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Role of CCR2 Genotype in the Clinical Course of Syncytium-Inducing (SI) or Non-SI Human Immunodeficiency Virus Type 1 Infection and in the Time to Conversion to SI Virus Variants

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The effect of a valine to isoleucine switch in the CCR2 first transmembrane domain (CCR2 64I) on the clinical course of human immunodeficiency virus type 1 (HIV-1) infection was analyzed in relation to the presence or absence of syncytium-inducing (SI) HIV-1 variants. Compared with persons with a wild-type genotype for CCR2 and CCR5, subjects with a CCR2-64I/1 or 64I/64I (but CCR5 wild-type homozygous genotype) had significantly delayed disease progression (relative hazard, 0.66; 95% confidence interval, 0.44–0.99) with a 1.5-fold slower CD4 T lymphocyte decline and a 1.2-fold lower RNA virus load. The delay in disease progression was more pronounced when only non-SI (NSI) HIV-1 variants were present and was not observed after conversion to SI HIV-1 in CCR2-64I/1 persons. In CCR2-64I/1 subjects, a higher conversion rate to and a higher prevalence of SI HIV-1 was observed. These findings suggest that the mechanism of action of the CCR2 polymorphism is mediated via CCR5-restricted NSI HIV-1 variants.

G protein-coupled seven-transmembrane receptors have been identified as the coreceptors for human immunodeficiency virus type 1 (HIV-1). Syncytium-inducing (SI), T cell line-adapted HIV-1 variants utilize the C-X-C chemokine receptor 4 (CXCR4), whereas macrophage-tropic, non-SI (NSI) HIV-1 variants use the C-C chemokine receptor 5 (CCR5), and some primary SI viruses may use both. Other receptors, including CCR2 and CCR3, also serve as coreceptors, albeit for a minority of HIV-1 variants [1, 2].

A specific 32-bp deletion in the CCR5 gene (CCR5 Δ32) has been associated with a reduced susceptibility for infection and delayed disease progression [3]. This has been confirmed in many cohorts, including the Amsterdam Cohort [4]. More recently, a valine to isoleucine switch in the first transmembrane domain of CCR2 (CCR2 64I) was shown to be associated with delayed disease progression [5–7].

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This study was part of the collaborative Amsterdam Cohort Studies on AIDS.

Written informed consent was obtained from all participants. In the conduct of clinical research, human experiment guidelines of the authors’ institutions were followed.

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Here, we analyze the role of CCR2 genotype in the clinical course of HIV-1 infection in Amsterdam Cohort Studies participants in relation to the presence or absence of SI HIV-1 variants. We also analyzed the conversion rate towards SI HIV-1 variants in relation to CCR2 genotype.

Methods

Study population. The study population, 364 Caucasian, homosexual men enrolled in the Amsterdam Cohort between October 1984 and March 1986, was previously described [4]. The censor date of our study was set at 1 January 1996. None of the participants received triple therapy during follow-up. Of the 364 participants, 131 seroconverted during the study. No differences in AIDS-free survival were found between persons who had documented seroconversion and seroprevalent persons by Kaplan-Meier ($P = .36$) and Cox proportional hazard analysis (relative hazard [RH], 1.17; 95% confidence interval [CI], 0.84–1.63) for seroconverter compared with seroprevalent persons. This result suggests a good estimation of the seroconversion date in the latter group. Therefore, we combined 131 persons with documented seroconversion and 233 seroprevalent subjects as one study group.

CCR2 genotyping. DNA samples were available for CCR2 genotyping from 344 (94%) of the 364 men. Genomic DNA was
analyzed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis with primers CCR2-1A (sense, positions 170–187 in CCR2, 5'-TTGTGGCAACATGG-ATGG-3') and CCR2-1Z (antisense, positions 279–298 in CCR2, 5'-GAGCCCAACATGGGAGAAT-3') [5]. Samples were amplified with 1 U of Taq polymerase (Promega, Madison, WI) in the provided buffer with a final MgCl₂ concentration of 3 mmol/L. PCR was done in a thermocycler (Uno II; Biometra, Göttingen, Germany) as follows: 5 min of denaturation at 95°C; 35 cycles of 30 s at 95°C, 30 s at 50°C, and 30 s at 72°C (slope 2°C/s), and 5 min of elongation at 72°C. PCR products were subjected to restriction analysis with BsoBI for 2 h at 60°C (New England BioLabs, Beverly, MA) and analyzed on a 4% Nusieve gel (2:2). Fifteen samples (11 CCR2 wild-type and 4 CCR2 64I heterozygous samples) were subjected to sequence analysis (Thermo sequenase-radiolabeled terminator cycle sequence kit; Amersham, Cleveland), which confirmed the CCR2 genotype as determined by PCR-RFLP analysis (not shown).

Virologic assays. HIV-1–positive peripheral blood mononuclear cells were cocultivated with MT2 cells every 3 months to detect SI HIV-1 variants [9]. Time of SI conversion was the calculated midpoint between last SI-negative and first SI-positive sample. SI phenotype data were available for 281 of 364 participants. Serum virus load was measured by use of a quantitative HIV-1 RNA nucleic acid–based sequence amplification (NASBA Virologic assays. HIV-1–positive peripheral blood mononuclear cells were cocultivated with MT2 cells every 3 months to detect SI HIV-1 variants [9]. Time of SI conversion was the calculated midpoint between last SI-negative and first SI-positive sample. SI phenotype data were available for 281 of 364 participants. Serum virus load was measured by use of a quantitative HIV-1 RNA nucleic acid–based sequence amplification (NASBA Virologic assays. 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Figure 1. CCR2 and CCR5 genotype and disease progression. Kaplan-Meier plots for time to AIDS (1987 definition; top) or time to death (bottom) for subjects classified as CCR2/CCR5-[64I/+] (thick lines), [Δ32/+] (thin lines), and [+/-] (intermediate lines). No. of men at risk are indicated for each time point. Relative hazards (RH), confidence intervals (CI), and log-rank P values are shown.

Significantly slower decrease in CD4 T lymphocytes was seen in CCR2/CCR5-[64I/+] (decrease of 53/mm³/year [P = .005]), [Δ32/+] (53/mm³/year [P = .004]), and [64I/Δ32] subjects (27/mm³/year [P = .02]) compared with CCR2/CCR5-[+/-] subjects (78/mm³/year). The median virus load »2 years after seroconversion in CCR2/CCR5-[+/-] subjects was 10^4 copies/mL (range, ≤10^10-10^6); this level was 1.3-fold higher (P = .41) than the viral RNA load in [64I/+] subjects (10^3 copies/mL [range, 10^0-10^9]), 2.5-fold higher (P = .02) than in [Δ32/+] subjects (10^6 copies/mL [range, 10^0-10^9]), and 20.0-fold higher (P = .008) than in the small group of CCR2/CCR5-[64I/Δ32] subjects (10^3 copies/mL [range, 10^0-10^9]).

CCR2 and CCR5 genotype in relation to HIV-1 biologic phenotype. At the end of the study, not considering subjects lost to follow-up, the total frequency of SI HIV-1 was higher in CCR2/CCR5-[64I/+] subjects (21 [52.5%] of 40) than in subjects categorized as [Δ32/+] (20 [37%] of 54), [64I/Δ32] (1 [1.9%] of 5), and CCR2/CCR5-[+/-] (61 [33.9%] of 180). The prevalence of SI HIV-1 in CCR2/CCR5-[64I/+] and [+/-] subjects was significantly higher than the prevalence in [+/-] persons (P = .03).

To analyze a possible difference in the rate of evolution to SI HIV-1, Kaplan-Meier survival analysis was performed with conversion to SI HIV-1 as an end point (figure 2, top). In the
Figure 2. CCR2 and CCR5 genotype and HIV-1 biologic phenotype. Kaplan-Meier plots for subjects classified as CCR2/CCR5-/+ (intermediate lines), [64I/+] (thick lines), and [Δ32/+] (thin lines) for time to emergence of SI HIV-1 variants (top), time to AIDS when only NSI variants were present (middle), and time to AIDS after emergence of SI HIV-1 (bottom). No. of men at risk are indicated for each time point. Relative hazards (RH), confidence intervals (CI), and log-rank P values are shown.
first 4.5 years after seroconversion, ~15% of subjects in each of the 3 genotypic groups converted to an SI HIV-1 variant. During the subsequent follow-up, however, CCR2/CCR5-[64I/+] subjects had an increased conversion rate. Median time to SI conversion was 8.5 years after seroconversion in CCR2/CCR5-[64I/+] subjects but 11.5 years in CCR2/CCR5-[Δ32+/+] and [+/-+] subjects. However, the difference in evolution to an SI phenotype between CCR2/CCR5-[64I/+] and [+/-+] subjects was not significant for the total follow-up period (P = .23). The previously described more rapid evolution to SI HIV-1 variants in the group with CCR5-[Δ32] heterozygotes compared with CCR5 Δ32 homozygotes [4] was still visible, although not statistically significant (P = .31).

The protective effects of CCR2 64I and CCR5 Δ32 were stronger only in the presence of NSI HIV-1 variants when compared with total follow-up (figure 2, middle; [64I/+] RH = 0.44 [95% CI, 0.22–0.90] vs. RH = 0.66 [95% CI, 0.44–0.99]; [Δ32+]: RH = 0.22 [95% CI, 0.11–0.42] vs. 0.40 [95% CI, 0.26–0.58]). Analysis of the period after the emergence of SI HIV-1 variants (figure 2, bottom) showed that CCR5 Δ32 heterozygosity was still associated with prolonged AIDS-free survival (P = .06; RH = 0.44 [95% CI, 0.20–0.97]). The protective effect of CCR2 64I remained evident but was no longer significant after the emergence of SI (P = .44; RH = 0.73, [95% CI, 0.34–1.54]).

Discussion

We confirmed a correlation between CCR2 genotype and prolonged AIDS-free survival, as originally described by Smith et al. [5]. In 344 HIV-1–seropositive participants from the Amsterdam Cohort Studies, a Cox proportional hazard analysis on CCR2/CCR5-[64I/+] genotype yielded an RH of 0.66 for progression to AIDS. This confirms the findings in various cohorts in which RH varied from 0.6 to 0.7 for the CCR2 64I heterozygous genotype [5, 6]. In accordance with a delayed progression to AIDS, we observed a slower CD4 cell decline and a lower serum viral RNA load in CCR2/CCR5-[64I/+] than in [+/-+] subjects.

The biologic mechanism by which the CCR2 64I genotype provides protection against disease progression is not known. Primary HIV-1 variants in general do not use CCR2 ([12, 13], A.M.R.H., unpublished data) excluding coreceptor dysfunctioning as a major explanation. More likely, this polymorphism influences the CCR5 pathway, directly or indirectly. It has been suggested that CCR2 64I may result in a diminished expression of CCR5. In support of this is the observation that the G to A mutation resulting in the CCR2 64I substitution is linked to a point mutation in the promotor region of the CCR5 gene [6]. However, this point mutation is located within a 1.9-kb intron that may be dispensable for optimal promotor activity [14, 15]. In agreement with this, neither preliminary data from Kostrikis et al. [6] or Rizzardi et al. [7] nor preliminary results from our laboratory (A.M.R.H.) support the hypothesis that CCR5 expression is reduced in CCR2 64I heterozygotes.

In this study, we showed a higher conversion rate to and a higher prevalence of SI HIV-1 variants among CCR2 heterozygotes than in wild-type homozygotes in contrast to CCR5 Δ32 heterozygotes in which SI conversion tends to be delayed. This suggests a different mechanism of action of CCR2 64I than of CCR5 Δ32 in delaying HIV-1 disease progression. The protective effects of both CCR2 64I and CCR5 Δ32 were stronger in the sole presence of NSI HIV-1 variants when compared with the total follow-up. This suggests that the mechanism of both protective polymorphisms is mediated via NSI HIV-1 variants, which in general are CCR5 restricted. Elucidating the mechanism of action of CCR2 64I might help to identify potential sites for therapeutic interventions in HIV-1 infection.

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