

*Short Report***T-Cell and Chemokine Receptor Variation in South Amerindian Populations**

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ABSTRACT The immune response of relatively small, endogamous populations is of special interest, because they may differ from those of large, ethnically diverse, urban groups. As a contribution to this area of investigation, we tested 99 individuals from two Brazilian native populations for two T-cell receptor gene segments (TCRBV3S1 and TCRBV18) and 241 subjects from eight tribes of this ethnic group in relation to the chemokine receptor CCR5Δ32 allele. Differences in TCRBV3S1 and TCRBV18 prevalences of the Amerindians in relation to European- and African-derived individuals were not marked. We confirmed the absence of the CCR5Δ32 allele in most groups, its presence in the Mura and Kaingang, probably because of European gene introgression. *Am. J. Hum. Biol.* 17:515–518, 2005. © 2005 Wiley-Liss, Inc.

The success of an immune response greatly depends on the ability of T and B cells to recognize and distinguish different antigens. Each T cell presents unique antigen-specific surface molecules (the T-cell receptor or TCR), which are responsible for antigen recognition. TCR is a heterodimeric molecule composed of α and β or, alternately γ and δ chains. Each chain has two regions, a constant region, encoded by a C gene segment, and a variable region. The variable region is responsible for peptide–MHC complex recognition. Several polymorphisms of TCR gene segments have been described (Charmley et al., 1993; Posnett et al., 1994; De Inocencio et al., 1995), most of which related to the variable regions of both the β and α chains and, consequently, potentially influenced the recognition of the peptide–MHC complex.

In this work, we investigated two biallelic polymorphisms from two different TCRBV (T-cell receptor beta variable) gene segments. The first is a single nucleotide polymorphism (C/T) located within the 23-bp spacer region of the TCRBV3S1 gene segment recombination signal sequence (RSS) region. Although the polymorphic site is located in the RSS nonconserved spacer region, this allelic variant is associated with the level of V β 3.1+ T cells in the peripheral

pool (Posnett et al., 1994). Homozygotes for allele 1 have a low frequency of V β 3.1+ T cells (approximately 1% of the total T cells), whereas homozygotes for allele 2 have a higher frequency of V β 3.1+ T cells (approximately 7.5%); heterozygotes present an intermediate frequency. The second polymorphism studied is located in the TCRBV18 gene segment and results from a nucleotide substitution (C/T) that creates a stop codon inside the gene segment. Individuals homozygous for the null allele are unable to produce TCR molecules using the TCRBV18 gene segment, resulting in the absence of V β 18+ T cells in the peripheral pool. A detectable level of functional V β 18+ T cells is observed in the peripheral

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compartment of individuals heterozygous for this polymorphism (Charmley et al., 1993).

We also analyzed an allelic variant of the CCR5 chemokine receptor gene, the CCR5 Δ 32 allele. CCR5 is the main cellular receptor for CCL3, CCL4, and CCL5 chemokines and is also an efficient coreceptor for HIV-1 macrophage tropic lineages (Feng et al., 1996). The CCR5 Δ 32 allelic variant is characterized by a 32-bp deletion that leads to a nonfunctional protein and, consequently, in CCR5 Δ 32 homozygous individuals, the absence of CCR5 protein expression. As a result, CCR5 Δ 32 homozygotes show a relative resistance to HIV-1 infection (Samson et al., 1996), and it was already suggested that this allelic variant could confer resistance to other intracellular pathogens. The allele average prevalence in Europe is of 10%, with a north-south gradient showing a range of 16–4%; it is, however, absent in Africans (Samson et al., 1996; Libert et al., 1998).

The objective of this work is to extend the information available in relation to these systems to Native Americans. Peculiarities of their reaction to diverse immunological insults are well known (see, for instance, Black [2004]), and polymorphic genetic variability may contribute to this distinctiveness.

SUBJECTS AND METHODS

DNA samples from the Tiriyo, Mura, Cinta Larga, Gavião, and Zoró Amazonian Indians, a group from the Paraguayan Gran Chaco (Lengua), another from the Paraguayan forest (Ach ), and the Kaingang from southern Brazil were studied. DNA was extracted from peripheral blood using the method described by Lahiri and Nurnberger (1991).

For the analysis of the TCRBV3S1 RSS polymorphism, DNA samples were amplified by PCR using the specific primers: 5'-CCTTGATGGCCTGTTTTTCAC-3' and 5'-GTGCCATCGGAGCCAGCAC-3' (Posnett et al., 1994). The primers were mixed with 1 μ L of DNA, 2.5 μ L 10 \times PCR buffer (containing 30 mM MgCl₂), 1 μ L dNTP solution (final amount of 10 mM each/reaction), and 0.2 μ L of Taq polymerase (5 U/ μ L). The samples were subjected to 36 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 51°C, and 2 min of extension at 72°C, preceded by 1 min at 94°C and followed by 5 min at 72°C in a DNA thermal cycler (MJ Research, Inc., Watertown, MA). The resultant 431-bp fragment was digested

with *Pvu*II, and the products were visualized by electrophoresis in a 1% agarose gel containing ethidium bromide. The two allelic variants located at the TCRBV3S1 RSS differ at only a single nucleotide position (C/T). This cytosine to thymine transition creates a *Pvu*II site inside the TCRBV3S1 RSS. When DNA amplified with specific primers is submitted to digestion with *Pvu*II, homozygotes for allele 1 can be identified by the presence of a single DNA band in agarose gel (431 bp), reflecting the absence of the restriction site. Homozygotes for allele 2 can be identified by the presence of a 352-bp band (the 79-bp fragment cannot be observed in a 1% agarose gel), whereas in heterozygotes two bands (431 and 352 bp) can be observed.

For the analysis of TCRBV18 polymorphism, the specific primers 5'-ATTTCATCAATG GCCAGCGAC-3' and 5'-GGAGCTTCTTAG AACTCAG-3' were used (Charmley et al., 1993). Polymerase chain reaction (PCR) was performed under the same conditions described for the TCRBV3 polymorphism in 40 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 60°C, and 2 min of extension at 72°C, preceded by 1 min at 94°C and followed by 5 min at 72°C. The TCRBV18 polymorphism analyzed is a single nucleotide substitution (a C–T base transition), leading to the introduction of a stop codon at the V β 18 gene segment sequence (allele 2) and simultaneously eliminating a restriction site for the *Kpn*I enzyme. Homozygotes for allele 2 can be identified by the presence of a single band in agarose gel (235 bp) when DNA amplified with specific primers is digested with *Kpn*I. DNA from homozygotes for allele 1 is identified by the presence of two bands (100 and 135 bp), whereas heterozygotes show three bands (of 235, 135, and 100 bp).

The CCR5 typing was performed by PCR using the specific primers 5'-GGTCTTCAT TACACCT-3' and 5'-AGGATTCCCGAGTA GCAGAT-3' (Chies and Hutz, 2003) under the following conditions: 1 μ L of DNA, 2.5 μ L 10 \times PCR buffer (containing 30 mM MgCl₂), 1 μ L dNTP solution (final amount of 10 mM each/reaction), and 0.2 μ L of Taq polymerase (5 U/ μ L). The samples were subjected to 40 cycles of 1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C, preceded by 1 min at 94°C and followed by 5 min at 72°C in a DNA thermal cycler (MJ Research, Inc., Watertown, MA). The PCR product was

subjected to electrophoresis on 3% agarose gel containing ethidium bromide and was visualized by ultraviolet irradiation. Amplification of the normal allele generates a 137-bp band, whereas the CCR5Δ32 allele generates a 105-bp band.

Allele frequencies were determined by counting. Genotype distributions were analyzed using contingency tables and the Fisher test. Hardy-Weinberg equilibrium was tested using a chi-square goodness-of-fit test (software SPSS 10.0 for Windows).

RESULTS AND DISCUSSION

The results concerning the TCRBV3S1 and TCRBV18 systems are shown in Table 1. TCRBV3S1*1 shows frequencies, respectively, of 65% and 74% among the Kaingang and Cinta Larga, which are not significantly different from those observed among European-derived (54%) or African-derived (68%) subjects from southern Brazil (Dresch et al., 2002). Non-Indian admixture varies slightly in these two tribes—Kaingang, 5%; Cinta Larga, 3% (Callegari-Jacques and Salzano, 1999) and cannot explain the TCRBV3S1*1 frequency difference. The TCRBV3S1 gene segment is involved with the immune responses in a variety of situations (for example, see Buhler et al., 2000; Probert et al., 2001). Therefore, it is of interest to verify that alleles that interfere with the level of Vβ3.1⁺ T cells do not show marked interpopulation differences.

Allele 2 (null) of the TCRBV18 gene segment shows a frequency of 15% among the

Kaingang but did not appear in the Cinta Larga. Previous studies in European-derived and African-derived persons from southern Brazil detected frequencies, respectively, of 19% and 6% (Dresch et al., 2002). It is quite intriguing that such a null allele could be found in relative high frequencies among different human populations. For instance, besides the previously cited data, Charmley et al. (1993) observed that 11% of a European-derived sample tested were homozygous for this null allele and, in a sample of individuals aged 80 years or more, with European ancestry, the TCRBV18 null allele frequency was as high as 24.6% (Peres et al., 2004). At first glance, one can imagine that a condition that leads to the absence of functional Vβ18⁺ T cells would be unfavorable, because it represents a “gap” in the T-cell repertoire. Nevertheless, because Vβ18⁺ T cells are involved in several autoimmune diseases and other deleterious immune responses (Fraser et al., 1999; Osman et al., 1999; Kircher et al., 2002), in some cases the absence of a given TCR can be advantageous to the individual. In this way, the null TCRBV18 allele could be favored, but its prevalence is too variable at present to suggest a meaningful hypothesis about its frequency.

Previous studies did not detect the CCR5Δ32 allele among Amerindian populations (Brazilian Tikuna, Baniwa, Kashinawa, Kanamari [Leboute et al., 1999]; Argentinian Chiriguano [Mangano et al., 2001]; Brazilian Tiriyo and Waiampi [Grimaldi et al., 2002]). Our observations reinforce these findings (Table 2). Its presence among the Mura (2%) and Kaingang (3%) may be due to European-derived gene introgression, because, as previously mentioned in the introduction, its average frequency in Europe is approximately

TABLE 1. TCRBV3S1 and TCRBV18 genotype and allele frequencies in two Brazilian Native populations

Systems, genotypes and alleles	Kaingang		Cinta Larga	
	N	%	N	%
TCRBV3S1				
1/1	32	45	10	53
1/2	28	39	08	42
2/2	11	16	01	5
Total	71		19	
Allele 1		65		74
TCRBV18				
1/1	52	73	28	100
1/2	16	23	0	0
2/2	3	4	0	0
Total	71		28	
Allele 2		15		0

TABLE 2. Frequency of the CCR5Δ32 allele in eight South American Native populations

Population	Geographic coordinates	Number of tested individuals	Frequency of the Δ32 allele
Tiriyo	1°57'N, 55°49'W	21	0
Mura	3°34'S, 59°12'W	47	0.02
Cinta Larga	9°50'-12°30'S, 59°10'-60°50'W	24	0
Gavião	10°10'S, 61°8'W	23	0
Zoró	10°20'S, 60°20'W	15	0
Lengua	23°S, 56°W	18	0
Aché	23°S, 58°W	22	0
Kaingang	27°S, 51-54°W	71	0.03

10%. Callegari-Jacques and Salzano (1999) estimated that the Mura should have 8% and the Kaingang 5% of non-Indian admixture.

Immunological susceptibility or resistance involves a complex set of intrinsic (genetic) and extrinsic (life histories, unique exposition to infectious agents) factors. The populations studied here are in different stages of non-Native acculturation, and additional studies involving their immune system may shed light in this process.

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