high as 350 K) of dimer row island formation in Si(100) has intriguing consequences. Besides providing a logical pathway from adatom adsorption and diffusion to growth of islands, these chain structures and their prevalence suggest that the smallest and most obvious stable structure on the surface, the ad-dimer residing on top of the dimer rows, does not participate in a fundamental way in the growth of larger dimer row islands. If this is so, it will require reevaluation (at least on semiconductor surfaces) of the concept of a critical nucleus for growth and of the many rate equation models for diffusion and growth on this surface, which all require the size of a critical nucleus as input. We no longer appear to have, at these temperatures, a well-defined critical or stable nucleus because growth proceeds from long-lived metastable structures that may have a variety of sizes.

REFERENCES AND NOTES

1. For a review and an extended list of references, see Z. Zhang and M. G. Lagally, *Science* **276**, 377 (1997).
2. Y.-W. Mo and M. G. Lagally, *Surf. Sci.* **248**, 313 (1991); Y.-W. Mo, J. Kleiner, M. B. Webb, M. G. Lagally, *Phys. Rev. Lett.* **66**, 1998 (1991); *Surf. Sci.* **268**, 275 (1992).
3. R. J. Hamers, U. Köhler, J. E. Demuth, *Ultramicroscopy* **31**, 10 (1989); Y.-W. Mo, R. Kariotis, D. E. Savage, M. G. Lagally, *Surf. Sci.* **219**, L551 (1989); J. Y. Tsao, E. Chason, U. Köhler, R. J. Hamers, *Phys. Rev. B* **40**, 11951 (1989).
4. J. A. Venables, *Philos. Mag.* **27**, 697 (1973). The "critical nucleus" is defined as that structural entity for which the addition of one more atom will for the first time reduce the free energy of the entity. It can be as little as one atom. The stable nucleus is the smallest structural entity with a negative free energy. It can be as little as two atoms.
5. Y.-W. Mo, R. Kariotis, B. S. Swartzentruber, M. B. Webb, M. G. Lagally, *J. Vac. Sci. Technol. A* **8**, 201 (1990).
6. P. J. Bedrossian, *Phys. Rev. Lett.* **74**, 3648 (1995).
7. G. Brocks and P. J. Kelly, *ibid.* **76**, 2362 (1996).
8. J. van Wingerden, A. van Dam, M. J. Haye, P. M. L. O. Scholte, F. Tuinstra, *Phys. Rev. B* **55**, 4723 (1997).
9. W. Wulfhekel, B. J. Hattink, H. J. W. Zandvliet, G. Rosenfeld, B. Poelsema, *Phys. Rev. Lett.* **79**, 2494 (1997).
10. V. Milman *et al.*, *Phys. Rev. B* **50**, 2663 (1994).
11. B. S. Swartzentruber, *ibid.* **55**, 1322 (1997).
12. Q.-M. Zhang, C. Roland, P. Boguslawski, J. Bernholc, *Phys. Rev. Lett.* **75**, 101 (1995).
13. J. A. Appelbaum, G. A. Baraff, D. R. Hamann, *Phys. Rev. B* **14**, 588 (1976).
14. X. R. Qin and M. G. Lagally, unpublished data.

15. We thank B. Swartzentruber for valuable advice and help. Supported by NSF (grant DMR93-04912) and by Sandia National Laboratory (grant AS-1168).

14 July 1997; accepted 14 October 1997

Vigorous HIV-1–Specific CD4$^+$ T Cell Responses Associated with Control of Viremia

Eric S. Rosenberg, James M. Billingsley, Angela M. Caliendo, Steven L. Boswell, Paul E. Sax, Spyros A. Kalams, Bruce D. Walker*

Virus-specific CD4$^+$ T helper lymphocytes are critical to the maintenance of effective immunity in a number of chronic viral infections, but are characteristically undetectable in chronic human immunodeficiency virus–type 1 (HIV-1) infection. In individuals who control viremia in the absence of antiviral therapy, polyclonal, persistent, and vigorous HIV-1–specific CD4$^+$ T cell proliferative responses were present, resulting in the elaboration of interferon-γ and antiviral β chemokines. In persons with chronic infection, HIV-1–specific proliferative responses to p24 were inversely related to viral load. Strong HIV-1–specific proliferative responses were also detected following treatment of acutely infected persons with potent antiviral therapy. The HIV-1–specific helper cells are likely to be important in immunotherapeutic interventions and vaccine development.

Infection with HIV-1 is characterized by a quantitative decline in the number of CD4$^+$ lymphocytes and a qualitative impairment of their function (*1*). Immunological abnormalities in T helper cell function occur early, during the asymptomatic phase of infection and before the loss in CD4$^+$ cell number (*2*). Loss of T cell function in vitro predicts progression to acquired immunodeficiency syndrome (AIDS) and a decrease in survival time (*3*). In addition to these general defects in lymphocyte function, infection typically fails to induce detectable HIV-1–specific proliferative responses (*4*). It is thought that CD4$^+$ helper cell responses play a key role in maintaining effective immunity in murine models of chronic viral infections (*5*). In HIV-1 infection, the lack of virus-specific proliferative responses is the most dramatic defect in the repertoire of the immune system. When such responses have been observed, they are typically weak, with stimulation indices (SIs) rarely greater than 5 (*6*).

Recently, a subset of HIV-1–infected persons who appear to successfully control virus replication in the absence of antiretroviral therapy has been identified. Despite infections of up to 18 or more years, these individuals maintain normal CD4$^+$ T cell counts, low to undetectable viral loads, and have no evidence of HIV-1–related disease manifestations (*7*). The persistence of vigorous cytolytic T lymphocyte (CTL) and humoral immune responses in some of these individuals suggests that the host immune response may be effectively containing viral replication (*8*). Given the demonstrated importance of virus-specific CD4$^+$ T helper cell responses in other chronic viral infections, we examined individuals with long-term nonprogressive infection for evidence of such responses directed against HIV-1.

Initial studies were performed in an HIV-1–infected hemophiliac (subject 161-J) with 18 years of documented seropositivity, a normal CD4$^+$ T cell count, and a viral load of <400 RNA molecules per milliliter of plasma, who had never been treated with antiretroviral agents. Consistent with previous studies (*9*), an extremely vigorous CTL memory response was detected, with more than 1 HIV-1–specific CTL per 200 peripheral blood mononuclear cells (PBMC) (*10*). Freshly isolated PBMC from this subject were exposed to whole soluble HIV-1 p24 and gp160 protein, resulting in vigorous virus-specific lymphocyte proliferation (Fig. 1A). Nearly identical results were obtained with HIV-1 antigens derived from baculovirus, yeast, and Chinese hamster ovary (CHO) cells, whereas control antigens derived from the same sources elicited no responses. The PBMC stimulated with p24 resulted in the most vigorous lymphocyte proliferation, with SI > 200 to baculovirus- and CHO-derived antigens. Envelope protein gp160 elicited a less intense but significant lymphocyte proliferative response to baculovirus- and yeast-derived antigen. The responses were mediated by the CD4$^+$ T lymphocyte subset, as demonstrated by loss of activity with depletion of CD4$^+$ cells (Fig. 1B). These virus-specific proliferative responses were highly repro-

E. S. Rosenberg, J. M. Billingsley, S. A. Kalams, B. D. Walker, Partners AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, and Harvard Medical School, Boston, MA 02114, USA.
A. M. Caliendo, Partners AIDS Research Center and Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA.
S. L. Boswell, Fenway Community Health Center, Boston, MA 02115, USA.
P. E. Sax, Partners AIDS Research Center and Division of Infectious Diseases, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115, USA.

*To whom correspondence should be addressed. E-mail: bwalker@helix.mgh.harvard.edu

ducible over the 2 years of observation (*11*), indicating that such responses can persist in the absence of detectable viremia and in the absence of disease progression.

Having observed a strong virus-specific proliferative response in a subject who is controlling viral replication, we next examined the association between plasma HIV-1 RNA viral load and lymphocyte proliferative responses in a cohort of individuals with a wide range of viral loads. Ten HIV-1–infected individuals with varying clinical histories and viral loads who had never been treated with antiretroviral therapy were evaluated (*12*). The relation between viral load and proliferative response to p24 antigen was examined by linear regression. The analysis demonstrated a highly significant inverse correlation (Fig. 2): Individuals with the strongest p24-specific proliferative responses had the lowest viral loads, and those individuals with higher HIV-1 viral loads had a markedly decreased ability to respond to p24 ($R = -0.80$, $P < 0.006$), whereas analysis of CD4 count versus viral load showed only a trend ($P < 0.06$). For eight of the individuals evaluated, assays were repeated on a second visit, and the results were similar. This inverse association was confirmed in a second independent cohort of 15 infected, untreated persons from San Francisco [$R = -0.78$, $P < 0.001$ (*13*)]. Comparison of proliferative responses to Env protein and viral load did not show a correlation, but significant Env-specific responses were seen exclusively in persons with low viral loads (*14*).

To ensure that the observed $CD4^+$ T cell responses were not just reflective of the duration of infection, we evaluated a long-term nonprogressing patient with a high viral load. This subject had documented HIV-1 infection for more than 10 years, a CD4 cell count of 900 cells/mm^3, and a viral load of 175,000 RNA molecules per milliliter of plasma. In this individual, SI to p24 and gp160 antigen remained at <2.5 even 9 months after the viral load was reduced to less than 400 RNA copies per milliliter of plasma by initiation of combination antiretroviral therapy. We also detected no evidence of HIV-1–specific proliferative responses to either p24 or gp160 in 15 seronegative lab workers and 20 individuals who had been repeatedly exposed sexually to HIV-1 but remain uninfected (p24: mean SI = 1.5, range = 0.5 to 4.8; gp160: mean SI = 1.2, range = 0.1 to 5.0).

To further characterize the HIV-1–specific lymphocyte responses, we examined the in vitro kinetics of lymphocyte proliferation over a 6-day period in two persons with robust responses. Both subjects demonstrated vigorous proliferative responses to both p24 and gp160, with detectable responses as early as day 2 (Fig. 3, A and B). In contrast, subjects with undetectable responses at day 6 likewise had undetectable responses at earlier times (*14*). Induction of proliferation was achieved with concentrations of p24 antigen as low as 0.05 μg/ml (*15*). Limiting dilution analysis revealed a frequency of p24-specific CD4 cells of greater than 1 per 10,000 PBMC for subject 161-J and greater than 1 per 19,000 PBMC for subject CTS-01 (*16*). Exposure to HIV-1 antigen resulted in the specific induction of interferon-γ production (Fig. 3C), indicating that the response is Th-1–like (*17*). Similarly, stimulation with viral antigen resulted in the production of chemokines RANTES and the macrophage inflammatory proteins MIP-1α and MIP-1β (Fig. 3D), which have been shown to mediate potent inhibition of macrophage tropic strains of HIV-1 (*18*). The p24-specific proliferation was not associated with the production of interleukin-4 (IL-4) or IL-10, and no IL-4, IL-10, or interferon-γ was detected in persons who lacked detectable proliferative responses to p24 (*19*). Proliferative responses to HIV-1 antigen were abrogated with the addition of 100 ng/ml of rIL-10 (*19*), suggesting that a Th-2–type cytokine environment may have a profound antiproliferative effect (*20*).

We further characterized two individuals with strong p24-specific helper cell responses to determine the dominant epitopes targeted. When overlapping 22–amino acid peptides spanning p24 were used, peptide-specific proliferative responses (conservatively defined as an SI > 10) were detected in both individuals, confirming the presence of virus-specific proliferative responses and demonstrating that multiple epitopes are targeted. The dominant response in subject 161-J was to a peptide in the NH_2-terminal portion of p24 [residues 133 to 154: PIVQNIQGQM-VHQAISPRTLNA (*21*)] with an SI of 83, whereas the dominant response in subject CTS-01 was to the peptide composed of residues 213 to 234 (DRVHPVHAGPI-APGQMREPRGS), with an SI of 102. Some peptides were targeted by both individuals, whereas other peptides eliciting SI > 10 were differentially recognized (*22*). For both subjects, whole protein generated higher stimulation indices than did any individual peptide, consistent with the polyclonal nature of the response. No significant proliferative responses to HIV-1 peptides were detected in HIV-1 seronegative control subjects (*23*).

We next examined the effect of antiviral therapy instituted during primary infection on the HIV-1–specific proliferative response, to determine if the inhibition of viral replication early in the course of in-

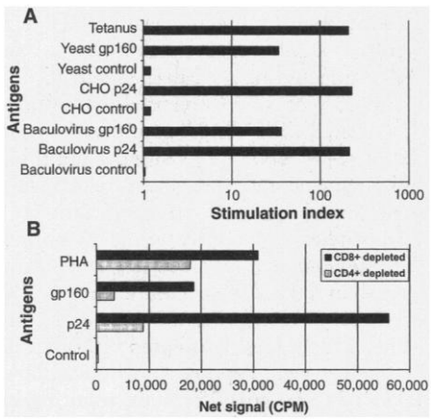

Fig. 1. HIV-1–specific $CD4^+$ proliferative responses to HIV-1 antigens. (**A**) In vitro lymphocyte proliferative responses were determined for PBMC from an asymptomatic HIV-1 infected subject (subject 161-J). PBMC were isolated by Ficoll-Hypaque density centrifugation and tested in a standard [^{3}H]thymidine uptake assay using the designated antigens derived from HIV-1 gp160 and p24, as well as control proteins (*30*). (**B**) HIV-1–specific proliferation is mediated by the $CD4^+$ lymphocyte subset. Aliquots of PBMC from the subject in (A) were depleted of either the $CD8^+$ or $CD4^+$ fraction with the use of antibody-coated flasks according to manufacturer's instructions (Applied Immune Sciences, Menlo Park, California) and were tested for proliferation to HIV-1 antigens and PHA (phytohemagglutinin). Efficiency of $CD8^+$ and $CD4^+$ depletions was 94.7 and 89.3%, respectively.

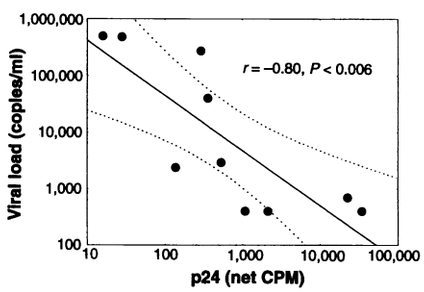

Fig. 2. p24-Specific $CD4^+$ lymphocyte proliferation is inversely correlated with HIV-1 plasma viral load. Proliferative assays and simultaneous plasma HIV-1 viral load measurements (solid circles) were performed on 10 individuals with documented HIV-1 infection with no prior antiretroviral therapy (four long-term nonprogressors and six persons with chronic infection of variable duration). The relation between viral load and proliferative response to p24 antigen was examined by linear regression performed by the Statistica software package (Statsoft, Tulsa, Oklahoma). In those subjects with an undetectable viral load, a value of 400 copies/ml (minimum detectable value) was assigned. The solid line represents the linear regression, and the dotted line represents the 95% confidence interval. Proliferation assays were performed as described in Fig. 1.

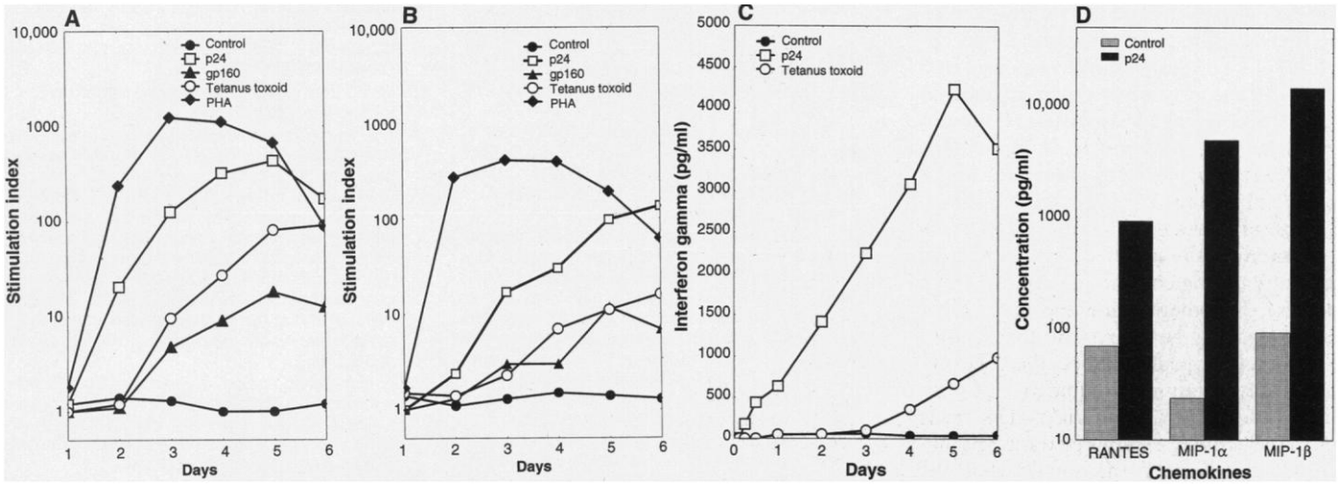

Fig. 3. Kinetics and cytokine patterns of HIV-1–specific proliferative responses in two individuals with long-term nonprogressive HIV-1 infection and low to undetectable HIV-1 viral loads. (**A** and **B**) The in vitro kinetics of subjects 161-J and CTS-01, respectively. Proliferation assays were harvested every 24 hours (*30*). Subject 161-J was described above; subject CTS-01 is a male who was infected sexually with HIV-1 who has never been treated with antiretroviral therapy, with 14 years of documented asymptomatic infection, an HIV-1 viral load of 700 copies/ml, and a CD4 count of 900 cells/mm^3. The corresponding ΔCPM values at peak proliferation for subject 161-J (day 5) were PHA = 42,451; p24 = 31,746; gp160 = 1269; and tetanus = 5145; and for subject CTS-01 (day 6) were PHA = 7728; p24 = 22,567; gp160 = 992; and tetanus = 1895. (**C**) In vitro kinetics of interferon-γ cytokine production were determined by stimulating PBMC from subject 161-J as described (*30*) and harvesting cell supernatants at the designated times. Cell culture supernatants were analyzed by enzyme-linked immunosorbent assay (ELISA) (Endogen, Cambridge, Massachusetts) for the presence of interferon-γ. (**D**) PBMC from subject 161-J stimulated with antigen and cell culture supernatants were collected on day 6. Supernatants were analyzed by ELISA for the presence of RANTES, MIP-1α, and MIP-1β (R&D Systems, Minneapolis, Minnesota).

fection allowed generation of this response. Three individuals diagnosed with acute HIV-1 retroviral syndrome (*24*) and treated with combination antiviral therapy before seroconversion all demonstrated the gradual generation of virus-specific proliferative responses as viral load was suppressed to undetectable levels (Fig. 4). With the initiation of aggressive antiviral therapy, the lowering of plasma viral load was strongly associated with the generation of a p24-specific proliferative response ($R = -0.85$, $P < 0.008$) (Fig. 4D). These results contrast with the reported lack of strong HIV-1–specific proliferative responses after initiation of potent antiviral therapy in chronic infection (*25*), and with the lack of detectable responses in a person tested 6 months after seroconversion who was not treated with antiviral therapy [subject JH-5300 (*12*)]. No untreated patients identified at the time of primary infection were available as additional controls, because all patients chose to follow recommendations to initiate antiviral therapy.

These data provide evidence that HIV-1 induces a strong HIV-1–specific proliferative response in persons who are controlling viremia in the absence of antiretroviral therapy and suggest that early, aggressive treatment of primary infection may facilitate the generation of these responses. Although disease progression in HIV-1 infec-

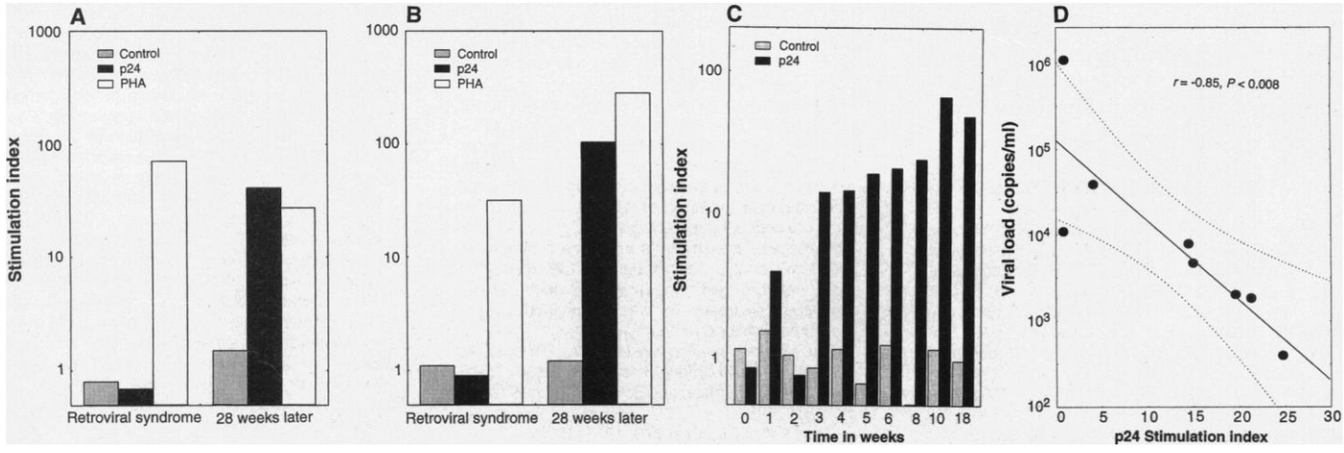

Fig. 4. Detection of HIV-1–specific proliferative responses after combination antiretroviral therapy during primary HIV-1 infection. (**A**, **B**, and **C**) Lymphocyte proliferation assays for three individuals diagnosed with primary HIV-1 infection and acute retroviral syndrome (*23*). All individuals began treatment with three drug combination therapy (including a protease inhibitor) at the time of diagnosis and had viral loads <400 copies/ml within 2 months of initiating therapy. (**D**) Gradual generation of a p24-specific proliferative response is associated with the lowering of HIV-1 viral load during primary HIV-1 infection. Proliferation assays were performed at baseline and every 7 to 10 days after the initiation of combination antiretroviral therapy for the subject in (C) (solid circles). The relation between viral load and p24 proliferative response was analyzed by linear regression; the solid line represents the linear regression, and the dotted lines represent the 95% confidence interval.

tion is likely to depend on several factors, the inverse association between p24-specific $CD4^+$ T cell proliferative responses and viral load is consistent with the hypothesis that these responses contribute to immunological control of virus replication. This hypothesis is supported by animal models of chronic viral infection, which have shown that the presence of functional $CD4^+$ cells is essential for the maintenance of effective immunity during chronic infections (*5*). In addition, cytomegalovirus-specific helper cells appear to be important for maintenance of cytomegalovirus-specific CTLs after adoptive transfer in humans (*26*). In HIV-1 infection, virus-specific $CD4^+$ cells may be selectively eliminated during primary infection, because these cells would be activated during a period of high-level viremia and therefore would be particularly susceptible to infection (*27*). Alternatively, such cells could be selectively eliminated by activation-induced cell death during the period of persistent high antigen load in primary infection (*28*). The mechanisms whereby $CD4^+$ cells contribute to the maintenance of effective antiviral immunity are not known but may relate to enhanced CTL precursor activity, increased production of antiviral cytokines, or augmentation of humoral immune responses.

Therapy with potent antiviral agents in persons with chronic infection has resulted in modest increases in CD4 cell function but has not resulted in the restoration of strong or persistent HIV-1–specific $CD4^+$ T cell proliferative responses (*25*). Whether benefit would be derived from the induction of virus-specific $CD4^+$ T cells in chronically infected individuals, in conjunction with control of viral load by antiviral drugs, deserves to be tested. Induction of strong HIV-specific $CD4^+$ proliferative responses have already been achieved with DNA vaccination in an animal model, suggesting that the means to test this are available (*29*).

REFERENCES AND NOTES

1. H. W. Murray, B. Y. Rubin, H. Masur, R. B. Roberts, *N. Engl. J. Med.* **310**, 883 (1984); J. S. Epstein *et al.*, *J. Infect. Dis.* **152**, 727 (1985); H. C. Lane *et al.*, *N. Engl. J. Med.* **313**, 79 (1985).
2. F. Miedema, *J. Clin. Invest.* **82**, 1908 (1988); J. Laurence, S. M. Friedman, E. K. Chartash, M. K. Crow, D. N. Posnett, *ibid.* **83**, 1843 (1989); M. Clerici *et al.*, *Nature* **339**, 383 (1989).
3. M. T. Roos *et al.*, *J. Infect. Dis.* **171**, 531 (1995); M. J. Dolan *et al.*, *ibid.* **172**, 79 (1995).
4. F. Miedema *et al.*, *Immunol. Rev.* **140**, 35 (1994).
5. M. Battegay *et al.*, *J. Virol.* **68**, 4700 (1994); M. Matloubian, R. J. Conception, R. Ahmed, *ibid.*, p. 8056; M. G. von Herrath, M. Yokoyama, J. Dockter, M. B. Oldstone, J. L. Whitton, *ibid.* **70**, 1072 (1996); A. R. Thomsen, J. Johansen, O. Marker, J. P. Christensen, *J. Immunol.* **157**, 3074 (1996); O. Planz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 6874 (1997).
6. B. Wahren *et al.*, *J. Virol.* **61**, 2017 (1987); J. A. Berzofsky *et al.*, *Nature* **334**, 706 (1988); J. F. Krowka *et al.*, *J. Clin. Invest.* **83**, 1198 (1989); R. D. Schrier *et al.*, *J. Immunol.* **142**, 1166 (1989); D. Schwartz *et al.*, *AIDS Res. Hum. Retroviruses* **10**, 1703 (1994); O. Pontesilli *et al.*, *ibid.*, p. 113.
7. B. F. Haynes, G. Pantaleo, A. S. Fauci, *Science* **271**, 324 (1996).
8. M. R. Klein *et al.*, *J. Exp. Med.* **181**, 1365 (1995); Y. Cao, L. Qin, L. Zhang, J. Safrit, D. D. Ho, *N. Engl. J. Med.* **332**, 201 (1995); G. Pantaleo *et al.*, *ibid.*, p. 209; C. Rinaldo *et al.*, *J. Virol.* **69**, 5838 (1995).
9. T. Harrer *et al.*, *J. Immunol.* **156**, 2616 (1996).
10. The PBMC were cultured at 250 to 16,000 cells per well in 24 replicate wells of a 96-well microtiter plate. To each well, 2.5×10^4 γ-irradiated (30 Gy) PBMC from an HIV-1 seronegative donor were added, along with CD3-specific monoclonal antibody 12F6 at 0.1 μg/ml. Fourteen days later, the wells were split and assayed for cytotoxicity on ^{51}Cr-labeled autologous B-LCL infected with vaccinia expressing HIV-1 gene products. The fraction of nonresponding wells was defined as the number of wells in which ^{51}Cr release did not exceed the mean plus three standard deviations of the spontaneous release of the 24 control wells over the number of the assayed wells. Activated cell frequency was calculated by the maximum likelihood method [F. de St. Groth, *J. Immunol. Methods* **49**, 11 (1982)].
11. The HIV-1–specific proliferative responses to p24 were tested 12 times over 24 months, with SIs ranging from 38 to 465 (mean, 134).
12. Subjects participating in this study were all documented to be HIV-1–positive by HIV-1/2 enzyme immunoassay and confirmed by protein immunoblot. They included four long-term nonprogressors with low viral loads and persons with progressive infection. Each individual signed an informed-consent agreement approved by the institutional review board. CD4 counts (and viral loads) for the 10 subjects: DF-2851, 243 cells/mm^3 (503,000 copies/ml); PJ-9202, 80 cells/mm^3 (485,000 copies/ml); JH-5300, 507 cells/mm^3 (279,000 copies/ml); MK-099, 420 cells/mm^3 (58,228 copies/ml); PG-9011, 1400 cells/mm^3 (2950 copies/ml); MK-089, 1099 cells/mm^3 (2396 copies/ml); CTS-01, 900 cells/mm^3 (700 copies/ml); BD-0971, 599 cells/mm^3 (<400 copies/ml); LS-5175, 1738 cells/mm^3 (<400 copies/ml); 161-J, 1400 cells/mm^3 (<400 copies/ml).
13. S. Kalams, E. Rosenberg, J. Billingsley, B. Walker, in preparation.
14. E. Rosenberg, J. Billingsley, B. Walker, unpublished observations.
15. Lymphocyte proliferation assays were performed as described in Fig. 1. Concentration of p24 was titrated to determine the lowest amount of antigen required to stimulate a p24-specific lymphocyte proliferative response. For subject 161-J, a p24 concentration of 0.05 μg/ml still elicited a response, whereas for subject CTS-01, the response was lost at concentrations <0.5 μg/ml.
16. The PBMC were cultured at seven concentrations ranging from 1×10^3 to 2×10^6 cells/ml in 24 replicate wells of 96-well U-bottom microtiter plates in the presence of HIV-1 p24, and in 12 replicate wells in the presence of control proteins. Plates were incubated and harvested as described in Fig. 1. For each cell concentration, the fraction of nonresponding wells was determined to be the fraction of wells with fewer counts per minute than the mean plus three standard deviations for the 12 control wells. Activated cell frequency was determined by the maximum likelihood method (*10*).
17. T. R. Mosmann, H. Cherwinski, M. W. Bond, M. A. Giedlin, R. L. Coffman, *J. Immunol.* **136**, 2348 (1986); T. R. Mosmann and S. Sad, *Immunol. Today* **17**, 138 (1996).
18. F. Cocchi *et al.*, *Science* **270**, 1811 (1995).
19. E. Rosenberg and B. Walker, unpublished observations.
20. M. Clerici *et al.*, *J. Clin. Invest.* **93**, 768 (1994).
21. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
22. Three p24 peptides were recognized by both individuals with SI > 10: residues 163 to 184 (AFSPEVIPMFSALSEGATPQDL), 243 to 264 (LQEQIGWMTNNPPIPVGEIYKR), and 263 to 284 (KRWIILGLNKIVRMYSPTSILD).
23. PBMC from seronegative persons resulted in SI < 3 for all p24 peptides.
24. The diagnosis was made on the basis of a negative HIV-1/2 enzyme immunoassay (Abbott Laboratories, Abbott Park, IL), the presence of HIV-1 viral RNA (AMPLICOR HIV MONITOR TEST, Roche Diagnostic Systems, Branchberg, NJ), and subsequent seroconversion documented by both HIV-1/2 enzyme immunoassay and protein immunoblot (Abbott Laboratories). Proliferation assays were performed at baseline (before initiation of antiviral therapy) and at designated intervals during therapy, with the use of baculovirus-derived p24 antigen at 5 μg/ml.
25. A. D. Kelleher, A. Carr, J. Zaunders, D. A. Cooper, *J. Infect. Dis.* **173**, 321 (1996); S. M. Schnittman and L. Fox, *AIDS Res. Hum. Retroviruses* **13**, 815 (1997); M. Connors *et al.*, *Nature Med.* **3**, 533 (1997); B. Autran *et al.*, *Science* **277**, 112 (1997).
26. E. A. Walter *et al.*, *N. Engl. J. Med.* **333**, 1038 (1995).
27. D. Weissman, T. D. Barker, A. S. Fauci, *J. Exp. Med.* **183**, 687 (1996); S. Stanley *et al.*, *N. Engl. J. Med.* **334**, 1222 (1996).
28. A. K. Abbas, *Cell* **84**, 655 (1996).
29. C. Lekutis and N. L. Letvin, *J. Immunol.* **159**, 2049 (1997); C. Lekutis, J. W. Shiver, M. A. Liu, N. L. Letvin, *ibid.* **158**, 4471 (1997).
30. Proliferation assays were performed by resuspending PBMC in RPMI 1640 medium containing 10% human AB serum, Hepes buffer, L-glutamine, and penicillin-streptomycin. Cells (1×10^5 cells per well) were cultured in six replicate wells of 96-well U-bottom plates in the presence of HIV-1 recombinant proteins, control proteins, tetanus toxoid, or medium alone. Six days later, the cells were pulsed with [^{3}H]-thymidine at 1.0 μCi per well, and uptake was measured 6 hours later with a scintillation counter (Packard Topcount, Packard Instruments, Meriden, CT). The HIV-1 p24 and gp160 proteins (Protein Science, Meriden, CT) are recombinant proteins derived from the *gag* or *env* gene of HIV-1 (NY-5 and LAV strains, respectively) produced in a baculovirus expression system and demonstrated 90 to 95% purity. These proteins were tested over a range of concentrations, with 0.5 μg/ml as the standard concentration. A mixture of baculovirus proteins was used as a control antigen at 0.015 μg/ml (equal to the baculovirus antigen concentration in the recombinant proteins), with comparable results obtained at 1.5 μg/ml. Yeast- and CHO-derived HIV-1 proteins were provided by Chiron (Emeryville, CA). The yeast-derived p24 Gag protein (residues 139 to 369) is recombinantly derived in a yeast expression system using the HIV-1 strain SF2. The gp120 was derived from HIV-SF2 expressed in CHO cells. These proteins demonstrated >90 and 94.8% purity, respectively. Each protein was used at a concentration of 0.5 μg/ml, and preparations of CHO and yeast proteins were used as controls for these antigens at 0.5 μg/ml. Tetanus toxoid (Connaught Laboratories, Willowdale, Ontario, Canada) was used at 2 μg/ml. For the recombinant HIV-1 proteins, a stimulation index (SI) was defined as the ratio of the mean counts per minute (CPM) of the HIV-1 protein wells to the mean CPM of the control protein wells. For tetanus toxoid, SI was defined as the ratio of the mean CPM of the stimulated wells to the mean CPM of six control wells containing PBMC and medium alone. For assays using CD4- or CD8-depleted PBMC, cells were cultured as described above in the presence of γ-irradiated (40 Gy) autologous PBMC and antigen.

31. We thank D. K. H. Wong for help and advice with statistical analysis, M. Hirsch , D. Cotton, V. Young, and C. Hay for review of manuscript, T. Flynn and L. Larosa for help with patients, and D. J. Ruhl for technical help. This project was supported by NIH grants R01-A128568, F32-A109738, R01-A136550, U19-A136611, and UO1-AI41531.

16 July 1997; accepted 10 October 1997