HIV-1 THERAPY

Sustained virologic control in SIV+ macaques after antiretroviral and α4β7 antibody therapy

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Antiretroviral drug therapy (ART) effectively suppresses replication of both the immunodeficiency viruses, human (HIV) and simian (SIV); however, virus rebounds soon after ART is withdrawn. SIV-infected monkeys were treated with a 90-day course of ART initiated at 5 weeks post infection followed at 9 weeks post infection by infusions of a primatized monoclonal antibody against the αβ7 integrin administered every 3 weeks until week 32. These animals subsequently maintained low to undetectable viral loads and normal CD4+ T cell counts in plasma and gastrointestinal tissues for more than 9 months, even after all treatment was withdrawn. This combination therapy allows macaques to effectively control viremia and reconstitute their immune systems without a need for further therapy.

Profound and durable suppression of HIV by antiretroviral therapy (ART) represents a major accomplishment in HIV-AIDS research. However, HIV persists in patients despite long-term ART therapy such that, once ART is withdrawn, virus invariably rebounds. Lifetime ART treatment is associated with toxicity (1), residual chronic inflammation, and the accelerated onset of diseases associated with aging (2, 3).

High levels of viral replication in gastrointestinal tissues (GITs) during acute infection lead to severe depletion of local CD4+ T cells (4), damage to the gut epithelium, and the rapid formation of persistent viral reservoirs. Generalized immune dysfunction and chronic immune activation follow. Even when administered days after infection, ART fails to fully reverse these insults (5). We reasoned that preventing HIV-susceptible cells from accessing GITs might reduce damage to the gut and the mucosal immune system in a way that would allow immune mechanisms to effectively control infection.

A principal pathway that CD4+ T cells use to traffic into GITs involves an interaction between integrin αβ7, expressed on CD4+ T cells, with mucosal vascular addressin cell adhesion molecule 1 (MadCAM-1), expressed primarily on high endothelial venules within GITs (6, 7). CD4+ T cells that express high levels of the αβ7 integrin (αβ7hi) are preferential targets of HIV and simian immunodeficiency virus (SIV) during acute infection (8–12). In order to disrupt trafficking of αβ7hi CD4+ T cells into GITs, we developed a recombinant rhesus monoclonal antibody against the heterodimeric form of αβ7 (αβ7mAb) that blocks αβ7 binding to MadCAM (13–15).

Administration of αβ7mAb before and during repeated low-dose intravaginal SIV challenge of rhesus macaques (RMs) leads to significant protection from transmission (13). In treated animals that became infected, GIT CD4+ T cells were preserved and GIT proviral DNA was reduced, and thus, virus-mediated damage to GITs was minimized.

Because ART provides only partial protection to GITs (5, 14), we considered the possibility that adding αβ7mAb might improve this protection. To this end, we conducted a study in genetically characterized (table SI, A to D) SIV-infected RMs that combined a 90-day course of ART, beginning 5 weeks post-infection with a series of eight infusions of αβ7mAb. This treatment strategy included five phases as outlined in fig. S1. In phase I (weeks 1 to 5), 18 RMs were infected intravaginally with a 200 median tissue culture infectious dose (TCID50) of SIVmac239. After 5 weeks, all 18 animals began a 90-day daily regimen of ART (phase II). During phase III (weeks 9 to 18), 11 animals received αβ7mAb once every 3 weeks (eight infusions total); 7 animals received nongroup-specific rhesus immunoglobulin G (IgG). During phase IV (weeks 18 to 32), ART was withdrawn, and αβ7mAb–IgG treatment was continued. In phase V (weeks 32 to 50), all treatment was terminated. Three out of 11 αβ7mAb–treated animals developed antibodies against the αβ7mAb (fig. S2) and were excluded from further analysis.

ART + αβ7mAb controls plasma and gut viral loads

All 15 animals showed similar peaks in viremia around weeks 2 to 3 (~2.9 × 108 copies/ml), and they all fully suppressed viremia by 3 weeks after ART initiation. The two groups developed divergent viral load (VL) patterns after ART was withdrawn (phase IV). In all seven IgG-treated animals, viremia rebounded to high levels (~107 copies/ml) within 2 weeks and maintained those levels out to week 50 (Fig. 1B). In contrast, two out of eight αβ7mAb–treated animals never rebounded, and the remaining six out of eight rebounded but then regained control of viremia within 4 weeks (Fig. 1A). Virologic control was robust in all eight αβ7mAb–treated animals, with either complete control or transient low-level blips of viremia. The difference in viremia between the two groups after discontinuation of ART was significant (P < 0.0001) (Fig. 1C). Virologic control in all eight αβ7mAb–treated animals persisted to week 81 (fig. S3), although the last infusion of αβ7mAb (half-life of ~14 days) was administered at week 32 (15).

Both treatment groups showed similar levels of proviral DNA in GITs during phases I and II. Immediately after cessation of ART, all monkeys showed increases in proviral DNA. High levels (20 to 40 copies/ng DNA) persisted in the seven IgG-treated animals (Fig. 1F). In contrast, in all eight αβ7mAb–treated macaques, proviral DNA decreased to levels at or below the level of detection by week 30 (Fig. 1D). The difference in the geometric means of the two groups (Fig. 1F) during phases IV and V was significant (P < 0.0001). Undetectable proviral DNA loads in all eight αβ7mAb–treated macaques persisted well after the final infusion of αβ7mAb (week 32). Thus, combining αβ7mAb therapy with short-term ART promoted persistent systemic and...
mucosal virologic control following discontinuation of all therapy.

**Rebound of CD4+ T cell subsets**

Blood and GIT mononuclear cells isolated from each phase of the study were analyzed by flow cytometry (table S2, A to C). During phase I, the absolute numbers of blood total CD4+ T cells and subsets showed a sharp decline (Fig. 2, A to D). After the first administration of α4β7 mAb (phase III), CD4 values diverged. The α4β7 mAb–treated animals, but not controls, showed marked increases in total CD4+ T cells and, notably, in the effector memory (EM) CD4+ T cell subset (> fourfold increase, \( P < 0.0001 \)) (Fig. 2D). These increases were sustained after discontinuation of the α4β7 mAb treatment (phase V). By week 50, total CD4+ T cell numbers approached preinfection levels.

Acute HIV-1 and SIV infections are characterized by a rapid depletion of CD4+ T cells in GItTs (4, 15–18). Therefore, we evaluated the fate of GIT CD4+ T cells (Fig. 2, E to H), with values expressed as the percent of CD4+ T cells within the gated population of CD45+ cells. We observed a sharp decline in total CD4+ T cells during the acute phase in both groups with CD45+/CD4+ T cells reaching their nadir by the end of phase I (Fig. 2E). In phase III, the CD4+ T cell profile of the two treatment groups diverged. The relative frequency of CD45+/CD4+ cells in the α4β7 mAb–treated animals gradually increased through phase V (\( P < 0.0001 \)). CD4+ T cell subsets showed an apparent recovery of both T central memory (CM)
and TEM cells in the αβ7 mAb–treated animals, with the EM recovering at a faster rate (Fig. 2, G and H). The relative proportion of naïve CD4+ T cells remained constant through phase V (Fig. 2F). The quality of this recovery was well reflected by increases in the frequencies of T helper 17 (TH17) and T helper 22 (TH22) subsets of CD4+ T cells in both GITs and blood (fig. S4) (19). Consistent with these results, immunohistological analysis of GIT sections after week 50 revealed abundant CD4+ T cells in αβ7 mAb–treated animals but not in controls (fig. S5).

To better understand the repopulation of gut tissues with CD4+ T cells, we used a newly developed antibody-targeted positron emission tomography (immuno-PET) combined with the computed tomography (CT) imaging technique (20). Around week 50 (phase V), four macaques from each treatment group were imaged with 64Cu-labeled F(ab)2 antibody against CD4. Although we had originally hypothesized that αβ7 mAb would inhibit CD4+ T cell trafficking to GITs, we instead observed repopulation of CD4+ T cells in a wide variety of immune tissues, including GITs (Fig. 3). This result suggests that the protective effect of αβ7 mAb in minimizing GIT viral load early in infection (Fig. 1, D to F) facilitated the repopulation of CD4+ cells throughout the systemic and mucosal immune systems. It is unclear whether the reconstitution of these immune sites resulted from the control of viremia, whether these immune competent components contributed to virologic control, or both.

**Phenotypic analysis of NK cells and other cell lineages**

Total natural killer (NK) cells in blood remained similar in both groups during phases I to IV (Fig. 4) and decreased during phase V but only in the IgG-treated group (Fig. 4A). We observed a decrease in the cytolytic subset (NKG2α+/CD8+/CD16+/CD56−) during phase III and IV in the αβ7 mAb–treated group, followed by an increase during phase V (Fig. 4B). Cytokine-synthesizing NK cells (NKG2α+/CD8+/CD16+/CD56−) increased during phase IV in αβ7 mAb–treated animals.
(Fig. 4C). By week 50, these values approached baseline. A related pattern was observed in GITs. In the α4β7 mAb-treated group, the proportion of the cytokine-synthesizing NK cell subset increased through phases IV and V (Fig. 4H), with corresponding decreases in the proportion of other NK cell subsets. These changes are noteworthy in two ways. First, among all NK cell subsets analyzed, α4β7 expression is highest (~40%) on cytokine-synthesizing NK cells. Second, these increases coincided with the appearance of virologic control in phase IV. Frequencies of NKp44+ innate lymphoid cells (ILCs) decreased in both treatment groups as early as phase I (fig. S6), which is consistent with the loss of ILCs in acute HIV infection (27). However, we observed a sustained increase in the frequency of ILCs in gut biopsies in ART + α4β7 mAb-treated animals beginning in phase III but not in controls. Note that vaccine-induced ILCs have been correlated with protection from SIV infection (28).

In the baseline. A related pattern was observed in GITs. Of note, measurements taken on weeks 15, 17, and 19 (phase III) differentiated NK cells, and (Fig. 4G) cytokine synthesizing NK cells, (Fig. 4H) terminally differentiated NK cells, and (Fig. 4I) intermediate NK cells. Monkeys treated with ART + α4β7 mAb (blue) and the ART + rhesus IgG (red). Frequencies of NK cell subsets in GITs were calculated as the percentage of total NK cells in the same sample.

**Identification of signature plasma cytokines**

Results presented above suggest that virologic control was immune mediated. To this end, we analyzed a panel of 20 immune or inflammatory markers (table S3) in plasma samples. To identify signatures for each of the five phases of the study, we used a partial least square discriminant analysis (PLSDA). PLSDA models were created for the five study phases by using measurements from each sample and time point, and variable importance projection scores were used to omit markers that did not contribute to group differentiation. Cross-validation was used to assess model performance. In phases I and II, we found no distinction between treatment groups, with a high cross-validation error between models (0.28 and 0.21, respectively) (fig. S12, A and B). In phases III to V, distinct signatures differentiated the two treatment groups, with low cross-validation errors in each phase (0.09, 0.02, and 0.01, respectively) (Fig. 5A). The differentiating signature varied in each of these phases. One common feature of all three was a comparative decrease in interleukin-21 (IL-21), granulocyte-macrophage colony-stimulating factor, soluble CD14 (sCD14), interferon-α (IFN-α), as well as RA; however, IFN-γ and transforming growth factor-β (TGF-β) were reduced. In phases IV and V, α4β7 mAb treatment was associated with increased IL-10 and RA, with comparative decreases of proinflammatory markers, including IL-1β, IFN-γ–induced protein (IP-10), complement-reactive protein (CRP), the coagulation biomarker D-dimer, and two markers, sCD163 and intestinal fatty acid–binding protein (I-FABP), associated with gut permeability (Fig. 5A and fig. S13).

**ART + α4β7 mAb restores plasma retinoic acid levels**

RA induces the expression of α4β7 (23, 24) and also plays an essential role in gut homeostasis (23, 25–27). Baseline RA levels decreased during acute infection in both groups (Fig. 5B). A similar response was observed in two uninfected monkeys afflicted with chronic diarrhea (fig. S14), suggesting that such declines are linked to gut inflammation. Of note, measurements taken on weeks 15, 17, and 19 (phase III) differentiated
the two groups, such that RA in the $\alpha_4\beta_7$ treatment group recovered to near-baseline levels. To understand whether these changes reflected the administration of $\alpha_4\beta_7$ mAb, independent of SIV infection, we treated two uninfected animals with $\alpha_4\beta_7$ mAb and observed no effect on serum RA (fig. S15). Indeed, we also observed a similar response in I-FABP levels in phase III (fig. S13). Finally, we carried out an exploratory analysis to identify additional biomarkers in phase III that might correlate with the degree of gut proviral DNA rebound in phase IV. Both positive and negative predictors of the magnitude of viral rebound in gut tissues were identified (fig. S16).

**Induction of gp120 V2-specific antibody responses**

Plasma and peripheral blood mononuclear cell (PBMC) samples were also screened for SIV-specific antibody responses and antibody-dependent cell-mediated cytotoxicity (ADCC). We found neither neutralizing antibodies nor differences in ADCC titers (fig. S17). In human and macaque vaccine trials, nonneutralizing antibodies directed against these convarable (V2) in Env glycoprotein 120 (gp120) correlated with reduced risk of acquisition (28, 29). We therefore characterized the specificity of the antibody responses by performing Pepscan enzyme-linked immunosorbent assay (ELISA) of sera from five animals in each group against linear peptides spanning the entire gp120 subunit (fig. S18). Serum samples from weeks 30 to 36 showed that five out of five $\alpha_4\beta_7$ mAb-treated macaques reacted, to varying levels, with two overlapping V2 peptides (peptides 26 and 27). None of the IgG-treated controls reacted to both of these peptides (two animals reacted to one peptide). We found no other consistent difference in epitope-specific reactivity between the two groups. We then evaluated antibody responses using a more sensitive surface plasmon resonance (SPR) assay. Sera from all 15 animals through the five treatment phases were reacted with both SIV gp120 and an SIV cyclic V2 peptide. Sera from both treatment

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**Fig. 5. Multivariate cytokine signatures and plasma levels of retinoic acid.** (A) Separate PLSDA models for phases III (left), IV (middle), and V (right). Score plots of latent variable 1 (LV1) versus LV2 (top). Monkeys treated with $\alpha_4\beta_7$ mAb (blue) and IgG (red). Corresponding loadings plots (bottom) with the multivariate cytokine signatures for each phase. (B) Plasma levels (means ± SD) of RA are displayed (pmol/ml) in the individual monkeys treated with $\alpha_4\beta_7$ mAb (blue) and IgG (red) through all five phases of the study (denotes unpaired t test, *P < 0.003).
groups showed similar reactivity to gp120 (mean values) (fig. S19). However, the two treatment groups differed in their reactivity to V2. Whereas eight out of eight α4β7 mAb–treated animals showed persistent reactivity to V2, only three out of seven IgG–treated animals reacted to V2 (Fig. 6A). We then fine-mapped this reactivity by competing serum reactivity from all eight animals with overlapping 15mer peptides spanning V2 (Fig. 6B). A peptide corresponding to the sequence KNFMGLKRDCKKEY (see Fig. 6 legend) reduced serum reactivity by ~80%. Alignment of this sequence with an HIV Thai 6 legend) reduced serum reactivity by ~80%.

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Editor's Summary

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