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HLA-C genotype and TCR vβ expression analysis in Mexican patients with Psoriasis

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Short title: HLA and TCR vβ analysis in psoriatic Mexican patients

Abbreviations: TCR vβ, T-cell receptor beta-chain variable; HLA, Human Leukocyte Antigen; RT-PCR, Reverse Transcriptase-Polymerase Chain Reaction; SSP-PCR, Sequence-Specific Primer-Polymerase Chain Reaction.

Abstract

Genetic background and T-cell expansion have been associated as the most important factors for psoriasis susceptibility in the Caucasian population. This study was performed to identify the T-cell receptor Vβ repertoire and HLA-Cw genotype in two Mexican groups with severe chronic plaque-type psoriasis. HLA-C typing was performed to detect the allele pattern associated with the disease by sequence-specific primer-polymerase chain reaction. In parallel, RT-PCR and Western blot were used for the identification of the TCR Vβ repertoire. We found a wide variety of HLA-C alleles displayed with a preference to HLA-Cw *07 as the most representative allele in the group of patients. TCR Vβ-2 and Vβ-7 clone-type frequencies were statistically significant (p of 0.0280) when compared to other TCR Vβ expressed in the two groups. We found notable differences both in the HLA-C genotype and TCR Vβ repertoire in the groups of patients studied. Since Mexican individuals are genetically different from the Caucasian population, we suggest that due to these differences the susceptibility to disease and activation of T-cells for a proper immune response may be affected.

Introduction

Psoriasis is a common, chronic, inflammatory skin disease, with a prevalence of over 1% in most populations; however among Caucasians it has a prevalence of 2-4% (Nevitt *et al.*, 1996). Raychaudhuri *et al.* (2001) reported the prevalence of psoriasis around the world including North, Central and South America. In Mexico, psoriasis is seen with a frequency similar to reports worldwide; nevertheless the population is characterized by a mixed ethnicity of native Mexican indigenous and white people denominated as Mestizo. In addition, we have noticed an important 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 (Chang *et al.*, 2005; Holm *et al.*, 2005; Yan *et al.*, 2008). *HLA-C, CDSN, CCHCR1, SEEK,* and *PSORS1C3* alleles have been described 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 (Higgins *et al.*, 1992) and subacute thyroiditis with the HLA-B35 allele (Kramer *et al.*, 2004). However, psoriasis is the only inflammatory disease that strongly associates with HLA-Cw*0602 (Nair *et al.*, 2006).

The pathogenesis of psoriasis still remains elusive; the type 1 T-cells have been involved through the secretion of their cytokines, which contributes to the epidermal hyperproliferation. It has been proposed that psoriasis is a disease of activated innate immunity to explain the relation between the environmental factors and the exacerbation of the psoriasis (Nickoloff *et al.*, 2004), but in the treatment of severe psoriasis, the good response to drugs blocking T-cell activation

supports the idea 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 each time, or different ones. Superantigens, unlike conventional antigens, activate T-cells expressing certain T-cell receptors, which possess a region highly variable 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.* (1999), although several authors have found differences in the expression of the TCR Vβ usage for the presentation of antigens to the MHC class II on antigen presenting cells (Chang *et al.*, 1994; Vollmer *et al.*, 2001; Hwang *et al.*, 2003). 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 in the analysis of the human leukocyte antigen (HLA) alleles Cw type and the frequency of the TCR Vβ usage in a group of native indigenous and Mestizo Mexican patients in their risk for psoriasis.

Results

Haplotype and allelic frequencies of HLA-C loci

The frequencies for each HLA-C allele identified by PCR-SSP are shown in Table 1. The alleles of these loci are not represented in all the tested subjects and could be found only rarely in some patients of the two groups studied. It can be observed that HLA-Cw*07*07 (30%) and HLA-

Cw*03*07 (20%) are the most frequent alleles in the psoriatic patients from group 1, but this was different in group 2: HLA-Cw*01*08* (10%), -Cw*03*12, -Cw*04*16, -Cw*04*12, -Cw*05*07, -Cw*06*08, -Cw*07*08.

TCR Vβ expression analysis

The hypervariable region of the TCR Vβ gene family was examined as reported by several authors (Ahangari *et al.*, 1997; Bour *et al.*, 1999; Fernandes *et al.*, 2005). PBMCs were used for the isolation of total mRNA. To obtain an overall scheme of the Vβ transcript expression, we included a set of 24 oligonucleotides for the RT-PCR experiments reported by Fernandes *et al.* (2005). Results are shown in Figure 1. A subset of regions within group 1 is observed, the Vβ2, Vβ7 and Vβ23 subfamilies were predominantly expressed. In contrast, the expression profile of group 2 shows a wider usage of Vβs, such as Vβ1, Vβ2, Vβ6, Vβ7, Vβ13S2, Vβ15, Vβ16, Vβ20 and Vβ23.

To confirm these results, the expression profile was analyzed at protein level with a selected panel of monoclonal antibodies against specific segments of the TCR Vβ receptors described in *Materials and Methods*. Western blot experiments were performed with crude extract of PBMCs. Results illustrate that all samples of group 1 displayed positive bands for all TCRs analyzed as shown in Figure 2. Only 50% of the samples from group 2 displayed a similar pattern.

A comparison of TCR Vβs expression between RT-PCR and Western blot analysis was done using Fisher's exact test to prove the association between the methods and the groups

studied. Statistical analyses showed TCR Vβ-2 and Vβ-7 were predominantly expressed in both groups with a p of 0.0280.

Discussion

Susceptibility to psoriasis has been investigated through the study of the MHC I genes supporting the allele Cw*0602 in pure Caucasian populations (Holm *et al.*, 2005; Nair *et al.*, 2006). Unlike those reports, we have shown 14 distinct risk alleles in the Mexican patients analyzed (Table 1). From the two groups studied, we identified a wider variety of alleles among Mestizo patients that have a Caucasian background (group 2), but the allele *06 is rarely present. A study performed by Fan *et al.* (Fan *et al.*, 2008), suggest that ethnic population might transmit distinctive susceptibility alleles because of the genetic heterogeneity of *PSORS1* locus. To date none of the alleles shown in Table 1 have been reported in association with psoriasis, even in the Chinese (Fan *et al.*, 2007; Fan *et al.*, 2008) or the Sardinian (Lio *et al.*, 2003; Orru *et al.*, 2005; Scola *et al.*, 2008) populations. We suggest that for the group of patients included in this study, Cw*07 is the most prevalent allele in association with the disease.

The TCR repertoire was studied in PBMC by RT-PCR and Western blot. Transcript analysis shows a preferential usage of the Vβ-2, -7 and -23 in group 1 (gray bars) and a larger usage in group 2 (black bars): Vβ-1, -2, -6, -7, -13S2, -15, -20 and -23 (Figure 1). Frequency analysis bearing TCR Vβ in PBMCs with the monoclonal antibodies here acquired, produced significant differences between the two groups. All samples of group 1 expressed every TCR Vβ clone type, while in group 2, only five patients produced the same results. Statistical analysis showed a $p<0.05$ using the X^2 test (Figure 2). 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 (Kay *et al.*, 1995; Yoshioka *et al.*, 1999; Menssen *et al.*, 2000). TCR Vβ-7 expression has been studied in psoriasis as well as in several normal tissues and other diseases (Hodges *et al.*, 1998; Nickoloff *et al.*, 2004; Dokouhaki *et al.*, 2006). Since all samples from group 1 were positive using Western blot in PBMC, we decided to examine random skin biopsies (n=6) by immunohistochemistry. We included apparently normal skin biopsies and lesions from the same patients. Infiltrating T-cells were detected in the non-lesional skin biopsies while in lesional skin, paucity of T-cells was observed (data not shown). This inconsistency has been reported by other authors (Komatsu *et al.*, 1996; Carlen *et al.*, 2007), and could be explained by the event dubbed as Köbner response (Hwang *et al.*, 2003).

Previous studies have reported selectivity of the clones displaying different TCR Vβ of infiltrated T-cells in psoriatic lesions: TCR Vβ-13 and -15 (Chang *et al.*, 1994); TCR Vβ-5.1, -11, -12, -13.1 and -16 (Ahangari *et al.*, 1997); TCR Vβ-3 and -13.1 (Bour *et al.*, 1999) and more recently TCR Vβ-3, -13S2 and -21 (Diluvio *et al.*, 2006). This evidence is bound to studies of patient specimens with streptococcal infections. In correlation to these data, Kansal *et al.* (2003) 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 entirely proved. We may speculate that similar T-cell events occur for the selection of the TCR Vβ in our study although the study for the identification of the antigen(s) involved by means of mass spectrometry would provide interesting data.

In the present report we have shown a major expansion of two TCR Vβs and a preferential HLA-C haplotype in the group of patients studied. These preliminary findings could lead to future tailor-made strategic treatments for the Mexican population.

Materials and methods

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 from ages between 24 to 74 years old presented active chronic plaque-type psoriasis (CPP). The diagnosis was based on clinical and histopathological data of 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 were reviewed and approved by the Ethics and Research Committee of the Central Hospital Dr. Ignacio Morones Prieto, Universidad Autonoma de San Luis Potosi in San Luis Potosi city, Mexico.

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.

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.

TCR Vβ expression analysis by RT-PCR

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 primers described by Fernandes *et al.* (Fernandes *et al.*, 2005). The RT-PCR products were run in 2% agarose gel and stained with ethidium bromide.

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, Vβ-7 (clone ZOE) recognizes V-beta-7.1, Vβ-11 (clone C21) recognizes the two known sequences PL3.12 and PH15, Vβ-13.1 (Immu222) recognizes the V beta-13.1 member, Vβ-17 (clone E17.5F3), Vβ-20 (clone ELL 1.4) and Vβ-22 (Immu546) recognizes at least the IGRb03 sequence.

TCR Vβ expression analysis by Western blot

The PBMCs cells and skin biopsies were homogenized by sonication and protein quantitation was performed by the Lowry method (Sigma). BSA was used for the standard curve and the absorption was measured at 590 nm. 50 µL of total protein extract at 5 µg/µL were 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 at a constant current of 2mA/cm 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 above described 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 the incubation with the secondary antibody anti-mouse IgG alkaline phosphatase-conjugated from 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.

Statistical analysis

The statistical analysis between the frequency of the six different TCR Vβ expressions produced by Western blot in patients from Group 1 and 2 was done using an X^2 test. Fisher's exact test was used to associate the expression of the TCR Vβs present in the two groups studied and the two methods performed by a 2x2 contingency table. Analysis was executed using the InStat software program v.3.0 (GraphPad 5.0 Software, San Diego, CA). Values of $p<0.05$ were considered as significant.

Conflict of interest

The authors state no conflict of interest.

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Tables

Table 1. HLA-C allele frequency of the two psoriatic groups by SSP-PCR

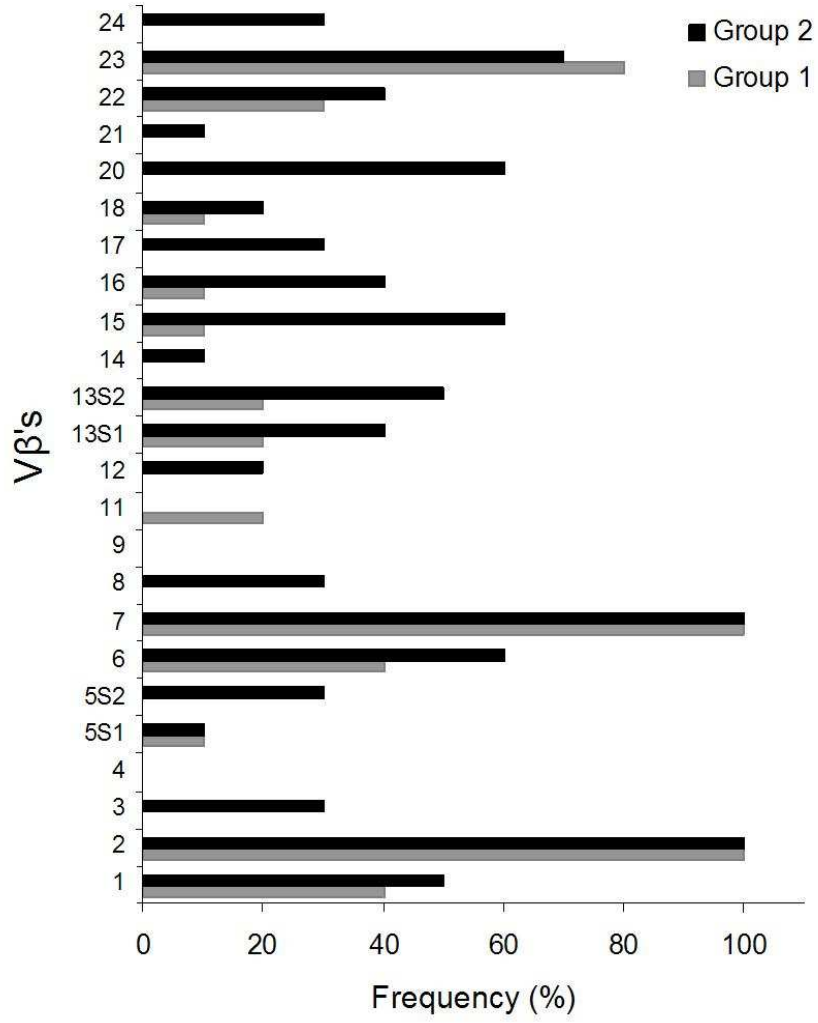
HLA-Cw*	Group 1[1] (%) (n=10)	Group 2[1] (%) (n=10)
*01*03	1 (0.1)	0
*01*08	0	1 (0.1)
*03*04	1 (0.1)	1 (0.1)
*03*07	2 (0.2)	0
*03*12	0	1 (0.1)
*04*07	1 (0.1)	1 (0.1)
*04*16	0	1 (0.1)
*04*12	0	1 (0.1)
*05*07	0	1 (0.1)
*06*08	0	1 (0.1)
*07*07	3 (0.3)	0
*07*08	0	1 (0.1)
*07*15	1 (0.1)	1 (0.1)
*08*08	1 (0.1)	0

[1]Group 1 corresponds to indigenous patients; Group 2, Mestizo patients.

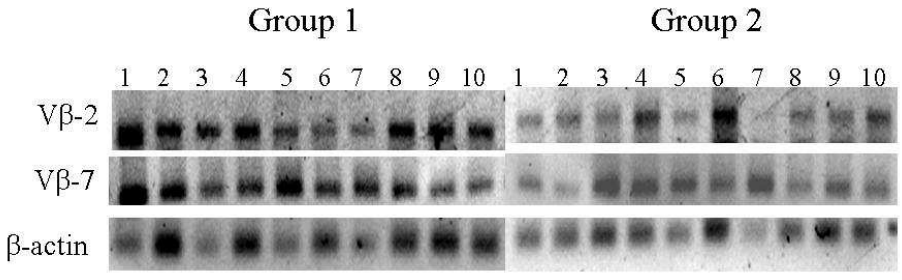
1 **Figures**

2 Figure 1.

a



b

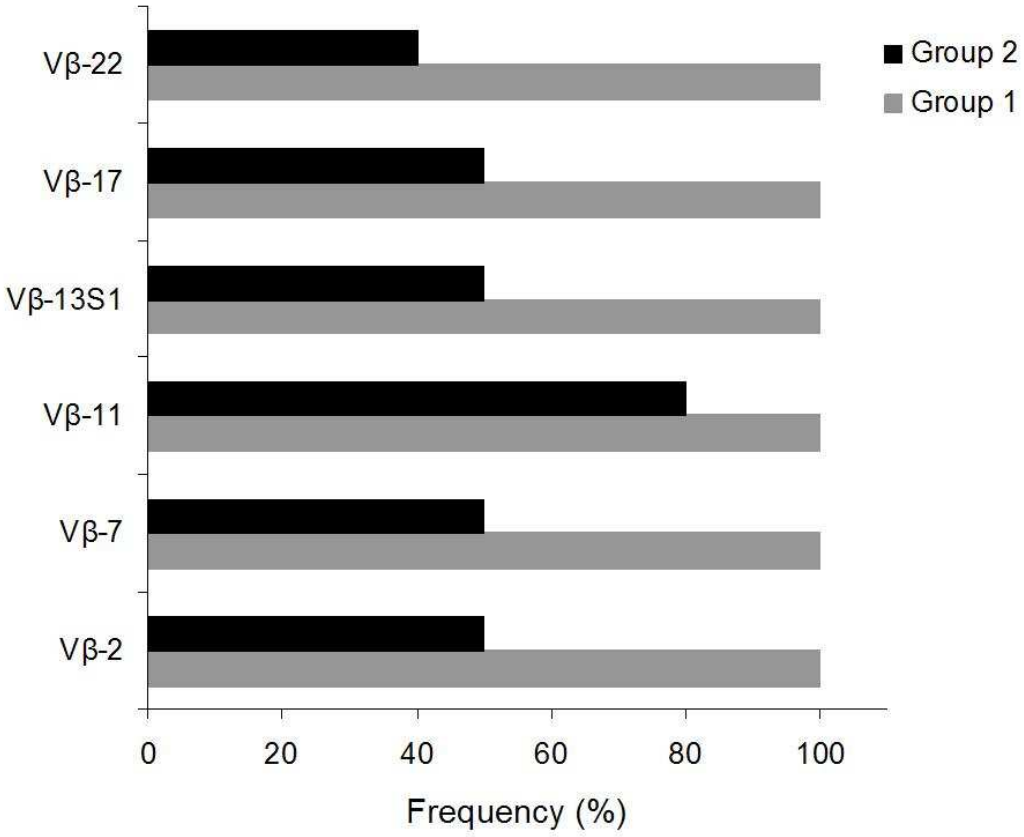


3

4

Figure 2.

a


Vβ-22
Vβ-17
Vβ-13S1
Vβ-11
Vβ-7
Vβ-2
Group 2
Group 1
0
20
40
60
80
100
Frequency (%)

b

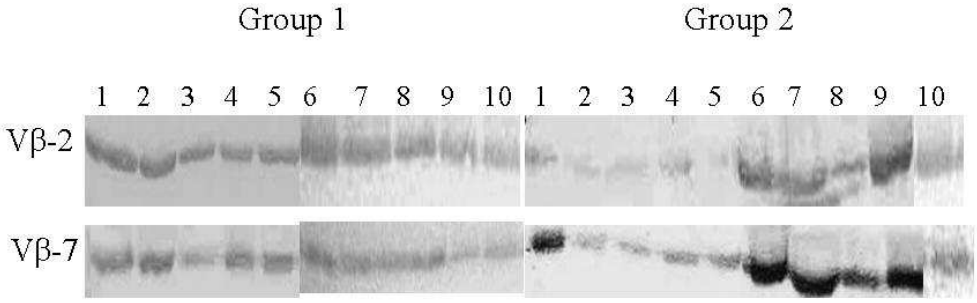

Group 1
Group 2
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6 7 8 9 10
Vβ-2
Vβ-7

Figure legends

Figure 1. (a) Transcript analysis of the 24 TCR Vβ chains in peripheral blood reported as frequency from Mexican native indigenous (group 1, gray bars) and Mestizo patients (group 2, black bars) with CPP lesions. (b) RT-PCR products were analyzed on 1% agarose gels. We show the amplification of TCR Vβ-2 and -7 and the β-actin as internal control.

Figure 2. (a) Detection of TCR Vβs (2, 7, 11, 13S1, 17 and 22) at protein level in peripheral blood derived from Mexican native indigenous (group 1, gray bars) and Mestizo patients (group 2, black bars). (b) TCR Vβ-2 and -7 blots detected with monoclonal antibodies as described in *Materials and Methods*.