Exposure to Phlebotomus papatasi and/or Leishmania major: Possible Etiologic Link to Tunisian Pemphigus

Ines Zaraa, Thouraya Boussoffara, Melika Ben Ahmed, Soumaya Marzouki, Nabiha Ben Hassouna, Myriam Kallel Sellami, Sondes Makni, Amel Ben Osman, Hechmi Louzir, Mourad Mokni

competent cutaneous permeability barrier (Cameron *et al.*, 2007). In this regard, decreased expression of elongases in AD-like skin lesions and subsequent failure to adequately produce long FAs might have contributed to the depletion of the ceramide[NS] species with very long-chain FAs that are more essential to CBF than those with shorter FAs (Janusova *et al.*, 2011). Interestingly, peroxisome proliferator activator receptors, liver X receptor, and the sterol regulatory element binding protein signaling pathways, which are important in the transcriptional regulation of elongases, also have critical roles in epidermal barrier function (Elias, 2005), which may be in line with the alteration of elongase and disrupted CBF in AD-like skin lesions.

In conclusion, we found that the profiles of FA lengths of ceramide species are different in AD-like skin lesions, i.e., the ceramides with very long FAs (≥C24) decreased whereas those with shorter FAs (≤C22) increased in AD-like skin lesions. The expression levels of elongases were downregulated in AD-like skin lesions, which might have impaired the synthesis of ceramide species with very long FAs and tilted the balance toward the dominance of ceramide species with shorter FAs over those with longer FAs in the SC of AD. These results suggest that the alteration in elongases as well as in the profile of FA lengths of ceramides could be useful for the diagnosis of AD. More importantly, the modulation of elongases might be effective in repairing damaged CBF in AD.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Yang-Hui Park[1,2], Won-Hee Jang[1], Jung A. Seo[1], Miyoung Park[1], Tae Ryong Lee[1], Young-Ho Park[1], Dae Kyong Kim[2] and Kyung-Min Lim[1]

[1]Amorepacific CO R&D Center, Yongin, Republic of Korea and [2]Department of Environmental & Health Chemistry, College of Pharmacy, Chung-Ang University, Seoul, South Korea
E-mail: kimlim@amorepacific.com or proteinlab@hanmail.net

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Exposure to *Phlebotomus papatasi* and/or *Leishmania major:* Possible Etiologic Link to Tunisian Pemphigus

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TO THE EDITOR

Epidemiological data in Brazil indicated a significant association between pemphigus and a frequent exposure to black flies (Lombardi *et al.*, 1992; Aoki *et al.*, 2004). Accordingly, recent records demonstrated that anti-desmoglein 1 (Dsg1) antibodies are present in endemic areas of Brazilian pemphigus foliaceus, not only in pemphigus subjects but also in patients with parasitic diseases transmitted by hematophagous vectors (onchocerciasis: 83%, Chagas disease: 58%, and leishmaniasis: 43%) (Diaz *et al.*, 2004). Diaz *et al.* (2004) suggested that saliva of the

Abbreviations: BP, bullous pemphigoid; Dsg1, desmoglein; HC, healthy control; L. major, Leishmania major*;* P. papatasi, Phlebotomus papatasi*; SGE, salivary gland extract; ZCL, zoonotic cutaneous leishmaniasis*

Table 1. Anti-Dsg1 and anti-SGE antibodies in the different study groups

	Dsg 1	ELISA	SGE		ELISA	
	Positive test, *n* (%)[1]	Mean ± SD (U ml^{-1})	IgG, *n* (%)[2]	Mean ± SD (OD)	IgG4	IgE
Pemphigus (*n*=31)	18 (58%)	74.7 ± 80.11	8 (26%)	0.152 ± 0.21	5/8	0/8
HC pemphigus (*n*=31)	2 (6.5%)	6.14 ± 12.38	2 (6.4%)	0.073 ± 0.03	0/2	0/2
ZCL (*n*=60)	8 (13.3%)	11.32 ± 25.13	32 (53%)	0.237 ± 0.22	10/10	5/10
HC ZCL (*n*=60)	1 (1.6%)	5.23 ± 5.96	37 (62%)	0.299 ± 0.3	9/10	6/10
BP (*n*=31)	NP		1 (3.2%)	0.056 ± 0.02	NP	NP

Abbreviations: Dsg, desmoglein; HC, healthy control; NP, not performed; OD, optical density; SGE, salivary gland extract; ZCL, zoonotic cutaneous leishmaniasis.
[1]Index value $\geqslant$20 U ml^{-1}.
[2]Relative OD$\geqslant$0.15.

hematophagous vector-containing cadherin proteins could be the sensitizing antigen. Interestingly, Dsg1 is a 160-kDa desmosomal core glycoprotein that belongs to the cadherin family (Culton *et al.*, 2008). As reported in pemphigus (Amagai, 2010), the antibody production during host immune response to the salivary antigen of sand flies is associated in humans with the production of Th2 cytokines (Marzouki *et al.*, 2011), contrasting to data reported in mice showing both Th1 and Th2 responses (Mbow *et al.*, 1998; Kamhawi, 2000; Valenzuela *et al.*, 2001). In the same way, restriction of IgG4 antibody response in pemphigus has been also reported in patients with parasitosis and individuals exposed to bee stings (Muller, 2005).

Comparable data have been accumulated recently in Tunisia where pemphigus is endemic. Accordingly, several environmental factors such as wasp, bee, and spider stings have been shown to be significantly associated with pemphigus (Bastuji-Garin *et al.*, 2002) The zoonotic cutaneous leishmaniasis (ZCL) due to *Leishmania major* (*L. major*) and transmitted by a hematophagous vector *Phlebotomus papatasi* (*P. papatasi*) is more prevalent in rural areas where a higher incidence of Tunisian endemic pemphigus has been also reported. Additional data demonstrated that anti-Dsg1 antibodies were more prevalent in subjects with visceral leishmaniasis (21.7%) (Kallel Sellami *et al.*, 2007). More recently, it has been shown that the anti-saliva antibodies developed by individuals living in endemic areas of ZCL in Tunisia were prominently of the IgG4 subclass (Marzouki *et al.*, 2011), as commonly reported for pathogenic anti-Dsg1 antibodies in pemphigus.

Thus, we aimed to investigate a possible etiological link between pemphigus and ZCL, related either to the parasitic agent *L. major* and/or to the saliva of hematophagous vector, *P. papatasi*. For this purpose, serum antibodies against Dsg1 and *P. papatasi* salivary gland extracts (SGEs) were investigated in 31 patients with pemphigus (25 from the North, and 6 from the Central and Southern parts of Tunisia) and 60 individuals with ZCL, living in an endemic area of *L. major*. The antibody reactivity was compared with that obtained from sera of two groups of Tunisian healthy controls (HCs), numbering 31 and 60 subjects, which were matched with pemphigus and ZCL patients, respectively, regarding geographical origin, sex, and age. A final group included 31 patients with bullous pemphigoid, which were matched with regard to geographical origin with pemphigus patients. Participants gave their written informed consent.

Anti-Dsg1 antibodies were detected by a commercial ELISA kit (MBL, Nagoya, Japan) according to the manufacturer's instructions. For all positive sera, immunoblot analysis was performed using epidermal extract, and IgG subclass was determined by ELISA as described (Kallel Sellami *et al.*, 2007). Specific anti-saliva IgG antibodies were investigated using an in-house ELISA as previously described (Marzouki *et al.*, 2011). The cutoff for the assay was the mean relative optical density of 31 healthy controls of pemphigus plus 2 SDs. The production of IgG4 and IgE antibodies against *P. papatasi* saliva was measured by ELISA. For sera with specific anti-saliva IgG antibodies, immunoblot analysis was performed using SGE as described (Marzouki *et al.*, 2011).

As shown in Table 1, the prevalence of anti-Dsg1 antibodies in the ZCL patients was significantly higher than that of the control group (χ^2, P=0.016). The median of the anti-Dsg1 antibodies index value did not differ significantly between the ZCL patients and HC (3.6 vs. 2.78; P=0.07). As expected in the pemphigus patients, IgG4 was the most common subclass of anti-Dsg1 antibodies (31/31 patients). Contrastingly, anti-Dsg1 antibodies detected in non-pemphigus groups were prominently of IgG2 (8 out of 11) and IgG1 (6 out of 11) isotypes, with an absence of the IgG4 subclass, a known feature in these conditions (Kallel Sellami*et al.*, 2004). Only one serum (from a ZCL patient) with a high anti-Dsg1 antibody index value (169.1 U ml^{-1}) reacted with the 160-kDa band of Dsg1 (data not shown). Altogether our findings are consistent with previous reports demonstrating more prevalent anti-Dsg1 antibodies in patients with leishmaniasis (Diaz *et al.*, 2004; Kallel Sellami *et al.*, 2007).

To better define the role of the vector agent, we analyzed the antibody response against SGE of *P. papatasi*. The prevalence of anti-SGE antibodies was significantly higher in the pemphigus group than in the HC (P=0.04) and bullous pemphigoid (P=0.013) groups

(Table 1). Among the pemphigus patients with anti-SGE antibodies, only one originated from an endemic area of *L. major* transmission. Furthermore, the serum level of anti-SGE antibodies was significantly higher in the pemphigus sera than in the HC ($P=0.046$) and bullous pemphigoid ($P=0.018$) sera (Table 1). Altogether, these data suggest that anti-SGE antibodies are more specifically found in pemphigus patients than in patients with other bullous diseases.

Consistent with previous reports (Rohousova *et al.*, 2005; Marzouki *et al.*, 2011), we showed that a high proportion of people living in an endemic area of *L. major* showed IgG antibodies against sand fly saliva, regardless of whether these individuals developed ZCL. In Tunisia, Marzouki *et al.* (2011) showed that IgG anti-saliva antibodies were prominently of the IgG4 subclass (76%). Similarly, we showed that all tested sera with anti-SGE antibodies from the ZCL group and most of the positive HC ZCL sera showed IgG4 subclass (Table 1). Interestingly, among the eight pemphigus patients with anti-saliva antibodies, five showed the IgG4 subclass. None of the two HC of pemphigus with IgG anti-saliva antibodies tested positive for the IgG4 subclass (Table 1).

Contrasting with the result of Marzouki *et al.* (2011), no difference in the levels of anti-SGE antibodies was noted between ZCL and HC ZCL groups (Table 1). Nevertheless, the presence of anti-Dsg1 antibodies was significantly higher in the ZCL group, suggesting that patients in contact with the parasite were more likely to develop anti-Dsg1 antibodies than individuals with high anti-SGE titers but not exposed to the parasite. However, one important datum may argue against such a hypothesis: none of the eight pemphigus patients with anti-saliva antibodies have a clinical history or biological features of cutaneous leishmaniasis (data not shown).

It is well accepted that IgE antibodies against mosquito saliva are commonly observed in the sera of people exposed to mosquito bites (Reunala *et al.*, 1994a, b; Peng *et al.*, 2002). They may indicate a classic type I hypersensitivity (Shan *et al.*, 1995), and are often associated with IgG4 antibodies (Reunala *et al.*, 1994a, b). Furthermore, the presence of IgG4 antibodies against *Aedes* was correlated to the production of IgE antibodies, suggesting that IgG was the result of an allergic reaction to mosquito bites (Brummer-Korvenkontio *et al.*, 1994; Reunala *et al.*, 1994a, b; Palosuo *et al.*, 1997; Remoue *et al.*, 2007). Consistent with the results of Marzouki *et al.* (2011), we demonstrated that nearly 50% of ZCL patients and the HC ZCL group demonstrated anti-IgE antibodies (Table 1), and no correlation was found between the presence of IgG4 and IgE antibodies (data not shown). Interestingly, none of the pemphigus patients or the HC pemphigus subjects showed IgE antibodies against SGE (Table 1). In addition, none of the pemphigus patients displayed clinical symptoms of atopy. Collectively, our data suggest that IgG4 antibody production does not result from an allergic phenomenon but are rather a specific feature of the immune response against *P. papatasi* saliva.

As reported by Marzouki *et al.* (2011), we showed that sera from people living in the endemic area of *L. major* reacted prominently with a 30-kDa protein and more inconsistently with six other salivary proteins (12, 15, 21, 28, 36, and 44 kDa) (Figure 1). In pemphigus patients, the immunoblot analysis confirmed, in six out of eight positive sera, the recognition of *P. papatasi* salivary proteins. In the latter patients, the antibody response was mainly directed toward 12 and 15 kDa (five cases) and to a lesser extent 30 kDa (three cases), 21 kDa (three cases), and 36 kDa (one case) (Figure 1).

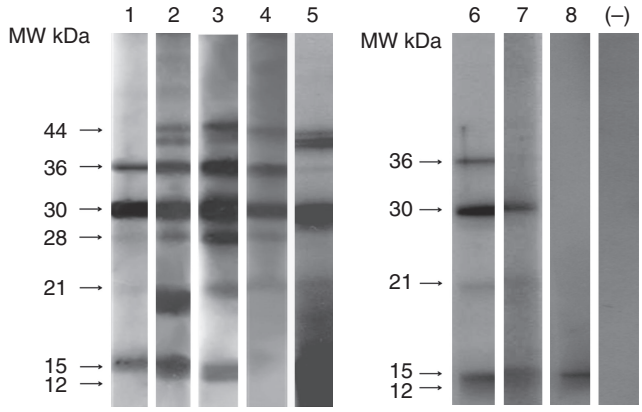

Figure 1.. Identification of the target proteins in the saliva of *Phlebotomus papatas*: immunoblot profiles of salivary antigens from a Tunisian strain of *P. papatasi* with positive sera from subject living in an endemic area of *L. major* (1–5), pemphigus patients (6–8), and negative (–) human sera are shown. The molecular weight (MW) is shown on the left of the panel. Sera form subject living in an endemic area of *L. major* revealed reactivity to various salivary proteins (12, 15, 21, 28, 30, 36, and 44 kDa). Pemphigus sera reacted mainly with 12, 15, 21, and 30 kDa salivary proteins.

Our findings further support that an environmental component may initiate the autoimmune response in pemphigus. A persistent exposure to *P. papatasi* may be a possible etiologic link to pemphigus in Tunisia. A direct implication of the *Leishmania* parasite could not, however, be excluded.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Inès Zaraa[1,2,3], Thouraya Boussoffara[4], Mèlika Ben Ahmed[2,5], Soumaya Marzouki[5], Nabiha Ben Hassouna[4], Myriam Kallel Sellami[2,6], Sondes Makni[2,6], Amel Ben Osman[1,2], Hechmi Louzir[2,4,5] and Mourad Mokni[1,2,3]

[1]Department of Dermatology, Hospital La Rabta, Tunis, Tunisia; [2]Faculté de Médecine de Tunis, Tunis El Manar University, Tunis, Tunisia; [3]Research Unit UR 24/04, Tunis, Tunisia; [4]Laboratory of Immunology, Vaccinology, and Molecular Genetics, Institut Pasteur de Tunis, Tunis, Tunisia; [5]Department of Clinical Immunology, Institut Pasteur de Tunis, Tunis, Tunisia and [6]Department of Immunology, Hospital La Rabta, Tunis, Tunisia
E-mail: inesrania@myway.com

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Desmoplakin Regulates Desmosome Hyperadhesion

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TO THE EDITOR

The skin is subjected to continuous physical stress. Keratinocytes resist mechanical stress by tethering the tension-bearing keratin intermediate filament cytoskeleton to sites of intercellular contact known as desmosomes (Green and Simpson, 2007; Garrod and Chidgey, 2008). The plakin protein desmoplakin (DP) is an obligate desmosomal constituent necessary for keratin anchorage at cell–cell contacts. Establishing and maintaining the DP–keratin association is essential for regulating desmosomal adhesive strength in both developing epidermis and adult stratified tissue (Vasioukhin *et al.*, 2001; Huen *et al.*, 2002).

Desmosome assembly is a calcium-dependent process, where major cytoplasmic plaque components of the desmosome (e.g., DP, keratin) coalesce with other desmosomal proteins (i.e., cadherins, armadillo proteins) at sites of nascent junction formation (Pasdar *et al.*, 1991). Desmosomal proteins accumulate at cell–cell borders over time to form robust intercellular junctions capable of resisting mechanical stress. More recently, it has been reported that as desmosomes mature over the course of several days, they no longer require calcium to maintain intercellular adhesion (Wallis *et al.*, 2000; Cirillo *et al.*, 2010). Calcium-independent desmosomes have been associated with a state of enhanced intercellular adhesive strength termed hyperadhesion (Cirillo *et al.*, 2010; Thomason *et al.*, 2010). It has been hypothesized that the acquisition of hyperadhesion is essential for the epidermis to resist the perpetual mechanical stress to which it is subjected (Garrod and Kimura, 2008).

The underlying signaling and structural properties that govern desmosome maturation and acquisition of hyperadhesion have only recently begun to be studied in greater detail. The data suggest that activation of protein kinase C (PKC) in hyperadhesive epithelial sheets of Madin-Darby canine kidney or HaCaT cells is sufficient to convert desmosomes from calcium-independent to calcium-dependent (Wallis *et al.*, 2000; Cirillo *et al.*, 2010). Likewise, inhibition of PKC signaling is

Abbreviations: DP, desmoplakin; Gly, glycine; PKC, protein kinase C; Ser, serine