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Cell Fusion Mediated by Interaction of a Hybrid CD4.CD8 Molecule with the Human Immunodeficiency Virus Type 1 Envelope Glycoprotein Does Occur after a Long Lag Time

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Several domains of CD4 have been suggested to play a critical role in events that follow its binding to the human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein (gp120-gp41). It has been reported previously that cells expressing a chimeric molecule consisting of the first 177 residues of human CD4 attached to residues from the hinge, transmembrane, and cytoplasmic domains of human CD8 did not form syncytia with HIV-1-infected cells (L. Poulin, L. A. Evans, S. Tang, A. Barboza, H. Legg, D. R. Littman, and J. A. Levy, J. Virol. 65:4893–4901, 1991). In contrast, we found that the hybrid CD4.CD8 molecule expressed in human cells did render them susceptible to fusion with cells expressing HIV-1_{IIIB} or HIV-1_{RF} envelope glycoproteins encoded by vaccinia virus recombinants, but only after long lag times. The lag time of membrane fusion mediated by the hybrid CD4.CD8 molecule was fivefold longer than that for the wild-type CD4 molecule. However, the rate of binding to and the affinity of soluble gp120 for membrane-associated CD4.CD8 were the same as for CD4. Both molecules were laterally mobile, as determined by patching experiments. Coexpression of the CD4.CD8 chimera with wild-type CD4 did not lead to interference in fusion but had an additive effect. Therefore, the proximal membrane domains of CD4 play an important role in determining the kinetics of postbinding events leading to membrane fusion. We hypothesize that the long lag time is due to the inability of the CD4.CD8-gp120-gp41 complex to undergo the rapid conformational changes which occur during the fusion mediated by wild-type CD4.

The binding of the membrane-associated human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein (gp120-gp41) to its membrane receptor, CD4, initiates a complex cascade of events which result in the fusion of the viral and cellular membranes and ultimately virus entry (20, 38). Cells which express gp120-gp41 also fuse with CD4 target cells; this fusion in some cases is dramatically manifested by the formation of giant cells (syncytia) (24, 37). While binding of gp120-gp41 to membrane-associated CD4 is needed for initiation of fusion, a number of factors determine the kinetics of the subsequent events required for merging of the membranes. It was found that many animal cells expressing human CD4 do not fuse with gp120-gp41-expressing cells (1, 2, 27) and that human cell components are needed to overcome the fusion block (8, 17).

It was suggested that the CDR3 loop in the first domain of CD4 also plays a role in postbinding events (reviewed in reference 35), but recent studies in which extensive mutations of this loop were made (7) questioned this hypothesis (7, 35, 36). Other domains of CD4, however, may play a role in postbinding events, as demonstrated by monoclonal antibodies (MAbs) directed to the second (9, 10, 29) or third (21) domain of CD4. These MAbs inhibited infection and fusion of CD4 cells without interfering with gp120 binding. It was shown that one of the antibodies, prebound to the second domain of soluble CD4 (sCD4), blocked sCD4-induced conformational changes in the HIV-1 envelope glycoprotein (29). Similar results were obtained with an antibody directed to the third domain of CD4 (35). Results from experiments with CD4 chimeric molecules support the notion that membrane-proximal CD4 domains affect fusion (32). It has been reported that cells expressing a hybrid molecule containing the first 177 residues of human CD4 attached to residues from the hinge, transmembrane, and cytoplasmic domains of human CD8 did not form syncytia with HIV-1-infected cells (32). However, cells expressing the chimeric CD4.CD8 hybrid were susceptible to HIV-1 infection, albeit at very low efficiency (32). This indicates that the CD4.CD8 molecules are fusion competent but that fusion or another postbinding event is greatly impaired.

We recently found that membrane fusion mediated by the CD4-gp120-gp41 interaction is initiated after a lag time, which varies from 1 to 2 min for virus-cell fusion (16) to 10 to 90 min for cell-cell fusion (12, 13, 34), and that the lack of syncytium formation does not necessarily prove lack of membrane fusion (12). The lag time was longer when gp120-gp41 was expressed at lower levels. In addition, the kinetics of syncytium formation closely resembled the kinetics of membrane fusion but was delayed (13). These observations led us to hypothesize that the lag time may be the critical parameter which is affected by the CD4 membrane-proximal domains.

In this study, we found that cells expressing the chimeric CD4.CD8 molecule do fuse with gp120-gp41-expressing cells, but only after long lag times. The long lag times were not due to impaired lateral mobility of the CD4.CD8 molecules or to different kinetics of binding to gp120. We hypothesize that the long delays in fusion are due to the inability of the CD4.CD8-gp120-gp41 complex to undergo the rapid conformational changes which occur during fusion mediated by wild-type CD4

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and involve bending of the hinge region between the second and third CD4 domains.

MATERIALS AND METHODS

Cells and reagents. The human cell line CEM was obtained from the American Type Culture Collection, Rockville, Md. The $CD4^-$ subclone 12E1 was derived from CEM cells by ethyl methanesulfonate mutagenesis and negative selection with OKT4A and complement as described previously (22). The CD4.CD8 construct was generated as described previously (3, 32). The A2.01 cell lines expressing wild-type CD4 (designated A2.01.T4) and the CD4.CD8 construct (A2.01.T4.T8) were grown in the presence of 0.8 mg of G418 per ml. All cell lines were propagated in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics. The recombinant vaccinia viruses vPE16 and VR222, encoding HIV-1_{IIIB} (18) and HIV-1_{RF} gp120-gp41, respectively, were provided by P. Earl, S. Merli, and B. Moss (National Institutes of Health [NIH], Bethesda, Md.) from the NIH AIDS Research and Reference Reagent Program. The vaccinia virus recombinant vCB3 encoding CD4 (8) was provided by C. Broder (NIH, Bethesda, Md.). Mouse anti-gp120 MAb 110.1, which binds with high affinity to gp120, was a generous gift from Shu Lok Hu (Bristol-Myers, Seattle, Wash.). Recombinant $gp120_{IIIB}$ and sCD4 were obtained from ABT (Cambridge, Mass.). The fluorescein isothiocyanate (FITC)-conjugated MAbs OKT4 and OKT4A were obtained from Ortho Diagnostics (Raritan, N.J.). The FITC-conjugated goat anti-mouse MAbs were obtained from Sigma (St. Louis, Mo.). The membrane- and water-soluble fluorescent dyes DiI and BCECF, respectively, were obtained from Molecular Probes (Eugene, Ore.).

Measurement of cell surface-bound gp120. Binding of gp120 to cells was measured by flow cytometry and analyzed as described before (22). Briefly, the cells were incubated with recombinant $gp120_{IIIB}$ ($rgp120_{IIIB}$) at 10 μg/ml for different periods of time at 37°C and then washed and stained with murine anti-gp120 MAb 110.1 and FITC-labeled goat anti-mouse antibody. The stained cells were analyzed by the Epic Profile (Coulter Electronics, Hialeah, Fla.). The relative fluorescence intensity (in fluorescence units [FU]) was calculated from a standard curve generated by using fluorescent beads with increasing intensities (Flow Cytometry Standards, Research Triangle Park, N.C.). The background values (in FU) due to autofluorescence and to nonspecific binding of antibodies were subtracted from the experimental FU values (31). The data are presented as the ratio of FU of bound gp120 to FU of saturated maximal bound gp120. In most cases, maximal bound gp120 was determined by incubation with 10 μg of gp120 per ml at 37°C for 2 h, because further increases in the concentration of gp120 or the incubation period did not lead to an increase in the amount of bound gp120.

Patching of CD4 and CD4.CD8 molecules. Cells expressing CD4 or CD4.CD8 were incubated with OKT4A (1 μg/ml) at 4°C for 1 h, washed twice, and incubated with FITC-conjugated goat anti-mouse polyclonal antibody (10 μg/ml) for 1 h at 4°C. They were then transferred to 37°C for 5 min and washed and fixed with 1% paraformaldehyde for 10 min at 4°C. The cells were then observed with a Videoscope intensifier (5×10^5 gain) attached to a video camera, and the image was enhanced by using Universal Imaging processing equipment.

Fusion and syncytium formation assays. Cells expressing gp120-gp41 were labeled by adding 4 μl of the lipid fluorescent dye DiI (3.5 mg/ml) to 10^6 cells in 1 ml of phosphate-buffered saline (15). Cells were washed three times with RPMI 1640 supplemented with 10% fetal calf serum and antibiotics to remove unbound DiI. In another experiment, cells expressing gp120-gp41 were labeled with a water-soluble dye, BCECF (12). The labeled cells were added to $CD4^+$ cells in microtiter plates (10^5 per well) at a labeled/unlabeled cell ratio of 1:100. The plates were placed in a CO_2 incubator at 37°C and taken out for observation of dye redistribution by fluorescence video microscopy, indicating fusion, 0.5 to 4 h after mixing. Cells were examined by either phase-contrast or epifluorescence through the 20X objective of a Nikon inverted microscope with a rhodamine G-2A filter block. To minimize photodynamic damage, incident excitation light was adjusted to the lowest possible level with neutral-density filters. The images were acquired and analyzed with a video imaging system, which consists of a multichannel intensifier plate, video camera, video monitor, and image processing software run on a personal computer (26, 33), and photographic prints were produced by a video printer. The fusion yield was defined as the number of cells with dye redistribution (doublets, triplets, etc.) relative to the total initial number of labeled cells (12). The nonspecific dye transfer which occurs in noninfected cells (commonly less than 10%) must be subtracted to obtain the fusion yield value (13).

The kinetics of membrane fusion was also measured by adding saturating concentrations of sCD4 (10 μg/ml) at different times after the HIV-1 envelope-expressing effector cells were mixed with target cells and then counting the number of syncytia 5 or 20 h later. This approach to measuring membrane fusion kinetics is based on previous observations (13, 34) that the number of syncytia formed in the presence of sCD4 added at different times after cell coculture is proportional to the fusion kinetics monitored by fluorescence dye redistribution. Presumably, sCD4 acts to inhibit further binding of membrane-associated gp120-gp41 and CD4 but cannot affect already formed fusion junctions. The subsequent formation of syncytia "amplifies" those fusion junctions and allows monitoring of the fusion kinetics.

Syncytia were defined as giant cells four times or more the diameter of uninfected single cells (25). They are bound by a single membrane and not disrupted by pipetting. In some experiments to distinguish between syncytia and cell aggregates, we drew 0.1 ml of cell suspension in and out 10 times with an Eppendorf pipette. This action completely disrupted the aggregates, and the syncytia were counted easily. In all experiments involving syncytium formation, the ratio of gp120-gp41-expressing cells to target cells was 1:1.

RESULTS

Cells expressing hybrid CD4.CD8 fuse with gp120-gp41-expressing cells but after longer lag times than cells expressing wild-type CD4. To address the question of whether cells expressing CD4.CD8 molecules fuse and whether the kinetics of fusion is affected by the CD4 membrane-proximal domains, we (i) counted the number of syncytia as a function of time, (ii) measured membrane fusion by redistribution of fluorescent dyes, and (iii) evaluated cell fusion by adding sCD4 at different times after the cells were cocultured. The level of surface expression of CD4 and CD4.CD8 by the A2.01 transfectants was measured by flow cytometry. The mean fluorescence was converted to FU by using standard fluorescent beads and normalized to the CEM cell line value, which was set at 100% (corresponding to approximately 1.7×10^4 molecules per cell [23]), as described previously (22). While both A2.01.T4 and A2.01.T4.T8 expressed fewer CD4 and CD4.CD8 (17 and 57%, respectively) than CEM cells (100%), threefold more CD4.CD8 than CD4 was expressed by A2.01.T4.T8 cells.

TABLE 1. Kinetics of syncytium formation by CD4.CD8 cells is much slower than that by CD4 cells[a]

Env and MOI[b] (PFU/cell)	Mean no. of syncytia ± SD			
	CEM cells, 6 h	A2.01.T4 cells, 6 h	A2.01.T4.T8 cells	
			6 h	24 h
$HIV\text{-}1_{IIIB}$				
3.3	410 ± 14	325 ± 42	94 ± 10	177 ± 4
1.1	228 ± 14	214 ± 39	65 ± 4	108 ± 12
$HIV\text{-}1_{RF}$				
3.3	284 ± 20	285 ± 17	46 ± 6	149 ± 14
1.1	240 ± 4	207 ± 15	16 ± 2	120 ± 6

[a] 12E1 cells were infected with recombinant vaccinia viruses encoding the genes for the $HIV\text{-}1_{IIIB}$ and $HIV\text{-}1_{RF}$ envelope glycoproteins and mixed with CD4 (CEM or A2.01.T4)- or CD4.CD8 (A2.01.T4.T8)-expressing cells. The ratio of the cells expressing gp120-gp41 to the target cells was 1:1, and the total number of cells was 10^5 per well in a 96-well microtiter plate. Values represent means ± standard deviations for triplicate cultures in each group.

[b] The MOI was determined by a plaque-forming assay on HeLa cells.

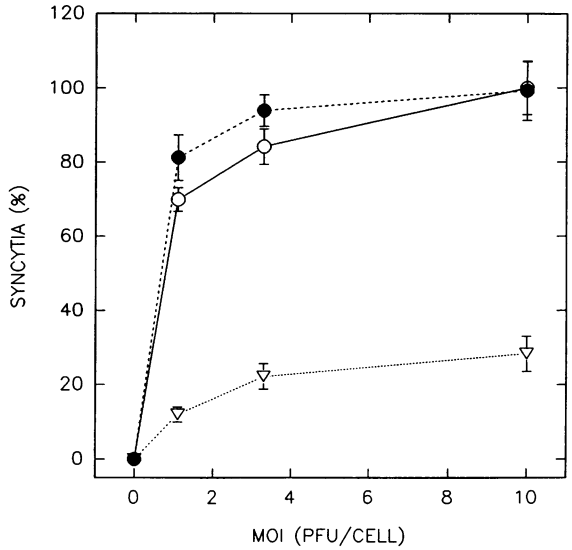

FIG. 1. Syncytium formation between 12E1 cells expressing the $HIV\text{-}1_{RF}$ envelope glycoprotein after infection with recombinant vaccinia virus at different MOIs and CD4- or CD4.CD8-expressing cells. Cells expressing gp120-gp41 (10^5 per well) were mixed with an equal number of CEM (○), A2.01.T4 (●), or A2.01.T4.T8 (▽) target cells, and syncytia were counted 6 h later. The number of syncytia is represented as a percentage of the maximal number at an MOI of 10 for CEM cells (443 ± 32 per well), assumed to be 100%.

We found that syncytium formation mediated by the interaction of CD4.CD8 with gp120-gp41 occurred with both $HIV\text{-}1_{IIIB}$ and $HIV\text{-}1_{RF}$ envelope-expressing effector cells, but only after prolonged coculture (Table 1). After 24 h, the number of syncytia formed by CD4.CD8-expressing cells (A2.01.T4.T8) was about half that formed by the A2.01.T4 cells in spite of the threefold-lower expression of surface CD4 receptors. However, it should be noted that the number of syncytia formed by the CD4-expressing cells after 24 h not only did not increase but actually decreased (15 to 30%), probably because of the destruction of some of the already formed syncytia.

To examine whether an increase in the surface level of gp120-gp41 would overcome the kinetic limitation imposed by the CD4.CD8 molecule, we infected the effector cells with recombinant vaccinia virus at increasing multiplicities of infection (MOIs). The difference between the number of syncytia formed with CD4- and CD4.CD8-expressing cells at early times of coculture (6 h) remained at all MOIs tested (Fig. 1).

The reduced number of syncytia formed by the CD4.CD8-expressing cells could be attributed to changes in the kinetics of membrane fusion or gp120 binding. To discriminate between these two possibilities, we measured the kinetics of early stages of membrane fusion leading to merging of the cell membranes by a fluorescent-dye redistribution assay (12) and an assay based on the use of sCD4 (34). CD4-negative cells (12E1) expressing vaccinia virus-encoded gp120-gp41 were labeled with the membrane-soluble fluorescent dye DiI and mixed with cells expressing either CD4 or CD4.CD8. While fluorescent-dye redistribution to the CD4-expressing CEM and A2.01.T4 cells was observed as early as 15 min and reached saturation at 3 to 4 h, DiI redistribution to CD4.CD8-expressing cells did not occur during the first hour (Fig. 2), and even at 4 h there were few fused cells (not shown). Similar differences in fusion kinetics were also observed with a water-soluble dye, BCECF; even after 3 h of coculture, the number of fused cells containing CD4.CD8 molecules was more than threefold lower than that for CD4-expressing cells (Table 2). Nonspecific dye transfer of DiI and leakage of BCECF precluded their use for rigorous monitoring of fusion for periods exceeding 4 h.

The fusion kinetics of cells expressing the CD4.CD8 molecule was also measured by a second assay, which allows monitoring of membrane fusion for longer periods. This assay is based on the ability of sCD4 to inhibit the early stages of cell fusion (13, 34). sCD4 (10 μg/ml) was added at different times after the cells were mixed. Syncytia were counted 6 and 24 h later. The ability of sCD4 to block syncytium formation by CEM and A2.01.T4 cells was maximal when sCD4 was mixed simultaneously with the cells (time zero) and declined by 50 to 60% when sCD4 was added 1 h after coculture. There was no inhibition when sCD4 was added after 2 h (Fig. 3). In contrast, addition of sCD4 to cocultures of cells expressing CD4.CD8 molecules 1 h after cell mixing completely blocked syncytium formation, and its addition as late as 5 h reduced the number of syncytia by 50% compared with the number without sCD4 (Fig. 3). These findings suggest a lag time in fusion of about 1 h, which is fivefold longer than the lag time for the CD4 cells (10 to 15 min). The rate of fusion after the lag time, which is defined as the number of fusion events per unit of time and reflects the variation in lag times within the cell population (11), was also about fivefold lower for the CD4.CD8-expressing cells than for the CD4-expressing cells.

These findings indicate that while the CD4.CD8 molecule is fusion competent, it requires prolonged periods of time to manifest its fusion potential.

gp120 binds to surface-associated CD4.CD8 molecules with the same kinetics as to surface-associated CD4 molecules. One possible explanation for the slower rate of syncytium formation by CD4.CD8-expressing cells is that the rate of binding to gp120 is slower. To check this possibility, we measured the rate of binding of recombinant gp120 to CD4.CD8- and CD4-expressing cells. We found that the rate of binding (Fig. 4) and the equilibrium isotherms (not shown) were indistinguishable for the two types of molecules. The calculated on and off rate constants at 37°C are $1.0 \times 10^5\ M^{-1}\ s^{-1}$ and $2.1 \times 10^{-4}\ s^{-1}$, respectively. These values are very close to the respective rate constants for sCD4 binding to gp120-gp41-expressing cells (14). The equilibrium dissociation constant was 2.1 nM, which is also very close to the value found for binding of sCD4 to gp120-gp41-expressing cells. We conclude that the kinetics of

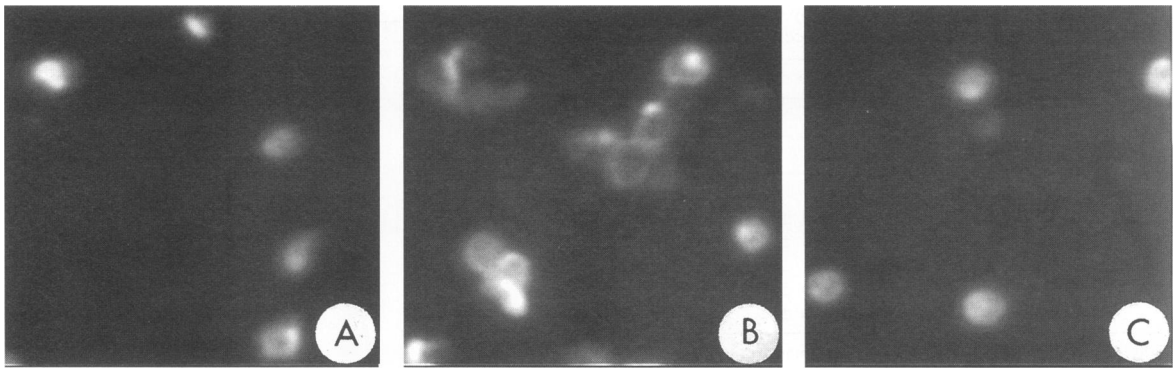

FIG. 2. Fusion of CD4 and CD4.CD8 cells with gp120-gp41-expressing 12E1 cells as monitored by fluorescent-dye redistribution. 12E1 cells expressing vaccinia virus-encoded gp120-gp41$_{IIIB}$ were labeled with the fluorescent dye DiI and mixed at a ratio of 1:100 with either (A) CD4-negative cells (12E1), (B) CD4-positive cells (CEM), or (C) CD4.CD8-expressing cells. The pictures were taken under epifluorescence 1 h after mixing. The dye redistribution seen in panel B is an indication of fusion. The dye has not redistributed in the other two panels, which indicates lack of fusion.

CD4-gp120 interaction is not affected by the CD4 membrane-proximal domains.

Lateral mobility of CD4.CD8 molecules is not impaired. While the kinetics of soluble receptor molecules may resemble the kinetics of binding of membrane-associated molecules, the kinetics of cell binding is also critically dependent on lateral mobility (4). In addition, the slow kinetics of fusion of A2.01.T4.T8 cells may be due to impaired lateral diffusion of the CD4.CD8 molecules, which may hinder the rate of formation of a putative fusion complex (5). Therefore, we compared the lateral mobility of CD4 and CD4.CD8 molecules by measuring their patching induced by antibodies. Figure 5 shows that staining with OKT4A followed by staining with FITC-conjugated goat anti-mouse antibody at 4°C and incubation for 5 min at 37°C resulted in patching of the CD4.CD8 molecules, identical to the patching of wild-type CD4 molecules on CEM cells. This means that the diffusion coefficient, D, is greater than 10^{-9} cm^2/s, as estimated by the formula $D = x^2/4t_D$, where x is a characteristic length of patch formation (of the order of 10 μm) and t_D is the time of incubation (5 min). It is interesting that a similar value (0.5×10^{-9} cm^2/s) was found for the wild-type CD4 molecule by fluorescence photobleaching recovery measurements (30). The average distance between two CD4.CD8 molecules is 100 nm (if the number of CD4.CD8 molecules is 10^4 per cell), and it will take less than 50 ms at $D > 10^{-9}$ cm^2/s for the two molecules to encounter each other by diffusion. Therefore, the lateral mobility of CD4.CD8 molecules is not a factor determining the differences in cell binding and the slow fusion kinetics of the CD4.CD8-expressing A2.01 cells.

CD4.CD8 molecule does not interfere in the fusion reaction between cells expressing wild-type CD4 and gp120-gp41-expressing cells. It was recently observed that fusion-deficient gp41 mutants can dominantly interfere with the fusion reaction mediated by wild-type gp120-gp41 molecules (19). It was proposed that this reflects the existence of a fusion complex

TABLE 2. Fusion of cells expressing CD4 and CD4.CD8 molecules, as observed by redistribution of the water-soluble fluorescent dye BCECF[a]

Cells	% of cells with dye redistribution	
	1 h	3 h
CEM	37 ± 3	42 ± 5
A2.01	5 ± 2	7 ± 3
A2.01.T4	23 ± 4	30 ± 5
A2.01.T4.T8	9 ± 2	15 ± 4

[a] The number of cells showing BCECF dye was normalized to the total number of labeled cells, assumed to be 100%. The dye redistribution to CD4-negative cells (A2.01) is the result of nonspecific dye transfer. The fusion yield can be calculated as the difference between the percentage of cells showing dye redistribution and redistribution due to nonspecific dye transfer (5 to 7% in this case).

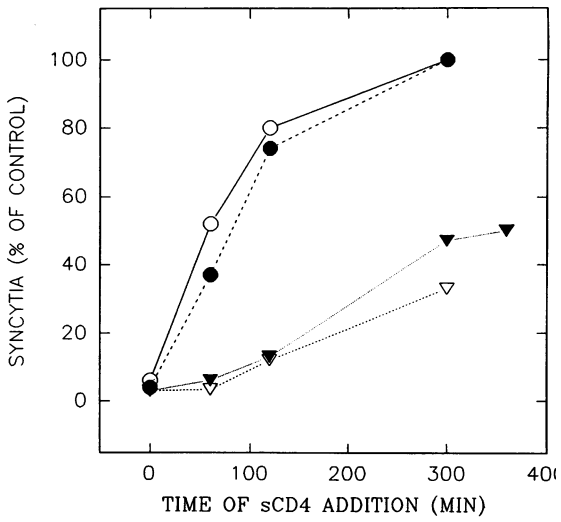

FIG. 3. Kinetics of cell fusion as measured by sensitivity to blocking by sCD4 added at different times after cell coculture. 12E1 cells expressing vaccinia virus-encoded gp120-gp41$_{IIIB}$ were mixed at a ratio of 1:1 with either CD4-positive CEM and A2.01.T4 cells (open and solid circles, respectively) or CD4.CD8-expressing cells (triangles). sCD4 (10 μg/ml, final concentration) was gently added to the cell coculture at the indicated times. Syncytia were counted after 5 h or, in the case of CD4.CD8-expressing cells, after 5 h (▽) and after 20 h (▼), and are represented as a percentage of the maximal number of syncytia for each cell line without sCD4 (626 ± 52 per well for CEM, 573 ± 38 for A2.01.T4, and 90 ± 6 for A2.01.T4.T8 after 5 h or 269 ± 7 measured after 20 h).

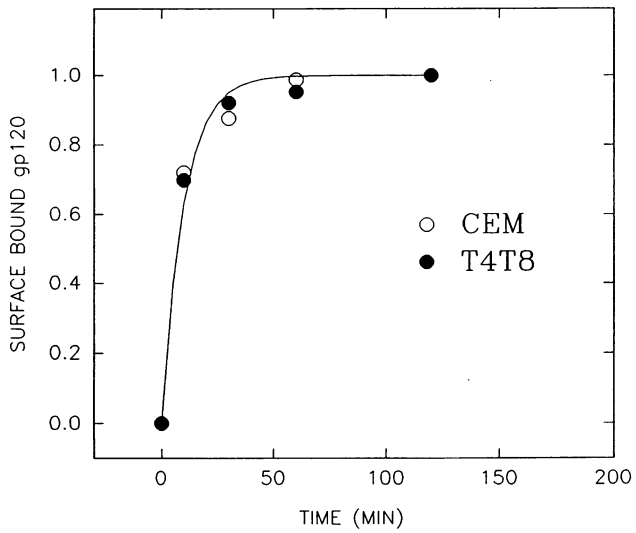

FIG. 4. Kinetics of rgp120 binding to cells expressing CD4.CD8 or CD4. CEM and A2.01.T4.T8 cells were incubated with 10 μg of $rgp120_{IIIB}$ per ml for the indicated periods of time at 37°C. They were then washed twice in cold buffer and stained with anti-gp120 antibody 110.1 and FITC-labeled goat anti-mouse antibody. Surface-bound gp120 was quantitated by flow cytometry as described in Materials and Methods. The amount of surface-bound gp120 is normalized to the maximal amount of bound gp120.

built by a certain number of envelope molecules (19). One might assume that the formation of a fusion complex by gp120-gp41 molecules requires interaction with an analogous complex of CD4 molecules and that fusion-deficient CD4 molecules may abrogate the function of such a complex. To find out whether the CD4.CD8 molecule exerts a dominant interference effect in such a putative complex, we coexpressed wild-type CD4, encoded by a recombinant vaccinia virus, in CD4.CD8-expressing cells. We did not observe any interference but rather an additive effect, which reflects the contribution of each molecule (CD4.CD8 and CD4) to the fusion process (Table 3).

DISCUSSION

The "nonbinding" domains of CD4 affect the fusion reaction by a mechanism which is presently unknown. It has been proposed that fusion may require an appropriate conformation of CD4 (32), an ability to undergo conformational changes needed for fusion (28), or oligomerization of CD4 (35). Alternatively, the membrane-proximal CD4 domains could affect fusion by exerting their effect indirectly through the binding domain (35).

This indirect effect could change the rate of gp120 binding to CD4 rather than its affinity. We previously showed that the rate of binding of sCD4 to gp120-gp41-expressing cells is strongly decreased at lower temperatures (14), and this may be in part responsible for the lack of fusion at low temperatures. However, this study shows that the rate of binding of gp120 to membrane-associated CD4.CD8 is the same as to CD4. The question still remains whether the rate of binding of membrane-associated gp120-gp41 to membrane-associated

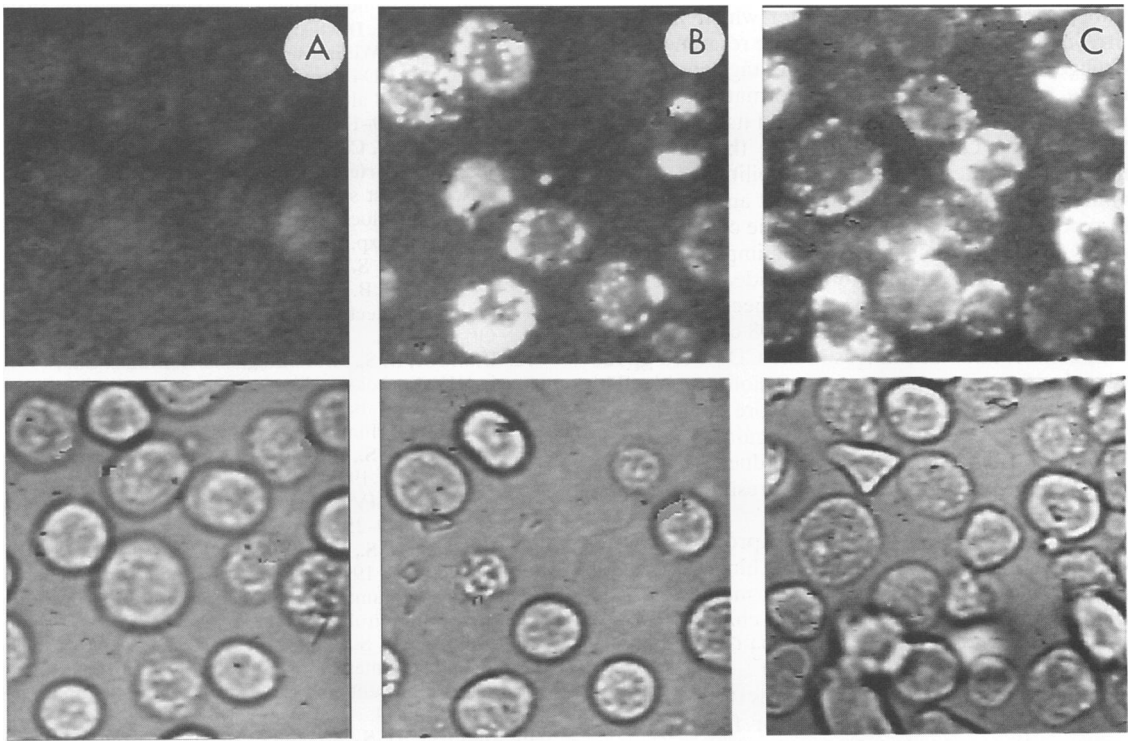

FIG. 5. Patching of CD4.CD8 molecules is not impaired. CEM and A2.01.T4.T8 cells were stained with OKT4A and then with FITC-conjugated goat anti-mouse antibody at 4°C for 1 h and transferred to 37°C for 5 min. They were then washed and fixed. Pictures were taken under epifluorescence (top panels) and bright field (bottom panels) illumination. (A) Control $CD4^-$ A2.01 cells, which show lack of nonspecific staining; (B) $CD4^+$ CEM cells; (C) $CD4.CD8^+$ A2.01.T4.T8 cells.

TABLE 3. Coexpression of CD4.CD8 with CD4 does not interfere dominantly with fusion[a]

Target cells	CD4 infection (MOI)	No. of syncytia
12E1	0	0
	1	440 ± 11
	10	662 ± 12
CEM	0	488 ± 12
A2.01.T4.T8	0	129 ± 16
	1	571 ± 26
	10	810 ± 15

[a] 12E1 effector cells were infected with recombinant vaccinia virus encoding the gene for the HIV-1_{IIIB} envelope at an MOI of 10 PFU/cell 16 h prior to coculture with the indicated target cells at a ratio of 1:1 and a total of 2×10^5 cells per well. The number of syncytia was counted 6 h after the coculture and is represented as an average for triplicate cultures per group.

CD4.CD8, which involves lateral diffusion of the interacting molecules (4), is slower than the rate of binding to membrane-associated CD4. The similar lateral mobilities of CD4.CD8 and CD4 molecules are one indication that this is unlikely. In addition, the adhesion rate of T cells is commonly dominated by adhesion molecules other than the CD4-gp120-gp41 interaction, and the binding step of the fusion reaction may not be significantly affected by differences in cell binding because of the multivalent nature of the interaction between surface-associated molecules. However, only further experiments can definitely resolve this issue.

We suggest that the long lag times of fusion mediated by the hybrid CD4.CD8 molecule are due to postbinding phenomena. A kinetic analysis of the fusion process in other viral systems (e.g., influenza virus) revealed that the lag time (delay) before fusion includes a commitment phase, after which the fusion process is irreversible (for a recent review, see references 5 and 6). It was suggested that the commitment stage involves the formation of a fusion complex and conformational changes leading to exposure of the fusion peptide and its insertion into the target membrane. One might speculate that because of their similar binding rates and lateral mobilities, CD4.CD8 molecules bind and form fusion complexes (if any) at the same rate as wild-type CD4 molecules. However, the conformational changes in the CD4.CD8 molecules may be impaired, leading to inefficient exposure of the fusion peptide or inefficient insertion into the target membrane. We speculate that the impairment in fusion kinetics of the CD4.CD8 cells is due to a slower rate of conformational changes in the CD4.CD8-gp120-gp41 complex, leading to exposure of the fusion peptide. This is in concordance with the recent data of Moore et al. (29) and Sattentau (35), which indicate that MAbs against the second and third domains of CD4 block sCD4-induced conformational changes of the gp120-gp41 molecule, presumably related to fusion.

It has been suggested that the fusion process requires bending of the CD4 molecule at the putative hinge-like region between domains 2 and 3 toward the target membrane (21). One might imagine that this conformational change occurs at an abnormally slow rate with the hybrid CD4.CD8 molecule. However, the recent finding that human cell components are required for membrane fusion (8, 17) indicates the existence of accessory molecules which are required for fusion (2, 27). Hence, an alternative mechanism for the involvement of the CD4 membrane-proximal domains in fusion is their interaction with those accessory molecules, which can be impaired by antibodies or CD8 segments.

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