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Class II HLA in Mexican patients with pemphigus vulgaris: a shared epitope for autoimmunity

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Abstract

Introduction: *Pemphigus is an autoimmune blistering disease of skin and mucous membranes characterized by presence of IgG antibodies against desmoglein 3, and 1. Desmoglein 3 and 1 are presented in pemphigus vulgaris and pemphigus foliaceous, respectively. Desmoglein are transmembrane proteins that form part of cellular junctions called desmosomes. Major histocompatibility complex class II molecules have been related to autoimmune disease; in pemphigus vulgaris, different human lymphocyte antigens (HLA) were associated among different ethnic groups, such as HLA-DR4, HLA-DR14, and HLA-DR1.* **Objective:** *to determine the allele HLA-DR genetic frequencies in Mexican patients with pemphigus.* **Method:** *Patients with clinical, histological, and immunofluorescence diagnosis monitored at the Dermatology Department of the Mexican General Hospital were included. DNA was extracted from blood samples and genetic recognition of HLA-DRβ1 was performed by polymerase chain reaction and hybridization. Forty-three patients with pemphigus were included: 35 (81.4%) women and eight men (18.6%) between 16 and 85 years old.* **Results:** *The HLA-DR14 and HLA-DR1 genetic frequencies were elevated among pemphigus patients and these alleles confer relative risk to pemphigus 2.2 and 3.3, respectively.* **Conclusion:** *These findings suggest that pemphigus vulgaris susceptibility is part of a general predisposition to present autoimmune diseases.* (Gac Med Mex. 2016;152:527-30)

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Introduction

Pemphigus vulgaris (PV) is an acquired autoimmune disease where IgG antibodies targeting desmosomal proteins produce intraepithelial blisters (Figs. 1 and 2). Desmoglein (Dsg) 3 is the main antigen[1], but 50% to 60% of patients have additional anti-Dsg 1 antibodies, which is pemphigus foliaceus (PF) antigen[2]. Pemphigus has been observed at all races and ages, predominating at 30-60 years age. It affects both genders, with slight predominance in females at a 1.6:1 ratio. Its frequency ranges from 0.5 to 3.2 per 100,000 population per year[3]. Mortality was 75% prior to the introduction of corticosteroids in 1950[4]. PV diagnosis is established by perilesional intact or clinically uncompromised skin histological study. Suprabasal acantholysis and blister formation are highly suggestive of PV (Fig. 2), but the diagnosis should be confirmed by direct immunofluorescence[5], where the characteristic

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Figure 1. *Patient with PV showing flaccid blisters and erosions* (pictures from the Hospital General Dr. Manuel Gea González Dermatology Department files).

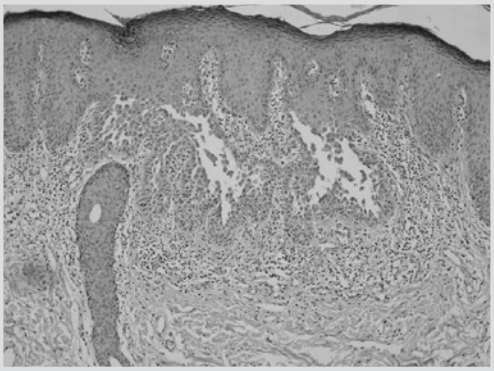

Figure 2. *Histopathology, H&E, 10x. Observe the formation of intraepithelial blisters, characterized by suprabasal acantholysis. Papillary dermis with abundant inflammatory infiltrate.*

IgG deposit on the epidermal intracellular space is observed (Fig. 2)[2]. Molecules that participate in the production of auto-antibodies are encoded by numerous genes, some of them located within a genetic system known as major histocompatibility complex (MHC)[6]. MHC genes' product plays a role in antigenic recognition by T-lymphocytes; this process occurs by recognition of antigenic determinants known as "epitopes". There are 3 classes of MHC genes: class I, class II and class III; the former two are encoded by the human leukocyte antigen (HLA) system[7]. These molecules act as restriction elements, which are essential for antigen recognition by CD8+ cytotoxic T-lymphocytes[8].

In the case of Mexican patients, in a previous study with a reduced number of participants, HLA-DR14 (DR6) was found to be more common in patients with pemphigus, especially PV, with regard to healthy control population[9]. Therefore, the purpose of our study was to broaden the number of patients and to determine HLA-DR-allele gene frequencies in Mexican patients with pemphigus.

Material and methods

A prospective, comparative, open-label, observational study was carried out. Forty-three patients who sequentially attended the Dermatology Department of the General Hospital *Dr. Manuel Gea González* over a period of 12 consecutive months, with clinical and histological diagnosis of pemphigus confirmed by direct immunofluorescence, were included. Informed consent was obtained from all patients, and all procedures were conducted in accordance with the principles expressed in the Declaration of Helsinki and the regulations of the ethics committee of the participating institutions. The controls meet the condition of being Mexican without a pemphigus diagnosis.

Genomic DNA was isolated from peripheral blood mononuclear cells using the salting-out technique[10]. HLA-DRβ1 genotyping was carried out by means of the polymerase chain reaction method with 0.125 U/µl of Taq DNA polymerase (Promega, Madison, WI), followed by hybridization with sequence-specific probes using the Dynal PCR-SSO equipment (Hoffman La-Roche). The information on the nucleotides and probes that were used was taken from the specifications for DR and DQ published in the 12th International Histocompatibility Workshop. Descriptive statistics was used for epidemiological data analysis. Gene frequencies were calculated based on the frequency of each gene or allele with regard to a total of 2N (one from the mother and other from the father). The analysis of each HLA allele association frequency was carried out by means of 2 x 2 contingency tables, and the chi-square test and Fisher's exact test were applied. The strength of association in the 2 x 2 tables was determined with the odds ratio, where a value above 1 is positive or susceptibility association, and below 1 it is negative or protective association.

Table 1. HLA-DRB1 of patients with pemphigus vulgaris and controls

DRB1	Cases (n = 86)		Controls (n = 198)			
	n	gf	n	gf	p	RR
DR4	27	0.313	47	0.237	NS	–
DR14	20	0.232	21	0.105	0.09	2.5
DR8	10	0.116	33	0.165	NS	–
DR13	3	0.034	10	0.05	NS	–
DR11	2	0.023	20	0.1	NS	–
DR1	13	0.151	10	0.05	0.08	3.3
DR16	2	0.023	5	0.025	NS	–
DR7	4	0.046	22	0.111	NS	–
Others	5	0.058	–	–	NS	–

gf: gene frequency; RR: relative risk.

Results

Forty-three patients clinically diagnosed with pemphigus with confirmation by histopathological study were included in the study, 41 of them (95.3%) with PV and 2 (4.7%) with PF. The age range was from 16 to 84 years at the moment of the study, and mean age was 44.26 ± 18.27, considering the age at the moment of pemphigus diagnosis, with a standard deviation of ± 18.27. The group included 35 females (81.4%) and 8 males (18.6%), with a female:male ratio of 4.375:1. Comorbidities were reported in eight patients, had concomitant skin disorders (one had psoriasis and another had acquired hand-foot hyperkeratosis); one female patient had been previously diagnosed with multiple sclerosis and 5 had type-2 diabetes mellitus. Possible triggering factors were inquired without any relationship being found between PV or PF onset and environmental factors. When HLA-DR gene frequencies in patients with pemphigus were compared with those in the general population, an increase in the HLA-DR14 and HLA-DR1 alleles gene frequency (gf) was found in the patients with pemphigus, with associated relative risk of 2.2 and 3.3, respectively (Table 1).

Discussion

There are different mechanisms that can be responsible for autoreactive T cells activation in autoimmune diseases. Autoimmunity-associated MHC alleles can confer susceptibility by affecting the development of the T-cell repertoire in the thymus and peripheral immune system or by presenting peptides of their own, against which an immune response is directed[11]. This study demonstrates the association of pemphigus with the HLA-DR14 (DR6) and HLA-DR1 alleles in Mexican patients and confirms reports in other populations such as non-Jewish Iranians[12], Ashkenazi Jews[13], Native Americans[14], Latin American Mestizos[15,16], Caucasians[17] and Turks[18], in whom the HLA-DR14 (DRb1*1401, *1405) and HLA-DR4 (DRβ*0402, *0406) alleles confer strong susceptibility for the development of PV. It should be noted that HLA-DR4 is one of the most common alleles in the Mexican population (0.237 gf in healthy controls), and even when it was found to be elevated in patients with pemphigus (0.313 gf), this difference was not statistically significant.

On the other hand, pemphigus onset and progression possibly depend on the interaction of several factors, some known and others yet to be discovered. These include genetic factors such as the already-mentioned HLA alleles, as well as other genes in the affected tissues that might confer higher susceptibility. In the particular case of pemphigus, evidence suggests that molecules that regulate desmogleins expression, such as the cholinergic receptors, are involved in the pathophysiology of the disease[19,20]. Therefore, individuals with genetic predisposition for pemphigus will develop the disease only if one or more additional factors are present. The nature of the majority of these factors is

currently unknown. However, some medications (penicillamine[21], chloroquine[22], imiquimod[23]), radiotherapy[24], viruses[25] and the use of cosmetics[26], among other factors[27], have been reported to act as triggers. From a pathophysiological point of view, we consider pemphigus to be a unique condition with a wide spectrum of clinical presentations, which range from oral PV, without skin involvement with anti-Dsg3 IgG, the mucocutaneous variant with anti-Dsg3 and Dsg1 IgG, to PF only with skin involvement and anti-Dsg1 IgG. Documented cases of transition from PV to PF[28,29], and vice versa, support this idea. On the other hand, specific alleles in the HLA-DRβ1 locus have been associated with autoimmune conditions such as Graves' disease, systemic lupus erythematosus[30,31], autoimmune insulin syndrome, rheumatoid arthritis, autoimmune chronic hepatitis[32] and multiple sclerosis[33]. In the specific case of rheumatoid arthritis, this condition was associated with a restricted number of HLA-DRβ1-locus alleles, especially DRB *0401, *0404 and *0101. Each one of these alleles shares homologous sequences at the third hypervariable region of the β chain, which is known as shared epitope[34]. Together with the above-described, reports on the coexistence of two autoimmune disorders in a single individual support the existence of a shared epitope for autoimmunity and, therefore, we can conclude that disease expression will depend on interaction with the environment, as well as on other yet to be discovered genetic determinants.

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References

1. Amagai M. Desmoglein as a target in autoimmunity and infection. J Am Acad Dermatol. 2003;48:244-52.
2. Harman K, Gratian M, Bhogal B, Challacombe S, Black M. A study of desmoglein 1 autoantibodies in pemphigus vulgaris: racial differences in frequency and the association with a more severe phenotype. Br J Dermatol. 2000;143:343-8.
3. Vega M, Villatoro V, Mosqueda A. Pénfigo vulgar: informe del manejo de 40 casos. Rev Mex Dermatología. 1998;42: 244-9.
4. Harman K, Albert S, Black M. Guidelines for the management of pemphigus vulgaris. Br J Dermatol. 2003;149:926-37.
5. Mutasin D, Adams B. Immunofluorescence in dermatology. J Am Acad Dermatology. 2001;45:803-22.
6. Trowsdale J, Ragoussis J, Campell RD. Map of the human MHC. Immunol Today. 1991;12:443-6.
7. Heinrichs H, Orr HT. HLA non-A, B, C class I genes: their structure and expression. Immunol Res. 1990;9:265-74.
8. Ploegh H, Orr HT, Strominger J. Major histocompatibility antigens: the human (HLA-A, B, C) and murine (H2-K, H2-D) class I molecules. Cell. 1981;42:287-99.
9. Vega-Memije M, Sáez M, Cortés-Franco R, Domínguez L, Granados J. Análisis de HLA-DR en pacientes mexicanos con pénfigo. Gac Méd Méx. 2001;137:535-40.
10. Miller S, Dykes D, Polesky H. A salting-out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.
11. Wucherpfenning K. Structural basis of molecular mimicry. J Autoimm. 2001;16:293-302.
12. Mobini N, Yunis E, Alper C, et al. Identical MHC markers in non-Jewish Iranian and Ashkenazi Jewish patients with pemphigus vulgaris: possible common central Asian ancestral origin. Hum Immunol. 1997;57:62-7.
13. Scharf S, Freidmann A, Steinman L, Brautbar C, Erlich H. Specific HLA DQB y HLA DRB1 alleles confer susceptibility to pemphigus vulgaris. Proc Natl Acad Sci. 1989;86:6215-9.
14. Hans-Filho G. dos Santo V, Katayama J, Aoki V, et al. An active focus of high prevalence of fogo selvagem on an Amerindiam reservation in Brazil. J Invest Dermatol. 1996;107:68-75.
15. Loewnthal R, Slomov Y, Gonzalez-Escribano M, et al. Common ancestral origin of pemphigus vulgaris in Jews and Spaniards: a study using microsatellite markers. Tissue Antigens. 2004;63:326-4.
16. Bordenave K, Griffith J, Hordes S, Williams T, Padilla R. The historical and geomedical immunogenetics of pemphigus among the descendants of Sepahrdic Jews in New Mexico. Arch Dermatol. 2001;137:825-6.
17. Ahmed A, Wagner R, Khatri K, et al. Major histocompatibility complex haplotypes and class II genes in non-Jewish patients with pemphigus vulgaris. Proc Natl Acad Sci. 1991;88:5056-60.
18. Birol A, Yavuzer R, Turkak H, Gürgey E. HLA-class 1 and 2 antigens in Turkish patients with pemphigus. Int J Dermatol. 2002;41:79-83.
19. Vu T, Lee T, Ndoye A, et al. The pathophysiological significance of non desmoglein targets of pemphigus autoimmunity. Development of antibodies against keratinocyte cholinergic receptor in patients with pemphigus vulgaris and pemphigus foliaceus. Arch Dermatol. 1998;134:971-80.
20. Botelho-Nogueira L, Quarantini L, Miranda-Scippa A. Use of anticholinergic drugs and worsening of pemphigus foliaceous in a patient with bipolar disorder. Rev Bras Psiquiatr. 2011;33:412-3.
21. Thurston J, Marks J, Trapnell D. Pemphigus foliaceus induced by penicillamine. Br Med J. 1976;11:1423-4.
22. Ghaffarpour G, Jalali MH, Yaghmaii B, Mazloomi S, Soltani-Arabshahi R. Chloroquine/hydroxychloroquine-induced pemphigus. Int J Dermatol. 2006;45:1261-3.
23. Bauza A, Del-Pozo L, Saus C, Martin A. Pemphigus-like lesions induced by imiquimod. Clin Exp Dermatol. 2009;34:e60-e62.
24. Orion E, Matz H, Wolf R. Pemphigus vulgaris induced by radiotherapy. JEADV. 2004;18:508-9.
25. Tayyebi N, Nahidi Y, Mahmoudi M, et al. Evaluation of coexistence of the human herpesvirus type 8 (HHV-8) infection and pemphigus. Int J Dermatol. 2010;49:780-3.
26. 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.
27. Tur E, Brenner S. Diet and pemphigus. In pursuit of exogenous factors in pemphigus and fogo selvagem. Arch Dermatol.1998;134:1406-10.
28. Tóth G, Pas H, Jonkman M. Transition of pemphigus vulgaris into pemphigus foliaceus confirmed by antidesmoglein ELISA profile. Int J Dermatol. 2002;41:525-7.
29. Tsuji Y, Kawazhima T, Yokoya K, et al. Clinical and serological transition from pemphigus vulgaris to pemphigus foliaceus demostrated by desmoglein ELISA system. Arch Dermatol. 2002;138:95-6.
30. Liu X, Wu Y, Yang Y, et al. Identical twins: one with anti-glomerular basement membrane glomerulonephritis the other with systemic lupus erythematosus. BMC Nephrol. 2013;14:1-5.
31. Furukawa H, Kawasaki A, Oka S, Ito I, Shimada K, Sugii S. Human leukocyte antigens and systemic lupus erythematous: a protective role for the HLA-DR6 alleles DRβ1*13:02 and *14:03. Plos one. 2014;9:e87792.
32. Roh E, Kim YA, Ku EJ, et al. Two cases of methimazol induced insulin autoinmune syndrome in Graves' disease. Endocrinol Metab. 2013;28: 55-60.
33. Zuñiga J, Yu N, Barquera R, et al. HLA class I and class II conserved extended haplotypes and their fragments or blocks in Mexicans: implications for the study of genetic diversity in admixed populations. Plos one. 2013;8:e74442.
34. Wicks I, McColl G, D'Amico A, Dougherty L, Tait B. Use of monoclonal antibodies to detect disease associated HLA-DRB1 alleles and the shared epitope in rheumatoid arthritis. Ann Rheum Dis. 1997;52:135-9.