions disappeared after successful treatment [42]. In a different series, dermal thickness as measured by ultrasound was decreased in patients treated with phototherapy [43] and topical imiquimod [44]. Due to its depth of penetration, which is a function of frequency, the usefulness of ultrasonography is somewhat limited to the skin and subcutaneous tissue. In this regard MRI is more advantageous and allows for better assessment of deeper structures such as the fascia and underlying muscle [45, 46]. As with ultrasonography, MRI can be resourceful in monitoring disease activity and response to treatment [47].

#### 5. Treatment

The treatment of morphea and skin disease in SSc is challenging, and its efficacy is difficult to assess owing to the absence of validated and standardized outcome measures. Nonetheless, numerous treatment modalities both systemic and topical have been investigated, the majority of which have been abandoned due to lack of response or have not been investigated in larger populations. However, among these interventions, methotrexate (MTX) alone or in combination with systemic steroids and phototherapy have been proven to be beneficial with stronger evidence to support their use.

5.1. Methotrexate. The effectiveness of methotrexate, primarily in conjunction with systemic steroids, has been validated by several retrospective studies. In the recent past, at least six prospective, including double-blind, randomized trials have confirmed the efficacy and safety of this therapeutic regimen. For example, in patients with juvenile morphea, clinical remission for a mean duration of 25 months was achieved with simultaneous use of MTX and prednisone [48]. In another study of pediatric patients with moderate to severe morphea, this combination strategy quickly resulted in clinical improvement, as determined by Modified LS Skin Severity Index, within two months of treatment [49]. Improvement in musculoskeletal involvement has also been observed in a prospective study of adults with deep morphea (mean age 52 years) [47]. When added to MTX and prednisone, imatinib, which inhibits fibroblast activity, halted the progression of skin disease and joint deformity in a 3-year-old patient [50]. MTX likely exerts its antifibrotic effects via inhibition of inflammatory cytokines such as IL-2, IL-4, IL-6, IL-8, and TNF-alpha and adhesion molecules such as ICAM-1 [7, 51, 52].

With regard to the use of MTX in SSc, in 2009 the European League Against Rheumatism/EULAR Scleroderma Trails and Research published recommendations for the management of the multiple manifestations of SSc, including cutaneous involvement. Based on two randomized controlled trials on patients with early diffuse SSc or limited SSc [53, 54], methotrexate was recommended as a firstline treatment for early diffuse SSc (Class A recommendation). Notwithstanding, MTX was superior to placebo in one of these studies [54], whereas the other showed only a trend favoring MTX, but it did not reach statistical significance. Two important considerations can be drawn from these conflicting observations. First, it is conceivable to hypothesize that, relative to morphea, the modest or lack of response to treatment with MTX in SSc is due to the fact that most studies for management of morphea included a combination of MTX and steroids. Second, with widespread involvement, the efficacy of MTX may be reduced or difficult to quantify. Placebo controlled trials assessing the benefits of combined MTX and systemic steroids for diffuse SSc are lacking.

Mycophenolate mofetil is reserved as a second line agent that could be used for treatment of localized and generalized morphea after failed response to MTX and/or phototherapy [55, 56]. Over the past decade, B-cell depletion therapy has gained special attention as a successful intervention for various immune-mediated diseases. Pertaining to its use for sclerodermoid conditions, there are conflicting results in patients with refractory sclerodermoid GVHD either showing improvement [17] or lack of response [57]. A recent case report showed resolution of localized scleroderma with rituximab [58]. Larger case series and prospective studies will help elucidate its potential use as a standard treatment.

5.2. Phototherapy. First documented in 1994 [59], phototherapy for treatment of morphea has since been widely used and studied. By virtue of their longer wavelength and thus deeper penetration, PUVA therapy and UVA1 are the cornerstone of light treatment for localized scleroderma. Its mechanism of action likely involves the combination of various effects such as alteration in cytokine and growth factors expression, modulation of endothelial dysfunction, induction of matrix metalloproteinases that degrade collagen, apoptosis of Langerhans cells and T cells, and inhibition of collagen synthesis [60–63]. The treatment course varies among clinical protocols, but it typically requires approximately 30 sessions before clinical, histological, and ultrasonographic improvement can be appreciated. Furthermore, clinical improvement continues beyond cessation of therapy; thus prolonged treatment is neither needed nor indicated. Phototherapy is effective in all Fitzpatrick skin prototypes and is generally well tolerated, with no serious side effects. The main caveats to the use of UVA1 are the need for prolonged exposure times, diminished effectives after repetitive treatment owing to increased pigmentation, and its availability at specialized centers only. Alternatively, narrowband UVB and broadband UVA can be used with satisfactory results [64– 67].

There is a paucity of data on the use of phototherapy for management of diffuse skin involvement in SSc, but PUVA and UVA1 have been reported to be effective. In a study of 18 patients with acrosclerosis, low dose UVA1 resulted in reduction of clinical score from 19.4 to 14.9; this was accompanied by elevation of dermal collagenase [68]. In a larger series of patients with different skin conditions amenable to treatment with UVA1, 12 patients with SSc/CREST had a moderate response (51-75% improvement) as determined by clinical assessment by the same physician before and after treatment [69]. Another study involving 3 patients with systemic scleroderma also reported improvement in the mRSS after UVA1 treatment [70]. Due to the small number of study subjects, evaluation of limited disease with involvement of the hands only, and the subjective (clinical) assessment of response to treatment, the results of these studies cannot be generalized.

On the other hand, provocative data from the basic research literature may be key in providing the foundation for the development of new therapeutic interventions for morphea and SSc. For instance, the tight-skin (Tsk (-/+)) model of SSc shows abnormal fibrillin-1 expression and chronic oxidative damage that may be responsible for impaired angiogenesis [71]. Circulating endothelial cells and EPCs from patients with SSc treated with iloprost, a synthetic analogue of the vasodilatory prostacyclin PGI2, exhibit upregulation of antiapoptotic genes and genes involved in wound healing [72]. Treatment with recombinant human erythropoietin resulted in resolution of a nonhealing digital ulcer and reduction in apoptotic rates of bone marrow endothelial cells [73] in a patient with SSc. In conclusion, the advent of new technology has furthered our understanding of the imbricated mechanisms behind the development of these debilitating and disfiguring conditions. Nonetheless, placebo-controlled trials exploring these newly discovered pathways are much needed to expand our treatment repertoire. This task is rather challenging because, by virtue of its heterogenous presentation, better measures of disease activity and outcomes are necessary to accurately evaluate evidence-based therapies. Fortunately, research in this area is underway.

#### **Conflict of Interests**

The authors declare that they have no conflict of interests.

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## Clinical Study Anal Involvement in Pemphigus Vularis

#### Somayeh Khezri,<sup>1</sup> Hamid-Reza Mahmoudi,<sup>1</sup> Seyedeh Nina Masoom,<sup>1</sup> Maryam Daneshpazhooh,<sup>1</sup> Kamran Balighi,<sup>1</sup> S. Hamed Hosseini,<sup>2</sup> and Cheyda Chams-Davatchi<sup>1</sup>

<sup>1</sup> Autoimmune Bullous Diseases Research Center, Department of Dermatology, Tehran University of Medical Sciences, Razi Hospital, Vahdate-Eslami Square, Tehran 11996, Iran

<sup>2</sup> Knowledge Utilization Research Center, School of Public Health, Tehran University of Medical Sciences, Tehran 1417613151, Iran

Correspondence should be addressed to Maryam Daneshpazhooh; maryamdanesh.pj@gmail.com

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*Background.* Pemphigus vulgaris (PV) is an autoimmune blistering disease of the skin and mucosa. Anal mucosa may be involved in PV, but the frequency and clinical profile are not fully ascertained. *Objective.* The aim was to investigate the involvement of the anal area in newly diagnosed PV patients. *Patients and Methods.* A total of 168 consecutive newly diagnosed PV patients were enrolled. Anal symptoms and signs, involvement of other body sites, and severity of disease were recorded. *Results.* A total of 47 out of 168 patients (27.9%) had involvement of the anal area. Anal involvement was significantly associated with PV lesions in ophthalmic (P = 0.03), nasal (P = 0.02), and genital mucosa (P < 0.001) but not the oral cavity (P = 0.24). There was a significant association between number of involved mucosal sites and anal involvement (P < 0.001). Anal involvement was associated with oral severity (P = 0.02). Constipation was the most frequent sign (91.5%). *Conclusion.* Anal involvement in PV seems to be more frequent than previously assumed. Routine anal examination is recommended even in asymptomatic patients as anal involvement appears to correlate with the severity of PV.

#### 1. Introduction

Pemphigus vulgaris (PV) is a rare, autoimmune, potentially fatal mucocutaneous bullous disease in which pathogenic autoantibodies are directed against the keratinocyte cell surface molecules desmoglein 3 (Dsg3) and to a lesser extent Dsg1 [1]. The incidence of this disease varies from 0.16 to 1.62 cases per 100,000 with increased incidence in Jews, Indians, and middle easterners [2]. PV is characterized by bullae that typically begin in the oral cavity and may spread to involve the skin. Other mucosal surfaces including conjunctiva, nasal mucosa, pharynx, larynx, epiglottis, esophagus, cervix, vagina, and penile mucosa may also be affected in the course of disease [3-8]. Anal involvement may also be seen in PV but its frequency and clinical profile are not fully ascertained yet [9-12]. The aim of this study was to investigate the involvement of the anal area in newly diagnosed PV patients presenting to the Autoimmune Bullous Diseases Research Center (ABDRC), Tehran, Iran, during a 15-month period.

#### 2. Patients and Methods

This prospective study included 168 consecutive patients newly diagnosed with PV, attending the ABDRC, between October 2009 and January 2011. The diagnosis of PV was based on the presence of clinical features of the disease, including mucocutaneous bullae and erosions along with histopathological (suprabasal cleft and acantholysis) and direct immunofluorescence (lattice-like intercellular epidermal IgG and/or C3 deposits) findings of the biopsy material [13]. Only patients with new-onset untreated disease were enrolled in this study and all subjects underwent physical examination of the anal area. Anoscopy was not performed.

The following information was collected on each patient: (1) age at onset of PV and gender; (2) anal symptoms and signs; (3) nonanal involvement; (4) severity of disease. The severity of skin and mucosal disease was rated based on a grading system proposed by Harman et al. [14] as follows: oral grading: Grade 0, without any lesion; Grade I, minor activity

	Total ( <i>n</i> = 168)	Patients with anal involvement (n = 47)	Patients without anal involvement (n = 121)	<i>P</i> value for differences between patients with and without anal involvement
Age y; mean ± SD	$44 \pm 12.6$	$45 \pm 13.3$	$44 \pm 12.3$	0.6
M : F ratio	1:1.21	1:0.96	1:1.33	0.4
Phenotype- <i>n</i> (%)				
Mucosal	8 (4.8)	2 (4.3)	4 (3.3)	
Mucocutaneous	141 (84)	43 (91.5)	98 (81)	0.08
Cutaneous	19 (11.2)	2 (4.3)	19 (15.7)	
Number (%) of patients with				
Oral involvement	137 (81.5)	41 (87.2)	96 (79.3)	0.24
Genital involvement	44 (26.2)	23 (48)	20 (16.5)	<0.001
Nasal mucosal involvement	38 (22.6)	17 (34)	22 (18)	0.02
Ophthalmic involvement	36 (21.4)	15 (32)	20 (16.5)	0.03

TABLE 1: Characteristics of pemphigus vulgaris patients with or without anal involvement.

SD: standard deviation; M: male; F: female; P value less than 0.05 was considered significant.

(up to three lesions); Grade II, moderate activity (more than three but less than 10 erosions or generalized desquamative gingivitis); Grade III, severe (more than 10 discrete erosions or extensive, confluent erosions, or generalized desquamative gingivitis with discrete erosions at other oral sites). Skin grading: Grade 0, without any lesions; Grade I, minor activity (less than five discrete lesions); Grade II, moderate activity (more than five but less than 20 discrete lesions); Grade III, severe (more than 20 discrete lesions or extensive, confluent areas of eroded skin). Only lesions within 2 cm or less from the anal orifice were considered as anal involvement.

Statistical analysis was performed by Student's *t*-test for differences in means of ages of patients with or without anal lesions. The chi-square test was used to analyze differences between involvement of different anatomical sites in patients with or without anal involvement. *P* value <0.05 was considered significant. Fisher's exact test was used wherever necessary.

#### 3. Results

A total of 168 newly diagnosed PV patients were examined, 92 patients (54.8%) were female, and 76 patients (45.2%) were male (M: F ratio = 1:1.21). Age distribution of PV was from 19 to 72 years with a mean  $\pm$  SD of 44  $\pm$  12.6 years. A total of 47 out of 168 patients (27.9%) had involvement of the anal area. The lesions were confined to the stratified epithelium of the anal region.

Table 1 shows characteristics of patients with or without anal involvement. Twenty-three of 47 patients with anal involvement were female (48.9%) and 24 patients (51.1%) were male (M : F ratio= 1:0.96). Forty-one (87.2%) out of 47 patients with anal involvement had concomitant oral lesions, while this figure was 79.3% (96 out of 121) for patients without anal involvement. The difference was not significant (P = 0.24). On the other hand, anal involvement was significantly associated with PV lesions in other mucosal sites including ophthalmic (P = 0.03), nasal (P = 0.02), and genital mucosa (P < 0.001).

Focusing on severity of oral disease, 54 cases (32.1%) were grade II followed by grade I (51 cases, 30.4%), grade III (32 cases, 19%), and grade 0 (31 cases, 18.5%). Significant association between severity of oral disease and anal involvement was seen (P = 0.02). Skin grading was as follows: 6 cases (3.6%) were grade 0, grade I (39 cases, 23.2%), grade II (56 cases, 33.3%), and grade III (67 cases, 39.9%). There was no association between anal involvement and severity of skin disease (P = 0.06).

Two of our patients (4.3%) showed anal lesions in the absence of involvement of other mucosal surfaces; 16 patients (34%) had involvement in one other mucosal site in addition to anal area, and 29 out of 47 patients with anal lesions (62%) showed involvement of PV in at least two other mucosal sites. There was a significant association between the number of involved mucosal sites and anal involvement (P < 0.001).

Table 2 shows clinical signs and symptoms reported by patients. Thirty out of 47 patients with anal lesions (64%) complained of anorectal symptoms, while 17 patients (36%) were symptom-free. Constipation was the presenting symptom in the majority of cases (73.8%) followed by pain on defecation (50%). Erosion was found in 43 patients (91.5%) and was the most common anal sign with a mean of 1.3 anal erosions per patient.

#### 4. Discussion

Our study shows greater frequency of anal involvement in PV patients than previous reports (27.9%). Although anal area is a well-known site of involvement in PV, the frequency of this involvement is not fully investigated and figures vary widely (2% [2], 9.3% [10, 11], and 16.5% [15]). There are several reasons for the underestimation of the incidence of anal PV: firstly there may be underreporting of cases, because patients are uncomfortable discussing anal symptoms and may attribute them to other causes such as fissures or hemorrhoids [10, 11]; secondly physicians may not routinely examine the anal area, and at last a significant number of lesions may be asymptomatic or subtle. We performed anal

TABLE 2: Symptoms and signs in patients with pemphigus vulgaris.

	Number (%) among total patients	% among patients with anal involvement
Symptoms		
Spontaneous pain	10 (6)	23.8
Bleeding	16 (9.5)	38
Pain on defecation	21 (12.5)	50
Constipation	31 (18.5)	73.8
Total	42 (25)	
Signs related to PV		
Erosion	43 (25.6)	91.5
Leukoedema	16 (9)	34
Perianal involvement	8 (4.8)	17
Pseudofissure	7 (4.2)	15
Ulcer	6 (3.5)	12.8
Vegetant	2 (1.2)	4.3
Bullae	1 (0.6)	2.1
Total	47 (27.9)	
Signs unrelated to PV		
Hemorrhoid	17 (10)	16 (34%)*
Prolapse	1 (0.6)	0**

\* One of the patients shows hemorrhoid in absence of other anal pathology in physical examination.

\*\*One patient showed prolapse in examination without any other anal pathology.

examination regardless of patients' symptoms and severity of disease.

In this study the presence of anal involvement correlated with the severity of mucosal disease as well as with the number of other involved mucosal sites. To our knowledge no study had ascertained anal involvement with the severity of PV. Most of our patients with anal involvement (62%) showed involvement of multiple other mucosal sites. The few patients described in the literature also tended to have involvement of PV at multiple sites, especially the oral mucosa. Epstein et al. reported a patient with widespread involvement at multiple mucosal and cutaneous sites [16]. Malik et al. [17] reported 16 patients with anal involvement and suggested that anal involvement generally occurs in the setting of extensive involvement of PV at other sites. Hotz et al. [15] studied 103 PV patients; seventeen of them had anal involvement. Signs in eleven patients included 9 cases of erosions, one pseudofissure, and one bulla. Regarding the association of anal involvement with the severity of PV, the inclusion of involvement of other mucosa including anal mucosa in any future criteria of severity of PV may be justified.

As expected, erosions were the most common anal sign. Pseudofissure—a linear erosion or ulcer not in a typical site for anal fissure (12 o'clock)—was also a frequent, noticeable finding. Hemorrhoids were found in 17 patients (10%) in anal examination. Interestingly, 16 out of 17 patients with hemorrhoids had anal PV; all of these 16 patients had erosions as the anal sign in their examination. Hemorrhoids are subject to repeated trauma during defecation, and it seems that they could be considered a vulnerable site for erosions in PV. Seventeen patients (10%) showed leukoedema described as pearly white appearance of the mucosa surrounding anal erosions. This has been previously described by Hotz et al. [15].

In conclusion, anal involvement in PV seems to be more frequent than previously assumed. Although most patients with anal lesions were symptomatic and had defecation problems, routine anal examination is recommended even in asymptomatic patients as it appears to correlate with the severity of PV.

#### **Conflict of Interests**

The authors declare that they have no conflict of interests.

#### **Authors' Contribution**

Somayeh Khezri and Hamid-Reza Mahmoudi made equal contribution to this research.

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## Review Article Genetics of Psoriasis and Pharmacogenetics of Biological Drugs

#### Rocío Prieto-Pérez,<sup>1</sup> Teresa Cabaleiro,<sup>1</sup> Esteban Daudén,<sup>2</sup> Dolores Ochoa,<sup>1</sup> Manuel Roman,<sup>1</sup> and Francisco Abad-Santos<sup>1,3</sup>

<sup>1</sup> Servicio de Farmacología Clínica, Hospital Universitario de la Princesa, Instituto Teófilo Hernando,

Universidad Autónoma de Madrid and Instituto de Investigación Sanitaria Princesa (IP), 28006 Madrid, Spain

<sup>2</sup> Servicio de Dermatología, Hospital Universitario de la Princesa, Instituto de Investigación Sanitaria Princesa (IP), 28006 Madrid, Spain

<sup>3</sup> Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, 28006 Madrid, Spain

Correspondence should be addressed to Francisco Abad-Santos; fabad.hlpr@salud.madrid.org

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Psoriasis is a chronic inflammatory disease of the skin. The causes of psoriasis are unknown, although family and twin studies have shown genetic factors to play a key role in its development. The many genes associated with psoriasis and the immune response include *TNF* $\alpha$ , *IL23*, and *IL12*. Advances in knowledge of the pathogenesis of psoriasis have enabled the development of new drugs that target cytokines (e.g., etanercept, adalimumab, and infliximab, which target TNF $\alpha$ , and ustekinumab, which targets the p40 subunit of IL23 and IL12). These drugs have improved the safety and efficacy of treatment in comparison with previous therapies. However, not all patients respond equally to treatment, possibly owing to interindividual genetic variability. In this review, we describe the genes associated with psoriasis and the immune response, the biological drugs used to treat chronic severe plaque psoriasis, new drugs in phase II and III trials, and current knowledge on the implications of pharmacogenomics in predicting response to these treatments.

#### 1. Introduction

Psoriasis is a chronic inflammatory disease of the skin which is characterized by the presence of erythematous scaly plaques [1]. The prevalence of psoriasis is 2-3% worldwide [2]. Psoriasis has a negative impact on the patient's health and quality of life, is associated with serious medical comorbidities, and affects the quality of life of family members [3, 4].

While the exact cause of psoriasis is unknown, genetic and environmental factors play an important role in its development [5].

The environmental factors that appear to influence the course of and the susceptibility to psoriasis include chronic infections, stress, low humidity, drugs (beta-blockers, lithium, antimalarial agents, and interferon), smoking, and obesity [6]. The role of genetics in the pathogenesis of the disease is well documented in family and twin studies [7]. Genetic factors have been well studied in candidate-driven gene-specific studies and in genomewide association studies (GWAS). The genome regions most strongly associated with the development of the disease are associated with the immune system. Interleukin 23 receptor (*IL23R*), *IL12B*, and the human leukocyte antigen Cw6 (*HLA-Cw6*) of the major histocompatibility complex have been strongly associated with psoriasis [8]. Several studies have described the important role of single-nucleotide polymorphisms (SNPs) in the promoter region of the tumour necrosis factor gene (*TNF* $\alpha$ ) [8].

Discovery of such consistent associations has enabled the development of new, more effective drugs with various targets, such as the p40 subunit of IL-12/23 (ustekinumab) and TNF $\alpha$  (infliximab, adalimumab, and etanercept) [1]. Other biological drugs are in phase III trials and include those targeting IL17 (ixekizumab and secukinumab) and the IL17 receptor (anti-IL17R) (brodalumab), all of which are administered subcutaneously [9]. Phase II clinical trials have demonstrated the efficacy and safety of inhibitors of Janus kinase (JAK) (tofacitinib) and phosphodiesterase 4 (PDE4) (apremilast) [3, 10–13], which are administered orally and may be less expensive than biological drugs.

Although these new drugs have improved tolerability and response to treatment, researchers must increase their knowledge of psoriasis in order to find additional options for oral treatment that are safer, more effective, and free of serious side effects. The influence of genetic polymorphisms on the response to biological drugs has been demonstrated in psoriasis [14, 15]; therefore, advances in pharmacogenetics would enable us to tailor treatment.

In this paper, we describe SNPs in genes associated with psoriasis and those associated with the immune response. We also review current knowledge on biological drugs and the impact of polymorphisms on the response to treatment of psoriasis.

#### 2. Genetics of Psoriasis

The immune system plays a key role in psoriasis. Macrophage activation triggers an immune response that releases TNF $\alpha$ , IL1 $\beta$ , IL12, and IL23 [8]. Psoriasis has been associated with genes involved in the immune response, namely, *TNF* $\alpha$ , *IL12B*, and *IL23R* [8]. However, there has also been associated with genes not involved in immune pathways, such as the early differentiation keratinization markers involucrin (*IVL*) and small proline-rich protein (*SPRR*). These genes are involved in atypical epidermal cellular organization and differentiation [16] and are upregulated in psoriasis [17]. A review of the genes and SNPs associated with psoriasis and the immune system is presented in Table 1.

T helper 17 (Th17) lymphocytes release IL22 and IL17 (Figure 1), which are highly expressed in psoriatic skin [18]. These lymphocytes also produce IL2, IFNy, and TNF $\alpha$ (Figure 1) [3]. The proinflammatory cytokine  $TNF\alpha$  plays a key role in the pathogenesis of psoriasis [19, 20]. Polymorphisms in the  $TNF\alpha$  gene may alter the release of this cytokine in healthy subjects [21]. A study performed in Caucasian patients with early-onset psoriasis showed a strong association with  $TNF\alpha$  polymorphisms (rs1800629 and rs361525) (Table 1) [19]. In this sense, a meta-analysis of 18 published case-control studies showed that when the GA + AA genotype was compared with the GG genotype, the risk of psoriasis increased for rs361525 and decreased for rs1800629 in  $TNF\alpha$ gene (Table 1) [22]. Kaluza et al. (2000) observed a decrease in TNF $\alpha$  production in peripheral blood mononuclear cells (47 cases and 43 controls) stimulated with mitogens in psoriatic patients who were A allele carriers of rs361525 (*TNF* $\alpha$  gene) compared to controls [23]. Moreover, the authors found an association between the A allele in rs361525 in the  $TNF\alpha$ gene and increased production of TNF $\alpha$  and early onset of psoriasis (Table 1) [24]. A study performed in an Egyptian population (46 cases and 96 controls) revealed an association

between SNPs in *TNF* $\alpha$  (GG allele in rs1800629) and psoriasis (P < 0.05) (Table 1) [25]. However, no significant differences were found in rs1800629 and rs361525 in this gene in Korean patients with psoriasis (n = 103) and controls (n = 125) [26].

Reich et al. (1999) analyzed rs361525 and rs1800629 in  $TNF\alpha$  gene in patients with type I psoriasis (onset before 40 years; n = 100) and type II psoriasis (onset beyond 40 years; n = 51) and in healthy controls (n = 123) (Table 1) [27]. The results showed that the rs361525\*A allele was more frequent and the rs1800629\* A allele was less frequent in patients with type I psoriasis than in controls (P = 0.0012 and P = 0.041, resp.), although no differences were found between these polymorphisms and type II psoriasis [27]. Nedoszytko et al. (2007) analyzed 166 patients with psoriasis (134 with type I and 32 with type II) and 65 healthy controls [28] and found similar results to those of Reich et al. [27], with a higher prevalence of the A allele in rs361525 and lower frequency of the A allele in rs1800629 (*TNF* $\alpha$  gene) in Caucasian patients than in controls (Table 1) [28]. A previous study performed in 99 Caucasian patients (64 with type I psoriasis and 35 with type II psoriasis) showed decreased frequency of the GG genotype and increased frequency of the GA genotype of rs361525 (TNF $\alpha$  gene) in patients with type I psoriasis compared with controls (n = 123) (Table 1) [29]. Therefore, the GG genotype in this SNP is associated with a lower risk of type I disease [29].

The inflammatory response in psoriasis is characterized by production of TNF $\alpha$ , as seen above, and production of IL1 $\beta$  (Figure 1) [24]. In fact, this proinflammatory cytokine is overexpressed in psoriatic lesions [30]. An *in vitro* study in peripheral blood mononuclear cells (231 cases and 345 controls) revealed an association between the CC genotype in rsl6944 in the *IL1* $\beta$  gene with increased production of IL1RA in response to lipopolysaccharide and IL10 and late-onset psoriasis (over 40 years) (Table 1) [24]. Johansen et al. (2010) observed that expression of IL1 $\beta$  was decreased 4 days after treatment with adalimumab (a human monoclonal antibody against TNF $\alpha$ ) [30].

IL23 regulates and stimulates the activation, differentiation, and survival of Th17 lymphocytes (Figure 1) [31, 32] and is highly expressed in psoriatic lesions [18]. IL12 induces the production of IFN $\gamma$  by Th1 (Figure 1) [33]. The p40 subunit of IL23 and IL12 is the therapeutic target of ustekinumab, a highly effective biological drug, thus suggesting that *IL12* and *IL23* play an important role in psoriasis [33–35]. Polymorphisms in *IL23R* and *IL12B* have been associated with susceptibility to psoriasis in both Caucasian [36, 37] and Asian patients [38, 39].

In Caucasians, a GWAS (1446 cases and 1432 controls) showed the combination of rs3212227 and rs6887695 in *IL12B* as a risk haplotype in psoriasis (Table 1) [37]. The authors also found an association between rs11209026 in the *IL23R* gene and psoriasis [37]. Capon et al. (2007) performed a study of 318 cases and 288 controls and found significant differences between the groups for rs3212227 in *IL12B* (P = 0.036) (Table 1) [40]. A subsequent GWAS with 1810 cases and 2522 controls found an association between SNPs in *IL23R* (rs7530511 and rs11209026) and *IL12B* (rs6887695 and rs3212227) and predisposition to psoriasis in Caucasian



FIGURE 1: Simplified representation of the main mediators of inflammation in psoriasis, the therapeutic targets of biological drugs, and oral alternatives currently under development. Th: helper T lymphocyte; LB: lymphocyte B; APOE: apolipoprotein E; TNF: tumor necrosis factor; IL: interleukin; RANTES, chemokine regulated on activation normal T cells expressed and secreted; CCL: chemokine Cys-Cys motif ligand; MIP: macrophage inflammatory protein; MCP: monocyte chemoattractant protein; PDE4: phosphodiesterase 4; cAMP: cyclic adenosine monophosphate; IFN: interferon; JAK: Janus kinase; STAT: signal transducer and activator of transcription; NF- $\kappa$ BIA: nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor; NF- $\kappa$ B/REL: nuclear factor kappa B/v-rel reticuloendotheliosis viral oncogene complex; a: infliximab, mouse variable region; b: adalimumab, human variable region; c: etanercept, Human TNRFp75 (TNRF2); (–) indicates inhibition and ( $\rightarrow$ ) indicates stimulation.

patients (Table 1) [36]. Smith et al. (2008) found similar results, associating these four SNPs with psoriasis [41], and Liu et al. (2008) identified an association between psoriasis and *IL23R* (rs11209026) and *IL12B* (rs6887695) (Table 1) [42]. Hüffmeier et al. (2009) analyzed the same four SNPs in 1114 patients and found a strong association between rs11209026 (*IL23R*) and rs3212227 (*IL12B*) and psoriasis (Table 1) [43]. Another recent study also associated rs11209026 in *IL23R* gene with psoriasis (Table 1) [2]. Other *IL12B* and *IL23R* susceptibility loci identified in GWAS in Caucasian patients include rs2201841 and rs2066808 (*IL23R*) and rs2082412 and rs2546890 (*IL12B*) (Table 1) [44, 45].

The SNPs rs11209026 in *IL23R* gene and rs3212227 in *IL12B* gene have also been studied in Japanese patients (143 cases and 100 controls), and the A allele (rs3212227) was more frequent in patients with psoriasis than in healthy subjects (Table 1) [46]. In a GWAS performed in a Thai cohort (206 cases and 144 controls), a marginally significant association was found between rs7530511 (*IL23R* gene) and psoriasis (Table 1) [38]; rs3212227 (*IL23R*) was also associated with the disease [38]. However, the authors did not find an association with rs6887695 in *IL12* gene [38]. A GWAS performed in a Chinese population (217 cases and 288 controls) identified other polymorphisms associated with psoriasis in *IL23R* (A allele rs11465817-A allele rs1343152 haplotype) and *IL12B* 

(rs6887695) (Table 1). The SNP in *IL12B* was replicated with 578 cases and 1422 controls, and the authors found a positive association with psoriasis [39].

Nair et al. (2009) found strong associations between psoriasis and other genes: *IL13*, which is involved in Th2 lymphocyte modulation (rs20541); TNF $\alpha$  interacting protein 3 (*TNFAIP3*) (rs610604, rs6920220, rs10499194, and rs5029939 [47, 48]) and TNFAIP3 interacting protein (*TNIP1*), which regulate the activity of nuclear factor kappa B (NF- $\kappa$ B) [33]; *IL1RN*, which inhibits the activity of IL1; and *HLA-C* (rs12191877), which is involved in inflammatory responses [44] (Table 1). In addition, rs610604 (*TNFAIP3*) and rs17728338 (*TNIP1*), but not rs2066808 (*IL23R*) and rs397211 (*IL1RN*), were associated with psoriasis in a casecontrol study (Table 1) [2].

Ellinghaus et al. (2010) studied the TNF receptor-associated factor 3 interacting protein gene (*TRAF3IP2*) and identified an association between 2 SNPs and psoriasis (rs13210247 and rs33980500) (Table 1) [45]. This association was confirmed by Hüffmeier et al. (2010) in 2040 German patients with psoriasis vulgaris [49]. *TRAF3IP2* encodes a protein that interacts with NF- $\kappa$ B/REL (v-rel reticuloendotheliosis viral oncogen) complexes and modulates IL17 pathways [45]. In another GWAS, rs240993 (*TRAF3IP2* gene) was associated with psoriasis in Caucasian patients (Table 1)

	TABLE 1: Single-nucleotide polymorphisms (SN	VPs) in genes as:	sociated witl	1 psoriasis.		
Gene	Role in immune system*	SNP	$MAF^{**}$	Minor allele	Population	References
		rs7530511	0.125	Т	Caucasian, Japanese, Chinese	[33, 34, 36 - 38, 41]
		rs2201841	0.275	C	Caucasian	[44, 45]
IL23R	Encodes a subunit of the receptor required for LL2A signaling. Inis protein associates constitutively with IAK2 and hinds to transcription activator STAT3	rs11209026	0.067	А	Caucasian	[2, 33, 34, 36, 37, 41-43]
		rs11465817	0.279	A	Chinese	[39]
		rs1343152	0.357	C	Chinese	[39]
		rs2066808	0.092	C	Caucasian	[44]
ILIO	Encodes a cytokine produced by monocytes and lymphocytes that downregulates the expression of Th1 cytokines and blocks NF- $\kappa$ B activity. It enhances B-cell survival, proliferation, and antibody production and regulates the JAK-STAT signaling pathway	rs1800896	0.467	Α	Caucasian, Egyptian	[25, 60]
	Encodes a proinflammatory cytokine produced by macrophages. TNF $lpha$ is	rs1800629	0.217	А	Caucasian, Egyptian, Korean	[19–22, 25–29]
INFA	implicated in multiple roles such as cell proliferation, differentiation, and apoptosis	rs361525	0.131	А	Caucasian	[19, 20, 22-24, 26-29]
		rs1799724	0.158	A	Caucasian	$[14]^{#}$
		rs6887695	0.217	Т	Caucasian, Chinese	[33, 36, 37, 39, 41, 42]
IL12B	II.12B is a cytokine expressed by activated macrophages that serves as an essential inducer of Th1 cell development	rs3212227	0.225	U	Caucasian, Japanese, Chinese	[33, 36-38, 40-43, 46, 103]
		rs2082412	0.225	А	Caucasian	[44]
		rs2546890	0.438	G	Caucasian	[45]
GBP6	Interferon induces GBP that hydrolyzes GTP to both GDP and GMP	rs928655	0.288	IJ	Caucasian	[42]
IL6	Encodes a cytokine that induces inflammatory responses through IL6R $\alpha$ and maturation of B cells	rs1800795	0.467	G	Egyptian	[25]
	Encodes a cytokine produced by activated Th2 that is involved in maturation and	rs20541	0.233	Τ	Caucasian	[2, 44, 61]
IL13	differentiation of B cells. ILl3 downregulates macrophage activity and inhibits the	rs848	0.242	Τ	Caucasian	[61]
	production of proinflammatory cytokines and chemokines	rs1800925	0.196	Т	Caucasian	[61]
		rs610604	0.408	C	Caucasian	$[2, 15, 44]^{\#}$
	TNF induces the expression of TNFAIP3, which inhibits NF- $\kappa B$ activation and	rs6920220	0.175	А	Caucasian	[33, 44, 47]
TNFAIP3	TNF-mediated apoptosis. TNFAIP3 is involved in cytokine-mediated immune and	rs10499194	0.175	Т	Caucasian	[33, 44, 47]
	inflammatory responses	rs5029939	0.042	G	Caucasian	[44, 47, 48]
		rs2230926	0.027	G	Caucasian	[15]#
IdINI	Encodes TNFAIP3 interacting protein 1, which plays a role in the regulation of NF- $\kappa B$ activation	rs17728338	0.075	Α	Caucasian	[2, 44]
ILIRN	ILIRN inhibits ILI and modulates immune and inflammatory responses	rs397211	0.164	G	Caucasian	[44]
		rs12191877	0.125	Τ	Caucasian	[44, 45, 51]
HLA-C	HLA class I molecules play a central role in the immune system by presenting	rs10484554	0.135	Н	Caucasian, Chinese	[2, 42, 104]
	peptides derived from endoplasmic reticulum lumen	rs1265181	0.258	C	Chinese	[35, 104]
		rs3134792	0.111	IJ	Caucasian	[105]

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	TABLE 1: Contin	nued.					
Gene	Role in immune system*	SNP	MAF**	Minor allele	Population	References	1
NIF_#BIA	Encodes a member of the NF-kB inhibitor family, which interacts with REL dimers	rs2145623	0.290	C	Caucasian	[45]	1
VIAN-INT	to inhibit NF- $\kappa$ B/REL complexes, which are involved in inflammatory responses	rs8016947	0.465	Т	Caucasian	[50]	
APOE	APOE plays a role in the proliferation of T lymphocytes and protects against some infections in matients with neoriasis [73]	rs429358	0.078	APOE*4	Caucasian	[75]	1
		rs7412		I	Caucasian	[75]	1
VDR	Encodes the nuclear hormone receptor for vitamin D3, which regulates immune response pathways	rs4516035	0.381	С	Caucasian	[20]	
$_{IFN\gamma}$	Encodes a soluble cytokine with antiviral, immunoregulatory, and antitumor properties, and it is a potent activator of macrophages	rs2430561	I	I	Caucasian	[54]	1
IL2	Encodes a cytokine that is important for the proliferation of T and B lymphocytes	rs2069762	I	1	Korean	[53]	1
IL4	IL4 is a pleiotropic cytokine involved in the modulation of Th2 immune responses. IL4 receptor also binds to IL13, which may contribute to many overlapping functions of this cytokine and IL13	rs2243250	0.137	Т	Korean	[53]	1
	Encodes a cytokine that regulates T-cell and natural killer activation and	rs2857261	0.431	ß	Chinese	[69]	
IL15	proliferation. <i>IL15</i> also induces the activation of JAK kinases, as well as the phosphorylation and activation of STAT3, STAT5, and STAT6	rs10519613 rs1057972	0.102	A	Chinese	[69] [69]	
TNFRSFIB	TNFRSFIB is a TNF $\alpha$ receptor that mediates the recruitment of antiapoptotic proteins	rs1061622	0.239	IJ	Caucasian, Japanese	[14]*	1
MCPI	<i>MCP1</i> encodes a cytokine characterized by two cysteines separated by a single amino acid that displays chemotactic activity for monocytes and basophils	rs1024611	0.305	IJ	Caucasian	[12]	1
1 1 ILU		rs3087243	0.460	Α	Caucasian	$[81]^{##}$	1
C1LA4	Encodes a protein which inhibits 1 cells	rs231775	0.389	IJ	Caucasian	$[81]^{##}$	
1 מחות	DEFB4 is a member of a family of microbicidal and cytotoxic peptides made by	rs2740091			Caucasian	[56]	1
DEFB4	neutrophils	rs2737532	I		Caucasian	[56]	
STAT4	In response to cytokines, the STAT proteins are phosphorylated and translocate to the cell nucleus, where they act as transcription activators. STAT transduces IL12, IIL23, and IFN type I signals in T lymphocytes and regulates the differentiation of Th cells	rs7574865	0.230	Ц	Caucasian	[72]	1
IL18	IL18 stimulates production of IFN $\gamma$ in Th1	rs187238	1	I	Japanese	[58]	1
11 10	IL19 is a member of the IL10 cytokine subfamily with a role in inflammatory	rs2243188	0.230	Α	Caucasian	[64, 68]	1
1117	responses	rs2243158	0.085	C	Caucasian	[64]	
06 11	Encodes a cytokine structurally related to IL10 and transduces its signal through	rs1713239	0.177	IJ	Chinese	[65]	1
0771	STAT3 in keratinocytes	rs2981572	I		Caucasian	[64, 66, 68]	
		rsl342642	0.314	А	Caucasian	[67]	1
11.20RA	Encodes a recentor for IL20, a cytokine that may be involved in enidermal function	rs1184860	I		Caucasian	[67]	
		rs1167846	0.246	Т	Caucasian	[67]	
		rs1167849	0.285	Α	Caucasian	[67]	
<i>FD D D D</i>	Encodes an aminopeptidase involved in trimming HLA class I-binding precursors	rs151823	0.093	А	Chinese	[52]	
1 11 11 11 11	so that they can be presented on HLA class I	rs27524	0.332	А	Caucasian	[20]	1
ILIB	Encodes a cytokine produced by activated macrophages which plays an important role in the inflammatory response	rs16944	0.358	A	Caucasian	[24]	

#### Autoimmune Diseases

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	TABLE I. COULD	manin				
Gene	Role in immune system <sup>*</sup>	SNP	$\mathrm{MAF}^{**}$	Minor allele	Population	References
		rs13210247	0.080	IJ	Caucasian	[45, 49]
	Fucodes a motein that interacts with TRAE moteins and nlave a central role in	rs33980500	I		Caucasian	[45, 49]
TRAF3IP2	innate immunity in response to pathogens, inflammatory signals, and stress	rs13196377	0.053	Α	Caucasian	[49]
	) - -	rs13190932	0.058	Α	Caucasian	[49]
		rs240993	0.250	Τ	Caucasian	[50]
IL28RA	Encodes a receptor complex that interacts with IL28A, IL28B, and IL29. The expression of these cytokines can be induced by viral infection	rs4649203	0.239	ß	Caucasian	[50]
TYK2	Encodes a member of the JAK protein family that promulgate cytokine signals by phosphorylating receptor subunits. TYK2 is a component of IFN I and II signaling pathways and may play a role in antiviral immunity	rs12720356	0.124	C	Caucasian	[50]
IFIHI	Encodes a protein that mediates induction of IFN response to viral RNA [83]	rs17716942	0.195	С	Caucasian	[50]
		rs4085613	0.403	Ц	Caucasian	[50]
	Encodes a protein that plays a role in skin barrier function [83]	rs4845454	0.403	C	Caucasian	[50]
LCE		rs1886734	0.407	Α	Caucasian	[20]
		rs4112788	0.403	А	Caucasian	[50]
		rs6701216	0.137	Τ	Caucasian	[42]
		rs4112788	0.417	Т	Chinese	[85]
ZNF313	Encodes a protein that is involved in T-cell activation [83]	rs2235617	0.432	IJ	Caucasian	[50]
		rs495337	0.430	А	Caucasian	[105]
* Data from ] transducer ar monophosph polypeptide ξ MCP: monoc receptor-asso ring finger pr	NCBI web page [57]; ** MAF: minor allele frequency for Caucasian population (data from Hapl d activator of transcription; ThI: type I helper T lymphocyte; TNF: tumor necrosis factor; GBP: gua ate; TNFAIP: TNF-alpha interacting protein; TNIP1: TNFAIP3 interacting protein; ILIRN: interla gene enhancer in B cells inhibitor, alpha; REL: v-rel reticuloendotheliosis viral oncogene; APOE: yte chemoattractant protein; CTLA4: cytotoxic T lymphocyte-associated protein 4; DEFB4: defens ciated factor 3) interacting protein; IRAK: interleukin-1 receptor-associated kinase; TYK: tyrosin- otein 114; <sup>#</sup> association between psoriasis and response to anti-TNF treatment; <sup>##</sup> haplotype GG of i	Map web page [10 mylate-binding pr leukin 1 receptor a apolipoprotein E sin beta 4A; IFN: e kinase; IFIH1: i rs3087243-rs2317;	[6] and Alfree otein; GTP: g untagonist; HI interferon; EF interferon ind 75 associated	d [107]). IL: interlet uanosine triphosph. LA: human leukocy in D receptor; TNF čAP: endoplasmic r uced with helicase i with psoriasis.	ukin; R: receptor; JAK: ate; GDP: guanosine dip te antigen; NF- <i>κ</i> BIA: n RSFI: tumor necrosis fa eticulum aminopeptida: C domain 1; LCE: late c	Janus kinase; STAT: signal hosphate; GMP: guanosine uclear factor of kappa light actor receptor superfamily; se; TRAF3IP: TRAF3(TNF ornified envelope; RNFI14:

TABLE 1. Continued

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[50]. In the GWAS performed by Ellinghaus et al. (2010), also in Caucasian patients, an association was identified between rs12191877 (*HLA-C*) and rs2145623 (nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor gene, NF- $\kappa$ BIA) and psoriasis (Table 1) [45]. Feng et al. (2009) performed a GWAS (1359 cases and 1400 controls) and showed rs12191877 (*HLA-C*) to be a high-risk SNP for psoriasis (Table 1) [51]. The SNP rs8016947 in NF- $\kappa$ BIA was associated with psoriasis (GWAS) (Table 1) [50].

Ellinghaus et al. identified new susceptibility loci [45], such as rs4649203 in *IL28RA* and rs12720356 in the tyrosine kinase 2 gene (*TYK2*) (Table 1) [50]. These authors also found an interaction between *HLA-C* and the endoplasmic reticulum aminopeptidase gene (*ERAP1*) (rs27524) [50]. In a Chinese population, another SNP in *ERAP1* (rs151823) was associated with early-onset psoriasis (less than 40 years) (GWAS, 8312 cases and 12919 controls) (Table 1) [52]. In a case-control study performed in patients with psoriasis (n = 1050; controls n = 1497), the SNPs rs8016947 (NF- $\kappa$ BIA), rs4649203 (*IL28RA*), rs12720356 (*TYR2*), and rs27524 (*ERAP1*) were not associated with the disease [2].

Activation of Th1 lymphocytes was associated with the production of cytokines such as IL2 and INFy [3, 18] (Figure 1). In a Korean population (114 patients and 281 controls), the rs2069762 (G allele) in IL2 conferred a risk of psoriasis, mainly in the late-onset group (Table 1) [53]. As for INFy, rs2430561 has been associated with susceptibility to psoriasis (78 cases versus 74 controls) (Table 1) [54]. Furthermore, production of IFNy was increased by DEFB4 (defensin beta 4A), a microbiocidal and cytotoxic peptide [55]. A significant association was found between rs2740091 and rs2737532 in DEFB4 and predisposition to psoriasis in Caucasian patients (498 cases and 577 controls) (Table 1) [56]. IL18 also stimulates IFNy production [57], and the presence of polymorphisms in the IL18 gene (rs187238) was associated with susceptibility to psoriasis in Japanese patients (Table 1) [58].

Th2 lymphocytes release IL4, IL6, IL10, and IL13 [3] (Figure 1). A study performed in 114 psoriasis patients and 281 controls from Korea showed that rs2069762 (G allele) in IL2 conferred a risk of developing the disease, mainly in lateonset psoriasis (Table 1) [53]. Moreover, the cytokines IL6 and IL10 seem to be important in the development of psoriasis [59]. In an Egyptian population (46 cases and 96 controls), an association was established between psoriasis and SNPs in IL6 (CC genotype in rs1800795) and IL10 (GG genotype in rs1800896) (Table 1) [25]. In addition, Craven et al. (2001) found differences in rs1800896 (IL10) genotype frequencies between patients with late-onset disease (n = 84) and controls (Table 1) [60]. However, results for the associations between rs1800896 in IL10 gene and psoriasis are controversial, since several studies did not find any differences between cases and controls for this SNP [27, 59]. IL13 is involved in the differentiation and maturation of B cells and differentiation and function of Th17 lymphocytes [33]. Julia et al. (2012) found an association between rs20541 in IL13 and psoriasis (Table 1) [2]. Moreover, the CCG haplotype of rs1800925rs20541-rs848 in IL13 was associated with susceptibility to psoriasis in a study performed in 1446 cases and 1432 controls

(Table 1) [61]. In contrast, Duffin et al. (2009) found these associations with psoriatic arthritis, but not with psoriasis [62], and other authors found that rs20541 and rs1800925 in *IL13* gene were involved in psoriatic arthritis but not in psoriasis [63].

Other cytokines and chemokines associated with psoriasis include IL19, IL20, IL15, and MCP1 (monocyte chemoattractant protein). Minor alleles of rs2243188 and rs2243158 in IL19 have a protective effect in patients with the disease (Table 1) [64]. In a case-control study (340 cases and 199 controls), the G allele in rs1713239 (IL20) was associated with psoriasis in a Chinese population (Table 1) [65]. Kingo et al. (2004) found an association between G allele carriers of rs2981572 (IL20) and predisposition to psoriasis in Caucasian patients (Table 1) [66]. Polymorphisms in the IL20 receptor (*IL20RA*) have also been associated with psoriasis (Table 1) [67]. Of note, the haplotype in IL19 and IL20 exhibited a susceptibility factor for the development of psoriasis [68]. IL15 induces the activation of the Janus kinase/signal transducer transcription activation factor (JAK/STAT) pathway and may trigger an immune response in psoriatic lesions [57, 69]. Polymorphisms in IL15 (rs2857261, rs10519613, and rs1057972) have been associated with psoriasis in a Chinese population (Table 1) [69]. However, in a Caucasian population, no clear association was found between rs1057972 and rs10519613 in IL15 gene and psoriasis [70].

MCP1 is a CC-type chemokine that plays a role in the recruitment of monocytes and T lymphocytes in inflammation [71]. Wang et al. (2008) found high serum levels of MCP1 in patients with psoriasis compared with controls [71]. The SNP rs10224611 (GG or AG genotype) in the *MCP1* gene may confer susceptibility to psoriasis (507 cases and 530 controls) (Table 1) [71].

Other genes associated with psoriasis include signal transducer and activator of transcription 4 (*STAT4*), apoliprotein E (*APOE*), vitamin D receptor (*VDR*), and cytotoxic T lymphocyte-associated protein 4 (*CTLA4*). Zervou et al. (2009) found a weak association between the T allele in rs7574865 (*STAT4*) and predisposition to psoriasis (Table 1) [72]. *APOE* may play a role in psoriasis by modifying the proliferation of mitogen-activated T lymphocytes and ensuring protection against some infections (Figure 1) [73]. Other authors have reported the APOE- $\varepsilon$ 4 allele to be a risk factor for the development of severe form of psoriasis [74]. In addition, 2 SNPs in the *APOE* gene (rs429358 and rs7412) have been associated with chronic plaque psoriasis and guttate psoriasis (Table 1) [75].

Several authors have demonstrated the role of *VDR* in the pathogenesis of psoriasis [76, 77]. Rucevic et al. (2009) described possible effects of *VDR* polymorphisms on the immune system, namely, immunomodulation, stimulation of cellular differentiation, and inhibition of proliferation [78]. The TaqI polymorphism (allele T) in *VDR* was associated with familial psoriasis in a Turkish population [79]. In addition, the A allele in rs451635 (*VDR* gene) was protective against susceptibility to nonfamilial psoriasis (Table 1) [76]. In contrast, Zuel-Fakkar et al. (2011) did not find any association between the polymorphisms ApaI and TaqI in *VDR* and psoriasis [77]. CTLA4 is a protein that downregulates activation of T lymphocytes [80]. The GG haplotype of rs3087243-rs231775 in *CTLA4* has been associated with psoriasis, but the analysis of these SNPs individually revealed no statistically significant associations (Table 1) [81]. Thus, in other studies, rs231775 in *CTLA4* gene was not associated with the disease in Korean [82] or Caucasian [80] populations.

Moreover, in a recent review the authors have emphasized other SNPs in genes associated with psoriasis (Table 1) [83]: interferon induced with helicase C domain 1 (IFIH1; rs17716942), late cornified envelope (LCE; rs4085613, rs4845454, rs1886734, rs4112788, rs6701216, and rs4112788), and ring finger protein 114 (RNF114; rs2235617 and rs495337). These genes have also been related with immune system (Table 1): *IFIHI* with response to viral infections, *LCE* with epidermal skin barrier function, and RNF114 with T-cell activation. Although, the SNP rs67011216 in LCE gene has been associated with psoriasis in a GWAS study of 223 patients with psoriasis (91 of them with psoriatic arthritis) [42], other authors did not find this association in patients with psoriatic arthritis (n = 1057 cases and n = 5575controls) [84]. Previously, Zhang et al. (2009) have found an association between rs4112788 in LCE gene and psoriasis in a GWAS performed in Chinese population [85]. A case-control study performed in patients with psoriatic arthritis has found this same association in Caucasian population [86].

In addition, Hébert et al. (2012) supported that the knowledge of risk genes for psoriasis may be useful to predict the response to treatment in patients with this disease [83].

In summary, the literature on the genes involved in immune system that participate in the pathogenesis of psoriasis indicates that *IL23R*, *IL10*, *TNF* $\alpha$ , *IL12B*, *GBP6*, *IL6*, *IL13*, *TNFAIP3*, *TNIP1*, *IL1RN*, *HLA-C*, *NF-\kappaBIA*, *APOE*, *VDR*, *IFN* $\gamma$ , *IL2*, *IL4*, *IL15*, *TNFRSFIB*, *MCP1*, *CTLA4*, *DEFB4*, *STAT4*, *IL18*, *IL19*, *IL20*, *IL20RA*, *ERAP1*, *IL1B*, *TRAF3IP2*, *IL28RA*, *TYK2*, *IFIH1*, *LCE*, and *ZNF313* play an important role in the development of this disease.

#### 3. Pharmacogenetics of Biological Drugs

3.1. Biological Drugs. The use of agents that block the action of TNF $\alpha$  (infliximab, etanercept, and adalimumab) has shown clear benefits in the treatment of patients with inflammatory diseases such as psoriasis [87]. TNF $\alpha$  induces the production of proinflammatory cytokines such as IL1 and IL6 (Figure 1), which in turn limits leukocyte migration and expression of adhesion molecules by endothelial cells and leukocytes. Neutralization of the biological activity of TNF $\alpha$ leads to an overall reduction in inflammation. Although anti-TNF $\alpha$  therapy is safe and well tolerated, some adverse events have been reported [88].

Advances in knowledge of the metabolic pathways involved in the pathogenesis of psoriasis and related diseases have led to the search for new therapeutic targets and the development of new biological drugs [10]. Such is the case of ustekinumab, a novel human immunoglobulin IgG1 $\kappa$  monoclonal antibody that binds strongly to the p40 subunit shared by IL12 and IL23 (Figure 1). This drug was designed to block

the inflammatory cascade of Th1 and Th17 lymphocytes, since the altered behavior of keratinocytes in psoriasis probably results in deregulation of these pathways (Figure 1) [89]. In general, ustekinumab was well tolerated [90].

As mentioned above, psoriasis is mediated by the Th1/Th17 response. New biological therapies—both anti-IL17 agents (ixekizumab and secukinumab) [91, 92] and anti-IL17R agents (brodalumab) [93]—are being developed for the treatment of moderate-to-severe plaque psoriasis (Figure 1). Anti-IL17 drugs are now in phase III trials and may become new alternatives to ustekinumab and anti-TNF therapy [9]. Findings for anti-IL17 and anti-IL17R drugs illustrate the importance of the role of IL17 in the pathogenesis of psoriasis [18, 94].

3.2. Other Treatments of Psoriasis in the Future. Biological drugs are well tolerated and improve the PASI-75 (Psoriasis Area and Severity Index reduction ≥75%) score at week 12 [88, 92, 93, 95, 96]. Their main disadvantages are that injectable administration may cause rejection in some patients. Orally administered alternatives—tofacitinib and apremilast—are being developed (Figure 1).

Tofacitinib is a small JAK1/3 inhibitor molecule that was developed to treat psoriasis and other inflammatory diseases (Figure 1) [97]. The JAK family plays a key role in signal transduction from cytokine receptor in lymphocytes to STAT, which is involved in immune responses (Figure 1) [10, 98].

Apremilast is a PDE4 inhibitor that increases levels of cyclic adenosine monophosphate (cAMP) (Figure 1), which activates the protein kinase A and modulates the cytokines involved in the immune response of psoriasis (decreases TNF $\alpha$ , IL23, and IFN $\gamma$  and increases IL10) [3]. PDE4 inhibitors cause anti-inflammatory activities [99], such as modulation of the synthesis and release of cytokines and chemokines from immune system cells. Stimulation with TNF $\alpha$  and IL1 $\beta$  can release several mediators: IL8, eotaxin-1, macrophage inflammatory protein  $1-\alpha$ (MIP1 $\alpha$ /CCL3), MCP1, and chemokine regulated on activation, normal T cells expressed and secreted (RANTES/CCL5) (Figure 1) [99]. PDE4 inhibitors also suppress the production of inflammatory mediators by Th1 (IL2, IFNy), Th2 (IL4), and macrophages (TNF $\alpha$ ) but increase IL10 synthesis (Figure 1) [99]. Phase II studies have shown an acceptable tolerability and safety profile [100]. Phase III clinical trials of apremilast are ongoing.

Below, we review a selection of pharmacogenetics studies evaluating the efficacy and safety profile of biological drugs.

3.3. Pharmacogenetics. Only two studies have reported the effect of polymorphisms on the response to drugs used to treat psoriasis. In the first, Tejasvi et al. (2012) evaluated associations between two SNPs in *TNFAIP3* (rs2230926 and rs610604) and the response to TNF therapy in a cohort from Michigan (n = 433 patients) and a cohort from Toronto (n = 199 patients), both comprising patients with psoriasis and psoriatic arthritis [15]. The SNP rs610604 in *TNFAIP3* gene had previously been associated with predisposition to psoriasis and psoriatic arthritis [101]. The authors showed

a favorable response to anti-TNF drugs (etanercept, infliximab, and adalimumab) and etanercept in carriers of the G allele of rs610604 in *TNFAIP3* in their Michigan cohort (OR = 1.5 and OR = 1.64, resp.) (Table 1). The T-G haplotype of rs2230926-rs610604 (*TNFAIP3*) was also associated with the response to anti-TNF in this cohort (Table 1). The authors did not find significant differences between rs610604 in *TNFAIP3* gene and adalimumab or infliximab analyzed individually or between the SNPs studied and the response to anti-TNF drugs in the Toronto cohort. The study presented the differences in the results between the two cohorts, stating that the reduced size of the Toronto cohort was a limitation of the study [15].

The other study was performed in 80 Greek psoriatic patients (43 women and 37 men) treated with adalimumab, infliximab, and etanercept. The authors analyzed five polymorphisms in three genes:  $TNF\alpha$  (rs361525, rs1800629, rs1799724), TNFRSF1A (rs767455), and TNFRSF1B(rs1061622) [14]. Genotyping data revealed an association with response to treatment after 6 months; the patients who achieved a reduction in the PASI score >75% were classified as responders and those with a reduction of  $\leq$ 50% were classified as nonresponders [14].

Vasilopoulos et al. [14] found an association between a polymorphism in TNF $\alpha$  (CC genotype for rs1799724; P = 0.027) and in TNFRSF1B (TT genotype for rs1061622; P =0.019) and a better response to anti-TNF treatment (Table 1). The statistical analysis of each agent separately revealed an association between these genotypes and a positive response to etanercept after 6 months of therapy (P = 0.002 and P = 0.001, resp.). However, these SNPs were not associated with a good response to infliximab or adalimumab. The authors explained these differences by the mode of action of biological drugs (etanercept binds to soluble  $TNF\alpha$ , and adalimumab and infliximab bind to transmembrane  $TNF\alpha$ ). The tests of association between the haplotype rs1799724rs1061622 (*TNF\alpha-TNFRSF1B* genes) and the response to anti-TNF drugs showed significant differences (P < 0.05) for CT, CG, and TG. It is important to note that Vasilopoulos et al. excluded rs361525 (TNFα), rs1800629 (TNFα), and rs767455 (TNFRSF1A) from the statistical analysis because of a deviation from the Hardy-Weinberg equilibrium [14]. Nevertheless, other authors have reported that a deviation in Hardy-Weinberg equilibrium indicates a real association between genotype and disease [102].

Before treatment of psoriasis can be personalized, more studies should investigate the polymorphisms presented in this review, as well as other polymorphisms and their possible association with drugs used in the treatment of psoriasis. One recent review reported a role for SNPs in psoriasisrelated autoimmune diseases (psoriatic arthritis, rheumatoid arthritis, and Crohn's disease) that could play a role in the response to anti-TNF drugs [8].

#### 4. Conclusions

Our review focused only on those polymorphisms associated with the immune system and psoriasis. Current knowledge is limited, and many other SNPs not associated with 9

immune system may be implicated in the development of psoriasis. Larger studies are necessary to obtain a better understanding of this complex disease, the pathways involved in its pathogenesis, and its pharmacogenetic implications in order to develop more effective and safer drugs that can be administered on a personalized basis.

#### **Conflict of Interests**

Esteban Daudén has the following conflict of interests: Advisory Board member, consultant, grants, research support, participation in clinical trials, honorarium for speaking, research support, with the following pharmaceutical companies: AbbVie (Abbott), Amgen, Astellas, Centocor Ortho Biotech Inc., Galderma, Glaxo, Janssen-Cilag, Leo Pharma, Novartis, Pfizer, MSD, and Celgene.

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## Review Article Mediators of Pruritus in Lichen Planus

#### Kalina Welz-Kubiak and Adam Reich

Department of Dermatology, Venereology and Allergology, Wroclaw Medical University, Ulica Chalubinskiego 1, 50-368 Wroclaw, Poland

Correspondence should be addressed to Adam Reich; adam.reich@umed.wroc.pl

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Lichen planus (LP) is an inflammatory mucocutaneous disease, showing a wide variety of clinical subtypes. The classic presentation of LP involves the appearance of polygonal, flat-topped, violaceous papules and plaques with reticulated white lines, termed "Wickham's striae". Cutaneous lesions tend to be extremely pruritic, and this symptom does not subside after common antipruritic treatment. Moreover, based on our previous pilot study, it could be stated, that itch is the most unpleasant and bothersome symptom of LP for majority of patients suffering from this disease. However, the underlying mechanisms of itch in lichen planus remain still unknown. In addition, there is no study on mediators of this sensation, but taking into account pathogenesis of LP there are some possible mediators implicated to transmit or modulate itch. In pathogenesis of LP important are such mechanisms as apoptosis, autoimmune reaction, and role of stress. With these pathways some, previously described in other diseases, itch mediators such as cytokines, proteases, and opioid system are connected. Whether these mechanisms are involved in pruritus accompanying LP requires further investigation. Limited knowledge of pruritus origin in lichen planus is responsible for the lack of the effective antipruritic treatments. Here, we describe possible mechanisms participating the pathogenesis of pruritus in lichen planus.

#### 1. Introduction

Lichen planus (LP) is a chronic inflammatory disease involving both the skin and mucous membranes. This is relatively rare disease, occurring in about 0.5% of general population, with the similar incidence in males and females; the disease rarely develops in children [1].

LP shows a wide variety of clinical manifestations, and numerous subtypes of LP have been described, showing variable lesion configuration and morphology, that is, eruptive LP, inverse LP, mucosal LP, lichen planopilaris, hypertrophic LP, bullous LP, actinic LP, annular atrophic LP, erosive LP, pigmented LP, perforating LP, invisible LP, and others. However, all types of LP have similar histology showing band-like lymphohistiocytic infiltrate at the dermoepidermal junction with vacuolar degeneration of the basal layer of epidermis. Necrotic keratinocytes (civatte bodies or cytoid bodies) are extruded into the papillary dermis. Irregular acanthosis may result in a saw-toothed appearance of dermoepidermal junction. Hyperorthokeratosis may also be seen but is rather considered as a feature of lichenoid drug eruption [2]. The classic clinical manifestation of LP involves the presence of polygonal, flat-topped, violaceous papules and plaques with reticulated white lines, termed "Wickham's striae". It is believed that Wickham's striae result from focal hypertrophy of granular layer of the epidermis. Furthermore, LP lesions may arise as an isomorphic response to trauma (Koebner phenomenon). The disease most commonly affects extremities, particularly the flexural areas of wrists and ankles. Oral involvement is present in about 30–70% of patients with LP. Lesions of oral LP most commonly appear as asymptomatic or tender, white, reticulated patches or plaques (reticulated form) or as painful erosions and ulcers (erosive form). LP of the genitalia most commonly presents with pruritus or hyperalgesia and may lead to vaginal discharge or hemorrhage.

Importantly, cutaneous lesions of LP tend to be extremely pruritic and this symptom usually does not subside after common antipruritic treatment. Our preliminary studies indicated that pruritus is the most important and bothersome symptom of the disease for the majority of patients suffering from LP [3, 4]. However, to date, the clinical characteristics and pathogenesis of pruritus in LP are nearly completely unknown.

Itch or pruritus is a cutaneous sensation different from pain. It is evoked by pruritogenic stimuli activating distinct subgroups of dedicated primary afferent C-fibers, including both histamine-sensitive and histamine-insensitive nonnociceptive polymodal nerve fibers, although nociceptive polymodal fibers are also involved to some extend [5-7]. Keratinocytes, leukocytes, mast cells, fibroblasts, endothelial cells, and cutaneous nerves may produce several endogenous pruritogens, including histamine, kinins, proteases, neurotrophins, some opioids, and cytokines [8]. Many of these mediators and modulators released at the periphery can directly activate the itch-sensitive C-fibers by specific receptors on the nerve endings or they can act indirectly by inducing the release of pruritogenic mediators and modulators from other cells [9]. Moreover interactions among them can exacerbate and strengthen itch sensation to promote chronic pruritic diseases [10].

Although the exact pathogenesis of LP is still not fully elucidated, here we would like to discuss some of possible pruritic mediators and mechanisms which may be involved in pruritus present in LP.

#### 2. Interleukin 31

LP results from an autoimmune reaction, and it is believed that cell-mediated autoimmunity directed against keratinocytes of basal layer results in the formation of subepithelial infiltrate, composed initially of CD4+ lymphocytes and, subsequently, CD8+ cytotoxic cells. Activated lymphocytes produce a variety of cytokines, and it seems very probable that at least some of these cytokines might also assist in the development of itch in LP.

Some previous studies suggested that interleukin 31 (IL-31) and its receptor components IL-31RA and OSMR could be a key cytokine pathway involved in itching which accompanies a number of inflammatory skin conditions, mostly atopic dermatitis [11–13]. IL-31 is a newly discovered, T-cellderived, short-chain member of the alpha-helical family of IL-6 cytokines. IL-31 receptors were found to be localized in dorsal root ganglia, but itch is rather induced by binding of this cytokine to receptors located on sensory neurons in the skin. Transgenic mice that overexpress IL-31 developed severe pruritus and pruritic skin lesions. In an AD-like murine model (NC/Nga mice), high IL-31 mRNA expression was associated with scratching behavior, while an anti-IL-31 antibody reduced scratching desire [12, 13].

It was also reported that TNF- $\alpha$ , a proinflammatory cytokine which plays an important role in the pathogenesis of LP and is elevated in the skin of patients with LP, may stimulate IL-31 expression, a phenomenon that might be responsible for escalation of itch sensation in LP [14, 15]. Ongoing studies should verify the hypothesis, whether IL-31 is indeed a key player involved in the pathogenesis of pruritus in LP and whether therapies directed against this cytokine will provide benefit for treated patients.

#### 3. PAR: Protease Activated Receptors

Elevated production of proinflammatory cytokines leads to increased expression of HLA-DR antigens, intercellular adhesion molecule (ICAM) and Fas receptors which cause apoptosis of keratinocytes. Activated T lymphocytes are attracted to the dermoepidermal junction, where they induce apoptosis of basal layer of keratinocytes; T-cell surface CD95L (Fas ligand) binds to CD95 (Fas) on the keratinocyte surface and activates the caspase cascade resulting in keratinocyte apoptosis [16]. These proteases, mainly of caspase family, play a crucial role in apoptosis, but it was also described that some enzymes released from apoptotic cells may activate protease activated receptors (PAR).

PAR belongs to the family of G-protein-coupled receptors. Activation of PAR is initiated by the cleavage of the N terminus of the receptor to generate a new tethered ligand terminus, which activates PAR itself. Synthetic peptides which have an amino acid sequence similar to the tethered ligand are also able to activate PAR. Four PAR subtypes PAR-1 to PAR-4, have been identified so far [17, 18]. Tryptase acts on PAR-2 and on PAR-1, but only at high concentration, trypsin on PAR-1, PAR-2, and PAR-4 but not on PAR-3; thrombin acts on PAR-1, PAR-3, and PAR-4 but not on PAR-2, while kallikreins (KLK), mainly KLK5 and KLK14 act only on PAR-2 [18, 19]. Remarkably, PAR-2 has been recently shown to be involved in chronic itch, suggesting that proteases from apoptotic cells may partake in pruritus pathogenesis [17]. One study also reported that mice which overexpressed epidermal KLK7 displayed massive itchy behavior [20]. Another study demonstrated that trypsin-induced scratching behavior in mice was inhibited by a PAR-2 blocking peptide, suggesting the role of serine protease/PAR-2 signaling in pruritus [21, 22]. PAR-2 is reported to interact synergistically with transient receptor potential (TRP) vanilloid type 1 (TRPV1), which belongs to the superfamily of TRP channels, thereby amplifying itch sensation. These findings suggest that serine protease inhibitors or PAR-2 antagonists might be a promising therapeutic tool for the management of itching in the future and could possibly help to break the vicious itchscratch cycle in pruritic dermatoses [23, 24]. Furthermore, expression of PAR has been shown to be increased in diseases with hypertrophic granular layer such as LP, and it is quite likely that PAR receptors are indeed involved in itch which accompanies LP.

#### 4. Toll-Like Receptors

Another potential mechanism possibly taking part in pathogenesis of LP is the activation of toll-like receptors (TLRs). TLRs have recently emerged as key sensors of invading microbes, acting through recognition of pathogen-associated molecular patterns (PAMP) [25]. Recognition of ligands by the TLR leads to a series of signaling events resulting in the induction of acute host responses necessary to kill pathogens. In addition to PAMP, TLRs bind endogenous molecules such as heat shock proteins. TLRs are also responsible for the induction of dendritic cell maturation, which is responsible and necessary for the initiation of adaptive immune responses by producing large amounts of various cytokines activating other components of immune system, mainly lymphocytes [26].

Some members of the TLR family are also involved in the pathogenesis of autoimmune and chronic inflammatory response. In addition, TLR7 was described as a novel receptor mediating itch sensation [27]. These receptors were found to be localized in dorsal root ganglia and on sensory neurons in the skin. Interestingly, their agonists relieved pruritus in laboratory experiments. Referring to the participation of TLRs in the pathogenesis of LP, it seems reasonable to evaluate their role as possible itch mediators in this disease.

#### 5. Opioid Receptors

Some authors suggested a close relationship between LP and emotional stress. Thus, it seems probable that the opioid system in the skin may be another potential player of pruritus in LP. It was supposed that activation of  $\mu$ opioid receptors induces pruritus, while activation of  $\kappa$ opioid receptors exerts an opposite effect [28]. However, the pathogenesis of opioid-induced itch is still not completely understood, albeit some mechanisms have been proposed. First refer to the influence of opioid system on the production of pruritogens or other cytokines in keratinocytes [29–31]. There have been numerous studies regarding the immune actions of opioids, and immune cells have been identified as their targets. Activation of  $\kappa$ -opioid receptors decreases the inflammatory response by downregulating several cytokines and chemokines. Meanwhile, activation of  $\mu$ -opioid receptors may induce a proinflammatory response [28]. Another mechanism of itch sensation is the activation of  $\mu$ - and/or  $\kappa$ opioid receptors directly on sensory neurons [32, 33]. Lately it was reported that some  $\mu$ -opioid receptor-immunoreactive nerve fibers expressed gastrin-releasing peptide, which may be a marker for itch-specific nerves [34, 35]. Future studies should indicate which mechanism is indeed involved in chronic pruritus pathogenesis and whether activation of opioid system results in itch accompanying LP.

#### 6. Conclusions

Summarizing, itch is an important and burdensome symptom of LP; however, this symptom has been poorly studied in LP. Pathogenesis of itch in LP is still indifferently understood, and there is no effective therapeutic modalities alleviating pruritus in patients suffering from this disease. We hope that in the near future new studies will be initiated to better characterize and understand itch in LP. We do believe that such studies may help in the development of new effective antipruritic strategies for LP.

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## *Review Article*

## p38 MAPK Signaling in Pemphigus: Implications for Skin Autoimmunity

Athanasios Mavropoulos,<sup>1,2</sup> Timoklia Orfanidou,<sup>2</sup> Christos Liaskos,<sup>2</sup> Daniel S. Smyk,<sup>2</sup> Vassiliki Spyrou,<sup>3</sup> Lazaros I. Sakkas,<sup>4</sup> Eirini I. Rigopoulou,<sup>4</sup> and Dimitrios P. Bogdanos<sup>1,2,4</sup>

<sup>1</sup> Cellular Immunotherapy and Molecular Immunodiagnostics, Institute for Research and Technology-Thessaly (I.RE.TE.TH), 41222 Larissa, Greece

<sup>2</sup> Institute of Liver Studies, Transplantation Immunology and Mucosal Biology,

King's College London School of Medicine at King's College Hospital, Denmark Hill Campus, London SE5 9RS, UK

<sup>3</sup> Department of Animal Production, Technological Educational Institute of Larissa, 41110 Larissa, Greece

<sup>4</sup> Department of Medicine, Faculty of Medicine, School of Health Sciences, University of Thessaly, Viopolis, 41110 Larissa, Greece

Correspondence should be addressed to Dimitrios P. Bogdanos; dimitrios.bogdanos@kcl.ac.uk

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p38 mitogen activated protein kinase (p38 MAPK) signaling plays a major role in the modulation of immune-mediated inflammatory responses and therefore has been linked with several autoimmune diseases. The extent of the involvement of p38 MAPK in the pathogenesis of autoimmune blistering diseases has started to emerge, but whether it pays a critical role is a matter of debate. The activity of p38 MAPK has been studied in great detail during the loss of keratinocyte cell-cell adhesions and the development of pemphigus vulgaris (PV) and pemphigus foliaceus (PF). These diseases are characterised by autoantibodies targeting desmogleins (Dsg). Whether autoantibody-antigen interactions can trigger signaling pathways (such as p38 MAPK) that are tightly linked to the secretion of inflammatory mediators which may perpetuate inflammation and tissue damage in pemphigus remains unclear. Yet, the ability of p38 MAPK inhibitors to block activation of the proapoptotic proteinase caspase-3 suggests that the induction of apoptosis may be a consequence of p38 MAPK activation during acantholysis in PV. This review discusses the current evidence for the role of p38 MAPK in the pathogenesis of pemphigus. We will also present data relating to the targeting of these cascades as a means of therapeutic intervention.

#### 1. Introduction

The skin represents the first organ of the body exposed to the external environment and thus serves as the primary barrier of the immune defense system. Its key role is to maintain protection against hazardous environmental threats such as microorganisms and viruses [1, 2]. The epidermis is the outmost cellular tissue of the skin and expresses several proteins that orchestrate the essential protective functions. Inflammatory mediators such as prostaglandins, histamines, cytokines, and chemokines are synthesized and secreted from keratinocytes regulating the skin's immune responses [3, 4].

When this epithelial barrier is compromised due to the deterioration of skin tissue integrity, patients are at high risk of fluid and electrolyte loss, as well as infection. If left untreated, conditions can be fatal. Even though the mechanisms of skin damage are essentially the same as the ones that control protective immunity and despite the evolution of sophisticated anti-inflammatory and tissue repair mechanisms, the formation of immune complexes against self-antigens (a hallmark of autoimmunity) initiates a sustained inflammatory response characterized by autoreactive immune cells, cytokines, and autoantibody production [5–8].

Pemphigus signifies a distinctive skin-specific acquired autoimmune disease characterized by intraepidermal blistering, which is induced by autoantibodies against desmosomal cadherins, desmoglein 1 (Dsg1), and Dsg3 [9–11]. Three typical variations are known and are classified as pemphigus vulgaris (PV), pemphigus foliaceus (PF), and some other variants distinguished by the degree of inflammation, the level of separation in the epidermis, and immunologic properties of autoantigens. PV-IgG-targeted cells and triggered signaling pathways are tightly linked to the secretion of cytokines and chemokines that initiate and perpetuate inflammation and subsequent tissue damage [12–14].

This review will discuss up to date evidence of the role of certain key signaling pathways such as p38 MAPK in the pathogenesis of pemphigus, with particular emphasis on the protagonist cells. These data may help us to better understand the signaling cascade pathways of other distinct immunemediated skin pathologies such as psoriasis [15–18].

#### 2. Pemphigus Vulgaris: An Ideal Model to Study Autoimmunity

PV is a potentially lethal autoimmune blistering disease that affects the skin and mucous membranes [23]. It is a relatively rare disease with an incidence of about 1-3.5 cases per 100,000 individuals annually worldwide, being more frequent amongst middle aged individuals. Despite its low frequency, research on PV has benefited from some distinct advantages fundamental for the elucidation of its molecular pathogenesis and the development of unique targeted therapies [24, 25]. Firstly, a distinct clinical pathology is easily observable; secondly, the molecular targets (autoantigens and reactive autoantibodies) are clearly defined; thirdly, some successful in vivo models are readily available; and lastly but not the least, the skin is easily accessible to topical and systemically delivered pharmaceutics. Detection of tissue-specific and serum autoantibodies and further characterization of their molecular specificity are mandatory for the diagnosis of autoimmune blistering diseases. For this purpose, various specific immunoassays, including immunofluoresence, enzyme-linked immunosorbent assay and immunoprecipitation, have been developed [26-28].

The trademark of PV histopathology is intraepidermal blistering-associated acantholysis. Acantholysis is defined as the loss of adherence between epithelial cells and structural components maintaining cell-cell and cell matrix adhesion in the skin and mucous membranes [29]. The junctions that facilitate cohesiveness between epithelial cells are termed desmosomes. Desmosomes are principally located in tissues that experience mechanical forces such as the skin and heart and function as anchor sites for cytoskeleton microfilaments [30]. The desmosomal proteins responsible for mediating cellular adhesion are called cadherins and include the Dsg and desmocollins (Dsc). These constitute the protein components of desmosomes that are responsible for mediating cellular adhesion [31]. In the epidermis, a total of four Dsg (Dsg1-4) and three Dsc (Dsc1-3) isoforms are expressed. Autoantibodies directed against the Dsgs are typical in PV, and several studies have demonstrated that antibodies directed against Dsg1 and Dsg3 induce acantholysis within the epidermis and mucous membranes [32-34]. In contrast, PF is characterized by antibodies directed against Dsg1 only [35]. Seminal studies by the group of Jensen and Lazarus have suggested that keratinocyte inflammatory responses are probably involved in PV-IgG-induced acantholysis [36, 37], but the direct link with Dsgs was not addressed at that time.

The expression of Dsg3 is mainly confined to stratified epithelia. In the epidermis, it is expressed throughout the basal and the spinous layers. Dsg3 inactivation is sufficient to induce the characteristic blistering pathology of PV patients [38, 39]. Mice injected with Dsg3-specific IgG purified from PV patients have been shown to develop acantholysis. Similarly, genetic deletion of Dsg3 or Dsc3 in mice results in a phenotype resembling PV in its mucosal-dominant form [40-42]. Dsg3-deficient littermates suffer from oral erosions and epidermal blistering in areas subjected to extensive mechanical stress. Recombinant Dsg3 can also be used to deplete patient sera of disease-causing antibodies, demonstrating that autoantibodies are indeed directed against Dsg3 and induce blistering. The exact mechanism of this histopathology is defined loss of keratinocyte adhesions by binding of anti-Dsg3 antibodies to Dsg3 through steric hindrance, internalization of Dsg3, changes in molecular integrity, and subsequent intracellular signal transduction [43].

Prevention of Dsg3 endocytosis and/or inhibition of downstream signaling pathways can prevent PV autoantibody-induced loss of adhesion in both cell culture and animal model systems [21]. Tyrosine kinase initiated pathways, protein kinase C (PKC), RhoA, and c-myc have all been implicated in the series of events leading to loss of adhesion in keratinocytes treated with PV IgG [44, 45]. Particularly convincing data has recently been published regarding the involvement of the p38 mitogen-activated protein kinase (MAPK) pathway, which has been linked to both Dsg3 endocytosis and the loss of keratinocyte adhesion in response to PV IgG [19, 21, 46-48]. Interestingly, the pathogenic activity of polyclonal PV IgG can be attributed to p38-MAPKdependent clustering and endocytosis of Dsg3, whereas pathogenic monoclonal Dsg3 antibodies can function independently of this pathway [49]. Detailed evidence is beginning to accumulate that activation of signaling molecules may have an important role in the ability of pathogenic pemphigus IgGs to induce blistering. In fact, both p38 and downstream mediators such as heat shock protein (HSP) 27 may be part of this important process [19].

#### 3. p38 MAPK Pathway Activation, Detection, and Relevance to Skin Autoimmunity

The p38 MAPK signaling pathway is a critical participator in the regulation of cellular and humoral autoimmune responses [50, 51]. Usually initiated by cellular stresses or inflammatory cytokines, its main task is to orchestrate cytokine gene expression including tumor necrosis factor (TNF)- $\alpha$  and interferon- $\gamma$  (IFN- $\gamma$ ) by means of transcriptional and posttranscriptional mechanisms such as stabilization of mRNA transcripts [52-56]. P38 MAPK is also important for cellular survival, proliferation, differentiation, and apoptosis [57]. An increasing number of studies have provided data demonstrating the significant role of this cascade in the pathogenesis of several immune-mediated diseases, including rheumatoid arthritis (RA), Sjögren's syndrome, systemic lupus erythematosus (SLE), autoimmune hepatitis (AIH), and psoriasis to name a few [58]. Defects in p38 MAPK pathway can explain the increased expression of proinflammatory cytokines seen in psoriasis [59]. For instance, there is increased TNF- $\alpha$  protein expression, but similar mRNA levels, in lesional compared with nonlesional psoriatic skin, demonstrating that TNF- $\alpha$  expression in psoriatic skin is regulated posttranscriptionally by p38 MAPK, and therefore p38 signaling pathway can be targeted therapeutically [60–62].

The activity of p38 MAPK can be studied in available experimental models and clinical material such as peripheral blood mononuclear cells (PBMCs) and pathological tissue from patients with autoimmune diseases [63]. The conventional techniques for p38 detection include western blotting, immunoprecipitation, and confocal immunofluorescence microscopy that detects the active p38 kinase translocated to the cell nucleus. However, these applications require large populations of homogenous cells, and the data obtained are not fully quantitative. Extended culturing of primary cells is also mandatory in order to maximize cell densities. It should be noted that extensive culturing periods, often in the presence of Interleukin (IL)-2 and other growth factors, can affect or bias signaling analyses that depend on interaction of cell receptors and phosphorylation of intracellular proteins.

Optimized protocols based on sensitive phospho flow cytometry has been recognised as promising alternatives for the investigation of the phosphorylation of p38 MAPK within different PBMCs [64-66]. Phospho-specific flow cytometry technology may help us to better understand the enigmatic role of this signalling cascade in the induction of autoimmunity, as well as its role in immunosuppressive-induced remission [67]. Its main advantage is that it delivers extremely rapid, sensitive, and fully quantifiable observations. Moreover, it allows multiparametric analysis of samples containing mixed subpopulations such as PBMCs. This is feasible upon successful combination of fluorochrome conjugated antibodies against surface markers such as anti-CD3 and anti-CD56 and intracellular epitopes such as phospho-p38 [63]. Therefore, direct analysis of rare populations becomes feasible, as does multiparametric detection of several epitopes within these cells. We have optimized methodology for the successful application of phosphor-specific flow cytometry in order to detect phosphorylated p38 MAPK within innate immune cells such as NK and NKT [63]. In addition, we have provided technical instructions permitting simultaneous flow cytometric measurement of p38 MAPK phosphorylation and intracellular cytokine production. This might be of special value in cases of autoimmunity or cancer where the unavailability of numerous cells from immune-compromised individuals hampers analysis of their biological responses.

Given the key role p38 MAPK signaling pathway plays in inflammatory responses through the production of cytokines and inflammatory mediators, its inhibition is considered to be a promising target for chronic inflammatory diseases [50, 68]. Several pharmaceutical companies have invested heavily on the development of agents specifically inhibiting p38 MAPK activation. An increasing number of novel p38 MAPK inhibitors have been used in experimental studies and clinical trials and have helped us to further define the role of p38 MAPK [69–71]. For instance the  $\alpha$ -selective p38 MAPK inhibitor, SCIO-469, acts as a topical anti-inflammatory agent via the p38 MAPK pathway to reduce neutrophil-induced

acute inflammation in the skin, and observations in clinical models suggest that selective p38 MAPK inhibition may be an effective therapeutic strategy to manage acute skin inflammation [72].

#### 4. p38 MAPK and Pemphigus Vulgaris

The p38 MAPK signaling pathway participates in chronic inflammatory skin pathologies, as p38 MAPK inhibitors reduce skin inflammation in various rodent models of human skin diseases [73]. For example, p38 MAPK inhibitors protect the epidermis against the acute damaging effects of ultraviolet irradiation by blocking apoptosis [74]. In addition, topical p38 MAPK inhibition reduces dermal inflammation and epithelial apoptosis in burn wounds [75, 76].

What is of particular importance is the finding of an autoantibody-mediated autoimmune skin disease where p38 MAPK is directly activated by the same autoantibodies and implicated in disease pathogenesis [12]. This occurs in PV and (regarding p38 MAPK) David Rubenstein's lab has demonstrated that pemphigus-IgG binding to keratinocytes augmented intracellular phosphorylation events [13, 19]. In their studies, cultured human keratinocytes with IgG purified from patients with PV activate the p38 MAPK and lead to phosphorylation of the small HSP 27, actin cytoskeleton reorganization, as well as the collapse of the intermediate actin microfilaments. In isolated tissue cultures where p38 MAPK was inhibited, the phosphorylation of HSP 27 and cytoskeleton reorganization was greatly diminished. The same group of investigators have generated a mouse model in which PV was passively transferred. Inhibiting p38 MAPK in these mice prevented the formation of blisters in vivo. It also appears that inhibiting p38 MAPK blocks the pathogenic IgG from inducing blistering in the skin in mouse models of PV. Importantly, p38 MAPK and HSP27 phosphorylation has also been observed in the epidermis surrounding the skin lesions of both psoriasis and PV [77, 78] (Figure 1).

Timing of p38 MAPK activation is critical for understanding the hierarchy of signaling events leading to acantholysis. Time course experiments demonstrated that the activities of Src and EGFRK peak at 30-60 min after exposure to PV IgG suggesting that engagement of Src/EGFRK is a critical step that generates signals from ligated antigens to the intracellular effectors such as p38 affecting keratinocyte adhesion and viability [46]. Phosphorylated p38 MAPK by antibodies from PV patients can be detected as early as 15 min; however, the majority of PV IgGs induced peak p38 activity after a prolonged incubation. In cultured keratinocytes, p38 knockdown abrogated desmosomal Dsg3 reduction by PV mAbs, whereas exogenous p38 activation caused internalization of Dsg3, Dsc3, and desmoplakin [21]. It was therefore suggested that p38 MAPK may not be essential for the loss of intercellular adhesion in PV but may function downstream to augment blistering via Dsg3 endocytosis.

Additionally, Marchencko et al. have demonstrated the circulation of antimitochondrial antibodies against various poorly defined mitochondrial autoantigens in sera from PV patients [47]. These autoantibodies appear to have the ability



FIGURE 1: The role of p38 MAPK in the induction of pemphigus vulgaris (PV). There are at least three potential p38-MAPK-related mechanisms involved in the pathogenesis and/or the progression of PV. (a) The binding of pathogenic autoantibodies targeting Dsg3 in keratinocytes initiates an array of signals leading to the activation of p38 MAPK cascade with subsequent phosphorylation of MAPKAPK (mitogen-activated protein kinase-activated protein kinases 2/3) and heat shock protein 27 (Hsp 27). The final outcome of these events is actin filaments reorganization and induction of acantholysis [19]; (b) p38 MAPK, MAPKAPK, and Hsp27 may form a complex (signalosome) that regulates the reorganization of actin filaments and the induction of acantholysis [20]; (c) studies in p38–/– keratinocytes demonstrate a p38 MAPK-independent blister formation. The subsequent activation of this pathway, however, can lead to *de novo* depletion of multiple desmosomal molecules, further facilitating spontaneous blister formation [21]. This latter hypothesis indicates that p38 MAPK signaling may not be responsible for the induction of PV but could play a role for the progression of the disease.

to penetrate keratinocytes and react with mitochondrial proteins. Data provided in the same study suggested that downstream signaling of antimitochondrial antibodies involves JNK and late p38 MAPK activation, further underlying the important role for these signaling cascades in non-Dsg3 or Dsg1 autoantibody-specific autoimmune blistering.

Whether pemphigus-IgG-augmented p38 signaling is directly or indirectly linked to the pathogenesis of pemphigus through the secretion of cytokines and chemokines that initiate or activate inflammatory events remains debatable [79, 80]. Natural killer (NK) cells are professionally programmed to induce rapid bursts of cytokines and chemokines during innate immune responses [81]. Recent studies on innate immunity have noticeably expanded our understanding of the function of NK cells in health and disease [82]. Besides their well-known functions in cancer and autoimmunity, significant contributions from NK cells to allergies, and various skin diseases have emerged. NK cells play an important part in skin autoimmunity, such as in psoriasis [83]. Evidence that NK may also participate in the pathobiology of pemphigus is beginning to emerge. Takahashi et al. showed that a higher percentage and number of NK cells are present in the peripheral blood of PV patients [84]. These expressed the CD69 cell surface marker indicating that the NK cells were activated and expanded in the peripheral blood of PV patients. They also exhibited lower levels of perforin and granzyme defects in their cytotoxic ability and increased levels of Interleukin 10 expression.

In another study by Stern et al., CD4+ T cells from the PBMCs and perilesional skin of PV patients were cocultured

with CD56+ CD3-NK cells from the PBMCs of the same patients [22]. In the presence of Dsg3 peptides, CD4+ T cells proliferated, indicating that NK cells functioned as antigenpresenting cells. Supernatants from these cocultures and serum from the same patients with active PV had statistically significantly elevated levels of IFN-y, IL-6, and IL-8, compared with controls indicating that the NK cells stimulated CD4+ T cells to produce proinflammatory cytokines in a similar manner to other autoimmune pathologies [85]. These data have led to the formulation of a hypothetical scenario for the p38 MAPK-induced pathogenesis of PV (Figure 2). In the microenvironment of the affected tissues, NK cells may present Dsg3 peptides to resident and circulating CD4+ T cells which proliferate and produce various cytokines. Both Dsg3-reactive Th1 and Th2 cells have been detected at similar frequencies in studied PV patients, yet the numbers of autoreactive Th1 cells exceeded those of Th2 cells in chronic active PV. The in-vivo-activated NK cells may also travel to lymph nodes, spleen, and bone marrow and stimulate B cells to produce high levels of pathogenic autoantibodies [8, 86].

NK cells are controlled by activating and inhibiting receptors. The killer-cell immunoglobulin-like receptors (KIRs) and their cognate HLA class I ligands are crucial for NK regulation. Activating KIR genes have been utilized in risk estimation for autoimmunity [87]. In a recent report, activating KIR and HLA Bw4 ligands have been demonstrated to be associated with decreased susceptibility to PF [88]. Activation of NK cells appears to be critical for the clinical manifestations and subsequent clinical stages of autoimmune blistering diseases. Based on the latest observations in the literature, a vital role of NK cells in the pathogenesis of PV is brought to light. However, more research is necessary in order to delineate the precise molecular mechanisms and activated signaling pathways in NK cells. There is currently no thorough information to the activation of p38 MAPK in NK and NKT cells from PF and PV patients.

#### 5. Targeting p38 MAPK in Pemphigus: Corticosteroids versus Monoclonal Antibodies

The mainstay of PV treatment is systemic administration of corticosteroids aiming at reducing inflammation and autoantibody production [89, 90]. PV patients have traditionally been treated with glucocorticoids and adjuvant immunosuppressive therapies. However, clinical remission has been achieved in only 30% of patients. Methotrexate, mycophenolate mofetil, or cyclophosphamide are typically introduced as steroid sparing agents, since many patients experience severe side effects from glucocorticoid-induced immunosuppression [91, 92]. Newer modalities of treatment, such as B-cell depletion therapy with rituximab, have begun to show some promise in such patients. Rituximab is increasingly used in patients with PV with inadequate response to conventional therapy [93, 94].

Corticosteroids dampen inflammatory responses, and this occurs at least in part by inducing rapid and prolonged expression of MAP kinase phosphatase 1 (MKP-1), which potently inactivates p38 MAPK [95]. Any attempt for discussing therapeutic inhibition of p38 MAPK pathway stems from the fact that natural negative feedback mechanisms exist to guarantee that, within cells, MAPKs are not activated ad infinitum. In this regard, MAP kinases can themselves induce different types of protein phosphatases that dephosphorylate and cease their function. These are termed dual-specificity phosphatases (DUSPs), and each member of the DUSP family has a unique set of properties including tissue distribution, subcellular localization, and precise substrate affinity and specificity [96]. For example, MKP-1 targets primarily p38 MAPK contrary to MKP-2 which preferentially dephosphorylates ERK and JNK [97-99]. The strength and duration of p38 MAPK activation are often the critical determinant of cellular responses regulated by the action of these phosphatases [100]. In psoriatic lesions the p38 MAPK negative feedback mechanism provided by DUSP1 seems to be defective. DUSP1 mRNA expression was demonstrated to be significantly downregulated in psoriatic skin lesions as compared with paired samples of nonlesional psoriatic skin [101]. Downregulation of DUSP1 may contribute to the sustained inflammatory response seen in psoriasis. However, there is no information available regarding the expression of MKP-1 in PV. Yet, inhibition of p38 MAPK by MKP-1-dependent mechanisms leads to downregulation of IL-8, one of the cytokines implicated in the pathogenesis of PV [22, 56, 98].

As previously mentioned, p38 MAPK inhibition prevented blistering in the murine model of PV. Early clinical studies focusing on targeting p38 MAPK in inflammatory disorders, such as rheumatoid arthritis, Crohn's disease, and psoriasis, raised significant safety concerns [70]. A novel allosteric p38 MAPK inhibitor, KC-706 (Kémia Inc), has been tested during a phase II multicenter, open-label trial in patients with active PV [102]. The safety and efficacy of KC-706 in accomplishing remission, while maintaining stable doses of corticosteroids, were monitored over a 3-month period. KC-706 had been administered orally to 15 patients with PV. Approximately half of the patients exhibited a partial response to treatment, while the remaining patients either failed to improve or deteriorated [102]. Disappointingly, the inhibitor had to be abandoned due to severe adverse reactions. Thus the notion that p38 MAPK signaling is directly involved in the pathogenesis of PV, and hence p38 MAPK inhibitors can be ideal treatment agents needed to be treated with caution. Nevertheless, there is a continuous generation of biologics targeting the p38 pathway in a more sophisticated manner aiming to avoid undesirable side reactions [14, 24, 48, 103]

Currently, there is an advent of certain other biologics and monoclonal antibodies that have shown particular potential in the treatment of PV. For example rituximab is a chimeric monoclonal antibody that targets the CD20 molecule on B cells resulting in their depletion [104]. Administration of rituximab has been approved for certain lymphomas, RA, chronic lymphocytic leukemia, and Wegener's granulomatosis [105, 106]. It is also used in certain cases to treat PV [107–109]. The rationale for the use of rituximab in patients with PV is based on its ability to deplete CD20+ B cells that presumably produce pathogenic antibodies. Antibodies



FIGURE 2: Schematic representation of the suggested interplay between natural killer (NK), CD4+ T, B cells, and polymorphonuclear cells (PMN) during pemphigus pathogenesis [22]. NK cells accumulate from the bloodstream to the epidermis and act as antigen-presenting cells by introducing desmoglein (Dsg3) peptides to CD4+ T cells. p38 MAPK phosphorylation within lymphoid subpopulations such as NK and CD4+ T cells induces expression of IFN- $\gamma$ , IL-6, and IL-8 that amplify the inflammatory response, MHC-II presentation, and autoantibody production by B cells. The activated T cells further enhance B-cell anti-Dsg3 secretion and presentation to epidermal cells. Anti-Dsg3 activates the p38 MAPK pathway within keratinocytes leading to MAPK-activated protein kinase 2 (MK-2) mediated heat shock protein 27 (Hsp27) phosphorylation, actin microfilament reorganization, and acantholysis.

against CD20 can activate complement and induce antibodydependent cellular cytotoxicity (ADCC) in B lymphocytes. In B lymphocytic leukemia cells, cross-linking of rituximab induced strong and sustained phosphorylation of p38 MAPK [104]. Introduction of the p38 inhibitor completely blocked signaling downstream of p38, which was evident by the absence of MK2 activity and significantly reduced the degree of anti-CD20-induced apoptosis. Therefore the chimeric anti-CD20 antibody rituximab induces apoptosis in B-cell chronic lymphocytic leukemia cells through a p38-mitogen-activated protein-kinase-dependent mechanism. Yet, in 2F7 non-Hodgkin's lymphoma cells, rituximab inhibited p38 MAPK and NF-kappaB activity and downregulated IL-10 expression via Sp1 [110]. Interestingly, other monoclonal antibodies such as the anti-TNF monoclonal antibody adalimumab (Humira) rapidly inhibit p38 MAPK activity in lesional psoriatic skin preceding clinical improvement [62, 101]. The activities of ERK1/2, MSK1/2, and MK2 and the levels of TNF- $\alpha$  were also reduced. The clinical benefits of anti-TNF antibodies adalimumab (Humira), etanercept (Enbrel), and infliximab (Remicade) in the treatment of PV have been previously reported [111, 112].

#### 6. Concluding Remarks

It has become apparent that p38 MAPK is involved in skin autoimmune diseases such as pemphigus. Nevertheless, its targeting can lead to completely unpredictable outcomes and certain adverse side effects depending on the different cells participating in skin autoimmune pathogenesis. PV pathogenesis is still disputed, and treatment remains perplexing [113].

Activating p38 MAPK in autoreactive B cells will enhance their apoptosis and cessation of autoantibody production.

As for keratinocytes, the *de facto* inflicted cell type in PV, a different strategy might be necessary since apoptosis and acantholysis appear to be tightly linked to each other. The time course of p38 MAPK activation, as well as the ability of inhibitors of p38 MAPK to block activation of the proapoptotic proteinase caspase-3, suggests that induction of apoptosis is a consequence of p38 MAPK activation during acantholysis in PV [13]. Therefore, acantholysis can be prevented by inhibitors of p38 MAPK signaling kinases, in addition to other known targets such as the mammalian target of rapamycin, Src, EGFR kinase, phospholipase C, calmodulin, and protein kinase C, as well as inhibitors of executioner caspases [114].

In PV, skin infiltrating NK, NKT, and T cells, prolonged p38 MAPK activity will favor an uncontrolled selfperpetuating inflammatory loop and generation of autoreactivity. If we are to induce apoptosis of autoreactive T cells, it may be wise to spare their assassins, namely, NK and NKTs. Therefore, careful monitoring of p38 signaling events within each cell type is of paramount importance. The advent of phospho-specific flow cytometry technology allows multiparametric analysis of rare populations and multiparametric detection of several epitopes within those cells. A typical example of how powerful this technology can become is the analysis of p38 phosphorylation within rare human CD3+ CD56+ cells [63]. We are currently capable of detecting p38/IFN-y double positive NK and NKT cells using optimized phospho-flow cytometry protocols. This has been tested in cells from healthy individuals and in peripheral blood NK and NKT cells from patients with AIH and primary biliary cirrhosis (PBC) [115–119]. In AIH, p38 MAPK pathway is activated in the NKT cells, and the magnitude of this activation parallels the disease activity status of the patient [67]. Phospho-p38 MAPK positive NKT cells were more frequent in patients tested at diagnosis than in patients with immunosuppressive drug-induced remission.

The holy grail of current research in PV and PF is to be able to achieve and maintain clinical remission without extensive use of corticosteroids. This is crucial since glucocorticosteroids can only block but not reverse acantholysis. Since p38 MAPK is phosphorylated in PV and is one of the targeted molecules by glucocorticoids, advancing our knowledge on the exact kinase phosphorylation/dephosphorylation events in every single cell involved can provide fresh impetus for further research into the precise role of this enigmatic signaling cascade in pemphigus and other autoimmune skin diseases. This will greatly facilitate to resolve debates on autoimmune molecular pathogenesis and open novel perspectives on successful targeted therapies.

#### Abbreviations

- AIH: Autoimmune hepatitis
- Dsc: Desmocollin
- Dsg: Desmoglein
- DUSP: Dual specificity phosphatase
- HSP: Heat shock protein
- IFN: Interferon
- IL: Interleukin

- LPS: Liposaccharide
- MAPK: Mitogen activated protein kinase
- PF: Pemphigus foliaceus
- PV: Pemphigus vulgaris
- RA: Rheumatoid arthritis
- Th1: T-helper 1
- TNF: Tumor necrosis factor.

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## Clinical Study

## **Pemphigus Vulgaris and Infections:** A Retrospective Study on 155 Patients

# Nafiseh Esmaili,<sup>1,2</sup> Hossein Mortazavi,<sup>1,2,3</sup> Pedram Noormohammadpour,<sup>2</sup> Majid Boreiri,<sup>2</sup> Tahereh Soori,<sup>4</sup> Iman Vasheghani Farahani,<sup>5</sup> and Mitra Mohit<sup>6</sup>

<sup>1</sup> Autoimmune Bullous Diseases Research Center, Tehran University of Medical Sciences, Tehran 1199663911, Iran

<sup>3</sup> Razi Hospital, Vahdat Islamic Square, Tehran 1199663911, Iran

<sup>5</sup> Sharif University of Technology, Tehran 1136511155, Iran

<sup>6</sup> Islamic Azad University, Tehran Medical Branch, Tehran 193951495, Iran

Correspondence should be addressed to Hossein Mortazavi; mortazma@sina.tums.ac.ir

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*Background*. Autoimmune process and immunosuppressive therapy of pemphigus vulgaris would predispose the patients to infections. *Aim*. We aimed to study the prevalence of infection and pathogenic agents in pemphigus vulgaris patients admitted to dermatology service. *Material and methods*. This retrospective study was conducted on 155 pemphigus vulgaris patients (68 males, 87 females) admitted to dermatology service between 2009 and 2011. In this study, the diagnosis of pemphigus vulgaris was confirmed by light microscopic and direct immunofluorescence findings. Data were collected through a questionnaire. *Results*. Of 155 pemphigus vulgaris patients, 33 had infection at admission and 9 acquired nosocomial infection. In addition, 37 cases of oral candidiasis and 15 cases of localized herpes simplex were recorded. Totally, 94 cases of infection were recorded. The occurrence of infection was significantly related to the severity of disease, number of hospital admissions, and presence of diabetes mellitus. The most common pathogenic germs isolated from cultures were *Staphylococcus aureus* and *Escherichia coli*. *Conclusion*. Severity of pemphigus vulgaris and diabetes were directly related with tendency to infections. Staphylococcus aureus and Escherichia coli were the most common pathogenic agents. Due to limitations of retrospective study, a prospective study is recommended.

#### 1. Introduction

Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are organ-specific autoimmune bullous diseases characterized by loss of cell adhesion (acantholysis) and blister formation [1, 2]. These dermatoses are proven to be induced by autoimmune phenomenon [1–3]. Considering this etiology, immunosuppressive therapies are the main treatments available for these disorders. Infections are important complications in these patients attributable to disruption of the epidermal barrier due to the disease itself and immunosuppression induced by treatment [4, 5].

There are many reports regarding predisposition to infections due to immunosuppressive therapy and the immunocompromised state of pemphigus patients [6, 7]. PV has a high prevalence (30 per 100,000 inhabitants) in Iran; in this regard, we have studied this autoimmune disease from many different points of view [8–10]. The aim of the present study was to determine the rate of infection and pathogenic agents in PV patients admitted to dermatology inpatients service through a retrospective study.

#### 2. Material and Methods

This retrospective study was performed on 155 PV patients (87 females, 68 males) admitted to the dermatology service of Razi Hospital of Tehran in Iran, between 2009 and 2011. The mean age of patients was  $41.66 \pm 13.29$  years (range: 14–70). Of 155 admitted PV patients, 104 patients (67.10%) were first

<sup>&</sup>lt;sup>2</sup> Department of Dermatology, Tehran University of Medical Sciences, Tehran 1199663911, Iran

<sup>&</sup>lt;sup>4</sup> Infectious Diseases Specialist, Razi Hospital, Tehran University of Medical Sciences, Tehran 1199663911, Iran

TABLE 1: Prevalence of all infections in PV patients.

Diagnosis	No. of patients (% of 155)	Treatment
Oral candidiasis	37 (23.87%)	Systemic itraconazole
Herpes simplex	15 (9.68%)	Systemic Acyclovir
Skin infection	16 (10.32%)	According to pathogenic agent
Urinary bacterial infection	13 (8.39%)	According to pathogenic agent
Pulmonary infection	13 (8.39%)	Empirical regarding infectious man suggestion
All	94 (60.65%)	_

TABLE 2: Frequency of infection regarding admission of patients (excluding oral candidiasis and herpes infections).

		Infection		
Admission	No infection	At admission	Nosocomial infection	Sum
First admission	85	15	4	104
Multiple admission	28	18	5	51

admitted and the 51 remaining patients (32.90%) had multiple admissions.

Patients with a clinical diagnosis of PV with compatible histopathology and direct immune fluorescence (DIF) findings confirming the clinical diagnosis of PV entered the study. Light microscopic and direct immunofluorescence findings in favor of pemphigus vulgaris were suprabasal bullae and acantholysis and IgG and C3 depositions in the intercellular regions of epidermis, respectively. The severity of PV was evaluated by a "severity index for pemphigus," namely, mild, moderate, and severe [11]. All of the PV patients contributing to this study (mild, moderate, and severe) were hospitalized in the dermatology wards of Razi Hospital regardless of severity of the disease. Accordingly, 43 patients (27.74%) had mild, 98 patients (63.23%) had moderate, and 14 patients (9.03%) had severe forms of the disease.

Regardless of the severity of pemphigus, the patients were treated with 2 mg/kg/day prednisolone and 2.5 mg/kg/day azathioprine [12]. In this study, 18 patients with mild disease with upper limit of the normal range of liver function tests (aspartate aminotransferase = 42 U/L, alanine transaminase = 41 U/L) or lower limit of the normal range of white blood cells count  $(4 \times 10^3/\text{microliter or less})$  who were not suitable for azathioprine adjuvant therapy were treated with 2 mg/kg/day prednisolone alone. 117 patients with mild, moderate, and severe disease (mild = 25, moderate = 88, and severe = 4) were treated with 2 mg/kg/day prednisoloneand 2.5 mg/kg/day azathioprine. Twenty PV patients with moderate and severe disease (moderate = 10, severe = 10) with upper limit of the normal range of liver function tests who were not suitable for azathioprine adjuvant therapy were treated with 2 mg/kg/day prednisolone and 2 g/day (4  $\times$ 500 mg/day tablet) mycophenolate mofetil [13].

TABLE 3: Frequency of infection regarding severity of PV patients (excluding oral candidiasis and herpes infections).

		Infection		
Severity	No infection	At admission	Nosocomial infection	Total infection
Mild	36	7	0	7
Moderate	71	20	7	27
Severe	6	6	2	8

The phenotype of PV recorded in order of frequency was mucocutaneous in 104 (67.10%), cutaneous in 30 (19.35%), and mucosal phenotype in 21 patients (13.55%).

Demographic data including age, gender, number of admissions, severity of the disease, underlying medical disorders such as history of diabetes and hypertension, treatment protocols, and infections recognized during the admission period were registered in the appropriate questionnaires. The ethical committee of Tehran University of Medical Sciences approved the study.

2.1. Statistical Analysis. Data were collected by questionnaire and analyzed by statistical software, SPSS. Chi-square test and Student's *t*-test were used for data analysis. *P* value less than 0.05 was assigned as statistically significant.

#### 3. Results

In total 94 cases of infections were recorded (Table 1). Fifty two patients had a clinical diagnosis of oral candidiasis and localized oral herpes simplex. Excluding these 52 patients, 42 patients had pulmonary, bacterial skin, and urinary infections.

Excluding oral candidiasis and herpes infections, with regard to the rate of infection in men and women (20/68 versus 22/87), there was no statistically difference between the two genders.

From the 104 first admitted PV patients, 19 patients (18.27%) had infections; while from the 51 patients with multiple admissions to hospital, 23 (45.10%) had infections (P < 0.001) (Table 2).

With regard to 42 patients with pulmonary, bacterial skin, and urinary infections, 33 patients had infections at admission (day 0 to 2), while 9 patients were infected from day 3 and thereafter. Namely, these 9 patients had hospital-acquired infection (nosocomial infection).

Severity of the disease is shown in Table 3. Regarding the severity of disease, the rates of infection between mild, moderate, and severe were significantly different (P = 0.011).

With regard to 42 patients with pulmonary, bacterial skin, and urinary infections, 16 patients had skin infection, 13 patients had urinary infections, and 13 patients had pulmonary infections (Table 1). The results of cultures of skin and urinary tract infections and percentage of resistant antibiotic to pathogenic agents are shown in Table 4.

Table 5 presents the data regarding the relationship between type of drug therapy regimen with frequency of

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Site of involvement	Germ	Percentage	Resistant to antibiotic	Percentage
			Penicillin	60%
			Cefazolin	40%
		Cephalexi Ampicillin Clindamyc	Cephalexin	26.7%
	Stathylococcus aurous	15 (03 7%)	Ampicillin	20%
Skin	Siuphylococcus uureus	15 (95.770)	Clindamycin	20%
			13.3%	
		PercentageResistant to antibioticPenicillin Cefazolin Cefazolin Cephalexin Ampicillin Clindamycin Vancomycin 		
			Cefotaxime	6.7%
	Other	1 (6.3%)	—	_
			Cefixime	57.1%
			Gentamicin	57.1%
			TMP	42.8%
	Escherichia coli	7 (53.8%)	Ampicillin	28.6%
		, (0010,0)	Amikacin	28.6%
			Ciprofloxacin	28.6%
Uripary tract			Cefotaxime	14.3%
ormary tract			Gentamicin	100%
	Pseudomonas	2 (15.4%)	Cefixime	50%
			Amikacin	50%
	Proteus	1 (78%)	Ampicillin	100%
	1701203	1 (7.070)	Cefixime	100%
	Other	3 (23.0%)	TMP	66.7%
	Other	5 (25.070)	Ampicillin	33.3%

TABLE 4: Pathogenic agents and percentage of resistance to antibiotics in order of frequency in skin and urinary tract infections of PV patients.

TABLE 5: Frequency of infections regarding regimen of immunosuppressive therapy (excluding oral candidiasis and herpes infections).

Type of drug therepy		Infection		Total infaction
Type of drug incrapy	No infection	At admission	Nosocomial infection	Total Infection
Prednisolone	14	4	0	4
Prednisolone + azathioprine	88	20	9	29
Prednisolone + mycophenolate mofetil	11	9	0	9

infections. The difference between 3 immunosuppressive therapy regimens was not significant (P = 0.151).

Of 155 patients, 14 patients were diabetic. The rate of infection in diabetic versus nondiabetic PV patients was 50% and 24.82%, respectively (P = 0.044).

In PV patients with nosocomial infection, the mean duration between admission to hospital and onset of infection was  $13.22 \pm 5.7$  day (range: 4–22). The mean dose of prednisolone at the time of nosocomial infection onset was  $55.8 \pm 17.9$  mg/day.

No mortality was recorded in this study.

#### 4. Discussion

PV is a well-known autoimmune disease [14]. Nowadays, the relationship between autoimmunity, immunodeficiency, and infection is well recognized. It is believed that autoimmunity and immunodeficiency are not separate entities, but rather some connection exists between them [15, 16]. On the other

hand, hospitalization in addition to immunosuppressive therapy would predispose the PV patients to infection.

Our search in the literature revealed some similar studies performed in other countries [6]. In our study, 60.6% of PV patients had infections, while in the study of Belgnaoui et al., 68% of patients had infections. Overall, our results are similar to the study of Belgnaoui et al. The small differences between the two studies may be due to differences in severity of disease and duration of hospitalization [6].

The study of Ljubojević et al. on 159 PV patients during 19 years revealed several complications associated with high doses of corticosteroids and immunosuppressive therapy [17]. These complications were as follows: skin infection in 26 patients (16.35%), sepsis in 9 patients (5.66%), and 14 patients (8.81%) died during the period of hospitalization. With regard to skin infection, the results of the Ljubojević et al. study are similar to those of the present study. The absence of sepsis and death in our study may be due to the small number of patients with severe PV and the shorter period of our study. In our study, the occurrence of infection had a direct relationship with disease severity, and the difference between mild and severe was significant. In Ljubojević et al.'s study, severe cutaneous and mucosal involvement was also consistent with a higher mortality rate [17].

Mourellou et al. followed 48 patients for 11 years; they concluded that complications and mortality rate of PV were related to the severity of PV. Our study is consistent with the study by Mourellou et al. [18].

In the current study, the rate of infection in PV patients with diabetes was significantly higher than in nondiabetics (P = 0.044). Belgnaoui et al. also reported more severe bacterial infection in diabetics PV patients [6].

In our study, the rate of infection in patients receiving immunosuppressive adjuvants plus systemic steroids was not significantly different from patients receiving corticosteroids alone. This means that all PV patients receiving corticosteroids (with or without adjuvant immunosuppressive) are prone to infections. It should be noted that we did not include the PV patients treated with rituximab (which may be a susceptibility factor for infection). Kim et al. found that there was no difference in prednisolone alone or prednisolone plus adjuvant with regard to prognosis and time to remission in PV patients [19].

Most bacterial skin infections detected in our patients were due to *Staphylococcus aureus*. In other studies in PV patients, skin infections due to *Staphylococcus aureus* have been reported as well [20]. In the study by Kanwar and Dhar, among the causes of 10 deaths of PV, sepsis was the most common cause and the responsible pathogenic agent in 4 cases was *Staphylococcus aureus* [21].

*Escherichia coli* was the most frequent cause of urinary tract infection in our study. Obviously, *Escherichia coli* is the most common cause of urinary tract infections in the general population [22].

In the current study, 9.68% of patients had localized herpes simplex infection, while in the study of Belgnaoui et al. 17% of patients had localized herpes infection [6]. In several reports, the herpes infection has been studied in PV patients [6, 23, 24]. Although high doses of corticosteroid and immunosuppressive therapy would cause patients to be prone to an extensive herpes simplex virus infection, in this study, we had only localized herpes simplex virus infections [24]. Previously, our group had studied the herpes simplex infection and PV in Iranian patients [10]. In that study we concluded that a herpes virus infection occasionally is responsible for exacerbation of PV [10].

In the current study, 23.87% of patients had oral candidiasis, while in the study of Belgnaoui et al. 30% of patients had oral candidiasis [6]. With regard to oral candidiasis, the result of the two studies is similar. Previously, laryngeal candidiasis has been reported in patients with PV, but in this study we had only localized oral candidiasis [25].

Infection rate had a positive significant relationship with the number of admission sessions. Patients with multiple admission sessions had a rate of infection approximately two times more than patients admitted for first time. Logically, patients with a more severe disease would have more admissions, and consequently the rate of infections would increase. Retrospective nature and relatively short period of the study (2 years) are major limitations of this project. Another limitation of the study is PV patients on different immunosuppressive adjuvant therapy included in this study. A prospective study with followup is recommended.

We concluded that PV patients with multiple admission sessions, diabetes mellitus, and severe disease are at higher risk of infection. According to a high rate of antimicrobial resistance, antibiograms are recommended for antibiotics therapy.

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