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CONCISE COMMUNICATIONS

Reduced Prevalence of the CCR5 Δ 32 Heterozygous Genotype in Human Immunodeficiency Virus–Infected Individuals with AIDS Dementia Complex

Ronald P. van Rij,¹ Peter Portegies,² Teysir Hallaby,³
 Joep M. A. Lange,³ Janny Visser,¹
 Ana-Maria de Roda Husman,¹
 Angélique B. van 't Wout,^{1,a} and Hanneke Schuitemaker¹

¹Department of Clinical Viro-Immunology, CLB, and the Laboratory of Experimental and Clinical Immunology of the University of Amsterdam; ²Department of Neurology and ³National AIDS Therapy Evaluation Centre (NATEC), Academic Medical Centre, Amsterdam, The Netherlands

Heterozygosity for a 32-bp deletion in the CCR5 gene (CCR5 Δ 32), which encodes the coreceptor for macrophage-tropic non-syncytium-inducing (NSI) human immunodeficiency virus type 1 (HIV-1) variants, results in a lower CCR5 expression and reduced NSI HIV-1 replication. Because infection of macrophages and microglial cells by NSI HIV-1 is considered to be instrumental for the development of AIDS dementia complex (ADC), we studied whether the CCR5 Δ 32 heterozygous genotype correlated with a reduced frequency of ADC. Two (4.1%) of 49 patients with ADC versus 27 (14.5%) of 186 AIDS patients without ADC were heterozygous for CCR5 Δ 32 ($P = .05$). In contrast, a point mutation in the first transmembrane domain of CCR2 (CCR2 64I) did not show this protective effect ($P = .57$). The reduced prevalence of the CCR5 Δ 32 allele among patients with ADC may indicate a reduced or absent reservoir of macrophage-tropic NSI HIV-1 in the brain of CCR5 Δ 32 heterozygotes.

Chemokine receptors have been identified as coreceptors for entry of human immunodeficiency virus type 1 (HIV-1) in human CD4⁺ T lymphocytes and monocytes. CC chemokine receptor (CCR) 5 is the principal coreceptor for macrophage-tropic, non-syncytium-inducing (NSI) HIV-1 variants, whereas syncytium-inducing (SI) HIV-1 can alternatively or in addition use CXC chemokine receptor 4 (CXCR4). Other receptors, including CCR2, CCR3, CCR8, and a variety of orphan receptors, can also serve as coreceptors, although only for a minority of HIV-1 variants (reviewed in [1]).

Heterozygosity for a 32-bp deletion in the CCR5 coding region (CCR5 Δ 32), which occurs in approximately 18% of the Caucasian population, has been associated with a reduced cell surface expression of CCR5, impaired NSI HIV-1 replication

in vitro [2], and a reduced virus load in vivo [3, 4]. Most likely as a consequence of these observations, CCR5 Δ 32 heterozygosity correlates with a delayed progression to AIDS [1, 3, 4] (and references therein). More recently, a valine-to-isoleucine switch in the first transmembrane domain of CCR2 (64I) was also shown to be associated with a delay in HIV-1 disease progression [5–7].

Infection of brain macrophages and microglial cells is considered to be instrumental in the development of AIDS dementia complex (ADC). These cells express several chemokine receptors but mainly support the infection of macrophage-tropic, NSI HIV-1 variants [8]. Zidovudine, which preferentially inhibits NSI, but not SI, HIV-1 replication [9], has a preventive and therapeutic effect on ADC [10]. These findings may imply that NSI HIV-1 infection in the brain plays an important role in the development of ADC.

Peripheral blood mononuclear cells (PBMC) from CCR5 Δ 32 heterozygous individuals show a reduced capacity to replicate macrophage-tropic NSI HIV-1 variants, compared with cells from CCR5 wild-type homozygous individuals [2]. In addition, CCR5 expression levels on monocytes/macrophages correlate positively with the infectability of NSI HIV-1 strains [11]. Therefore, we hypothesized that CCR5 Δ 32 heterozygosity may protect against the development of ADC and anticipated a reduced prevalence of the CCR5 Δ 32 heterozygous genotype in patients with ADC.

Methods

Study population. A case-control study was designed, consisting of 49 AIDS patients diagnosed with ADC between 1984 and

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

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^a Current affiliation: Department of Microbiology, University of Washington, School of Medicine, Seattle (A.B.vW.).

Reprints or correspondence: Hanneke Schuitemaker, Dept. of Clinical Viro Immunology, CLB, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands (J_Schuitemaker@clb.nl).

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1995 and a control group consisting of 186 patients who died of AIDS without developing ADC. Patients from both the case and control groups were either attending the Academic Medical Centre AIDS clinic or were homosexual participants of the Amsterdam Cohort Studies on AIDS. None of the patients received triple therapy. The characteristics of the groups are shown in table 1. Median survival after AIDS diagnosis in the control group was significantly longer than the median time to develop ADC after AIDS diagnosis ($P = .005$, Mann Whitney U test), indicating that the survival of the control group could have been long enough to develop ADC.

CCR2 and CCR5 genotyping. The CCR2 and CCR5 genotypes of the subjects were determined by a polymerase chain reaction (PCR)-restriction fragment length polymorphism method and a PCR-based method, respectively, as described elsewhere [3, 7].

Isolation of biological virus clones and determination of coreceptor usage. HIV-1 biological clones were isolated by cocultivation of patient cells with healthy donor, phytohaemagglutinin-stimulated PBMC under limiting diluting conditions, as described elsewhere [12]. Coreceptor usage was tested by use of human astrogloma U87 cells, stably expressing CD4 and one of the HIV-1 coreceptors CCR1, CCR2, CCR3, CCR5, or CXCR4. All coreceptor encoding cell lines were maintained in Iscove's medium supplemented with 10% fetal calf serum, 5 $\mu\text{g}/\text{mL}$ polybrene, 100 U/mL penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin, and 1 $\mu\text{g}/\text{mL}$ puromycin and selected regularly with 200 $\mu\text{g}/\text{mL}$ g418 for CD4 expression.

Of each cell line, 10^2 cells/well were seeded in a 96-well plate and after 24 h were inoculated with at least 10^2 TCID₅₀ per isolate in a total volume of 200 μL . After 24 h, the cells were washed twice with PBS to remove unbound virus and incubated in 200 μL of medium. At day 7, the cells were transferred to 24-well plates and maintained in 1 mL of fresh medium. Supernatant was collected at 7, 14, 21, and 28 days after infection, and virus replication was analyzed by a p24 antigen-capture ELISA.

All cell lines expressed CD4 on the cell surface and CCR1, CCR2, CCR3, CCR5, and CXCR4 expression on the corresponding U87 cell lines was confirmed by flow cytometry or reverse transcription-PCR (data not shown). A U87 cell line stably expressing CD4, but no HIV-1 coreceptor, was included in all experiments as a control. This cell line was maintained in the same medium as the coreceptor encoding cell lines but without puromycin. None of the tested HIV-1 virus clones was able to infect the control cell line (data not shown).

Results

The prevalence of the CCR5 $\Delta 32$ genotype was investigated among 49 individuals diagnosed with ADC and 186 AIDS patients who never developed ADC. Characteristics of the study population are presented in table 1. In the non-ADC group, 27 individuals (14.5%) were CCR5 $\Delta 32$ heterozygotes. A significantly lower prevalence of the CCR5 $\Delta 32$ heterozygous genotype was observed among the group of patients with ADC (2 individuals, 4.2%; $P = .05$, χ^2 test). CCR5 $\Delta 32$ heterozygosity was associated with protection against the development of ADC, with an odds ratio of 0.25 (95% confidence interval, 0.06-1.09). In contrast, the prevalence of the CCR2 64I heterozygous genotype was not significantly different among the ADC group and the non-ADC control group (14.3% and 11.3%, respectively; $P = .57$, χ^2 test). Because of the strong protective effect of zidovudine on the development of ADC, the control group may contain patients who, if untreated, would have developed ADC. However, excluding patients with a history of zidovudine treatment ($n = 48$; 13.3% CCR5 $\Delta 32$ heterozygotes) from the control group did not change the outcome of our study (data not shown).

Two individuals developed ADC despite a CCR5 $\Delta 32$ heterozygous genotype. We reasoned that these individuals might carry viruses with an altered coreceptor usage, thereby circumventing the reduced expression of CCR5. We analyzed coreceptor usage of biological virus clones isolated from these patients and 2 ADC patients with a CCR5 wild-type homozygous genotype. In a previous study, we showed that HIV-1 variants isolated from the last available peripheral blood samples closely resembled the variants isolated postmortem from various non-lymphoid tissues, including the brain and cerebrospinal fluid (CSF) [12]. Therefore, to optimally reflect the virus variants present in the brain and CSF at the onset of ADC, we isolated virus from the PBMC sample as close as possible to ADC diagnosis. As shown in table 2, all 4 patients, irrespective of their CCR5 genotype, carried NSI HIV-1 variants that exclusively used CCR5 for entry. An SI HIV-1 variant, which was

Table 1. Characteristics of the study population.

Characteristic	ADC patients (case) ($n = 49$)	Non-ADC AIDS patients (control) ($n = 186$)	P
AIDS diagnosis (calendar year) [median (range)]	May 1987 (7/84-10/95)	Apr 1989 (3/85-11/95)	—
Survival after AIDS (mo.) [median (range)]	15.3 (0.7-74.8)	11.8 (0.1-80.6)	.08 ^a
ADC diagnosis after AIDS (months) [median (range)]	6.7 (0.0-50.6)	NA	.005 ^{a,b}
CD4 count at AIDS (CD4 cells/ μL) [median (range)] ^c	155 (10-540)	105 (10-720)	.23 ^a
CCR2 64I/+ prevalence [n (%)]	7 (14.3)	21 (11.3)	.57 ^d
CCR5 $\Delta 32$ /+ prevalence [n (%)]	2 (4.1)	27 (14.5)	.05 ^d

NOTE. ADC, AIDS dementia complex; NA, not applicable; CCR, CC chemokine receptor.

^a Case and control groups were compared by use of the Mann Whitney U test.

^b Time to develop ADC after AIDS diagnosis among the cases was compared with survival after AIDS diagnosis in the control group.

^c Patients were excluded when CD4 counts were not available within 6 months to AIDS diagnosis.

^d Case and control groups were compared by use of the χ^2 test.

Table 2. Coreceptor usage of biological human immunodeficiency virus type 1 clones obtained from AIDS dementia complex (ADC) patients with the CC chemokine receptor 5 (CCR5) wild-type homozygous and $\Delta 32$ heterozygous genotypes.

Patient	Time after AIDS (months)	Time after ADC (months)	No. of clones obtained	No. of clones tested	Coreceptor designation
CCR5 +/+ ^a					
ADC-2	33	0	26	15	R5, R5X4
ADC-4	9	0	2	2	R5
CCR5 $\Delta 32$ /+ ^b					
ADC-25	34	0	39	15	R5
ADC-26	13	12 ^c	10	10	R5

^a CCR5 +/+, CCR5 wild-type homozygote.

^b CCR5 $\Delta 32$ /+, CCR5 $\Delta 32$ heterozygote.

^c From this patient, no samples closer to the diagnosis of ADC were available.

isolated from 1 of these individuals, could additionally use CXCR4.

Discussion

We have shown here that CCR5 $\Delta 32$ heterozygosity is associated with protection against the development of AIDS dementia complex. Our findings are in contrast with the findings of Barroga et al. [13], who did not observe a protective effect of CCR5 $\Delta 32$ on the development of neurological disorders in AIDS patients. However, besides patients with ADC, patients with mild, HIV-1-associated cognitive motor disorders were included in that study. These disorders might be secondary to opportunistic infections in the brain (e.g., toxoplasmosis) that are neither direct effects of the presence of HIV-1 in the brain nor indicative for future development of ADC [14].

Two individuals developed ADC despite a CCR5 $\Delta 32$ heterozygous genotype. These patients with ADC, however, did carry NSI HIV-1 variants that exclusively used CCR5 for entry. Although a close resemblance of viral sequences in nonlymphoid tissues and peripheral blood was observed in a previous study [12], we cannot rule out the possibility that brain-derived virus may differ from viral clones obtained from the peripheral blood. Flow cytometry analyses showed that CCR5 expression levels were similar on the CD4⁺ lymphocytes of these patients, compared with cells from healthy CCR5 $\Delta 32$ heterozygotes (data not shown).

Together, these results exclude an altered coreceptor usage and a relatively high CCR5 expression as an explanation for the development of ADC, despite their CCR5 $\Delta 32$ heterozygous genotype. Whether the NSI variants in the patients with heterozygous CCR5 $\Delta 32$ who developed ADC have adapted to the low CCR5 expression by increased affinity for this coreceptor, thus explaining the development of ADC, remains to be established.

The role of CCR3 in microglia infection is still controversial. None of the virus clones studied here had the capacity to replicate in the U87-CCR3 cell line, which may indicate that HIV-1 CCR3 usage is not a prerequisite for the development of ADC.

The underlying mechanism for the protection against ADC is currently unknown. One could argue that the relatively low serum virus load in CCR5 $\Delta 32$ heterozygotes could account for the reduced prevalence of ADC, a clinical feature that has been associated with a high virus load in the CSF [15]. However, no protective effect on the development of ADC was observed for the CCR2 64I mutation, while this genotype has also been associated with a reduced viral burden [5, 6].

Alternatively, protection against ADC associated with CCR5 $\Delta 32$ might be due to a reduced expression of CCR5 and consequently an impaired replication of NSI HIV-1 variants, also in macrophages of CCR5 $\Delta 32$ heterozygotes. This might result in a reduced NSI load in monocytes/macrophages and consequently a reduced transport of NSI HIV-1 into nonlymphoid tissues and/or a lower de novo synthesis of NSI HIV-1 variants by macrophages in these tissues. Both of these mechanisms might result in a smaller macrophage-tropic HIV-1 reservoir in nonlymphoid tissues in CCR5 $\Delta 32$ heterozygotes.

Our findings may have implications for the complete eradication of HIV-1, for which the macrophage-tropic HIV-1 reservoir may be considered one of the major obstacles. Our data suggest that this important viral reservoir seems to be limited in CCR5 $\Delta 32$ heterozygotes; therefore, antiretroviral treatment may be most successful in these patients.

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