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REVIEW

Update on immunogenetics of Tunisian endemic pemphigus foliaceus

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Abstract

Pemphigus foliaceus (PF) is an autoimmune blistering skin disease characterized by the presence of bullous skin lesions, the absence of mucous tissue involvement, and the production of auto-antibodies directed against a keratinocyte transmembrane protein localized in the desmosome and member of the cadherines, desmoglein 1. These pathogenic auto-antibodies are responsible for the intra-epidermal formation of blisters through the loss of keratinocyte adhesion, the so-called acantholysis process. The endemic form of PF observed in the south of Tunisia is characterized by a significantly higher incidence rate compared to the sporadic form in northern countries, occurrence mainly in young women and the absence of cases during childhood. Tunisian endemic PF is an ideal research model for the decryption of the puzzle of genetic and environmental factors and their interactions in the development of autoimmune diseases. In this review, we will summarize recent findings regarding the epidemiologic and immunologic features of Tunisian PF and its genetic and environmental factors.

KEYWORDS

autoimmune blistering disease, desmoglein, genetic polymorphism, HLA, Prolactine, TLR

1 | INTRODUCTION

Autoimmune diseases (AIDs) are the result of a complex interplay between genetic factors, environmental triggers, and regulatory aberrations of the immune response. The identification of the factors involved in the pathogenesis of AIDs can lead to the discovery of new therapeutic targets and consequently to more effective treatment and preventive strategies.[1]

Most of AIDs are polygenic complex diseases where the genetic component is the result of additive and interactive effect of small individual effects of a large number of genes, each contributing gene is both not necessary nor sufficient to disease susceptibility.[2]

Pemphigus foliaceus (PF) is an autoimmune blistering skin disease characterized by the presence of bullous skin lesions, the absence of mucous tissue involvement, and the production of auto-antibodies directed against a keratinocyte transmembrane protein localized in the desmosome and member of the cadherines, desmoglein 1 (Dsg1).[3,4] These pathogenic auto-antibodies (-Abs) are responsible for the intra-epidermal formation of blisters through the loss of keratinocyte adhesion, the so-called acantholysis process.[5]

PF occurs in a sporadic or endemic form in different areas of the world. The endemic form is found mainly in Brazil and some other Latin American countries[6,7] and in Tunisia (North Africa). In 1993, Morini et al.[8] reported, for the first time, the endemic Tunisian form in the south of the country. This form is characterized by several features, namely, a significantly higher incidence rate compared to northern countries (6.7 cases/million/yr), the occurrence mainly in young women and the absence of cases during childhood.[9]

Endemic PF is an ideal research model for decrypting the puzzle of genetic and environmental factors and their interactions in the development of AIDs: (i) it is an organ specific AID and the immune response is directed against a well-defined autoantigen; (ii) auto-antibodies (Abs) are pathogenic and directly responsible of the tissue injury and blister formation; and (iii) the prevalence of the disease in the endemic regions is relatively high and environmental and genetic factors can be studied over a long period of time in these regions/populations.[10,11]

In this review, we will summarize recent findings regarding the epidemiologic and immunologic features of Tunisian PF and its genetic and environmental factors.

Abbreviations: Abs, Antibodies; AIDs, Autoimmune Diseases; Dsg, Desmoglein; FBAT, Family-Based Association Test; HC, Healthy Controls; HSP, Heat Shock Protein; LD, Linkage Disequilibrium; PAMP, Pathogen Associated Molecular Pattern; PCR-RFLP, PCR-Restriction Fragment Length Polymorphism; PF, Pemphigus Foliaceus; PRRs, Pattern Recognition Receptors

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2 | EPIDEMIOLOGIC AND IMMUNOLOGIC FEATURES

Anti-Dsg1 Abs can be detected with variable frequencies in healthy household contacts and individuals living in the endemic areas, their presence is now considered as a serologic abnormality that may precede the occurrence of the disease. We conducted a case-control study including 90 PF patients and 270 healthy controls (HC) to characterize the prevalence and geographic distribution of anti-Dsg1 Abs, which helped us to better identify the endemic regions where to focus on the search for environmental and genetic factors contributing to the disease.

In this study, HC were recruited from all parts of the country and were age, gender, and location matched to PF patients (3 controls/patient). Among the 90 PF patients (40 from the north and 50 from the south), 75 (83.33%) had anti-Dsg1 Abs. The epidemiologic features characterizing the Tunisian endemic form of PF were clearly expressed in the southern regions where a more important sex ratio imbalance (9:1 vs. 2.3:1 female/male), a lower mean age of disease onset (33.5 vs. 45 yr), and a higher proportion of patients from rural origin (6:1 vs. 1:2.5 rural/urban) were observed.[12]

Among the 270 HC, twenty (7.4%) had Abs against Dsg1. Even though the difference was not statistically significant, a north–south gradient was observed with a higher rate in the south (9.23% vs. 5.71%). Anti-Dsg1 seropositivity was associated with an important imbalance in gender and a lower mean age. A significant association was observed between farming and the presence of anti-Dsg1 Abs in HC.

Among the 203 healthy relatives of the 40 PF patients from the south, 32 (15.75%) were anti-Dsg1 positive; the frequency of anti-Dsg1 positive sera reached 22% in the rural area of Moknessy (Sidi Bouzid) and 17% in the southern rural sub-regions of Gabès and Sfax, namely, Mereth and Jebeniana. In all studied subregions, the prevalence of anti-Dsg1 Abs was higher in PF relatives than in the general population, which strongly argues for the participation of genetic factors in the occurrence of the disease.

These data strengthen those previously reported by Bustuji-Garin et al. and confirm that PF occurs in two different forms in Tunisia, a sporadic form in the north and an endemic form in the south. PF endemic zones are typically found in southern areas with rural lifestyle, farming as occupation, and daily close contact with ruminants. Three endemic foci of PF in southern Tunisia, which extend over an area of around 200 km have thereby been located, Moknessy, Mereth, and Jebeniana (Fig. 1), where the existence of 7 PF multiplex families were identified.[13] Each of these 7 multiplex families included at least 2 PF patients and a variable number of patients with other blistering diseases and healthy relatives with anti-Dsg1 Abs.[14]

The presence of anti-Dsg1 Abs in the serum of healthy subjects raises the question of whether they are producing the same Abs as patients, which is actually very unlikely because the pathogenicity of patients' anti-Dsg1 Abs has been largely proved including by their capacity to transfer the disease to unaffected animals,[15] or if they are producing different Abs (different class and/or sub-class, different variable regions and specificity/antigen recognized). The analysis of the class and subclass of Abs produced by patients and healthy subjects showed that most of anti-Dsg1 Abs detected in Tunisian southern healthy subjects belonged to the IgG2 subclass, whereas patients had mainly detectable IgG4 followed by IgG2 subclass.[12] This result suggests that progression from the preclinical to the clinical phase of the disease is closely associated with subclass switching,[16] from IgG2 to IgG4 subclass in the Tunisian population. Furthermore, we have demonstrated, in collaboration with the group of Prof. Masakuchi Amagai (Keio University, Tokyo, Japan), that patients' anti-Dsg1 Abs recognize the N-terminal domains (EC1, EC2, and EC3) of the mature and precursor forms of Dsg1, whereas those of healthy relatives and controls recognize the C-terminal domains (EC4 and EC5) of the precursor form only.[17]

All these findings allow us to suggest that exposure to unknown environmental factors is enough for 17–22% of people living in endemic areas to produce a nonpathogenic antibody response, IgG2 Abs directed against the C-terminal domains of the precursor form of Dsg1. Genes' susceptibility alleles would be required for epitope spreading and class switch leading to the production of pathogenic IgG4 Abs directed against the N-terminal domains of the precursor and mature forms of Dsg1and the development of the disease (Fig. 2).

3 | GENETIC FEATURES

The small number of familial cases made family-based approaches like genome-wide study not applicable. We therefore used the candidate gene strategy through case-control studies for the search of susceptibility genes and eventual genetic interactions (epistasis) between them. Genes involved either in pemphigus physiopathology or in the immune response and its regulation were studied as candidate genes.[18]

To limit the risk of false positive associations encountered in such studies due to the genetic heterogeneity of the selected populations and ethnically unmatched cases and controls, we selected HC matched (up to 3 HC per patient) in gender, age (±5 yr) and geographic origin to the patients (Table 1).

3.1 | Autoantigen genes

Anti-Dsg1 Abs play a crucial role in the initiation of the autoimmune response in PF.

Involvement of autoantigen gene polymorphisms has been reported in several AID. *DSG1*gene is polymorphic and is a candidate gene predisposing to PF. Two types of polymorphic markers were identified. The first is made of a variant haplotype of five mis-sense mutations located in the part of the gene encoding the fourth and fifth extracellular domains of the protein and was not found to be associated with PF. The second marker consists of a single silent T to C transition at position 809. An increased frequency of the homozygous genotype C/C was observed in Tunisian and in French Caucasian PF patients compared to controls.[19,20] Although, association studies indicate that *DSG1* could be involved either in PF susceptibility or in the production of anti-Dsg1 Abs, the role of these *DSG1* polymorphisms in PF physiopathology remains yet questionable.

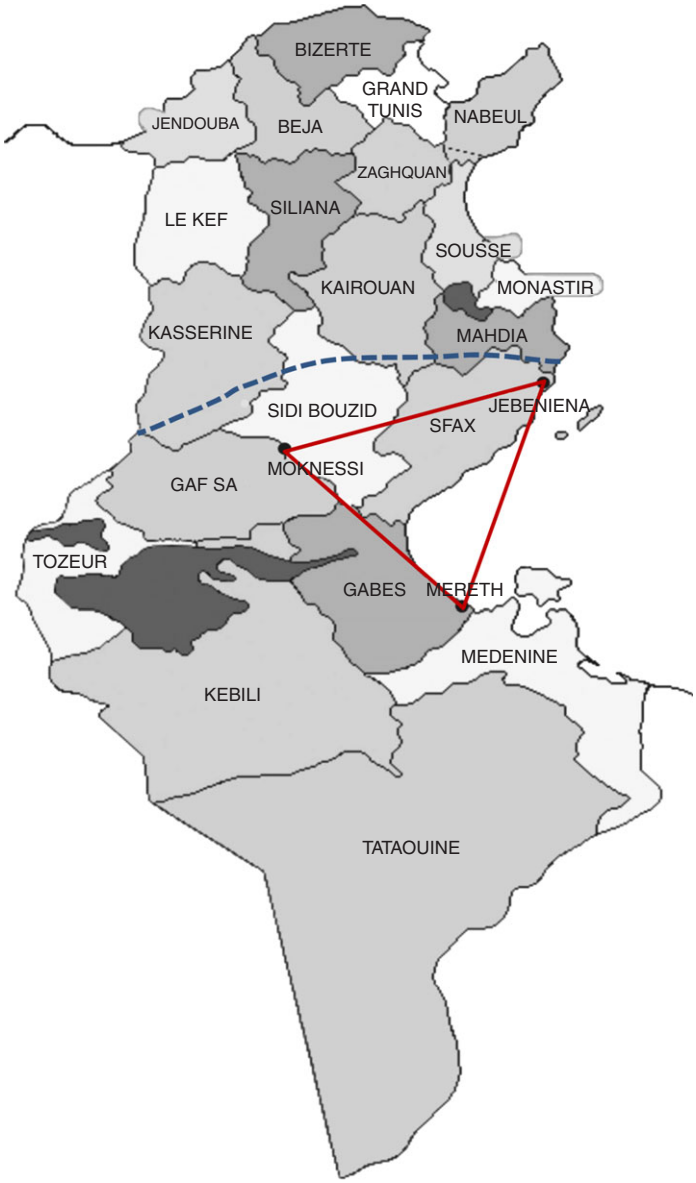

FIGURE 1 Tunisia is divided into 24 administrative regions called governorates (4 for the capital Tunis). More than three-fourths of the population lives in the cities and coastal regions of the north and east of the country and more than one-third of the total the surface (mainly in the south) is occupied by the desert (the Sahara). The blue hatched line marks the separation between the north where occurs the sporadic form and the south where occurs the endemic form of pemphigus foliaceus. The 3 endemic foci identified in the south, namely, the localities of Jebeniana, Moknessy, and Mereth, are connected by the red triangle

3.2 | Human leucocyte antigens

Human leukocyte antigens (HLA) present auto-antigens to specific T lymphocytes and thus play a crucial role in the initiation and development of the autoimmune response, cellular as well as humoral. The polymorphism of HLA loci, especially HLA DR/DQ molecules, has been largely studied and proved to contribute to a large part of the genetic susceptibility in most of AIDs (reviewed in Seldin[21]). Besides the alleles previously reported to confer susceptibility to PF (DRB1*04 and DQB1*0302),[22–24] our case-control study revealed DRB1*03 as an allele of susceptibility to Tunisian PF.[25] This striking association was observed with a weak significance in the whole selected PF population originating from both the north and the south of Tunisia. This was a surprising finding as DRB1*03 has never been reported to be associated with the endemic or sporadic form of PF, but was in contrast shown to be negatively associated with the Brazilian endemic form of PF. So, to eliminate confounding factors that may provide spurious results and to confirm or reject this association, we proceeded to the analysis of HLA class II alleles distribution in more homogeneous PF populations (patients originating from the north, where occurs the sporadic form, and those from the south, where occurs the endemic form, separately in 2 groups). The results showed that DRB1*03 was no more associated with PF in the north, whereas it was associated with a very high significance in the southern group. Moreover, the HLA DR/DQ familial transmission study has confirmed the case-control results. Thus, the DRB1*03 allele has been considered as the main and characteristic susceptibility allele of the endemic Tunisian form of PF. It is important to note that DRB1*04 was confirmed as susceptibility allele with a much higher significance in the south than in the north.

It is well established that the structure of DRB1 chains account for their ligand specificity. Interestingly, the DRB1*03 chain shared similar amino acid binding characteristics at crucial anchor positions with the β chains of the DR4 susceptibility alleles, indicating that the DRB1*03 molecule can accommodate and present Dsg1 peptide(s) in the same manner as other susceptibility alleles.

In the group of 20 seropositive HC, no significant association was found between the expression of HLA class II susceptibility alleles

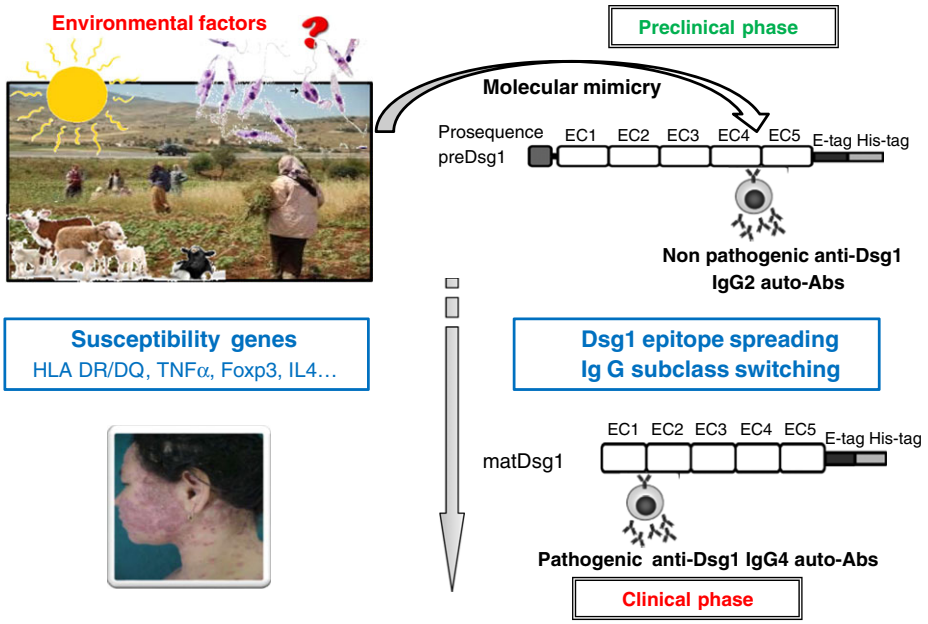

FIGURE 2 **Individuals living in the endemic areas of pemphigus foliaceus in south Tunisia are exposed to environmental factors such as sunlight, contact with ruminants, parasitic, and other infections.** In these conditions, the normal anti-infectious antibody response will elicit in some of them, via the molecular mimicry mechanism, the production of nonpathogenic IgG2 antibodies recognizing the C terminal extracellular domains (EC3 to EC5) of the precursor form of desmoglein 1 (Dsg1). Among these healthy individuals with anti-Dsg1 antibodies, some of the young women carrying the susceptibility genes will undergo the epitope spreading mechanism and Ig G subclass switching of the anti-Dsg1 antibodies leading to the production of pathogenic IgG4 auto-antibodies directed against the N terminal extra cellular domains EC1 and EC2 of the mature and precursor forms of Dsg1 and the development of the disease

TABLE 1 Studied genetic markers in Tunisian pemphigus foliaceus

Target	Chromosome region	Genes/markers	Polymorphisms	Pathway
Autoantigen	18q12.1	*DSG1*	T to C transition at position 809	Tolerance
Human leukocyte antigens	6p21.32	HLA DR/DQ	DRB1/DQB1	Antigen presentation
	6p21.3–21.4	HLA STR	D6S291, D6S273, *TNFa*, *MICA* D6S265 and D6S276	Inflammation TNFα
	6p21.33	*HSP70*	*HSPA1L*, *HSPA1A*, and *HSPA1B*	Oxidative stress
Th2 cytokines	5q31.1	IL4 IL4R IL13 IL13RA2	rs2243250 rs4787948, rs3024530 and rs3024622 rs1881457 and rs205412 rs535036	IL-4 signaling antibodies class switch
Tregs	Xp11.23	*FoxP3*	rs3761547, rs3761548, rs3761549, rs2294021 and $(GT)_n$	Tolerance
IL23/Th17	1p31.3	*IL23R*	rs1884444, rs7517847, rs11209026 and rs10889677	Inflammatin immune response Th17-derived cytokines
	6p12.2	*IL17A*, *IL17F*	rs3748067 and rs2275913, rs763780	
	22q11.1	*IL17RA*	rs4819554	
	1q21.3	RORγt STAT3	rs9645406 rs744166	

and the production of anti-Dsg1 Abs; in contrast, the majority of them (18/20) bore at least one negatively associated allele, which constitutes a strong argument to the protector effect of these negatively associated alleles.

Taken together, these findings support the view that HLA system is not involved in the initial breakage of tolerance to Dsg1, but controls the transition from an asymptomatic and nonpathogenic autoantibody response to the production of pathogenic Abs and the clinical phase of the disease (Fig. 2).

3.3 | Other candidate genes

Besides *DSG1* and HLA class II, we have analyzed the polymorphism of many other genes selected for their role in the immune response and its regulation and/or in the physiopathology of PF.

In the HLA region, we have analyzed 6 polymorphisms of microsatellite loci at 6p21.3–21.4 spanning HLA: D6S291, D6S273, *TNFa*, *MICA*, D6S265, and D6S276 in 81 PF patients compared to 123 HC recruited from the south of Tunisia.[26] Three short tandem repeat

alleles from the *TNFa* locus were associated with the disease: the *TNFa*/2 and, at a lower level, the *TNFa*/5 as susceptibility alleles and the *TNFa*/6 as a protective one. Furthermore, the expression of the *TNFa*/2-TNFa/5 and D6S265*11-D6S265*11 genotypes was found to confer susceptibility to PF. As the *TNFa* locus is located in the HLA region near DR/DQ genes, this association with *TNFa* polymorphism could be explained by its linkage disequilibrium (LD) with the DRB1 locus. Interestingly, no significant LD was found between *TNFa*2/*TNFa*5 alleles and DRB1*03/DRB1*04 alleles after multivariant and multiple regression analyses. For the more, we have recently reported that the rs1800629>A located in 5′UTR of the *TNFa* gene confers susceptibility to PF.[27] Taken together, these findings argue for an independent role of the *TNFa* gene in the susceptibility to Tunisian endemic PF.

TNFa polymorphisms could be implicated in the ethiopathogenesis of Tunisian endemic PF by the induction of a high TNFα production, a key pro-inflammatory cytokine that could play a mediator role in PF by increasing epithelial damage and maintaining the chronic activation of the $CD4^+$ T-cell infiltrates in skin lesions.

The *HSP70* (heat shock protein 70) gene is also located within the HLA region on chromosome 6. Intracellular HSP proteins support the folding and the transport mechanisms of other proteins under physiologic conditions and following physical or chemical stress. HSP70 has an anti-oxidative effect and plays an important role in protecting cells from stress-induced apoptosis. We have analyzed polymorphisms of *HSP70* genes by PCR-restriction fragment length polymorphism (PCR-RFLP) in 80 Tunisian patients with PF, 160 matched HC, and 147 related healthy subjects.[28] Different approaches were used to test the selected 3 SNPs: case-control study, family study, and haplotype analysis. A significant and interesting susceptible effect was observed with the *HSPA1L*>T allele and the *HSPA1L*>T/T, *HSPA1A*>C/C, and *HSPA1B*>G/G genotypes. Even though the multivariant regression analysis could not exclude a possible LD between HSP70 genes and HLA-DR/DQ alleles, these associations are very interesting given the high temperature and UV radiation intensity that characterize the endemic regions of PF in the south of Tunisia and the decreased effects of the observed mutations on the HSP chaperon protein production or its interaction with the target proteins, which may lead to an impaired cell response to stress and have an effect on epidermis under heat or UV radiation exposure.

Taking into account the importance of the IL4/IL13axis in the switch to the immunopthogenic IgG4 subclass of anti-Dsg1 auto-antibodies, we have conducted a familial and a case-control studies including 80 Tunisian patients, 147 related subjects, and 160 matched HC.[29] We investigated seven nucleotide polymorphisms by PCR-RFLP technique: rs2243250 in the promoter region of *IL4* gene, rs4787948, rs3024530, and rs3024622 in the *IL4R* gene, rs1881457, and rs205412 SNPs in the *IL13* gene, and rs535036 in the *IL13RA2* gene. The T allele and TT genotype of the IL4–590 C/T gene polymorphism were significantly increased in the PF patients group compared to HC. This association was confirmed with the family-based association test (FBAT). Interestingly, the serum IL-4 levels were significantly increased in patients with the TT genotype compared to CT or CC genotypes. On the other hand, a weak positive and a negative association were found with C/C and C/G genotypes of the rs3024622, respectively, making of this *IL4-R* gene polymorphism a reliable marker for susceptibility beside the *IL4* gene especially that a gene–gene interaction and epistasis was noted between the 2 genes with an increased risk to develop PF for the T-A-C-A combination of rs2243250-rs4787948-rs3024622-rs3024530 polymorphisms. Regarding the IL13and its receptor, no association was found between the studied polymorphisms and PF.

Treg cells play a crucial role in establishing and maintaining self-tolerance and immune homeostasis. The transcription factor FoxP3, which is a specific marker of Treg cells, plays a key role in their development and function. The *FOXP3*gene is located on chromosome Xp11.23 within an area of AIDs linkage and genetic polymorphism in *FOXP3* gene has been proved to be implicated in the pathogenesis of several AIDs, particularly disorders with a female predominance. We analyzed the polymorphism of 4 SNPs (rs3761547, rs3761548, rs3761549, and rs2294021) and a $(GT)_n$ microsatellite in the intronic and promoter regions of *FOXP3*gene in 98 PF patients and 182 matched HC, all women.[30] According to the epidemiologic features of the disease, patients were classified into two groups: an endemic group ($n = 33$) and a sporadic one ($n = 65$). In the whole population, the C allele of the rs3761549C>T polymorphism and its homozygous genotype, and the C allele of the rs2294021C>T polymorphism were found to be associated with the susceptibility to PF. Interestingly; not only these associations were maintained in the endemic group, but the weak positive association observed in the whole population with the A allele of the rs3761548>A/C polymorphism was reinforced, and a new association was revealed with GG genotype of the rs3761547A>G polymorphism. Furthermore, the haplotype rs3761547>G-rs3761548>A-rs3761549>C-$(GT)_{15}$-rs2294021C>T composed of the susceptibility alleles was confirmed as a susceptibility haplotype distinctly and in more pronounced manner in the endemic group. These findings underline once more the particular genetic background of the Tunisian endemic PF and provide evidence for the implication of the *FOXP3* gene in the pathogenesis of the disease.

Because of the crucial role of Th2 cytokines in the switch to the pathogenic subclass of anti-Dsg1 auto-Ab, PF has long been considered as a Th2 disease. However, Dsg1-responsive Th1 and Th2 cells were simultaneously detected in PF patients' blood, and more recently, significant high frequencies of $CD4^+IL17^+$ cells in pemphigus patients' PBMCs and skin biopsies were reported, particularly in acute onset and active chronic stages.[31,32] The Th17 differentiation from naïve Th cells requires specific cytokines such as TGF-β, Il6 and IL23, and the SNP located at position -174 of the IL6 gene was reported to be associated with endemic Brazilian PF.[33] These findings incriminating the Th17 cells in the pathogenesis of pemphigus prompted us to evaluate the eventual genetic contribution of the IL23/Th17 genes' polymorphisms in Tunisian PF especially that these cells have been implicated in several other AIDs. So, in a case-control study on 115 PF patients and 201 matched HC, we analyzed the polymorphism of 4 SNPs in the *IL23R* gene on chromosome 1: rs1884444, rs7517847, rs11209026, and rs10889677, two SNPs in the *IL17A*gene on chromosome 6: rs3748067 and rs2275913; one SNP in each of *IL17F* gene on chromosome 6: rs763780; *IL17RA*

gene on chromosome 22: rs4819554, *RORγt* gene on chromosome 1:rs9645406 and *STAT3* gene: rs744166 within chromosome 17.[27]

Among the 4 Tag SNPs genotyped in the *IL23R* gene, only rs11209026>G was found to be strongly associated to PF susceptibility. Moreover, it was in strong LD with the other genotyped SNPs generating the susceptibility haplotype rs1884444>T-rs7517847>T-rs11209026>G-rs10889677>A. This variant, located between the transmembrane domain and putative JAK2 binding site in the cytoplasmic portion of IL-23R protein, is extremely conserved across different species. Consequently, a change in the highly conserved Arg to Gln might have functional consequences in the IL-23R transduction pathway and explain its association with several other AIDs. On the other hand, we found significant associations with genotyped SNPs in the 6p12.2 region of chromosome 6: rs3748067>C in*IL17A*and rs763780>C in *IL17F*. Taken together, these findings give a strong argument supporting the genetic contribution of IL23/Th17 pathway genes variants in PF pathogenesis.

In summary, our results confirmed the contribution of several genes in the genetic susceptibility to Tunisian PF (*HLA DR/DQ*, *Dsg1*, *TNFα*, *IL4*, *IL4R*) and revealed that of many others (*FoxP3*, *IL23R*, *IL17A*, *IL17F*, *HSP70*) (Fig. 3). However, it is clear that the genetic component of PF' pathogenesis is much more complex and many of the genes involved have still to be identified. In this regard, new approaches such as high throughput analyses of gene expression should be of a great help, genes overexpressed in lesional skin biopsies and/or PBMC of patients at active stage compared to HC should be selected as candidate genes for the genetic polymorphism analyses and could represent new targets for therapy and/or diagnoses.[34]

4 | ENVIRONMENTAL FACTORS

The link between genetic and environmental factors in the pathogenesis of complex diseases such as cancer and AIDs has long been unknown.[35,36] The spectacular development and results of epigenetic research over the last years have made it possible to understand and establish this link: epigenetic mechanisms serve as a link between environmental stimuli and genetic factors. Indeed, epigenetic modifications such as DNA methylation, histone modification are critical in chromatin remodeling and regulation of gene expression and cumulative evidence have shown that environmental factors can induce epigenetic modifications, thereby regulating gene expression without changes in the nucleotide sequence.[37,38]

Recent evidence suggest that epigenome is dynamic and changes in response to the environment. The identified environmental exposures have been classified into 3 broad categories: 1—chemical agents, including air polluants, industrial chemicals and solvents, tobacco, and personal care products; 2—physical agents such as ionizing radiation and sunlight; and 3—biologic agents such as microbes and microbiote, foods and diet, toxins.[39]

As for most of other AIDs, environmental factors play a crucial role in the development of PF. The involvement of environmental factors in Tunisian PF is supported by several arguments. A first epidemiologic study demonstrated that Tunisian PF is significantly associated with certain traditional activities such as traditional cosmetics (such as "henna") and contact with ruminants.[40] As previously described in Peru, we observed a significant association between living in rural conditions with farming as main occupation and the presence of Abs against Dsg1 and/or the development of endemic PF,[12] suggesting that this activity may expose individuals living in the endemic areas to particular environmental factors present in these south regions. In this regard, if the climate conditions in south Tunisia are quite different from those observed in South American endemic regions, the living conditions of the target populations are very similar because they are in close contact with animals and have farming activities.[41,42]

Based on the observations of Diaz et al. on the possible role of a salivary antigen from the sand fly as initial target for the Ab response in *Fogo Selvagem*,[43] we searched for the presence of anti-Dsg1 Abs in the sera of patients with *hydatidosis* and *leishmaniasis*, two parasitic diseases that are predominant in rural areas where a high incidence of Tunisian endemic PF is observed.[44] As expected, anti-Dsg1 Abs were present in 93.22% of PF patients (55/59) and 6.57% of HC (10/152). Interestingly, these Abs were also present in 40% (14/35) and 21.7% (5/23) of the hydatidosis and leishmaniasis sera, respectively. These anti-Dsg1 Abs were mainly from IgG2 subclass in hydatidosis patients and from IgG3 subclass in leishmaniasis, but never from IgG4 subclass. Among all Dsg1 positive sera from parasitic group, only one serum from a patient with hydatidosis showed intercellular staining on monkey esophagus and reacted with the 160 kDa band of Dsg1 in immunoblot analysis, whereas another serum from a patient with leishmaniasis only showed the characteristic aspect in immunofluorescence, suggesting that these Abs detected in our parasitic patients should be directed against cryptic, conformational epitopes of the EC5 domain of Dsg1. On the other hand, the prevalence of Abs against parasitic antigens in sera samples from PF patients did not differ significantly from the control group.

These results confirm that anti-Dsg1 Abs are prevalent in Tunisian patients with parasitic diseases.[44] Unlike the Brazilian endemic PF where the initial environmental factor seems to be well identified, much more research is still needed to clarify this issue in the Tunisian endemic PF.

Whether biological or physicochemical, environmental factors will interact with the immune system and induce a first innate immune response and/or a disturbance of oxidative stress. TLRs are the best characterized and most studied class of the innate immunity receptors known as pattern recognition receptors (PRRs). Each PRR detects one or more pathogen associated molecular pattern (PAMP), a conserved molecular motif shared by a large number of microbes and absent from eukaryotic cells.

Taking into account the presumed role of several microbes in the pathogenesis of pemphigus and the assumed role of the colonizing microbiota of the skin in eliciting and perpetuating conditions promoting its development, we analyzed the expression of 3 TLRs, TLR2, 3, and 4, with keratinocytes in skin biopsies of PF patients compared to HC.[45] TLR 2 and TLR4 are extracellular/membrane receptors, whereas TLR3 is an intracellular/endosomal receptor. TLR2 interacts with peptidoglycan, a component of all bacterial cell walls, as well as additional constituents of Gram-positive bacteria, Mycobacteria and

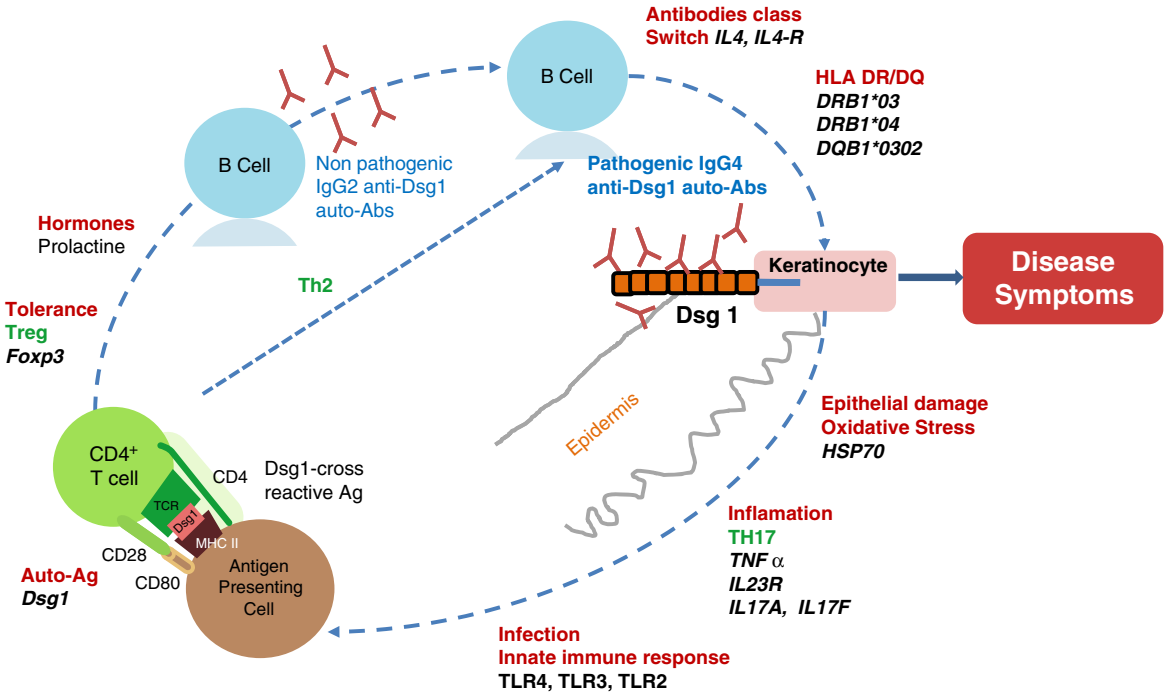

FIGURE 3 **Tunisian endemic pemphigus foliaceus patients are typically young women living in poor rural localities of the south of the country.** The epithelial damage caused by the high temperature and intense UV radiation characterizing these sunny regions is exacerbated by decreased activity of the HSP70, which results in an impaired cell response to stress, oxidative stress, and stress-induced apoptosis. Bacterial and parasitic infections are common in these poor rural regions with lack of hygiene. The innate immune response induced is associated with high TNF-α production and overexpression of TLRs 2, 3, and 4 in the epidermis. The inflammatory conditions hence created in the epidermis are maintained and reinforced by the IL17 A/F pro-inflammatory cytokines produced by the Th17 cells. TNF-α, not only maintain activation of CD4$^+$ T cells, but also increase expression of presenting (HLA class II) and co-activating (CD 80, CD 86) molecules on Langerhans cells and other antigen presenting cells enabling them to present immunogenic Dsg1 (and/or Dsg1cross reactive exogenous antigens) issued peptides to specific CD4$^+$ T helper cells. In the context of a failure of the Treg compartment (Fox P3 mutations, among others), anti-Dsg1 specific CD4$^+$ T helper cells are activated, their proliferation being enhanced by higher production of Prolactin. These T helper cells activate specific autoreactive B cells to produce IgM and IgG2 nonpathogenic anti-Dsg1 antibodies. The IL4 produced by the activated autoreactive Th2 cells allows the subclass switching to IgG4. On the other hand, and in the presence of HLA class II and other unknown susceptibility genes, the epitope spreading mechanism takes place and the autoreactive anti-Dsg1 B cells now produce pathogenic IgG4 anti-Dsg1 auto-antibodies that bind the N terminal domains of desmoglein 1 and contribute to the acantholysis process leading to the appearance of disease symptoms

fungi. TLR3 recognizes double stranded RNA, a component of the life cycle of most viruses. As for TLR4, its more important ligand is the LPS (lipopolysaccharide), a major component of Gram-negative bacterial outer membrane.

Immunochemistry analyses of the 43 patients' and 20 HC' (individuals who underwent plastic surgery) skin biopsies showed a significant overexpression of the 3 TLRs throughout the epidermis in biopsies of patients' skin lesions. This overexpression seems to be a dynamic process, as the keratinocytes are known to be very proliferative in PF. The significant increase of these TLRs simultaneously may merely reflect the complicated environmental conditions of women in the southern rural regions of Tunisia, who encounter a multitude of pathogenic micro-organisms during their lifetime. Even though the specific link with a particular environmental trigger have to be established, the association observed between TLR4 diffuse expression and the production of anti-Dsg1 Abs could be in line with a potential role of a specific TLR4 ligand in the ethiopathogenesis of Tunisian endemic PF.

Reactive oxygen species (ROS) are highly reactive and transient molecules generated during normal metabolism and indispensable as mediators in many cellular processes. Under physiologic conditions, their production is controlled by a large number of enzymatic and nonenzymatic anti-oxidant factors, which act as protective systems. The oxidative stress is the consequence of an impairment of the delicate balance between the ROS generation and the anti-oxidant defense resulting in DNA, proteins, and lipids damages. The oxidative damage of molecules has been involved in the pathogenesis of a large number of diseases and an increasing attention has been focused on the role of ROS during inflammatory response. Furthermore, the immune response in pemphigus is accompanied by a strong inflammatory reaction and PF patients in the endemic regions of south Tunisia are exposed to high sunlight and UV radiation. Hence, we undertook to study the oxidative state in the sera and skin biopsies of PF patients. The sera levels of malondialdehyde (MDA) were significantly higher in the group of PF patients ($n = 42$) than in controls ($n = 78$), indicating an increase of lipid peroxidation (LPO) in patients, whereas nonsignificant decrease of protein oxidation measured using thiol (SH) serum levels was observed in patients.[46] On the other hand, a significant rise of catalase activity (CAT) was observed in patients compared to controls and the anti-Dsg1 Abs titers were significantly correlated with the CAT activity. As for skin biopsies, increased levels of MDA and

conjugated dienes (CD), superoxide dismutase (SOD), and CAT activities were observed in lesional, perilesional, and normal biopsies of patients ($n = 13$) compared to healthy individuals ($n = 7$) indicating a significant increased activity of LPO and antioxidant defense systems in patients' biopsies[47]; however, the measurement of protein oxidation showed a significant decrease of SH level in lesional, perilesional, and normal biopsies of patients compared to HC, attesting the protein oxidation in pemphigus patients' biopsies. Collectively, these findings strongly support the involvement of oxidative stress in the pathogenesis of PF (Fig. 3). The increase of protein oxidation and lipid peroxidation could explain the immunologic features of the disease and the role of inflammation in its onset/aggravation, suggesting a potential utility of anti-oxidants such as vitamins in the therapeutic management of PF.

5 | HORMONAL FACTORS

Most of AIDs are known to be more frequent in women than in men, but the female predominance in endemic Tunisian PF is remarkable as approximately 80% of patients are women with a women/men ratio reaching 12/1.[27,30] The predictable clinical improvement of the disease during pregnancy and worsening in postpartum suggest the involvement of hypothalamic-pituitary-gonadal (HPG) axis and hormonal factors in the etiopathogenesis of PF. On the other hand, many previous studies showed that hormones, especially female hormones, can interact with the immune system and influence immune responses of many AIDs that occur predominantly in women.

Among the hormones that could be involved in the pathogenesis of PF, we analyzed the role of the follicle-stimulating hormone (FSH), the luteinizing hormone (LH), the prolactin (PRL), and the estradiol (E2). A case-control study was conducted during one year on a group of 14 women PF patients (10 premenopausal and 4 postmenopausal) and a group of 14 matched HC. Serum concentrations of FSH, LH, PRL, E2, IgE, and IL4 were measured in the blood samples taken the third day of the periodic cycle.[48]

Significant higher mean serum levels of PRL were observed in PF patients compared to HC and two patients (14.28%) had hyperprolactinemia (rate above normal range), whereas in the control group no one had hyper-prolactinemia. We also found a positive correlation between (i) serum PRL levels and serum LH, IgE, and IL-4 levels; (ii) serum IL-4 levels and serum LH, FSH, and IgE levels; and (iii) serum LH and FSH levels in PF patients. The correlation between the PRL concentrations and IL4 and IgE serum levels suggest that PRL may enhance antibody switching but this have to be confirmed in larger cohorts. Although its limitation, due to a relatively small number of patients and controls, this preliminary study revealed interesting results supporting the implication of female hormones in the pathogenesis of PF (Fig. 3).

6 | CONCLUSION

Tunisian endemic PF represents an interesting research model on autoimmunity. Even though great progress has been made in characterizing its epidemiologic and immunologic features and identifying its genetic and environmental factors, a lot remains to be done to elucidate the key steps of the pathogenesis of this complex disease. Recent developments in epigenetic and microbiota research as well as new techniques like high throughput analyses can be of great help in solving the puzzle of the interplay between environmental, genetic, and immunologic factors in the etiopathogenesis of this AID and consequently the development of new therapeutic tools.

AUTHORSHIP

M.H. contributed to the conceptualization of writing original draft, review, editingand revision. A.O. contributed to the conceptualization of investigation, formal analysis, writing original draft. M.A. and T.H. contributed to the conceptualization.

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DISCLOSURES

The authors declare no conflicts of interest.

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