Analysis of Genetic Polymorphisms in CCR5, CCR2, Stromal Cell–Derived Factor–1, RANTES, and Dendritic Cell–Specific Intercellular Adhesion Molecule–3–Grabbing Nonintegrin in Seronegative Individuals Repeatedly Exposed to HIV-1

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To determine the influence of host genetics on human immunodeficiency virus (HIV) type 1 infection, we examined 94 repeatedly exposed seronegative (ES) individuals for polymorphisms in multiple genes and compared the results with those for 316 HIV-1–seropositive and 425 HIV-1–seronegative individuals. The frequency of homozygous C-C chemokine receptor (CCR) 5–D32 was higher in ES (3.2%) than in HIV-1–seropositive individuals (0.0%; P = .012). However, the CCR5-59029A, CCR2-64I, stromal cell–derived factor (SDF)–1–3′A, RANTES (regulated on activation, normally T cell–expressed and –secreted)–403A, and RANTES-28G polymorphisms were not associated with resistance to HIV-1 infection. Furthermore, we identified novel variants in the DC-SIGN (dendritic cell–specific intercellular adhesion molecule–3–grabbing nonintegrin) repeat region and observed that heterozygous DC-SIGN reduced the risk of HIV-1 infection (3.2% in ES individuals vs. 0.0% in HIV-1–seropositive individuals; P = .011).

Despite high-risk behavior and/or multiple exposures to HIV-1, some individuals remain seronegative or uninfected. It has been demonstrated extensively that genetic polymorphisms in host genes affect the risk of HIV-1 acquisition and the progression of disease [1, 2]. Individuals homozygous for CCR5-D32 are highly resistant to HIV-1 infection [1, 2]. CCR2-64I affects the progression of HIV-1 disease but not acquisition of infection [3], whereas several studies have shown that CCR2-64I homozygosity is associated with a reduced risk of acquiring HIV-1 infection [4]. Homozygous CCR5-59029A is associated with an accelerated progression to AIDS [5]. RANTES-403G/A and -28C/G have been shown to influence progression of disease [6] but may have different effects on transmission of HIV-1 in different populations. Stromal cell–derived factor (SDF)–1–3′A is not associated with transmission of HIV-1.

Dendritic cells (DCs) are among the first cells encountered by HIV-1 during sexual transmission. HIV-1 may use DCs as carriers to gain entry into lymph nodes and, subsequently, to infect CD4+ T cells [7]. DC-SIGN (DC-specific intercellular adhesion molecule–3–grabbing nonintegrin) is able to bind the HIV-1 surface protein, gp120, with high affinity and in a CD4-independent manner [7, 8]. DC-SIGN is abundantly expressed primarily on DCs, including those derived from monocytes and those located beneath the genital surface. DC-SIGNR (DC-SIGN–related molecule) shows the same function as DC-SIGN [9], and both of them are organized into 3 domains: an N-terminal cytoplasmic region, a neck region containing 7 repeats of the 23-aa sequence, and a C-terminal domain with homology to C-type lectins [9, 10]. DC-SIGNR is polymorphic in the repeat region in white populations, whereas no similar polymorphisms have been identified in DC-SIGN [9].

In the present study, we screened 94 exposed seronegative (ES) individuals for known polymorphisms that may alter susceptibility to HIV-1 infection, including CCR5-D32, CCR5 promoter polymorphisms, CCR2-64I, SDF-1–3′A, RANTES-403A, and RANTES-28G, and compared their prevalence in ES individuals with those in 316 HIV-1–seropositive and 425 HIV-1–seronegative individuals. We confirmed that CCR5-D32 is associated with resistance to HIV-1 infection and identified novel variations in the DC-SIGN repeat region that may contribute to the resistance to HIV-1 infection of ES individuals.

Subjects and methods. Three cohorts of 835 subjects were recruited for the present study. The 94 ES individuals have been enrolled at the Fred Hutchinson Cancer Research Center Vaccine Trials Unit since 1993 because of their multiple exposures to HIV-1.

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to HIV-1 through unprotected sexual activity with known HIV-1–seropositive partners [11]. Details for study eligibility and additional epidemiologic features of this cohort have been described elsewhere [11].

The HIV-1–seropositive cohort includes 230 HIV-1 seroconverters who have been recruited from the University of Washington Primary Infection Clinic (PIC) since 1993, 29 HIV-1–infected long-term nonprogressors, and 57 HIV-1–seropositive individuals without a documented date of seroconversion. Details for study enrollment and follow-up of PIC subjects have been described elsewhere [12]. The HIV-1–seronegative cohort includes participants recruited from the Fred Hutchinson Cancer Research Center Vaccine Trials Unit and studies of men who have sex with men, as described elsewhere [13]. None of the 425 HIV-1–seronegative subjects had record of repeated high-risk exposures to HIV-1. Informed consent was obtained from patients, and human experimentation guidelines of the US Department of Health and Human Services and those of the authors’ institutions were followed in the use of clinical samples.

A multiplex polymerase chain reaction (PCR) strategy was used for genotyping CCR5-Δ32, CCR5-59029A, CCR2-64I, and SDF-1–3A [14]. A PCR–restriction fragment–length polymorphism method was used for genotyping RANTES-403 and RANTES-28 [15]. The sequences of cDNAs to mRNAs spanning the entire coding regions of CCR1, CCR5, and RANTES were determined by direct sequencing.

The DC-SIGN repeat region in exon 4 was amplified from genomic DNA with the following pairs of primers: 5′-CCACTTTTGGGCAGGAC-3′ (1F1) and 5′-AGCAACTCACCACACACAGA-3′ (1R), designed from GenBank sequence AF209479. PCR amplification was performed in a volume of 30 μL containing 0.25 mmol/L dNTPs, 1.0 μmol/L each primer, 2.5 mmol/L MgCl2, and 1.0 U of Expand High Fidelity PCR System Enzyme in 1X reaction buffer (Roche). The cycle conditions were 5 min at 94°C, followed by 40 cycles of 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C, and then 1 cycle of 7 min at 72°C. Alleles were distinguished by use of 3% agarose gel electrophoresis with ethidium-bromide staining.

Pearson’s χ2 test was used to determine whether the distribution of genotypes or alleles is independent of the ES, HIV-1–seronegative, and HIV-1–seropositive cohorts. The exact P value was estimated to the Monte Carlo accuracy, with a 99% confidence interval (CI) based on 10,000 tables sampled. For CCR5-Δ32, CCR5-59029A, CCR2-64I, SDF-1–3A, RANTES-403A, and RANTES-28G, a 3 × 3 contingency table was used. For RANTES compound genotypes, a 3 × 6 table was used to test whether the distribution is independent. Because no individuals with a homozygous DC-SIGN repeat region mutation were found, a 2 × 3 table was used to test the distribution of DC-SIGN repeat region genotypes. All the above analyses were performed by use of StatXact-5 (Cytel Software).

The association between possession of a genotype or allele and risk of acquiring HIV-1 was evaluated by use of Fisher’s exact test, and the odds ratio (OR) and 95% CI were calculated. Because there were no DC-SIGN repeat region variations and no individuals homozygous for CCR5-Δ32 in the HIV-1–seropositive cohort, their ORs could not be calculated by use of a general method. In this case, StatXact-5 automatically added 0.5 to each cell to calculate the OR for continuity correction. The χ2 test, a less exact but widely known statistical analysis, was also performed with Yates’s correction for 2 × 2 table analysis. All P values were 2-sided. These analyses were performed by use of PRISM (version 4.0; GraphPad Software), InStat (version 3.0; GraphPad Software), and StatXact-5.

Results. We examined CCR5-Δ32 in 94 ES, 425 HIV-1–seronegative, and 316 HIV-1–seropositive individuals. The frequencies of individuals homozygous for CCR5-Δ32 among the ES, HIV-1–seronegative, and HIV-1–seropositive cohorts were 3.2%, 0.7%, and 0.0%, respectively, whereas the frequencies of individuals heterozygous for CCR5-Δ32 were 23.4%, 16.0%, and 17.4%, respectively (figure 1). A χ2 test for independence showed that there was a significant difference in the distribution of CCR5-Δ32 genotypes among the ES, HIV-1–seronegative, and HIV-1–seropositive cohorts (P = .008 [99% CI, .006-.010]). The frequency of individuals homozygous for CCR5-Δ32 was significantly higher in the ES cohort than in the HIV-1–seropositive cohort (P = .012, by Fisher’s exact test; OR, 24.21

Figure 1. Frequency of CCR5-Δ32 genotypes in total populations among exposed seronegative (ES), HIV-1–seronegative (HIV-1−), and HIV-1–seropositive (HIV-1+) individuals. Analysis with the χ2 test showed a significant difference in the distribution of CCR5-Δ32 genotypes among ES, HIV-1−, and HIV-1+ individuals (P = .008). Analysis with Fisher’s exact test showed that the frequency of individuals homozygous for CCR5-Δ32 was significantly higher in the ES than in the HIV-1+ cohort (P = .012). Analysis with the χ2 test also yielded a similar P value (P = .013).
Because no HIV-1–seropositive individuals were homozygous for CCR5-Δ32, the OR was calculated using the addition of 0.5 to each cell. The χ² test was also performed and yielded a P value (P = .013) similar to that calculated by Fisher’s exact test. Because CCR5-Δ32 is observed almost exclusively among individuals of European descent, we further analyzed CCR5-Δ32 genotypes in white individuals only. Again, the distribution of CCR5-Δ32 genotypes was associated with the ES, HIV-1–seronegative, and HIV-1–seropositive cohorts (P = .046; 99% CI, 0.041–0.052) among whites. Individuals who were homozygous for CCR5-Δ32 were significantly more common in the ES cohort than in the HIV-1–seropositive cohort (P = .025, by the χ² test). These results, which are consistent with those of previous studies [1, 2], indicate that being for homozygous CCR5-Δ32 is associated with resistance to HIV-1 infection in ES individuals.

The neck region of DC-SIGN between the C-terminal domain and the transmembrane domain is formed by repeats of 69 bp that encode repeating units of 23 aa. Analysis of this region in our 3 cohorts of 835 individuals revealed, on the basis of the number of repeats (range, 6–8 repeats), the presence of 3 alleles and 3 genotypes of 6/7, 7/7, and 7/8. The allele frequencies found in all studied populations were 0.24% for allele 6, 99.52% for allele 7, and 0.24% for allele 8. As shown in table 1, allele 7 is regarded as the wild type, whereas alleles 6 and 8 are regarded as mutations. Our data demonstrate that polymorphisms in the DC-SIGN repeat region are rare, which may be why a previous study did not demonstrate such polymorphisms in the DC-SIGN repeat region in 150 white individuals [9] (table 1). Cloning and sequencing of each allele indicated that the difference among alleles is due to a deletion or insertion of 69 nt encoding the multiple 23 aa repeats (data not shown).

We found that 3 (3.2%; all are white) of 94 ES individuals and 5 (1.2%; 3 white and 2 of other ethnic origins) of 425 HIV-1–seronegative individuals were heterozygous for the DC-SIGN repeat region (table 1). However, none of the 316 HIV-1–seropositive individuals studied had variants in the DC-SIGN repeat region. Because individuals who are homozygous for CCR5-Δ32 are highly resistant to HIV-1 infection, they were excluded from further analyses. Because we only found wild-type and heterozygous mutations, a 2 × 3 table was used to test the overall distribution of DC-SIGN repeat region genotype. We observed a significant difference in the overall distribution of DC-SIGN repeat region genotypes among the ES, HIV-1–seronegative, and HIV-1–seropositive individuals (P = .011 [99% CI, .008–.014]). ES individuals were significantly more likely to have more variations in the DC-SIGN repeat region, compared with HIV-1–seropositive individuals (P = .011, by Fisher’s exact test; OR, 25.03 [95% CI, 1.28–489.56]) (P = .025, by the χ² test). Because no DC-SIGN repeat region variation was found in the HIV-1–seropositive cohort, the OR was calculated by adding 0.5 to each cell with StatXact-5 software. These results suggested that individuals heterozygous for DC-SIGN repeat alleles may be associated with reduced susceptibility to HIV-1 infection in ES individuals.

We also examined CCR5-59029A, CCR2-64I, SDF-1–3'A, RANTES-403A, and RANTES-28G in 64 ES, 356 HIV-1–seronegative, and 236 HIV-1–seropositive individuals. Again, we excluded the individuals who were homozygous for CCR5-Δ32 from further statistical analyses. By use of the χ² test, we found no significant difference in the distribution of these genotypes among the ES, HIV-1–seronegative, and HIV-1–seropositive individuals in all populations or in white individuals only (data not shown). Taken together, our results are consistent with those of most previous studies [1, 2], suggesting that none of the CCR5-59029A, CCR2-64I, SDF-1–3'A, RANTES-403A, and RANTES-28G polymorphisms is associated with the probability of acquiring of HIV-1 infection.

We sequenced the CCR5 promoter region from 52 ES individuals, the CCR5-coding region from 36 ES individuals, the CCR1-coding region from 39 ES individuals, and the RANTES-
coding region from 14 ES individuals. Aside from the reported mutation/variants, no additional variations were found in the coding regions of these coreceptors sequenced.

Discussion. It has been shown that polymorphisms in multiple human genes have significant effects on the progression to AIDS or death after acquisition of HIV-1 infection. However, studies of the effect of genetic polymorphisms on protection against HIV-1 infection have been less substantial. We examined a cohort of ES individuals for known polymorphisms—such as CCR5-32, CCR5-59029A, CCR2-64I, SDF-1-3A, RANTES-403A, and RANTES-28G, as well as the coding regions of CCR1, CCR5, and RANTES—and compared the results with those for control cohorts of HIV-1–seronegative and HIV-1–seropositive individuals. We found that individuals who are homozygous for CCR5-32 are significantly more common in the ES cohort, compared with the HIV-1–seropositive and HIV-1–seronegative cohorts, which is consistent with the results of other studies and demonstrates the importance of homozygous CCR5-32 in protection against HIV-1 infection [1, 2].

In searching for additional genetic polymorphisms that may affect HIV-1 infection, we identified novel variations in the DC-SIGN repeat region. Of the 835 individuals we tested, all 8 individuals with DC-SIGN repeat region variations were from the HIV-1–seronegative cohort: 3 were ES individuals, and 5 were HIV-1–seropositive individuals. Compared with HIV-1–seropositive individuals, a higher prevalence of DC-SIGN variations that may exert effects on resistance to HIV-1 infection was observed among ES individuals, suggesting that an association exists between DC-SIGN variation and resistance to HIV-1 infection in ES individuals. This observation warrants further studies in larger cohorts.

In summary, homozygous CCR5-32 accounts for the resistance to HIV-1 infection in a small proportion (3 of 94) of ES individuals. None of the polymorphisms of CCR5-59029A, CCR2-64I, SDF-1-3A, RANTES-403A, and RANTES-28G was associated with protection against HIV-1 infection in our ES cohort. Variations in the DC-SIGN repeat region may also have a protective effect on transmission of HIV-1. The rare frequencies of both homozygous CCR5-32 and DC-SIGN variants in ES stress the need to search for other potential genetic variations that may exert effects on resistance to HIV-1 infection in ES individuals.

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References