２．実用新案登録
無し

３．その他
無し

II．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

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Ⅲ．研究成果の刊行物・別刷

HIV-1 Infection *Ex Vivo* Accelerates Measles Virus Infection by Upregulating Signaling Lymphocytic Activation Molecule (SLAM) in CD4+ T Cells

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Measles virus (MV) infection in children harboring human immunodeficiency virus type 1 (HIV-1) is often fatal, even in the presence of neutralizing antibodies; however, the underlying mechanisms are unclear. Therefore, the aim of the present study was to examine the interaction between HIV-1 and wild-type MV (MVwt) or an MV vaccine strain (MVvac) during dual infection. The results showed that the frequencies of MVwt- and MVvac-infected CD4+ T cells within the resting peripheral blood mononuclear cells (PBMCs) were increased 3- to 4-fold after HIV-1 infection, and this was associated with a marked upregulation of signaling lymphocytic activation molecule (SLAM) expression on CD4+ T cells but not on CD8+ T cells. SLAM upregulation was induced by infection with a replication-competent HIV-1 isolate comprising both the X4 and R5 types and to a lesser extent by a pseudotyped HIV-1 infection. Notably, SLAM upregulation was observed in HIV-infected as well as -uninfected CD4+ T cells and was abrogated by the removal of HLA-DR+ cells from the PBMC culture. Furthermore, SLAM upregulation did not occur in uninfected PBMCs cultured together with HIV-infected PBMCs in compartments separated by a permeable membrane, indicating that no soluble factors were involved. Rather, CD4+ T cell activation mediated through direct contact with dendritic cells via leukocyte function-associated molecule 1 (LFA-1)/intercellular adhesion molecule 1 (ICAM-1) and LFA-3/CD2 was critical. Thus, HIV-1 infection induces a high level of SLAM expression on CD4+ T cells, which may enhance their susceptibility to MV and exacerbate measles in coinfected individuals.

The attenuated measles virus (MV) vaccine has greatly reduced the morbidity and mortality of measles in industrialized countries. However, measles is still a leading cause of death among children in developing countries, especially in sub-Saharan Africa, where almost 90% of global pediatric human immunodeficiency virus type 1 (HIV-1) infections occur (http://apps.who.int/ghodata/). Because both HIV-1 and MV cause immunosuppression, it is conceivable that coinfection with HIV-1 and MV increases the risk of disease progression (17). In fact, an observational study of hospitalized children in Zambia showed that the fatality rate increased among HIV-1-infected children with measles (18).

The low levels of neutralizing antibodies in HIV-1-infected children may explain the high mortality of measles. The measles vaccine is only weakly immunogenic in HIV-1-infected children, inducing only low levels of neutralizing antibody, which decline rapidly (17). However, a recent large-scale prospective study in Zambia conducted by Moss et al. reported a good initial antibody response to measles vaccine in both HIV-1-infected and -uninfected children (19). Moreover, to understand the impact of HIV-1 infection on the clinical manifestation of measles, Permar et al. conducted a study using MV-vaccinated or -unvaccinated rhesus monkeys that are chronically infected with a simian immunodeficiency virus (24). They monitored the virological and immunological status of the monkeys after MV challenge and found that the clinical manifestation of measles occurs even in monkeys with high titers of vaccine-induced MV neutralizing antibody.

This finding implies that the presence of neutralizing antibody alone is not sufficient protection from measles in HIV-1-infected individuals. Therefore, it is highly likely that an as yet unknown factor(s)/mechanism(s) affected by HIV-1 is involved in the exacerbation of measles in HIV-1-infected individuals.

Some studies analyzed the interaction between MV and HIV-1 *in vitro*. Garcia et al. studied HIV-1 replication in peripheral blood mononuclear cells (PBMCs) coinfected with MV and found that MV-induced inhibition of lymphocyte proliferation suppresses HIV-1 replication, but without any apparent increase in the production of chemokines or any other soluble factors in coinfected cultures (7). In a more recent study, the same group demonstrated that the cell cycle progression of T cells, which is required for efficient HIV-1 replication, was blocked by MV (8); however, it is still not clear whether HIV-1 affects MV infection directly or indirectly.

Understanding the impact of HIV-1 infection on MV infection

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and replication is important both for developing successful strategies for measles eradication and for HIV-1 control. The receptor for wild-type MV has been identified to be signaling lymphocytic activation molecule (SLAM; also known as CD150), and attenuated vaccine strains can utilize both SLAM and CD46 (4). Recently, nectin4 was also identified as an MV receptor that is important for MV to spread into epithelial cells and release viral particles from the apical membrane into the lumen of the respiratory tract (20, 21). However, SLAM remains a major receptor of MV in lymphoid organs and plays an important role in a systemic MV infection. Therefore, the aim of the present study was to investigate the course of MV infection in HIV-1-infected PBMCs *ex vivo* at the level of the individual cell, focusing on SLAM expression. The results presented here showed that HIV-1 replication in resting PBMCs induces the upregulation of SLAM expression on CD4$^+$ T cells in a manner that is dependent on cell-to-cell contact, resulting in higher levels of MV infection.

MATERIALS AND METHODS

Cell preparation. Human peripheral blood samples were collected from healthy donors after receiving written informed consent. Sample collection was approved by the institutional ethical committee of the National Institute of Infectious Diseases (Tokyo, Japan). PBMCs were separated by Ficoll-Hypaque density gradient centrifugation (Lymphosepal; IBL, Gunma, Japan). T cells were isolated using a total T cell enrichment kit (StemCell Technologies, Vancouver, BC, Canada), after depletion of CD14$^+$ cells. For monocyte depletion, CD14$^+$ cells were depleted from PBMCs using magnetically activated cell sorting (MACS) with CD14 microbeads (Miltenyi Biotec, Cologne, Germany). For B cell and HLA-DR$^+$ cell depletion, PBMCs were incubated with biotin-labeled anti-CD19 (BioLegend, San Diego, CA) and biotin-labeled anti-HLA-DR (BioLegend) antibodies, respectively, followed by positive selection using anti-biotin tetrameric antibody complex (TAC), magnetic colloid, and an EasySep magnet (all from StemCell Technologies).

Preparation of virus stock. To prepare HIV-1 clones HIV-1$_{NL-E}$, HIV-1$_{NLAD8-E}$, and HIV-1$_{NL-D}$, human 293T embryonic kidney cells seeded at a density of 7 × 10^6 cells/15-cm dish were transfected with 30 μg of pNL-E, pNLAD8-E, and pNL-D, respectively, using the calcium phosphate precipitation method as described previously (29). To prepare HIV-1 pseudotyped with vesicular stomatitis virus glycoprotein (HIV-1/VSV-G), 293T cells were cotransfected with 36 μg of pNL-EdENV (pNL-E with an *env*-inactivating mutation) and 4 μg of pVSV-G per 7 × 10^6 cells. At 2 days posttransfection, the culture supernatant was collected, filtrated, and frozen at −80°C. The amounts of viruses in each culture supernatant were measured using an in-house HIV-1 Gag p24 enzyme-linked immunosorbent assay.

To prepare green fluorescent protein (GFP)-expressing wild-type (IC323-EGFP) (12) or vaccine strain (GFP-MVAIK) (6) MV, 1 × 10^7 human SLAM-expressing Vero cells (Vero/hSLAM) cells were infected with 1 × 10^5 PFU of each virus (multiplicity of infection [MOI] = 0.01) for 2 h, washed, and then grown in Dulbecco's modified Eagle medium (DMEM; Gibco, Carlsbad, CA) supplemented with 2% heat-inactivated fetal bovine serum (FBS). Infected cells were harvested when approximately 80% cytotoxicity was observed. Cells were then frozen and thawed three times and sonicated for 10 s to release the cell-bound viruses. The titer of measles virus was measured using a plaque assay, described in the following section.

Titration of MV. MV was titrated as described previously (26). In brief, monolayers of 2 × 10^5 Vero/hSLAM cells grown in 12-well plates were infected with serially diluted wild-type MV (MVwt) or an attenuated MV vaccine strain (MVvac). After removal of viruses, the cells were overlaid with DMEM supplemented with 2% methylcellulose and 2% FBS. At day 5 postinfection, cells were stained with 5% neutral red, the numbers of plaques were counted, and the numbers of PFU/ml were calculated.

HIV-1 and MV infection. For HIV-1 infection, untreated PBMCs or PBMCs depleted of either CD14$^+$ monocytes, CD19$^+$ B cells, or HLA-DR$^+$ cells or purified T cells (1 × 10^6 cells) were infected with 100 ng of p24 of either HIV-1$_{NL-E}$, HIV-1$_{NL-D}$, HIV-1$_{NLAD8-E}$, or HIV-1/VSV-G for 2 h, washed three times with RPMI 1640 (RPMI), and then cultured in RPMI supplemented with 5% heat-inactivated human serum (P-RPMI), penicillin (100 μg/ml), and streptomycin (100 μg/ml). At day 3 postinfection, the culture medium was changed to 5% P-RPMI supplemented with interleukin-2 (IL-2; 50 U/ml) and then cultured for 2 days.

For MV infection, HIV-1$_{NL-D}$-infected and -uninfected PBMCs (1 × 10^6 cells) were mock infected or infected with 5 × 10^4 PFU of either MVwt or MVvac for 2 h at day 5 after HIV infection, washed three times with RPMI, and then cultured in 5% P-RPMI for 2 days.

Flow cytometry. Cells were stained with a suitable combination of fluorescence-labeled monoclonal antibodies (MAbs): Pacific Blue-labeled anti-CD4 (eBioscience, San Diego, CA), allophycocyanin (APC)-Cy7-labeled anti-CD8 (eBioscience), peridinin chlorophyll protein-labeled anti-CD3 (R&D Systems, Inc., Minneapolis, MN), APC-labeled anti-CD14 (R&D Systems), phycoerythrin (PE)-labeled anti-SLAM (eBioscience), and PE-Cy7-labeled anti-CD19 (BioLegend). Dead cells were visualized using a LIVE/DEAD fixable dead cell stain kit (Invitrogen, Carlsbad, CA). HIV-1- and/or MV-infected cells were analyzed using flow cytometry (FACSCant II flow cytometer; BD Bioscience, Pharmingen, CA) and a FACSDiva flow cytometer (BD Bioscience) or Flowjo software (Tree Star, San Carlos, CA).

Detection of cytokines. The levels of gamma interferon (IFN-γ), IL-1β, IL-2, IL-5, IL-10, tumor necrosis factor alpha (TNF-α), and TNF-β in the culture supernatant of the HIV-1-infected or mock-infected PBMC cultures were measured using a FlowCytomix human Th1/Th2 11plex kit (Bender MedSystems, Vienna, Austria), according to the manufacturer's protocol, at day 5 postinfection. The minimum detection levels for each cytokine were as follows: IFN-γ, 1.6 pg/ml; IL-1β, 4.2 pg/ml; IL-2, 16.4 pg/ml; IL-4, 20.8 pg/ml; IL-5, 1.6 pg/ml; IL-6, 1.2 pg/ml; IL-8, 0.5 pg/ml; IL-10, 1.9 pg/ml; IL-12 p70, 1.5 pg/ml; TNF-α, 3.2 pg/ml; and TNF-β, 2.4 pg/ml. Results were calculated using FlowCytomix Pro software (Bender MedSystems).

Transwell assay. HIV-1-infected or -uninfected PBMCs were cultured in the top chamber of a transwell plate (pore size, 0.4 μm; Costar; Corning, Corning, NY). HIV-1-uninfected PBMCs were placed on the bottom chamber and cultured for 5 days.

Blocking of antibodies against cell adhesion molecules. An isotype control IgG1 (PeproTech Inc., Rocky Hill, NJ) or serial dilutions of anti-leukocyte function-associated molecule 1α (anti-LFA-1α) or anti-LFA-3 MAbs (serially diluted from 10 μg/ml) were added to HIV-1-infected PBMC cultures just after 2 h of infection to analyze the effect of cell-to-cell contact on SLAM upregulation. Anti-LFA-1α and anti-LFA-3 MAbs were prepared from hybridomas kindly provided by Hideo Yagita (Juntendo University, Tokyo, Japan).

Statistical analysis. Because of the limited sample size, each experiment was performed once per donor. Data obtained from less than three donors were excluded from the statistical analysis. The significance of the data was evaluated by the Mann-Whitney U test, by the Tukey multiple-comparison test, or by use of the Pearson correlation coefficient on the basis of the normality and variance of the data using GraphPad Prism software (version 4.0; GraphPad Software, San Diego, CA). P values of <0.05 were considered statistically significant.

RESULTS

HIV-1 infection enhances the infectivity of coinfected MV *ex vivo*. First, the consequences of coinfection of PBMCs with HIV-1 and MV were analyzed at the single-cell level using flow cytometry. DsRed-expressing (DsRed$^+$) HIV-1$_{NL-D}$- or mock-infected PBMCs were coinfected with GFP-expressing (GFP$^+$) MVwt or
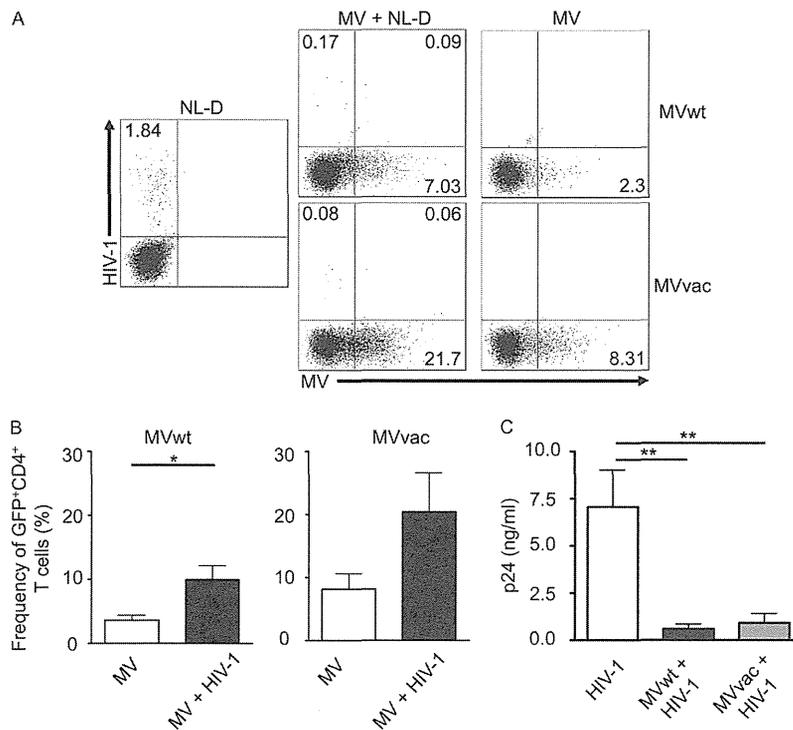
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A

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C

FIG 1 Effect of HIV-1 infection on MV infection in an *ex vivo* HIV-1/MV coinfection model. PBMCs were coinfected with HIV-1$_{NL-D}$ and/or either MVwt or MVvac, and the infected cells were analyzed. (A) Representative flow cytometry plots showing MVwt- or MVvac-infected CD4$^+$ T cells. (B) Cumulative data showing the frequency of MVwt- or MVvac-infected CD4$^+$ T cells. The bars represent the mean ± SEM ($n = 7$). *P* values were calculated using the Mann-Whitney U test. *, $P < 0.05$. (C) Levels of p24 antigen in culture supernatants of HIV-1 and/or MV-infected PBMCs. The bars represent the mean ± SEM ($n = 5$). *P* values were calculated using one-way analysis of variance followed by the Tukey multiple-comparison test. **, $P < 0.01$.

MVvac at day 5. Two days later, HIV- and/or MV-infected cells were analyzed. As shown in Fig. 1A, HIV-1$_{NL-D}$-infected and MV-infected cells were identified as DsRed$^+$ and GFP$^+$ cells, respectively. In the case of MVwt infection, the frequency of MVwt-infected CD4$^+$ T cells within the HIV-1$_{NL-D}$-infected PBMC population (9.96% ± 2.45%) was significantly higher than that in the MV-only-infected PBMC population (3.60% ± 0.77%) ($P = 0.0175$; $n = 7$; Fig. 1B, left). Likewise, in the case of MVvac infection, the frequency of MVvac-infected CD4$^+$ T cells tended to be higher within the HIV-1$_{NL-D}$-infected PBMC population (20.42% ± 6.20%) than within the MV-only-infected PBMC population (8.14% ± 2.45%), although the result was not statistically significant ($P = 0.1158$; $n = 7$; Fig. 1B, right). These results indicated that HIV-1 infection enhances MV infection in PBMCs. It should be noted that the HIV-infected CD4$^+$ T cell population disappeared upon MV infection (from 1.84% to 0.26% and 0.14% for MVwt and MVvac, respectively), and the doubly infected CD4$^+$ T cell population was rarely visible (Fig. 1A). Although we used the same MOI, the percentage of MV-infected T cells was always higher in MVvac infection than in MVwt infection. This is probably due to the wider tropism of MVvac, which utilizes both SLAM and CD46 as receptors (4).

The level of HIV-1 Gag p24 in the culture supernatant was also significantly reduced by coinfection with either MVwt or MVvac compared with that observed after infection with HIV-1$_{NL-D}$ alone ($P < 0.01$; $n = 5$; Fig. 1C). These results are consistent with those reported previously; i.e., MV infection inhibits the replication of HIV-1 (7, 8, 10).

SLAM expression on CD4$^+$ T cells is induced by HIV-1 infection. Because both MVwt and MVvac utilize SLAM as a receptor (5, 22), SLAM expression was examined in HIV-1-infected PBMCs. PBMCs were infected with either CXCR4-tropic HIV-1$_{NL-E}$, CCR5-tropic HIV-1$_{NLAD8-E}$, or HIV-1/VSV-G, all of which express GFP, and were then cultured for 5 days without additional stimulation (apart from the addition of IL-2). It is noteworthy that, regardless of HIV-1 infection, SLAM expression was slightly increased under these culture conditions at day 5 (basal increase), and this basal increase varied among individuals (5.17 ± 2.63% at day 0 to 8.81% ± 2.00% at day 5; $n = 7$). Therefore, the net increase in the frequency of SLAM^{hi} CD4$^+$ or SLAM^{hi} CD8$^+$ T cells was calculated by subtracting their respective basal levels of increase. Induction of SLAM expression on CD8$^+$ T cells was low and did not increase statistically significantly with HIV-1 infection (Fig. 2A, bottom, and B, right). However, importantly, both the level of SLAM expression and the frequency of SLAM^{hi} CD4$^+$ T cells increased after infection with HIV-1, and increased SLAM expression was observed in HIV-1-infected (GFP$^+$) as well as in uninfected CD4$^+$ T cells (Fig. 2A, top). Because the net increase in SLAM expression varied between donors, PBMCs from 10 donors were examined. The frequency of SLAM^{hi} CD4$^+$ T cells markedly increased in the HIV-1$_{NL-E}$-infected cultures (8.79% ± 1.47%), while the frequency in HIV-1/VSV-G-infected cultures increased only slightly (2.52% ± 0.58%). This difference between HIV-1$_{NL-E}$- and HIV-1/VSV-G-infected cultures was statistically significant ($P < 0.01$; $n = 10$; Fig. 2B). Of note, HIV-1/VSV-G-infected
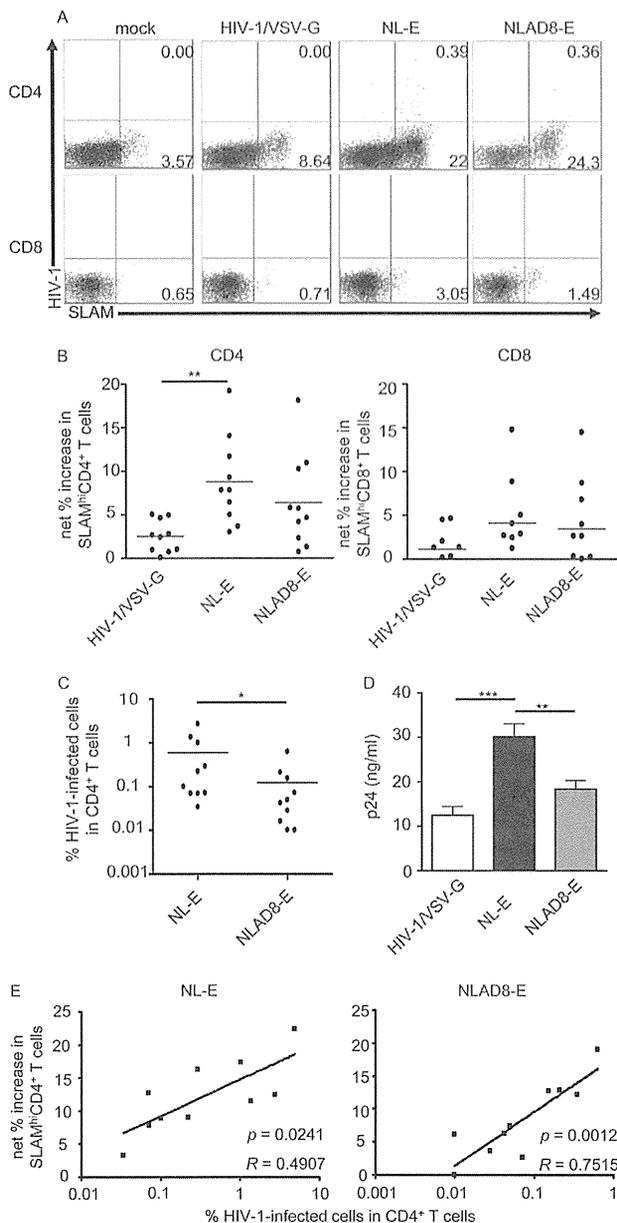
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(GFP$^+$) cells were scarcely detectable under these conditions, probably reflecting the low transduction efficiency of VSV-pseudotyped lentivirus in unstimulated T cells. A marked upregulation of SLAM expression induced by HIV-1$_{\mathrm{NLAD8-E}}$ infection was also observed in some donors, but the difference between HIV-1$_{\mathrm{NLAD8-E}}$ and HIV-1/VSV-G-infected cultures was not statistically significant (Fig. 2B). This probably reflects the variable number and low frequency of CCR5$^+$ CD4$^+$ T cells (5 to 10% of CD4$^+$ T cells), which are a target of CCR5-tropic HIV-1$_{\mathrm{NLAD8-E}}$, in donor PBMCs. As expected, the frequency of HIV-1$_{\mathrm{NL-E}}$-infected CD4$^+$ T cells (0.88% ± 0.28%) was higher than that of HIV-1$_{\mathrm{NLAD8-E}}$-infected CD4$^+$ T cells (0.19% ± 0.01%) ($P = 0.0433$; $n = 10$; Fig. 2C). In parallel with the high frequency of SLAM$^{\mathrm{hi}}$ CD4$^+$ T cells, the levels of p24 were the highest in the culture supernatants of HIV-1$_{\mathrm{NL-E}}$-infected cultures compared to other HIV-1-infected cultures (Fig. 2D). There was a significant correlation between the frequency of HIV-1-infected CD4$^+$ T cells and that of SLAM$^{\mathrm{hi}}$ CD4$^+$ T cells in both HIV-1$_{\mathrm{NL-E}}$-infected ($R = 0.4907$, $P = 0.0241$; $n = 10$) and HIV-1$_{\mathrm{NLAD8-E}}$-infected ($R = 0.7517$; $P = 0.0012$; $n = 10$) cultures (Fig. 2E).

To determine whether the replication of HIV-1 was required for SLAM upregulation, PBMCs were infected with a 20-fold higher dose of HIV-1/VSV-G. The frequency of GFP$^+$ CD4$^+$ T cells and SLAM$^{\mathrm{hi}}$ CD4$^+$ T cells under these conditions was identical to that seen in HIV-1$_{\mathrm{NL-E}}$-infected PBMCs (see Fig. S1 in the supplemental material). Taken together, these results indicated that HIV-1 replication is not essential but that higher and/or persistent levels of HIV-1 are involved in the upregulation of SLAM expression on CD4$^+$ T cells.

All subsequent studies were carried out using CXCR4-tropic HIV-1$_{\mathrm{NL-E}}$.

SLAM upregulation by HIV-1 infection is not caused by direct infection of CD4$^+$ T cells. Despite the fact that HIV-1 infection enhanced SLAM expression on CD4$^+$ T cells, upregulation was more obvious in HIV-1-uninfected CD4$^+$ T cells (Fig. 2A). To further test the importance of direct HIV-1 infection of CD4$^+$ T cells for SLAM upregulation, T cells were enriched from PBMCs. PBMCs and T cells were separately infected with HIV-1$_{\mathrm{NL-E}}$, and the expression of SLAM on CD4$^+$ T cells was analyzed after 5 days of culture. A representative result from six individuals is shown in Fig. 3A, and plots from all six individuals are shown, with averages, in Fig. 3B. The majority of HIV-1$_{\mathrm{NL-E}}$-infected CD4$^+$ T cells in the purified T cell cultures were SLAM-dull (Fig. 3A), and the net increase in the frequency of SLAM$^{\mathrm{hi}}$ CD4$^+$ T cells (2.87% ± 1.04%) was much lower than that in the PBMC cultures (13.00% ± 3.72%) ($P < 0.01$; $n = 6$; Fig. 3B). To examine in more detail which cell population within the PBMCs contributed to the upregulation of SLAM by HIV-1 infection, whole PBMCs and those depleted of either CD14$^+$ monocytes, CD19$^+$ B cells, or HLA-DR$^+$ cells were infected with HIV-1$_{\mathrm{NL-E}}$. Cell depletion was evaluated by flow cytometry (Fig. 3C). The removal of HLA-DR$^+$ cells resulted in depletion of both monocytes and B cells (Fig. 3C, lower right). Of note, a minor population of CD123-, CD141-, and/or CD303-expressing cells (peripheral dendritic cells [pDCs] and conventional dendritic cells [DCs]) were also removed by depletion of HLA-DR$^+$ cells (data not shown). There were no significant differences in the increase in SLAM$^{\mathrm{hi}}$ CD4$^+$ T cells among monocyte- and B cell-depleted PBMCs compared to whole PBMCs following HIV-1 infection in four donors; the relative increases in monocyte- and B cell-depleted PBMCs compared to
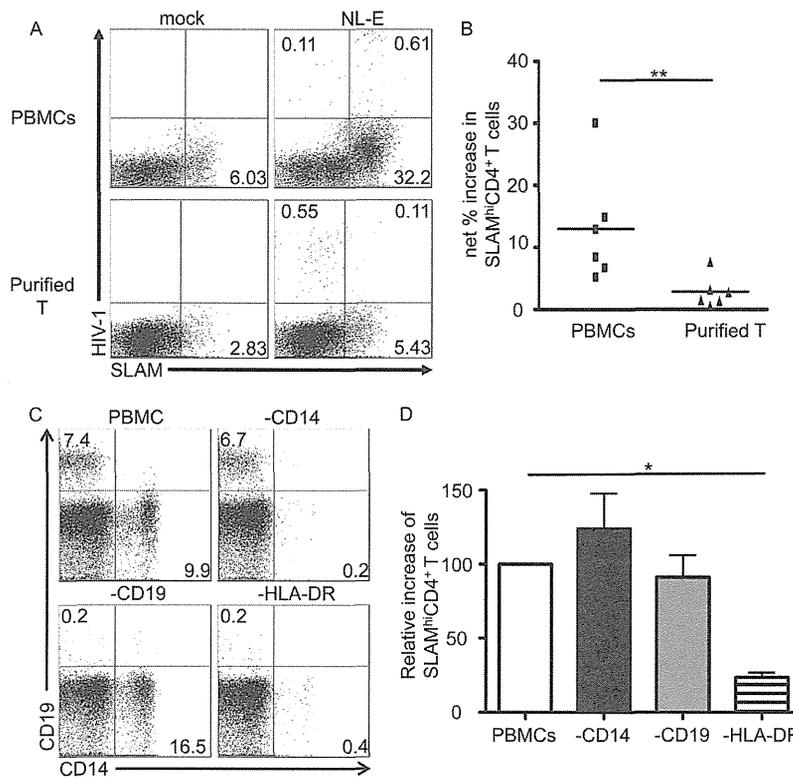
FIG 2 SLAM expression on CD4$^+$ T cells within the HIV-1-infected PBMC population. (A and B) PBMCs were infected with HIV-1/VSV-G, HIV-1$_{\mathrm{NL-E}}$, or HIV-1$_{\mathrm{NLAD8-E}}$ and SLAM expression on CD4$^+$ T cells was analyzed. (A) Representative flow cytometry plots showing SLAM expression on CD4$^+$ T cells and CD8$^+$ T cells. (B) Cumulative data showing the percent increase in the frequency of SLAM$^{\mathrm{hi}}$ CD4$^+$ and SLAM$^{\mathrm{hi}}$ CD8$^+$ T cells from 10 donors. P values were calculated using one-way analysis of variance followed by the Tukey multiple-comparison test. **, $P < 0.01$. (C) Cumulative data showing the frequency of HIV-1-infected CD4$^+$ T cells from 10 donors. P values were calculated using the Mann-Whitney U test. *, $P < 0.05$. (D) Levels of p24 antigen in culture supernatants of HIV-1-infected PBMCs. The bars represent the mean ± SEM ($n = 10$). P values were calculated using one-way analysis of variance followed by the Tukey multiple-comparison test. **, $P < 0.01$; ***, $P < 0.001$. (E) Correlation between the frequency of HIV-1$_{\mathrm{NL-E}}$- and HIV-1$_{\mathrm{NLAD8-E}}$-infected CD4$^+$ T cells and the percent increase in the frequency of SLAM$^{\mathrm{hi}}$ CD4$^+$ T cells from 10 donors. Correlation statistics were analyzed using the Pearson correlation.

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FIG 3 Comparison of the levels of SLAM upregulation on CD4$^+$ T cells induced by HIV-1 infection in the presence or absence of non-T cells. (A and B) Purified T cells and PBMCs were separately infected with HIV-1$_{NL-E}$. (A) Representative flow cytometry plots showing SLAM expression on CD4$^+$ T cells. (B) Cumulative data showing the percent increase in SLAM^{hi} CD4$^+$ T cells. The bars represent the mean ± SEM ($n = 6$). P values were calculated using the Mann-Whitney U test. **, $P < 0.01$. (C and D) PBMCs were infected with HIV-1$_{NL-E}$ after removal of monocytes, B cells, or HLA-DR$^+$ cells. (C) Representative flow cytometry plot evaluating the depletion of monocytes, B cells, or HLA-DR$^+$ cells. (D) Cumulative data showing the relative increase in the frequency of SLAM^{hi} CD4$^+$ T cells. The increase in the frequency of SLAM^{hi} CD4$^+$ T cells by HIV-1 infection in the PBMC population was set to 100%. The bars represent the mean ± SEM ($n = 4$). P values were calculated using one-way analysis of variance followed by the Tukey multiple-comparison test. *, $P < 0.05$.

whole PBMCs (set at 100%) were 124.1% ± 23.68% and 91.19% ± 14.84%, respectively ($n = 4$). In contrast, SLAM expression was significantly repressed in HLA-DR$^+$ cell-depleted PBMCs following HIV-1 infection (relative increase, 23.65% ± 3.13%; $n = 4$). Taken together, these results indicated that a population of HLA-DR-expressing cells, including DCs, but not monocytes and B cells, is involved in the upregulation of SLAM by HIV-1 infection.

Role of cytokines in induction of SLAM expression during HIV infection. SLAM expression on T cells and DCs is upregulated by IFN-γ (9) and IL-1β (14), respectively. Therefore, the levels of 11 cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, TNF-α, and TNF-β) in the culture supernatants of HIV-1$_{NL-E}$-infected and -uninfected PBMCs were measured at day 5. The results showed that the production of IFN-γ, IL-1β, and TNF-α in HIV-1$_{NL-E}$-infected PBMCs was significantly higher than that in uninfected PBMCs ($P = 0.0006, 0.0023,$ and 0.0041, respectively; $n = 4$; Fig. 4A). The levels of IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, and TNF-β were low or undetectable, irrespective of HIV-1 infection (data not shown). SLAM upregulation on CD4$^+$ T cells was not affected, when HIV-1$_{NL-E}$-infected PBMCs were cultured in the absence or presence of anti-IFN-γ blocking MAb (data not shown). Furthermore, SLAM upregulation was not observed in PBMC cultures in the presence of recombinant IFN-γ (data not shown).

To test the potential contribution of any soluble factors produced in HIV-1-infected PBMC cultures, we performed a transwell assay. HIV-1$_{NL-E}$-infected PBMCs were seeded into the top chamber of the transwell, and uninfected PBMCs were placed in the bottom chamber. The cells were then cultured for 5 days. A representative result is shown in Fig. 4B. SLAM was markedly upregulated in HIV-1$_{NL-E}$-infected PBMCs in the top chamber (net increase, 32.81%; Fig. 4B, upper right), whereas upregulation was less obvious in uninfected PBMCs in the bottom chamber (net increase, 3.35%; Fig. 4B, lower right). The result was reproduced using PBMCs from eight separate donors, and the difference was statistically significant (bottom chamber, 1.07% ± 0.80%; top chamber, 9.08% ± 4.60%; $P < 0.001$; $n = 5$; Fig. 4C). Thus, these data clearly show that soluble factors produced by HIV-1 infection make a minimal contribution (if any) to SLAM upregulation on CD4$^+$ T cells. Rather, cell-to-cell contact would appear to be the most important factor.

Importance of costimulatory molecules for SLAM upregulation on CD4$^+$ T cells. SLAM expression on T cells is induced by T cell receptor (TcR) stimulation with anti-CD3 antibody (3). In addition, Sheng and colleagues showed that LFA-3/CD2 interaction and, particularly, CD2 signaling are necessary but not sufficient for CD4$^+$ T cell activation (25). We showed earlier that in PBMCs, HLA-DR$^+$ cells are largely responsible for the upregulation of SLAM after HIV-1 infection (Fig. 3C). Previously, we
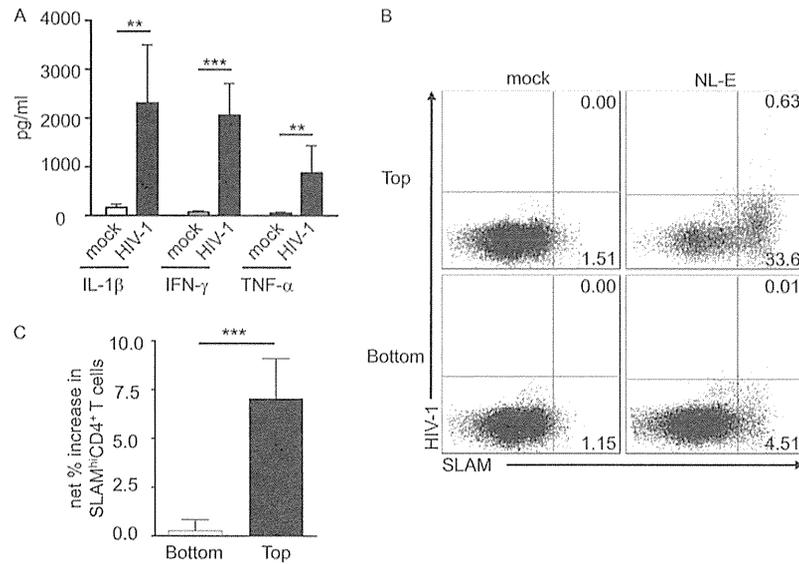
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FIG 4 Impact of soluble factors on SLAM upregulation by HIV-1-infected CD4$^+$ T cells. (A) PBMCs were infected with HIV-1$_{NL-E}$, and the cytokine levels in the culture supernatants were measured. The bars represent the mean $\pm$ SEM ($n = 7$). P values were calculated using the Mann-Whitney U test. **, $P < 0.01$; ***, $P < 0.001$. (B and C) HIV-1$_{NL-E^-}$ and mock-infected PBMCs were cultured in the top chamber and uninfected PBMCs were placed in the bottom chamber of a transwell plate. (B) Representative flow cytometry plots showing SLAM expression on CD4$^+$ T cells. (C) Percent increase in the frequency of SLAM^{hi} CD4$^+$ T cells. The bars represent the mean $\pm$ SEM ($n = 8$). P values were calculated using the Mann-Whitney U test. ***, $P < 0.001$.

showed that HIV-1 replication and expansion are associated with the activation of CD4$^+$ T cells through cell-to-cell contact with monocyte-derived DCs via costimulatory molecules such as LFA-1/intercellular adhesion molecule 1 (ICAM-1) and LFA-3/CD2 (27). We next tested the effect of blocking antibodies that inhibited these interactions on SLAM expression on CD4$^+$ T cells. HIV-1$_{NL-E}$-infected PBMCs were cultured in the absence or presence of blocking MAbs against LFA-1α and LFA-3. As

shown in Fig. 5A, increased SLAM expression on CD4$^+$ T cells within the HIV-1$_{NL-E}$-infected PBMC population was inhibited by anti-LFA-1α MAb (48.81% $\pm$ 18.12%; $n = 5$) as well as by anti-LFA-3 MAb (86.58% $\pm$ 6.93%; $n = 5$), although the effect of anti-LFA-1α MAb was less pronounced and was not statistically significant at 10 μg/ml (Fig. 5B). Nevertheless, both anti-LFA-1α and anti-LFA-3 MAbs inhibited SLAM upregulation in a dose-dependent manner (Fig. 5C), and the in-
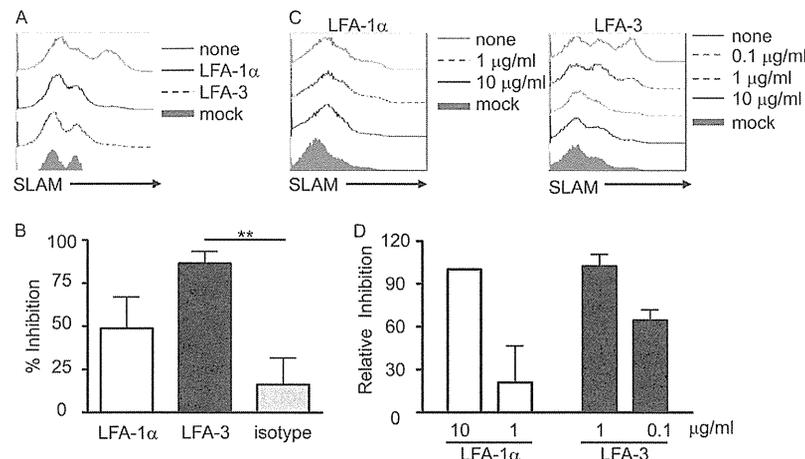

FIG 5 Role of cell-to-cell contact in SLAM upregulation on HIV-1-infected CD4$^+$ T cells. (A and B) HIV-1$_{NL-E}$-infected PBMCs were cultured in the presence of 10 μg/ml of anti-LFA-1α or anti-LFA-3 MAbs or isotype control IgG1. (A) Representative histogram showing SLAM expression on CD4$^+$ T cells. (B) Percent inhibition of the increase in the frequency of SLAM^{hi} CD4$^+$ T cells. The frequency of SLAM^{hi} CD4$^+$ T cells upregulated by HIV-1 infection was arbitrarily designated 100%. The bars represent the mean $\pm$ SEM ($n = 5$). P values were calculated using one-way analysis of variance followed by the Tukey multiple-comparison test. **, $P < 0.01$. (C and D) HIV-1$_{NL-E}$-infected PBMCs were cultured in the presence of serially diluted concentrations of anti-LFA-1α or anti-LFA-3 MAbs. (C) Representative histogram showing SLAM expression on CD4$^+$ T cells cultured with anti-LFA-1α (left) or anti-LFA-3 (right) MAbs. (D) Relative inhibition of the increase in the frequency of SLAM^{hi} CD4$^+$ T cells. The percent inhibition in the frequency of SLAM^{hi} CD4$^+$ T cells by 10 μg/ml of anti-LFA-1α or anti-LFA-3 MAbs was arbitrarily designated 100%. The bars represent the mean $\pm$ SEM ($n = 3$).

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hibitory effect of anti-LFA-3 MAb was observed at concentrations as low as 0.1 μg/ml (Fig. 5D).

To confirm whether the inhibition of HIV-1-associated SLAM upregulation by these blocking antibodies also resulted in reduced MV infectivity in CD4$^+$ T cells, HIV-1$_{NL-D}$-infected PBMCs cultured in the presence of blocking antibodies were infected with MVwt ($n = 2$). As expected, although the frequency of MVwt-infected CD4$^+$ T cells was increased by HIV-1 infection (from 1.30% to 4.51% for donor 1 and from 2.59% to 7.63% for donor 2), the frequency of MVwt-infected cells was reduced by anti-LFA-1α (1.21% and 5.01% for donor 1 and donor 2, respectively) and more strongly by anti-LFA-3 (1.34% and 3.63% for donor 1 and donor 2, respectively) (see Fig. S2 in the supplemental material). These data clearly indicate that SLAM upregulation and the resulting increase in MV-infected CD4$^+$ T cells are mediated by cell-to-cell contact through the interaction of costimulatory molecules that are highly expressed on HLA-DR$^+$ DC subsets in HIV-1-infected PBMC cultures.

DISCUSSION

The present study shows that HIV-1 infection enhanced MV infection in CD4$^+$ T cells. Interestingly, we observed that the frequencies of MVvac-infected CD4$^+$ T cells were higher than those of MVwt in both HIV-1-infected and -uninfected PBMCs. This difference may be explained by different receptor usage (4) and by differences in polymerase activity between the wild-type and vaccine strains (1) used in this study. In addition, both strains of MV inhibited HIV-1 replication (Fig. 1C), which is consistent with previous reports (7, 8, 10). Although the precise mechanism(s) underlying HIV-1 suppression by MV is not completely understood, the reduction of p24 antigen observed in the culture supernatant occurred in parallel with the elimination of HIV-1-infected cells after MV infection. It is speculated that MV infection causes apoptosis of HIV-1-infected cells directly through the expression of viral nucleoprotein and hemagglutinin proteins (2, 13, 15, 28) or through the induction of cell G$_0$/G$_1$ arrest (8), although no doubly infected CD4$^+$ T cells were detected in the culture system used in the present study. Alternatively, considering the fact that HIV-1-infected CD4$^+$ T cells are already activated, they may be hyperactivated by MV, resulting in activation-induced cell death (11).

Because MV utilizes SLAM as a receptor, it is very likely that the enhanced MV infection observed in this ex vivo MV and HIV-1 coinfection model was due to HIV-1-induced upregulation of SLAM. Meroni et al. showed that SLAM expression on CD4$^+$ T cells ex vivo is diminished during the early phase of HIV infection (16). In addition, SLAM expression on CD4$^+$ T cells was different in patients recently and chronically infected with HIV-1 (16). SLAM expression on CD4$^+$ T cells from HIV-1-infected individuals may fluctuate depending on the activation state of the immune system in vivo. It is important to note that most CD4$^+$ T cells within the ex vivo PBMC population were in the resting state and that SLAM expression was transiently downregulated soon after the initiation of culture (unpublished observation). SLAM is expressed on activated cells, and chronic hyperactivation is a characteristic feature of HIV-1 infection (11). It was assumed that CD4$^+$ T cells in the PBMC cultures were not hyperactivated and were, rather, akin to the cells within the lymphoid organs, in which a variety of antigen-presenting cells (APCs) and T cells are in contact with each other and where HIV-1 replication/expansion

occurs. Therefore, it is possible that SLAM expression is upregulated in lymphoid organs during HIV-1 infection, which may enhance the infectivity of MV.

One of the aims of the present study was to examine the mechanism(s) by which HIV-1 infection enhances SLAM expression on CD4$^+$ T cells. IFN-γ upregulates SLAM expression on T cells in patients with tuberculosis (9, 23). However, neither IFN-γ nor any other soluble factors played a major role in the SLAM upregulation observed in this study. It is possible that SLAM upregulation by IFN-γ is a specific feature of certain T cells reactive to Mycobacterium tuberculosis. Nevertheless, the low level of SLAM upregulation induced in T cell culture may be mediated by cytokines, including IFN-γ.

In the present study, blocking experiments showed that cell-to-cell contact (presumably DCs to CD4$^+$ T cells) via LFA-1/ICAM-1 and LFA-3/CD2 interactions enhanced SLAM expression on CD4$^+$ T cells (Fig. 5). Inhibition of the LFA-3/CD2 interaction led to a more marked abrogation of SLAM expression than inhibition of the LFA-1/ICAM-1 interaction. It is noteworthy that a previous study also showed that the LFA-3/CD2 interaction was more important than the LFA-1/ICAM-1 interaction for antigen-dependent DC-T cell synapse formation (27). Therefore, CD2 costimulatory signals, in addition to TcR signals, may be involved in SLAM upregulation. Potential candidate APCs that interact with CD4$^+$ T cells to upregulate SLAM on CD4$^+$ T cells in HIV-infected PBMC cultures could be HLA-DR$^+$ DCs.

In conclusion, the precise mechanism(s) by which MV exacerbates the disease outcomes in HIV-1-infected individuals remains unknown. The present study, which employed a PBMC-based ex vivo HIV-1 and MV coinfection model, showed that increased susceptibility to MV infection involves induction of a high level of SLAM expression by HIV-1 infection via cell-to-cell contact. This is the first report showing a direct relationship between HIV-1 infection and SLAM expression. The high mortality and morbidity of measles in children coinfected with HIV-1 and MV may be due to upregulation of SLAM expression on CD4$^+$ T cells, which presumably occurs within lymphoid organs through T cell contact with DCs during HIV-1 infection. Further in vivo coinfection studies in a macaque model should help to clarify these outstanding issues.

ACKNOWLEDGMENTS

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