HIV TREATMENT

Lack of therapeutic efficacy of an antibody to $\alpha_4\beta_7$ in SIVmac251-infected rhesus macaques

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Sustained virologic control of human immunodeficiency virus type 1 (HIV-1) infection after discontinuation of antiretroviral therapy (ART) is a major goal of the HIV-1 cure field. A recent study reported that administration of an antibody against $\alpha_4\beta_7$ induced durable virologic control after ART discontinuation in 100% of rhesus macaques infected with an attenuated strain of simian immunodeficiency virus (SIV) containing a stop codon in *nef*. We performed similar studies in 50 rhesus macaques infected with wild-type, pathogenic SIVmac251. In animals that initiated ART during either acute or chronic infection, anti-$\alpha_4\beta_7$ antibody infusion had no detectable effect on the viral reservoir or viral rebound after ART discontinuation. These data demonstrate that anti-$\alpha_4\beta_7$ antibody administration did not provide therapeutic efficacy in the model of pathogenic SIVmac251 infection of rhesus macaques.

The development of therapeutic strategies that lead to sustained virologic remission in the absence of antiretroviral therapy (ART), defined as a functional cure, represents a major goal of HIV-1 cure research (*1*–*3*). Expression of the gut-homing integrin $\alpha_4\beta_7$ is up-regulated on a subset of memory $CD4^+$ T cells and promotes trafficking to gastrointestinal-associated lymphoid tissue (GALT), where these cells are a preferred site of early viral replication (*4*–*7*). As such, $\alpha_4\beta_7$ has been explored as a potential therapeutic target for the prevention and treatment of HIV-1 infection. It has recently been reported that administration of an antibody against $\alpha_4\beta_7$ in ART-suppressed, simian immunodeficiency virus (SIV)–infected rhesus macaques led to virologic suppression to undetectable levels in 100% of animals after ART withdrawal (*8*). Although not reported, this previous study used an attenuated strain of SIVmac239 containing a stop codon in *nef* to reduce viral replication during acute infection. On the basis of these findings, a clinical trial has been initiated to evaluate the therapeutic efficacy of anti-$\alpha_4\beta_7$ antibody administration in HIV-1–infected humans (NCT02788175). To evaluate the generalizability of these preclinical findings in a more commonly used SIV model, we evaluated the therapeutic efficacy of anti-$\alpha_4\beta_7$ antibody administration in rhesus macaques infected with wild-type, pathogenic SIVmac251.

We conducted two studies in a total of 50 SIVmac251-infected rhesus macaques that initiated ART during either early acute infection (study 1; $N = 36$) or late chronic infection (study 2; $N = 14$). In study 1, 36 rhesus macaques were infected by the intrarectal route with 500 MID_{50} wild-type, pathogenic SIVmac251 (Fig. 1A) (*9*). Preformulated ART consisting of tenofovir disoproxil fumarate, emtricitabine, and dolutegravir (TDF/FTC/DTG; Gilead) (*10*, *11*) was initiated on day 35 after infection and was continued until day 126, consistent with the previously published anti-$\alpha_4\beta_7$ antibody therapy protocol (*8*). On day 63 (4 weeks after initiation of ART), animals started receiving one of three antibody infusions, which were administered every 3 weeks for a total of eight infusions (N =12 animals per group). Group 1 received 50 mg/kg of anti-$\alpha_4\beta_7$ antibody (*8*) (clone A4B7, primatized ACT1, MassBiologics), group 2 received 5 mg/kg anti-$\alpha_4\beta_7$ antibody (clone A4B7, MassBiologics), and group 3 received 50 mg/kg isotype control antibody (clone DSPR1, MassBiologics). On day 126 (13 weeks after initiation of ART), after the fourth anti-$\alpha_4\beta_7$ antibody infusion, ART was discontinued in all animals as per the previously published protocol (*8*) (Fig. 1A).

In study 2, a similar treatment protocol was performed in 14 SIVmac251-infected rhesus macaques that initiated ART (TDF/FTC/DTG) during chronic infection. Rhesus macaques were infected with wild-type, pathogenic SIVmac251, and ART was initiated after 1 year of chronic infection. Animals were then virologically suppressed with ART for 6 months before the initiation of the antibody infusions (Fig. 1B). Chronically infected rhesus macaques had setpoint viral loads of 3 to 5 log SIV RNA copies/ml before ART initiation (fig. S1). Six months after initiation of ART, 50 mg/kg anti-$\alpha_4\beta_7$ antibody (group 1; clone A4B7, MassBiologics; $N = 7$ animals) or 50 mg/kg isotype control antibody (group 2; clone DSPR1, MassBiologics; $N = 7$ animals) infusions were started on day 0 and were administered every 3 weeks for a total of eight infusions (Fig. 1B). ART was discontinued on day 63 after the fourth anti-$\alpha_4\beta_7$ antibody infusion. Two macaques were euthanized before ART discontinuation: one in group 1 because of the development of clinical AIDS shortly after ART was initiated and one in group 2 because of an anaphylactic reaction after administration of the control DSPR1 antibody. These two animals were therefore not included in the analysis. $CD4^+$ T cell numbers from all animals were monitored over the course of these studies and showed modest declines after ART discontinuation, particularly in the chronically treated animals in study 2 (fig. S2).

We measured serum anti-$\alpha_4\beta_7$ antibody concentrations by enzyme-linked immunosorbent assay (ELISA) before and 1 day after each infusion. In study 1, anti-$\alpha_4\beta_7$ antibody concentrations in animals that received the 50 mg/kg dose reached a median peak concentration of 2.86 log μg/ml (range 2.53 to 3.09 log μg/ml) after each infusion, followed by a decline to a median of 1.72 log μg/ml 3 weeks after each infusion (Fig. 1C). Antibody concentrations were comparable after all eight infusions without the development of suppressive antidrug antibodies (ADA) (fig. S3A). In study 2, antibody concentrations mirrored those observed in study 1, with a median peak antibody concentration of 2.97 log μg/ml (range 2.79 to 3.29 log μg/ml) after each infusion, followed by a decline to a median of 1.74 log μg/ml 3 weeks after each infusion, and antibody concentrations were comparable for all eight infusions (Fig. 1D) without the development of suppressive ADA (fig. S3B).

Plasma viral loads for all macaques were measured by reverse transcription polymerase chain reaction (*10*). In study 1, peak viremia for all macaques on day 14 after SIVmac251 infection was a median of 7.43 log SIV RNA copies/ml (range 6.42 to 8.16), was comparable among groups, and was similar to our previous experience with this challenge virus (*12*, *13*) (Fig. 2, A to C). As expected, peak viral loads in these animals were ~1.5 logs higher than those achieved with the attenuated SIV containing a stop codon in the *nef* gene (*8*). Initiation of ART led to virologic control in all study groups, with viremia largely suppressed by day 56 and fully or nearly fully suppressed by the time of ART discontinuation on day 126. After discontinuation of ART, virologic rebound was observed in all macaques in all groups (Fig. 2, A to C). Peak plasma rebound viremia had a median of 4.87 log SIV RNA copies/ml (range 4.07 to

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6.37) in the 50 mg/kg anti-$\alpha_4\beta_7$ antibody group 1 (Fig. 2A); 4.15 log SIV RNA copies/ml (range 3.41 to 6.67) in the 5 mg/kg anti-$\alpha_4\beta_7$ antibody group 2 (Fig. 2B); and 4.91 log SIV RNA copies/ml (range 3.48 to 5.89) in the sham group 3 (Fig. 2C). Plasma setpoint viral loads did not show significant differences among the treatment groups on day 364 (Fig. 2D), indicating that the anti-$\alpha_4\beta_7$ antibody infusions did not lead to virologic control after ART discontinuation using this protocol (*8*) in SIVmac251-infected rhesus macaques.

We next assessed the potential impact of anti-$\alpha_4\beta_7$ antibody treatment on viral DNA in peripheral blood mononuclear cells (PBMCs), lymph nodes (LNs), and colorectal biopsies (CRs) in these animals on days 56, 126, and 210 (Fig. 2E). No significant differences in viral DNA levels were observed in these anatomical compartments in the groups that received anti-$\alpha_4\beta_7$ antibody compared with the sham group on day 126 (Fig. 2F), consistent with the lack of impact on viral rebound after ART discontinuation. Discontinuation of ART led to an increase in viral DNA in all groups on day 210, as expected.

In study 2, all animals rebounded by day 21 after ART discontinuation (study day 84) (Fig. 3, A and B). Spontaneous virologic control after a low amount of virus replication was observed in one monkey in the control group. Peak plasma viral loads after ART discontinuation had a median of 5.77 log SIV RNA copies/ml (range 3.18 to 7.12) in the 50 mg/kg anti-$\alpha_4\beta_7$ antibody group 1 (Fig. 3A) and 4.90 log SIV RNA copies/ml (range 2.27 to 6.01) in the sham group 2 (Fig. 3B). No significant differences were observed in setpoint viral loads between the anti-$\alpha_4\beta_7$ antibody group and the control group on day 364 (Fig. 3C). Viral DNA levels on days 63 and 147 in the PBMC, LN, and CR compartments also showed no differences between the anti-$\alpha_4\beta_7$ antibody group and the sham group on day 63 (Fig. 3, D and E). Discontinuation of ART led to an increase in viral DNA in both groups on day 147, as expected.

Although there was no correlation between treatment arms and setpoint viral loads in either study, pre-ART viral loads strongly correlated with setpoint viral loads after ART discontinuation at day 364 in both studies [study 1: $R = 0.53$, $P = 0.0006$, Fig. 4A; study 2: $R = 0.74$, $P = 0.0078$, Fig. 4B; Spearman's rank-correlation tests]. These data suggest that the viral loads before ART initiation were critical determinants in defining setpoint viral loads after ART discontinuation, consistent with our prior observations (*10*, *12*).

We used next-generation deep sequencing before ART initiation and after ART discontinuation in all animals in both studies to look for evidence of Env immune selection pressure (table S1). In study 1, we observed the emergence of V4 loop deletions and single-site polymorphisms in both the anti-$\alpha_4\beta_7$ antibody groups and the control group, but there was no association with the treatment arm. In study 2, we observed multiple V4 loop deletions, insertions, and polymorphisms in both groups, also with no association with the treatment arm. These data suggest that V4 mutations emerged during SIVmac251 replication but did not reflect selection pressure related to anti-$\alpha_4\beta_7$ antibody treatment.

Cellular immune responses to Env, Gag, and Pol, as measured by interferon-gamma ELISPOT assays (*14*), were comparable among groups in study 1 (fig. S4A) and study 2 (fig. S4B). Moreover, SIV Env-specific antibody responses,

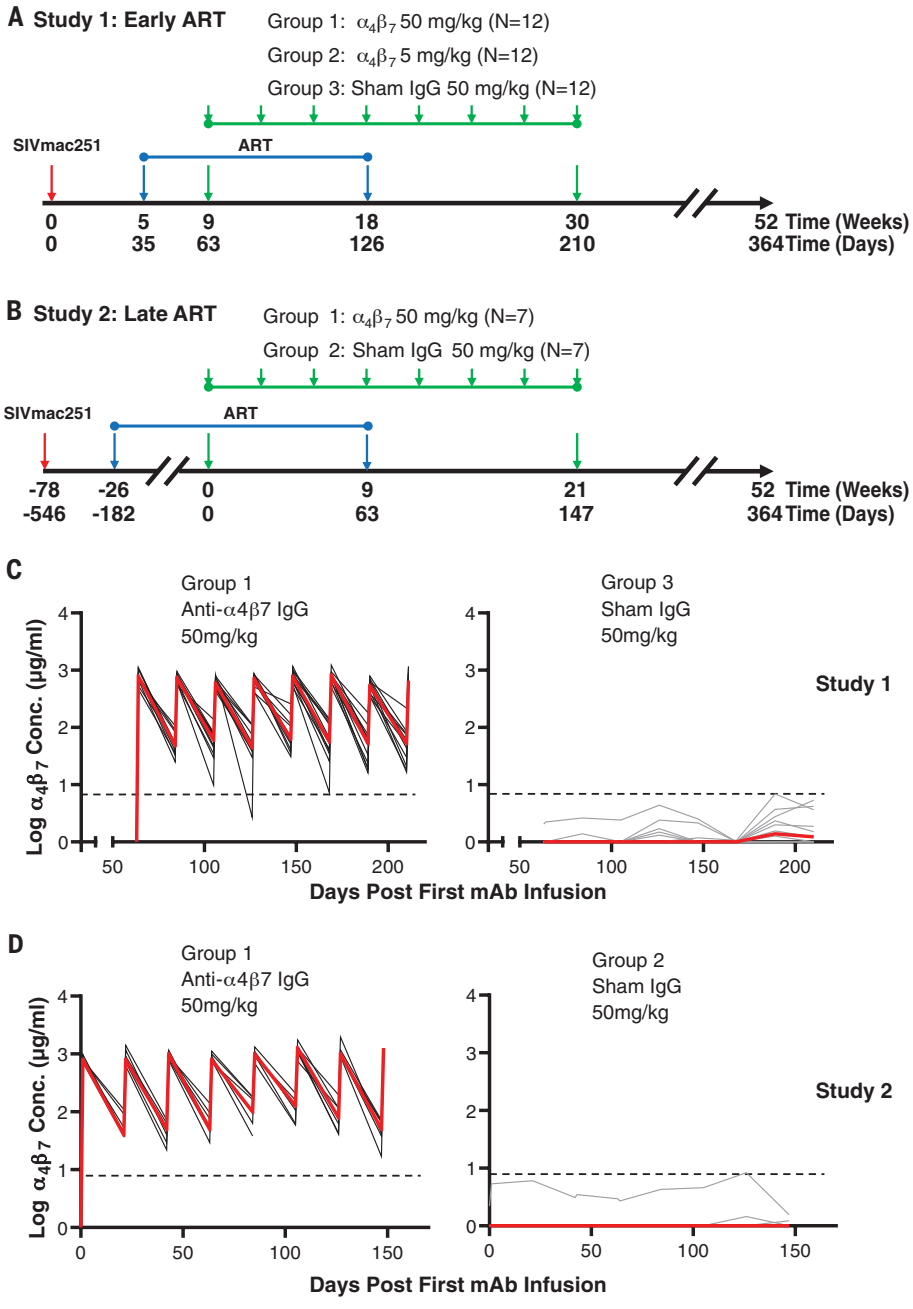

Fig. 1. Study design and antibody pharmacokinetics. Shown are schematic overviews of (**A**) study 1, early ART ($N = 36$ animals), in which ART treatment was initiated on day 35 of acute infection, and (**B**) study 2, late ART ($N = 14$ animals), in which ART was initiated after 1 year of chronic infection. SIVmac251 infection is shown with a red arrow. Initiation and discontinuation of daily ART are indicated by blue arrows. Eight antibody infusions for each group are indicated by green arrows. Serum log anti-$\alpha_4\beta_7$ antibody concentrations (in micrograms per milliliter) are shown before and after each antibody infusion in (**C**) study 1 and (**D**) study 2. Black lines represent anti-$\alpha_4\beta_7$ antibody concentrations in each individual monkey, with median concentrations shown in red. Limit of detection is 1 μg/ml.

as measured by ELISA (*13*), were comparable among groups in study 1 (fig. S5A) and study 2 (fig. S5B). Cellular and humoral immune responses decreased during ART suppression and then increased after ART discontinuation, as expected.

The experiments described here involve two studies in SIVmac251-infected rhesus macaques (table S2) that began ART during either acute or chronic infection and were designed with a comparable protocol to the published therapeutic anti-$\alpha_4\beta_7$ antibody study, including the same source, clone, dose, and regimen for the anti-$\alpha_4\beta_7$ antibody (*8*). In contrast to that prior study, we used the more common model of wild-type, pathogenic SIVmac251 instead of attenuated SIVmac239 with a stop codon in *nef* (*15*). The importance of this difference was evident in vivo by the higher peak viral loads after infection and the slower virologic control after ART initiation in the present study (Figs. 2 and 3) compared with the previous study (*8*). After ART discontinuation, exceptional virologic control was reported in 100% of macaques in the prior study (*8*). By contrast, we observed rapid viral rebound in all anti-$\alpha_4\beta_7$ antibody–treated animals that was indistinguishable from that of controls (Figs. 2 and 3). Moreover, we did not observe any impact of anti-$\alpha_4\beta_7$ antibody administration on viral DNA in PBMCs, LNs, or CRs. Our data demonstrate that anti-$\alpha_4\beta_7$ antibody administration using this protocol (*8*) had no discernable impact on cellular and humoral immune responses, viral DNA, or viral RNA after ART discontinuation in animals infected with wild-type, pathogenic SIVmac251.

Concurrent studies from Di Mascio *et al.* (*16*) and Iwamoto *et al.* (*17*) also demonstrate that anti-$\alpha_4\beta_7$ antibody infusions had no effect in the original model of attenuated SIVmac239 with a stop codon in *nef* (*8*). The reasons for these differences from the previously published study (*8*) remain unclear. However, we did observe that peak viral loads before ART initiation strongly correlated with viral rebound after ART discontinuation, as we observed in prior studies that evaluated broadly neutralizing antibodies (*18*). Taken together, these studies suggest that anti-$\alpha_4\beta_7$ antibody administration is not a viable strategy to target the viral reservoir or to induce virologic remission in SIVmac251-infected rhesus macaques.

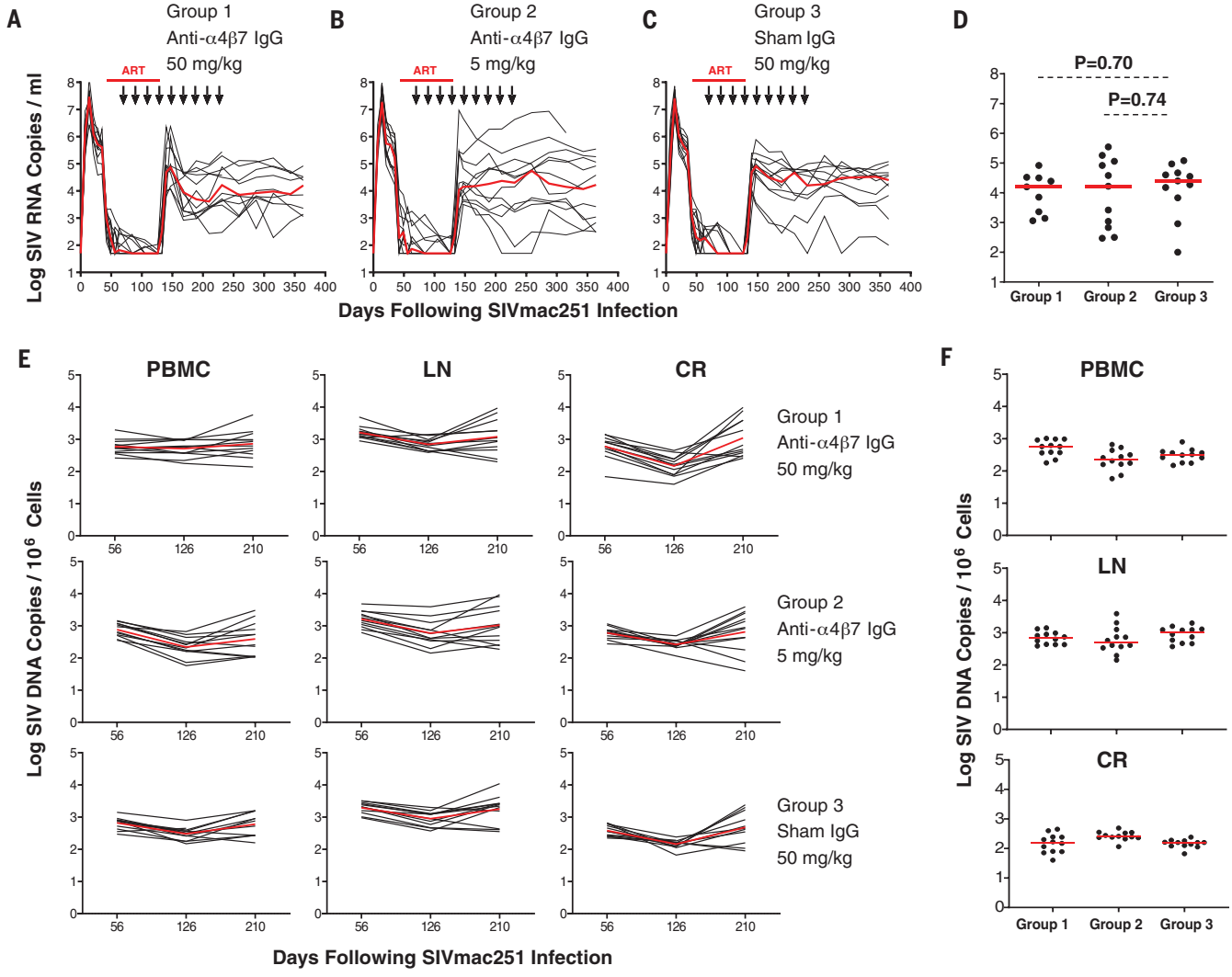

Fig. 2. Viral loads and viral DNA in anti-$\alpha_4\beta_7$ antibody–treated rhesus macaques that initiated ART during acute SIVmac251 infection (study 1). Plasma viral loads in macaques treated with (**A**) 50 mg/kg anti-$\alpha_4\beta_7$ antibody ($N = 12$), (**B**) 5 mg/kg anti-$\alpha_4\beta_7$ antibody ($N = 12$), or (**C**) sham immunoglobulin G (IgG) ($N = 12$). (**D**) Setpoint plasma viral loads on day 364. (**E**) Viral DNA levels in PBMCs, LNs, and CRs in macaques treated with 50 mg/kg anti-$\alpha_4\beta_7$ antibody, 5 mg/kg anti-$\alpha_4\beta_7$ antibody, or sham IgG. (**F**) Viral DNA levels on day 126 in PBMCs, LNs, and CRs immediately before ART discontinuation. Plasma viral loads are shown as log SIV RNA copies/ml (limit of detection, 100 copies/ml). Viral DNA is shown as log SIV DNA copies per 1×10^6 cells (limit of detection, 3 copies/10^6 cells). Red lines indicate median values. Differences between groups were calculated by paired Student's *t* tests.

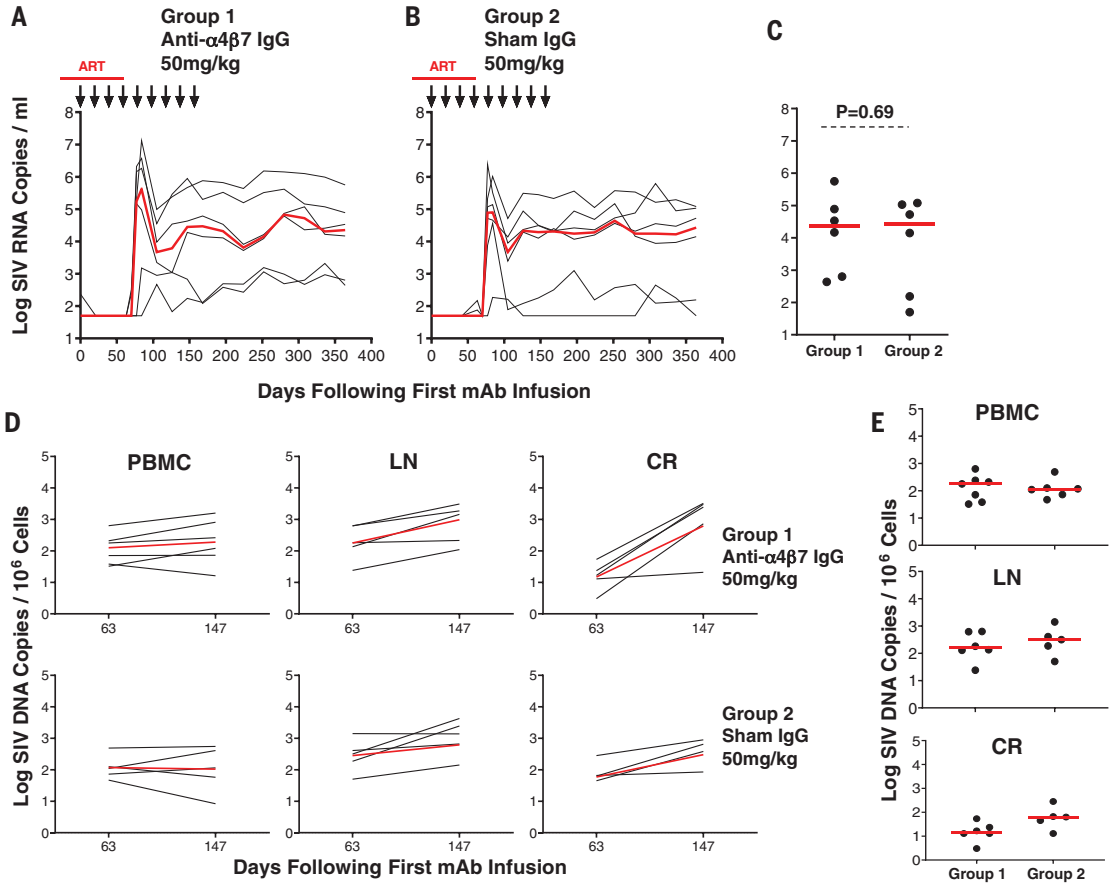

Fig. 3. Viral loads and viral DNA in anti-$\alpha_4\beta_7$ antibody–treated rhesus macaques that initiated ART during chronic SIVmac251 infection (study 2). Plasma viral loads in macaques treated with (**A**) 50 mg/kg anti-$\alpha_4\beta_7$ antibody (N = 7) or (**B**) sham IgG (N = 7). (**C**) Setpoint plasma viral loads on day 364. (**D**) Viral DNA levels in PBMCs, LNs, and CRs in macaques treated with 50 mg/kg anti-$\alpha_4\beta_7$ antibody or sham IgG. (**E**) Viral DNA levels on day 63 in PBMCs, LNs, and CRs immediately before ART discontinuation. Plasma viral loads are shown as log SIV RNA copies/ml (limit of detection, 100 copies/ml). Viral DNA is shown as log SIV DNA copies per 1 × 10^6 cells (limit of detection, 3 copies/10^6 cells). Red lines indicate median values. Differences between groups were calculated by paired Student's t tests.

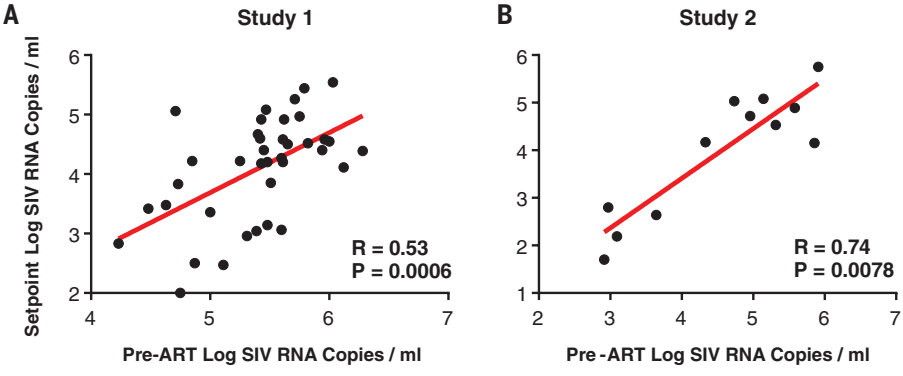

Fig. 4. Viral load correlations. Correlations between pre-ART peak viral loads and setpoint viral loads after ART discontinuation on day 364 in (**A**) study 1 and (**B**) study 2 analyzed with Spearman's rank-correlation tests.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S5
Tables S1 and S2

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Science

Lack of therapeutic efficacy of an antibody to $\alpha_4\beta_7$ in SIVmac251-infected rhesus macaques

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An antibody is not the antidote

An HIV therapeutic that would give long-term remission without sustained antiretroviral therapy (ART) is a long-term goal. Byrareddy *et al.* [*Science* **354**, 197 (2016)] reported that treating simian immunodeficiency virus (SIV)–positive macaques with an antibody against integrin $\alpha_4\beta_7$ during and after ART results in sustained virologic control after stopping all treatment. Three studies in this issue question the reproducibility of that result. Di Mascio *et al.* sequenced the virus used in the 2016 study and found that it was a variant with a stop codon in the *nef* gene rather than a wild-type virus. Abbink *et al.* used the same antibody for $\alpha_4\beta_7$ as before but tested control of a more commonly used pathogenic virus. Iwamato *et al.* used the same *nef*-stop virus as in the earlier paper but combined the antibody against the integrin with an antibody against the SIV envelope glycoprotein, which also blocks viral binding of the integrin. None of these three new studies found that treating with the antibody had any effect on virologic control after stopping ART treatment.

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