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Resistance to Replication of Human Immunodeficiency Virus Challenge in SCID-Hu Mice Engrafted with Peripheral Blood Mononuclear Cells of Nonprogressors Is Mediated by $CD8^+$ T Cells and Associated with a Proliferative Response to p24 Antigen

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High levels of resistance to challenge with human immunodeficiency virus type 1 SF162 were observed in animals engrafted with peripheral blood mononuclear cells of four long-term nonprogressors (LTNPs). Resistance was abrogated by depletion of $CD8^+$ T cells in vivo and was observed only in LTNPs with proliferative responses to p24. In a subgroup of nonprogressors, $CD8^+$ T cells mediated restriction of challenge viruses, and this response was associated with strong proliferative responses to p24 antigen.

Although patients with normal $CD4^+$ T-cell counts and low levels of virus in plasma are a heterogeneous group, a small subgroup of patients with truly nonprogressive human immunodeficiency virus (HIV) infection likely hold important clues to the basis of an effective immune response to HIV. It now appears clear that a large fraction of patients previously considered long-term nonprogressors (LTNPs) ultimately show a decline of $CD4^+$-T-cell numbers. Members of a small subpopulation (<0.8% of HIV-infected individuals) show no signs of progression over a 10-year period (12, 22, 23, 36). Extensive studies have demonstrated strong cellular and humoral HIV-directed responses in LTNPs (2, 6, 7, 15, 18, 29, 31, 32). Regardless of the host or virus factors involved in nonprogression in these patients, a clear demonstration of immunity-mediated resistance to challenge virus and targets of such a response within HIV would enhance development of an effective HIV vaccine. Recently we established a human HIV-peripheral blood mononuclear cell (PBMC)-SCID mouse model, a modification of the method developed by Mosier et al. (13, 26, 28), to study the PBMC of infected patients (5). We determined whether PBMC of LTNPs support replication of patients' autologous viruses in this model and further whether these PBMC mediate restriction of challenge-virus replication.

Engraftment of CB-17 SCID mice and sample collection were performed as previously described (5). Animals were challenged intraperitoneally with HIV_{SF162} on day 7 and sacrificed on day 21. To deplete $CD8^+$ T cells, on day 6 animals received 0.2 mg of 7ptF9 anti-CD8 monoclonal or 833ICG isotype control antibody (Coulter, Hialeah, Fla.). In preliminary experiments the 7ptF9 antibody was not blocked by the detecting antibody to CD8. Because there is no substantial lymphopoiesis, 7ptF9 treatment resulted in high-level (>98%) depletion of $CD8^+$ T cells throughout the experimental period. Proviral DNA and plasma viral RNA assays were performed using the Perkin-Elmer (Foster City, Calif.) model 7700 sequence detector. Dunnett's test for multiple comparisons was used to compare the percentages of $CD4^+$ T cells and the Wilcoxon two-sample test was used with the Bonferroni multiple-testing correction to compare levels of virus in plasma and provirus in spleen between groups of animals. In vitro cultures were performed as previously described (3). Standard enzyme-linked immunosorbent assays were used to quantify the CC chemokines MIP-1α, MIP-1β, and RANTES (R&D Systems, Minneapolis, Minn.) or p24 (Coulter). Standard ^{51}Cr-release assays (37) and proliferation assays (33) were performed as previously described.

All patients have been infected for greater than 13 years (Table 1). Two patients typically classified as LTNPs (27, 35) were included as controls. These two patients (patients 1 and 2) had levels of HIV RNA in plasma of <500 to 14,650 copies/mm^3 at three or four time points over the past 4 years of study. Patients 3 to 6 consistently had plasma HIV RNA levels of <50 copies/ml and no recovered virus in $CD8^+$-T-cell-depleted cocultures or in UV-irradiated cultures (9).

In a previous study, engraftment of CB-17 SCID mice with PBMC from HIV-infected patients resulted in rapid replication of patient-derived (autologous) viruses (5). Plasma viremia peaked on day 10 and was associated with depletion of $CD4^+$ T cells to 5% of human cells. This pattern was repeatedly observed in animals engrafted with PBMC of seven patients with progressive disease (not shown). However, in some experiments there was no replication of the patients' autologous viruses in animals engrafted with cells from two true LTNPs (patients 3 and 6). This observation permitted the

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TABLE 1. Clinical data of study patients[a]

Patient[b]	Route of infection	Yr of diagnosis	No. of $CD4^+$ T cells/mm^3	No. of $CD8^+$ T cells/mm^3	HIV RNA in plasma (copies/ml) by:	
					bDNA	RT-PCR
1	Homosexual sex	1985	902	714	<500–14,650	26,400
2	Homosexual sex	1986	729	2,478	<500–14,080	3,470
3	Homosexual sex	1985	941	1,015	<500	<10
4	Homosexual sex	1985	774	774	<500	<10
5	Homosexual sex	1985	914	656	<500	<10
6	Received Factor VIII for hemophilia	1986	734	672	<500	<10

[a] Flow cytometry values indicated were determined at the time of donation. Levels of virus RNA in plasma were determined using a branched chain DNA (bDNA) assay with a sensitivity in plasma of 500 copies/ml and by real-time RT-PCR with a sensitivity of 10 copies/ml.

[b] All patients had not received antiretroviral agents during or prior to the study period except as indicated. Patient 2 was receiving zidovudine (AZT) monotherapy during the study. Patient 3 received IFN-α–AZT (January 1990 to December 1995) or IFN-α–AZT–didanosine (DDI) (January 1996 to December 1996) as part of a National Institute of Allergy and Infectious Diseases protocol. He did not receive antiretroviral agents during the study period and has remained off antiretroviral agents and without viremia since that time.

study of restriction of challenge-virus replication in animals engrafted with PBMC of these patients. In preliminary experiments, upon challenge with 5 to 125 50% tissue culture infectious doses ($TCID_{50}$) of HIV_{LAV}, $CD4^+$ T cells were maintained in animals engrafted with cells from patients 3 and 6 (not shown). We then determined if similar resistance would be observed upon challenge with the macrophage-tropic primary isolate HIV_{SF162}. In animals engrafted with cells from an uninfected donor, significant depletion of $CD4^+$ T cells ($P = 0.05$) and increases in levels of virus in plasma ($P < 0.03$) and of proviral DNA ($P = 0.03$) were observed at the 5- to 125-$TCID_{50}$ doses when results were compared to results with unchallenged animals (Fig. 1). In both challenged and unchallenged animals engrafted with PBMC from patients 1 and 2, virus replication and $CD4^+$-T-cell depletion were similar to those previously observed in animals engrafted with PBMC from progressors (5).

In contrast, animals engrafted with the cells from three of the four LTNPs (patients 4 to 6) did not replicate autologous viruses above levels of detection. Although unchallenged animals engrafted with cells from patient 3 had a lower percentage of $CD4^+$ T cells than those of patients 4 to 6, no $CD4^+$ T-cell depletion from this lower baseline was detected in challenged animals. No depletion of $CD4^+$ T cells was observed in the majority of groups of animals engrafted with PBMC of patients 3 to 6 over a broad range of challenge doses when levels were compared to levels in unchallenged animals. Significant depletion of $CD4^+$ T cells was found only in animals engrafted with the cells of patient 6 and challenged with 125 $TCID_{50}$ ($P = 0.05$). No significant increase in virus in plasma was detected in challenged animals engrafted with PBMC of patients 3 to 6 when levels were compared with levels in unchallenged animals. Although several animals engrafted with the PBMC of patient 4 had plasma viremia, the distribution of these values was not significantly greater than that of unchallenged animals when values were corrected for multiple comparisons ($P = 0.07$). Significant increases in provirus in spleen were observed only in animals engrafted with PBMC of patient 4 and challenged with 125 $TCID_{50}$ ($P < 0.003$). Sequence analysis of proviral DNA from the spleens of two of these animals confirmed that the virus detected was HIV_{SF162}.

In separate experiments, the effect of $CD8^+$-T-cell depletion on restriction of virus replication was investigated (Fig. 1). In animals engrafted with cells from patient 3 or 5 and depleted of $CD8^+$ T cells, challenge virus replicated to levels comparable to those of animals engrafted with PBMC from an uninfected control. This replication resulted in significant increases in virus in plasma ($P = 0.05$) and provirus in spleen ($P = 0.03$) when such levels were compared to levels in 25-$TCID_{50}$-challenged, nondepleted animals. Because $CD8^+$-T-cell depletion increases the percentage of $CD4^+$ T cells in the peritoneal wash, comparisons were made with similarly depleted, unchallenged animals. Increased virus replication in $CD8^+$-T-cell-depleted animals resulted in only partial $CD4^+$-T-cell depletion in those animals engrafted with the cells of patient 3 but significant depletion in the mice engrafted with cells from patient 5 ($P < 0.03$).

Because it is possible that restricted challenge-virus replication might not be entirely due to a $CD8^+$-T-cell response but also to restriction of replication at the level of the $CD4^+$ T cell, the ability to replicate virus in the absence of $CD8^+$ T cells was investigated in vitro. In cultures depleted of $CD8^+$ T cells (<1% residual $CD8^+$ T cells), no residual restriction of the replication of HIV_{SF162} or HIV_{LAV} was observed (not shown). The lack of a deletion within the CCR5 gene was confirmed by restriction fragment length polymorphism analysis. We also investigated whether patients 3 to 6 could be distinguished from patients 1 and 2 in standard assays of $CD8^+$ T-cell-mediated HIV-specific immunity. PBMC of patients 3 to 6 did not show a greater ability to suppress replication of HIV_{SF162} or HIV_{LAV} than those of patients 1 and 2. Similar results were obtained with 5, 50, and 125 $TCID_{50}$ of HIV_{SF162} or HIV_{LAV} or when $CD8^+$ T cells were depleted and then repleted at various ratios (100:0 to 100:100 CD4/CD8 ratios) (not shown). No differences in the levels of the CC chemokines MIP-1α, MIP-1β, and RANTES were found in the supernatants of cultures. No difference in the magnitudes of the direct cytotoxic T lymphocyte (CTL) responses was detected in patients 1 and 2 when their responses were compared to those of patients 3 to 6. PBMC of uninfected patients and patients with detectable virus in plasma (patients 1 and 2) showed no proliferative response to p24 (Fig. 2). In contrast, patients 3 to 6 had strong responses to p24 (stimulation index, 10 to 140) that were abrogated by $CD4^+$-T-cell depletion in vitro.

The data from this study establish this resistance phenotype in a unique subgroup of nonprogressors that likely make up less than 0.8% of the HIV-infected population. These patients are characterized by nonprogressive disease, a level of virus in plasma below 50 copies/ml, a lack of readily detectable virus by culture, a lack of autologous virus replication in the SCID-Hu mouse, and strong proliferative responses to HIV antigens. Data from this study as well as previous epidemiologic data (12, 22, 23, 30, 33, 36) indicate that each of these characteristics is quite rare among patients with slowly progressive or

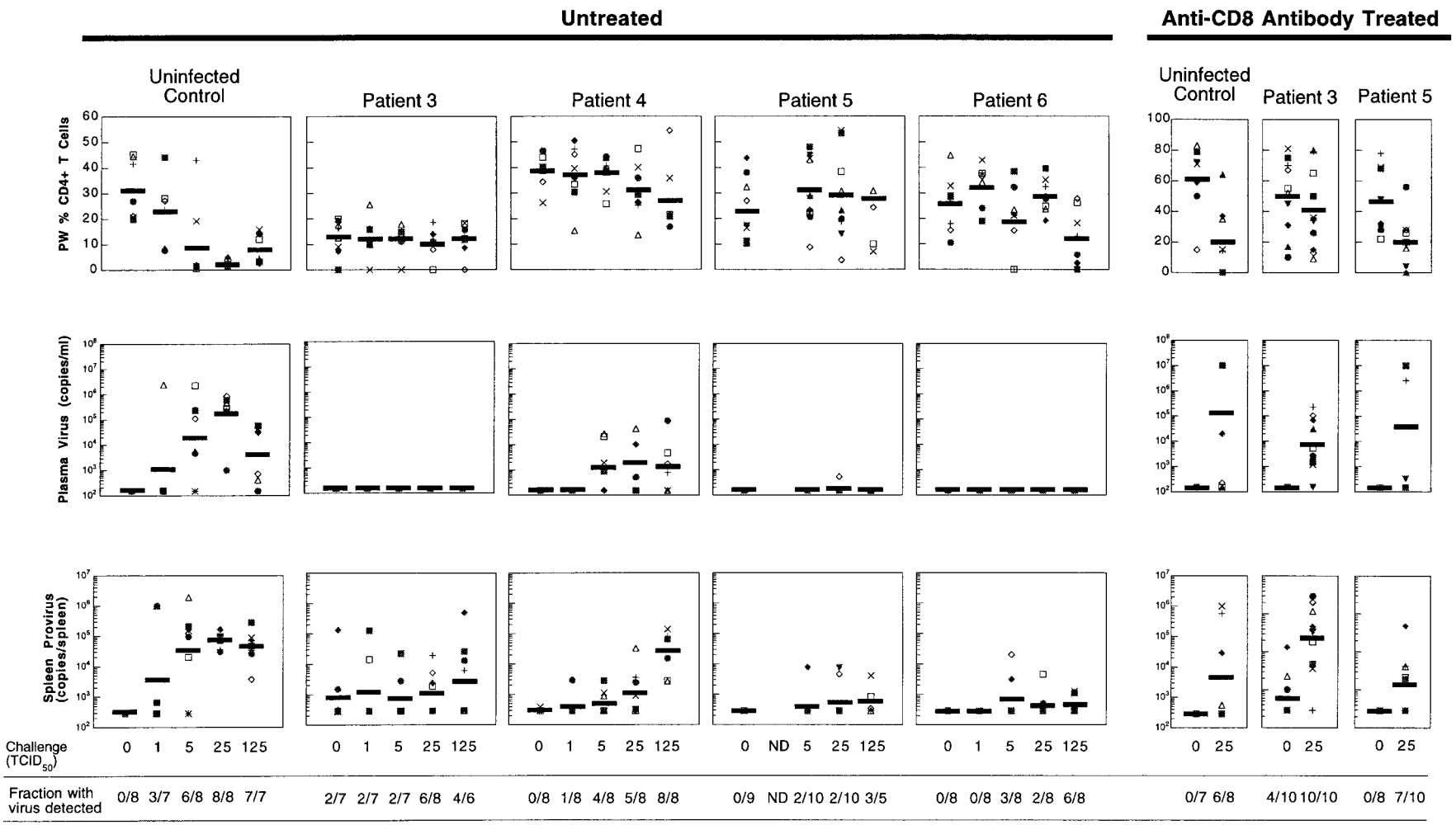

FIG. 1. Changes in $CD4^+$-T-cell numbers and levels of HIV-1 in animals engrafted with human PBMC and challenged with 1 to 125 $TCID_{50}$ of HIV_{SF162}. Values for similarly prepared animals that received a human $CD8^+$-T-cell-depleting antibody on day 6 (1 day prior to challenge) are also shown. Values shown are those at the time of sacrifice (day 21). The percentages of human cells within the peritoneal wash (PW) which are $CD4^+$ are shown in the top panels. Levels of virus in plasma and provirus in spleen determined by real-time PCR are shown in the two lower panels. The percentage of $CD4^+$ T cells and levels of virus RNA in plasma and provirus DNA in spleen indicated by a given symbol within a column correspond to the same animal. The fraction of animals with detectable virus refers to the number of animals with virus RNA in the plasma or provirus in the spleen, or spleen coculture, divided by the number of animals in that group.

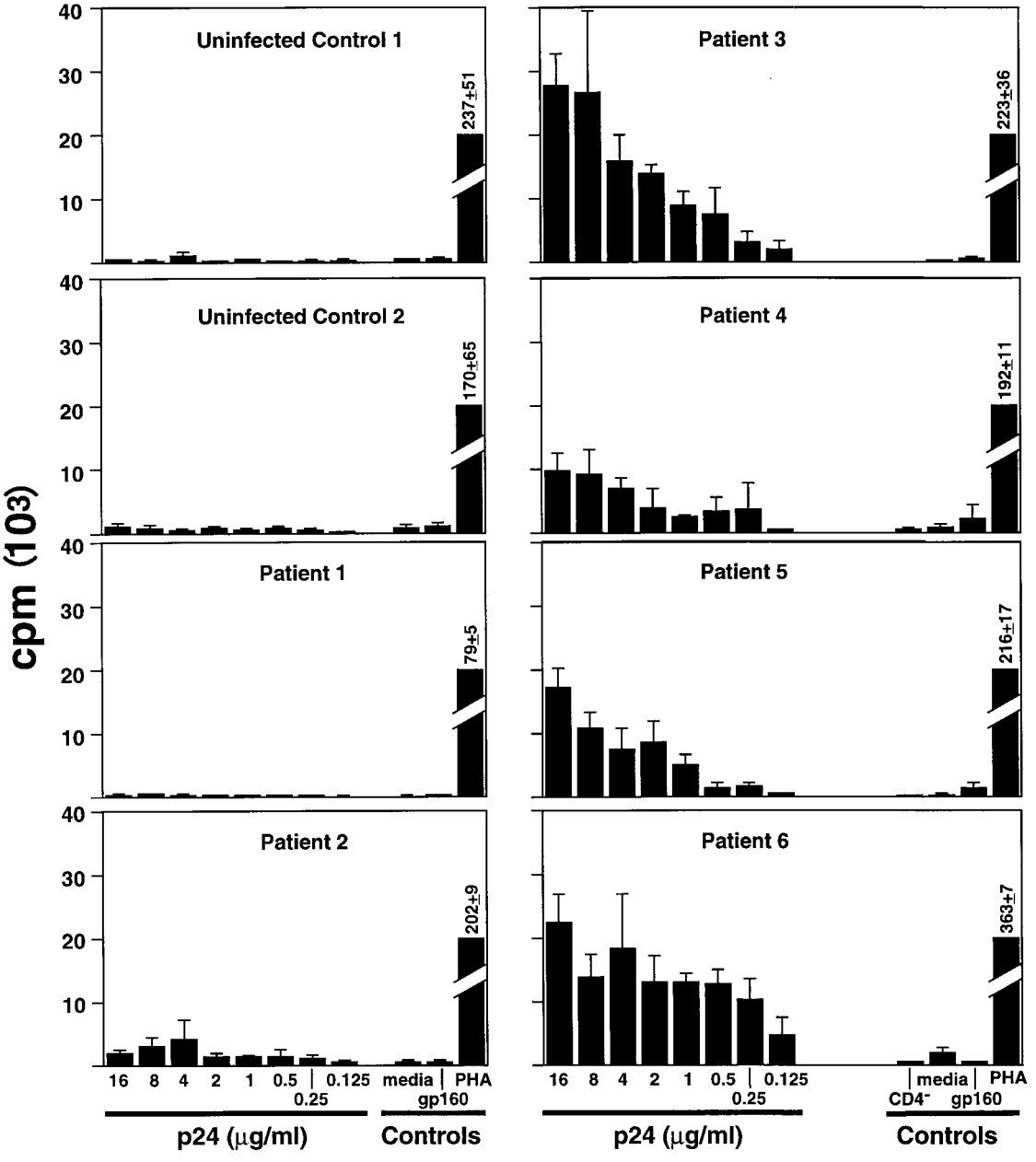

FIG. 2. In vitro proliferation to HIV p24 antigen. PBMC (10^5) from two uninfected controls and patients 1 to 6 were incubated with the indicated antigens or media in a standard [H^3]thymidine uptake assay. Results of triplicate cultures are shown. For patients 3 to 6, controls included $CD4^+$-T-cell-depleted PBMC stimulated with 10 μg of p24 per ml. PHA, phytohemagglutinin.

nonprogressive disease, yet each of these was associated with the subgroup described here. Although the in vivo phenotype has been repeatedly observed over the past 4 years of study of these patients, no in vitro correlate which clearly distinguished patients 3 to 6 from progressors or other nonprogressors was found until the proliferative responses to HIV antigens in such patients and experimental animals was recently described (10, 11, 33).

It should be stressed that these data do not directly indicate the mechanism by which patients 3 to 6 have avoided progressive disease. The model presented is not a model of nonprogression; rather, it is a model of immunity-mediated restriction of a challenge virus. These data do extend some recent data documenting the role of $CD8^+$ T cells in restricting virus replication in macaques chronically infected with simian/human immunodeficiency virus or simian immunodeficiency virus (16, 24, 34). It should also be noted that the SCID-Hu mouse model does not directly model all of the aspects of resistance to challenge observed in virus infection of a natural host. In the SCID-Hu mouse model, the lack of lymphopoiesis and moderate lymphocyte activation leads to rapid virus replication and $CD4^+$-T-cell depletion. The lack of virus replication and $CD4^+$-T-cell depletion in many animals engrafted with cells from patients 3 to 6 suggests that the form of resistance mediated by the $CD8^+$ T cells of these patients is quite potent.

We did not detect higher levels of HIV-specific $CD8^+$-T-cell-mediated immunity by in vitro assays of PBMC from patients 3 to 6 compared to those of patients 1 and 2. In the PBMC of patients 1 and 2, who meet the widely used criteria of nonprogressors (27, 35), high levels of suppressive and direct CTL were observed, yet these responses were not associated with restriction of autologous virus replication in the mouse model. However, the CTL and $CD8^+$-T-cell repletion assays used are crude and nonquantitative and thus might have underestimated greater HIV-specific $CD8^+$-T-cell responses in patients 3 to 6. By a more quantitative assay, the numbers of antigen-specific $CD8^+$ T cells detected by antigen-specific accumulation of intracellular gamma interferon (IFN-γ) of patients 3 to 6 were similar to those of patients 1 and 2 and other patients with progressive disease (J. Gea-Banacloche, unpublished data), consistent with one other recent report (8). It should also be noted that each of these assays measures responses to laboratory test strains and might not measure dominant responses to the in vivo virus strain. It is possible that the measured responses then underestimate the responses to the circulating viruses of patients 3 to 6. It is also possible that maintenance of $CD4^+$-T-cell help in patients 3 to 6 results in the maintenance of high-avidity, HIV-specific $CD8^+$ T cells, which is not adequately measured by these assays (1, 4, 14, 17, 19–21, 25).

Further definition of the mechanisms of $CD8^+$-T-cell-mediated restriction of virus replication in the patients described in this study is likely to hold important clues to the parameters that should be measured or mechanisms that might be exploited as part of prophylactic or therapeutic vaccines for HIV. If such mechanisms are determined in vitro, the model described here may allow a further demonstration of their relationship with restriction of challenge-virus replication.

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