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HLA-Cw and TCR vβ analysis in twenty Mexican patients with psoriasis

Communication

Leticia Santos[1*], Ma Fabiola León-Galvan[1], Cornelia Fuentes-Ahumada[2], Erika Nahomy Marino-Marmolejo[1], Antonio De León-Rodríguez[1], Ana Paulina Barba De La Rosa[1], Rebeca Ceballos-Salazar[3], Bertha Torres-Alvarez[2], Benjamín Moncada-González[2]

[1] *Division of Molecular Biology, Institute for Scientific and Technological Research of San Luis Potosi (IPICYT), A.C., 78216 San Luis Potosi, Mexico*

[2] *Departmento de Dermatología, Hospital Central Dr. Ignacio Morones Prieto, San Luis Potosi, Mexico*

[3] *Departmento de Microbiología, Centro de Ciencias Basicas, Universidad Autonoma de Aguascalientes, Aguascalientes, Mexico*

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Abstract: Genetic background and T-cell expansion have been confirmed as the most important factors leading to psoriasis susceptibility in the Caucasian population. This study was performed to identify the T-cell receptor Vβ repertoire and HLA-Cw genotype in twenty Mexicans of two different ethnicities with severe chronic plaque-type psoriasis. HLA-Cw typing was performed to detect the allele pattern by SSP-PCR. In parallel, RT-PCR and Western blot were used for the identification of the TCR Vβ expression in peripheral blood cells. We identified a variety of HLA-Cw alleles in this group of patients distinct from the widely known HLA-Cw 0602 Caucasian allele. Moreover, TCR Vβ-2 and Vβ-7 clone-type frequencies were different and statistically significant ($P = 0.0280$). We speculate that because of diverse genetic backgrounds, the susceptibility to disease and activation of T-cells for a proper immune response could be specific; therefore, the findings might contribute to the elucidation of the pathogenesis in psoriatic Mexican patients.

Keywords: *T-cell receptor beta-chain variable region • Human Leukocyte Antigen • Sequence-specific primer-polymerase chain reaction • Psoriasis*

1. Introduction

Psoriasis is a chronic, inflammatory skin disease, with a prevalence of over 1% in most populations; however, among Caucasians, it has a prevalence of 2%–4% [1]. Raychaudhuri *et al.* [2] have reported the prevalence of psoriasis in countries around the world, including North, Central and South America. In Mexico, psoriasis is seen with a frequency similar to worldwide reports; nevertheless, the population is characterized by a mixed ethnicity of indigenous native Mexican and white people, referred to Mestizo. In addition, we have recently noticed a rare prevalence of this disease in a purely indigenous population in a subtropical area of the state of San Luis Potosi in Mexico. To date, only a few studies have been conducted in the search for the genetic background of non-Caucasian ethnic groups in association with psoriasis risk [3,4]. *HLA-C* [5], *CDSN, CCHCR1, SEEK,* and *PSORS1C3* alleles have been investigated by genetic linkage analyses to elucidate the association between the major histocompatibility complex (MHC) class I region and the susceptibility locus (*PSORS1*). Other inflammatory diseases are associated with polymorphisms at MHC class I genes, for example, ankylosing spondylitis with the HLA-B27 allele [6] and subacute thyroiditis with the HLA-B35 allele [7]. However, psoriasis is the only inflammatory disease that strongly associates with HLA-Cw*0602 [8]. The pathogenesis of psoriasis remains elusive: type 1

* *E-mail: lsantos@ipicyt.edu.mx*

T-cells have been involved through the secretion of their cytokines that contributes to epidermal hyperproliferation. To explain the relation between environmental factors and exacerbation of the psoriasis, it has been proposed that psoriasis is a disease of activated innate immunity [9]. However, in treatment of severe psoriasis, the favorable response to drugs blocking T-cell activation supports the notion that T-cell activation plays a key role in the inflammatory reaction. The antigen could be either internal (autoimmune disease) or external. In addition, it is not known whether in different clinical types of the disease the responsible antigen is the same in each occurrence, or different. Superantigens, unlike conventional antigens, activate T-cells expressing certain T-cell receptors, which possess a highly variable region known as the variable β region (TCR Vβ). The importance of this region and its role in autoimmune diseases has been determined by Bour *et al.* [10], although several authors have found differences in the expression of the TCR Vβ activity for the presentation of antigens to the MHC class II on antigen presenting cells [11–13]. Preferential usage of certain T-cell receptors by the lymphocytic infiltrate in psoriasis might indicate the involvement of one or several antigens in the pathogenesis of psoriasis. Thus, a significant change in the pattern of Vβ expression is likely to occur in T-cells responding to such stimuli.

We present our findings for the analysis of the human leukocyte antigen (HLA) alleles Cw type and the frequency of the TCR Vβ usage in association with psoriasis in each group of native indigenous and Mestizo Mexican patients.

2. Material and Methods

2.1. Patients

This study included ten native indigenous patients of the subtropical region of San Luis Potosi State (Group 1) and ten Mestizo patients living in the capital city of San Luis Potosi (Group 2). The patients, whose ages were between 24 to 74 years, presented active chronic plaque-type psoriasis (CPP). The diagnosis was based on clinical and histopathological data from the skin lesions. All patients signed consent letters under the Declaration of Helsinki, and the use of all samples and the experimental procedure for this study was reviewed and approved by the Ethics and Research Committee of the Central Hospital Dr. Ignacio Morones Prieto, Universidad Autonoma de San Luis Potosi, Mexico.

2.2. Samples

A 2-mm biopsy of the psoriatic skin lesion and another from a distant, apparently normal, zone were obtained from each person. All specimens were immediately frozen in liquid nitrogen and then transferred to -80°C until use. Peripheral blood was obtained from each patient, and the heparinized peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation by the Ficoll-Hypaque method (Sigma). Cells were washed twice with sterile PBS, snap frozen, and stored at -80°C until use.

2.3. HLA genotyping and data analysis

Genomic DNA was extracted from PBMCs using the UltraClean™ Blood DNA Isolation Kit (Non-Spin, MO BIO Laboratories Inc.). The HLA-Cw genotype was analyzed by sequence-specific primer-polymerase chain reaction (SSP-PCR) in the Transplantation Laboratory / HLA Laboratory of the Haartman Institute at the University of Helsinki, Finland. Genotype frequencies of different haplotypes were obtained by direct counting and we reported frequencies of each allele.

2.4. TCR Vβ expression

2.4.1. RT-PCR analysis

The PBMCs were homogenized in Trizol (Invitrogen) and RNA isolation was done according to the manufacturer's instructions. The final RNA pellet was resuspended in 20 µl of RNase-free diethyl-pyrocarbonate-treated water. The isolated RNA was reverse transcribed using oligo-dT primer and SuperScript II RNase H Reverse Transcriptase (Invitrogen). For amplification of the cDNAs, we have used the set of 24 pair of primers described by Fernandes *et al.* [14]. The RT-PCR products were run in 2% agarose gel and stained with ethidium bromide.

2.4.2. Antibodies

The following murine anti-human monoclonal antibodies were purchased from Biodesign International: Vβ-2 (clone MPB2D5), which recognizes all alleles of the single member of V beta-2 family; the Vβ-7 (clone ZOE) recognizes V-beta-7.1; the Vβ-11 (clone C21) recognizes the two known sequences PL3.12 and PH15; and the Vβ-13.1 (Immu222) recognizes the V beta-13.1 member. The Vβ-17 (clone E17.5F3), the Vβ-20 (clone ELL 1.4), and Vβ-22 (Immu546) recognize at least the IGRb03 sequence.

2.4.3. TCR Vβ expression analysis by Western blot

The PBMCs cells and skin biopsies were homogenized by sonication; protein quantitation was performed by the Lowry method (Sigma). BSA was used for the standard

Table 1. HLA-Cw genotype in healthy Mexican individuals (organ donors).

Cw allele 1	Cw allele 2	% Frequency	n=129
*-	*03	1.6	2
*-	*04	3.1	4
*-	*05	0.8	1
*-	*07	0.8	1
*01	*02	0.8	1
*01	*03	3.1	4
*01	*04	3.1	4
*01	*07	1.6	2
*02	*03	2.3	3
*02	*04	0.8	1
*02	*06	0.8	1
*02	*07	1.6	2
*03	*-	3.1	4
*03	*01	0.8	1
*03	*02	1.6	2
*03	*03	0.8	1
*03	*04	2.3	3
*03	*05	1.6	2
*03	*06	2.3	3
*03	*07	10.1	13
*03	*08	2.3	3
*04	*-	4.7	6
*04	*02	2.3	3
*04	*03	4.7	6
*04	*04	0.8	1
*04	*05	3.9	5
*04	*06	0.8	1
*04	*07	3.9	5
*04	*08	3.1	4
*04	*09	1.6	2
*04	*10	0.8	1
*05	*02	0.8	1
*05	*03	0.8	1
*05	*04	1.6	2
*05	*07	1.6	2
*06	*01	0.8	1
*06	*02	1.6	2
*06	*03	1.6	2
*06	*04	2.3	3
*06	*08	0.8	1
*07	*03	2.3	3
*07	*04	5.4	7
*07	*06	1.6	2
*07	*07	0.8	1
*07	*08	3.1	4
*08	*03	1.6	2
*08	*04	0.8	1
*09	*01	0.8	1
*09	*04	0.8	1

curve, and the absorption was measured at 590 nm. 50 µl of total protein extract at 5 µg/µl concentration was mixed with 4x loading buffer and heated at 95°C for 5 minutes before loading onto a 12.5% SDS polyacrylamide gel. Electrophoresis was subjected to a 2 mA/cm constant current at room temperature. Separated proteins were transferred onto nitrocellulose membranes (Amersham) using a Bio-Rad Semi-Dry Electrophoretic Transfer Cell following the manufacturer's instructions. The membranes were incubated with a panel of monoclonal antibodies described in the previous subsection against TCR Vβ diluted 1:5,000 in blocking solution for 1–2 hours. Excess of antibody was removed by several washing steps prior to incubation with the secondary antibody anti-mouse IgG alkaline phosphatase-conjugated (Sigma) diluted 1:10,000. Lastly, the membranes were washed and developed with 10 mL of developing solution containing 66 µl NTB (Sigma) and 33 µl BCIP (Sigma) until color appeared. The reaction was stopped with 10 mL stop solution.

2.4.4. Statistical analysis

The statistical analysis between the frequency of the six different TCR Vβ expressions produced by Western blot in patients from Groups 1 and 2 was done using a chi-squared test. Fisher's exact test was used to associate the expression of the TCR Vβs present in the two groups; the two methods were performed using a 2x2 contingency table. Analysis was executed using the GraphPad InStat 5.0 program (GraphPad Software Inc., San Diego, CA). Values of $p<0.05$ were considered significant.

3. Results

3.1. HLA-C expression

We have previously reviewed the HLA-C allele frequencies in healthy Mexican donors (Table 1). We then compared the frequencies identified in this study by PCR-SSP (Table 2). The alleles of these loci were not represented in the tested subjects and could be found only rarely in some patients of the two groups studied. We observed that HLA-Cw*07*07 and HLA-Cw*03*07 are repeated alleles in the psoriatic patients from Group 1, but this was different from those in Group 2: HLA-Cw*01*08*, -Cw*03*12, -Cw*04*16, -Cw*04*12, -Cw*05*07, -Cw*06*08, -Cw*07*08.

Table 2. HLA-Cw allele type of the two psoriatic groups by SSP-PCR.

Group 1* Patient Code	HLA-Cw	Group 2† Patient Code	HLA-Cw
MP H7	*01 *03	MP SLP9	*01 *08
MP H8	*03 *04	MP SLP1	*03 *04
MP H2	*03 *07	MP SLP3	*03 *12
MP H5	*03 *07	MP SLP8	*04 *07
MP H6	*04 *07	MP SLP7	*04 *12
MP H3	*07 *07	MP SLP2	*04 *16
MP H9	*07 *07	MP SLP6	*05 *07
MP H10	*07 *07	MP SLP5	*06 *08
MP H11	*07 *08	MP SLP4	*07 *08
MP H4	*07 *15	MP SLP10	*07 *15

**Group 1, indigenous patients*
†Group 2, Mestizo patients

3.2. TCR Vβ expression

The hypervariable region of the TCR Vβ gene family was examined as reported by several authors [10,13,14] (Table 3). In a subset of regions within group 1, the Vβ2 and Vβ7 subfamilies were predominantly expressed. In contrast, the expression profile of Group 2 shows a wider range of Vβs. To validate these results, the expression profile was also analyzed at the protein level by Western blot. Results illustrate that samples of Group 1 displayed positive bands for most of all TCRs analyzed (Table 4). This activation has an expression pattern different from that in Group 2.

A comparison of TCR Vβs expression between RT-PCR and Western blot analysis was done using Fisher's exact test to examine the association between the methods and the groups studied. Statistical analysis showed TCR Vβ-2 and Vβ-7 were mainly expressed in both groups (p = 0.0280).

4. Discussion

Susceptibility to psoriasis has been investigated through the study of the MHC I genes supporting the allele Cw*0602 in purely Caucasian populations [4,8]. We are interested in an association study, particularly in the ten psoriatic indigenous patients, because they are unique cases found in our dermatology practice. Unlike the evidence, we have found 14 distinct risk alleles in the Mexican patients analyzed (Table 2). As expected, we identified a wider variety of alleles among Mestizo patients who have a Caucasian background (Group 2); a more conserved pattern is seen among the ten indigenous psoriatic patients (Group 1).

Our data was further analyzed against: 1) an HLA-Cw database of 129 healthy Mexican Mestizo individuals who are candidates for organ donation (49 alleles), and 2) a literature report of 167 individuals from distinct regions of Mexico (43 Cw alleles) [15]. As shown in Table 1, the haplotype is very variable, with a 10.1% highest allele frequency (Cw *03*07). Gorodesky *et al.* reported *0401/2 as the highest allele frequency (15.9%). Studies performed by Fan *et al.* [16] suggest that an ethnic population might transmit distinctive susceptibility alleles because of the genetic heterogeneity of the *PSORS1* locus. To date, none of the alleles shown in Table 2 have been reported in association with psoriasis, not even for Chinese or Sardinian populations [17]. We observed that, for the group of patients included in this study, Cw*07 could be a prevalent allele in association with the disease; however, these results must be confirmed in a sufficiently powered study with a representative number of patients.

The TCR repertoire was studied in PBMC using RT-PCR and Western blot. Transcript analysis shows a preferential usage of the Vβ-2 and -7 in Group 1 and a larger usage in Group 2 (Table 3). Frequency analysis of TCR Vβ in PBMCs produced significant differences between the two groups. All samples from Group 1 expressed every TCR Vβ clone type, whereas in Group 2, only 5 patients produced the same results. Statistical analysis showed a $p<0.05$ using the chi-squared test (Table 4). Statistical analysis, including transcript and protein results, suggests two major restricted T-cell expansions: TCR Vβ-2 and TCR Vβ-7. Evidence of the TCR Vβ-2 expression bias has been already reported for T-cells that are destined to migrate to the skin [18], and TCR Vβ-7 expression has been studied in psoriasis, as well as in normal tissues and other diseases [9,19]. Since the majority of the samples from Group 1 were positive to TCRs using Western blot in PBMC, we decided to examine random skin biopsies

Table 3. RT-PCR analysis of TCR Vβ expression in patients with psoriasis in the two groups studied.

Group 1*												Group 2†										
TCR type	1	2	3	4	5	6	7	8	9	10	F‡	1	2	3	4	5	6	7	8	9	10	F*
Vβ-1			+			+				N	20%		+		+	+	+		+		+	60%
Vβ-2	+	+	+	+	+	+	+	+	+	N	100%	+	+	+	+	+	+	+	+	+	+	100%
Vβ-3	+									N	10%	+			+		+				+	40%
Vβ4						+				N	10%									+		10%
Vβ-5S1										N	0%									+		10%
Vβ-5S2										N	0%							+		+		20%
Vβ-6		+				+	+			N	30%				+	+	+					30%
Vβ-7	+	+	+	+	+	+	+	+	+	N	100%	+	+	+	+	+	+	+	+	+	+	100%
Vβ-8	+									N	10%				+		+					20%
Vβ-9						+				N	10%											0%
Vβ-11										N	0%											0%
Vβ-12	+									N	10%					+						10%
Vβ-13S1										N	0%						+					10%
Vβ-13S2	+					+		+		N	30%	+			+	+	+		+	+	+	70%
Vβ-14										N	0%		+		+		+		+		+	50%
Vβ15							+			N	10%											0%
Vβ-16	+									N	10%					+		+				20%
Vβ-17										N	0%											0%
Vβ-18							+		+	N	20%				+							10%
Vβ-20										N	0%		+		+		+		+	+		50%
Vβ-21										N	0%	+			+			+				30%
Vβ-22										N	0%											0%
Vβ-23										N	0%											0%
Vβ-24										N	0%											0%

**Group 1, indigenous patients*
†Group 2, Mestizo patients.
N, Non processed sample
‡F, frequency

(n=6) by immunohistochemistry. We included apparently normal skin biopsies and lesions from the same patients. Infiltrating T-cells were detected in the non-lesion skin biopsies, whereas in lesion skin a paucity of T-cells was observed (data not shown). This inconsistency has been reported by other authors, [20] and could be explained by the event referred to as the Koebner response [12].

Previous studies have reported selectivity of the clones displaying different TCR Vβ of infiltrated T-cells in psoriatic lesions: TCR Vβ-13 and -15 [11], TCR Vβ-5.1, -11, -12, -13.1 and -16 [21], TCR Vβ-3 and -13.1 [10], and more recently TCR Vβ-3, -13S2 and -21 [22]. This evidence is bound to studies of patient specimens with streptococcal infections. In correlation with these data, Kansal *et al.* [23] reported a T-cell expansion expressing TCR Vβ-4, -7 and -8 triggered by a superantigen induced by a specific streptococcal protease. The mechanism has been extensively discussed for psoriasis onset; however, the superantigen theory has not been satisfactorily proved. We assume that similar T-cell events occurred for the selection of the TCR Vβ in this work, although a study that identifies the amino acid sequence(s) antigen(s) involved using mass spectrometry would provide us a more complete scenario.

5. Conclusions

The data obtained suggest that both groups of patients with psoriasis analyzed here presented in their majority the HLA-Cw*07 as the risk allele. Moreover, the different alleles we found suggest that other genetic factors mapping in the MHC region may interact with *PSORS1*.

The analysis of the TCR Vβ gene repertoire in these two groups of patients was also performed to reveal particular immunodominant expansions that might involve antigen-driven T-cell responses with probable pathogenetic/pathophysiologic impact. Determination of the frequency (proportion) of individual Vβ families allows recognition of the poly-, oligo- or monoclonal pattern of responses in a given T-cell pool. We demonstrated that the over-expanded Vβ families (Vβ-2 y Vβ-7 skewing)

Table 4. Western blot analysis of TCR Vβ expression in patients with psoriasis from the two groups studied.

	Group 1*, patient no.											Group 2†, patient no.										
TCR type	1	2	3	4	5	6	7	8	9	10	F*	1	2	3	4	5	6	7	8	9	10	F*
Vβ-2	+	+	+		+	+	+	+	+	+	90%	+	+	+	+		+		+	+	+	80%
Vβ-7	+	+	+	+	+	+	+	+	+	+	100%	+	+	+	+	+	+	+	+	+	+	100%
Vβ-11	+	+	+	+		+			+	+	80%	+	+		+			+	+	+	+	70%
Vβ-13.1	+	+	+	+	+	+		+	+	+	90%	+	+	+	+			+				50%
Vβ-17	+	+	+	+	+	+		+	+	+	90%							+	+	+	+	40%
Vβ-22	+	+		+	+	+	+	+	+	+	90%							+	+		+	30%

**Group 1, indigenous patients*
†Group 2, Mestizo patients
‡F, frequency

suggest oligo/polyclonal T-cell expansions. The accumulation of these skewed T-cell populations into both groups of Mexican patients may reveal a preferential homing process, because they have different genetic backgrounds and their exposure to environmental antigens is assumed to be entirely different. This identification could provide evidence for the antigenic stimulus associated with the pathophysiology of the disease, and thus might contribute to the elucidation of the pathogenesis in Mexican psoriatic patients, or in patients with a similar genetic background, to facilitate their treatment.

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