A Common CD4 Gene Variant Is Associated with an Increased Risk of HIV-1 Infection in Kenyan Female Commercial Sex Workers

Julius O. Oyugi,¹,* François C. M. Vouriot,¹,4 Judie Alimonti,² Stephen Wayne,¹ Ma Luo,¹ Allison M. Land,¹ Zhujun AO,¹ Xiaojian Yao,¹ Rafick P. Sekaly,⁴ Lawrence J. Elliott,² J. Neil Simonsen,³ T. Blake Ball,¹,⁵ Walter Jaoko,⁵ Julius O. Oyugi,¹,a Françoise C. M. Vouriot,¹,a Judie Alimonti,³ Stephen Wayne,¹ Ma Luo,¹ Allison M. Land,¹ Zhujun AO,¹ Xiaojian Yao,¹ Rafick P. Sekaly,⁴ Lawrence J. Elliott,² J. Neil Simonsen,³ T. Blake Ball,¹,⁵ Walter Jaoko,⁵

Departments of ¹Medical Microbiology and ²Community Health Sciences, University of Manitoba, and ³National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, and ⁴University of Montreal, Montreal, Canada; ⁵Department of Medical Microbiology, University of Nairobi, Kenya

Background. It has been predicted that CD4 C868T, a novel CD4 single-nucleotide polymorphism (SNP) that has been found to be highly prevalent among Africans, changes the tertiary structure of CD4, which may alter susceptibility to human immunodeficiency virus (HIV) infection.

Methods. Participants were from a Kenyan cohort and included 87 uninfected and 277 HIV-1–infected individuals. DNA sequencing was used to determine CD4 genotype. A2.01 cells expressing similar levels of either wild-type CD4 or CD4-Trp240 as well as peripheral blood mononuclear cells from uninfected donors were infected with HIV-1IIIB or a Kenyan primary HIV-1 isolate. HIV-1 p24 enzyme-linked immunosorbent assay was used to determine the outcome of infection.

Results. CD4 C868T was found to be significantly more prevalent among HIV-1–infected participants than among HIV-1–uninfected participants (P = .002), and C868T was associated with an increased incidence of HIV-1 infection as well (P = .005, log-rank test; P = .009, Wilcoxon test), with an odds ratio of 2.49 (P = .009). Both in vitro and ex vivo models demonstrated a significant association between CD4 C868T and susceptibility to HIV-1 infection (P < .001 and P = .003, respectively).

Conclusion. Overall, the present study found a strong correlation between CD4 C868T and susceptibility to HIV-1 infection. Given the high prevalence of both HIV infection and CD4 C868T in African populations, the effect of this SNP on the epidemic in Africa could be dramatic.

Susceptibility to HIV-1 infection is determined by many factors, including host genetics. Several studies have reported a strong association between genetic polymorphisms and HIV infection or disease progression [1, 2]. Most of these studies have investigated polymorphisms in the genes that encode chemokines and chemokine receptors, such as CCR5Δ32, CCR2-64I, and stromal cell–derived factor (SDF) 1–3A [3]. The best-described polymorphism is CCR5Δ32 in the CCR5 gene, which has been associated with protection from HIV infection and delayed disease progression [4]. Single-nucleotide polymorphisms (SNPs) are the most common type of genetic variations and have been associated with many human diseases [5]. Nonsynonymous SNPs in receptor genes may alter protein folding and, therefore, affect its normal functioning via several mechanisms, including disruption of receptor-ligand binding [6, 7].

CD4 is a membrane glycoprotein that is mainly expressed on T lymphocytes, monocytes, and dendritic cells. Although generally assumed to be nonpolymorphic, the CD4 gene in fact contains a number of synon-
ymon polymorphisms [8, 9], some of which have been used for forensic work [10]. Bach et al. [11] first reported the presence of a common nonsynonymous SNP among people of African descent. The initial identification of the C868T SNP in the CD4 gene stemmed from the observation that some people of African descent had CD4 molecules that were unable to bind to a monoclonal antibody directed at an epitope in the third domain of CD4, called OKT4 [12]. The frequency of the C868T allele is high (20%) among African Americans and is much lower (<1%) among white persons [13].

Sequence analysis of the CD4 gene in individuals whose cells were nonreactive to anti-OKT4 revealed a sequence that is identical to normal CD4 except for a cytosine-to-thymidine substitution at nucleotide position 868 [14]. This substitution results in an amino acid change from arginine to tryptophan in residue 240 within the third domain of CD4. Therefore, C868T encodes a protein called CD4-Trp240 [15]. Kyte-Doolittle hydrophobicity analysis has predicted that replacement of the basic arginine residue with the more hydrophobic tryptophan at the center of a β sheet would have a significant effect on the tertiary structure of CD4 [15]. Evidence from CD4 structure and mutagenesis studies have shown that the redox state of a disulfide bond in the second domain of CD4 and the alteration in the third domain of CD4 can have an effect on the binding of HIV gp120 to the first domain of CD4 [16, 17].

It is plausible, therefore, that the C868T polymorphism may affect the tertiary structure of the molecule and alter either the binding of gp120 to CD4 or the normal function of CD4 in the activation of T cells. Either one could have an effect on susceptibility to HIV infection or disease progression. The present study examined the epidemiological association between C868T and susceptibility to HIV-1 infection in a cohort of female commercial sex workers in the Pumwani district of Nairobi, Kenya. The molecular mechanism underlying this association was also investigated using an in vitro T cell system as a model of HIV infection as well as peripheral blood mononuclear cells (PBMCs) from subjects with known CD4 genotypes. We observed that the CD4 C868T polymorphism was significantly associated with HIV-1 prevalence and incidence in this cohort. We also demonstrated that a cell line expressing CD4-Trp240 was more susceptible to infection with HIV-1 than and a Kenyan primary HIV-1 isolate than were cells expressing wild-type CD4. This result was confirmed in ex vivo cultures of PBMCs from subjects with different CD4 genotypes. Taken together, these data suggest that a polymorphism in the CD4 gene that is present at a high frequency in populations of African descent is a significant risk factor for HIV-1 infection.

**METHODS**

**Study subjects.** The present study was performed retrospectively in a cohort of commercial sex workers from the Pumwani district of Nairobi, Kenya. The study was approved by the ethics review committee of the University of Manitoba and the Kenyatta National Hospital. This cohort was established in 1985 to study the risk factors associated with heterosexual transmission of HIV in commercial sex workers [18, 19]. It is an open cohort that enrolls participants every year. Once enrolled, the participants come back twice a year for follow-up. At each visit, blood samples are obtained for HIV-1 serological analysis and determination of CD4 T cell counts; demographic and behavioral data are also obtained. CD4 genotyping was performed on 364 DNA samples obtained from adult females in this cohort, of whom 87 were not infected with HIV-1 and 277 were either infected with HIV-1 at enrollment or subsequently seroconverted. For ex vivo analyses of HIV infection, samples from donors from Winnipeg, Canada, who were at low risk for HIV infection were used. Blood was obtained from each donor for CD4 genotyping only after informed consent had been obtained.

**DNA isolation.** DNA for polymerase chain reaction (PCR) and sequencing procedures was isolated from at least 5 × 10⁶ PBMCs by means of the QIAamp DNA Mini Kit (Qiagen). The procedures were performed in accordance with the manufacturer’s instructions.

**CD4 sequence analysis.** All CD4 sequence genotyping was performed in a blinded fashion, and each run included samples with known CD4 genotypes. DNA samples were used to amplify by PCR the region of the CD4 gene containing nucleotide position 868. An ~300-bp region was amplified using the CD4 sense primer 5’-GGTCTCTCCCTCCCACCTCGCCCTT-3’ and the CD4 antisense primer 5’-CCAGGTTCACTCTTCGATGCAAC-3’. These primers were designed using the published CD4 sequence (GenBank accession number NM000616). The PCR was performed in 50-μL volumes containing 5 U/μL recombinant Taq DNA polymerase (Invitrogen Life Technologies) on a thermocycler (MJ Research), under the following conditions: 94°C for 2 min; followed by 30 cycles of 94°C for 30 s, 64°C for 30 s, and 72°C for 30 s; and final elongation at 72°C for 7 min. The PCR products were then purified using filter devices (Millipore) and resuspended in 20 μL of ddH₂O. Cycle sequencing was then

<table>
<thead>
<tr>
<th>CD4 868 genotype</th>
<th>Negative (n = 87)</th>
<th>Positive (n = 277)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (homozygous wild type)</td>
<td>66 (75.9)</td>
<td>191 (69.0)</td>
</tr>
<tr>
<td>CT (heterozygous)</td>
<td>12 (13.8)</td>
<td>77 (27.8)</td>
</tr>
<tr>
<td>TT (homozygous variant)</td>
<td>9 (10.3)</td>
<td>9 (3.2)</td>
</tr>
<tr>
<td>CT frequency</td>
<td>0.138</td>
<td>0.278</td>
</tr>
<tr>
<td>P for HWE</td>
<td>.012</td>
<td>.861</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated. For the comparison between HIV-1-negative and HIV-1-positive subjects, P = .002 (3 × 2 x² test). HWE, Hardy-Weinberg equilibrium.
performed on these PCR products, using ABI Prism Big Dye Terminator Mix (version 3.0) with AmpliTaq DNA polymerase (Applied Biosystems). Both strands were sequenced for all samples, using the primers 5'-TTCTCCTTCCACTCGCCTTT-3’ (forward sequence) and 5'-TCCTGTTTTCGCTTCAAGGGCC-3’ (reverse sequence). The cycle-sequencing procedure was conducted on a thermocycler (MJ Research), under the following conditions: 96°C for 3 min, followed by 60 cycles of 96°C for 30 s, 50°C for 30 s, and 60°C for 4 min. The samples were precipitated and washed, and the pellets were resuspended in 20 μL of high-grade formamide (Applied Biosystems). The samples were sequenced on an ABI Prism 3100 automated sequencer and analyzed (Applied Biosystems). Heterozygote sequences were identified by the appearance of C and T peaks overlapping at nucleotide position 868, and the sense and antisense sequences had to be concordant.

**PBMC preparation.** Fresh whole blood was obtained from 6 HIV-1–uninfected blood donors, 3 of whom were heterozygous for and 3 of whom were homozygous for the wild-type CD4 genotype. PBMCs were isolated by layering the mixture onto ficoll-hypaque (Bio-Lynx). The PBMC layer was collected after centrifugation. The cells were resuspended to the desired cell concentration in RPMI 1640 containing 10% fetal calf serum.

**Generation of stably transfected CD4 cell lines.** Plasmids encoding wild-type CD4 or CD4-Trp240 were created from cDNA from a heterozygous individual and cloned into pBlue-script, and sequences were confirmed. CD4 genes were cloned into the mammalian expression vector pDEST 12.2 (Invitrogen) via the ligase reaction. Clones were confirmed by DNA sequencing and then used to transfect a CD4-negative T cell line, A2.01, in the presence of Lipofectamine 2000 reagent (Invitrogen). Antibiotic-resistant A2.01 cells expressing CD4 molecules were

---

**Table 2.** Association between epidemiological data and CD4 868T genotype.

<table>
<thead>
<tr>
<th>Category</th>
<th>CD4 868 genotype</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date-dependent variable, mean (SD), year</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>1960.6 (7.0)</td>
<td>1960.9 (6.4)</td>
</tr>
<tr>
<td>Time of beginning commercial sex work</td>
<td>1986.4 (5.5)</td>
<td>1987.1 (5.0)</td>
</tr>
<tr>
<td>Time of cohort enrollment</td>
<td>1990.7 (3.2)</td>
<td>1991.0 (3.1)</td>
</tr>
<tr>
<td>Tribal origin, no.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>106</td>
<td>50</td>
</tr>
<tr>
<td>Group 2</td>
<td>74</td>
<td>24</td>
</tr>
<tr>
<td>Groups 3–18</td>
<td>36</td>
<td>22</td>
</tr>
</tbody>
</table>

<sup>a</sup> P values were calculated by Student’s t test for date-dependent variables and by the χ² test for tribal origin.
selected by means of G418 medium (1 mg/mL). These cells were stained with Leu-3a and anti-OKT4.

Cell lines and viruses. The anti-OKT4 monoclonal antibody was a gift from R. Sekaly. The A2.01 cell line was obtained from J. Hildreth through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health. HIV-1 was obtained from R. Gallo through the AIDS Research and Reference Reagent Program. HIV-1IIIB was obtained from R. Gallo through the AIDS Research and Reference Reagent Program. HIV-1Ba-L was obtained from S. Gartner, M. Popovic, and R. Gallo through the AIDS Research and Reference Reagent Program. The Kenyan primary viral isolates were a gift from A. Land [20]. A vesicular stomatitis virus G (VSV-G)–pseudotyped strain (HIV-1 pNL4.3 Luc+/Env) was donated by X. Yao [21].

Viral stocks and infection. HIV-1IIIB, HIV-1Ba-L, and primary Kenyan HIV-1 isolate ml1956 were grown in phytohemagglutinin-stimulated PBMCs from HIV-1–seronegative blood donors. The cells were cultured for 10 days before the culture supernatant was harvested and frozen at −80°C. TCID50 was calculated by the method of Reed and Muench [22]. For the infection assay, 2 × 10^6 A2.01 cells expressing similar levels of either CD4 isoform were resuspended in 1 mL of RPMI 1640 containing 10% fetal calf serum and incubated at 37°C for 3 h with either HIV-1IIIB at an MOI of 0.001 or ml1956 virus at an MOI of 0.01. After virus adsorption, the cells were washed, resuspended, and incubated at 37°C for 6 days. Culture supernatants and cells were harvested on days 1, 3, and 6, and HIV-1 infection was determined by p24 ELISA. PBMCs from HIV-1–uninfected donors were infected with HIV-1Ba-L by a similar method.

Infection of cells expressing different CD4 isoforms with a VSV-G–pseudotyped strain. The VSV-G–pseudotyped strain HIV-1 pNL4.3 Luc+/Env (which consists of HIV-1 as backbone but has the HIV env gene replaced with the VSV env gene and the HIV nef gene replaced with the VSV luciferase gene) was used to...
infect cells at 2 cpm/cell. The cultures were incubated at 37°C for 3 h, washed, aliquoted in a 96-well plate, and incubated at 37°C for 48 h. Infection with HIV-1 was determined by p24 ELISA and by luciferase assay.

**Luciferase assay.** HIV-1–infected cells were harvested at 48 h and lysed with 100 μL of lysis buffer. Cell lysates were resuspended in 100 μL of luciferase substrate buffer (Promega), and the reaction was read using a microplate luminometer (EG&G Berthold).

**Statistical analysis.** The χ² test and Fisher’s exact test were used for comparisons of categorical variables. Kaplan-Meier plots were generated to assess the difference in time to seroconversion between individuals with and those without CD4 C868T; the log-rank and Wilcoxon tests were applied. Logistic regression multivariate analysis was also performed for comparisons between groups. P < .05 was considered to indicate statistical significance.

### RESULTS

**Association between CD4 C868T and HIV-1 prevalence.** To determine whether the presence of the CD4 C868T allele correlated with HIV-1 prevalence, 364 CD4 genotyped samples were analyzed (table 1). The prevalences of the CD4 C868T allele among individuals possessing at least 1 copy of CD4 868T were 24.1% and 31.0% in the HIV-1-negative and HIV-1-positive groups, respectively. When the 2 groups were compared with respect to CD4 genotype status, the CD4 C868T allele was associated with a higher prevalence in the HIV-1–positive group (P = .002, χ² test).

**Association between CD4 C868T and HIV-1 incidence.** The higher frequency of the CD4 C868T variant among HIV-1–positive subjects suggested that a study of HIV incidence was warranted. Kaplan-Meier analysis (figure 1) was performed on data from a subset of the HIV-1–infected group (n = 48) for whom the time to seroconversion was known. Because only 3 individuals homozygous for CD4 868T had available data on seroconversion, they were not plotted on the graph. The analysis showed that heterozygous subjects seroconverted sooner than those with the wild-type CD4 allele (P = .005, log-rank test; P = .009, Wilcoxon test). The mean time to seroconversion for individuals with the wild-type allele was 2889 days (7.92 years), compared with 1236 days (3.39 years) for heterozygous individuals. These results were confirmed by logistic regression analysis, which showed that the CD4 868T state was associated with seroconversion (odds ratio, 2.49 [95% confidence interval, 1.26 – 4.91]; P = .009).

**Confounding variables.** To eliminate the effects of possible confounders, time of cohort enrollment, time of beginning commercial sex work, and tribal origin were compared between CD4 genotypes (table 2). None of the potential confounding factors were shown to be correlated with CD4 genotype.

**Association between CD4 C868T and in vitro susceptibility to HIV-1 infection.** To investigate the molecular mechanism underlying the association between CD4 C868T and susceptibility to HIV-1 infection, an in vitro infection model was used. A significantly higher amount of p24 was produced by HIV-1 Env–infected cells expressing CD4-Trp240 than by HIV-1 Env–infected cells expressing wild-type CD4 (for day 3, P = .026; for day 6, P < .001) (figure 2). Similar results were found for cells infected with the Kenyan primary HIV-1 isolate—cells expressing CD4-Trp240 produced significantly higher levels of p24 than did cells expressing wild-type CD4 (P = .001) (figure 3). These results suggest that cells expressing CD4-Trp240 are more susceptible to HIV-1 infection than are cells expressing wild-type CD4.
Assessment of the ability of the A2.01 cell line to support HIV-1 infection independently of CD4 isoform. The difference in susceptibility to HIV-1 infection seen between cells expressing different CD4 isoforms could be due to differences in the CD4 molecules or to other factors associated with the cell line used. To investigate the source of the difference, pseudotyped HIV-1 that infects cells independently of CD4 isoform was used for infection. Results from both p24 ELISA and the luciferase assay indicated no statistically significant differences in susceptibility to HIV-1 infection between the 2 CD4 isoforms, suggesting that cells expressing CD4-Trp240 are more susceptible to HIV-1 infection (figure 4).

Association between CD4 C868T and ex vivo susceptibility to HIV-1 infection. Although in vitro experiments are excellent for testing the effect that CD4-Trp240 has on cellular susceptibility to HIV infection, ex vivo assays using PBMCs isolated from human subjects better represent the natural setting. To investigate whether CD4-Trp240 is associated with susceptibility at the ex vivo level, PBMCs obtained from donors were infected with HIV-1 without prestimulation. A significantly higher amount of HIV-1 p24 was produced by PBMCs from donors with CD4 C868T than by PBMCs from donors with wild-type CD4, suggesting that individuals with this CD4 isoform are more susceptible to HIV-1 infection than are those with the wild-type isoform \( (P = .003) \) (figure 5).

DISCUSSION

The finding that a polymorphism in an HIV-1 receptor is involved in altering susceptibility to infection is not surprising, given that it has been shown for a number of other polymorphisms \[2, 3, 23–27\]. However, the present study is the first to demonstrate that a SNP in the primary HIV receptor, CD4, is associated with increased susceptibility to HIV-1 infection at the population level as well as in in vitro and ex vivo models.

This study found a correlation between the presence of CD4 C868T and the prevalence of HIV-1 infection among highly exposed commercial sex workers from Nairobi, Kenya. Given that prevalence is defined as the proportion of persons with a particular disease in a given population and that incidence is defined as the number of new cases that occur during a specified period in a given population, the presence of CD4 C868T should correlate with both HIV-1 incidence and prevalence. Because an incidence study measures the earliest indication of disease, incidence would more accurately reflect the association between the CD4 C868T variant and HIV disease. Therefore, the high incidence of HIV infection (figure 1) among subjects with CD4 C868T supports the role it plays in the increase in susceptibility to HIV-1 infection.

The strength of an association between a SNP and a disease is dependent on the sample size achieved. In this study, a sample size of 364 women was used. The study size was large enough to reveal effects generated by this particular genetic polymorphism. Cohorts of smaller and similar sizes have been used to reveal the prognostic values of many polymorphisms with allele frequencies similar to the frequency of CD4 C868T, including the CCR5Δ32, CX3CR1, and MDR-1 variants as well as IL4-589T and SDF1–3′A \[24, 27–29\]. For example, the allele frequencies in some of these populations were 0.044–0.14 for CCR5Δ32 and 0.21 for SDF1–3′A \[30\], whereas CD4 C868T has an allele frequency of 0.17 in our cohort. The study cohort as a whole was found to be in Hardy-Weinberg equilibrium for CD4 C868T, the prevalence of HIV-1 infection among highly exposed commercial sex workers from Nairobi, Kenya. Given that prevalence is defined as the proportion of persons with a particular disease in a given population and that incidence is defined as the number of new cases that occur during a specified period in a given population, the presence of CD4 C868T should correlate with both HIV-1 incidence and prevalence. Because an incidence study measures the earliest indication of disease, incidence would more accurately reflect the association between the CD4 C868T variant and HIV disease. Therefore, the high incidence of HIV infection (figure 1) among subjects with CD4 C868T supports the role it plays in the increase in susceptibility to HIV-1 infection.

The strength of an association between a SNP and a disease is dependent on the sample size achieved. In this study, a sample size of 364 women was used. The study size was large enough to reveal effects generated by this particular genetic polymorphism. Cohorts of smaller and similar sizes have been used to reveal the prognostic values of many polymorphisms with allele frequencies similar to the frequency of CD4 C868T, including the CCR5Δ32, CX3CR1, and MDR-1 variants as well as IL4-589T and SDF1–3′A \[24, 27–29\]. For example, the allele frequencies in some of these populations were 0.044–0.14 for CCR5Δ32 and 0.21 for SDF1–3′A \[30\], whereas CD4 C868T has an allele frequency of 0.17 in our cohort. The study cohort as a whole was found to be in Hardy-Weinberg equilibrium for CD4 C868T, whereas the HIV-1-negative group was found to be in disequilibrium when the population was divided by HIV status \( (P = .012) \). It is possible that this disequilibrium was due to an
enrichment of the less-susceptible CD4 C868C genotype in the HIV-1–negative group, which was highly exposed to HIV through commercial sex work.

There are several mechanisms that could explain the role played by CD4 C868T in the increased susceptibility to HIV-1 infection. First, it is possible that those with and those without the variant differed in their exposure to HIV or in their genetic background. Given that the risk of HIV acquisition is dependent on several factors—including condom use and HIV prevalence among clients, which varied as a function of time over the 18 years of the study—we sought to determine whether there were differences in the study subjects at the level of exposure to HIV. We observed no differences in age, duration of being a commercial sex worker, or time of enrollment into the cohort, suggesting similar levels of exposure (table 2). We also observed no association between the CD4 variant and any particular tribal group, suggesting that it is not simply a marker of ethnic genetic variation. The lack of association with tribal origin also discounts any tribal differences in sex practices or exposure to HIV that could account for the association with HIV incidence. Second, demographic or behavioral variations could explain the differences in susceptibility to HIV infection. To date, however, we have found no demographic or behavioral link with the CD4 C868T polymorphism [18].

Although our epidemiological data showed a strong association between CD4 C868T and increased susceptibility to HIV-1 infection, further investigations were warranted to dissect the molecular mechanism behind this association. Using in vitro and ex vivo models, we have shown that cell lines expressing CD4-Trp240 were more susceptible to HIV-1 infection than were cells expressing wild-type CD4. At the cellular level, susceptibility to HIV infection is dependent on 2 things, namely, (1) factors associated with virus entry and (2) host factors associated with virus replication. Regarding the first set of factors, because HIV must bind to CD4 and a chemokine receptor before entering into a cell, a higher binding affinity to CD4 could greatly enhance its entry. Therefore, a nonsynonymous SNP in CD4 could alter the binding affinity to HIV gp120, allowing HIV to infect these cells more efficiently.

Nonsynonymous SNPs in receptor genes that can alter the receptor functions (including binding affinity to their ligands) have been reported before. The A118G SNP found in the μ-opioid receptor gene has been reported to result in a receptor protein that binds with higher affinity to its ligand, β-endorphin [31–33]. Other relevant examples include SNPs found in genes encoding the HIV coreceptors and their ligands. In some studies, M280—an allele with 2 nonsynonymous SNPs found in CX3CR1—has been reported to be associated with the risk of HIV infection [33, 34]. That nonsynonymous SNPs in receptor genes have been described and found to alter the binding affinity to a ligand supports the possibility that CD4 C868T alters the binding affinity to gp120. However, further studies are required to confirm this hypothesis.

Regarding host factors associated with virus replication, the increased susceptibility to HIV-1 infection that results from CD4 C868T could be due to an alteration in the function or functions of one of the host cellular proteins required for HIV-1 replication. An alteration in the function of such a protein could be due to a change in signals transduced through the interaction between CD4-Trp240 and gp120. One of the proteins that this interaction could affect is p56lck. Signaling through the CD4 molecule plays an important role in the T cell receptor signaling pathway, which is responsible for the activation state of a T cell. Given that HIV preferentially infects activated CD4 T cells, if the expression of CD4-Trp240 affects the cellular activation state, it could create more target cells for HIV. Further work investigating this hypothesis is required.

It is also possible that CD4 C868T may not be the true cause of the effects observed in the present study but, rather, is in linkage disequilibrium with the actual predisposing allele, much like the haplotype of CCR2b-64I and CCR5 promoter alleles [35]. Although findings from the in vitro model used in the present study support the concept that the altered CD4 protein has a direct effect, further studies are required to address the nature of the mechanism and to determine whether it is functionally independent of other unidentified factors.

In conclusion, this study has demonstrated a strong association between the genetic marker CD4 C868T and the increased incidence and prevalence of HIV-1 infection. It has also shown that cells expressing CD4-Trp240 are more susceptible to HIV-1 infection than are cells expressing wild-type CD4. Given the high prevalence of both HIV infection and CD4 C868T among African populations, the effect of this SNP on the epidemic in Africa could be dramatic. Studies in other cohorts that have differing modes of HIV transmission and ethnic backgrounds are warranted. A detailed study of the CD4 C868T polymorphism and the role it plays in enhancing susceptibility to infection could help to further elucidate the mechanism of HIV-1 entry, in particular the complex interaction among gp120, CD4, and the coreceptors. This information is important for understanding current CCR5 therapies and for the development of new antiretroviral drugs.

Acknowledgments

We thank Drs. Jane Evans and Teresa Zelinski for critical review of the manuscript, Sue Ramadhani for technical assistance, and Mary Cheang for help in statistical analysis.

References


CD4 Polymorphism and HIV-1 Infection • JID 2009:199 (1 May) • 1333