JOURNAL OF VIROLOGY, May 1990, p. 2149–2156
0022-538X/90/052149-08$02.00/0

Vol. 64, No. 5

Human Immunodeficiency Virus Envelope Glycoprotein/CD4-Mediated Fusion of Nonprimate Cells with Human Cells

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Received 3 November 1989/Accepted 24 January 1990

Human immunodeficiency virus (HIV) infects human cells by binding to surface CD4 molecules and directly fusing with the cell membrane. Although mouse cells expressing human CD4 bind HIV, they do not become infected, apparently because of a block in membrane fusion. To study this problem, we constructed a recombinant vaccinia virus that can infect and promote transient expression of full-length CD4 in mammalian cells. This virus, together with another vaccinia recombinant encoding biologically active HIV envelope glycoprotein gp160, allowed us to study CD4/gp160-mediated cell-cell fusion in a wide variety of human and nonhuman cells in the absence of other HIV proteins. By using syncytium formation assays in which a single cell type expressed both CD4 and gp160, we demonstrated membrane fusion in lymphoid and nonlymphoid human cells but not in any of the 23 tested nonhuman cell types, derived from African green monkey, baboon, rabbit, hamster, rat, or mouse. However, in mixing experiments with one cell type expressing CD4 and the other cell type expressing gp160, all of these nonhuman cells could form CD4/gp160-mediated syncytia when mixed with human cells; in 20 of 23 cases, membrane fusion occurred only if the CD4 molecule was expressed on the human cells whereas in the other three cases, CD4 could be expressed on either one of the fusing partners. Interestingly, in one mouse cell line, CD4-dependent syncytia formed without a human partner, but only if a C-terminally truncated form of the HIV envelope glycoprotein was employed. Our results indicate that nonhuman cells are intrinsically capable of undergoing CD4/gp160-mediated membrane fusion, but this fusion is usually prevented by the lack of helper or the presence of inhibitory factors in the nonhuman cell membranes.

Acquired immune deficiency syndrome is caused by human immunodeficiency virus (HIV), a retrovirus with a narrow host range that infects mainly helper T lymphocytes and cells from monocyte-macrophage lineage expressing the CD4 cell surface glycoprotein. Considerable data suggest that the CD4 molecule itself serves as the receptor for HIV. Thus, infection of T cells with HIV can be inhibited by monoclonal antibodies (MAbs) directed against the CD4 molecule (5, 9, 18). In addition, the formation of a molecular complex between the HIV envelope glycoprotein and human CD4 has been demonstrated (17). Moreover, originally CD4-negative human cell lines can be infected with HIV after they are transformed to express CD4 on their cell surface (14).

After binding to CD4 on the target cells, HIV is internalized via direct, pH-independent fusion of the viral and cell membranes (16, 20). Although CD4 expression on a target cell seems to be sufficient for HIV attachment, the fusion process appears to be more complex. For example, mouse cells transformed with human CD4 can bind HIV but are not infected by the virus (14). This block seems to occur in the internalization process, since mouse cells have been shown to support HIV replication after transfection with HIV provirus (10). The results suggest that other cellular factors, in addition to CD4, may play a role in the internalization of HIV. These factors could either be helper molecules expressed in human, but not in mouse, cells or inhibitory components present in nonhuman cells.

Another phenomenon, closely related to HIV entry into susceptible cells, is syncytium formation. The formation of multinucleated giant cells was initially observed in HIV-infected helper T lymphocytes (19). Like infection, this process also can be blocked by certain antibodies to CD4 or gp160 (12, 22). Moreover, cells that express gp160 and no other HIV proteins form syncytia when cocultured with uninfected human CD4-bearing cells (11). In this study, we analyzed the CD4/human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein-mediated membrane fusion in a variety of lymphoid and nonlymphoid cell lines from several animal species. For this purpose, human CD4 or the HIV envelope glycoprotein gp160 was transiently expressed on human and nonhuman cells with recombinant vaccinia virus vectors. By monitoring syncytium formation after mixing CD4-bearing and gp160-expressing cells in tissue culture, we could rapidly screen a variety of cell lines for their fusion capacities. These data demonstrate a much more stringent requirement for the CD4-bearing cell than for the cell expressing gp160.

MATERIALS AND METHODS

Cells. The majority of cell lines used in this study were obtained from the American Type Culture Collection (Rockville, Md.), where the species of origin was verified by isoenzymology technique. The other cell lines were obtained from the following sources: A2.01 cells from K. Clouse (Department of Microbiology, Georgetown University, Washington, D.C.); RL-5, 6056, and 6516 from M. Gordon (Laboratory of Immunogenetics [LIG], National Institute of Allergy and Infectious Diseases [NIAID], Bethesda, Md.); BW 5147, SP-2, A.20, and P.815 cells from J. Yewdell (Laboratory of Viral Diseases [LVD], NIAID); E36 cells from S. O'Brien (Frederick Cancer Research Facility, Fred-

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erick, Md.); P.815-A3 from W. Biddison (Neuroimmunology branch, National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, Md.); and P.815/DR from E. Long (LIG, NIAID). Suspension cell medium contained RPMI 1640 (Quality Biologicals, Rockville, Md.) supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.), 2 mM L-glutamine, 10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], and antibiotics. Growth medium for adherent cell lines contained Dulbecco modified Eagle medium (Quality Biologicals) supplemented with 10% fetal calf serum, 2 mM glutamine, and antibiotics. Cells were maintained at 37°C in 5% CO_2 atmosphere. All of the cell lines were used within the first three passages from their arrival.

Recombinant vaccinia viruses. Recombinant vaccinia virus vEB-8, which directs the expression of the full-length human CD4 molecule under the control of the bacteriophage T7 promoter, was obtained by inserting the *Eco*RI-*Bam*HI fragment of the CD4 cDNA (15) into plasmid pEB-2 (1) and then using the resulting plasmid (pEB-8) to generate recombinant thymidine kinase-negative virus plaques by standard methods (13). Expression of CD4 is achieved upon coinfection of cells with vEB-8 and vTF7-3, which contains the bacteriophage T7 RNA polymerase gene under the control of the vaccinia P7.5 promoter (6). vPE16 is a recombinant vaccinia virus encoding a correctly processed full-length HIV-1 envelope glycoprotein gp160, whereas vPE17 directs the expression of a processed, truncated form of gp160 with a 104-amino-acid deletion in the cytoplasmic tail of the gp41 subunit (P. Earl and B. Moss, manuscript in preparation). In both vPE16 and vPE17, the HIV *env* gene is under the control of the vaccinia virus P7.5 promoter. vPE5 is a vaccinia virus which, upon coinfection of cells with vTF7-3, directs the expression of a secreted form of gp120, the external subunit of the HIV-1 envelope glycoprotein. As a control vaccinia virus, we used vSC8, encoding β-galactosidase under the control of the P11 promoter (3).

Transient expression of CD4 and gp160 on animal cells. Suspension cells were washed once with medium and suspended at 10^7/ml, and recombinant vaccinia viruses were added at a multiplicity of 10 PFU per cell. After a 1-h adsorption period, cells were diluted to a density of 5×10^5/ml and placed in a CO_2 incubator for 10 to 14 h. Adherent cells were trypsinized, washed twice with suspension cell medium, and infected in suspension.

Immunofluorescence. To measure cell surface expression of gp160 after vaccinia virus infection, cells were washed once with phosphate-buffered saline (PBS; Quality Biologicals)–0.1 mM NaN_3 and incubated for 30 min at 4°C with murine MAb 902 A1 directed against gp120 (4). After the cells were washed with PBS-NaN_3, bound antibodies were detected with fluorescein isothiocyanate (FITC)-labeled rabbit antiserum against murine immunoglobulin G (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). To detect surface CD4 expression, cells were washed and incubated for 30 min at 4°C with FITC-conjugated OKT4 antibody (Ortho Diagnostics Inc., Raritan, N.J.). For a gp120 binding assay, washed CD4-expressing cells or control cells were incubated for 30 min at 4°C with or without soluble gp120, prepared by concentrating the culture supernatant from vPE5- and vTF7-3-coinfected BSC-1 cells to 1/10 of the original volume. Similarly, concentrated supernatant from BSC-1 cells infected only with vTF7-3 was used as a control in some assays. Bound gp120 was detected by sequential incubations with 902 A1 MAb and FITC-conjugated anti-mouse immunoglobulin G as in the gp160 immunofluorescence assay. As a control antibody for 902 A1, we used H18-L10, a mouse MAb of the same isotype as 902 A1, directed against influenza virus hemagglutinin glycoprotein (a generous gift from J. Yewdell, LVD, NIAID). Surface fluorescence was quantitated by a FACS 440 flow cytometer.

Syncytium formation assay. Cells infected with recombinant vaccinia viruses and incubated for 10 to 14 h were washed twice with PBS and suspended at 10^6/ml in cell suspension medium. One-half of a milliliter of gp160-expressing cells was then mixed with 0.5 ml of CD4-bearing cells in 24-well tissue culture plates (Costar, Cambridge, Mass.). In control experiments, CD4- or gp160-expressing cells were substituted with cells infected with vSC8. In some experiments, adherent cells were coinfected with several recombinant vaccinia viruses and were screened for syncytia at 20 h after infection. For fusion inhibition studies, cells expressing CD4 or gp160 were preincubated for an hour with soluble CD4 (comprising the four extracellular domains of the protein; a generous gift from The UpJohn Co., Kalamazoo, Mich.) or with MAbs OKT4 or OKT4A (Ortho Diagnostics) before the fusion partner was added to the culture. In coculture studies, syncytia appeared at 1 to 3 h after mixing and they were documented by an inverted microscope (Labovert; E. Leitz Inc., Rockleigh, N.J.).

FITC labeling of viable cells. FITC labeling of viable, vaccinia virus-infected cells was performed by the method of Butcher and Weissman (2). Briefly, 200 μl of FITC-stock solution (270 μg/ml; Sigma Chemical Co., St. Louis, Mo.) was added to 10^7 cells suspended in 1 ml of 50% PBS–45% RPMI–5% fetal calf serum. After a 20-min incubation at 37°C, 3 ml of cold cell suspension medium was added and the cells were pelleted through a 6-cm cushion of fetal calf serum and washed twice with PBS. Labeled cells retained their viability and fluorescence for at least 48 h in tissue culture.

RESULTS

HIV-mediated syncytium formation of human cells. The wide host range of vaccinia virus, as well as the ability of infected cells to correctly process surface glycoproteins encoded by recombinant vaccinia viruses, made this vector system ideal for our studies. Previously, it was shown that human cells infected with a recombinant vaccinia virus encoding the HIV-1 envelope glycoprotein gp160 could fuse with human lymphocytes bearing CD4 on their cell surface (11). To increase the versatility of the analysis, we constructed a new vaccinia virus encoding the full-length human CD4 molecule.

Initial experiments demonstrated the surface expression of CD4 or gp160 when the CD4-negative A2.01 human T-cell line was infected with recombinant vaccinia viruses vTF7-3 plus vEB-8 or vPE16, respectively. Strong surface expression of CD4 or gp160 was observed at 12 h after infection by an indirect immunofluorescence cell sorting technique (Fig. 1a and b). When infected A2.01 cells expressing full-length gp160 were mixed with infected A2.01 cells expressing CD4, large syncytia appeared in culture within 1 to 3 h (Fig. 2a). As predicted, syncytium formation could be inhibited by soluble recombinant CD4 (Fig. 2d) or OKT4A (Fig. 2b) but not by OKT4 (Fig. 2c) MAb. Substituting a control vaccinia virus for either the virus encoding gp160 (Fig. 2e) or the virus encoding CD4 (Fig. 2f) resulted in no syncytium formation. The results were essentially similar when a nonlymphoid human cell line, TK^-143, was infected and used instead of A2.01 in these experiments (data not shown).

HIV-mediated syncytium formation between human and

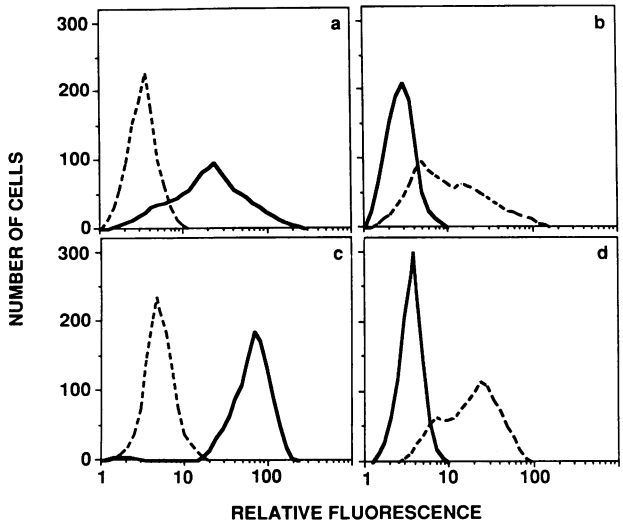

FIG. 1. CD4 or gp160 expression in human A2.01 (a and b) or mouse P.815 (c and d) cells after infection with recombinant vaccinia viruses vEB-8 plus vTF7-3 (a and c) or with vPE16 (b and d). Surface protein expression was detected either with OKT4 MAb (solid line) or with a MAb directed against gp160 (dotted line) by a FACS 440 flow cytometer as described in Materials and Methods.

nonhuman cells. We next infected a variety of lymphoid and nonlymphoid animal cell lines with recombinant vaccinia viruses encoding CD4 or gp160 (Table 1). Significant protein expression on the cell surface was observed by immunofluorescence at 10 to 14 h after the infection of all cell lines tested. Examples of CD4 and gp160 expression in P.815 mouse mastocytoma cells are shown in Fig. 1c and 1d, respectively. The levels of expression of both of these surface proteins were at least the same in the mouse cells as in human cells.

To address questions concerning species specificity of HIV envelope-mediated membrane fusion, we conducted mixing experiments with gp160-expressing and CD4-bearing animal and human cells. Results obtained with human, baboon, African green monkey, rabbit, rat, and mouse cell lines are summarized in Table 1. As shown, all of the tested cell lines expressing gp160 formed syncytia when mixed with CD4-positive human cells. In each case, syncytium formation was significant yet usually less efficient than when only human cells were mixed. As with human-human syncytia, specificity was indicated by inhibition of syncytium formation by soluble CD4 or OKT4A MAb. In the majority of cases, cell fusion was unidirectional, i.e., it only occurred if CD4 was expressed on human cells (Table 1; an example is shown in Fig. 3). Table 1 indicates only three exceptions to this generalization; one rabbit, one rat, and one murine CD4-bearing cell line formed syncytia when mixed with human cells expressing gp160. However, no syncytia were observed if both gp160 and CD4 were expressed on any of the nonhuman cells. In the one case examined, this restriction was not alleviated by coexpression of human major histocompatibility complex class I (P.815-A3) or class II (P.815/DR) proteins in mouse cells.

To rule out the possibility that the syncytia actually contained only human cells and arose from superinfection of CD4-bearing human cells with recombinant viruses released from the gp160-expressing nonhuman cells, we repeated some of the mixtures with FITC-labeled animal cells. FITC-labeled, gp160-expressing P.815 mouse cells mixed with unlabeled CD4-positive human cells formed strongly fluorescing syncytia (Fig. 4). In contrast, if control vaccinia virus-infected, FITC-labeled P.815 cells were added to a culture containing both CD4-bearing and gp160-expressing human cells, only individual FITC-positive cells were observed and all the syncytia were nonfluorescing. Furthermore, the cell fusion was already evident at 1 h after mixing the cells, 7 h earlier than was observed when A2.01 cells were purposely coinfected with the CD4- and gp160-expressing viruses (data not shown). Finally, no fusion at all was observed if the A2.01 cells were first infected with the CD4-expressing virus and then exposed 12 h later to the gp160-expressing virus (data not shown); we attribute this result to a virus exclusion phenomenon. These results verify that the syncytia observed in cocultures of human and nonhuman cells did indeed contain nonhuman cells and could not have arisen by the spread of the vaccinia virus vector.

In only one circumstance did we observe HIV envelope glycoprotein/CD4-mediated fusion of animal cells without a human partner. It had previously been observed that the expression of the truncated form of HIV envelope glycoprotein by recombinant vaccinia virus vPE17 in CD4-positive human lymphocytes resulted in enhanced syncytium formation as compared with the native envelope glycoprotein expression by vPE16 (P. Earl, unpublished observation). Consistent with this, CD4-bearing human cells formed syncytia with vPE17-infected animal cells more rapidly than with vPE16-infected animal cells. However, although syncytia were also larger in the former case, the requirement for a human cell as a fusing partner, as well as the unidirectionality to this membrane fusion, could still be demonstrated in all but one of the nonhuman cell types tested. Interestingly, mouse RAG renal adenocarcinoma cells, coinfected with vPE17, vEB-8, and vTF7-3, readily formed syncytia without a human partner (Fig. 5). The specificity of RAG fusion was confirmed by inhibition of syncytium formation with soluble CD4 (2 μg/ml) or OKT4A MAb (0.5 μg/ml) (data not shown).

Human CD4 on animal cells can bind to HIV envelope glycoprotein. In our syncytium formation experiments, fusion generally did not occur when CD4 was expressed on animal cells. To exclude the possibility that this was due to improper presentation of the gp120 binding site of CD4 on animal cells, we analyzed binding of soluble gp120 to P.815 cells expressing human CD4. gp120 binding to these murine cells was comparable to gp120 binding of CD4-bearing human A2.01 cells, as detected by incubation of the cells with conditioned medium containing vaccinia-encoded soluble gp120, followed by αgp120 Mab and FITC-labeled secondary antibody (Fig. 6a and b). The control experiment (Fig. 6c) indicates that no staining occurred when conditioned media lacking gp120 or a control MAb were employed or when P.815 cells were infected with a control vaccinia virus not expressing human CD4.

DISCUSSION

Our aim in this study was to analyze the cell specificity for CD4/HIV envelope glycoprotein-mediated fusion. Since the internalization of HIV occurs via direct fusion of viral and cell membranes (16, 20), cell-cell fusion (syncytium formation) is probably mediated through a similar if not identical process (11). With recombinant vaccinia viruses encoding either human CD4 or HIV gp160, we could rapidly screen a large number of human and nonhuman cells for their fusion characteristics. With this approach, our initial findings were

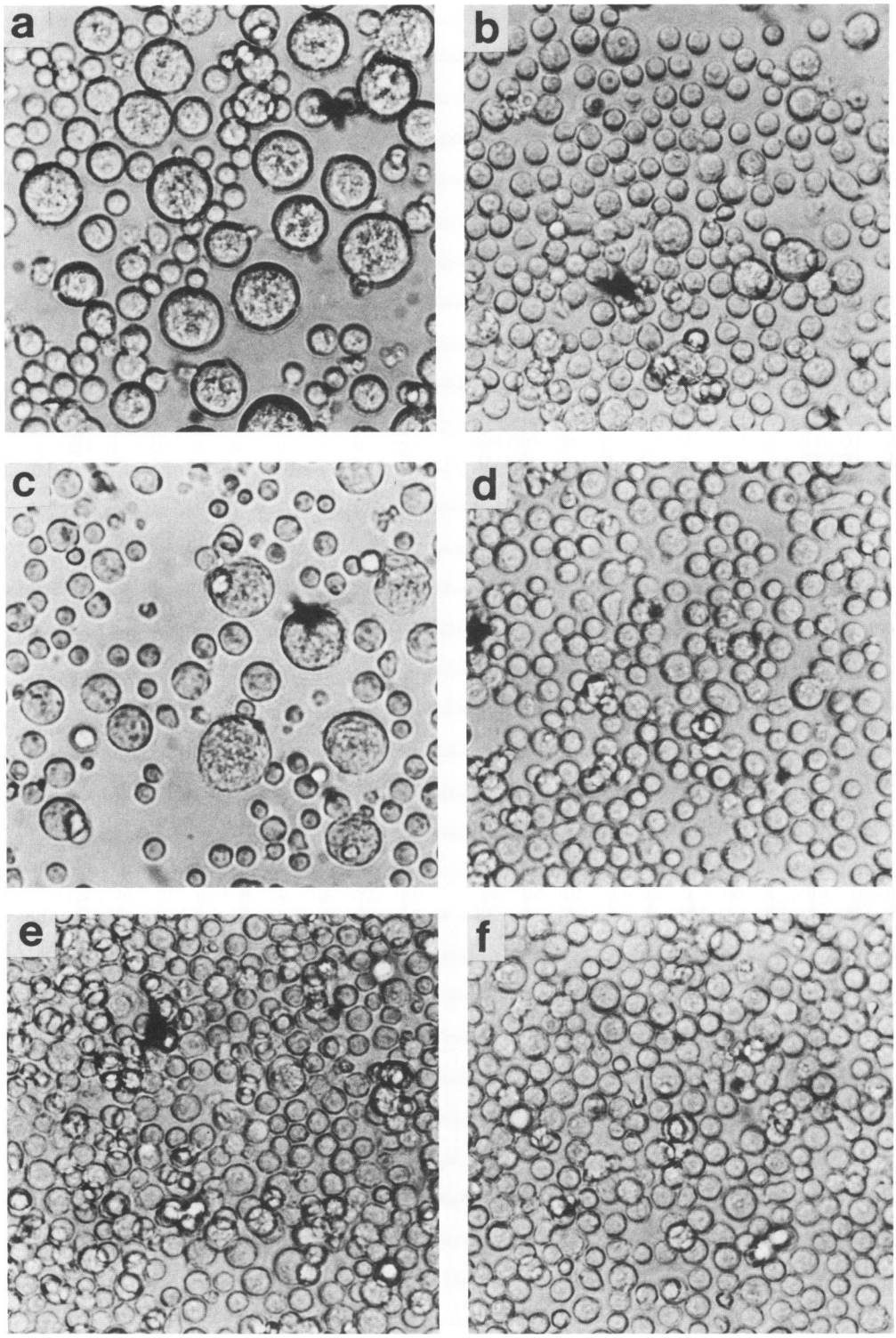

FIG. 2. Syncytium formation in cocultures of recombinant vaccinia virus-infected human A2.01 cells. Cells coinfected with vEB-8 plus vTF7-3 were mixed with vPE16-infected cells in the presence of no inhibitory agent (a), 0.5 μg of OKT4A per ml (b), 0.5 μg of OKT4 per ml (c), or 2 μg of soluble CD4 per ml (d). Panel e shows a coculture containing cells infected with vEB-8 plus vTF7-3 and cells infected with vSC8. Panel f shows a mixture of vSC8- and vPE16-infected cells. Micrographs (magnification, ×256) were taken 3 h after mixing.

TABLE 1. Syncytium formation between human and nonhuman cells expressing CD4 and HIV-1 gp160 encoded by recombinant vaccinia virus vectors

Cell line			Syncytium formation[a]		
Name	Origin	Type	Cell line/gp160 + A2.01/CD4	Cell line/CD4 + A2.01/gp160	Cell line/gp160 + cell line/CD4
A2.01	Human	T lymphocyte	+	+	+
TK143$^-$	Human	Fibroblast	+	+	+
CV-1	AGM	Kidney	+	−	−
26 CB-1	Baboon	Lymphoblast	+	−	−
6056	Rabbit	Macrophage	+	−	−
6516	Rabbit	T lymphocyte	+	−	−
RL-5	Rabbit	T lymphocyte	+	+	−
RK13	Rabbit	Kidney	+	−	−
E 36	Hamster	Lung	+	−	−
Y3-Ag1.2.3	Rat	Plasma cell	+	+	−
BW 5147	Mouse	T lymphocyte	+	−	−
A-20	Mouse	B lymphocyte	+	−	−
SP-2	Mouse	Plasma cell	+	−	−
PU 5-1.8	Mouse	Macrophage	+	+	−
P.815	Mouse	Mast cell	+	−	−
P.815-A3	Mouse	Mast cell (+HLA-A3)	+	−	−
P815/DR	Mouse	Mast cell (+HLA-DR)	+	−	−
NIH 3T3	Mouse	Fibroblast	+	−	−
MC 57	Mouse	Fibroblast	+	−	−
CL-7	Mouse	Fibroblast	+	−	−
L-A9	Mouse	Fibroblast	+	−	−
STO	Mouse	Fibroblast	+	−	−
TCMK-1	Mouse	Kidney	+	−	−
RAG	Mouse	Kidney carcinoma	+	−	−
CMT-93	Mouse	Rectum carcinoma	+	−	−

[a] Giant cell formation at 3 h after mixing the following: cell lines (left column) expressing gp160 plus human lymphocytes (A2.01) bearing CD4 (cell line/gp160 + A2.01/CD4), cell lines (left column) bearing CD4 plus human lymphocytes (A2.01) expressing gp160 (cell line/CD4 + A2.01/gp160), or cell lines (left column) expressing gp160 and cell lines (left column) bearing CD4 (cell line/gp160 + cell line/CD4) in tissue culture. A positive (+) result indicates that the culture contained cells with a diameter larger than three times that of a single cell. A negative (−) result indicates that the culture contained only single cells. AGM, African green monkey.

consistent with previous HIV infectivity studies. Thus, CD4-bearing lymphoid and nonlymphoid human cells readily fused to human cells expressing HIV gp160. In contrast, in 20 out of 23 animal cell lines derived from African green monkey, baboon, rabbit, hamster, rat, or mouse, CD4-bearing nonhuman cells did not form syncytia with human cells expressing the HIV envelope glycoprotein.

Several general models can be invoked to explain the failure of murine or other nonhuman cells to internalize HIV. One possibility is that there is an intrinsic incompatability between the cell membranes of different species which prevents fusion. Alternatively, there may be a specific surface component, in addition to CD4, that must interact with the HIV envelope glycoprotein in order for membrane fusion to occur, and this component may be present on human cells but not on mouse cells. As still another possibility, mouse cells may possess a specific inhibitor of the HIV gp160/CD4-mediated fusion process. Finally, rather than invoking the involvement of specific helper or inhibitory factors, it is possible that successful membrane fusion between gp160- and CD4-bearing cells depends on a variety of specific and nonspecific factors, including the inherent membrane compatability of the fusion partners, as well as multiple molecular interactions that additively affect the strength and duration of membrane contact.

Within this conceptual framework, the data presented here suggest several important points. We feel that inherent interspecies membrane incompatability cannot explain the failure of nonhuman cells bearing CD4 to fuse with human cells expressing gp160. First, the block to fusion was observed when such a membrane incompatibility could not exist, i.e., when the CD4-bearing and the gp160-expressing nonhuman cells were of the same type. Moreover, for each of the animal cell lines tested, fusion did occur if the animal cell expressed the HIV envelope glycoprotein and the human cell expressed CD4. The capability of the nonhuman cells to fuse with human cells in this unidirectional manner argues strongly against an inherent fusion incompatability.

One explanation for the unidirectionality of fusion is a requirement for a second human protein that interacts with the HIV-1 envelope protein. Arguing against this explanation, however, are the following three exceptions: a rabbit, a rat, and a mouse cell line that can fuse with a human cell regardless of which partner expresses CD4. Further arguing against the involvement of a specific helper protein in CD4/gp160-mediated membrane fusion is the previous report that mouse-human T-cell hybrids, containing all human chromosomes and properly expressing CD4, were not infectable by HIV (21). In support of this finding, we analyzed CD4/gp160-mediated syncytium formation in 11 hamster-human and mouse-human somatic cell hybrids (generous gifts from W. McBride, National Cancer Institute, Bethesda, Md., and S. O'Brien, Frederick Cancer Research Facility), most of which contained the nearly full complement of human chromosomes. When the hybrid cells were induced to express CD4 encoded by a recombinant vaccinia virus, fusion with either human or hybrid cells expressing gp160 did not occur (data not shown).

Another explanation for the unidirectionality of nonhuman cell-human cell fusion is related to differences in the lipid composition of cell membranes. According to this model, the fusion peptide of gp41 can only interact with human mem-

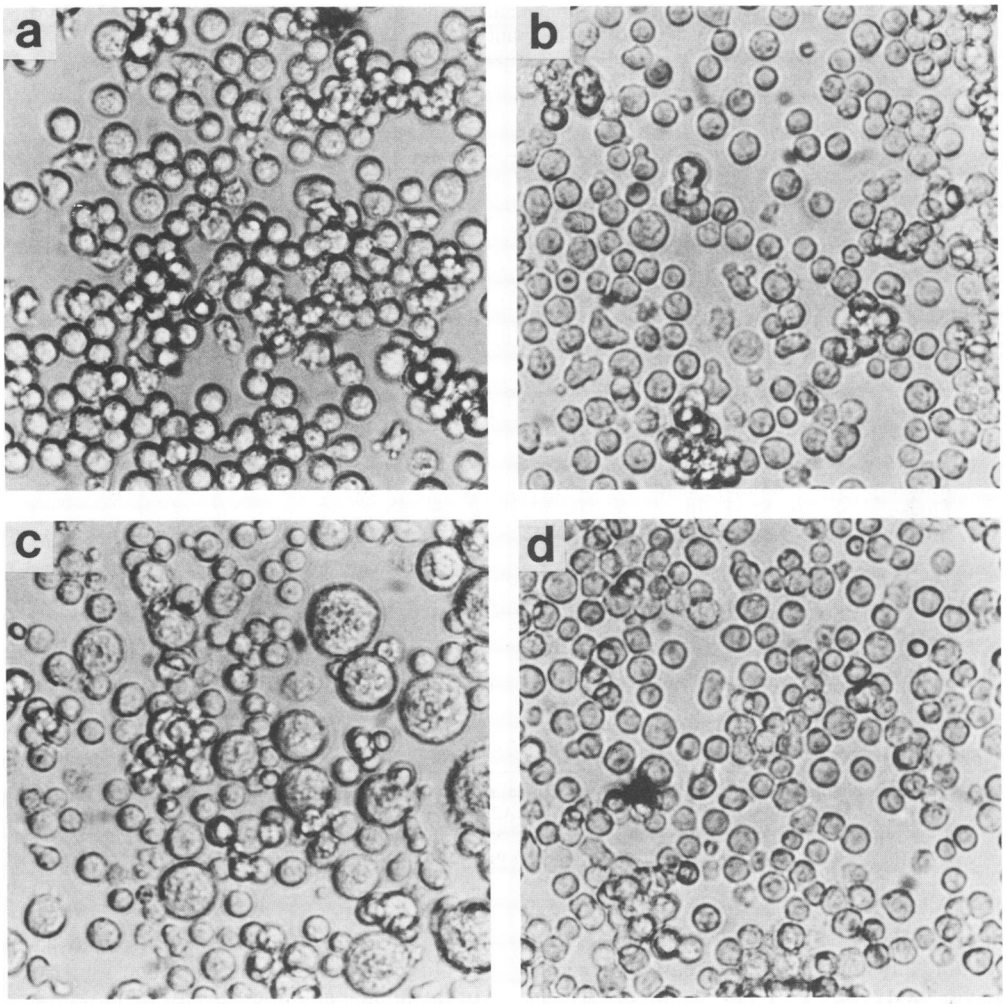

FIG. 3. Syncytium formation in a coculture containing gp160- and CD4-expressing mouse P.815 cells (a), CD4-expressing P.815 and gp160-expressing A2.01 cells (b), or gp160-expressing P.815 and CD4-expressing human A2.01 cells in the absence (c) and presence (d) of 2 μg of recombinant soluble CD4 per ml. Micrographs (magnification, ×256) were taken 3 h after mixing the cells.

branes. As before, however, this model cannot account for the three exceptions to unidirectionality or the results with RAG mouse cells. In the latter case, human cells were not required for syncytium formation provided that the cytoplasmic domain of gp41 was absent. It is possible that the mobility of the envelope glycoprotein in the membrane of the RAG cell is adversely affected by the cytoplasmic domain.

Several lines of evidence demonstrate that these exceptional results with RAG, Y3-Ag1.2.3, PU 5-1.8, and RL-5 cell lines were truly characteristic of nonhuman cells and were not simply artifacts resulting from mistaken identification of these cells as nonhuman cells. First, unlike human cells, these cells failed to form syncytia when gp160-expressing and CD4-bearing cells of the same type were mixed. Second, the species of origin of RAG, Y3-Ag1.2.3, and PU 5-1.8 was verified at the American Type Culture Collection and the cells were used for fusion assays quickly after their shipment from the American Type Culture Collection. Third, in Southern hybridization, a radiolabeled probe recognizing human specific ALU sequences (BLUR-8; a generous gift from Maxine Singer, Laboratory of Biochemistry, National Cancer Institute) failed to hybridize to chromosomal DNA isolated from these cells (data not shown).

Regarding a possible requirement for accessory surface components, it has been argued that coexpression of CD4 and human major histocompatability complex class II molecules may be required for simian immunodeficiency virus infection of target cells in tissue culture (8). Our findings suggest that this cannot be a sufficient explanation for the block of fusion of CD4-bearing mouse cells with HIV gp160-expressing mouse or human cells, since the block occurred even where the former also expressed major histocompatability complex class I or class II molecules (Table 1; P.815-A3 and P.815/DR). It has also been proposed that the adhesion molecule LFA-1 is involved in the fusion process, since anti-LFA-1 antibodies specifically inhibit syncytium formation between chronically HIV-infected T cells and phytohemagglutinin-stimulated lymphoblasts (7). However, LFA-1 cannot be an essential component, since HeLa cells transformed to express human CD4 can indeed be infected with HIV (18), even though they do not express LFA-1.

In view of all of the above consideration, we feel it is best to consider the CD4/gp160 fusion problem as a multifaceted process in which successful fusion may depend on several factors whose influences may vary between different cell types. Thus, in certain cases, fusion may be limited not by

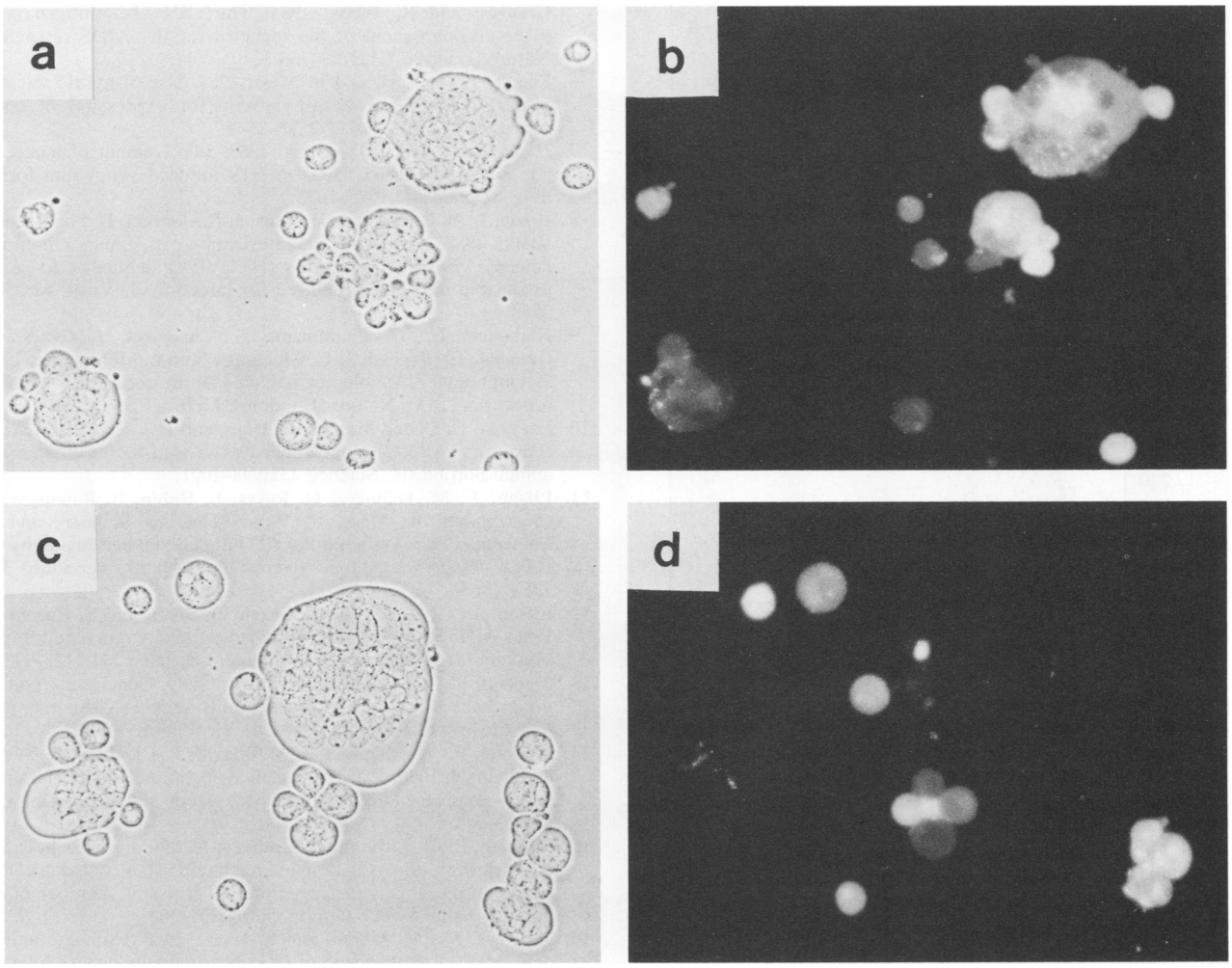

FIG. 4. Light (a and c) or fluorescence micrographs (b and d) of syncytia formed during a 2-h coculture of CD4-bearing human A2.01 cells, coinfected with vTF7-3 and vEB-8, and gp160-expressing, FITC-labeled murine P.815 cells, infected with vPE16 (a and b) or in a coculture containing both gp160 and CD4-bearing A2.01 cells mixed with FITC-labeled P.815 infected with control vSC8 vaccinia virus (c and d). Magnification, ×256.

the mere presence of CD4 and the envelope glycoprotein but by other interrelated membrane properties, such as auxiliary adhesion components, surface charge, membrane fluidity, and the mobility of specific surface molecules within the membrane. The vaccinia-based expression system provides a powerful tool for further investigating these parameters since it allows the expression of CD4 and gp160, in conjunction with other relevant surface proteins, in a broad range of

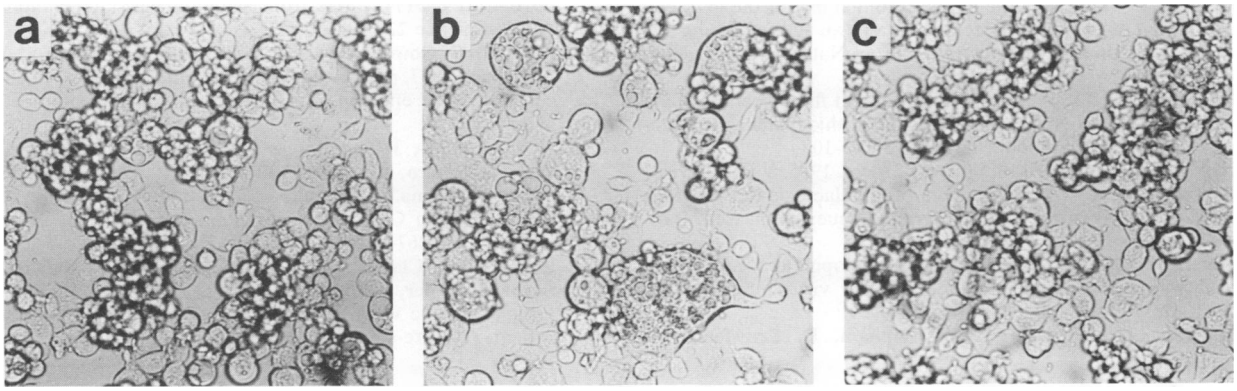

FIG. 5. Syncytium formation of RAG cells coinfected with recombinant vaccinia viruses vPE17 and vSC8 (a); vPE17, vEB-8, and vTF7-3 (b); or vEB-8, vTF7-3, and vSC8 (c). Micrographs (magnification, ×128) were taken 20 h after infection.

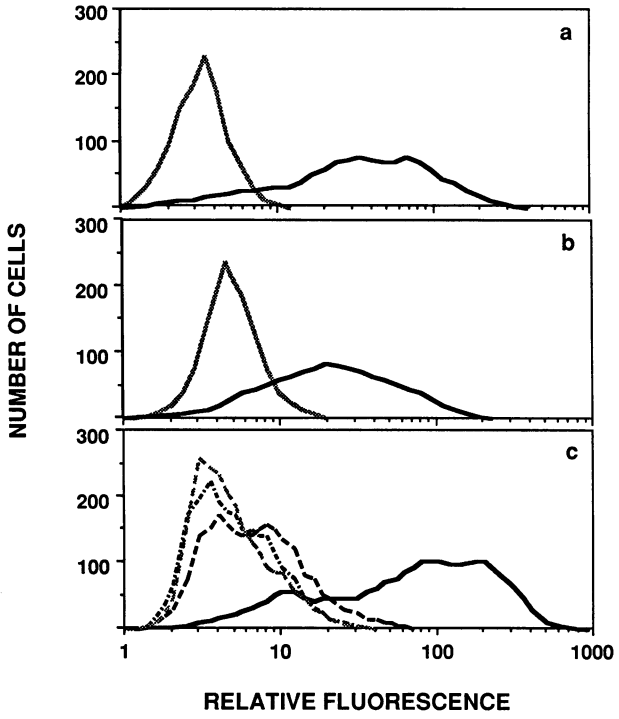

FIG. 6. Binding of soluble gp120 to human A2.01 (a) or mouse P.815 cells (b and c) expressing human CD4. (a and b) Cells were coinfected with recombinant vaccinia viruses vEB-8 and vTF7-3, washed and treated with soluble gp120 (——) or PBS (⋯⋯), followed by incubations with 902 A1 anti-gp120 MAb and FITC-conjugated secondary antiserum. (c) A separate experiment, where mouse P.815 cells were infected with vEB-8 plus vTF7-3 (——) or vTF7-3 only (– – –) and stained with sequential incubations with soluble gp120, anti-gp120 MAb, and FITC-conjugated antiserum against mouse immunoglobulin G. Additional controls include P.815 cells coinfected with EB-8 and vTF7-3 and stained with a mock gp120 preparation and anti-gp120 MAb (· · · ·) or with soluble gp120 followed by control MAb H18-L10 (·····).

cell types. This provides an opportunity to test the effects of expression of auxiliary adhesion components, as well as mutations such as cytoplasmic domain deletions, that may influence membrane mobility and the capacity of CD4/gp160 interaction to promote membrane fusion.

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