Published in final edited form as:
Clin Immunol. 2003 September ; 108(3): 234–240.

CCR5 promoter polymorphism determines macrophage CCR5 density and magnitude of HIV-1 propagation in vitro

Janelle R. Salkowitz[a], **Shannon E. Bruse**[b], **Howard Meyerson**[c], **Hernan Valdez**[d], **Donald E. Mosier**[e], **Clifford V. Harding**[f], **Peter A. Zimmerman**[b,*], and **Michael M. Lederman**[d,*]

[a]Case Western Reserve University, Center for AIDS Research, Department of Medicine, Division of Infectious Diseases, School of Medicine/WG7, 2109 Adelbert Road, Cleveland, OH 44106-4984, USA

[b]Case Western Reserve University, School of Medicine, W147D, The Center for Global Health & Diseases, 2109 Adelbert Road, Cleveland, OH 44106-4983, USA

[c]Department of Pathology, University Hospital of Cleveland, 11100 Euclid Avenue, Cleveland, OH 44106, USA

[d]Case Western Reserve University and University Hospitals of Cleveland, Center for AIDS Research, Department of Medicine, Division of Infectious Diseases, 2061 Cornell (Foley Building), Cleveland, OH 44106-4984, USA

[e]The Scripps Research Institute, Department of Immunology-IMM7, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

[f]Case Western Reserve University, Department of Pathology, Biomedical Research Building, Rm. 925, 2109 Adelbert Road, Cleveland, OH 44106-4943, USA

Abstract

The common CCR5 promoter polymorphism at position –2459 (A/G) has been associated with differences in the rate of progression to AIDS, where HIV-1-infected individuals with the CCR5 –2459 G/G genotype exhibit slower disease progression than those with the A/A genotype. Mechanisms underlying the relationship between these polymorphisms and disease progression are not known. Here through in vitro infection of peripheral blood mononuclear cells obtained from healthy Caucasian blood donors with macrophage-tropic HIV-1 isolates we observed low, medium, and high viral propagation in association with G/G, A/G, and A/A promoter genotypes, respectively. Flow cytometric analysis of unstimulated CD14+ monocytes from these same donors revealed a similar hierarchy of CCR5 receptor density in association with promoter genotypes. Finally, PBMC from persons with the G/G promoter polymorphism produced higher levels of β-chemokines after in vitro stimulation. Thus, the CCR5 –2459 (A/G) promoter polymorphism determines CCR5 expression and predicts the magnitude of HIV-1 propagation in vitro. These findings may provide important insight regarding the regulation of mechanisms that influence the rate of HIV-1 propagation and progression to AIDS.

Introduction

Entry into host cells by the human immunodeficiency virus type 1 (HIV-1) can be supported by the binding of viral envelope glycoproteins to both the CD4 receptor and to a seven-

*Corresponding author. Fax: +1-216-368-4825 (P.A. Zimmerman) or +1-216-844-5523 (M.M. Lederman). MXL6@po.cwru.edu (M.M. Lederman).

transmembrane chemokine coreceptor. The major chemokine coreceptors for HIV-1 are CCR5 for macrophage tropic (R5-tropic) and CXCR4 for T-cell-tropic (X4-tropic) HIV-1 isolates. New HIV-1 infections in humans are established by R5-tropic isolates even though both R5- and X4-tropic forms may coexist in the same inocula [1]. There is reason to propose that alterations in CCR5 expression could influence disease transmission and progression because people with a homozygous deletion of 32 basepairs in the CCR5 gene open reading frame (CCR5 Δ32) do not express CCR5 and are highly resistant to infection with HIV-1 [2–8] and people who are heterozygous for this deletion mutation tend to have both lower levels of plasma HIV-1 RNA and a more favorable course of disease [9,10].

The common polymorphism of the CCR5 promoter –2459 is also assoociated with delayed progression to AIDS in HIV-1 infected homosexual men [11]; HIV-1-infected persons with the CCR5 –2459 G/G polymorphism had slower progression to AIDS than those who had the A/A promoter variant [11–13]. Interestingly, constructs with the G promoter polymorphism had less promoter activity than did constructs with the A- polymorphism when linked to a cholamphenical acetyltransferase (CAT) reporter gene [11]. Nonetheless, the mechanism whereby the –2459 polymorphism affects the course of HIV-1 infection is not clear. Because the CCR5 –2459 promoter single nucleotide polymorphism (SNP) is associated with clinical outcome in HIV-1-infected people [11,14–16] and HIV-1 replication is a key determinant of outcome [17], we asked if these polymorphisms might determine the expression of CCR5 and the magnitude of HIV-1 replication in vitro.

Materials and methods

Study population

PBMC from 18 Caucasiàn healthy laboratory and health care workers not at risk for HIV-1 infection were analyzed for expression of CCR5, for susceptibility to in vitro infection with HIV-1 laboratory strains JR-FL, and for β-chemokine production. Persons with the CCR5 Δ32 allele were excluded from this study. All studies were performed following protocols approved by the University Hospitals of Cleveland Institutional Review Board (IRB).

Cell preparation and culture conditions

Whole blood drawn into sodium heparin-containing tubes (Becton–Dickenson, Mountain View, CA) was either prepared on site or shipped overnight before preparation. Peripheral blood mononuclear cells (PBMC) were purified by Ficoll–Hypaque (Pharmacia, Piscataway, NJ) density sedimentation and were cryopreserved in 90% fetal bovine serum (FBS; Summit Biotechnologies, Fort Collins, CO), and 10% dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO). Frozen PBMC were thawed, washed gently, and cultured overnight in a polypropylene culture tube in complete medium (CM) consisting of RPMI 1640 (BioWhittaker, Inc., Walkersville, MD) supplemented with 20% heat-inactivated FBS, 10 mM Hepes (BioWhittaker), 10 mM L-glutamine (BioWhittaker), 100 U/ml penicillin, and 100 μg/ml streptomycin (BioWhittaker) at 37°C in a humidified 5% CO_2-enriched incubator before functional studies were performed.

DNA preparation and genotyping of CCR5 ORF and CCR5 promoter

One hundred thousand PBMC were pelleted and stored at −70°C. DNA was purified using the QiaAmp Blood Kit (Qiagen, Santa Clairita, CA). PCR amplification was performed in a solution (25 μl) containing 2.5 pmoles of the appropriate positive-strand and negative-strand primer; 67 mM Tris–HCl (pH 8.8); 6.7 mM $MgSO_4$; 16.6 mM $(NH_4)_2SO_4$; 10 mM 2-mercaptoethanol; 100 μM dATP, dGTP, dCTP, and dTTP; 2.5 units of thermostable DNA polymerase (PE Applied Biosystems, Foster City, CA); and 10–50 ng of purified human genomic DNA. Oligonucleotide primers used to direct amplification of CCR5 ORF products

(wt = 312 bp; Δ32 = 280 bp) include 62000 + 5′-GTC TTC ATT ACA CCT GCA GCT CTC-3′ and 62311- 5′-GGT CCA ACC TGT TAG AGC TAC TGC-3′. Amplicons for the CCR5 ORF were visualized on 2% agarose gels following electrophoresis in 1X TBE and stained with a 1:10,000 dilution of SYBR Gold (Molecular Probes, Eugene, OR) and detected using a Storm 860 scanner (Molecular Dynamics, Sunnyvale, CA) as described previously [18]. Persons determined to have the CCR5 Δ32 allele were excluded from this study.

Genotyping for *CCR5 promoter, CCR5* Δ*32*, and *CCR2 64V* and -*I* alleles was performed as described previously [18,19]. Post polymerase chain reaction (PCR) ligase detection reaction (LDR) analyses were used to genotype *CCR5* promoter SNPs–*2733*, -*2554*, -*2459*, -*2135*, -*2132*, -*2086*, and -*1835.* Complete details of this genotyping strategy have been described in Kawamura et al. [20].

Lymphocyte proliferation

PBMC were cultured in quadruplicate round-bottom microtiter wells at a concentration of 10^5 cells/0.1 ml CM. Lymphocyte proliferation was assayed in medium alone or medium supplemented with increasing concentrations of PHA (Sigma) and IL-2 (Cellular Products, Inc., Buffalo, NY) (0.15/0.15, 0.31/0.31, 0.63/0.63, 1.25/1.25, 2.5/2.5, 5/5, and 10/10 μg/ml PHA/%IL-2, respectively). After 3 days of culture, 1 μCi [^{3}H]thymidine (ICN Pharmaceuticals, Inc., Costa Mesa, CA) was added to all wells. Cells were harvested 18 h later using a semiautomated cell harvester and [^{3}H]thymidine incorporation was assayed using automated gas scintillation spectrometry. Lymphocyte proliferation was recorded in counts per minute, and 10 and 50% of maximal stimulation was defined for each individual based on the analysis of the stimulation response curves. It should be noted that for all subjects, although peak proliferation varied, the concentrations of stimuli that resulted in 10 and 50% of maximal stimulation were remarkably consistent (0.4 μg/ml PHA, 0.4% IL-2 and 1.25 μg/ml PHA, and 1.25% IL-2 respectively).

Viral stocks and in vitro infections

HIV-1 laboratory isolate JR-FL was obtained from the AIDS Reference and Reagent Program (Rockville, MD) and was propagated in PHA blasts obtained from healthy controls using standard methodologies [21]. Culture supernatant was analyzed for HIV-1 p24 antigen concentration by ELISA (Coulter, Miami, FL) and the $TCID_{50}$ of each virus isolate was determined by limiting dilution using PHA blasts.

Infectivity assays were performed as follows: PBMC were stimulated at 10 and 50% of maximal-stimulation conditions (0.4 μg/ml PHA, 0.4% IL-2 and 1.25 μg/ml PHA, and 1.25% IL-2 respectively). After 48 h, cells were centrifuged and incubated for 2 h with the HIV-1 isolate at a multiplicity of infection of 0.1. The cells were washed 3 times and resuspended in CM supplemented with IL-2 (0.4% for 10% or 1.25% for 50% maximal stimulation). One hundred thousand cells in 300 μl CM were plated in quadruplicate microtiter wells. Supernatants were collected and replaced with fresh CM at 1, 3, 5, 7, and 11 days postinfection and were analyzed for HIV-1 p24 antigen concentration by ELISA (Coulter).

β-chemokine production

PBMC were incubated at a concentration of 10^6 cells/ml at 10, 50, or 100% of optimal stimulation conditions or without stimulation for 48 h. Supernatants were assayed for MIP-1α, MIP-1β, and RANTES by ELISA (R&D Systems, Minneapolis, MN).

CCR5 expression

PBMC were incubated without stimulation in CM for 24 h to allow for recovery from cryopreservation. Cells were washed and then resuspended in CM at 1×10^6 cells/ml and cultured for 48 h in a multiwell culture plate. Cells were removed from the culture wells by pipetting up and down, whereas the wells were gently scraped with the pipette tip. Cells were then were centrifuged at 400*g*, washed, and resuspended in 200 μl PBS. Recovery was ≥70% with ≥95% viability. PBMC were incubated with monoclonal antibodies CD4-PerCP/CD14-FITC/IgG_1-PE, CD4-PerCP/CD14-FITC/CCR5-PE (Pharmingen, San Diego, CA) for 30 min at room temperature and then were washed twice and fixed with 1% paraformaldehyde in PBS and analyzed immediately using a FACScan (Becton–Dickenson, San Jose, CA). The monoclonal antibody directed against the chemokine receptor CCR5 was labeled with fluorochrome at a ratio of 1 molecule of phycoerythrin per antibody molecule. Known ratios of QuantiBRITE-PE beads (Becton–Dickenson, San Jose, CA) were analyzed using the same instrument settings as used in the assay. The geometric mean fluorescence for the control IgG2a-PE was subtracted from the geometric mean fluorescence of the CCR5-PE-positive cells and was converted into the number of PE molecules bound per CD4+ T lymphocyte and CD14+ monocyte [22]. Monocytes and lymphocytes were analyzed separately using CELLQuest software (Becton–Dickenson) collecting at least 10,000 events for each analysis.

Statistical analysis

All statistical tests were performed using STATVIEW 5.0.1 software (SAS Institute, Inc., Cary, NC).

Results and discussion

Since CCR5 promoter polymorphisms at –2459 were previously associated with magnitude of linked reporter gene activity [11], we asked if this SNP influenced the density of CCR5 on $CD4^+$ mononuclear cell subsets. By flow cytometric analyses, we observed that the density of CCR5 was lower on unstimulated $CD14^+$ monocytes from healthy individuals with the –2459 G/G and A/G (10,378 ± 4,289 receptors/cell) genotypes compared to A/A (16,411 ± 7,334 receptors/cell) genotypes (Fig. 1; $P < 0.05$ two-sided *t* test). In contrast, the densities of CCR5 on total resting $CD4^+$ T cells and $CD4^+$ T cells stimulated with PHA and IL-2 were comparable in persons with the G/G, A/G, and A/A genotype (data not shown).

To explore further the potential significance of these findings, we asked if the magnitude of viral replication in vitro was related to surface expression of CCR5. After activation of target PBMC with PHA and IL-2, supernatants were collected at 1, 3, 5, 7, and 11 days postinfection and were analyzed for HIV-1 p24 antigen concentration by ELISA. To more closely approximate conditions reflecting stimulation conditions in vivo, we utilized suboptimal concentrations for mitogen and IL-2 [18]. Following suboptimal stimulation and incubation with the R5-tropic virus JR-FL, PBMC obtained from –2459 G/G donors produced fewer HIV-1 p24 antigen in culture than did PBMC obtained from A/A donors (Fig. 2A). Specifically, after 11 days in culture, PBMC obtained from 4 people with the G/G genotype produced 1.2 ± 1.7 (mean ± SE) ng p24 antigen/ml, whereas PBMC obtained from 6 people with the A/G genotype produced on average 15.7 ± 12.8 ng p24 antigen/ml and PBMC obtained from 5 people with the A/A genotype produced on average 109 ± 105.7 ng/ml ($P < 0.05$, Kruskall–Wallis test). Although the same hierarchical relationships between viral propagation and donor genotypes were observed using greater (near optimal) concentrations of mitogen and IL-2, differences in HIV-1 p24 antigen production associated with CCR5 –2459 genotypes were not significant (Fig. 2B). In these conditions, PBMC obtained from persons with the –2459 G/G genotype produced 54.3 ± 34.6 ng p24 antigen/

ml, A/G PBMC produced 61 ± 40.3 ng/ml, and A/A PBMC produced 131 ± 90.2 ng/ml ($P < 0.7$, Kruskal–Wallis test). It should be noted that p24 antigen production by PBMC from A/A donors was similar at both suboptimal and near optimal stimulation conditions, whereas PBMC from G/G and A/G donors produced more virus after stimulation in near optimal conditions than after suboptimal stimulation ($P < 0.001$, Mann–Whitney test). These results suggest that higher levels of CCR5 expression driven by the CCR5 –2459A allele may permit high level HIV-1 propagation irrespective of stimulation conditions, whereas higher level stimulation is required to support HIV-1 replication in the presence of the –2459G allele.

Other studies have shown correlations between disease progression and the CCR2-64 I/V amino acid substitution and between CCR5 promoter haplotype/haplotype combinations (diplotype) [10,19,23–28]. We were unable to demonstrate any relationship between these diplotypes and the in vitro phenotype; however, these additional analyses may have been limited by the small numbers of individuals in the diplotype in vitro phenotype categories observed in this study.

Because β-chemokines may block HIV propagation in vitro [29,30] and heightened β-chemokine expression also has been seen in high-risk HIV-seronegative persons [31,32], we wanted to assess the relationship between β-chemokine production and genetic regulation of CCR5 expression. For these studies, we assayed cell culture supernatants for MIP-1α, MIP-1β, and RANTES by ELISA (R&D Systems, Minneapolis, MN, USA) after 48 h of cultivation without stimulation or after stimulation with PHA and IL-2 at suboptimal, near-optimal, and optimal stimulation conditions. At near-optimal stimulation conditions, PBMC obtained from people with the G/G genotype appeared to produced more MIP-1α and more MIP-1β (18,084 ± 2,074 pg/ml and 13,063 ± 864 pg/ml, respectively; Fig. 3A and B) than did PBMC obtained from people with the A/G genotype (14,288 ± 4,830 pg/ml and 12,179 ± 3,864 pg/ml) or with the A/A genotype (10,119 ± 2,970 and 9,398 ± 3,147). There were no differences among the groups in production of MIP-1α and MIP-1β by unstimulated cells or cells stimulated at suboptimal conditions (data not shown) and no differences in RANTES production for any stimulation conditions (Fig. 3C and data not shown).

Our results suggest that the CCR5 –2459G allele (in contrast to the CCR5 –2459A allele) is associated with both lower CCR5 expression on circulating $CD14^+$ monocytes and with lower levels of macrophage-tropic (JR-FL) HIV-1 propagation in vitro. The lower level of CCR5 expression related to the CCR5 –2459 genotype has been shown to be an important determinant of the magnitude of HIV-1 replication in vivo [10]. These results taken together suggest the plausibility of CCR5 expression being rate limiting in cellular susceptibility to HIV-1 propagation. Although $CD4^+$ T cells are the major targets for HIV replication, in our in vitro system we were unable to show a relationship between CCR5 promoter genotype and CCR5 density on $CD4^+$ T cells either unstimulated or after stimulation with PHA and IL-2. This may be related to the recognized heterogeneity of CCR5 expression in different phenotypically defined $CD4^+$ T-cell populations [33]. Additional studies are necessary to examine the factors underlying CCR5 expression on these selected $CD4^+$ T-cell sub-populations according to CCR5 –2459 genotype. As levels of viremia are closely associated with prognosis of HIV-1 infection, results from our study suggest that delay in progression to AIDS associated with the CCR5 –2459G allele may result from lower expression of CCR5 on susceptible cell populations [11,14–16].

To further investigate the mechanisms by which CCR5 expression influences viral propagation and disease progression, we found that limiting the concentration of factors (PHA and IL-2) conventionally used for in vitro HIV-1 propagation improves the ability to resolve the molecular interactions contributing to HIV-1 pathogenesis. As the CCR5 –

2459G allele appears to require greater stimulation to promote HIV-1 replication, it may be possible to exploit further the polymorphism residing in the CCR5 promoter or to develop strategies to reduce CCR5 expression that will suppress and/or prevent HIV-1 infection.

Acknowledgments

We thank David T. McNamara for valuable technical assistance during the course of this investigation. We thank Laurin Kasehagen for assistance and advice on statistical analyses and Philip Murphy for helpful criticisms. All studies were performed following protocols approved by the University Hospitals of Cleveland Institutional Review Board (IRB). This work was supported by Grants AI 36219, AI 43645, and AI 51649 from the National Institutes of Health.

References

1. Zhu T, Mo H, Wang N, Nam DS, Cao Y, Koup RA, Ho DD. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. Science. 1993; 261:1179. [PubMed: 8356453]
2. Dean M, Carrington M, Winkler C, Huttley GA, Smith MW, Allikmets R, Goedert JJ, Buchbinder SP, Vittinghoff E, Gomperts E, Donfield S, Vlahov Kaslow D, Saah A, Rinaldo C, Detels R. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CCR5 structural gene. Science. 1996; 273:1856. [PubMed: 8791590]
3. Huang Y, Paxton WA, Wolinsky SM, Neumann AU, Zhang L, He T, Kang S, Ceradini D, Jin Z, Yazdanbakhsh K, Kunstman K, Erickson D, Dragon E, Landau N, Phair J, Ho DD, Koup R. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat Med. 1996; 2
4. O'Brien TR, Winkler C, Dean M, Nelson JA, Carrington M, Michael NL, White GC 2nd. HIV-1 infection in a man homozygous for CCR5 delta 32. Lancet. 1997; 349:1219. [letter] [see comments]. [PubMed: 9130945]
5. Samson M, Libert F, Doranz BJ, Rucker J, Liesnard C, Farber CM, Saragosti S, Lapoumeroulie C, Cognaux J, Forceille C, Muyldermans G, Verhofstede C, Burtonboy G, Georges M, Imai T, Rana S, Yi Y, Smyth RJ, Collman RG, Doms RW, Vassart G, Parmentier M. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature. 1996; 382:722. [see comments]. [PubMed: 8751444]
6. Smith MW, Dean M, Carrington M, Huttley GA, O'Brien SJ. CCR5-delta 32 gene deletion in HIV-1 infected patients. Lancet. 1997; 350:741. discussion 742 [letter; comment]. [PubMed: 9291930]
7. Wang B, Palasanthiran P, Zeigler J, Cunningham A, Saksena NK. CCR5-delta 32 gene deletion in HIV-1 infected patients. Lancet. 1997; 350:742. [letter; comment]. [PubMed: 9291932]
8. Zimmerman PA, Buckler-White A, Alkhatib G, Spalding T, Kubofcik J, Combadiere C, Weissman D, Cohen O, Rubbert A, Lam G, Vaccarezza M, Kennedy PE, Kumaraswami V, Giorgi JV, Detels R, Hunter J, Chopek M, Berger EA, Fauci AS, Nutman TB, Murphy PM. Inherited resistance to HIV-1 conferred by an inactivating mutation in CC chemokine receptor 5: studies in populations with contrasting clinical phenotypes, defined racial background, and quantified risk. Mol Med. 1997; 3:23. [PubMed: 9132277]
9. Valdez H, Purvis SF, Lederman MM, Fillingame M, Zimmerman PA. Association of the CCR5 delta 32 mutation with improved response to antiretroviral therapy. J Am Med Assoc. 1999; 282:734.
10. Tang J, Shelton B, Makhatadze NJ, Zhang Y, Schaen M, Louie LG, Goedert JJ, Seaberg EC, Margolick JB, Mellors J, Kaslow RA. Distribution of chemokine receptor CCR2 and CCR5 genotypes and their relative contribution to human immunodeficiency virus type 1 (HIV-1) seroconversion, early HIV-1 RNA concentration in plasma, and later disease progression. J Virol. 2002; 76:662. [PubMed: 11752157]
11. McDermott DH, Zimmerman PA, Guignard F, Kleeberger CA, Leitman SF, Murphy PM. CCR5 promoter polymorphism and HIV-1 disease progression. Multicenter AIDS Cohort Study (MACS). Lancet. 1998; 352:866. [PubMed: 9742978]

12. Knudsen TB, Kristiansen TB, Katzenstein TL, Eugen-Olsen J. Adverse effect of the CCR5 promoter –2459A allele on HIV-1 disease progression. J Med Virol. 2001; 65:441. [PubMed: 11596075]

13. Clegg AO, Ashton LJ, Biti RA, Badhwar P, Williamson P, Kaldor JM, Stewart GJ. The Australian Long-Term Non-Progressor Study Group, CCR5 promoter polymorphisms, CCR5 59029A and CCR5 59353C, are under represented in HIV-1-infected long-term non-progressors. AIDS. 2000; 14:103. [PubMed: 10708279]

14. An P, Martin MP, Nelson GW, Carrington M, Smith MW, Gong K, Vlahov D, O'Brien SJ, Winkler CA. Influence of CCR5 promoter haplotypes on AIDS progression in African-Americans. AIDS. 2000; 14:2117. [PubMed: 11061652]

15. Martin MP, Dean M, Smith MW, Winkler C, Gerrard B, Michael NL, Lee B, Doms RW, Margolick J, Buchbinder S, Goedert JJ, O'Brien TR, Hilgartner MW, Vlahov D, O'Brien SJ, Carrington M. Genetic acceleration of AIDS progression by a promoter variant of CCR5. Science. 1998; 282:1907. [In Process Citation]. [PubMed: 9836644]

16. Ometto L, Bertorelle R, Mainardi M, Zanchetta M, Tognazzo S, Rampon O, Ruga E, Chieco-Bianchi L, De Rossi A. Polymorphisms in the CCR5 promoter region influence disease progression in perinatally human immunodeficiency virus type 1-infected children. J Infect Dis. 2001; 183:814. [PubMed: 11181160]

17. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science. 1996; 272:1167. [PubMed: 8638160]

18. Salkowitz JR, Purvis SF, Meyerson H, Zimmerman P, O'Brien TR, Aledort L, Eyster ME, Hilgartner M, Kessler C, Konkle BA, White GC 2nd, Goedert JJ, Lederman MM. Characterization of high-risk HIV-1 seronegative hemophiliacs. Clin Immunol. 2001; 98:200. [PubMed: 11161976]

19. Smith MW, Dean M, Carrington M, Winkler C, Huttley GA, Lomb DA, et al. Contrasting genetic influence of CCR2 and CCR5 variants in HIV-1 infection and disease progression. Science. 1991; 277:959. [PubMed: 9252328]

20. Kawamura T, Gulden FO, Sugaya M, McNamara DT, Borris DL, Lederman MM, Orenstein JM, Zimmerman PA, Blauvelt A. R5 HIV productively infects Langerhans cells and infection levels are regulated by compound CC R5 polymorphisms. Proc Natl Acad Sci, USA. 2003 in press.

21. Division of AIDS, NIAID. DHHS. DAIDS Virology Manual for HIV Laboratories. Vol. NIH-97-3828. NIH; Washinton, DC: 1997. p. 46

22. Iyer SB, Hultin LE, Zawadzki JA, Davis KA, Giorgi JV. Quantitation of CD38 expression using QuantiBRITE beads. Cytometry. 1998; 33:206. [PubMed: 9773881]

23. Ioannidis JP, Rosenberg PS, Goedert JJ, Ashton LJ, Benfield TL, Buchbinder SP, Coutinho RA, Eugen-Olsen J, Gallart T, Katzenstein TL, Kostrikis LG, Kuipers H, Louie LG, Mallal SA, Margolick JB, Martinez OP, Meyer L, Michael NL, Operskalski E, Pantaleo G, Rizzardi GP, Schuitemaker H, Sheppard HW, Stewart GJ, Theodorou ID, Ullum H, Vicenzi E, Vlahov D, Wilkinson D, Workman C, Zagury JF, O'Brien TR. Effects of CCR5-Delta32, CCR2-64I, and SDF-1 3′A alleles on HIV-1 disease progression: an international meta-analysis of individual-patient data. Ann Intern Med. 2001; 135:782. [PubMed: 11694103]

24. Kostrikis LG, Huang Y, Moore JP, Wolinsky SM, Zhang L, Guo Y, Deutsch L, Phair J, Neumann AU, Ho DD. A chemokine receptor CCR2 allele delays HIV-1 disease progression and is associated with a CCR5 promoter mutation. Nat Med. 1998; 4:350. [PubMed: 9500612]

25. Michael NL, Louis LG, Rohrbaugh AL, Schultz KA, Dayhoff DE, Wang CE, Sheppard HW. The role of CCR5 and CCR2 polymorphisms in HIV-1 transmission and disease progression. Nat Med. 1997; 10:1160. [PubMed: 9334732]

26. Gonzalez E, Bamshad M, Sato N, Mummidi S, Dhanda R, Catano G, Cabrera S, McBride M, Cao XH, Merrill G, O'Connell P, Bowden DW, Freedman BI, Anderson SA, Walter EA, Evans JS, Stephan KT, Clark RA, Tyagi S, Ahuja SS, Dolan MJ, Ahuja SK. Race-specific HIV-1 disease-modifying effects associated with CCR5 haplotypes. Proc Natl Acad Sci USA. 1999; 96:12004. [PubMed: 10518566]

27. Mulherin SA, O'Brien TR, Ioannidis JP, Goedert JJ, Buchbinder SP, Coutinho RA, Jamieson BD, Meyer L, Michael NL, Pantaleo G, Rizzardi GP, Schuitemaker H, Sheppard HW, Theodorou ID, Vlahov D, Rosenberg PS. Effects of CCR5-Delta32 and CCR2-64I alleles on HIV-1 disease

progression: the protection varies with duration of infection. AIDS. 2003; 17:377. [PubMed: 12556692]

28. Mangano A, Gonzalez E, Dhanda R, Catano G, Bamshad M, Bock A, Duggirala R, Williams K, Mummidi S, Clark RA, Ahuja SS, Dolan MJ, Bologna R, Sen L, Ahuja SK. Concordance between the CC chemokine receptor 5 genetic determinants that alter risks of transmission and disease progression in children exposed perinatally to human immunodeficiency virus. J Infect Dis. 2001; 183:1574. [PubMed: 11335892]
29. Akhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA. CC-CKR-5: a RANTES, MIP-1a, MIP-1b receptor as a fusion co-factor for macrophage-tropic HIV-1. Science. 1996; 272:1955. [PubMed: 8658171]
30. Cocchi F, DeVico A, Garzino-Demo A, Arya S, Gallo R, Lusso P. Identification of RANTES MIP-1 and MINP-1 as the major HIV-suppressive factors produced by $CD8^+$ T cells. Science. 1995; 270:1811. [PubMed: 8525373]
31. Paxton WA, Liu R, Kang S, Wu L, Gingeras TR, Landau NR, Mackay CR, Koup RA. Reduced HIV-1 infectability of CD4+ lymphocytes from exposed-uninfected individuals: association with low expression of CCR5 and high production of beta-chemokines. Virology. 1998; 244:66. [PubMed: 9581779]
32. Zagury D, Lachgar A, Chams V, Fall LS, Bernard J, Zagury JF, Bizzini B, Gringeri A, Santagostino E, Rappaport J, Feldman M, O'Brien SJ, Burny A, Gallo RC. C-C chemokines, pivotal in protection against HIV type 1 infection. Proc Natl Acad Sci USA. 1998; 95:3857. [PubMed: 9520457]
33. Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. Proc Natl Acad Sci USA. 1997; 94:1925. [see comments]. [PubMed: 9050881]

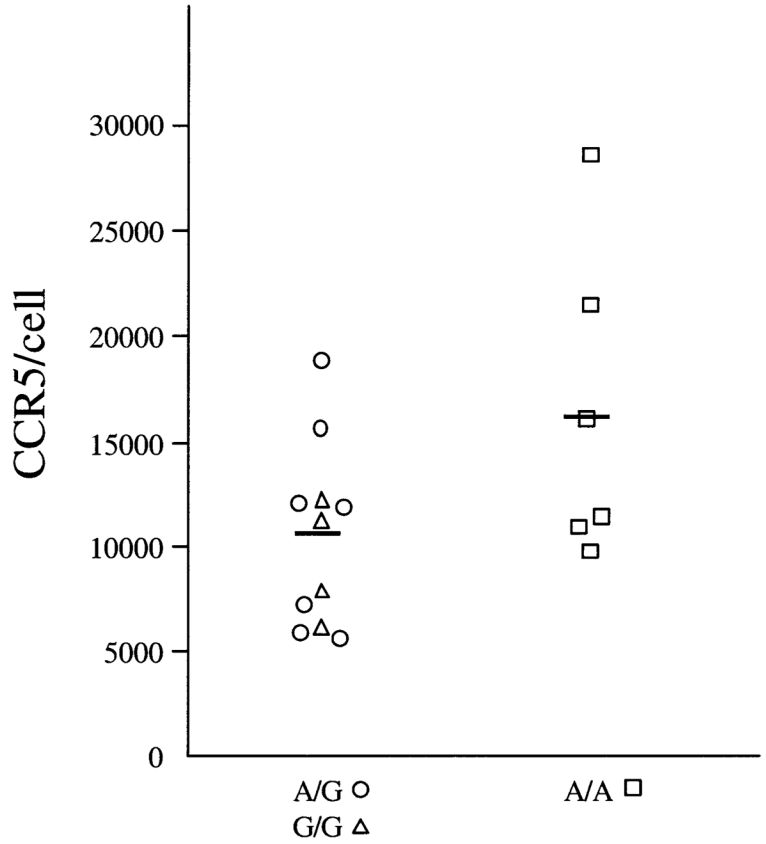

Fig. 1.

CCR5 expression on CD14+ monocytes. The number of chemokine receptors/cell from 16 uninfected Caucasian blood donors is shown on the *y* axis. The number of G/G (△) and A/G (○) donors were 10 (4 and 6, respectively), and the number of A/A donors was 6 (□).

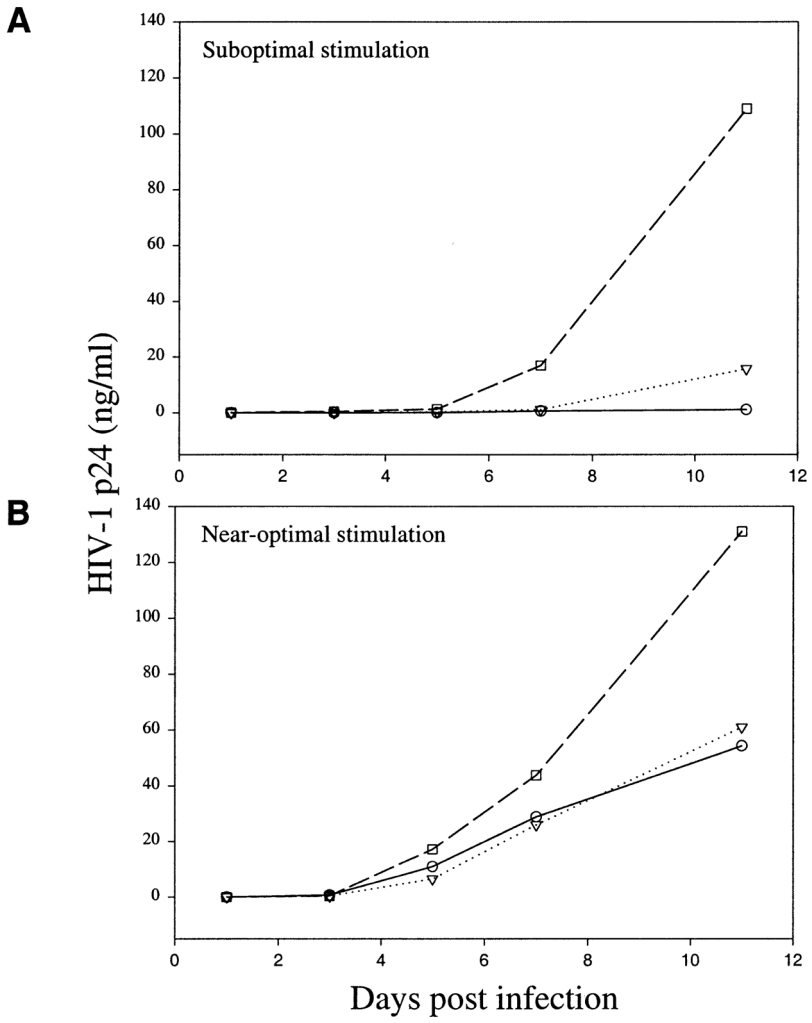

Fig. 2.

CCR5 –2459 SNP and in vitro infection with R5-tropic HIV-1 isolate JR-FL. Magnitude of HIV-1 p24 antigen produced (*y* axis) over time (*x* axis). (A) Suboptimal stimulation of PBMC obtained from four people with the G/G genotype (○) produced on average 1.2 ± 1.7 (mean ± SE) ng HIV-1 p24 antigen/ml, PBMC obtained from six people with the A/G genotype (▽) produced on average 15.7 ± 12.8 ng/p24 antigen/ml, PBMC obtained from five people with the A/A genotype (□) produced on average 109 ± 105.7 ng/ml ($P < 0.05$, Kruskall–Wallis test). (B) Near-optimal stimulation of PBMC from persons with the G/G genotype produced 54.3 ± 34.6 ng p24 antigen/ml; A/G PBMC produced 61 ± 40.3 ng/ml; A/A PBMC produced 131 ± 90.2 ng/ml ($P < 0.7$, Kruskal–Wallis test) over 11 days in culture.

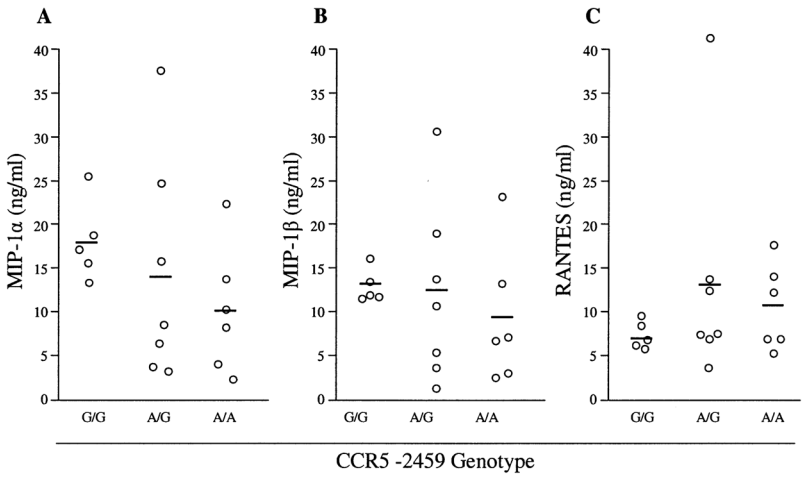

Fig. 3.

CCR5 (–)2459 SNP and supernatant β-chemokine production. In vitro production of β-chemokines at near-optimal stimulation conditions: (A). MIP-1α, (B) MIP-1β, and (C) RANTES. The number of G/G donors was 5, A/G donors was 7, and A/A donors was 6.