Journal of Medical Virology 64:398–401 (2001)

Lack of Effect of Chemokine Receptor CCR2b Gene Polymorphism (64I) on HIV-1 Plasma RNA Viral Load and Immune Activation Among HIV-1 Seropositive Female Workers in Abidjan, Côte d'Ivoire

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The prevalence of the CCR2b–V64I mutation among human immunodeficiency virus (HIV)-seropositive and -seronegative female workers and the potential effect of heterozygosity of this mutation on HIV-1 plasma RNA viral load and markers of immune activation were assessed. CCR2b–V64I was detected by polymerase chain reaction, followed by restriction enzymes analysis; plasma viral load was measured by the Amplicor HIV-1 monitor assay and $CD4^+$ T-cell counts and markers of immune activation by standard three-color FACscan flow cytometry. Of the 260 female workers, 56 (21.5%) were heterozygous for CCR2b–V64I, and 8 (3%) were homozygous. Of the 99 HIV-seronegative female workers, 19 (19.2%) were heterozygous for the CCR2b–V64I mutation compared with 37 (23%) of the 161 HIV-seropositive FSW ($P = 0.47$). In a univariate analysis of viral load among HIV-seropositive FSW, no difference was noted between those heterozygous for or without the mutation; both groups had plasma viral loads of 5.0 $\log_{10}$ copies/ml. After controlling for the effects of $CD4^+$ T-cell counts in a multivariate analysis, no significant difference was observed between the groups in viral load or in markers of immune activation. The data suggest that the presence of the CCR2b mutation has no effect on HIV-1 plasma viral load and markers of immune activation in our study population. The finding that the frequency of this mutation is similar in HIV-seropositive and -seronegative female workers suggests that its presence is not associated with increased risk of HIV infection. ***J. Med. Virol. 64:398–401, 2001.***

KEY WORDS: CCR2b; HIV-1; viral load; immune activation

INTRODUCTION

Recently, chemokine receptors have been shown to act as co-receptors for human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) during viral entry into target cells. A 32-base pair (bp) deletion mutation (Δ32) in the gene encoding the CCR5 receptor protects from HIV infection and delays the onset of AIDS and death in Caucasian populations [Dean et al., 1996; Huang et al., 1996]. A mutation that leads to the substitution of an isoleucine by a valine at position 64 in the first transmembrane region of the CCR2b receptor (CCR2b–V64I) gene has also been reported [Smith et al., 1997]. Depending on ethnic group, about 10–25% of the general population possess the latter mutation, but its role in protecting against HIV infection or delaying disease progression is controversial. Some studies have shown that the CCR2b–V64I mutation can lower the risk of progression to AIDS [Smith et al., 1997, Kostrisis et al., 1998; Van Rij et al., 1998]. In a Kenyan study of HIV-seropositive female workers, the frequency of the CCR2b–V64I mutation was significantly higher among female workers whose infection progressed slowly to AIDS compared with those whose infections progressed rapidly [Anzala et al., 1998],

Grant sponsor: Division of HIV/AIDS Prevention, National Center for HIV, STD, and TB Prevention; Grant sponsor: Centers for Disease Control and Prevention, Atlanta, GA.

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Accepted 6 November 2000

however, others studies have failed to demonstrate any protective effect of CCR2b–V64I mutation on disease progression [Schinkel et al., 1999; Daar et al., 1999; Eugen-Olsen et al., 1998; Michael et al., 1997] or on markers of disease progression [Eugen-Olsen et al., 1998; Michael et al., 1997]. One study in Scotland even suggested that heterozygosity for CCR2b–V64I mutation was a risk factor for heterosexual transmission of HIV infection to women [Lockett et al., 1999].

Very limited data exist on the prevalence and role of CCR2b–V64I in HIV infection in Africa. We assessed whether this mutation is more frequent among HIV-seronegative than seropositive female workers in Abidjan, and whether it has any effect in the seropositive female workers on markers of disease progression: HIV-1 plasma RNA viral load, $CD4^+$ T-cell counts, and cellular markers of immune activation.

MATERIALS AND METHODS

Between September 1996 and June 1997, consenting female workers were enrolled consecutively at a confidential clinic in Abidjan. Whole blood was collected into EDTA tubes (Becton Dickinson, San José, CA) containing sodium citrate gel and density media. Within 6 hr of collection, plasma was separated from cells by centrifugation at 200*g*, aliquots were stored at −70°C. HIV-1 antibody status was determined by a testing algorithm based on a combination of enzyme-linked immunosorbent assay (ELISA) [Nkengasong et al., 1998, 1999]. HIV-1 RNA viral load was quantified in plasma by the Amplicor HIV-1 Monitor test v1.5 (Roche Diagnostic System, Branchburg, NJ). All assays were carried out as recommended by the manufacturers. A three-color flow cytometric measurement by FACscan flow cytometer (Becton Dickinson, San Jose, CA) was carried out on fresh peripheral whole blood within 4 hr of blood collection. Aliquots of cells were stained with commercially available monoclonal antibodies (Becton Dickinson). The markers analyzed were $CD4^+$, CD8, CD25, CD38, CD45RO, CD4RA, and HLA-DR. CD25, CD38, and HLA-DR were used to quantify cellular activation, and CD45RO and CD45RA were used to quantify memory and naïve cells, respectively.

For CCR2b–V64I genotyping, genomic DNA was analyzed by use of polymerase chain reaction (PCR) with primers CCR2-F3 (forward, position 170–187 in CCR2b, 5′-ATGCTGTCCACATCTCGTTC-3′) and CCR2-1Z (antisense, position 279–298 in CCR2b, 5′-GAGCCCACAATGGGAGAGTA-3′). Samples were amplified with 2.5 U of *Taq* polymerase in $1 \times$ PCR buffer (Perkin–Elmer, Roche Diagnostics Systems, Branchburg, NJ) with a final $MgCl_2$ concentration of 1.5 mM. PCR conditions for CCR2b genotyping were 10 min of denaturation at 94°C; 35 cycles of 1 min at 94°C, 1 min at 59°C, 10 min at 72°C,and 10 min of elongation at 72°C in a thermocycler (Perkin–Elmer Gene Amp PCR system 9600). After PCR, the products were cleaved enzymatically with the FokI (New England Bio Labs, MA) for 2 hr at 37°C and run on a 2% agarose gel.

A simple comparison of two groups was carried out by the nonparametric Mann-Whitney or Kruskal-Wallis test. A multiple linear regression for each variable corrected for CD4 values was performed using the STATA program. The level of significance for all analyses was set at $P = 0.05$.

RESULTS

Characteristics of the Study Population

Of the 260 female workers, 161 (61.9%) were HIV-seropositive (119 HIV-1, 3 HIV-2, and 39 dually seropositive for HIV-1 and HIV-2) and 99 (38%) were HIV-seronegative. Median age was 27 years (interquarile range [IQR], 21–33). Among the 151 seropositive female workers with available $CD4^+$ T-cell counts and viral load measurement, the median $CD4^+$ T-cell count was 703 (IQR, 395–960); 16 (10.6%) had cell counts < 200 cells/μl, 39 (25.8%) had cell counts of 200–499, and 96 (63.6 %) had cell counts > 499 cell/μl. Their median viral load was 5.0 $\log_{10}$ copies/ml (IQR, 4.0–5.0).

Prevalence of CCR2b–V64I Mutation and Effect on Plasma RNA Viral Load and Markers of Immune Activation

Of the 260 female workers, 56 (21.5%) were heterozygous, and 8 (3%) were homozygous for *CCR2b–V64I* mutation. Nineteen (19.2%) of the 99 HIV seronegative female workers were found to be heterozygous for CCR2b–V64I, compared with 37 (23%) of 161 seropositive female workers ($P = 0.47$). Plasma viral load levels and markers of immune activation among HIV seropositive female workers were then examined to determine whether heterozygosity for CCR2b–V64I-affected markers of disease progression. In a univariate analysis, median plasma viral load was 5.0 $\log_{10}$ copies/ml among the 37 (23%) female workers heterozygous for the mutation and among the 117 (73%) without the mutation. Viral load was 4.0 $\log_{10}$ copies/ml among female workers ($n = 5$) who were homozygous for the mutation compared with those who were heterozygous (5.0 $\log_{10}$ copies/ml) or without any mutation (5.0 $\log_{10}$ copies/ml)($P = 0.21$). The median $CD4^+$ T-cell count was 715 (IQR, 366–1,047) for the 32 female workers heterozygous for the mutation, compared with 680 (IQR, 398–948) among the 114 female workers without the mutation ($P = 0.83$). In a multivariate analysis controlling for $CD4^+$ T-cell counts, no significant differences in viral load or markers of immune activation were observed between the groups (Table I).

DISCUSSION

In Abidjan, the prevalence of the CCR2b–V64I mutation was similar among HIV-seronegative and -seropositive female workers; however, heterozygosity for this mutation did not affect plasma viral load and immune activation markers. Limited studies have been

TABLE I. Effect of CCR2641 Mutation on Viral Load, CD4 Cell Count, and Cellular Parameters of Immune Activation in HIV-1-Infected Female Workers in Abidjan, Côte d'Ivoire

			Median percentages of activation markers				
			$CD8^+$ T cells (n)			CD^+ T cells (n)	
	CD4 cell counts (cells/μl)	Viral load (log_{10} copies/ml)	$HLA\text{-}DR^+$	$CD45RA^-$	$CD38^+$	$CD45RO^+$	$HLA\text{-}DR^+$
CCR2 (wild-type)	680 (114)	5.0 (117)	45 (119)	53 (119)	94 (119)	67 (119)	30.5 (119)
CCR2641	715 (32)	5.0 (37)	45.5 (32)	49 (32)	91.5 (32)	67.5 (32)	30.1 (32)
P-value	0.83	0.34	0.79	0.72	0.49	0.83	0.83

n=number of patients analyzed.

published on the role of the CCR2b–V64I mutation in HIV infection in Africa. The 21.5% heterozygosity for CCR2b–V64I found in our study is similar to that reported among female workers both in Kenya [Anzala et al., 1998] and in Europe [Lockett et al., 1999] and the United States [Michael et al., 1997]. By investigating in detail markers of immune activation, we confirm and extend other cross-sectional studies that failed to correlate the presence of CCR2b–V64I and markers of disease progression [Eugen-Olsen et al., 1998; Michael et al., 1997].

These findings are at variance with some cross-sectional and prospective studies that have shown a protective effect of CCR2b–V64I in markers of disease progression [Smith et al., 1997; Kostrisis et al., 1998]. Discrepancies between these results are possibly attributable to differences in study design; it has been proposed that the protective effect of this mutation is masked unless seroincident, rather than seroprevalent, cohorts are studied [Smith et al., 1997; Kostrisis et al., 1998; Michael et al., 1997; Smith et al., 1997]. Moreover, any beneficial effect of this mutation could be minimized by the very high immune activation status observed among both HIV-positive and HIV-negative female workers (Table I). Immune activation is critical in HIV pathogenesis in that it may increase numbers of available activated $CD4^+$ T cells that serve as susceptible targets for HIV in vivo and thus can increase plasma HIV-1 RNA viral load levels [Fauci 1996].

Lockett et al. [1999] reported a surprising finding that the presence of CCR2b–V64I mutation was a risk factor for heterosexual transmission of HIV-1 to women in Scotland. However, in our study, we did not find any difference in the prevalence of CCR2b–V64I among HIV-positive and HIV-negative female workers. Prospective studies are needed to gain further insights into the role of CCR2b–V64I mutation in an African population.

In summary, heterozygosity for CCR2b–V64I mutation was not found to be a risk factor for HIV infection in female workers in Abidjan, and it appeared to have no effect on viral load and the markers of immune activation in this population.

ACKNOWLEDGMENTS

The authors thank Kabran Nguessan, Emmanuel Boateng, Marie-Yolande Borget, and Maran Mathieu for technical assistance.

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