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Keywords: Pemphigus foliaceus; Toll-like receptor; Tunisia

Abbreviations: PAMPs: Pathogen-associated Molecular Patterns; PRRs: Pattern Recognition Receptors; TLR(s): Toll-like receptor(s); PGN: Peptidoglycan; LPS: Lipopolysaccharide; PF: Pemphigus Foliaceus; auto-Abs: auto-antibodies; Dsg1: Desmoglein 1; KC(s): Keratinocyte(s); PBS: Phosphate Bu er Saline solution; HSP60: Heat

associated with certain traditional activities [20], and (iv) nally, the potential e ect of parasites such as leishmania and hydatidosis in the aetiopathogenesis of endemic PF has been suggested [21,22].

Taken together, these reports provide substantial evidence that the initiation and/or exacerbation of skin lesions could be triggered by microbial organisms. Since the epidermis constitutes the rst barrier to invasive pathogens and TLR2, 3 and 4 were shown to be a variety of immune functional receptors on KCs [23], it is thus conceivable that certain microorganisms could participate in the disease process through interaction with KC's TLRs, and the subsequent activation of antigen-presenting cells and KCs, and the adaptive immune system.

e aim of this study was to investigate and compare the expression of TLR2, 3 and 4 by KCs in PF and normal skin biopsies, and then to correlate TLR expression with anti-Dsg1 auto-Ab titers a direct marker of the autoimmune response.

Materials and Methods

Subjects

is study was performed on para n-embedded skin biopsies obtained from 43 PF patients recruited from endemic southern Tunisian areas and attending the Dermatology Department of Hedi Chaker University Hospital (Sfax, Tunisia). e diagnosis of PF was established according to the standard clinical, histological and immunological criteria of the disease [24]. All specimens were taken from PF patients with active disease and before treatment (T0).

Twenty skin specimens were obtained from normal individuals that underwent plastic surgery. None of them su ered from autoimmune or in ammatory diseases. All patients and controls were recruited from southern Tunisian areas, belonging to the same socioeconomic population stratum and exposed to similar environmental conditions. Informed consent was obtained from both patients and controls. is study was approved by the Ethical Committee of the Habib Bourguiba University-Hospital of Sfax, Tunisia (protocol number of ethical committee: 4/12).

Immunohistochemistry

For immunohistological analysis, tissue specimens were xed in 10% formalin buf ered at pH 7•O for 24 h and paraf n-embedded. TLR2, TLR3 and TLR4 expression was detected using anti-TLR specic rabbit polyclonal IgG (Santa Cruz Biothechnology Inc®, Santa Cruz, CA, USA), diluted 1:100 in phosphate-bu ered saline (PBS) containing 5% skim milk. For immunohistochemical staining, tissue sections were treated with microwave. Nonspecic binding of Abs was blocked by incubating sections with bu ered casein solution for 1 h at room temperature (Power Block Universal Blocking Reagent, Bio Genex®, San Ramon, CA, USA). Sections were then incubated with the TLR specicanti-sera for 1 h at room temperature. As second step reagent, a Biotin/Streptavidin-peroxidase detection system (HRP Detection System, Lab vision®, Fremont, CA, USA) was used according to the instructions of the manufacturer with 3-amino-9 ethylkarbazole (AEC)

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