RESEARCH ARTICLE

Mucosal integrin α4β7 blockade fails to reduce the seeding and size of viral reservoirs in SIV-infected rhesus macaques

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Abstract

Cellular viral reservoirs are rapidly established in tissues upon HIV-1/SIV infection, which persist throughout viral infection, even under long-term antiretroviral therapy (ART). Specific integrins are involved in the homing of cells to gut-associated lymphoid tissues (GALT) and inflamed tissues, which may promote the seeding and dissemination of HIV-1/SIV to these tissue sites. In this study, we investigated the efficacy of prophylactic integrin blockade (α4β7 antibody or α4β7/α4β1 dual antagonist TR-14035) on viral infection, as well as dissemination and seeding of viral reservoirs in systemic and lymphoid compartments post-SIV inoculation. The results showed that blockade of α4β7/α4β1 did not decrease viral infection, replication, or reduce viral reservoir size in tissues of rhesus macaques after SIV infection, as indicated by equivalent levels of plasma viremia and cell-associated SIV RNA/DNA to controls. Surprisingly, TR-14035 administration in acute SIV infection resulted in consistently higher viremia and more rapid disease progression. These findings suggest that integrin blockade alone fails to effectively control viral infection, replication, dissemination, and reservoir establishment in HIV-1/SIV infection. The use of integrin blockade for prevention or/and therapeutic strategies requires further investigation.

KEYWORDS

α4β7/α4β1, HIV-1/SIV, integrin, viral reservoir

1 | INTRODUCTION

Integrins represent one of the most important families of cell adhesion receptors that mediate cell-cell and cell-extracellular matrix interactions. Integrins are heterodimeric transmembrane proteins composed of stable non-covalent associations between an α and β subunit. Of these, the α4 subunit (CD49d) couples with either β1 or β7 subunits, generating (for example) α4β1 and α4β7 integrins. $\alpha_4\beta_1$ integrin (very late antigen-4, VLA-4) is mostly expressed on leukocytes (except neutrophils), mediating recruitment to inflamed tissue sites through interaction with its ligand

vascular cell adhesion molecule-1 (VCAM-1).[1-3] Integrin α4β7 (Lymphocyte Peyer's patch adhesion molecule, LPAM) is predominantly expressed on a subset of T and B cells and functions as a homing receptor, mediating leukocyte homing to the gut-associated lymphoid tissues by interaction with its ligand MAdCAM-1.[4-8] Given these integrins are involved in the homing and trafficking of leukocytes to inflammatory sites and gastrointestinal (GI) tract, blockade of these integrins has been proposed as a treatment for multiple sclerosis, asthma, allergic conjunctivitis, type 1 diabetes, and inflammatory bowel disease (IBD, including ulcerative colitis and Crohn's disease).[3,9]

The HIV-1 envelope can bind integrin α4β7 and signals through the activation of LFA-1, in which the V1V2 domain of gp120, mimicking the structure of MAdCAM-1, interacts with α4β7 on CD4 + T cells,[10,11] resulting in the formation of virological synapses and subsequent cell-to-cell spreading of HIV-1.[12-17] The infected cells generally display a transitional memory Th17 phenotype with preferential expression of CCR6, α4β7, and α4β1,[18,19] serving as HIV-1/SIV target cells for viral infection and transmission,[20-22] consistent with our and another report that Integrin α4β7 expression on peripheral blood CD4(+) T cells predicts HIV-1 acquisition and disease progression outcomes.[23,24] All of these findings suggest the $\alpha_4\beta_7$ integrin plays role in HIV-1 infection and trafficking of virus-infected CD4 + T cells to mucosal tissues. In support of this, several investigations have reported that infusion of a recombinant rhesus mAb against $\alpha_4\beta_7$ (RM-Act-1) in acute SIV infection can sustain virologic control and preserve mucosal CD4 + T cells,[25-29] achieving a "functional cure" in SIV + macaques on cART. However, recent evidence indicates that blocking α4β7 integrin does not improve virologic control in HIV-1/SIV infection.[30-34] Since HIV-1 infected cell foci and systemic infection are established within hours to days following primary exposure,[35,36] here we evaluated whether early blockade of both α4β7 and α4β1 integrins could suppress virus infection and limit the seeding or size of viral reservoirs in systemic and lymphoid tissues in rhesus macaques infected with SIV as early intervention or prophylaxis treatment.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

All animals in this study were housed at the Tulane National Primate Research Center in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International standards. All studies were reviewed and approved by the Tulane University Institutional Animal Care and Use Committee under protocol number P0049. Animal housing and studies were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH, AAALAC #000594) and with the recommendations of the Weather all report; "The use of non-human primates in research." All clinical procedures were carried out under the direction of a laboratory animal veterinarian. All procedures were performed under anesthesia using ketamine, and all efforts were made to minimize stress, improve housing conditions, and to provide enrichment opportunities (eg, objects to manipulate in cage, varied food supplements, foraging and task-oriented feeding methods, interaction with caregivers and research staff).

2.2 | Animals and virus

A total of 14 adult Indian-origin rhesus macaques (*Macaca mulatta*) were intravenously inoculated with 100 TCID50 SIVmac251 and utilized to examine plasma viral load, immune cells, and cell-associated viral DNA/RNA. Of these, nine were untreated controls, other animals received intravenous infusion of anti-rhesus $\alpha_4\beta_7$ mAb (50 mg/kg at day 7 prior to SIV inoculation and day 21 post-SIV inoculation, n = 3; or same time schedule in chronically SIV-infected animal #LJ78, n = 1) or oral treatment of TR-14035 up to 2-weeks post-SIV inoculation (3mg/kg, MedChemExpress; n = 3). Blood and lymph node biopsies from three animals were collected at different time points post-SIV inoculation, processed into single-cell suspensions, and analyzed by flow cytometry and quantitative PCR.

2.3 | Tissue collection and phenotyping

Flow cytometry for surface staining was performed using standard protocols.[37] Cells were stained with: CD3 (SP34), CD4 (SK3), CD8 (SK1), β7 (M293), and LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Invitrogen, Grand Island, NY). Isotype-matched controls were included in all experiments. All antibodies and reagents were purchased from BD Biosciences Pharmingen (San Diego, CA) unless otherwise noted. Samples were resuspended in BD Stabilizing Fixative (BD Biosciences) and acquired on a FACS FORTESSA (Becton Dickinson, San Jose, CA). Data were analyzed with Flow jo software (Tree Star, Ashland, OR).

2.4 | Measurement of plasma level of anti-$\alpha_4\beta_7$ mAb

Anti-α4β7 levels were measured by ELISA. Recombinant human integrin α4β7 protein (R&D systems) were coated overnight at 1 μg/mL in PBS in high binding 96 well plates (Costar)

and then blocked with a solution of 1% Tween-20 (Sigma) and 5% non-fat dry milk (Rockland) in PBS for 2 hours. Serially diluted recombinant anti-α4β7 Ab (NHP reagents) or plasma samples diluted at 1/5000 were added to the plate for 1 hour. Plates were then washed five times with PBS/1% Tween-20 and incubated 1 hour with mouse anti-monkey IgG-HRP (Southern Biotech). After five washes, the TMB substrate (Southern Biotech) was applied for 20 minutes. The reaction was stopped with 100 μL/well TMB stop solution before reading OD at 450 nm on an ELISA plate reader (Synergy H4, BioTek).

2.5 | Genomic DNA and total RNA extraction

Fresh peripheral blood mononuclear cells (PBMC) were isolated from EDTA-treated venous blood by density gradient centrifugation with Lymphocyte Separation Medium (MP Biomedicals, Santa Ana, CA). Lymph nodes and rectal biopsies were collected at designated time points and processed to extract total genomic DNA and cellular RNA by AllPrep DNA/RNA Mini Kit (Qiagen) according to the manufacturer's instructions. Viral RNA in plasma was directly isolated using the QIAamp Viral RNA Mini Kit (Qiagen).

2.6 | Quantification of plasma viral load and cell-associated SIV RNA transcripts

The extracted RNA was reverse transcribed into cDNA using a SuperScript III first-strand synthesis system (Invitrogen) according to the manufacturer's protocol. RT reactions were performed in a thermocycler at 25.0°C for 5 minutes, 50.0°C for 60 minutes, followed by an enzyme inactivation step at 70.0°C for 15 minutes. For the quantification of targets, all primer/probe sets were synthesized by Integrated DNA Technologies IDT (Coralville, IA, USA) to target the SIVmac239 gag region (Forward primer: GTC TGC GTC ATC TGG TGC ATT C; Reverse primer: CAC TAG GTG TCT CTG CAC TAT CTG TTT TG; and probe: FAM-CTT CCT CAG TGT GTT TCA CTT TCT CTT CTG CG-BHQ-1). Plasma viral loads were measured by real-time PCR as we previously described.[38] cDNA from cell-derived RNA was used to quantify unspliced RNA transcripts by digital droplet PCR (QX100 Droplet Digital qPCR system, Bio-Rad) with minor modification as previously published.[39] Samples were run in duplicate in a 20 μL volume containing Supermix, 250 nM primers, 900 nM probe, and 2 μL of undiluted cDNA as following cycling conditions: 10 minutes at 95°C, 40 cycles of 94°C/30 seconds and 63°C/60 seconds, followed by final 98°C for 10 minutes. Droplets were analyzed by the

QuantaSoft software in the absolute quantification mode. Copies of SIV transcripts expressed as copies per one million cells were measured and normalized to cellular input, as determined by copies of genomic CCR5 (single-copy rhesus macaque CCR5 DNA per cell).[40-44] The limit of detection (LOD) was based on three or more replicates and calculated using GenEx 5 (www.multid.se).

2.7 | Quantification of cell-associated SIV DNA

To ensure that the quantification of total SIV DNA, 2-LTR DNA, and integrated proviral DNA was comparable, a series of specific standards (plasmids containing SIV U5 DNA, 2-LTR DNA junction[45] or CCR5 DNA fragment) were prepared to perform nested PCR. Since HIV-1 preferentially integrates into regions of the chromosome close to *Alu* repeats, two *Alu* primers are used to amplify the segments of integrated proviral DNA.[46] Two-step PCR amplification was run in parallel to quantify viral DNA as described.[47-50] Briefly, the preamplification reactions were performed using SIV long terminal repeat primer and two outward Alu primers, or primer pairs of U5 (Forward primer: AGG CTG GCA GAT TGA GCC CTG GGA GGT TC; Reverse primer: CCA GGC GGC GAC TAG GAG AGA TGG GAA CAC; probe: FAM-TTC CCT GCT AGA CTC TCA CCA GCA CTT GG-BHQ-1) on 7900HT Sequence Detectors (Life Technologies). The reaction conditions were performed as following: 25 μL of the reaction mix, containing 1X PCR buffer, 0.2 mM dNTPs, 2 mM MgCl₂, 0.8 μM of each primer, and 0.5 U Taq DNA polymerase (Invitrogen Life Technologies), was programmed to perform a 5 minutes hot start at 95°C, followed by 20 cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 30 seconds and extension at 72°C for 3 minutes. 2.5 μL of these amplicons were further amplified in triplicate with each primer/probe pairs by real-time PCR reaction using 40 cycles at 95°C for 15 seconds and 63°C for 1 minute. The highly reproducible calibration curves were generated by plotting Cq values against log-transformed concentrations of serial standard. Internal standard curves were also generated using the known copy number of target plasmids (1-500 copies) diluted in cellular DNA from SIV naïve RMs. The calibration curves and the internal regression curves were used for interpolating initial copies of each target in unknown samples. A non-template control (NTC) and extracted cellular DNA from the HUT78/SIVmac239 cell line (positive control) were included in the qPCR reactions. As described above, quantification of SHIV RNA/DNA was expressed as copies per 1 million cells, in which cell numbers were determined by copies of genomic CCR5 DNA per cell.
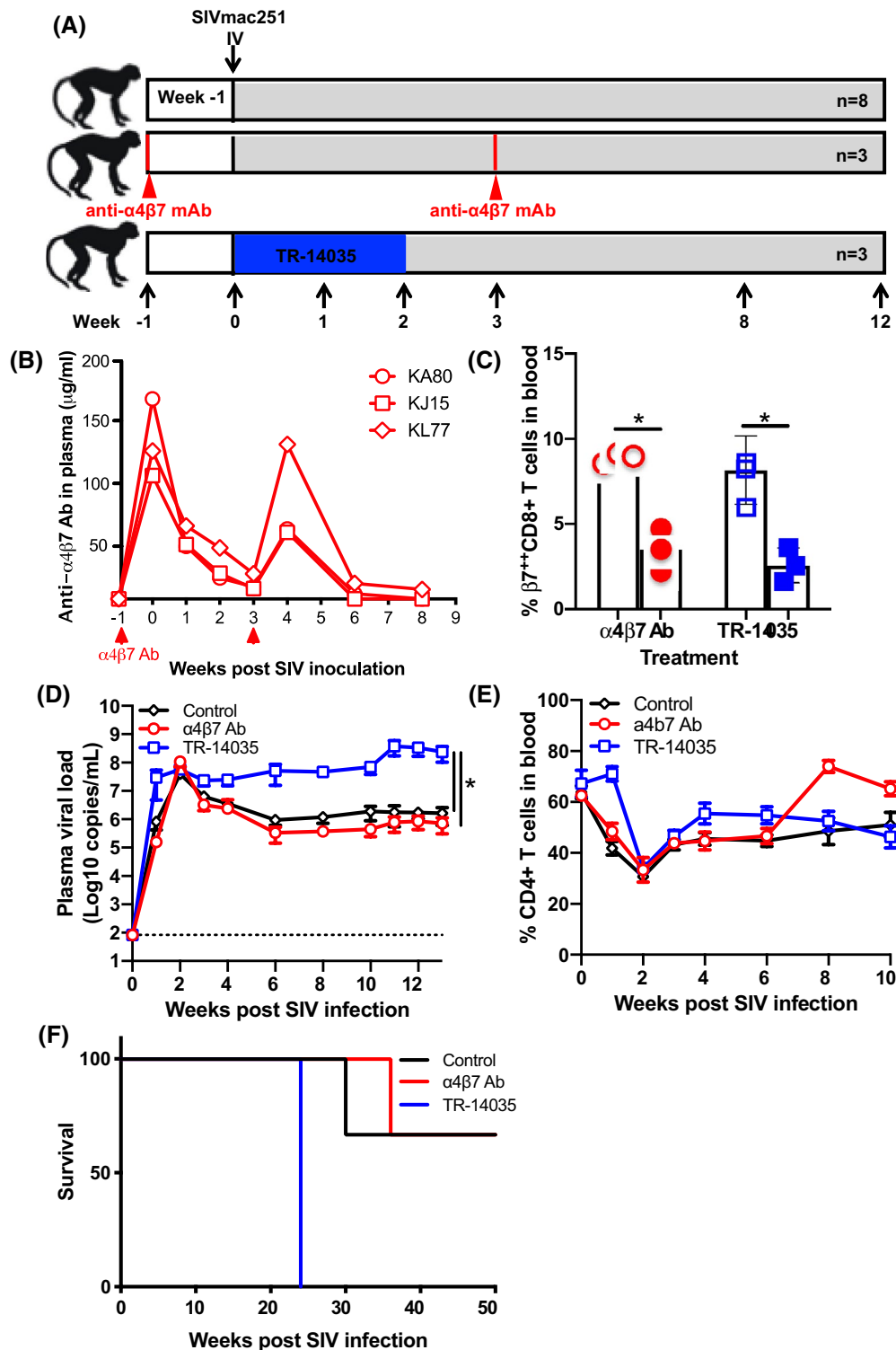
FIGURE 1 Efficacy of prophylactic integrin blockade on the viral acquisition and disease progression in rhesus macaques inoculated with SIV. A, Schematic representation of the study protocol and sampling in SIV inoculated macaques given intravenous anti-$\alpha_4\beta_7$ monoclonal antibody or oral TR-14035 ($\alpha_4\beta_7/\alpha_4\beta_1$ dual antagonist) treatment in early stages of SIV inoculation; B, Pharmacokinetics of plasma $\alpha_4\beta_7$ Ab levels after intravenous infusion; C, Detection of $\beta_7$high on CD8 + T cells from integrin inhibitor-treated or untreated animals at week 1 ($\alpha_4\beta_7$ Ab treatment or control) or week 3 (TR-14035 treatment or control) by flow cytometry; D, Plasma viral load in macaques post-SIV inoculation; E, Changes in peripheral CD4 + T cells in macaques post-SIV inoculation; F, Survival of macaques post-SIV inoculation. $\alpha_4\beta_7$ Ab, n = 3; TR-14035, n = 3; untreated controls, n = 8. Note that orally TR-14035 treated SIV-inoculated animals showed high viremia and rapid disease progression. Error bars indicate means ± SEM. Paired *t* tests were used to compare groups. *, $P < .01$

2.8 | Statistics

Statistical analyses were performed by non-parametric Mann-Whitney t test (two tailed) using GraphPad Prism 4.0 (GraphPad Software, SanDiego, CA). Significant statistic differences are indicated by asterisks (*$P < .05$,). The data are presented as the mean and s.e.m.

3 | RESULTS

3.1 | Integrin blockade at early stage post-SIV inoculation fails to suppress viral infection

Integrins play essential roles in lymphocyte homing and may mediate the trafficking of HIV-1 infected cells into tissues, and if so, integrin blockade would be expected to limit virus spread and seeding of various tissues, resulting in the reduction of plasma viremia and preservation of mucosal CD4 + T cells. In addition to anti-$\alpha_4\beta_7$ Ab, the small molecule TR-14035 can block both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins, and its therapeutic potential is being tested in inflammatory diseases.[51-53] To evaluate the potential efficacy of integrin blockade , SIV-infected animals were treated by intravenous infusion of anti-rhesus $\alpha_4\beta_7$ mAb at −7 and 21 post-SIV inoculation or orally administered TR-14035 daily for 2-weeks after SIV inoculation (Figure 1A). Consistent with previous reports,[25] $\alpha_4\beta_7$ mAb levels in plasma peaked after one week of antibody infusion, and gradually declined afterward (Figure 1B). Since β7high CD4 + T cells are usually depleted after SIV infection,[23] CD8 + T cells were used to directly assess the degree of β7 blockade from the inhibitors. The results showed both $\alpha_4\beta_7$ mAb and TR-14035 could bind/block integrin β expression, as indicated by a significant reduction of peripheral β7high CD8 + T cells in animals treated by $\alpha_4\beta_7$ Ab or TR-14035, as determined by flow cytometry using anti-β7 staining and analysis (Figure 1C). These data show that both integrin inhibitors could successfully bind and block integrin β expression following in vivo administration. However, although plasma $\alpha_4\beta_7$ Ab levels were at high levels at the time of SIV infection, $\alpha_4\beta_7$ Ab blockade did not substantially prevent SIV infection or prevent replication after infection as both the dynamics and magnitude of plasma viremia in anti-$\alpha_4\beta_7$ Ab treated animals were essentially equivalent to controls. Unexpectedly, treatment with $\alpha_4\beta_1/\alpha_4\beta_7$ dual antagonist (TR-14035) led to higher viremia throughout SIV infection as evidenced by significantly higher levels of viral RNA in plasma compared $\alpha_4\beta_7$ Ab treated or untreated controls. Further, integrin inhibitor administration also did not prevent peripheral CD4 + T cell depletion from animals on day 14 after SIV inoculation (Figure 1D and E). Finally, and in concert with the higher viremia in the TR-14035 treated group, these animals also showed more rapid disease progression to

AIDS (Figure 1F). These findings suggested that these integrin inhibitors lack significant prophylactic or treatment efficacy in early SIV infection.

3.2 | Treatment of Integrin inhibitors does not suppress viral replication and reservoir seeding in macaques inoculated with SIV

We next examined levels of cell-associated SIV RNA/DNA in peripheral blood and lymphoid tissues of macaques post-SIV inoculation, with or without integrin inhibitor treatment ($\alpha_4\beta_7$ Ab or TR-14035). As shown in Figure 2A-D, multiple viral parameters in PBMCs, including unspliced SIV RNA, multiply spliced SIV RNA (tat/rev), total SIV DNA, and two-long-terminal-repeat (2-LTR) circular SIV DNA were detected as early as day 7 after SIV inoculation in all animals, regardless of treatment. High levels of cell-associated SIV RNA/DNA were maintained throughout SIV infection. In addition to unintegrated viral DNA and 2-LTR DNA, total HIV-1/SIV DNA also includes integrated proviral DNA, which is the more accurate marker of viral reservoirs in HIV-1/SIV infection, and represents the major obstacle for cure strategies in patients even when plasma viremia is undetectable.[54-56] Our data showed that proviral DNA was detectable by 7 days of inoculation and was maintained at considerable levels (Figure 2E), suggesting that integrin inhibitor blockade did not limit viral DNA integration in PBMCs. Notably, there were no significant differences in cell-associated SIV RNA/DNA levels between treated animals and untreated controls. These data further confirm that the administration of integrin inhibitors does not suppress SIV infection at the cell level.

3.3 | Effects of prophylactic $\alpha_4\beta_7$ Ab infusion on viral reservoir seeding in lymphoid tissues of macaques inoculated with SIV

Integrins mediate migration and homing of peripheral/local leukocytes, including initial HIV-1/SIV viral target cells that become infected in mucosal tissue sites, which may then home to and seed distal gut-associated lymphoid tissues, promoting viral replication and amplification in tissues. Thus, a prophylactic blockade of mucosal homing integrins was expected to reduce the size of viral reservoirs. As indicated in Figure 1A and B, animals received anti-rhesus $\alpha_4\beta_7$ Ab by intravenous injection before SIV inoculation (day −7 days), and the levels of plasma $\alpha_4\beta_7$ Ab reached peaked by day 7 when they were intravenously challenged with SIV. In addition to peripheral blood, we also examined cell-associated SIV DNA/RNA in lymph nodes and rectal biopsies in SIV-inoculated macaques. The results showed that all SIV RNA
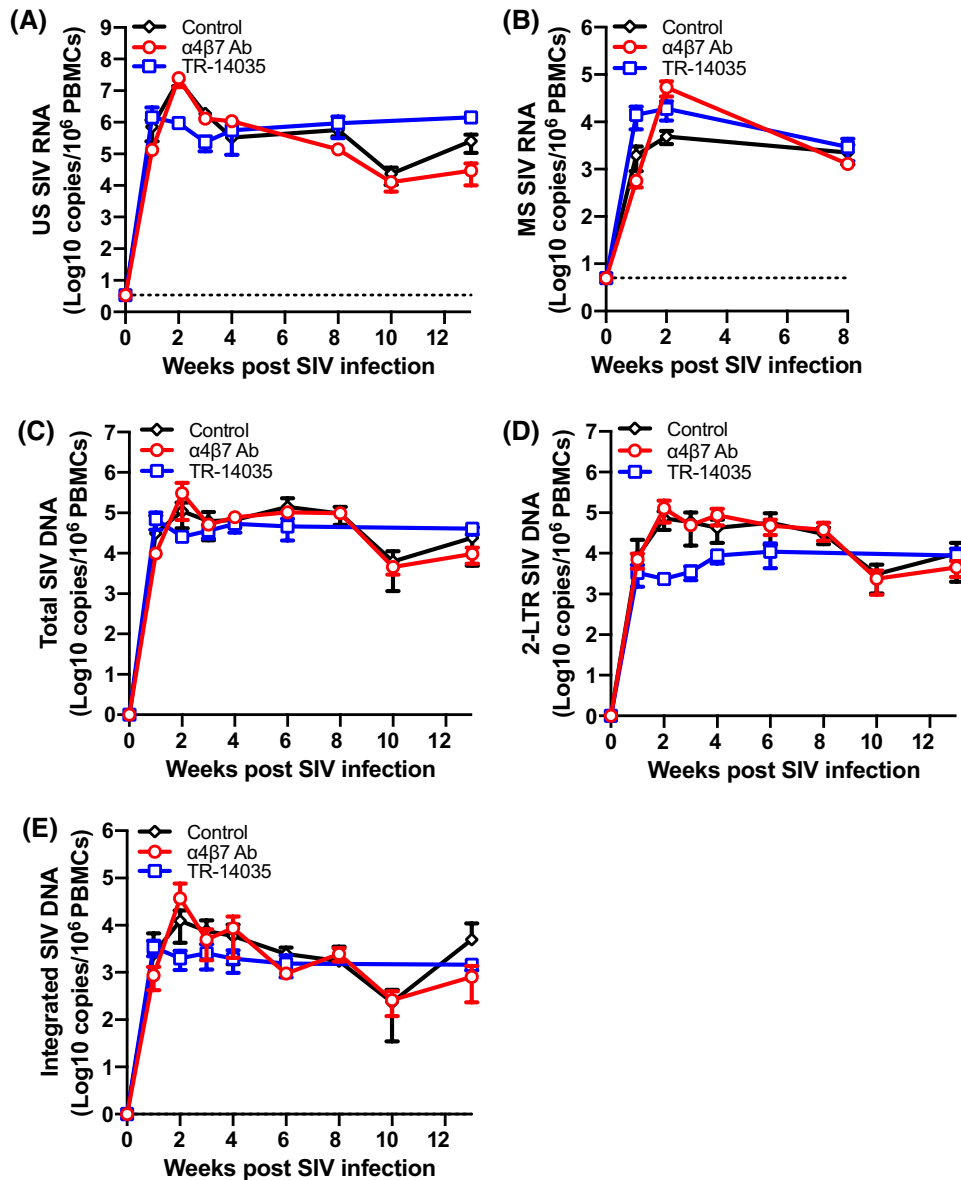
FIGURE 2 Effects of prophylactic integrin blockade on viral replication and reservoir seeding in peripheral blood mononuclear cells of rhesus macaques inoculated with SIV. Dynamics of unspliced SIV RNA (US SIV RNA, A), multiply tat/rev spliced SIV RNA (MS SIV RNA, B), total SIV DNA (C), circular 2-LTR SIV DNA (D), or integrated proviral SIV DNA (E) in PBMCs of animals treated with $\alpha_4\beta_7$ Ab or TR-14035, or untreated controls. Note that the dynamics of viral replication and proviral DNA in peripheral blood showed no significant differences between groups. Error bars indicate means ± SEM

and SIV DNA, including US SIV RNA, MS SIV RNA, total SIV DNA, 2-LTR SIV DNA, and proviral DNA, could be detected within 7 days, and levels were stably maintained in lymph nodes throughout SIV infection. Similar to PBMCs, these were no significant differences in individual SIV RNA/DNA levels between $\alpha_4\beta_7$ Ab treated and untreated animals infected with SIV (Figure 3A-E). Further rectal CD4 + T cells and cell-associated SIV levels in rectal tissues in $\alpha_4\beta_7$ Ab treated or untreated animals were compared 3 months after SIV inoculation and as shown in Figure 3F, there were also no significant differences between these groups, including percentages of rectal CD4 + T cells, and/or SIV RNA/

DNA levels. In summary, prophylactic $\alpha_4\beta_7$ Ab infusion failed to suppress viral reservoir seeding in both lymph nodes and gut-associated lymphoid tissues (GALT) of rhesus macaques intravenously challenged with SIV.

4 | DISCUSSION

Although long-term antiretroviral therapy significantly reduces the number of HIV-1 infected cells, residual cellular reservoirs containing proviral DNA persist, and promote viral rebound upon antiretroviral therapy interruption,
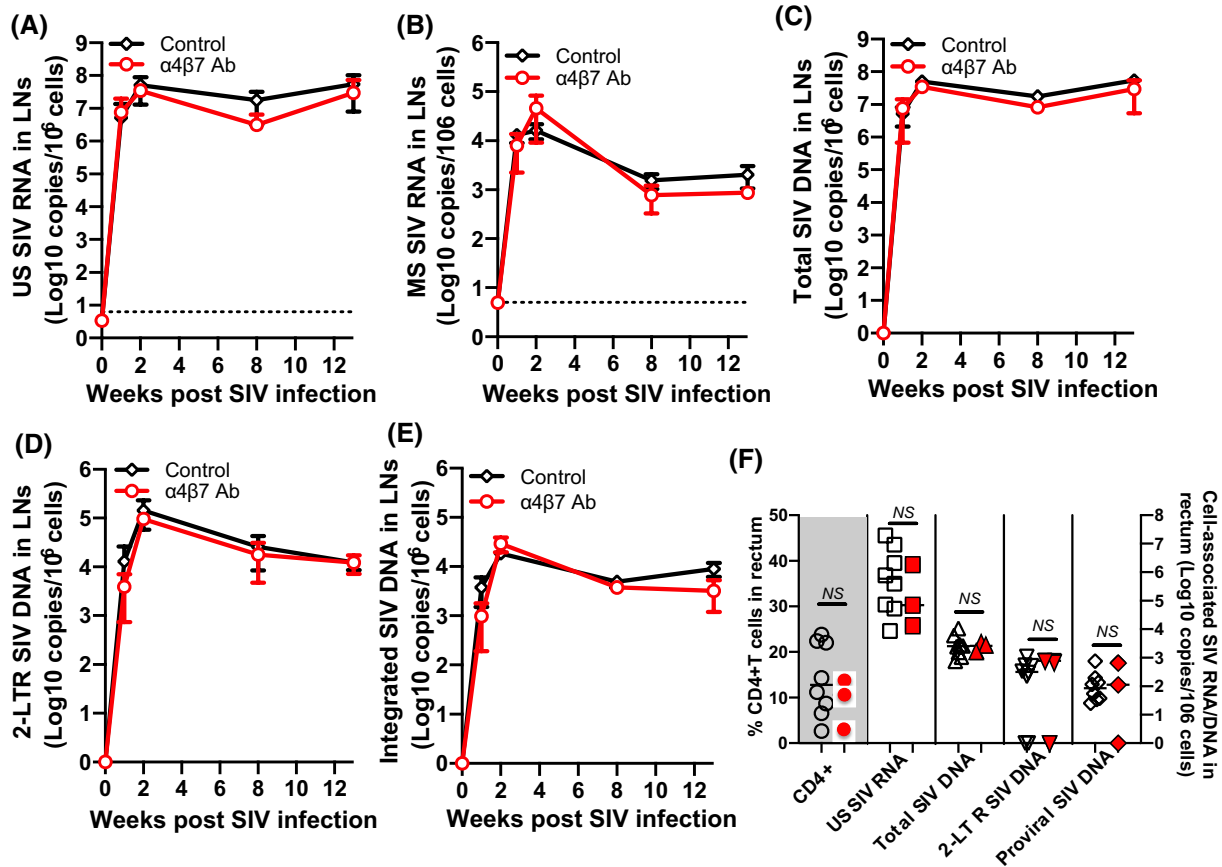
FIGURE 3 Effects of prophylactic $\alpha_4\beta_7$ Ab infusion on seeding and size of viral reservoirs in lymph nodes and rectum of rhesus macaques inoculated with SIV. Longitudinal changes in unspliced SIV RNA (US SIV RNA, A), multiply tat/rev spliced SIV RNA (MS SIV RNA, B), total SIV DNA (C), circular 2-LTR SIV DNA (D), or integrated proviral SIV DNA (E) in auxiliary lymph nodes of animals treated with $\alpha_4\beta_7$ Ab, compared with untreated controls. (F) Changes in rectal CD4 + T cells and cell-associated SIV RNA/DNA in rectal lymphocytes of macaques with or without early $\alpha_4\beta_7$ Ab treatment at 3 mo post-SIV inoculation. Note prophylactic $\alpha_4\beta_7$ Ab treatment did not preserve gut-associated mucosal CD4 + T cells or reduce viral reservoirs in either lymph nodes or rectum. Error bars indicate means $\pm$ SEM. NS. No significances were determined by two-tailed paired t tests

which is the major hurdle to a cure for HIV-1 infection. Since integrins, such as $\alpha4\beta7/\alpha4\beta1$, are engaged in the migration, trafficking, and homing of local cells to distal lymphoid tissues and are likely involved in the dissemination of HIV-1 infected cells and seeding of tissue reservoirs, here, we evaluated the efficacy of prophylactic and preventive integrin blockade ($\alpha4\beta7$ Ab or $\alpha4\beta7/\alpha4\beta1$ dual antagonist) on viral infection, replication, and reservoir seeding in systemic and lymphoid tissues of SIV-infected macaques. Consistent with recent reports, these results showed integrin blockade does not suppress viral infection or limit the size of viral reservoirs in blood or lymphoid tissues, as indicated by equivalent levels of plasma viral loads and cell-associated SIV DNA/RNA to untreated controls. Surprisingly, treatment of TR-14035 (a compound with dual $\alpha4\beta7/\alpha4\beta1$ inhibitor activity) resulted in even higher viremia throughout SIV infection and more rapid disease progression. These findings suggest that integrin blockade alone is insufficient to prevent or control HIV-1/SIV infection and reservoir seeding. Prophylactic

or therapeutic usage of integrin inhibitors in combination with antiretroviral therapy requires further investigation in HIV-1 + patients.

In the HIV-1/SIV life cycle, the virus produces unspliced RNA (~9-Kb) and two class sizes (early ~2-Kb and late ~4-Kb RNA), which are involved in viral replication, integration, translation, virion assembling, and release.[57,58] Various viral nucleic acids, representing different infectious status and clinical significance,[59-62] can be measured: HIV-1 gag RNA transcripts represent *bona fide* HIV-1 unspliced RNA during viral replication[63,64]; multiply spliced HIV-1/SIV tat/rev RNA express functional proteins for viral replication and production[65]; extrachromosomal two-long-terminal-repeat (2-LTR) circular viral DNA are bystander products of unintegrated HIV-1/SIV DNA[62]; and; 4) a pool of integrated proviral DNA is believed to contribute to viral rebound after ART cessation.[66] Therefore, cell-associated viral RNA or DNA assays may be used as potential indicators to estimate the size of HIV-1/SIV reservoirs.

Integrins, composed of α and β subunits, interact with the extracellular matrix and ligands expressed on leukocytes, regulating cell adhesion, migration, and homing to distant tissue sites. Of these, α4β7 and α4β1 typically play major roles in homing or trafficking of leukocytes to the GALT or inflamed tissues. Functional integrins may also involve the trafficking of virus-infected CD4 + T cells to distal mucosal lymphoid tissues, resulting in reservoir seeding in various tissues. It is also known that the HIV-1 envelope can bind integrin α4β7 on CD4 + T cells,[10,11] promoting HIV-1 spreading by cell-to-cell virological synapses.[12-16] Given the functions of integrins, we hypothesized early integrin blockade could help prevent HIV-1 infection or at least reduce the seeding and size of viral reservoirs in systemic and lymphoid compartments. Early studies demonstrate that α4β7 blocking antibody, combined with ART, could maintain low to undetectable viral loads and normal CD4 T cell counts in the peripheral blood and gastrointestinal tissues, even after all treatment is withdrawn.[25,27-29,67] However, our data indicate that prophylactic $\alpha_4\beta_7$ blockade essentially fails to prevent SIV infection and CD4 + T cell depletion in macaques intravenously inoculated by SIV, consistent with recent reports that blocking α4β7 integrin does not improve virologic control in either HIV-1 or SIV infection.[30-34] Possible explanations for these discrepancies could be differences in the viral stocks, dose of virus (high vs low-dose), routes of inoculation (eg, intravenous vs intrarectal challenge), timing of the treatments, or short-, long-term α4β7 treatment, yet we show high levels of anti-a4b7 antibody were still present at the time of virus inoculation (Figure 1). We also tested the therapeutic efficacy of α4β7 Ab on levels of viremia and reservoir size in one chronically SIV-infected animal, and still observed no significant impact on plasma viral loads or cell-associated SIV RNA/DNA levels (Suppl Figure 1). Our data showed that both α4β7 or α4β7/α4β1 blockade did not increase viremia and cell-associated SIV RNA in peripheral blood and lymphoid tissues during treatment, speculating latency reactivation may not be induced, albeit lack of direction evidence. Despite integrins are not a marker of the viral reservoir per se, integrin but not limited to α4β7 expressed on CD4 + T cells may be a target for latency reactivation,[68] effects of integrin blockade need to be further investigated. The integrin blockade is being tested as a treatment for multiple sclerosis, asthma, and inflammatory bowel disease.[9,69,70] Notably, the preferential pairing of β1 with the α4 subunit (α4β1) suppresses α4β7 expression and regulates the localization of memory CD4 + T cells in intestinal sites.[71] Further, neither the α4 small molecule inhibitor (ELN) nor anti-α4 Ab (natalizumab) block the replication of HIV-1/SHIV since the α4 subunit is not an essential cofactor for HIV-1 attachment and infection.[72,73] We thus investigated whether an $\alpha_4\beta_1/\alpha_4\beta_7$ dual antagonist (TR-14035) was able to better suppress SIV infection and limit the reservoir size in SIV-infected animals

when treated very early. As shown in Figures 1 and 2, TR-14035 clearly could bind and block β7 subunit expression, albeit its effects on other subunits are unknown. Surprisingly, oral treatment of TR-14035 up to two weeks after SIV inoculation did not prevent animals from virus infection or high levels of replication. In contrast, TR-14035 treatment consistently resulted in higher viremia as compared with α4β7 Ab treated or untreated animals and led to more rapid disease progression. Although speculative, TR-14035 treatment may compromise CD8 + T cell responses in tissues since α4β1 integrin is also essential for CD8 T-cell migration and function,[74,75] dampening Th2 responses,[76,77] homeostasis and immunity by cell-to-cell communication.[78,79] Our data also indicated that TR-14035 treatment reduced the frequency of peripheral CD20 + B cells beginning at ~1 month after SIV infection, compared with α4β7 Ab blockade or untreated controls (data not shown). However, the reasons behind its exacerbating effects on viremia remain unknown. Nonetheless, these findings suggest that integrin blockade may not be a useful treatment for the prevention or treatment of early HIV-1 infection.

DISCLOSURES

AUTHOR CONTRIBUTIONS

We thank Meagan Watkins and Eunice Vincent for animal scheduling and sample processing. WZ performed quantitative PCR and analyze the data; JS, AF, PC, and XW assisted with experiments; RSV provided advice and revisions; HX designed the experiments, analyzed the data, and wrote the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.