Human Immunodeficiency Virus Type 1 Disease Progression, CCR5 Genotype, and Specific Immune Responses

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The correlation among the presence of a 32-bp deletion in the CC-chemokine receptor 5 (CCR5) gene, disease progression, and human immunodeficiency virus type 1 (HIV-1)-specific immune responses was analyzed for a cohort of 79 Caucasian HIV-1-infected patients. The CCR5 genotype (CCR5/CCR5 = wild type/wild type or Δ32CCR5/CCR5 = 32-bp deletion/wild type) in peripheral blood mononuclear cells was determined by PCR, followed by sequencing of both wild-type and Δ32CCR5 gene fragments. HIV-1-specific humoral responses to gp41 and V3MNP peptides were determined by enzyme immunoassays. The prevalence of the Δ32CCR5 allele was lower among patients with rapid progression (progression to AIDS or to a CD4 cell count of <200 × 10^6/liter in less than 9 years; P < 0.01) compared to that for 42 patients with slow progression (no AIDS and CD4 cell count of >200 × 10^6/liter after at least 9 years from infection) or to that for 25 non-HIV-1-infected Swedish blood donors (P < 0.05). No differences were observed in the wild-type CCR5 sequences between the different groups of patients. For three analyzed patients, the 32-bp Δ32CCR5 gene deletions were identical. The antibody titers against gp41 and a V3MNP peptide in patients with the Δ32CCR5/CCR5 genotype were not significantly different from those in pair-matched CCR5/CCR5 controls. However, in 13 analyzed patients, a stronger serum neutralizing activity was associated with the Δ32CCR5/CCR5 genotype. Thus, a CCR5/CCR5 genotype correlates with a shortened AIDS-free HIV-1 infection period and possibly with a worse neutralizing activity, without an evident influence on the antibody response to two major antigenic regions of HIV-1 envelope.

Two major coreceptors, essential for the entry of human immunodeficiency virus type 1 (HIV-1) into CD4-positive cells, were recently described. The fusin, or CXC receptor 4 (CXCR-4), is required for the infectivity of T-cell-tropic syncytium-inducing HIV-1 strains (3). The CC-chemokine receptor 5 (CCR5) gene (20) is essential for the infectivity of macrophage-tropic (M-tropic) non-syncytium-inducing HIV-1 strains (1, 9, 10) by binding the viral gp120 V3 loop (6). It was recently shown that a homozygous 32-bp deletion in the CCR5 (Δ32CCR5/Δ32CCR5) gene causes truncation and loss of CCR5 receptor expression on lymphoid cells, reducing the permissiveness of cells for HIV-1 infection. This mostly results in protection of the host against infection with HIV-1 (17, 23, 26). A heterozygous CCR5 allele in the same gene pair (Δ32CCR5/CCR5), although not protective against HIV-1 infection, may change the rate of HIV-1-related disease progression (8, 15, 21, 22). In fact, a higher prevalence of this genotype has been reported for HIV-1-infected long-term nonprogressors (LTNPs) (11, 12). Additional, lesser known factors, such as switching between different receptor usages (7), are likely to be important during HIV-1 infection and disease progression. The prevalence of the Δ32CCR5 allele in the Caucasian population varies from 4 to 12% in Europe with a 15% peak in Denmark and Iceland (11, 18). The prevalence in HIV-1-infected and uninfected individuals in Sweden has not yet been reported. Furthermore, it is not known whether all Δ32CCR5 deletions are identical or whether certain sequences or mutations within the CCR5 gene correlate with disease progression.

The humoral immune response to HIV-1 has been extensively studied. It has been shown elsewhere that HIV-1-infected patients with a long-term nonprogressive course or slow disease progression display significantly different humoral responses than do those with a more rapid disease progression (4, 5, 16, 24, 28). It is not known whether the humoral HIV-1-specific responses are in any way correlated with the CCR5 genotype.

MATERIALS AND METHODS

Patients. Peripheral blood mononuclear cells (PBMC) were obtained from a cohort of 79 HIV-1-infected Swedish or Italian patients attending the Division of Infectious Diseases at Huddinge Hospital, Huddinge, Sweden (n = 64), or the Department of Infectious and Tropical Diseases at the University of Rome “La Sapienza,” Rome, Italy (n = 15), respectively. The cohort is composed of patients with a defined HIV-1 seroconversion time (n = 13) or a first positive HIV-1 test documented before 1988. Patients were divided into rapid and very rapid progressors versus slow progressors and LTNPs on the basis of their individual AIDS-free intervals (time from seroconversion or from first HIV-positive test to reach Centers for Disease Control and Prevention [CDC] stage C or 3) and their CD4+ cell counts in February 1997. The median time for progression in the whole cohort was 9.2 years. The patients considered rapid progressors were those with an AIDS-free period of less than 9 years (n = 37), including 12 very rapid progressors (AIDS-free interval shorter than 5 years). Slow progressors were all the remaining individuals (n = 42; 29 Swedish and 13 Italian patients) including 23 LTNPs, these being defined by the contemporary presence of 9 or more years of documented HIV-1 infection, a CD4+ cell count always above 500 per µl of blood, no antiretroviral therapy, and no HIV-related symptoms. Twenty-five HIV-1-seronegative Swedish blood donors were used as controls. Serum samples from 36 non-AIDS patients of the cohort (26 Swedish and 10 Italian patients; 47% in CDC stage A1, 39% in A2, and 14% in B1-B2), collected from October 1995 to February 1997, were also used for antibody measurement and neutralization testing.

Enzyme immunoassays and neutralization activity test. The levels of antibodies to a peptide corresponding to the V3 loop of HIV-1MN (U.S.-European consensus; RKKIHGPSQAY) and to a peptide corresponding to an antigenic region of HIV-1 gp41 (GIWGCSKLYCITAVPWNAS) were determined exactly as described previously (5). The 90% inhibitory activity (neutralizing
activity) of patient sera toward clinical primary Swedish and Italian macrophage-tropic HIV-1 isolates was measured as previously described (24).

PCR. A 183 bp fragment of the CCR5 gene was amplified by a previously described PCR (17) from PBMC genomic DNA. Wild-type 183 bp CCR5 gene fragments were distinguished from the CCR5 genes with an internal 32-bp deletion (Δ32CCR5) (17) by electrophoresis separation. For sequencing, a 340-bp fragment from the CCR5 gene was amplified by a separate PCR, with the primers 5'-CTCCGTAGACACTGATAGGTA-3' and biotinylated 5'-CAGAGCCCTGTCGGCTTCTCTT-3'. The amplification was performed in a volume of 50 μl with 10 μl of sample (lysed PBMC or extracted DNA from PBMC), 20 pmol of each primer, 0.5 mM MgCl2, and 125 μmoles of each deoxynucleoside triphosphate per reaction. After an initial 5 min at 94°C, 30 cycles of 94°C for 30 s, 62°C for 45 s, and 72°C for 90 s were performed. The PCR products were excised from the gels and were separately sequenced. The 32-bp sequence motif within the amplified wild-type CCR5 gene fragments correlates with the rate of disease progression.

Correlation between CCR5 genotype and HIV-1-specific humoral responses. To analyze the correlation between the CCR5 genotypes and HIV-1-specific humoral responses, titers of antibodies to HIV-1 V3 and gp41 peptides were determined by enzyme immunoassays for 12 patients with a Δ32CCR5/CCR5 genotype and for 24 CCR5/CCR5 controls (12 of them pair matched with the patients). No significant differences in V3 and gp41 antibody titers were observed with respect to the CCR5 genotype (Table 2).

In a subgroup of eight Swedish and five Italian patients, the serum neutralizing activity toward 5 to 10 primary M-tropic HIV-1 isolates was measured. The geometric mean neutralizing titer for the 2 Δ32CCR5/CCR5 subjects was significantly higher than that for the 11 subjects with the CCR5/CCR5 genotype (1.22 versus 1.14; P = 0.025). In this group, the corre-
The recent discovery that cellular chemokine receptors are required for HIV-1 entry into CD4-positive cells has improved the understanding of HIV-1 pathogenesis (6, 9, 10, 17, 23). It has been shown elsewhere that both the natural receptor ligands and their antagonists may interfere with HIV-1 infection of permissive cells in vitro (25). It has been proposed elsewhere that the presence of a heterozygous 32-bp deletion in the CCR5 gene may partially protect against progression to AIDS (13). Thus, this result has to be interpreted with caution. In conclusion, we could confirm that the Δ32CCR5 allele is more frequent among HIV-1-infected patients with a long-term AIDS-free infection period compared to those who rapidly progress to AIDS. No differences were seen in comparing LTNPs with the remaining slow progressors. A high prevalence of the Δ32CCR5 allele was found in our group of Swedish blood donors. Moreover, in the present study possible correlations between HIV-1-specific immune responses and the Δ32CCR5/CCR5 genotype were not clearly observed.

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