

be a type II variant, but the involvement of pre-existing capillaritis and sparing of normal skin appears to be unique.

Department of Dermatology,
Central Manchester and Manchester Children's
University Hospital NHS Trust,
Manchester Royal Infirmary,
Oxford Road, Manchester M13 9WL, U.K.
E-mail: vishalmadan@doctors.org.uk

V. MADAN
J.T. LEAR

References

- 1 Mouton RW, Jordaan HF, Schneider JW. A new type of minocycline-induced cutaneous hyperpigmentation. *Clin Exp Dermatol* 2004; **29**:8–14.
- 2 Pandit S, Hadden W. Black pigmentation of bone due to long-term minocycline use. *Surgeon* 2004; **2**:236–7.
- 3 Birkedal C, Tapscott WJ, Giadrosich K et al. Minocycline-induced black thyroid gland: medical curiosity or a marker for papillary cancer? *Curr Surg* 2001; **58**:470–1.
- 4 Gerson DM, Robinson M. Black pigmentation of atherosclerotic plaques associated with chronic minocycline therapy. *Cardiovasc Pathol* 2006; **15**:168–70.
- 5 Fakhfakh AC, Humbert P, Aubin F. Cutaneous pigmentation induced by minocycline: ultrastructural analysis and X-ray microanalysis. *Ann Dermatol Venerol* 1992; **119**:975–9.
- 6 Sant'Ambrogio S, Connelly J, DiMaio D. Minocycline pigmentation of heart valves. *Cardiovasc Pathol* 1999; **8**:329–32.

Conflicts of interest: none declared.

Anti-desmoglein-1 antibodies are prevalent in Tunisian patients with hydatidosis and leishmaniasis

DOI: 10.1111/j.1365-2133.2006.07687.x

SIR, Pemphigus is an autoimmune blistering disease mediated by pathogenic antibodies directed against desmoglein (Dsg) 1 and Dsg3.^{1,2} In Tunisia, pemphigus has particular epidemiological features. It occurs as an endemic form with an incidence rate of 6.7 cases per million per year. Case series suggest that pemphigus, especially the foliaceus form (PF), is frequent among young Tunisian women with an incidence rate reaching 20 cases per million in rural areas. This could be related to a risk factor more prevalent and/or more often expressed in these areas.^{3,4}

We have reported previously that anti-Dsg1 antibodies were prevalent in Tunisian healthy subjects, which argues for the role of a still unknown environmental factor in the occurrence of Tunisian endemic pemphigus.⁵ An epidemiological case-control study demonstrated that several environmental factors were significantly associated to Tunisian endemic pemphigus such as traditional cosmetics, Turkish baths and cutting up

raw poultry.⁶ However, infectious factors have not been yet investigated.

Recent data demonstrated that in endemic areas of Fogo selvagem, anti-Dsg1 antibodies were found not only in patients with PF, but also in patients with parasitic diseases, thus suggesting a possible involvement of these microorganisms in the aetiopathogenesis of pemphigus.⁷

In Tunisia, hydatidosis and leishmaniasis are two endemic parasitoses that are predominant mainly in rural areas where a higher incidence of Tunisian endemic pemphigus has also been reported.⁸ Based on these observations, we aimed to search for a link between these two parasitoses and Tunisian pemphigus. For this purpose, we searched for antibodies against Dsg1 and Dsg3 in sera samples of parasitic patients. Dsg reactivity was compared with a group of Tunisian normal subjects.

Serum samples were obtained from 58 patients with parasitic disease, 35 with hydatidosis and 23 patients with visceral leishmaniasis. We also obtained sera from 59 patients with PF and 152, age-, sex- and origin-matched, Tunisian normal individuals. Anti-Dsg1 and anti-Dsg3 antibodies were detected by a commercial enzyme-linked immunosorbent assay (ELISA) (MBL, Nagoya, Japan) using recombinant ectodomain of Dsg1 and Dsg3, respectively. The assay method was performed according to the manufacturer's instructions. The cut-off values were set at 14 and 7 for anti-Dsg1 and anti-Dsg3 ELISAs, respectively. Index values ranging from the cut-off value to 20 were considered as weakly positive. For all positive sera, we completed with (i) IgG subclass studies by ELISA, using antihuman IgG1, IgG2, IgG3 and IgG4 murine monoclonal antibodies (Sigma, Steinheim, Germany); (ii) indirect immunofluorescence on monkey oesophagus using a fluorescein-conjugated antihuman IgG antibody (The Binding Site, Birmingham, U.K.); and (iii) immunoblot analysis using human epidermal extract as described.⁹ We also tested patients with PF and controls for antileishmania and antihydatid fluid antibodies using an 'in house' ELISA as described.^{10,11} Plates were coated with hydatid fluid or soluble leishmania extract and cut-off values were calculated as the mean plus 2 SD of assay values obtained from Tunisian healthy subjects.

For Dsg1 ELISA, 55 (93.22%) patients with PF had positive values, as expected and observed in other studies. Ten sera from healthy individuals (6.57%) were also positive for anti-Dsg1 antibodies. In the infectious disease group, we found positive results in 14 of the 35 hydatidosis sera (40%) and five of the 23 visceral leishmaniasis sera (21.7%). The prevalence of anti-Dsg1 in these two groups was significantly higher than that of the control group ($P < 10^{-4}$ and $P = 0.04$, respectively). Index values were higher than 20 for 11 sera from the hydatidosis group, and for two sera from the visceral leishmaniasis group (Fig. 1a).

For Dsg3 ELISA, only three sera from patients with hydatidosis (8.5%) and four sera from patients with visceral leishmaniasis (17.3%) were positive. The prevalence of antibodies directed against Dsg3 was significantly higher ($P = 0.004$) in sera from the visceral leishmaniasis group than the control

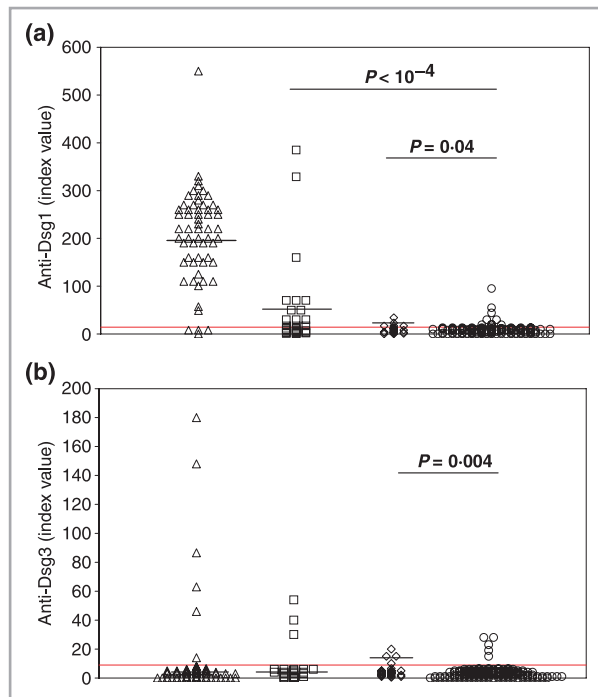


Fig. 1. Analysis of sera from patients with pemphigus foliaceus (Δ), hydatidosis ($\square$), visceral leishmaniasis ($\diamond$) and normal subjects ($\circ$) in ELISA using the recombinant ectodomain of desmoglein (Dsg) 1 (a) and of Dsg3 (b). The red line shows the cut-off value. The mean index value of each group is indicated.

group (3%). However, all index values were weakly positive in the visceral leishmaniasis group (Fig. 1b).

IgG subclass studies showed that anti-Dsg1 antibodies found in patients with hydatidosis were IgG2 in all cases. In two patients, additional IgG1 and IgG3 anti-Dsg1 antibodies were also detected. In visceral leishmaniasis patients, IgG3 was the most common subclass, followed by IgG1 and IgG2. Similar subclass distribution was noted for anti-Dsg3 antibodies (Table 1).

Table 1 Reactivity of IgG subclasses with desmoglein 1 and 3 in patients with hydatidosis and visceral leishmaniasis

IgG subclasses	Hydatidosis (n = 35)	Visceral leishmaniasis (n = 23)
Dsg1 ELISA		
IgG	14	5
IgG1	1 (7%)	1 (20%)
IgG2	14 (100%)	1 (20%)
IgG3	1 (7%)	2 (40%)
IgG4	0 (0%)	0 (0%)
None	0 (0%)	1 (20%)
Dsg3 ELISA		
IgG	3	4
IgG1	1 (33%)	0 (0%)
IgG2	3 (100%)	1 (25%)
IgG3	0 (0%)	3 (75%)
IgG4	0 (0%)	0 (0%)
None	0 (0%)	0 (0%)

Among all positive sera (for anti-Dsg1 and or anti-Dsg3) from parasitic patients, only two sera, from a patient with hydatidosis and a patient with visceral leishmaniasis, showed intercellular staining on monkey oesophagus with a titre of 1:10. By immunoblot analysis on epidermal extract, only one serum from a patient with hydatidosis reacted with the 160-kDa band of Dsg1.

The prevalence of antibodies against parasitic antigens in sera samples from patients with PF did not differ significantly from the control group. For antihydatid fluid antibodies, similar prevalences (4%) were detected in patients with PF and in controls. A higher prevalence of anti-soluble leishmania extract antibodies was observed (10% in patients with PF vs. 5% in controls); however, the difference was not statistically significant.

In this study, we demonstrated that a significant number of patients with parasitic diseases, such as leishmaniasis and hydatidosis, possess anti-Dsg1 and/or anti-Dsg3 antibodies when tested against the ectodomain of desmogleins using both highly specific and sensitive antibodies. These antibodies belong to IgG1, IgG2 and IgG3 subclasses but were never IgG4, which is the most common in Tunisian patients with pemphigus.⁵ Recent data demonstrated that in endemic areas of Fogo selvagem, anti-Dsg1 antibodies are prevalent in patients with parasitic diseases, thus suggesting a possible involvement of these microorganisms in the aetiopathogenesis of pemphigus.⁷ In this study, Diaz *et al.*⁷ found that 83% of patients with onchocerciasis, 43% of patients with leishmaniasis and 58% with Chagas disease have anti-Dsg1 antibodies. Epitope mapping studies revealed that all positive sera recognized epitopes on the EC5 (extracellular 5) domain of Dsg1. The authors hypothesized that this domain might contain the initial epitope(s) that trigger(s) the autoimmune response in pemphigus. In our study, the majority of positive sera gave negative staining by indirect immunofluorescence and immunoblot analysis. Several explanations may account for the discrepancy between results given by ELISA and other immunological techniques. Firstly, the solid phase ELISA may be more sensitive than other assays for detecting anti-Dsg antibodies. Secondly, and as reported by Li *et al.*,¹² antibodies against anti-EC5 gave negative staining by indirect immunofluorescence due to the cryptic nature of the EC5 domain. We can hypothesize that anti-Dsg1 antibodies detected in our parasitic patients might be directed against cryptic, conformational epitopes on the EC5 domain. Further studies using an immunoprecipitation assay with recombinant proteins are needed to confirm this point.

The second finding of this study is that pemphigus patients had negative serological tests for hydatidosis and leishmaniasis. Similar findings were also observed in Fogo selvagem sera, which did not recognize parasitic antigens. Diaz *et al.* hypothesized that saliva of the haematophagous vector containing cadherin proteins could be the sensitizing antigens and not the parasite itself.⁷ Although there is no haematophagous vector for hydatidosis, this parasite could use 'cadherin-like' molecules in the initial phase of invasion

(oncosphere stage) to cross the intestinal barrier. It is of note that many arguments are in line with a potential role of hydatidosis in the aetiopathogenesis of pemphigus. Firstly, these two diseases are endemic in rural areas and occur more frequently in women. Interestingly, contact with ruminants, which are hosts for *Echinococcus granulosus*, was found to be a risk factor for Tunisian endemic pemphigus.⁶ Secondly, hydatidosis seems to offer an adequate microenvironment for IgG4 isotype switching.¹³

In conclusion, our results confirm that anti-Dsg1 antibodies are prevalent in patients with parasitic diseases, suggesting a possible link of these environmental factors to Tunisian endemic pemphigus. Additional experiments and follow-up of subjects involved in the present study are needed to confirm a direct link between these parasitoses and Tunisian endemic pemphigus.

Acknowledgments

This work was supported by a grant from Le Comité Mixte Pour La Coopération Universitaire Franco-Tunisienne. We thank Pr Louzir H for kindly providing leishmania extract.

Laboratoire d'Immunologie and	M. KALLEL SELLAMI
‡Service de Dermatologie,	M. ZITOUNI*
Hôpital La Rabta 1007, Tunis, Tunisia	W. TOMBARI*
†Laboratoire d'Immunologie,	M. BEN AYED†
Hôpital Habib Bourguiba, Sfax, Tunisia	O. ABIDA†
§Service de Dermatologie,	L. LAADHAR*
Hôpital Charles Nicolle, Tunis, Tunisia	M. MOKNI‡
¶Service de Dermatologie,	B. FEZZA§
Hôpital Hédi Chaker, Sfax, Tunisia	H. TURKI¶
Service de Dermatologie,	I. MOKHTAR
Hôpital Habib Thameur, Tunis, Tunisia	A. BEN OSMAN‡
††INSERM U519, Institut Fédératif	R. KAMOUN MOHAMED§
de Recherche Multidisciplinaire	P. JOLY††
sur les Peptides (IFR23), Faculté Mixte	F. TRON††
de Médecine et de Pharmacie, Rouen, France	D. GILBERT††
	H. MASMOUDI†
	S. MAKNI*

(MEMBERS OF THE 'FRANCO-TUNISIAN GROUP OF SURVEY AND RESEARCH ON TUNISIAN ENDEMIC PEMPHIGUS')

Correspondence: M. Kallel Sellami

E-mail: maryam_kallel@yahoo.com

References

- Koulu L, Kusumi A, Steinberg MS et al. Human autoantibodies against a desmosomal core protein in pemphigus foliaceus. *J Exp Med* 1984; **160**:1509–18.
- Ogawa MM, Hashimoto T, Konohana A et al. Immunoblot analysis of Brazilian pemphigus foliaceus antigen using different antigen sources. *Arch Dermatol Res* 1990; **282**:84–8.
- Morini JP, Jomaa B, Gorgi Y et al. Pemphigus foliaceus in young women. An endemic focus in the Sousse area of Tunisia. *Arch Dermatol* 1993; **129**:69–73.
- Bastuji Garin S, Souissi R, Blum L et al. Comparative epidemiology of pemphigus in Tunisia and France: unusual incidence of pemphigus foliaceus in young Tunisian women. *J Invest Dermatol* 1995; **104**:302–5.
- Kallel Sellami M, Ben Ayed M, Mouquet H et al. Anti-desmoglein 1 antibodies in Tunisian healthy subjects: arguments for the role of environmental factors in the occurrence of Tunisian pemphigus foliaceus. *Clin Exp Immunol* 2004; **137**:195–200.
- Bastuji Garin S, Turki H, Mokhtar I et al. Possible relation of Tunisian pemphigus with traditional cosmetics: a multicenter case-control study. *Am J Epidemiol* 2002; **155**:249–56.
- Diaz LA, Arteaga LA, Hilario-Vargas J et al. Anti-desmoglein-1 antibodies in onchocerciasis, leishmaniasis, and Chagas disease suggest a possible etiological link to Fogo selvagem. *J Invest Dermatol* 2004; **123**:1045–51.
- Ben Rachid MS, Ben Ammar R, Redissi T et al. Geography of major parasitosis in Tunisia. *Arch Inst Pasteur Tunis* 1984; **61**:17–41.
- Hashimoto T, Ogawa MM, Konohana A, Nishikawa T. Detection of pemphigus vulgaris and pemphigus foliaceus antigens by immunoblot analysis using different antigen sources. *J Invest Dermatol* 1990; **94**:327–31.
- Maalej IA, Chenik M, Louzir H et al. Comparative evaluation of ELISAs based on ten recombinant or purified Leishmania antigens for the serodiagnosis of Mediterranean visceral leishmaniasis. *Am J Trop Med Hyg* 2003; **68**:312–20.
- Rickard MD, Honey RD, Brumley JL, Mitchell GF. Serological diagnosis and post-operative surveillance of human hydatid disease. The enzyme-linked immunosorbent assay (ELISA) using various antigens. *Pathology* 1984; **16**:211–15.
- Li N, Aoki V, Hans-Filho G et al. The role of intramolecular epitope spreading in the pathogenesis of endemic pemphigus foliaceus (Fogo selvagem). *J Exp Med* 2003; **197**:1501–10.
- Aceti A, Pennica A, Teggi A et al. IgG subclasses in human hydatid disease: prominence of the IgG4 response. *Int Arch Allergy Immunol* 1993; **102**:347–51.

Conflicts of interest: none declared.

Needle-free anaesthesia prior to botulinum toxin type A injection treatment of palmar and plantar hyperhidrosis

DOI: 10.1111/j.1365-2133.2007.07691.x

SIR, Botulinum toxin type A (BTX-A) has emerged as a new technique to treat intractable cases of focal hyperhidrosis (HH).¹ Injections on the palms and soles, although effective, are very painful and need some form of anaesthesia. Peripheral nerve blockade, the standard method of anaesthesia used, may have serious drawbacks,² particularly when used repeatedly. Needle-free anaesthesia may offer an alternative solution.³ The technique involves the use of needle-free injection of 0.02–0.04 mL of 2% lidocaine without adrenaline prior to BTX-A injection with needle. The mixture of lidocaine and BTX-A does not jeopardize toxin potency.⁴

The device used to achieve needle-free anaesthesia is the MED-JET® MBX (Medical International Technologies,