

CONCISE COMMUNICATION

Chemokine Receptor Gene Polymorphisms and Risk of Human T Lymphotropic Virus Type I Infection in Jamaica

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Polymorphisms of some chemokine receptor genes and their ligands are associated with susceptibility and progression of human immunodeficiency virus infection. This study assessed whether these variants are also responsible for susceptibility to infection with human T lymphotropic virus (HTLV) type I. Frequencies of *CCR5-Δ32*, *CCR2-64I*, and *SDF-1-3'A* genotype among 116 HTLV-I-positive and 126 HTLV-I-negative persons of African descent in Jamaica were 1.0%, 14.9%, and 5.4%, respectively. The association of HTLV-I infection with the most common variant, *CCR2-64I*, was examined in 532 subjects. Thirteen (5.4%) of 241 HTLV-I-negative subjects were homozygous for *CCR2-64I*, versus 3 (1.0%) of 291 HTLV-I-positive subjects ($P = .005$). Among HTLV-I carriers, provirus load and antibody titer were not significantly different in persons with *CCR2-+/64I* or *CCR2-+/+*. These findings suggest that *CCR2-64I*, or alleles in linkage disequilibrium with it, may affect the risk of HTLV-I infection in a recessive manner.

Some members of the CC and CXC families of transmembrane chemokine receptors serve as coreceptors for the entry of human immunodeficiency virus (HIV) into host cells [1, 2]. Allelic variants in these genes and their ligands are associated with an altered susceptibility to HIV infection and disease progression [2, 3]. A 32-bp deletion in the *CCR5* gene (*CCR5-Δ32*) is found in 10% of white persons but is rare (<2%) among other racial groups [1–3]. An amino acid change at position 64 in *CCR2* (*CCR2-64I*) is common in all racial groups with allele frequencies of 10%–15% [4]. A single base substitution in the 3' untranslated region of stromal-derived factor 1 (*SDF-1-3'A*), a ligand for the T

cell-tropic HIV coreceptor *CXCR4*, is found in 16%–26% of whites, Hispanics, and Asians, 6% of African Americans, and 3% of native Africans [2, 5]. *CCR5-Δ32/Δ32* homozygosity confers resistance against HIV infection, whereas *CCR5-+/Δ32* heterozygosity delays HIV disease progression [2]. *CCR2-+/64I* heterozygosity and *SDF-1-3'A/3'A* homozygosity also are thought to delay HIV disease progression [4, 5].

Human T lymphotropic virus (HTLV) type I is endemic in southwest Japan, the Caribbean, and parts of Africa [6]. Although HTLV-I and HIV have similar genomic structure and share many biologic properties including T cell tropism, the receptors for HTLV-I entry into host cells remain elusive. A number of cell surface molecules, including tetraspanin CD82, VCAM-1, and hsc-70, play a role in fusion with viral envelope glycoproteins [7, 8]. Although any of these molecules may serve as potential coreceptors, evidence for a functional role as a primary receptor for HTLV-I is lacking. Because chemokine receptors are also used as portals of entry by certain intracellular pathogens other than HIV [9], it is possible they may play a role in HTLV-I infection.

Methods

Study subjects. Subjects for the present analyses were selected from 2 epidemiologic cohorts in Jamaica. The Food Handlers Study screened > 13,000 food service license applicants from 13 parishes for HTLV-I antibody between March 1985 and May 1986 [10]. From

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Informed consent was obtained from all study participants. Study protocol followed human experimentation guidelines of the US Department of Health and Human Services and institutional review boards of the National Cancer Institute and the University of the West Indies.

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this cohort, 214 HTLV-I–positive and 226 age- and sex-matched HTLV-I–negative subjects residing in 3 parishes (Kingston, St. Andrew, and Clarendon) were enrolled in a nested case-control study between January 1987 and April 1998. The Mother-Infant Study screened >9000 pregnant women for HTLV-I antibody at 2 antenatal clinics in Kingston between January 1989 and August 1990 [11]. In total, 212 HTLV-I–positive and 127 HTLV-I–negative women, similar in age distribution, were enrolled in a prospective follow-up of their offspring.

For the initial analysis of gene frequency, we selected 242 participants (31 men and 211 women) from the Food Handlers Study on the basis of their African ancestry and availability of DNA specimens. For the subsequent analysis of *CCR2* polymorphism and HTLV-I infection, 532 subjects were included. In addition to the 242 food handlers, we selected an additional 290 women from the Mother-Infant Study on the basis of their African ancestry and availability of DNA specimens. Because the frequencies of *CCR2-64I* genotype were similar in both studies, we were able to combine the 2 populations.

Laboratory methods. Peripheral blood samples were cryopreserved and stored at -70°C until use. HTLV-I positivity was determined by ELISA (Dupont) and confirmed by Western blot (Cambridge-Biotech). HTLV-I antibody titers were determined by ELISA with the end-point dilution method (Genetic Systems or Cambridge-Biotech).

HTLV-I provirus load was measured by a real-time automated polymerase chain reaction (PCR) method. DNA was prepared from 10^6 viably frozen peripheral blood mononuclear cells (PBMC). In all, $10\ \mu\text{L}$ of DNA ($\sim 1\ \mu\text{g}$) was amplified for 45 cycles with AmpliTaq Gold polymerase with the ABI PRISM sequence detection system and TaqMan PCR reagent (PE Applied Biosystems). Provirus load was normalized by the number of human endogenous retrovirus (ERV)–3 copies to adjust for variation in DNA recovery and amplification efficiency.

The conditions for PCR amplification and the sequencing of the primers for *CCR5-Δ32* [2, 3], *CCR2-64I* [4], and *SDF-1-3'A* [5] genotypes have been described elsewhere. The *CCR5-Δ32* genotype was determined by PCR by using primers 5'-CTTCATTACACCTGCAGCTCTC-3' (forward) and 5'-CTCACAGCCCAGTGCGACTTCTTCT-3' (reverse), which flank the 32-bp deletion. The *CCR2-64I* and *SDF-1-3'A* genotypes were determined by PCR amplification followed by restriction fragment–length polymorphism. For *CCR2*, the primers were CCR2F3 5'-ATGCTGTCCACATCTCGTTC-3' (forward) and CKR2-1Z 5'-GAGCCCACAATGGGAGAGTA-3' (reverse). Amplified material was digested with *FokI* (New England BioLabs) according to the manufacturer's conditions. For *SDF-1*, the primers were SDF-1-3' untranslated region (UTR) 5'-CAGTCAACCTGGGCAAAGCC-3' (forward) and SDF-1-3' UTR 5'-AGCTTTGGTCTGAGAGTCC-3' (reverse). Amplified product was digested with *MspI* (New England BioLabs).

In vitro infection of PBMC from 6 study subjects with known *CCR2* genotypes (*CCR2-+/+* and *CCR2-64I/64I*, 3 each) was done as described elsewhere [12]. PBMC were cocultivated with an equal number of lethally irradiated (6000 rad) HTLV-I– and HTLV-II–infected cell lines. Previously established HTLV-I–infected T cell lines SP, MT2, and HUT102 and HTLV-II–infected T cell line AI-1050 were grown in C-RPMI supplemented with 10% interleukin

(IL)–2 and used for cocultivation [13]. After 24 h, cells were extensively washed and cultured at 10^6 viable cells/mL in C-RPMI, supplemented with 10% IL-2. The culture supernatants were collected at 7-day intervals for 60 days and stored at -70°C until further analysis. The HTLV antigen production in the culture supernatants was determined by use of a p19 Gag antigen capture assay after 60 days (Zepto Metrix).

Statistical analyses. Frequencies of each allele variant by HTLV-I status were compared with the likelihood χ^2 or Fisher's exact tests. The odds ratios (ORs) for the association of HTLV-I positivity with the presence of the chemokine receptor variant were calculated with EpiInfo version 6.04 (Centers for Disease Control and Prevention). The 95% confidence intervals were exact where the expected number of observations per cell was <5 . Among HTLV-I carriers, the mean HTLV-I provirus load and anti-HTLV-I titer were compared by the Kruskal-Wallis test. Statistical significance was based on 2-sided tests ($P = .05$).

Results

Among 242 subjects from the Food Handlers Study included in the analysis of gene frequencies, 5 (2.1%) were *CCR5-+/Δ32* and none were *CCR5-Δ32/Δ32* (table 1). Of the 242 subjects, 22 (9.1%) were *SDF-1-+/3'A* and 2 (0.8%) were *SDF-1-3'A/3'A*. Therefore, the allele frequencies of *CCR5-Δ32* and *SDF-1-3'A* were 1.0% (5 of 484) and 5.4% (26 of 484), respectively, which were too low to allow further evaluation with confidence.

Table 1. Association of *CCR2-64I*, *CCR5-Δ32*, and *SDF-1-3'A* genotypes with human T lymphotropic virus (HTLV) type I infection among Jamaican adults of African ancestry.

| Genotype | All subjects ^a | HTLV-I positive | HTLV-I negative | OR (95% CI) ^b |
|--------------|---------------------------|-----------------|-----------------|--------------------------|
| <i>CCR2</i> | | | | |
| +/+ | 372 (69.9) | 204 (70.1) | 168 (69.7) | 1.00 |
| +/64I | 144 (27.1) | 84 (28.9) | 60 (24.9) | 1.15 (0.77–1.73) |
| 64I/64I | 16 (3.0) | 3 (1.0) | 13 (5.4) | 0.19 (0.3–0.71) |
| Total | 532 (100) | 291 (100) | 241 (100) | |
| <i>CCR5</i> | | | | |
| +/+ | 237 (97.9) | 113 (97.4) | 124 (98.4) | 1.00 |
| +/Δ32 | 5 (2.1) | 3 (2.6) | 2 (1.6) | 1.65 (0.19–20.0) |
| Δ32/Δ32 | 0 | 0 | 0 | UD |
| Total | 242 (100) | 116 (100) | 126 (100) | |
| <i>SDF-1</i> | | | | |
| +/+ | 218 (90.1) | 106 (91.4) | 112 (88.9) | 1.00 |
| +/3'A | 22 (9.1) | 10 (8.6) | 12 (9.5) | 0.88 (0.34–2.29) |
| 3'A/3'A | 2 (0.8) | 0 | 2 (1.6) | UD |
| Total | 242 (100) | 116 (100) | 126 (100) | |

NOTE. Data are no. (%) of subjects. CI, confidence interval; OR, odds ratio; UD, undefined; +/+, wild type for each genotype; +/64I, +/Δ32, or +/3'A, heterozygous variants; 64I/64I, Δ32/Δ32, or 3'A/3'A, homozygous variants.

^aTotal subjects analyzed for *CCR2* genotype are sum of 242 subjects from Food Handlers Study and 290 subjects from Mother-Infant Cohort Study. Total subjects analyzed for *CCR5* and *SDF-1* genotypes were 242 subjects from Food Handlers Study.

^bOR compares frequency of HTLV-I positivity by zygosity by using +/+ as the referent group. CIs are exact where expected observations are <5 .

The allele frequency of *CCR2-64I*, on the other hand, was 14.9% (72 of 484); 60 subjects were *CCR2-+/64I* and 6 were *CCR2-64I/64I*.

Because the frequency of the *CCR2-64I/64I* variant appeared lower among 116 HTLV-I–positive subjects than in 126 HTLV-I–negative subjects (0 vs. 4.8%; $P = .06$) in the Food Handlers Study, we expanded this analysis to a larger number of subjects. Among 532 subjects included in the analysis of *CCR2* genotype and HTLV-I infection from the 2 study populations combined, 16 (3.0%) were *CCR2-64I/64I* homozygotes (table 1). The frequencies of *CCR2-64I/64I* homozygosity were 1.0% among HTLV-I–positive subjects and 5.4% among HTLV-I–negative subjects. Persons with *CCR2-64I/64I* homozygosity were thus significantly less likely to be HTLV-I positive (OR, 0.19; $P = .005$). *CCR2-+/64I* heterozygosity, however, did not appear to confer protection against HTLV-I infection (OR, 1.15; $P = .47$), suggesting a recessive effect.

We next examined whether persons homozygous for *CCR2-64I/64I* would confer resistance to HTLV-I infection. Table 2 summarizes combined data from all infection experiments. Replication kinetics of cocultivated PBMC revealed initial drop in antigen production (due to residual irradiated cell lines), followed by increase in antigen production around days 40–50 of culture [12]. Lymphocytes from all 3 subjects with *CCR2-64I/64I* were infected with HTLV-I when cocultivated with the HUT102 cell line. SP and MT2 cell lines also infected lymphocytes of ≥ 1 of these subjects. Furthermore, all lymphocytes became infected with HTLV-II when cocultivated with the AI-1050 cell line. These data provide evidence that lymphocytes from 3 persons with *CCR2-64I/64I* could be infected with HTLV-I and HTLV-II, as were the lymphocytes from persons with *CCR2-+/+*.

We compared levels of HTLV-I provirus load and antibody titer by *CCR2* genotype among HTLV-I–positive subjects in our series. HTLV-I provirus load (\log_{10} copies/ 10^5 cells) was similar between persons with *CCR2-+/64I* and persons with *CCR2-+/+* (3.19 vs. 3.41, $P = .46$). Likewise, the mean titer for HTLV-I antibody was not significantly different between subjects with *CCR2-+/64I* and those with *CCR2-+/+* (3.62 vs. 3.64 in \log_{10} ; $P = .82$). The mean provirus load of 2 persons with *CCR2-64I/64I* homozygosity appeared slightly lower than the mean provirus load among persons with *CCR2-+/+* (2.89 vs. 3.41; $P = .57$), but the number of *CCR2-64I/64I* homozygous subjects was too small to draw any conclusions. None of the HTLV-I carriers in our series had clinical disease associated with HTLV-I; thus, evaluation of an association between *CCR2* genotype and disease progression was impossible.

Discussion

The *CCR2-64I* variant forms heterodimers with *CCR5* and *CXCR4*, which reduce the amount of HIV coreceptors and thus the risk of HIV transmission [14]. In the present investigation, persons with *CCR2-64I/64I* genotype were significantly less

likely to be infected with HTLV-I, although 3 of the 16 *CCR2-64I/64I* homozygotes were infected with HTLV-I. The results of in vitro infection experiments confirm that both HTLV-I and -II can infect lymphocytes, regardless of the *CCR2* genotype (table 2), suggesting that *CCR2* is not a required receptor for HTLV infections. The observed incomplete penetrance for the effect of *CCR2-64I* variant against HTLV-I infection raises the possibility that the blockage of HTLV-I infection likely requires the presence of other cofactors. Gene polymorphisms in linkage disequilibrium with *CCR2-64I* may be responsible for the observed association. One example may be the *CCR-927T* variant, which is associated with slow progression of HIV-1 disease [15]. Alternatively, persons with *CCR2-64I/64I* may clear HTLV-I infection or fail to mount an HTLV-I antibody response.

The observed protective effect of *CCR2-64I/64I* homozygosity against HTLV-I infection provides an interesting parallel to that observed with *CCR5-Δ32/Δ32* in the context of HIV infection. Because *CCR2-64I* is a common variant in all racial groups, a lower frequency of *64I/64I* genotype, compared with *+/+* wild type, may be found among HTLV-I carriers in other ethnic groups, including Japanese persons.

In our analysis, we examined the association of *CCR2* genotype and the viral pathogenesis by using provirus load and antibody titer as intermediate markers of HTLV-I disease among HTLV-I carriers. No significant difference was found across *CCR2* genotype, suggesting that the *CCR2-+/64I* heterozygosity is unlikely to be a strong predictor of HTLV-I disease progression in our population.

Of note, the risk of HTLV-I infection in adults is usually higher in women than in men because the virus is more likely to be transmitted by males to females by sexual contact. Our inability to take this into account warrants caution in interpretation of our data from this predominantly female cohort. However, oversampling of female subjects is unlikely to have caused bias in our study, be-

Table 2. Cocultivation of human T lymphotropic virus (HTLV) type I–positive (SP, MT2, and HUT102) and HTLV-II–positive (AI-1050) cell lines and lymphocytes from HTLV-I–negative persons with *CCR2-64I/64I* and *CCR2-+/+* genotypes.

| Subject | <i>CCR2</i> genotype | HTLV-infected cell lines | | | |
|---------|----------------------|--------------------------|-----|--------|---------|
| | | SP | MT2 | HUT102 | AI-1050 |
| 1 | <i>+/+</i> | – | – | ND | + |
| 2 | <i>+/+</i> | + | + | ND | + |
| 3 | <i>+/+</i> | + | – | + | + |
| 4 | <i>64I/64I</i> | – | – | + | + |
| 5 | <i>64I/64I</i> | + | + | + | + |
| 6 | <i>64I/64I</i> | + | – | + | + |

NOTE. *CCR2-+/+* and *64I/64I* denote wild type and homozygote for *CCR2-64I* variant, respectively. +, Successful HTLV-I or -II infection of lymphocytes of the study subjects after 60 days of cocultivation with HTLV-positive cell line; ND, not done.

cause segregation of chemokine receptor genotype is not sex linked. In addition, frequency of *CCR2* variant did not vary across sex in our cohort, nor did the inverse association between *CCR2* variant and HTLV-I infection differ by sex. Moreover, we had no reason to believe that *CCR2* genotype is associated with survival of the subject in one sex but not the other.

One limitation of the present study was the lack of accurate assessment of exposure to HTLV-I. To confirm or refute our hypothesis, comparisons must be made between HTLV-I carriers and HTLV-I-exposed uninfected persons. Identifying markers for persons who resist this retrovirus would facilitate prevention strategies against this chronic infection and its disease outcomes. The role of chemokine receptor gene polymorphisms in HTLV-I infection and the disease pathogenesis also warrants investigation of this intriguing association in other ethnic groups.

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