JOURNAL OF VIROLOGY, Oct. 1994, p. 6332–6339
0022-538X/94/$04.00+0

Vol. 68, No. 10

Involvement of Human Leukocyte Antigen Class I Molecules in Human Immunodeficiency Virus Infection of CD4-Positive Cells

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Received 28 February 1994/Accepted 29 June 1994

We have studied the putative roles of human immunodeficiency virus (HIV)-associated and cell surface-expressed major histocompatibility complex class I (MHC-I) molecules in the course of the HIV life cycle by the combined use of MHC-I molecule-positive and MHC-I molecule-negative virus particles and MHC-I molecule-positive and MHC-I molecule-negative $CD4^+$ human cells. We found (i) that several anti-MHC-I monoclonal antibodies neutralize cell infection by direct interaction with HIV-associated MHC-I antigens, (ii) that these HIV-associated MHC-I antigens are however dispensable for cell infection, and (iii) that the cell surface-expressed MHC-I molecules are unnecessary for productive infection of $CD4^+$ human cells. These results clarify further the functions of MHC-I molecules during the HIV life cycle.

Human immunodeficiency virus (HIV) is the primary etiological agent of AIDS and associated diseases (4, 15). Understanding the physiopathology of AIDS, designing new drugs aimed at controlling HIV disease progression, and developing an HIV vaccine might require a better knowledge of the role(s) played by cell surface molecules during the viral life cycle. Because of its essential function during the binding of HIV to T cells and during HIV-induced syncytium formation, the CD4 molecule has been the most studied among these molecules (13, 23, 27). However, apart from CD4, other T-cell surface molecules are likely to be involved at different steps of the viral life cycle.

Approaches that use monoclonal antibodies (MAb) to screen for molecules which could play a role in the life cycle of the virus have allowed the identification of several candidates that appear on the surfaces of T cells. In addition to anti-CD4 MAb (9, 13, 23, 27, 30, 35), anti-CD3 (27), anti-HLA-DR (2, 13), and anti-LFA1/CD11a-CD18 MAb (21, 41) have been reported to be able to interfere with the replicative cycle of the virus. These MAb were all found to be able to inhibit HIV entry and/or syncytium formation. We first reported data indicating that anti-β2m MAb (10, 14) and several anti-HLA class I heavy chain MAb (11) have particular anti-HIV properties; these anti-major histocompatibility complex class I (MHC-I) MAb do not prevent syncytium formation, but they cause a drastic decrease in virus production when added to the culture medium of peripheral blood mononuclear cells immediately after exposure of the cells to HIV type 1 (HIV-1) (10, 11). However, this inhibitory effect was not evidenced when CEM cells were infected with HIV-1 and treated with anti-MHC-I MAb. We proposed that this inhibitory effect could be the consequence of an interference with the replicative cycle of HIV-1 through a process linked to T-cell activation. More recently, the neutralization of HIV-1 by anti-MHC-I MAb on the AA-2 CL1 cell line has been described by Arthur et al. (2), and these authors suggested that virion-associated cellular antigens, including the MHC-I molecules, can play a role in the infection process. Accordingly, MHC-I molecules could play different roles during the HIV life cycle.

Questioning the exact role(s) of virus-associated MHC-I molecules and cell surface-expressed MHC-I molecules during the HIV life cycle became urgent, particularly in light of the debate surrounding the role of cellular antigens, including MHC-I, that, when bound to virions, could confer protection against simian immunodeficiency virus (SIV) to macaques and protection against HIV-1 grown in human cells to chimpanzees (1, 7, 24, 25, 34, 40). By using MHC-I molecule-positive ($MHC\text{-}I^+$) and MHC-I molecule-negative ($MHC\text{-}I^-$) HIV to infect $MHC\text{-}I^+$ and $MHC\text{-}I^-$ cells either in the presence or in the absence of anti-MHC-I MAb, we have studied here the roles played by virion-bound and cell surface-expressed MHC-I molecules. We found that neither virion-bound MHC-I molecules nor cell surface-expressed MHC-I molecules play a major role in infection.

MATERIALS AND METHODS

MAb and recombinant proteins. Anti-HLA-DR MAb B8-12 (immunoglobulin G2b [IgG2b]) and anti-HLA class I MAb B9-12/B9-12-1 (IgG2a) were provided by C. Mawas (Institut National de la Santé et de la Recherche Médicale U119, Marseille, France). Anti-HLA class I MAb RL4-24-6 (IgG1) and anti-β2m MAb HC11-151-1 (IgG1) were obtained in our laboratory (11, 14). Anti-β2m MAb B1-1G6 (IgG2a) (14) was produced by Immunotech (Marseille, France). The main characteristics of the anti-HLA class I and anti-β2m MAb used in this study have been described previously (10, 11, 14). Anti-CD4 MAb 13B8-2 (IgG1) that inhibits HIV transcription (9) was produced by Immunotech. Anti-CD4 MAb OKT4A that inhibits the binding of HIV to CD4 and OKT4 was purchased from Ortho Diagnostic Systems (Raritan, N.J.). Anti-HIV-2 $p26^{gag}$ MAb MO9-42-2 was obtained in our laboratory (36). This MAb binds both HIV-2 $p26^{gag}$ and HIV-1 $p24^{gag}$ (37). Neutralizing anti-$HIV\text{-}1_{LAI}$ $gp120^{env}$ MAb 110-H (IgG1) and anti-$HIV\text{-}2_{ROD}$ $gp105^{env}$ MAb 125-F (IgG1), which bind the V3 loop, were provided by F. Nato and F. Traincard (Hybridolab, Paris, France) under an Agence National de Recher-

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ches sur le SIDA agreement. Purified (90%) HIV-1 recombinant envelope glycoprotein 160 (gp160) and gp120 produced in mammalian cells (BHK) infected with recombinant vaccinia virus were obtained from Transgène S.A. (Strasbourg, France) and soluble CD4 (sCD4) was provided by D. Klatzman (Paris, France) under Agence National de Recherches sur le SIDA agreements.

Cells. The $CD4^+$ lymphoblastoid CEM cell line was purchased from the American Type Culture Collection (Bethesda, Md.). The DAUDI cell line was provided by C. Mawas (Institut National de la Santé et de la Recherche Médicale U119). The $CD4^+$ DAUDI cell line was constructed as previously described (8) by infection of DAUDI cells with amphotropic retrovirus particles encoding a CD4 cDNA inserted downstream from the Moloney murine leukemia virus long terminal repeat as well as a neomycin-resistant selectable marker. All cell lines were cultured to a density of 5×10^5 cells per ml in RPMI 1640 medium (Axcell-Novotec, Lentilly, France) supplemented with 1% PSN antibiotic mixture, 1% glutamine, and 10% fetal calf serum (ATGC-Biotechnologie, Noisy-le-Grand, France) at 37°C in a 5% CO_2 atmosphere. The $CD4^+$ DAUDI cell cultures were fed twice weekly with RPMI 1640 containing 20% fetal calf serum and 2 mg of G418 (Gibco) per ml.

Virus production. Stock solutions of HIV-1_{LAI} (formerly LAV-1_{BRU}) and HIV-2_{ROD} strains from supernatants of chronically infected CEM cells or CD4-transfected DAUDI cells were prepared as previously described (8) and kept frozen at −80°C until use. All virus stock solutions were titrated for infection of the different cell types to be used in this study. One hundred microliters of a dilution of the virus stock solution corresponding to 100 50% tissue culture infective doses ($TCID_{50}$) was used for infection assays.

ELISA. Wells of a 96-well enzyme-linked immunosorbent assay (ELISA) plate (Nunc, Paisley, Scotland) were coated at 4°C for 18 h with 100 μl of a solution containing 1 μg of either heat-inactivated virus treated with Triton X-100 or recombinant gp160 in 100 mM sodium carbonate buffer (pH 9.6). After the wells were washed, nonspecific binding was blocked by a 4-h incubation with phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA; Sigma, St. Louis, Mo.) and 0.02% NaN_3. MAb diluted to a concentration of 10 μg/ml in PBS–0.2% BSA–0.02% NaN_3 was reacted with the coated antigens for 1 h at 20°C. The plates were washed three times with PBS containing 0.05% Tween 20. Bound Ig were detected by adding 100 μl of goat anti-mouse IgG heavy-plus-light-chain peroxidase conjugate (diluted 10^3-fold; Immunotech) for 1 h and then washing the plates and subsequently incubating them with *O*-phenylenediamine (Sigma, Main, Mo.) as the substrate.

Flow cytometric analysis. The two distinct protocols described below were used.

(i) Protocol A. Fifty microliters of gp120 at a concentration of 5 μg/ml was incubated for 18 h at 4°C with 50 μl of anti-HLA class I MAb (RL4-24-6 at 100 μg/ml), anti-β2m MAb (HC11-151-1 at 100 μg/ml), sCD4 (20 μg/ml), or medium alone, and this mixture was then added to 10^5 CEM cells. After a 30-min incubation at 4°C, the cells were washed three times with PBS–0.2% BSA–0.1% NaN_3 and bound gp120 was detected by using a pool of human HIV^+ sera (50 μl of a 1/100 dilution of a pool of 10 sera reacting with gp120 in Western blot [immunoblot] analysis) followed by 50 μl of a 1/25 dilution of biotinylated sheep anti-human Ig (Amersham) and 50 μl of a 1/50 dilution of fluoresceinated avidin (Immunotech). After being stained, cells were fixed in PBS containing 2% formaldehyde, and fluorescence intensity was measured on an EPICS PROFILE cytofluorometer (Coulter, Coultronics, Margency, France).

(ii) Protocol B. Cells (10^5) were incubated for 30 min at 4°C with saturating concentrations of anti-HLA class I MAb (RL4-24-6), anti-β2m MAb (B1-1G6), or anti-CD4 MAb (OKT4A at 5 μg/ml or OKT4 at 10 μg/ml). After the cells were washed three times with PBS–0.2% BSA–0.1% NaN_3, 50 μl of gp120 at a concentration of 5 μg/ml was added to the cell suspension. Bound gp120 was detected as described above.

HIV infection assay. Cells (5×10^5) were incubated for 30 min at 4°C in a flat-bottomed 96-microwell plate (Costar) with 100 μl of virus stock solution corresponding to 100 $TCID_{50}$. Thereafter, cells were washed five times and cultured in 24-microwell plates (Costar). The amount of virus produced by the cells was monitored twice a week by measuring the reverse transcriptase activity in 1 ml of cell-free culture supernatant by using a synthetic template primer which permitted the RT to neosynthesize radioactive DNA, as previously described (10). Depending on the viral life cycle step analyzed, MAb at a saturating concentration (100 μg/ml for anti-HLA class I and anti-β2m MAb, 10 μg/ml for anti-CD4 MAb, 10 μg/ml for anti-HIV gp^{env} MAb 110-H, or 50 μg/ml for anti-HIV gp^{env} MAb 125-F) either were added to virus for 30 min at 4°C before the virus was incubated with target cells and were also added to the cell culture medium at the same concentration (neutralization assay), were added to cells for 30 min at 4°C prior to exposure of the cells to virus (inhibition of binding and/or fusion assay), or were added to the culture medium only after cells had been exposed to HIV for 30 min at 4°C and unbound virions had been eliminated by extensive washing of the cells (postbinding assay).

RESULTS

Neutralization of HIV-1 and HIV-2 infection of CEM cells by HLA class I- and β2m-specific MAb. The neutralization produced by anti-MHC class I MAb was evaluated by incubating HIV with MAb before adding HIV to the CEM cells. As shown in Fig. 1a, graph A, when HIV-1_{LAI} was treated with anti-HLA class I MAb (RL4-24-6 or B9-12), anti-β2m MAb (HC11-151-1 or B1-1G6), or anti-HIV-1_{LAI} $gp120^{env}$ neutralizing MAb (110-H), RT activity remained negative on day 14 postinfection. Virus production was evidenced in the control culture on day 7 postinfection. Similarly, when HIV-2_{ROD} (Figure 1a, graph B) was treated with anti-HLA class I MAb, anti-β2m MAb, or anti-HIV-2_{ROD} $gp105^{env}$ neutralizing MAb (125-F), the RT activity remained negative on day 14 postinfection, whereas virus production was evidenced in the control culture on day 7 postinfection. In contrast to the complete neutralization of binding observed with sCD4 treatment, the anti-MHC-I MAb RL4-24-6 and B1-1G6 did not neutralize the binding of the recombinant HIV-1_{LAI} $gp120^{env}$ molecule to CEM cells (Fig. 2). In addition, these anti-MHC-I MAb did not bind the recombinant HIV-1_{LAI} $gp160^{env}$ molecule (see Fig. 4). Altogether, these data indicate that the neutralizing effect illustrated in Fig. 1a is unlikely to be related to an antigenic cross-reaction between MHC-I and the viral envelope.

To confirm that the absence of virus particles in culture supernatants of CEM cells exposed to anti-MHC-I MAb-treated HIVs was related to HIV neutralization and not to an effect of the MAb on the cells, CEM cells were treated with a saturating concentration of anti-HLA class I MAb (RL4-24-6 or B9-12) or anti-β2m MAb (HC11-151-1 or B1-1G6) before they were exposed to HIVs. Under these experimental conditions the productive infection kinetics found with HIV-1_{LAI}

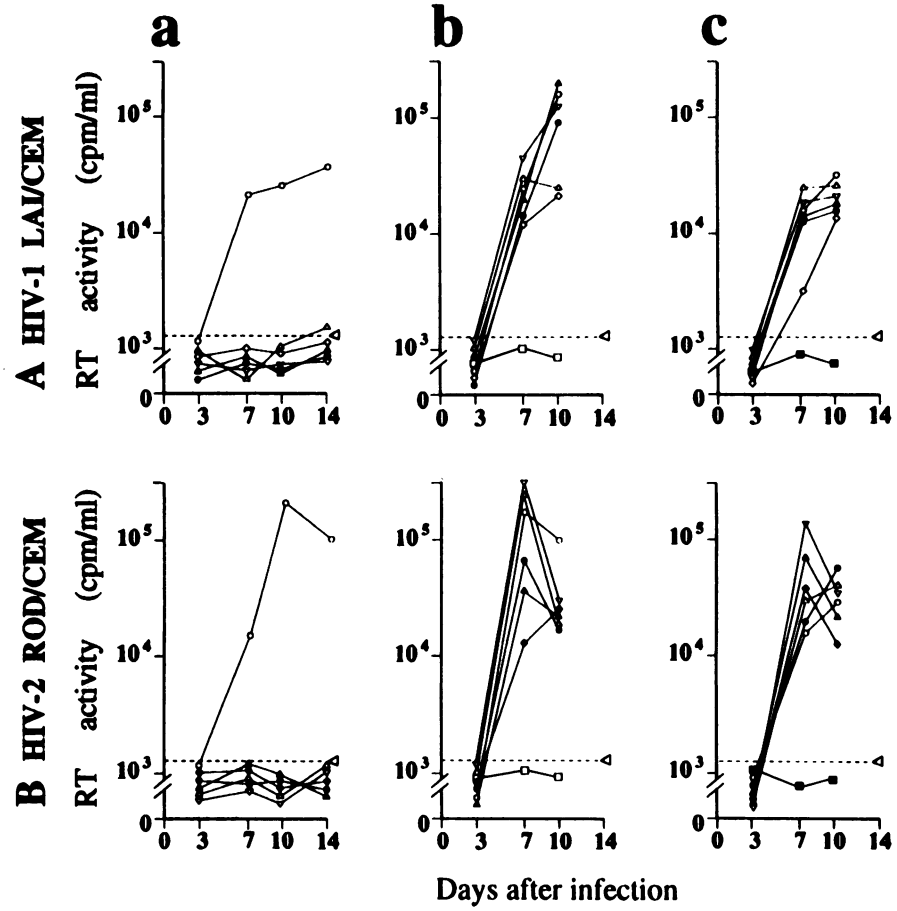


FIG. 1. Effect of anti-MHC-I MAb on the infection of CEM cells by HIV. Three different experimental protocols of infection were used, and virus production was monitored by measuring the RT activity in culture supernatants. RT activity of $<1.5 \times 10^3$ cpm/ml (values below the dashed lines) was considered negative. Several MAb were used in these experiments: anti-HLA class I MAb RL4-24-6 (●) and B9-12 (▲), anti-β2m MAb B1-1G6 (▽) and HC11-151-1 (△), anti-CD4 MAb 13B8-2 (■) and OKT4A (□), and anti-V3 loop MAb 110-H (◇) and 125-F (◆). ○, control infection performed in the absence of MAb. (a) Neutralization of HIV-1_{LAI} (graph A) and HIV-2_{ROD} (graph B) infection of CEM cells by HLA class I-, β2m-, and gp^{env}-specific MAb. Viruses (100 $TCID_{50}$) grown in CEM cells (HIV-1 LAI/CEM and HIV-2 ROD/CEM) were incubated for 30 min at 4°C with MAb before they were added to CEM cells. Cells were cultured in the presence of the MAb. (b) Treatment of CEM cells with HLA class I-, β2m-, CD4-, and gp^{env}-specific MAb before exposure to HIV-1_{LAI} (graph A) or HIV-2_{ROD} (graph B). CEM cells were incubated for 30 min at 4°C with MAb before exposure to 100 $TCID_{50}$ of HIVs grown in CEM cells (HIV-1 LAI/CEM and HIV-2 ROD/CEM). Cells were then cultured in the absence of MAb. (c) Postbinding treatment with HLA class I-, β2m-, CD4-, and gp^{env}-specific MAb of CEM cells exposed to HIV-1_{LAI} (graph A) or HIV-2_{ROD} (graph B). CEM cells were incubated for 30 min at 4°C with 100 $TCID_{50}$ of viruses grown in CEM cells (HIV-1 LAI/CEM and HIV-2 ROD/CEM). Cells were then cultured in medium containing MAb.

(Fig. 1b, graph A) and HIV-2_{ROD} (Fig. 1b, graph B) were similar for cells treated with anti-MHC-I MAb and for control cells that were untreated. Under similar experimental conditions OKT4A, an anti-CD4 MAb that blocks gp120-CD4 binding (5), completely inhibited the production of virus in CEM cells. Moreover, when anti-HLA class I MAb (RL4-24-6 or B9-12), anti-β2m MAb (HC11-151-1 or B1-1G6), or anti-HIV neutralizing MAb were added to the culture medium of CEM cells exposed to HIV-1_{LAI} (Fig. 1c, graph A) or HIV-2_{ROD} (Fig. 1c, graph B), no delay in the production of virus was evidenced with respect to controls which consisted of cells infected with HIVs and cultured in the absence of MAb. Under similar experimental conditions 13B8-2, an anti-CD4 MAb that blocks viral transcription (5), completely inhibited the production of virus in CEM cells.

These results indicate that anti-HLA class I and anti-β2m antibodies neutralize the infection of CEM cells by HIV-1 and HIV-2 grown in MHC-I^+ $CD4^+$ cells by interacting with the virus-associated MHC-I molecules.

HLA class I and β2m proteins associated with HIV do not play a central role in infection. We first attempted to produce virus particles lacking expression of HLA class I and β2m on their viral envelopes. To this end, HIV-1_{LAI} and HIV-2_{ROD} (grown in CEM cells) were used to infect CD4-transfected (MHC-I^- $CD4^+$) DAUDI cells. As shown in Fig. 3, production of HIV-1 and HIV-2 was evidenced on day 7 postinfection in the supernatant of CD4-transfected DAUDI cells, whereas RT activity in the culture supernatant of untransfected DAUDI cells (phenotype, MHC-I^- $CD4^-$) exposed to HIV-1 or HIV-2 remained negative on day 14. Cytofluorometric studies demonstrated that the CD4-transfected DAUDI cells remained negative for expression of HLA class I and β2m antigens (the RL4-24-6 and HC11-151-1 epitopes, respectively) but showed an HLA-DR (B8-12 epitope) induction

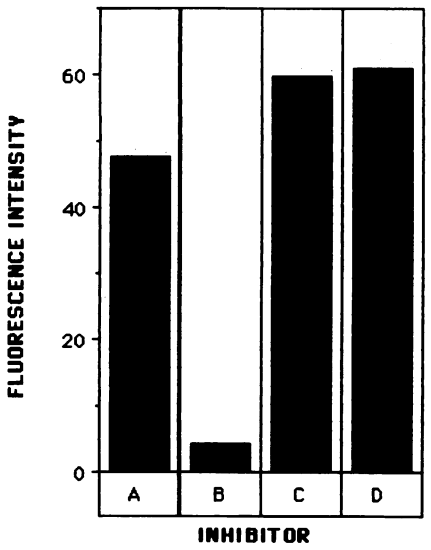


FIG. 2. Anti-MHC-I MAb fail to neutralize the binding of gp120 to CEM cells. gp120 was incubated for 18 h at 4°C with medium alone (lane A), sCD4 (lane B), anti-HLA class I MAb RL4-24-6 (lane C), or anti-β2m MAb B1-1G6 (lane D), and this mixture was added to CEM cells. Bound gp120 was detected by using anti-gp120 antibodies and a fluoresceinated probe as described in Materials and Methods.

after infection with HIVs (data not shown). Finally, the MHC-I phenotypes of cell-free virus particles produced in MHC-I$^-$ CD4$^+$ DAUDI cells and CEM cells were analyzed by ELISA. As shown in Fig. 4, HIVs produced in MHC-I$^-$ CD4$^+$ DAUDI cells lacked expression of HLA class I and β2m molecules, whereas HIVs grown in CEM cells expressed HLA class I and β2m molecules.

To determine whether virus-associated MHC-I molecules are necessary for infection, these MHC-I$^-$ HIVs were used for infection of CEM cells. As shown in Fig. 5A, MHC-I$^-$ HIV-1_{LAI} infected the CEM cells, as evidenced by the positive RT activity detected in the culture supernatant of these cells on day 7 postinfection. Moreover, when MHC-I$^-$ HIV-1_{LAI} particles were treated with anti-HLA class I MAb (RL4-24-6 or B9-12) or anti-β2m MAb (HC11-151-1 or B1-1G6), RT activity was also evidenced on day 7 postinfection. Under similar experimental conditions, the anti-HIV-1_{LAI} gp120^{env} neutralizing MAb (110-H) was able to inhibit the infection of CEM cells by MHC-I$^-$ HIV-1_{LAI}. Similarly, production of virus particles was evidenced when MHC-I$^-$ HIV-2_{ROD}, treated or not with anti-HLA class I MAb or anti-β2m MAb, was used to infect CEM cells (Fig. 5B). Only the anti-HIV-2_{ROD} gp105^{env} neutralizing MAb (125-F) was able to protect cells from infection with this HIV-2 preparation.

These results indicate that virus-associated HLA class I and β2m molecules are dispensable for productive infection of MHC-I$^+$ CD4$^+$ cells and that anti-MHC-I MAb-induced neutralization of MHC-I$^+$ HIVs results from interaction between MAb and the virus-associated MHC-I molecules.

Cell surface-bound HLA class I and β2m proteins are unnecessary for productive infection of CD4$^+$ cells. It seemed obvious that HLA class I and β2m molecules on the surfaces of target cells play no role in the infection process, since infection of MHC$^-$ CD4$^+$ DAUDI cells by HIVs grown in CEM cells is possible and virus particles are released by these cells (Fig. 3) (8). This theory was further confirmed by three sets of experiments. First, MHC-I$^-$ HIV-1_{LAI} and MHC-I$^-$ HIV-2_{ROD}

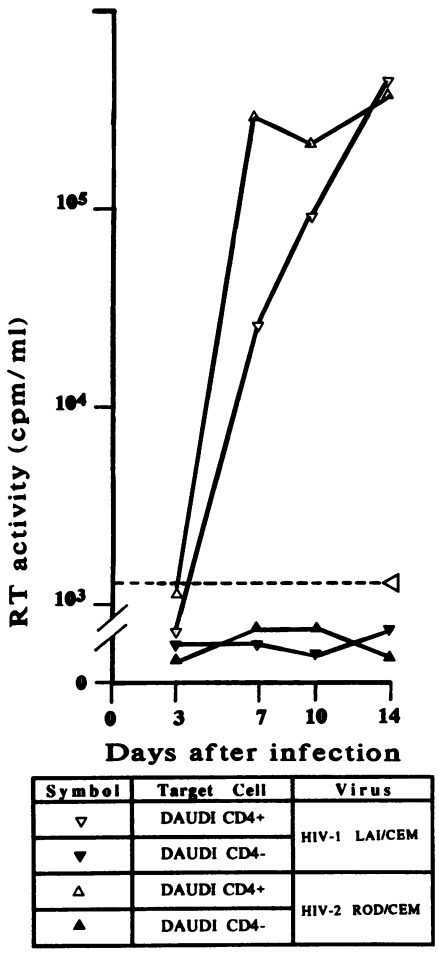


FIG. 3. Infection of MHC-I$^-$ cells by HIV-1_{LAI} and HIV-2_{ROD}. MHC-I$^-$ CD4$^-$ DAUDI cells and CD4-transfected (MHC-I$^-$ CD4$^+$) DAUDI cells were incubated for 30 min at 4°C with 100 $TCID_{50}$ of viruses grown in CEM cells. Virus production was monitored by measuring the RT activity in culture supernatants (see the legend to Fig. 1 for details).

were used to infect MHC-I$^-$ CD4$^+$ DAUDI cells. As shown in Fig. 6, MHC-I$^-$ HIVs infected MHC-I$^-$ CD4$^+$ DAUDI cells but failed to infect MHC-I$^-$ CD4$^-$ DAUDI cells. Second, as shown in Fig. 7, when CEM cells were treated with a saturating concentration of anti-CD4 MAb OKT4 or anti-MHC-I MAb (RL4-24-6 or B1-1G6) before exposure to recombinant gp120 (Fig. 7, lanes C, D, and E, respectively), the gp120 was able to bind the CEM cells, whereas it failed to bind CEM cells treated with OKT4A (Figure 7, lane B). Third, when CEM cells were treated with a saturating concentration of anti-MHC-I MAb before being exposed to virus, the productive infection kinetics was similar to that seen with untreated cells (Fig. 1b).

These results demonstrate that cell surface expression of HLA class I and/or β2m molecules is unnecessary for the productive infection of CD4$^+$ human cells.

DISCUSSION

The main purpose of this study was to clarify the function(s) of MHC-I molecules bound to particles or expressed on the surfaces of human cells during HIV infection of CD4-positive cells. We demonstrated that although MHC-I molecules are

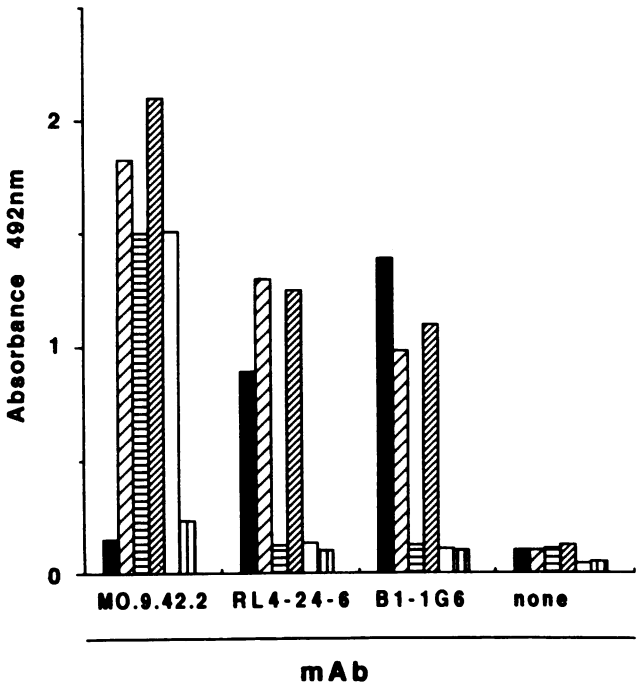
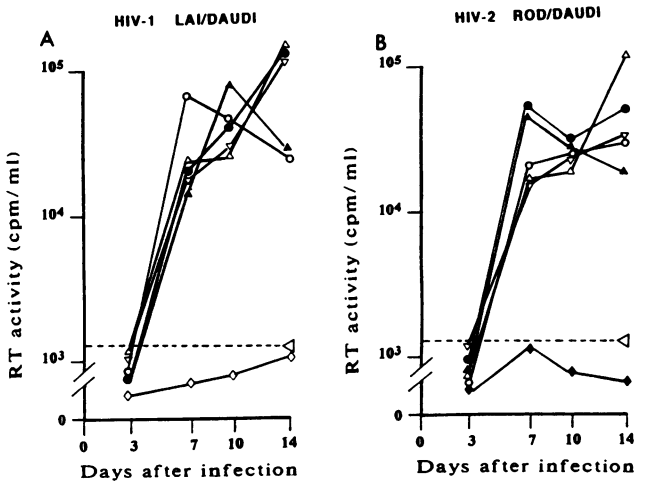


FIG. 4. MHC-I phenotyping of HIV-1_{LAI} and HIV-2_{ROD} virions grown in CEM cells or CD4-transfected DAUDI cells. HIV-1_{LAI} and HIV-2_{ROD} grown in CEM cells (▨ and ▨, respectively) or CD4-transfected DAUDI cells (▤ and □, respectively), control antigen prepared from uninfected CEM cells (■), or HIV-1_{LAI} gp160^{env} (▥) was used at 10 μg/ml to coat the solid phase. The presence of specific epitopes for HIV-1 p24^{gag}/HIV-2 p26^{gag} (MO9-42-2), HLA class I (RL4-24-6), and β2m (B1-1G6) in these samples was evaluated by indirect ELISA.



Symbol	mAb	Specificity
○	none	
◇	110-H	HIV-1 LAI env
◆	125-F	HIV-2 ROD env
●	RL4-24-6	HLA Class I
▲	B9-12	
△	HC11-151-1	β2m
▽	B1-1G6	

FIG. 5. Failure of HLA class I- and β2m-specific MAb to neutralize MHC-I$^-$ HIV-1_{LAI} (A) and MHC-I$^-$ HIV-2_{ROD} (B) infection of CEM cells. Viruses (100 $TCID_{50}$) grown in CD4-transfected DAUDI cells (HIV-1 LAI/DAUDI and HIV-2 ROD/DAUDI) were incubated with MAb before they were added to CEM cells. Cells were cultured in the presence of the same concentrations of the MAb. Virus production was monitored by measuring the RT activity (see the legend of Fig. 1 for details).

dispensable to the productive infection of human cells, MAb directed at these molecules can inhibit the life cycle of a virus by neutralization of viral particles.

The fact that cellular materials, including nucleic acids, enzymes, and a variety of other components, such as MHC products, are incorporated into budding virions has been known for a long time (3). HIVs, which mature at the cell surface, do not escape this rule; cell surface antigens such as CD3, CD4, CD5, CD11a, CD18, CD25, CD30, CD43, CD54, CD63, CD71, HLA class I, and HLA-DR have been detected as integral components of cell-free virions or have been found in virus preparations as contaminants that were difficult to remove (2, 16, 17, 20, 22, 28, 33).

The observation that anti-MHC-I MAb neutralize HIVs has recently led Arthur et al. (2) to speculate about the possibility that HIVs use not only their own envelope components but also host-derived adhesion molecules, including MHC-I molecules, in binding and subsequent infection of target cells. We report here that several anti-HLA class I and β2m-specific MAb neutralize the in vitro infection of T cells by HIV-1 and HIV-2 grown in MHC-I$^+$ CD4$^+$ human cells. At variance with the neutralizing anti-HLA heavy chain MAb M38 that binds HIV-1 gp120^{env} (6, 19, 26), the neutralizing MAb directed against the different MHC-I epitopes in our study did not prevent the binding of gp120 to CEM cells, nor did they bind the HIV-1_{LAI} gp160^{env}. Moreover, we found that these MAb did not neutralize MHC-I$^-$ HIV particles, indicating that the presence of HIV-associated MHC-I molecules is required for these anti-MHC-I MAb to mediate neutralization. This extends our understanding of anti-MHC-I MAb-mediated neutralization; it is likely that anti-MHC-I MAb neutralize HIV particles by a mechanism akin to opsonization. Moreover, we found that MHC-I$^-$ HIV particles can infect different human cell lines (e.g., CEM cells and CD4-transfected DAUDI cells), indicating that HIV-associated MHC-I molecules are dispensable for the binding of the virus to human cells and the entry of the virus into these cells.

We next investigated whether MHC-I molecules on the surface of the target play a role in binding and infection. We found (i) that HIVs grown in CD4-transfected DAUDI cells lack expression of MHC-I molecules on their envelopes and that MHC-I$^-$ HIV-1 and HIV-2 particles productively infect CD4-transfected DAUDI cells, (ii) that anti-MHC-I MAb do not inhibit the binding of viral envelope glycoproteins to CD4, and (iii) that anti-MHC-I MAb do not protect CEM cells from infection. These results extend our previous data (8) and indicate that the MHC-I molecules on the surfaces of the target cells do not play a major role in fusion or in productive infection. Altogether, these results argue against the involvement of MHC-I molecules in adhesion patches made up of viral and cellular molecules located between viruses and cells as proposed by Arthur et al. (2). However, it remains possible that in the absence of one of the virus-associated cellular antigens or cell surface-expressed molecules, the other mole-

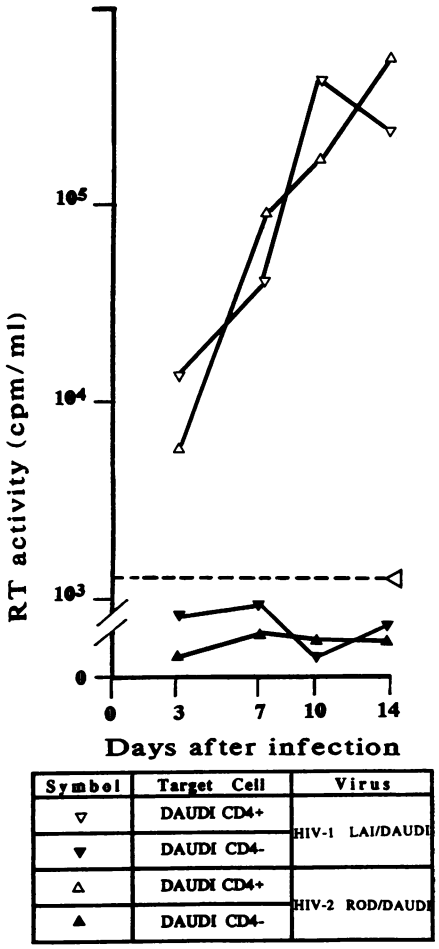


FIG. 6. Infection of MHC-I$^-$ cells by MHC-I$^-$ HIV-1_{LAI} and MHC-I$^-$ HIV-2_{ROD}. MHC-I$^-$ CD4$^-$ DAUDI cells and MHC-I$^-$ CD4$^+$ CD4-transfected DAUDI cells were incubated for 30 min at 4°C with 100 $TCID_{50}$ of HIVs grown in CD4-transfected DAUDI cells. Virus production was monitored by measuring the RT activity (see the legend to Fig. 1 for details).

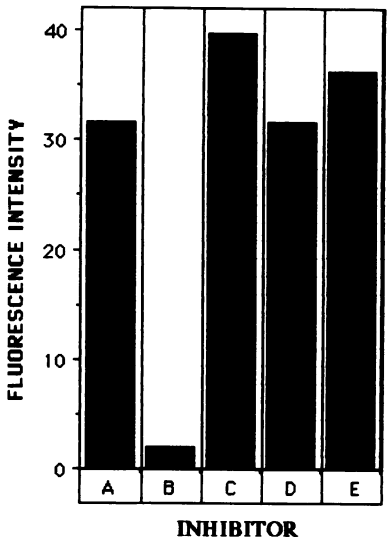


FIG. 7. Anti-MHC-I MAb fail to inhibit the binding of gp120 to CEM cells. The binding of soluble gp120 to CEM cells was evaluated either directly (lane A) or following the incubation of cells with a saturating concentration of anti-CD4 MAb OKT4A (lane B) or OKT4 (lane C), anti-HLA class I MAb RL4-24-6 (lane D), or anti-β2m MAb B1-1G6 (lane E). Fluorescence intensity was recorded by using the linear channels 0 to 1024.

cules participating in the putative patch compensate for the lack of that compound, allowing binding and fusion to occur. However, one important issue resides in the fact that the adhesion patch model should be regarded with respect to the HIV-associated antigen patch's molecular counterreceptor found on the target cell. In the case of a circulating CD4$^+$ T lymphocyte, MHC-I should not be expected to play a role (32). Adhesion molecules like HLA-DR, CD4, LFA-1 (CD11a/CD18), and I-CAM (CD54), molecules which could find their molecular counterreceptors on the surfaces of target cells, would be more relevant to this model than MHC-I molecules. It is noteworthy that MAb reacting with either HLA-DR (2, 13) or CD11a/CD18 (21, 41) are known for interfering with the viral replicative cycle. Further investigations will be required to understand the roles of these and other host cell surface molecules in the HIV life cycle.

One possible drawback in vaccine development is that the variability and rapid mutation of HIVs could limit the efficacy of vaccines made from standard laboratory strains of HIVs or their subunits. The fact that cellular materials (including MHC-I) have been shown to be able to contribute to the immunogenicity of whole virus vaccine preparations tested in animal models (1, 7, 12, 24, 25, 29, 31, 34, 38–41) and that MAb specific for MHC-I conserved structures (monomorphic epitopes) have been shown to neutralize HIV in vitro may be considered a new advance in the quest for conserved antigens that are expressed on the virion surface and able to induce protective immune responses against HIV. However, the concept of potential synergy between viral antigens and host antigens as a mechanism for provoking protective immune responses against lentiviruses is based on observations made with models whose conditions were far from physiologic (i.e., they involved monkeys infected with SIV-1 or HIV-1 grown in human cells). In these models, the relationship between the host cell origins of the viruses used for vaccination and those of the viruses used for challenge is of great importance for the achievement of protection. Indeed, when vaccinated monkeys are challenged with SIV grown in monkey cells, they are infected, even though they had previously been protected against SIV grown in human cells (7, 39). In humans the circulating cell-free viruses harbor the MHC-I antigens encoded by the patient's MHC-I genes. In the absence of any autoimmune disease, it is unlikely that virion-bound host syngenic MHC-I molecules could represent targets for the host's immune system. Accordingly, the possibility that an HLA molecule per se can induce a protective immune response against HIV in humans is low. It might be hazardous to purposely manipulate such host antigen recognition in humans. A breakdown of immunological tolerance to MHC molecules could generate autoreactive antibodies and/or effector T cells that would likely be responsible for selective immune dysfunction, dysfunction perhaps equivalent to that seen in HIV-infected patients (18).

ACKNOWLEDGMENTS

We thank Claude Mawas, Farida Nato, and François Traincard for providing us with MAb and Véronique Robert-Hebmann for her help

in the purification of MAb. We also thank Jean-Claude Chermann, Pierre Corbeau, Dominique Dormont, and Quentin Sattentau for stimulating discussions and Paul Clapham for critically reviewing the manuscript.

This work was supported by the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the Agence Nationale de Recherches sur le SIDA (ANRS-91077) and the Association pour la Recherche sur le Cancer (ARC-6055). M.B. is a fellow of the Agence Nationale de Recherches sur le SIDA.

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