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Tropical medicine rounds

Antidesmoglein 1 and 3 antibodies in healthy subjects of a population in the Peruvian high amazon

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Abstract

Background The objective of this study was to determine the presence of anti-Dsg1 and Dsg3 antibodies in healthy subjects of the high Peruvian Amazon (Tuemal, Rodriguez de Mendoza province, department of Amazonas) to establish the theoretical presence of environmental factors or triggers in the area.

Materials and methods Cross-sectional study. The study population included persons of any age or gender, clinically healthy, who were evaluated by a dermatologist to confirm the absence of blistering diseases. Blood samples were analyzed by indirect immunofluorescence (IIF), immunoprecipitation (IP), anti-Dsg1 IgM antibody (Ab) enzyme-linked immunosorbent assay (ELISA), as well as anti-Dsg1 and anti-Dsg3 IgG Ab ELISA.

Results Participants included 21 healthy subjects comprised of 61.9% males and 38.1% females; 47.6% had a positive anti-Dsg1 Ab ELISA for total IgG (or any subclasses). IIF detected antibodies against intercellular spaces in one subject. Anti-Dsg1 Ab IP was mildly positive in 33.3% of the subjects. Anti-Dsg1 IgG subclasses found positive were: IgG1 (19.0%), IgG2 (33.3%), and IgG3 (28.6%); none of the samples were positive for anti-Dsg1 Ab IgM ELISA, and 23.8% of the subjects were positive for anti-Dsg3 Ab ELISA. The age distribution was similar for subjects positive for anti-Dsg1 and anti-Dsg3 Ab ELISA, with higher frequencies found among the 20–29 and 40–49 year-old age groups.

Conclusion A fraction of healthy subjects of the high Peruvian Amazon developed anti-Dsg1 and anti-Dsg3 antibodies, demonstrating the possible presence of environmental factors for endemic pemphigus (EP) at a higher altitude than previously described.

Introduction

Endemic pemphigus foliaceus (EPF) and endemic pemphigus vulgaris (EPV) are the only autoimmune diseases known to occur in delineated areas with an endemic pattern.[1–9] The etiology of both diseases remains unknown; however, the action of one or many environmental triggers has been proposed.[10–12] A preponderant role has been attributed to the sting of hematophagous insects, particularly the genus *Simullium*.[12–16] According to this hypothesis, exposure of genetically predisposed healthy subjects in endemic areas to a hematophagous insect bite (or other environmental trigger) would cause development of anti-Dsg1 antibodies (during a preclinical phase) and subsequent clinical development of the disease with bullous or blistering lesions.[10,11]

The first reported case of anti-Dsg 1 antibodies in healthy subjects of an endemic area of Brazil was described by Warren in 2000.[17] The authors of the study used ELISA to detect antibodies in a sample of 70 patients with EPF, 372 healthy subjects that lived in endemic areas of Brazil (Limao Verde and surrounding areas), and 126 healthy subjects of Japan and the United States, where the disease is not endemic. The study demonstrated that the prevalence of anti-Dsg1 antibodies in healthy subjects of Limao Verde was 55%, while the prevalence in the less-endemic areas surrounding Limao Verde was 19%. In comparison, the prevalence of anti-Dsg1 antibodies in healthy subjects of nonendemic areas was only 2%. A later study about the development of anti-Dsg1 antibodies in healthy subjects of an endemic area of Tunisia was made by Kallel-Sellami *et al.*[18] who found that, of a sample of 179 healthy blood

donors, 17% were positive for anti-Dsg1 Abs, with IgG2 subclass expressed in all positive donors. IgG1 or IgG3 subclasses were occasionally found accompanying a positive IgG2 positive result. There have also been reports of anti-Dsg3 Abs in healthy subjects living in endemic areas of Brazil[19] and Peru.[20]

The first study[21] regarding the presence of anti-Dsg1 Abs in people living in endemic areas of the Peruvian Amazon was published by our team in 2003. In that study, 41 healthy subjects of the Campo Verde district, widely regarded as one of the endemic areas of EPF in Peru, were screened for anti-Dsg Abs. A prevalence of 31.7% of anti-Dsg Abs was found among the subjects of Campo Verde; in comparison, healthy controls (of the city of Pucallpa) showed a prevalence of only 12.1%. A later study of this same population showed that at 4-year follow-up,[20] 3 healthy subjects of Campo Verde had developed anti-Dsg1 Abs, raising the prevalence to 39.0%.

The majority of EPF reports occur in endemic areas in the range of 500–800 m a.s.l.[12,22]; however, between 2001 and 2003, Peruvian dermatologists practicing in the high Amazon of the Department of Amazonas evaluated 10 cases of pemphigus foliaceus (PF) in young adults.

The objective of this study was to determine the presence of anti-Dsg1 and Dsg3 antibodies in healthy subjects of the town of Tuemal (Rodriguez de Mendoza province, Department of Amazonas, Peru), located at 1200 m elevation, to establish the theoretical presence of environmental factors for endemic pemphigus (EP) at such altitudes.

Materials and methods

Cross-sectional study performed in July 2004. Included in the study were persons of any age or gender who had no history nor current diagnosis of blistering diseases. People with any systemic disease or who worked or travelled to a different community at least twice a week were excluded from the study. Of the 27 households that were found eligible, 21 households elected to participate (Fig. 1). One person from each household was selected through simple randomization.

Healthy subjects were evaluated by a dermatologist to confirm the absence of any blistering disease. Blood samples were analyzed using indirect immunofluorescence (IIF), immunoprecipitation (IP) with recombinant Dsg1 and Dsg3, and ELISA IgG anti-Dsg1 and anti-Dsg3 according to the technique described by Ishi *et al.*[23] and Amagai *et al.*[24] The use of these tests has been reported in a previous publication by our group.[5] Epidemiologic and laboratory data were recorded in a previously validated instrument.[20,21]

Blood sampling

Blood samples were preserved at −20 °C at the Clinical Research Institute at Universidad Nacional Mayor de San Marcos (Lima, Peru) and transported according to protocol to the dermatologic research laboratories of the University of North Carolina at Chapel Hill (USA) for appropriate processing.

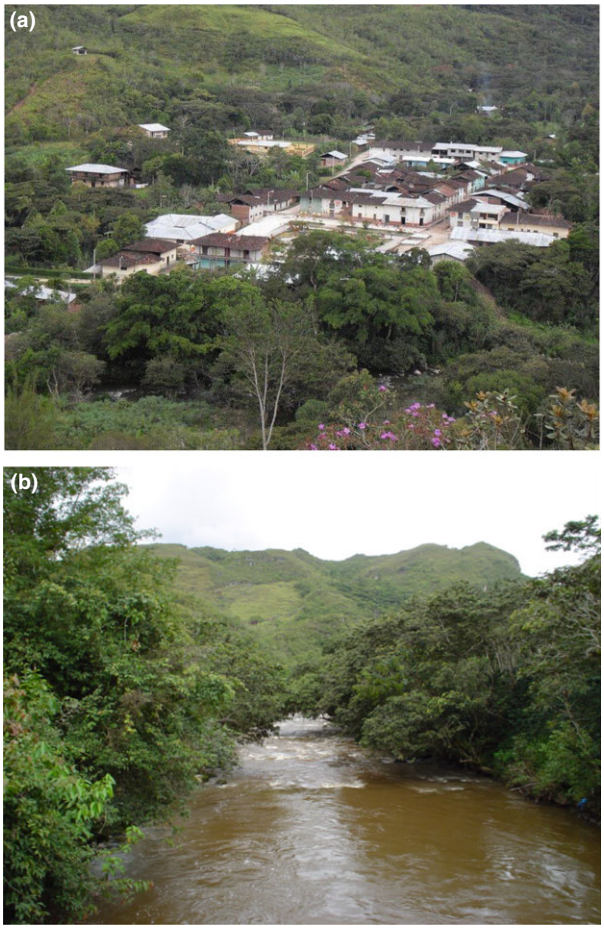


Figure 1 Town of Tuemal located at 1200 m a.s.l. (a) and the nearby San Antonio River (b)

Statistical analysis

Single variable statistics were performed based on obtained frequencies, percentages, measures of central tendency, and dispersion.

Ethical concerns

All serological studies were performed at the University of North Carolina at Chapel Hill, and the study was approved by the board members of research and ethics of the Clinical Research Institute at Universidad Nacional Mayor de San Marcos, Lima, Peru. The subjects who had a positive result for anti-Dsg1 and anti-Dsg3 Abs were informed about their risk to develop EPF and were encouraged to avoid exposure to hematophagous insects and other possible triggers.

Results

Twenty-one healthy subjects participated in this study. The median of age was 28 years old, and the range was between 7 and 82 years; 61.9% of the participants were male, and 38.1% were female. The most common occupation was farmer (61.9%), and most common residence type was mud walls with a tin roof. The characteristics of the subjects and their households are shown in Table 1.

A total of 47.6% (10/21) of healthy subjects were positive for IgG (or any of its subclasses) anti-Dsg1 Abs. IIF detected antibodies against the intercellular spaces in only one of the healthy subjects when monkey esophagus was used as a substrate, and none were detected when normal human skin was used. IP anti-Dsg1 was slightly positive in 33.3% of the subjects. Anti-Dsg1 IgG subclasses detected were: IgG1 (19.0%), IgG2 (33.3%), and IgG3 (28.6%); none were positive for IgG4. All evaluated subjects were negative for IgM anti-Dsg1 Abs by ELISA (Table 2, Graph 1).

We determined that 23.8% of evaluated healthy subjects were positive by ELISA for anti-Dsg3 Abs with index values greater than 100 in 3 of 5 positive subjects. IP anti-Dsg3 was slightly positive in only one subject (Table 2).

Table 1 Epidemiologic characteristics of healthy subjects of the town of Tuemal

Charateristic	Frequency	%
Age (years)		
0–9	2	9.5
10–19	4	19.1
20–29	5	23.8
30–39	2	9.5
40–49	4	19.1
50–59	2	9.5
60 or more years	2	9.5
Sex		
Male	13	61.9
Female	8	38.1
Occupation		
Farmer (agriculture)	10	47.6
Merchant	4	19.1
Student	3	14.3
Housewife	2	9.5
Rancher	2	9.5
Exposure to hematophagous insects		
Yes	15	71.4
No	6	28.6
House material		
Mud	17	81.0
Cement	2	9.5
Wood	2	9.5
Roof material		
Tin	18	85.7
Wood	3	14.3

The age group distribution was similar for anti-Dsg1 Abs and anti-Dsg3 Abs, with a higher frequency of positive results among the ages of 20–29 and 40–49 years (Graph 2).

Discussion

This study shows that Amazonic populations like the one of Tuemal, located above 800 m a.s.l., can develop anti-Dsg1 and anti-Dsg3 antibodies. In this research study, close to half of the studied subjects in Tuemal had a positive result for anti-Dsg1 IgG and a fifth for anti-Dsg3 IgG, despite residing at close to 1200 m a.s.l.

The prevalence of anti-Dsg1 Abs in healthy subjects of Tuemal was 47.6%, which is higher than that reported in one our previous studies in a nonendemic area for EPF in the Peruvian Amazon (12.1%).[21] This prevalence is also higher than the ones reported in healthy subjects in France, the United States, and Japan.[17,18]

Moreover, the prevalence of anti-Dsg3 Abs found in Tuemal (23.8%) was higher than that reported in urban nonendemic areas of EPF in Brazil such as Sao Paulo (1%) and Campo Grande (3%). Unfortunately, there are no population studies of antidesmoglein 3 in Peru.

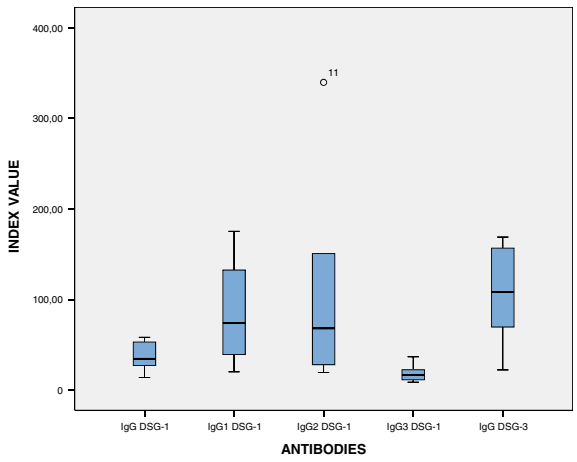
Both types of antibodies presented with a similar age group distribution, showing peaks in the third and fifth decades of life. This distribution could indicate that exposure to triggers, particularly hematophagous insects, happens during the most active and productive ages and thus are possibly related to occupational exposure, particularly farming and agriculture, as it has been previously observed in Pueblo Libre (Ucayali, Peru).[20,21] In contrast with Pueblo Libre (180 m a.s.l.) and Terena Reservation in Limao Verde (Brazil, 260 m a.s.l.), the households of Tuemal offered more protection against hematophagous insects, making exposure in the home comparatively less likely. Anti-Dsg 1 IgG4 Abs was negative in all studied subjects of Tuemal, which indicates that none of them were developing the preclinical phase of EPF.

The prevalence of anti-Dsg 1 IgM abs from Pueblo Libre was 25.0% and in some rural areas of Brazil was even up to 42%[25]; however, none of the healthy subjects of Tuemal tested positive for anti-Dsg1 IgM Abs. The fact that the endemic areas of Brazil had a higher prevalence of IgM antibodies could indicate a greater antigenic stimulation than Tuemal, perhaps explained by the difference in environmental conditions related to altitude. Longitudinal studies are necessary to determine whether changes in the prevalence of antibodies against desmoglein 1 and 3 in healthy subjects from Tuemal is similar to that one previously reported in an endemic area of EPF by our group, which increased in a 4-year period.[20]

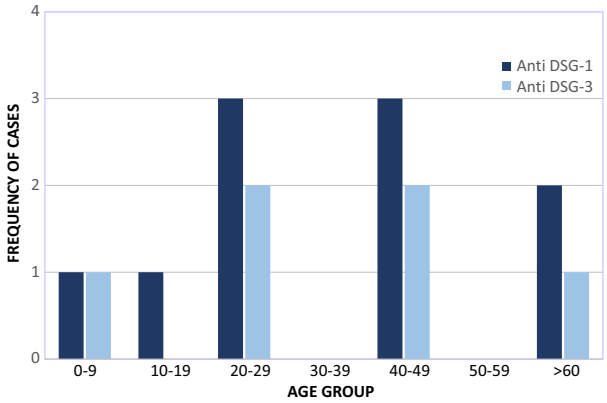
The variability in the detection of antibodies against desmoglein 1 and 3 using different immunological techniques has been addressed in previous studies.[5,19] The sera of the healthy

Table 2 Anti-Dsg1 and anti-Dsg3 antibodies in healthy subjects of the town of Tuemal (Amazonas, Peru)

Códe	IIF NHS	IIF ME	IP DSG1	ELISA IgM DSG1	ELISA IgG DSG1	ELISA IgG1 DSG1	ELISA IgG2 DSG1	ELISA IgG3 DSG1	ELISA IgG4 DSG1	IP DSG3	ELISA IgG DSG3
TM1	Neg	1;80	1+	Neg	52.99	175.32	339.62	22.43	Neg	Neg	156.75
TM2	Neg	Neg	Neg	Neg	Neg	58.31	19.52	Neg	Neg	Neg	Neg
TM3	Neg	Neg	1+	Neg	35.45	Neg	68.16	36.81	Neg	Neg	168.87
TM4	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	11.26	Neg	Neg	Neg
TM6	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM7	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM8	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM9	Neg	Neg	Neg	Neg	Neg	Neg	32.23	Neg	Neg	Neg	Neg
TM10	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM11	Neg	Neg	1+	Neg	58.17	22.69	Neg	6.81	Neg	1+	108.33
TM12	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM13	Neg	Neg	1+	Neg	13.89	Neg	Neg	8.71	Neg	Neg	Neg
TM14	Neg	Neg	1+	Neg	33.38	Neg	150.84	Neg	Neg	Neg	Neg
TM15	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM16	Neg	Neg	1+	Neg	Neg	Neg	23.93	Neg	Neg	Neg	22.26
TM17	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM18	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM19	Neg	Neg	1+	Neg	27.18	89.69	150.45	16.60	Neg	Neg	69.58
TM20	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
TM21	Neg	Neg	Neg	Neg	Neg	20.05	Neg	Neg	Neg	Neg	Neg



Graph 1 Index values of anti-desmoglein 1 and 3 (ELISA) antibodies in positive healthy subjects



Graph 2 Positive results for anti-Dsg1 and anti-Dsg3 (ELISA) antibodies according to age group

subjects might contain insufficient quantities of pathogenic antibodies, or the antibodies in sera may be directed against particular epitopes that are not well exposed on the native protein or denatures by the immunofluorescence or immunoblotting techniques.

Many of the people studied reported frequent exposure to hematophagous insects. This is consistent with other studies showing that the district of Omia (where Tuemal is located) is endemic for other vector-related diseases such as leishmaniasis[26] and Chagas disease,[27] both of which could have a role in the pathogenesis of EPF.[28]

A limitation of this study was the sample size; however, this limitation does not significantly affect the conclusions of this study because of the randomization and high prevalence of anti-Dsg1 and anti-Dsg3 Abs.

In conclusion, a fraction of the healthy subjects of the Tuemal, located at 1200 m of elevation developed anti-Dsg1 and anti-Dsg3 antibodies, demonstrating the possible presence of environmental factors for endemic pemphigus (EP) at such altitude.

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