Interleukin-1 Receptor Antagonist Gene Polymorphism and Circulating Levels of Human Immunodeficiency Virus Type 1 RNA in Brazilian Women

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Interleukin-1 receptor antagonist (IL-1ra) gene polymorphisms in 83 human immunodeficiency virus (HIV)-seropositive women were evaluated. Fourteen of the subjects (16.9%) were homozygous for IL-1ra allele 2 (IL-1RN*2). These women had a lower median level of HIV RNA than did women homozygous for allele 1 (IL-1RN*1) ($P = 0.01$) or heterozygous for both alleles ($P = 0.04$). Among 46 subjects not receiving antiretroviral treatment, HIV levels were also reduced in IL-1RN*2 homozygous individuals ($P < 0.05$). There was no relation between IL-1ra alleles and CD4 levels.

The interleukin-1 receptor antagonist (IL-1ra) is a naturally occurring inhibitor of IL-1 α and - β . Although devoid of biological activity, IL-1ra competes with IL-1 for binding to IL-1 receptors (6). The gene coding for IL-1ra is polymorphic (20). A region within the second intron contains a variable number of 86-bp tandem repeats. Most individuals are either homozygous for allele 1 (IL-1RN*1), which contains four tandem repeats, or heterozygous for IL-1RN*1 and allele 2 (IL-1RN*2), which contains two repeats. IL-1RN*2 homozygous individuals are a distinct minority in every population examined to date (3, 10, 12, 20). For individuals with chronic inflammatory disorders, the IL-1RN*2 genotype has been associated with proinflammatory responses more severe and more prolonged than those of other IL-1ra genotypes (12, 14, 21). While homozygosity for IL-1RN*2 may result in an increased susceptibility to chronic inflammation, this genotype may be beneficial in the immune defense against infection by promoting a prolonged Th1 cell-mediated immune response.

IL-1ra has been shown to block the induction of human immunodeficiency virus (HIV) replication in vitro (7, 8, 15), and antiretroviral treatment resulted in increased levels of circulating IL-1ra (18). The balance between IL-1ra and IL-1 concentrations may be important for the modulation of HIV production by monocytes in vivo (7). The relationship between IL-1ra gene polymorphism and HIV-1 infection has not been previously examined and is the subject of the present investigation.

Eighty-six consecutive HIV-seropositive women being seen at an AIDS clinic in Sao Paulo, Brazil, comprised the study population. Forty-six of these women were evaluated prior to the initiation of any antiretroviral treatment while the remaining 40 women were evaluated after treatment with combina-

tions of reverse transcriptase inhibitors, inhibitors of HIV assembly and protease inhibitors. Sixty-four of the study subjects were white, 14 were black, 7 were of mixed racial background, and 1 was of unknown heritage. None of the subjects had used antibiotics or anti-inflammatory medication for at least 30 days prior to testing. This study was approved by the Clinical and Ethical Committee of Hospital das Clinicas, University of Sao Paulo, Sao Paulo, Brazil.

The concentration of HIV-1 RNA in plasma was determined by the HIV-1 Amplicor Monitor Assay (Roche Diagnostics). The lower limit of detection was 400 copies/ml of plasma. The circulating CD4 lymphocyte concentration in plasma was measured by standardized flow cytometry.

Specimens were obtained from the endocervix with a cotton swab and placed into Amplicor collection tubes (Roche Diagnostics). The tubes were frozen at -20°C and shipped to Cornell, New York, New York, on dry ice. The specimens were diluted in Amplicor dilution buffer and analyzed for IL-1ra gene polymorphisms by PCR (20), as previously described (11, 12).

The relation between discrete variables was analyzed by Fisher's exact test. Continuous variables were analyzed by the Kruskal-Wallis test for nonparametric data. A P value of <0.05 was considered significant.

The majority of women tested (49 [57.0%]) were IL-1RN*1 homozygous, 20 (24.1%) were IL-1RN*1/IL-1RN*2 heterozygous, and 14 (16.2%) were IL-1RN*2 homozygous. Three women (3.5%) possessed other rare allelic combinations. Two black women were IL-1RN*1/IL-1RN*3 heterozygous and one white woman was IL-1RN*1/IL-1RN*4 heterozygous. These last three subjects were not evaluated further.

Among the total population tested, HIV-1 RNA concentrations varied according to the IL-1ra genotype (Table 1). The IL-1RN*2 homozygous women had fewer copies of circulating HIV-1 RNA per milliliter than did the IL-1RN*1 homozygotes ($P = 0.01$) or the IL-1RN*1/IL-1RN*2 heterozygotes ($P = 0.04$).

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TABLE 1. Relation between IL-1ra genotype and concentration of circulating HIV-1 RNA

Genotype	No. of patients	Median no. of copies of HIV-1 RNA/ml (range)
1,1	49	2,790 (<400–570,000)
1,2	20	1,550 (<400–200,000)
2,2	14	<400 (<400–46,000) ^a

^a Values were significantly lower than those for the 1,1 ($P = 0.01$) and 1,2 ($P = 0.04$) genotypes.

Among the 83 women tested, 46 were evaluated prior to initiation of antiretroviral treatment and 37 were tested following treatment. The relationships between levels of circulating HIV RNA and IL-1ra genotype were therefore evaluated separately in the two populations (Fig. 1). In the untreated patients, the levels of circulating HIV RNA were lowest in the IL-1RN*2 homozygotes, significantly different from levels in the IL-1RN*1 homozygotes ($P < 0.05$) and the IL-1RN*1/IL-1RN*2 heterozygotes ($P = 0.04$). Among women receiving combination antiretroviral treatment, median numbers of HIV-1 RNA copies per milliliter were reduced compared to those of the untreated group regardless of IL-1ra genotype. The levels of HIV-1 RNA in the IL-1RN*2 homozygotes were

still the lowest, but differences from the other genotypes no longer reached statistical significance.

In contrast to the results obtained with HIV-1 concentration, there was no relation between IL-1ra genotype and circulating CD4 lymphocyte concentrations in the untreated patients. The median (range) levels of CD4 were 444 (77 to 1,175) for IL-1RN*1 homozygotes, 343 (60 to 827) for IL-1RN*1/IL-1RN*2 heterozygotes, and 520 (445 to 810) for IL-1RN*2 homozygotes. This lack of a relationship remained the same following antiretroviral therapy (data not shown).

The majority of our subjects (90.6%) were classified as having either stage A1 or stage A2 HIV disease (1). There was no relation between stage of disease and IL-1ra genotype. Fifty percent of IL-1RN*2 homozygotes, 47.6% of IL-1RN*1/IL-1RN*2 heterozygotes, and 48.8% of IL-1RN*1 homozygotes were at stage A1.

There was a relationship between IL-1ra genotype and race. The frequency of the IL-1RN*2 allele was 34.1% among the white women as opposed to only 8.3% in the nonwhite population ($P = 0.001$). Of the 14 women homozygous for IL-1RN*2, 12 were white, 1 was black, and 1 was of mixed race. However, there was no significant difference in median levels of circulating HIV-1 RNA between untreated whites (2,500 copies/ml) and nonwhites (1,600 copies/ml). Further investigations with larger sample sizes are warranted to determine whether the low frequency of IL-1RN*2 in nonwhites may negatively influence the rate of HIV-1 proliferation in this population.

The distribution of IL-1ra genotypes in the HIV-seropositive women studied was similar to that seen previously in HIV-seronegative women from the state of Sao Paulo, Brazil (11), and from European and American populations (3, 12, 20). A lowered prevalence of IL-1RN*2 in a black African population (2) and in an African-American population (16) has also been previously reported. Although the numbers of subjects were small and additional studies with a larger number of patients are necessary for confirmation, the women in the present investigation who were homozygous for IL-1RN*2 had a strikingly lower concentration of circulating HIV-1 than did the other women. This occurred in the absence of any relation between IL-1ra genotype and CD4 lymphocyte concentration or stage of disease. This IL-1ra genotype might improve immunological defenses against microbial infections by virtue of elevated and/or prolonged proinflammatory immune responses. Whether the lower levels of circulating HIV associated with the IL-1RN*2 genotype will result in a reduced rate of sexual and/or neonatal transmission of HIV and the progression of HIV infection to AIDS remain interesting unexplored possibilities.

Previous investigations have identified a relationship between polymorphism in the β -chemokine receptor 5 gene and HIV transmission and progression to AIDS (9). Recently, a polymorphism in the gene coding for tumor necrosis factor alpha has also been shown to possibly influence HIV progression (13). It remains to be determined whether the IL-1ra polymorphism identified here may further influence disease outcome in HIV-infected individuals with these other polymorphisms.

The mechanism whereby IL-1ra genotype influences levels of HIV-1 RNA remains to be determined. Individuals who are

FIG. 1. Relation between IL-1ra genotype and concentration of circulating HIV-1 RNA. Levels of HIV-1 RNA and IL-1ra genotypes were determined for 46 women who had never received antiretroviral therapy and 37 women who were already undergoing treatment. The horizontal bars indicate medians.

IL-1RN*2 homozygotes have been shown to produce higher levels of IL-1ra in vitro than do individuals with the other IL-1ra genotypes (5, 19). However, IL-1 β production is also increased (19), leading to a net decrease in the IL-1ra/IL-1 β ratio. This would result in a relative deficiency in the capacity to terminate a proinflammatory reaction, a prolongation of a Th1 cell-mediated immune response, and an increased capability of defending against microbial infections. In HIV-infected individuals, the preservation of Th1-mediated immunity has been shown to retard HIV disease progression (4, 17). In addition, HIV may preferentially replicate in Th2 T lymphocytes instead of Th1 cells (17). Whether Th1-mediated immunity against HIV is, in fact, prolonged in IL-1RN*2 homozygotic individuals and whether this is related to a lowered HIV viral load in the circulation remains to be established.

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