Relative resistance to HIV-1 infection of CD4 lymphocytes from persons who remain uninfected despite multiple high-risk sexual exposures

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Some individuals remain uninfected with human immunodeficiency virus type-1 (HIV-1) despite multiple high-risk sexual exposures. We studied a cohort of 25 subjects with histories of multiple high-risk sexual exposures to HIV-1 and found that their CD8⁺ lymphocytes had greater anti-HIV-1 activity than did CD8⁺ lymphocytes from nonexposed controls. Further studies indicated that their purified CD4⁺ lymphocytes were less susceptible to infection with multiple primary isolates of HIV-1 than were CD4⁺ lymphocytes from the nonexposed controls. This relative resistance to HIV-1 infection did not extend to T-cell line-adapted strains, was restricted by the envelope glycoprotein, was not explained by the cell surface density of CD4 molecules, but was associated with the activity of the C-C chemokines RANTES, MIP-1α, and MIP-1β. This relative resistance of CD4⁺ lymphocytes may contribute to protection from HIV-1 in multiply exposed persons.

Some individuals lack evidence for infection with human immunodeficiency virus type 1 (HIV-1) despite multiple sexual contacts with HIV-1 infected partners[1]. This lack of infection in a proportion of exposed individuals may reflect a stochastic event, as the risk of infection from a single sexual contact is estimated to be low[2,3]. Epidemiological studies suggest, however, that some individuals are truly resistant to HIV-1 infection[1,4]. Host factors potentially affecting the efficiency of sexual transmission of HIV-1 include the HLA or TAP alleles expressed by individuals[5–9]. Several reports have documented that peripheral blood mononuclear cells (PBMC) from different donors are not equally infectible with HIV-1 (ref. 10–13), raising the possibility that relative resistance of PBMC to HIV-1 infection *in vitro* might be associated with resistance to transmission[14,15]. Virologic factors, including the syncytium-inducing (SI) or non-syncytium-inducing (NSI) phenotype of the virus may also affect the efficiency of sexual transmission[16,17].

Some exposed-uninfected individuals possess immune responses that are HIV-1-specific and HLA class I (ref. 18–20) or class II (ref. 21) restricted. The presence of non-HLA-restricted virus-suppressive CD8⁺ lymphocytes within exposed-uninfected individuals has also been suggested, but not proven[1,22]. The presence of such antiviral responses within exposed-uninfected individuals may reflect prior infection or immunization.

To obtain a better understanding of the mechanisms involved in resistance to sexual transmission of HIV-1, we studied the ability of CD4⁺ and CD8⁺ lymphocytes from a diverse cohort of sexually exposed individuals to affect HIV-1 infection *in vitro*.

Study population

A highly selected cohort of 25 HIV-1 seronegative subjects with histories of multiple high-risk sexual exposures to HIV-1 were studied. The cohort could be subdivided into three groups based on risk behaviors; homosexual men who reported sex with multiple HIV-1-infected partners (EU1–EU7), homosexual men who reported sex with predominantly a single HIV-1-infected partner (EU8–EU12), and heterosexual individuals reporting sex predominantly with a single HIV-1-infected partner (EU13–EU25).

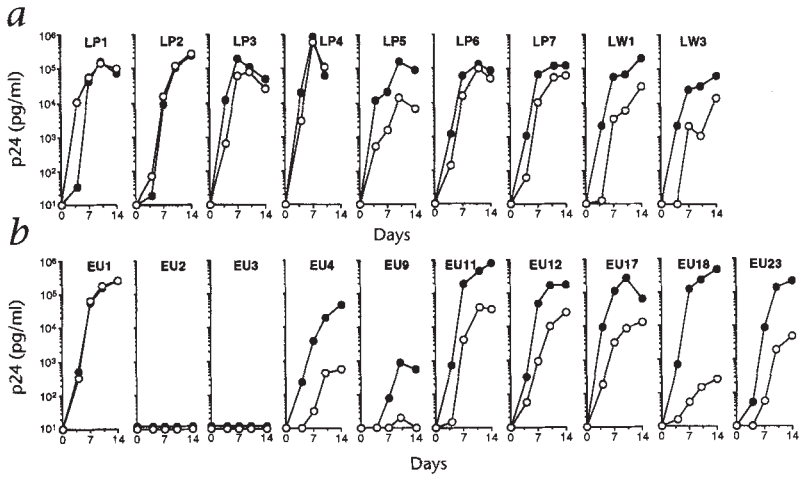

Fig. 1 $CD8^+$ Lymphocyte-mediated inhibition of JR-CSF replication. p24 production after inoculation with 3000 $TCID_{50}$ of JR-CSF for $CD4^+$ lymphocytes alone (●) or $CD4^+$ lymphocytes in the presence of an equal number of $CD8^+$ lymphocytes (○) is shown. *a*, Cells from nonexposed control individuals; *b*, cells from the exposed-uninfected individuals.

Among the homosexual men reporting multiple sexual partners, most were extensively exposed through receptive anal intercourse early in the HIV epidemic. Among the individuals reporting exposure to predominantly a single HIV-infected partner, most were in long-term relationships involving unprotected sexual intercourse over many years during which time several partners succumbed to AIDS. All subjects were HIV-1 negative by commercially available enzyme-linked immunosorbent assay (ELISA) tests and by diagnostic polymerase chain reaction (PCR) (sensitivity of two HIV copies per microgram DNA)[23]. Blood from unexposed controls was either supplied as leukopac preparations from random blood donors (LP1–LP17) or was drawn from seronegative laboratory workers (LW1–LW7) who reported only low-risk sexual activity.

$CD8^+$ lymphocyte inhibition of HIV-1 replication

We assessed the ability of $CD8^+$ lymphocytes to inhibit the replication of the JR-CSF strain of HIV-1 in autologous $CD4^+$ lymphocytes from nonexposed control individuals (Fig. 1*a*) and exposed-uninfected individuals (Fig. 1*b*). In eight of the nine nonexposed control subjects, the addition of $CD8^+$ lymphocytes resulted in ≤1 log reduction in p24 production by day 14. In the exposed-uninfected individuals, however, six out of the ten individuals showed an inhibition in p24 production of ≥1 log, with one showing ≥3 logs of inhibition (EU18). To better quantify this inhibition mediated by $CD8^+$ lymphocytes we performed the assay using serial fivefold dilutions of the stimulated $CD8^+$ lymphocytes (1 million to 1600 $CD8^+$ lymphocytes). No significant inhibition of virus replication was observed unless more than 2×10^5 $CD8^+$ lymphocytes were added (data not shown).

There was complete absence of detectable viral replication in the $CD4^+$ lymphocytes of two exposed-uninfected individuals (EU2, EU3) despite an inoculum of 3000 $TCID_{50}$ (median tissue culture infectious dose). This led us to consider that $CD4^+$ lymphocytes from exposed-uninfected subjects might be poorly infectible with HIV-1.

$CD4^+$ infectibility and efficiency of viral replication

Purified $CD4^+$ lymphocytes from control and exposed-uninfected individuals were assayed for infectibility by JR-CSF (Fig. 2*a*). $CD4^+$ lymphocytes from the control subjects all demonstrated consistent kinetics of p24 production. In contrast, whereas the $CD4^+$ lymphocytes from some exposed individuals showed virus replication similar to the control group, others showed almost no virus replication. Overall, the differences in p24 production

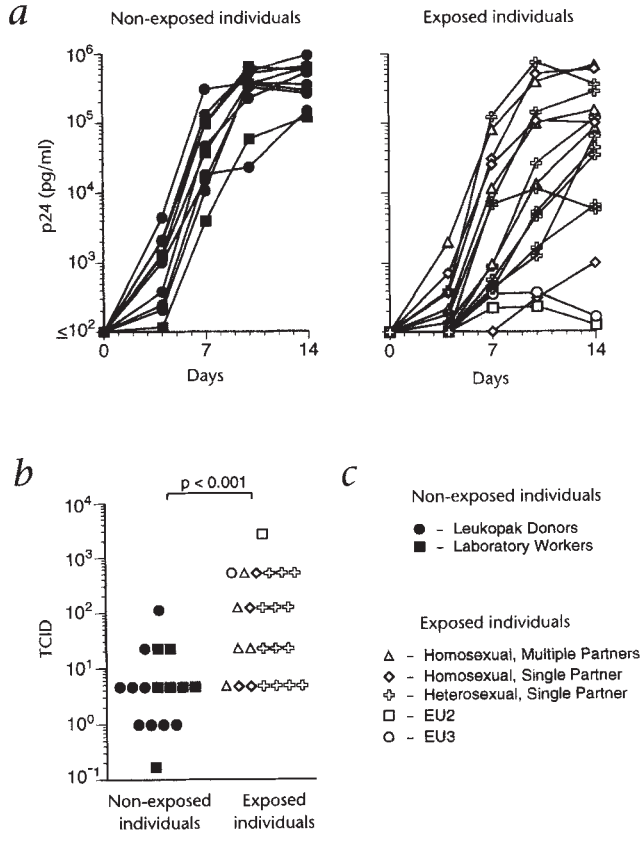

Fig. 2 Infectibility of purified $CD4^+$ lymphocytes by HIV-1. Cells were inoculated with 600 $TCID_{50}$ of HIV-1 JR-CSF (*a*) or serial fivefold dilutions of JR-CSF from 3000 to 0.2 $TCID_{50}$ (*b*) on day 0, and p24 production was measured over 14 days. *a*, *left*, Nonexposed controls; *right*, exposed-uninfected individuals. *b*, The number of TCID required to establish infection of $CD4^+$ lymphocytes from exposed-uninfected or nonexposed control subjects. *c*, Key to symbols used to identify each group of exposed-uninfected subjects or nonexposed control subjects. In addition, the two exposed-uninfected subjects with the most resistant $CD4^+$ lymphocytes (EU2 and EU3) are identified with separate symbols. Because of the availability of PBMC, $CD4^+$ lymphocytes from all subjects were not run in all assays.

between the CD4⁺ lymphocytes from the two groups were significant whether determined by p24 levels on any given day, or by area under the curve ($P < 0.01$). No significant differences in p24 production were noted between CD4⁺ lymphocytes from the three subgroups of exposed-uninfected individuals. We were also unable to identify specific sexual practices or histories that would identify subjects with the most resistant CD4⁺ lymphocytes.

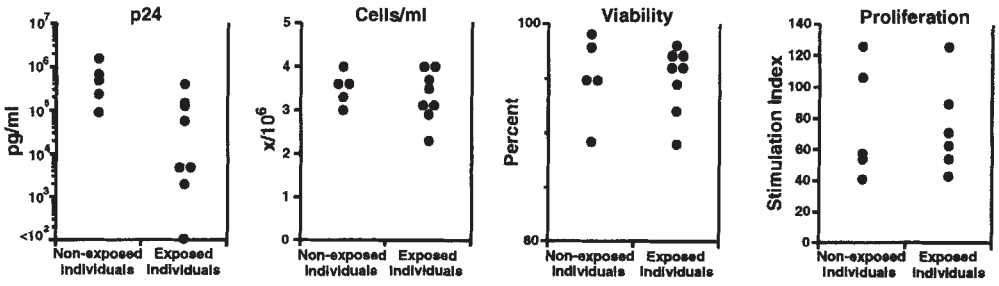

Fig. 3 Comparison of p24 production, cell counts, viabilities and proliferation in CD4⁺ lymphocytes from five unexposed subjects and eight exposed-uninfected subjects. The p24 data are from day 7 after infection of 2×10^5 CD4⁺ lymphocytes with 600 $TCID_{50}$ of JR-CSF. Cell counts and viabilities are from day 7 of parallel uninfected wells. Stimulation indices are calculated by tritiated thymidine incorporation in the presence or absence of PHA for 3 days. The numbers of cells that were available only allowed cells from six of the eight exposed-uninfected subjects to be assessed for proliferation to PHA by tritiated thymidine incorporation.

Serial fivefold dilutions of JR-CSF (3000 $TCID_{50}$ to 0.2 $TCID_{50}$) were used to determine the inoculum of virus required to establish infection in the CD4⁺ lymphocytes from the two groups (Fig. 2*b*), and a statistically significant difference was noted ($P < 0.001$). Between 0.1 and 120 TCID were required to establish infection in the nonexposed controls, as opposed to between 5 and 3000 for the exposed-uninfected individuals. Again relative resistance to infection did not segregate with any of the subgroups of exposed-uninfected individuals.

Control experiments were performed using CD4⁺ lymphocytes from five nonexposed and eight exposed-uninfected subjects to ensure that the observed differences in infectibility were not due to differences in CD4⁺ lymphocyte proliferation or viability (Fig. 3). There were no differences in total T-cell counts, viabilities, or stimulation indices (by tritiated thymidine incorporation) between the wells containing CD4⁺ lymphocytes from the two groups of individuals, despite the differences in levels of p24 production. In addition, there was no correlation between levels of p24 production and the total T-cell counts, viabilities, or stimulation indices of cells within the culture wells. Differences between the HIV-1 replication efficiency of purified CD4⁺ lymphocytes persisted when fresh, cryopreserved, or overnight-shipped samples were used (data not shown).

Susceptibility to other virus isolates

To investigate the mechanism of the resistance, CD4⁺ lymphocytes from two exposed-uninfected subjects (EU2 and EU3) and two nonexposed individuals (LW4 and LW5) were inoculated with 600 $TCID_{50}$ each of nine different virus isolates (Fig. 4*a*). Purified CD4⁺ lymphocytes from individuals EU2 or EU3 were resistant to infection with the primary isolates of HIV-1 (JR-CSF, 92U657, GT, and AD-6) but not the T-cell line-adapted strains (NL4-3 and SF2), indicating that the relative resistance of their CD4⁺ lymphocytes to HIV-1 infection was restricted to primary isolates.

A series of closely related viruses were used to map the viral determinants involved in overcoming the resistance to infection of CD4⁺ lymphocytes from subjects EU2 and EU3. SF162R3H is a virus produced by serial passage of the primary isolate SF162 into HUT-78 cells[24]. These two viruses differ by at least three amino acids within the envelope glycoprotein[24]. When two of these amino acid changes (within the V3 loop, I307R and A312V), were reintroduced into a clone of SF162 (SF162dbl), they were sufficient to alter the phenotype (NSI to SI) of the virus[24]. These two amino acid changes were also sufficient to overcome the resistant phenotype of the CD4⁺ lymphocytes from study subjects EU2 and EU3 (Fig. 4*a*). These results indicate that the resistance to infection of CD4⁺ lymphocytes from some exposed-uninfected subjects is restricted by the envelope glycoprotein of HIV-1.

The density of CD4 molecules on the cell surface may affect efficiency of HIV-1 infection in an envelope-restricted manner[25,26].

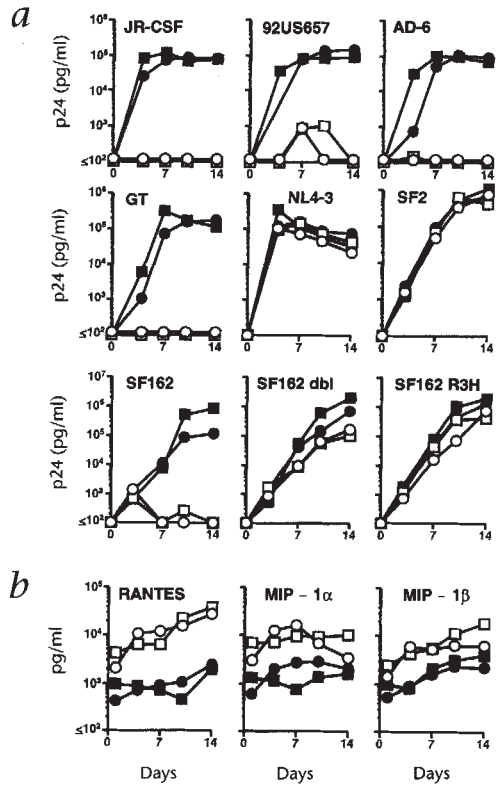

Fig. 4 Kinetics of production of p24 antigen and chemokines from purified CD4⁺ lymphocytes inoculated with various strains of HIV-1. Cells were inoculated with 600 $TCID_{50}$ of each virus isolate on day 0. Purified CD4⁺ lymphocytes were from two nonexposed subjects LW4 (●) and LW5 (■), and two exposed-uninfected subjects EU2 (□) and EU3 (○). *a*, p24 antigen production; *b*, production of RANTES, MIP-1α and MIP-1β, from cultures inoculated with SF162.

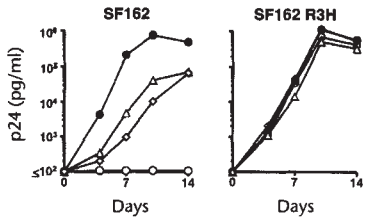

Fig. 5 Transfer of resistance by coculture of sensitive and resistant cells or the addition of recombinant chemokines. PHA-activated CD4+ lymphocytes from LW4 (●), EU3 (○), both (△), or LW4 plus 200 ng/ml of recombinant RANTES, MIP-1α and MIP-1β (◇) were inoculated with 600 $TCID_{50}$ of SF162 or SF162R3H, and p24 production was measured over 14 days. Recombinant chemokines were replenished on day 7.

However, no differences were noted in the mean fluorescence intensity of CD4 on either fresh or stimulated cells from EU2 and EU3 compared with LW4 and LW5, indicating that the block to infection in exposed-uninfected individuals is not mediated by the surface density of CD4 (data not shown).

C-C chemokines and resistance to infection

The C-C chemokines RANTES, MIP-1α and MIP-1β have been shown to inhibit replication of primary isolates, but not T-cell line-adapted strains of HIV-1 (ref. 27). Because these chemokines can be produced by multiple cell types, we tested whether the phytohemagglutinin (PHA)-activated CD4+ lymphocytes from EU2 and EU3 produced more of these chemokines than did the CD4+ lymphocytes from LW4 and LW5. As shown in Fig. 4*b*, greater amounts of all three chemokines were secreted from relatively resistant CD4+ lymphocytes than from sensitive CD4+ lymphocytes. We next tested whether the HIV-1 envelope-restricted resistance to infection could be transferred to sensitive CD4+ lymphocytes in cell-mixing experiments, and whether this could be mimicked by the addition of chemokines. Within CD4+ lymphocytes from LW4, replication of SF162, but not SF162R3H, could be inhibited >90% either by adding CD4+ lymphocytes from EU2 or by the addition of 200 ng/ml of recombinant RANTES, MIP-1α and MIP-1β (Fig. 5). These results indicate that the envelope-restricted HIV-1 inhibitory activity of these chemokines mimics the resistance observed in CD4+ lymphocytes from exposed-uninfected individuals.

Discussion

These studies demonstrate that CD4+ lymphocytes from some individuals who are at risk of acquiring HIV-1 infection through high-risk sexual activity are relatively resistant to HIV-1 infection *in vitro*. The resistance is limited to primary isolates of HIV-1 and does not extend to T-cell line-adapted strains, possibly explaining why this finding has not been reported more frequently. The resistance to HIV-1 infection *in vitro* is relative, not absolute. Even CD4+ lymphocytes that are the most resistant to HIV-1 infection in our study (from EU2 and EU3) are infectible if given a high enough inoculum of a primary isolate or a low inoculum of a T-cell line-adapted strain of HIV-1. We therefore caution that our findings are not consistent with absolute resistance to infection *in vivo*, but rather a relative resistance of CD4+ lymphocytes to infection *in vitro*. Because the infectious inoculum associated with sexual exposure is likely to be low[2,3], a small relative resistance *in vitro* may translate into substantial resistance *in vivo*.

The resistance that was observed in purified populations of CD4+ lymphocytes does not involve the action of contaminating CD8+ lymphocytes. The virus inhibitory activity in CD8+ lymphocytes from the exposed-uninfected subjects was only apparent when an equal number of CD8+ lymphocytes were added to the culture wells, and not when fivefold less were added. Therefore, small numbers of contaminating CD8+ lymphocytes cannot explain the decreased p24 antigen production from the cultures of purified CD4+ lymphocytes from exposed-uninfected subjects (Fig. 2*a*).

It is known from the results presented here and those of others, that CD4+ lymphocytes have a range of infectibilities by HIV-1 (ref. 10–13). If the infectibility of one's CD4+ lymphocytes correlates with the risk of acquiring HIV-1 infection through sexual exposure, it would be expected that there would be a high prevalence of individuals with relatively resistant CD4+ lymphocytes among individuals who fail to become infected despite multiple sexual exposures. Those with more susceptible CD4+ lymphocytes would have a greater risk of infection and therefore would be more likely to be eliminated from the uninfected group. This is fundamentally what we have observed in our study (Fig. 2*b*). However, based on previously observed rates of HIV-1 transmission in heterosexual couples[28], selective pressure exerted by exposure to HIV-1 may not be sufficient to explain the differences in infectibilities of CD4+ lymphocytes between the exposed and unexposed groups. Other factors may necessarily be involved.

What is the mechanism of the observed resistance to infection *in vitro*? We know that the relative resistance to infection is restricted by the envelope glycoprotein (Fig. 4*a*) and is not a function of the density of CD4 on the cell surface. Relatively resistant CD4+ lymphocytes therefore possess some characteristics of transformed T-cell lines, at least in terms of their abilities to be infected by HIV-1. They produce more RANTES, MIP-1α and MIP-1β than do sensitive CD4+ lymphocytes (Fig. 4*b*). The envelope-restricted resistance can be transferred from relatively resistant to sensitive cells and can be mimicked by the addition of recombinant chemokines to sensitive cells (Fig. 5). These findings suggest that chemokine production by some or all CD4+ lymphocyte clones in exposed-uninfected individuals may be operative in relative resistance to infection *in vitro*. Whether the increased production of HIV-1-inhibitory chemokines in exposed-uninfected individuals is in response to HIV-1 exposure remains to be determined.

It has previously been shown that T-helper type 1 cell clones of CD4+ lymphocytes are relatively resistant to HIV-1 infection[29] and can suppress HIV-1 replication in bystander cells[30]. It is therefore possible that some of the effect we are measuring is related to the T-helper cell profiles in our subjects, potentially in response to their exposure to HIV-1. In addition, other mechanisms may be involved. Polymorphism within CD4 (ref. 31, 32) or expression of putative second receptor molecules[33–37] could be operative in the resistance. We also cannot rule out an envelope-mediated postentry block, as has been described in lentivirus infection of macrophages[38].

Our results are consistent with previous descriptions of HLA class I- and class II-restricted HIV-1-specific immune responses in exposed-uninfected subjects[18–21]. After sexual exposure to lentiviruses, Langerhans cells within the mucosa may become infected, but dissemination may not always follow[39]. The block to HIV-1 infection in exposed-uninfected individuals could therefore be at the point of dissemination to CD4+ lymphocytes, rather than infection of mucosal Langerhans cells. The transient infection of mucosal cells could provide the milieu in which the

HLA class I- and class II-restricted responses could be generated in exposed-uninfected individuals.

Relative resistance of CD4+ lymphocytes to HIV-1 infection may also be associated with rates of disease progression. If an individual with relatively resistant CD4+ lymphocytes were to become systemically infected, that individual might have a high likelihood of long-term survival[40,41]. Indeed, CD4+ lymphocytes from some long-term survivors replicate JR-CSF less efficiently than do CD4+ lymphocytes from uninfected subjects[40]. The lack of resistance to SI viruses could also explain the rapid rise in viral load and loss of CD4+ lymphocytes that occurs when patients with stable disease courses acquire SI variants[42]. Therefore, in addition to viral and immune parameters, the *in vitro* susceptibility of CD4+ lymphocytes to HIV-1 infection should be included when considering factors that affect HIV-1 transmission and rates of disease progression.

Methods

Virus isolates. Isolates of HIV-1 used in this study include NL4-3 (ref. 43) , JR-CSF (ref. 44), AD-6 (from an acute seroconvertor)[45], GT (from an individual who transmitted HIV-1 to his sexual partner), 92US657 (ref. 46), SF162, SF162dbl (previously called I307R/A312V), SF162R3H (previously called R3H), and SF2 (ref. 24). Isolates NL4-3, SF162dbl, SF162R3H, and SF2, were SI and T-cell line tropic as determined by syncytium-induction in MT-2 cells[42]. All others were NSI and non-T-cell line tropic. Virus stocks were prepared by expansion on PHA-activated PBMC. Infectious titers were determined by serial dilution on PHA-stimulated PBMC from random donors[40,47,48].

HIV-1 inhibitory activity mediated by CD8+ lymphocytes. Ficoll-Hypaque isolated PBMC were enriched for CD4+ lymphocytes (>96% purity) by removal of CD8+ lymphocytes using anti-CD8 immunomagnetic beads (DYNAL, Great Neck, New York)[40]. The CD4+ lymphocytes were stimulated for 3 days in culture medium (RPMI 1640 supplemented with antibiotics and 10% fetal bovine serum) containing interleukin-2 (100 units/ml) (Hoffmann–LaRoche, Nutley, New Jersey) and PHA (5 μg/ml). CD8+ lymphocytes recovered from the beads were stimulated for 3 days with anti-CD3 monoclonal antibody, irradiated allogeneic feeders and interleukin-2 (ref. 40). One million CD4+ lymphocytes were cultured in the presence or absence of varying numbers of CD8+ lymphocytes (1 million to 1600 cells in serial fivefold dilutions) and inoculated with 3000 median tissue culture infectious doses ($TCID_{50}$) of JR-CSF (multiplicity of infection (MOI) = 0.003). After 24 h the free virus was removed, and the cultures were carried for a further 14 days with feeding and p24 sampling on days 4, 7, 10 and 14. Measurement of p24 antigen levels was by immunoassay (Abbott Laboratories, Abbott Park, Illinois).

CD4+ lymphocyte susceptibility to HIV-1 infection. HIV-1 infection of CD4+ lymphocytes was performed on cryopreserved samples. PBMC were stimulated with PHA for 3 days after which the CD8+ lymphocytes were removed. Isolates of HIV-1 were added to 2×10^5 activated CD4+ lymphocytes at an inoculum of 600 $TCID_{50}$ (MOI = 0.003), or where cell numbers allowed, at serial fivefold dilutions down to 0.2 $TCID_{50}$. Cultures were carried for 14 days with feeding and p24 sampling on days 4, 7, 10 and 14. The lowest inoculum that produced >100 pg of p24 after 14 days in culture was taken as the end-point dilution required to establish infection[47]. In selected cases triplicate wells of CD4+ lymphocytes were cultured for three days before the addition of 1 μCi of tritiated thymidine (Du Pont NEN, Wilmington, Delaware). Radionucleotide incorporation was determined by scintillation counting 18 h later. Cell counts and viability testing by trypan blue exclusion were also performed.

Density of CD4 antigen on the cell surface. Unstimulated or 3-day PHA-activated PBMC were reacted with anti-CD3 monoclonal antibody (UCHT1, Pharmingen, San Diego, California) labeled with R-phycoerythrin and monoclonal antibody Leu3a (Becton Dickinson, San Jose, California) labeled with fluorescein isothiocyanate. Results were obtained by five-parameter, three-color analysis on a FACS Vantage (Becton-Dickinson) after 488 nm excitation.

Experiments involving chemokines. Levels of RANTES, MIP-1α, and MIP-1β were measured by immunoassay (R&D Systems, Minneapolis, Minnesota). Recombinant RANTES, MIP-1α and MIP-1β were purchased (R&D Systems) and added at 200 ng/ml each to cultures of PHA-activated CD4+ lymphocytes. Cultures were supplemented with recombinant chemokines every 7 days.

Statistical analysis. Mann-Whitney U-test was used to calculate differences between experimental and control groups.

Acknowledgments

We are indebted to Project Achieve for patient referral, the patients themselves for their dedication to the study, M. Gately of Hoffmann–LaRoche for providing IL-2, J. Wong of the Massachusetts General Hospital for providing anti-CD3 monoclonal antibody, C. Cheng-Mayer and A. Trkola for providing virus isolates, J. Abrams and K. Grijalva for data coordination, A. Whetsell for technical assistance, C. Andrews, D.D. Ho and J.P. Moore for critical comments on the manuscript, and W. Chen for preparation of figures. Supported by grants and contracts (AI 30358, AI 35522, AI 45218, AI 95013) from the National Institutes of Health, the Center for AIDS Research of New York University School of Medicine (AI 27742), and the Aaron Diamond Foundation. R.A.K. is an Elizabeth Glaser Scientist of the Pediatric AIDS Foundation.

RECEIVED 22 NOVEMBER 1995; ACCEPTED 27 FEBRUARY 1996

1. Detels, R. *et al.* Resistance to HIV-1 infection. *J. Acquir. Immun. Defic. Syndr.* **7**, 1263–1269 (1994).
2. DeGruttola, V., Seage, G.R., Mayer, K.H. & Horsburgh, C.R. Infectiousness of HIV between male homosexual partners. *J. Clin. Epidemiol.* **42**, 849–856 (1989).
3. Prevots, D.R., Ancelle-Park, R.A., Neal, J.J. & Remis, R.S. The epidemiology of heterosexually acquired HIV infection and AIDS in Western industrialized countries. *AIDS* **8**, S109–S117 (1994).
4. Taylor, R. Quiet clues to HIV-1 immunity: Do some people resist infection? *J. NIH Res.* **6**, 29–31 (1994).
5. Kaslow, R.A. *et al.* A1, Cw7, B8, DR3 HLA antigen combination associated with rapid decline of T-helper lymphocytes in HIV-1 infection: A report from the Multicenter AIDS Cohort Study. *Lancet* **335**, 927–930 (1990).
6. Steel, C.M. *et al.* HLA haplotype A1 B8 DR3 as a risk factor for HIV-related disease. *Lancet* **1**, 1185–1188 (1988).
7. Kilpatrick, D.C., Hague, R.A., Yap, P.L. & Mok, J.Y. HLA antigen frequencies in children born to HIV-infected mothers. *Dis. Markers* **9**, 21–26 (1991).
8. Just, J. *et al.* Genetic risk factors for perinatally acquired HIV-1 infection. *Paediatr. Perinat. Epidemiol.* **6**, 215–224 (1992).
9. Klein, M.R. *et al.* Associations between HLA frequencies and pathogenic features of human immunodeficiency virus type 1 infection in seroconverters from Amsterdam cohort of homosexual men. *J. Infect. Dis.* **169**, 1244–1249 (1994).
10. Wainberg, M.A., Blain, N. & Fitz-Gibbon, L. Differential susceptibility of human lymphocyte cultures to infection by HIV. *Clin. Exp. Immunol.* **1987**, 136–142 (1987).
11. Cloyd, M.W. & Moore, B.E. Spectrum of biological properties of human immunodeficiency virus (HIV-1) isolates. *Virology* **174**, 103–116 (1990).
12. Williams, L.M. & Cloyd, M.W. Polymorphic human gene(s) determines differential susceptibility of CD4 lymphocytes to infection by certain HIV-1 isolates. *Virology* **184**, 723–728 (1991).
13. Spira, A. & Ho, D.D. Effect of different donor cells on human immunodeficiency virus type 1 replication and selection in vitro. *J. Virol.* **69**, 422–429 (1995).
14. Ometto, L. *et al.* Viral phenotype and host-cell susceptibility to HIV-1 infection

as risk factors for mother-to-child HIV-1 transmission. *AIDS* **9**, 427–434 (1995).
15. Lederman, M.M. *et al.* Human immunodeficiency virus (HIV) type 1 infection status and in vitro susceptibility to HIV infection among high-risk HIV-1-seronegative hemophiliacs. *J. Infect. Dis.* **172**, 228–231 (1995).
16. Roos, M.T.L. *et al.* Viral phenotype and immune response in primary human immunodeficiency virus type 1 infection. *J. Infect. Dis.* **165**, 427–432 (1992).
17. Zhu, T. *et al.* Genotypic and phenotypic characterization of HIV-1 in patients with primary infection. *Science* **261**, 1179–1181 (1993).
18. Rowland-Jones, S.L. *et al.* HIV-specific cytotoxic T-cell activity in an HIV-exposed but uninfected infant. *Lancet* **341**, 860–861 (1993).
19. Langlade-Demoyen, P., Ngo-Giang-Huong, N., Ferchal, F. & Oksenhendler, E. Human immunodeficiency virus (HIV) nef-specific cytotoxic T lymphocytes in noninfected heterosexual contacts of HIV-infected patients. *J. Clin. Invest.* **93**, 1293–1297 (1994).
20. Rowland-Jones, S. *et al.* HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. *Nature Med.* **1**, 59–64 (1995).
21. Clerici, M. *et al.* Cell-mediated immune response to human immunodeficiency virus (HIV) type-1 in seronegative homosexual men with recent sexual exposure to HIV-1. *J. Infect. Dis.* **165**, 1012–1019 (1992).
22. Mackewicz, C. & Levy, J.A. $CD8^+$ cell anti-HIV activity: Nonlytic suppression of virus replication. *AIDS Res. Hum. Retrovir.* **8**, 1039–1050 (1992).
23. Whetsell, A.J. *et al.* Comparison of three nonradioisotopic polymerase chain reaction-based methods for detection of human immunodeficiency virus type 1. *J. Clin. Microbiol.* **30**, 845–853 (1992).
24. Harrowe, G. & Cheng-Mayer, C. Amino acid substitutions in the V3 loop are responsible for adaptation to growth in transformed T-cell lines of a primary human immunodeficiency virus type 1. *Virology* **210**, 490–494 (1995).
25. Kabat, D., Kozak, S.L., Wehrly, K. & Chesebro, B. Differences in CD4 dependence for infectivity of laboratory-adapted and primary patient isolates of human immunodeficiency virus type 1. *J. Virol.* **68**, 2570–2577 (1994).
26. Brand, D., Srinivasan, K. & Sodroski, J. Determinants of human immunodeficiency virus type 1 entry in the CDR2 loop of the CD4 glycoprotein. *J. Virol.* **69**, 166–171 (1995).
27. Cocchi, F. *et al.* Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by $CD8^+$ T cells. *Science* **270**, 1811–1815 (1995).
28. O'Brien, T.R. *et al.* Heterosexual transmission of human immunodeficiency virus type 1 from transfusion recipients to their sex partners. *J. Acquir. Immune Defic. Syndr.* **7**, 705–710 (1994).
29. Maggi, E. *et al.* Ability of HIV to promote a TH1 to TH0 shift and to replicate preferentially in TH2 and TH0 cells. *Science* **265**, 244–248 (1994).
30. Vyakaram, A., Matear, P.M., Martin, S.J. & Wagstaff, M. Th1 cells specific for HIV-1 gag p24 are less efficient than Th0 cells in supporting HIV replication, and inhibit virus replication in Th0 cells. *Immunology* **86**, 85–96 (1995).
31. Hodge, T.W., Sasso, D.R. & McDougal, J.S. Humans with OKT4-epitope deficiency have a single nucleotide base change in the CD4 gene, resulting in substitution of TRP240 for ARG240. *Hum. Immunol.* **30**, 99–104 (1991).
32. Ghabanbasani, M.Z. *et al.* Possible association of CD3 and CD4 polymorphisms with insulin-dependent diabetes mellitus (IDDM). *Clin. Exp. Immunol.* **97**, 517–521 (1994).
33. Callebaut, C., Krust, B., Jacotot, E. & Hovanessian, A.G. T cell activation antigen, CD26, as a cofactor for entry of HIV in $CD4^+$ cells. *Science* **262**, 2045–2050 (1993).
34. Bhat, S., Spitalnik, S.L., Gonzalez-Scarano, F. & Silberberg, D.H. Galactosyl ceramide or a derivative is an essential component of the neural receptor for human immunodeficiency virus type 1 envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA* **88**, 7131–7134 (1991).
35. Patel, M. *et al.* Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. *AIDS Res. Hum. Retrovir.* **9**, 167–174 (1993).
36. Roderiquez, G. *et al.* Mediation of human immunodeficiency virus type 1 binding by interaction of cell surface heparan sulfate proteoglycans with the V3 region of envelope gp120-gp41. *J. Virol.* **69**, 2233–2239 (1995).
37. Stefano, K.A. *et al.* Replication of a macrophage-tropic strain of human immunodeficiency virus type 1 (HIV-1) in a hybrid cell line, CEMx174, suggests that cellular accessory molecules are required for HIV-1 entry. *J. Virol.* **67**, 6707–6715 (1993).
38. Mori, K., Ringler, D.J. & Desrosiers, R.C. Restricted replication of simian immunodeficiency virus strain 239 in macrophages is determined by env but is not due to restricted entry. *J. Virol.* **67**, 2807–2814 (1993).
39. Spira, A.I. *et al.* Cellular targets of infection and route of viral dissemination following an intravaginal inoculation of SIV into rhesus macaques. *J. Exp. Med.* **183**, 215–225 (1996).
40. Cao, Y., Qing, L., Zhang, L.Q., Safrit, J.T. & Ho, D.D. Virological and immunological characterization of long-term survivors of HIV-1 infection. *N. Engl. J. Med.* **332**, 201–208 (1994).
41. Schrager, L.K., Young, J.M., Fowler, M.G., Mathieson, B.J. & Vermund, S.H. Long-term survivors of HIV-1 infection: Definitions and research challenges. *AIDS* **8**, S95–S108 (1994).
42. Connor, R.I., Mohri, H., Cao, Y. & Ho, D.D. Increased viral burden and cytopathicity correlate temporally with $CD4^+$ T-lymphocyte decline and clinical progression in HIV-1 infected individuals. *J. Virol.* **67**, 1772–1778 (1993).
43. Adachi, A. *et al.* Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J. Virol.* **59**, 284–291 (1986).
44. Koyanagi, Y. *et al.* Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. *Science* **236**, 819–822 (1987).
45. Koup, R.A. *et al.* Temporal association of cellular immune responses with the initial control of viremia in primary HIV-1 syndrome. *J. Virol.* **68**, 4650–4655 (1994).
46. Moore, J.P. *et al.* Exploration of antigenic variation in gp120 from clades A through F of human immunodeficiency virus type 1 by using monoclonal antibodies. *J. Virol.* **68**, 8350–8364 (1994).
47. Reed, L.J. & Muench, H. A simple method of estimating 50 percent endpoints. *Am. J. Hyg.* **27**, 493–499 (1938).
48. Koup, R.A. & Ho, D.D. Quantitative culture assay for HIV-1 in peripheral blood. in *Techniques in HIV Research* (eds Aldovini, A. & Walker, B.D.) 107–112 (Stockton, New York, 1990).