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Association Study of CCR5 Delta 32 Polymorphism Among the HLA-DRB1 Caucasian Population in Northern Paraná, Brazil

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Chemokines are important determinants of early inflammatory response. The CC chemokine receptor 5 (CCR5) delta 32 variant results in a nonfunctional form of the chemokine receptor and has been implicated in a variety of immune-mediated diseases. In the present study, polymerase chain reaction (PCR) for genomic deoxyribonucleic acid (DNA) samples, using specific CCR5 oligonucleotide primers surrounding the breakpoint deletion, detected a 225-basepair (bp) product from the normal CCR5 allele and a 193-bp product from the 32 bp deletion allele. Human leukocyte antigen (HLA) class II (DRB1) typing was performed by PCR-sequence-specific primer (PCR-SSP). The aim of this study was to evaluate the association of HLA-DRB1 and CCR5 genetic polymorphisms. To evaluate the frequency distributions of CCR5 delta 32 polymorphisms in a Brazilian population and their association with allelic distribution of HLA genes, DRB1; a total of 120 Caucasian individuals from northern Paraná, Brazil, were tested. The CCR5/CCR5 genotype was found in 108 individuals (90%) and only one carried the CCR5 delta 32 allele homozygous genotype (0.0238), while 12 (10%) carried the CCR5 delta 32 allele heterozygous genotype. The observed frequency for the CCR5 delta 32 allele was 0.05 in the population studied. The results revealed a CCR5 delta 32 allele occurrence with HLA-DRB1*01 and DRB1*04 ($P<0.05$). It is possible that HLA-DRB1*01 and DRB1*04 alleles could be associated with the delta 32-bp deletion of CCR5. J. Clin. Lab. Anal. 22:229–233, 2008. © 2008 Wiley-Liss, Inc.

Key words: CCR5 delta 32 allele; Caucasian; HLA-DRB1; PCR-SSP

INTRODUCTION

Chemokines induce cell migration and activation by binding to specific G-protein-coupled cell-surface receptors on target cells (1). Chemokines are important determinants of early inflammatory response (2,3). Physiologically, chemokine receptors are expressed on different types of leukocytes and the receptors mediate the chemotaxis of T-cells and phagocytes to areas of inflammation (4).

The CC chemokine receptor 5 (CCR5) gene product is a member of the seven-transmembrane spanning (7-TMS) (5), G-protein-coupled receptor family that present response to their normal beta-chemokine ligands (RANTES, MIP-1alpha, MIP-1beta) and are involved in the chemotaxis of leukocytes toward sites of inflammation (6,7). A mutant allele of the beta-chemokine receptor gene CCR5 bearing a 32-basepair (bp) deletion (denoted as delta CCR5 or CCR5 delta 32), with a homozygous genotype leads to truncation and loss of the receptor on the cell surface (8,9).

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Viruses predominantly use one, or occasionally both, of the major coreceptors CCR5 and CXCR4, although other receptors, including CCR2B and CCR3, function as minor coreceptors. The mutant allele CCR5 delta 32 is remarkable because homozygous individuals are resistant to macrophage-tropic human immunodeficiency virus (HIV)-1 lineage infection (9–11). This allele is frequent in Caucasians, whereas it is rare or absent in major ethnic groups, such as native Africans, Asians, and Amerindians (7,10–13).

The inability of beta chemokines to bind to CCR5 causes significant defects in the chemotaxis mediated by them. Chemokine receptor 5 plays an important role in the recruitment of monocytes and encodes a cell-surface chemokine receptor molecule that serves as a coreceptor for macrophage-tropic strains of HIV type 1 (HIV-1) (9,14).

Considerable variation in the global distribution of CCR5 delta 32 has been observed. Heterozygosity and homozygosity for CCR5 delta 32 occurs in almost 10–15% and 1% of the Caucasoid ethnic group, respectively (15). Immunological and genetic factors were studied in HIV-exposed but persistently seronegative (HEPS) female sex workers from Chiang Rai, northern Thailand and human leukocyte antigen (HLA)-specific cytotoxic T lymphocytes HLA-A11, chemokine-related factors may act synergistically to determine HIV resistance in CCR5 delta-negative females (16).

The HLA system is a subset of human major histocompatibility complex (MHC) genes called class I and class II histocompatibility genes, characterized by an extensive degree of allelic polymorphism (17,18). The antigen presenting cells (APC) displaying foreign proteins in class II MHC molecules were recognizing by cluster domain (CD)4 T cells. These mechanisms are very important for the activation of immunological responses (19).

The class I and II MHC molecules play an important role in association with diseases, mainly autoimmune, conferring susceptibility or protection to the individual for determined diseases. Association with HLA antigens has been described in diverse models of human diseases in mice, such as diabetes mellitus, celiac disease, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis, among others (17,20–22).

The aim of this study was to analyze the possible association of CCR5delta32 polymorphism and the HLA-DRB1 allele in the Brazilian Caucasian population living in the region of north Paraná, Brazil.

MATERIALS AND METHODS

Study Population

Following approval from the Human Ethics Committee from Londrina State University and Maringá State University, peripheral blood was collected from 120 Caucasian individuals from north of Paraná-Brazil. The individuals are blood healthy donors with negative serology for HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV).

HLA Typing Analysis

Genomic deoxyribonucleic acid (DNA) from 120 Caucasian donors was obtained by PurigeneTM DNA Purification Kit (Gentra Systems, Minneapolis, MN). The Micro SSPTM 384 System DNA Typing trays (One Lambda, Inc., Los Angeles, CA) were used to type HLA class I and II allele. The amplified DNA fragments were analyzed by electrophoresis in a 2.5% agarose gel and visualized with ethidium bromide and exposure to ultraviolet (UV) fluorescence. Interpretation of the polymerase chain reaction–sequence-specific primer (PCR-SSP) result was based on the presence or absence of a specific amplified DNA fragment.

DNA Amplification to CCR5-PCR

DNA was analyzed by PCR using specific primers for CCR5: CCR5.1 (sense, 5′-ACC AGA TCT CAA AAA GAA-3′) and CCR5.2 (antisense, 5′-CAT GAT GGT GAA GAT AAG CCT CA-3′) (GenBank accession number: AF009962).

Samples were amplified using the kit buffer plus 1.5 mmol/L Taq polymerase (Invitrogen, Life Technologies, Carlsbad, CA). PCR conditions were performed for 5 min denaturation at 94°C, 35 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C, and 10 min elongation at 72°C in a thermocycler (PCR-Sprint Hybaid, Guelph, Ontario, Canada). All assays included positive control for 225-bp and 193-bp ladder controls and 100-bp commercial ladder (Invitrogen-USA). PCR products of 225 and 193 bp were analyzed by electrophoresis in a 3% agarose gel and visualized using UV fluorescence after staining with ethidium bromide. A negative control amplification containing no added DNA was included in each experiment reaction and no PCR product was detected in this reaction.

Statistical Analyses

Allele frequencies of HLA and genotype distributions of CCR5 were determined by counting. Hardy-Weinberg equilibrium was tested using a chi-squared goodness of fit test. The significance of CCR5 delta 32 and HLA associations was considered when $P < 0{,}05$ through the t-test in the Program Statistical 6.0. (Stat Soft Inc., Sao Paulo, Brazil).

RESULTS

In this work, analysis of CCR5 deletion polymorphism in the HLA-DRB1 Caucasian population of

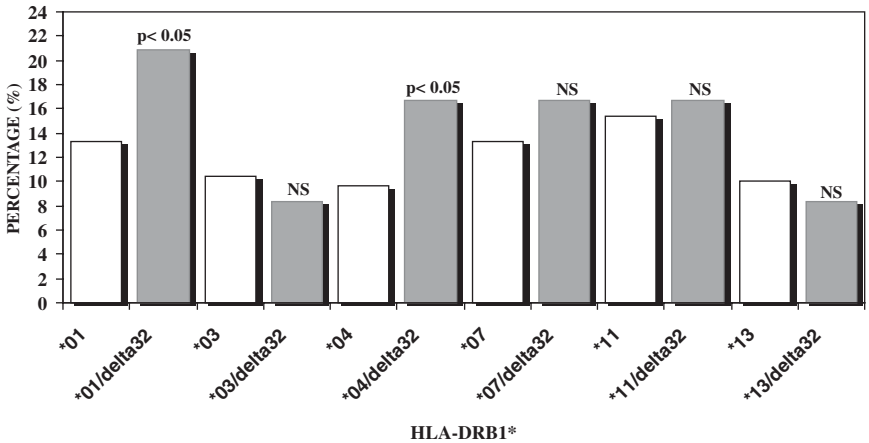

Fig. 1. Analysis of CCR5/delta32 polymorphism in HLA-DRB1 Caucasian population from north of Paraná. White bar: HLA-DRB1* Caucasian population. Gray bar: Caucasian individuals with CCR5/delta32 allele. All the alleles to CCR5/delta32 were compared with normal occurrence for HLA-DRB1 in the population. Percentage of CCR5/delta32 allele in HLA-DRB1*01 and DRB1*04 individuals were significant ($P<0.05$).

northern Paraná, Brazil, demonstrated the prevalence of the CCR5/CCR5 genotype in 108 individuals (90%), with delta32 carrier deletion presented by 12 Caucasian individuals (10%). The delta32/delta32 homozygous genotype was presented by only one individual. The observed frequency of CCR5/delta 32 allele was 0.05. For all samples the genotype did not differ from the theoretical distribution given by the Hardy-Weinberg equilibrium. The delta 32 allele is common and is present in different frequencies among Caucasians (10,11) but has not been found in people of African (7) or Asian ancestry (23).

HLA class II polymorphism (Fig. 1) was investigated and the occurrence of DRB1*11 (15.42%), DRB1*07 (13.34%), DRB1*01 (13.34%), DRB1*03 (10.0%), DRB1*13 (10.0%), and DRB1*04 (9.6%) was demonstrated.

In the context of CCR5 delta 32 allele association, 20.84% (5/24) DRB1*01 individuals presented the deletion allele for CCR5 ($P<0.05$) and 16.67% (4/24) DRB1*04 individuals presented the CCR5delta32 allele ($P<0.05$).

The results demonstrate that the distribution of CCR5 delta 32 in HLA-DRB1*03, DRB1*07, DRB1*11, and DRB1*13 individuals was compatible with the occurrence of these alleles in the Caucasian population. DRB1*07 and DRB1*11 alleles presented an occurrence of 16.67% (4/24), and DRB1*03 and DRB1*13 an occurrence of 8.33% [(2/24), but no statistical difference was found.

DISCUSSION

Homozygous defect in the gene encoding the CCR5 delta 32-bp deletion in the region corresponding to the second extracellular loop of CCR5, encoded a severely truncated molecule that fails to reach the cell surface. The CCR5 delta 32 variant results in a nonfunctional form of the chemokine receptor and has been implicated in a variety of immune-mediated diseases (8). This defective allele bearing CCR5 delta 32 has claimed particular attention, since homozygous deletion is highly but not totally protected from HIV-1 infection (24,25).

Certain studies investigating various ethnic groups demonstrate that the delta 2 allele is common among Caucasians, with a high frequency (>5%) of CCR5/delta 32 heterozygosity among European populations, in Britain, Spain, Ireland, and Cyprus, among Ashkenazi Jews (7,10,11,26), in Italy (27), Greece (28), France, Germany, and Denmark (29); intermediate and low-level (2–5%) delta 32 allele frequencies occur in the Middle East and the Indian subcontinent, among Hispanics (7,9), Argentine Amerindians (30), Puerto Rican Hispanics and Hawaiians (26) and by contrast, an inability to detect the delta 32 allele occurs among indigenous non-European populations, in Mexico, Japan (3), Africa (23), Oceania, among native Americans (7), and Asians and other Pacific Islander groups (23,26).

According to the Brazilian Institute of Geography and Statistics (IBGE), in Brazil as a whole, 53.7%, 6.2%, 38.4%, and 1.7% of the population were identified as White, Black, Brown, and others, respectively (31). Among the urban Brazilian population, Passos and Picanco (32) demonstrated a 7% frequency of CCR5/delta32 heterozygous individuals, the frequency of the delta CCR5 mutant allele in this population is 0.035; however, no homozygous delta CCR5 individual has been detected thus far. The CCR5delta 32 allele is absent among Brazilian Amazon tribe indigenous

populations (Tikuna, Baniwa, Kashinawa, and Kanamari) (33). Oliveira et al. (34) has demonstrated 9% of delta 32-bp carriers in the Brazilian healthy subjects. In the present data concerning the Caucasian population of northern Paraná, distribution of the CCR5/delta32 deletion genotype was detected in 10% of Caucasian individuals, the observed frequency of CCR5/delta 32 allele was 0.05, and the delta32/delta32 genotype was presented by only one individual. This is additional evidence regarding the contribution of the CCR5/delta 32 allele to the genetic mix of the Brazilian population, which is characterized by intense ethnic admixture.

It is known that the most common alleles among the population studied in southern region of Paraná, Brazil, are HLA-A*02, B*35, and DRB1*11 (35), and in the northeastern State of Pernambuco the allelic group HLA-B*15 is the most frequent (18). Verification showed that the most common allele occurrences of HLA-A*02, DRB1*11, and DRB1*07 were compatible with the occurrence of this allele in the Caucasian population and provide an additional contribution to studies concerning the genetic structure of the southern Brazilian population.

This is the first report of the CCR5 delta 32 allele associated with HLA alleles in the Caucasian population of northern Paraná, Brazil, where DRB1*01 and DRB1*04 individuals presented CCR5delta32 allele deletion, showing a statistical difference.

The interpopulation differences in delta 32-bp deletion of CCR5 and HLA haplotype frequency provide evidence regarding the distribution of many immunological molecules that could develop different responses in the host. It is possible that HLA-DRB1*01 and DRB1*04 allele could be associated with delta 32-bp deletion of CCR5. Further studies are required for a clearer understanding of Caucasian gene mixing in the Brazilian population, its relation to HLA alleles and to establish how these polymorphisms could influence susceptibility or resistance to diseases. Once there is small number of Δ32 allele carriers examined, further studies are needed to clarify this possible association of DRB1 with delta 32-bp deletion of CCR5

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