JOURNAL OF VIROLOGY, Aug. 1992, p. 4794–4802
0022-538X/92/084794-09$02.00/0

Vol. 66, No. 8

Complementation of Murine Cells for Human Immunodeficiency Virus Envelope/CD4-Mediated Fusion in Human/Murine Heterokaryons

TATJANA DRAGIC,[1] PIERRE CHARNEAU,[2] FRANÇOIS CLAVEL,[2] AND MARC ALIZON[1]*

INSERM U332, Institut Cochin de Génétique Moléculaire, 22 rue Méchain, 75014 Paris,[1] and Unité d'Oncologie Virale, Département SIDA et Rétrovirus, and CNRS URA 1157, Institut Pasteur, 75724 Paris Cedex 15,[2] France

Received 11 March 1992/Accepted 6 May 1992

Murine cell lines expressing human CD4 are resistant to the fusogenic effect of the human immunodeficiency virus (HIV) envelope. Consequently, they cannot be infected by HIV or form syncytia with HIV envelope-expressing cells. Murine cells could either lack human-specific cofactors necessary for the CD4/envelope-mediated membrane fusion or express inhibitors of this process. To address this question, we have tested the ability of heterokaryons made from CD4-expressing murine cells and human cells to undergo HIV envelope-mediated fusion. We have devised a rapid and specific assay based on the induction of *lacZ* expression, in which membrane fusion events with HIV-infected cells can be detected by a simple histochemical technique. CD4-positive murine/human heterokaryons, but not murine/simian heterokaryons, were found able to fuse with HIV envelope-expressing cells. In these experiments, the fusion resistant phenotype of murine-CD4 cells could be complemented by human cellular factors.

Entry of the human immunodeficiency virus (HIV) into its target cells is a complex process initiated by a high-affinity interaction between the viral surface glycoprotein (gp120) and CD4, the first molecule to be identified as a retroviral receptor (12, 24, 30). These observations were confirmed by gene transfer experiments in which several human cell lines could be infected by HIV-1 after they were engineered to express CD4 (27). By contrast, murine cell lines expressing human CD4 could not be infected by HIV-1 or vesicular stomatitis virus (VSV) pseudotypes bearing HIV-1 envelope glycoprotein, despite a seemingly correct gp120-CD4 interaction (27). More recently, other murine cell lines as well as rabbit, feline, and simian cell lines expressing human CD4 were also found to be resistant to HIV-1 entry (7). Interestingly, the same observation was also made for two human cell lines, U87 and SCL1 (6). In every case, the block to HIV infection appears to be at the level of virus entry.

The molecular events that follow the CD4-gp120 interaction and lead to uncoating and entry of the capsid in the target cell are only partly understood. A key step is a pH-independent membrane fusion between the HIV envelope and the cell membrane (28, 29, 39). The fusogenic properties of the HIV envelope are also responsible for the formation of syncytia between human CD4 cells and HIV-infected or HIV envelope-expressing cells (25, 37). Nonhuman cell lines engineered to express CD4 do not form syncytia with cells expressing gp160, the HIV envelope precursor (3). Resistance to the envelope fusogenic effect is the most probable cause of the block to HIV entry in CD4-positive cell lines.

There is no intrinsic inability for cells from different species to fuse, and human CD4 cells readily formed syncytia with rodent or other animal cell lines expressing gp160 (3). The unidirectionality of the CD4/HIV envelope-mediated fusion suggests a control by cellular factors distinct from CD4 and acting in concert with it. These factors could be human-specific molecules participating in the HIV envelope fusogenic activity, but it can also be envisioned that murine and other cells that cannot be infected by HIV express inhibitors of the fusion process. Indeed, CD4-expressing rodent-human somatic cell hybrids have been found to be resistant to HIV entry or syncytium formation with envelope-expressing cells (3, 40). Identification of cellular factors controlling HIV entry has obviously elicited much interest, but to date it has not been possible to provide conclusive evidence for one or the other mechanism.

A direct way to address the problem would be to phenotypically mix fusion-resistant CD4-bearing cells and fusion-permissive human cells. After their membranes have transiently been disrupted by polyethylene glycol (PEG) treatment, two or more cells can reassociate to form a heterokaryon in which the cytoplasms and membranes are mixed, but the nuclei remain individualized and functional (for reviews, see references 23 and 33). Heterokaryons have a limited viability and are not cycling, and they should not be able to support retroviral integration and replication (10). However, the ability to undergo CD4/envelope-mediated fusion can be rapidly tested in assays independent of HIV replication. To detect fusion events between heterokaryons and HIV envelope-expressing cells, we have devised a simple assay based on the transactivation of a bacterial β-galactosidase gene (*lacZ*) placed under the control of the long terminal repeat (LTR) of HIV-1. Fusion events with effector cells expressing the HIV-1 envelope and transactivator *tat* result in a dramatic increase of *lacZ* expression and can be detected in situ as blue foci in a simple histochemical assay. Using this test, we found that murine/human heterokaryons expressing CD4 were able to fuse with cells expressing the HIV envelope.

MATERIALS AND METHODS

Maintenance of cell lines. Adherent cells were propagated in Dulbecco's modified Eagle medium (DMEM) supple-

* Corresponding author.

mented with antibiotics (penicillin and streptomycin), 2 mM glutamine, and 10% newborn calf serum (for NIH 3T3 and derivatives) or 10% fetal calf serum (for COS7, HeLa, and derivatives). Cells growing in suspension were propagated in RPMI 1640 plus antibiotics, glutamine, and 10% fetal calf serum.

CD4-expressing cells. CD4 surface expression was analyzed with a fluorescence-activated cell sorter (FACS) (FACScan; Becton Dickinson), using phycoerythrin-conjugated monoclonal antibody (MAb) Leu3A (Becton Dickinson) as recommended by the supplier. HeLa-CD4 cells (27) were obtained from R. Weiss (Chester Beatty Institute, London, England); 3T3-CD4 cells were made by infection of 3T3 cells with the retroviral vector pMA245 (5), containing a human CD4 cDNA placed under control of the simian virus 40 early promoter, and a dihydrofolate reductase (*dhfr*) cDNA (36) under control of the murine leukemia virus LTR. Plasmid DNA was transfected by the calcium phosphate technique in the Ψ CRE helper-free ecotropic packaging cell line (13), and the cell-free supernatant was harvested 48 h later for infection of 3T3 cells as described previously (13). Clones were selected in complete medium plus 0.2 μM methotrexate (Calbiochem). CD4 expression in the 3T3-CD4 clone 14 used in this study was stable and not modified by further transfection and selection steps necessary to generate the LTR-*lacZ* derivative, SC6 (Fig. 1a).

HIV envelope-expressing cells. The surface expression of HIV envelope was analyzed by FACS, using the anti-gp120 MAb 110-4 (26), and by syncytium formation assay on HeLa-CD4 cells as described previously (27). Chronically HIV-1-infected H9/IIIB (32) and 8E5 (16) cells carrying a reverse transcriptase-defective HIV-1 genome have been described elsewhere.

***tat*-expressing cells.** The Jurkat-tat cell line has been described elsewhere (35). HeLa-tat cells were obtained by calcium phosphate transfection of HeLa cells with the expression vector pMA243, which has the capacity to encode the viral proteins Tat, Rev, Vpu, and Env. pMA243 was derived from an infectious HIV-1_{LAI} provirus, pLAI-3 (31), reconstructed from the fully sequenced HIV-1_{LAI} isolate (43), previously known as HIV-1_{BRU} or LAV/HTLV-III. To obtain pMA243, pLAI-3 was deleted from *Sph*I (position 993) to *Nco*I (position 5257), and *dhfr* was substituted for the first 255 nucleotides of *nef* as in the R7dhfr HIV vector (41). pMA243 also carries the 31-bp deletion (positions 301 to 331) previously shown to reduce the efficiency of HIV-1 RNA packaging (8). Clones of HeLa cells carrying pMA243 were selected in complete medium plus 2 μM methotrexate.

LTR-*lacZ* cells. Construction of the HeLa-LTRlacZ cells and their CD4 derivatives is described elsewhere (5). These cells were stably transfected with an LTR-*lacZ* plasmid (pMA175) in which the bacterial β-galactosidase gene (2) is placed under the control of a *Sca*I-*Hin*dIII fragment (positions −139 to +82) of the LTR of the HIV-1_{LAI} isolate (43). The same LTR-*lacZ* construct was used to generate 3T3-CD4-LTRlacZ and COS7-LTRlacZ. 3T3-CD4 cells were cotransfected with plasmids pMA175 and PGK-his, in which the histidinol dehydrogenase gene (*hisD*) (18) is under the control of the mouse phosphoglycerate kinase promoter (1), and clones were selected in the presence of 0.5 mM histidinol (Sigma) in DMEM without histidine as described previously (18). COS7 cells were cotransfected with plasmids pMA175 and PGK-hygro, in which the hygromycin B resistance gene (17) is under control of the phosphoglycerate kinase promoter, and clones were selected in the presence of 0.3 mg of hygromycin B (Calbiochem) per ml. LTR-*lacZ*-containing clones were tested by PEG fusion with HeLa-tat cells (see Fig. 4).

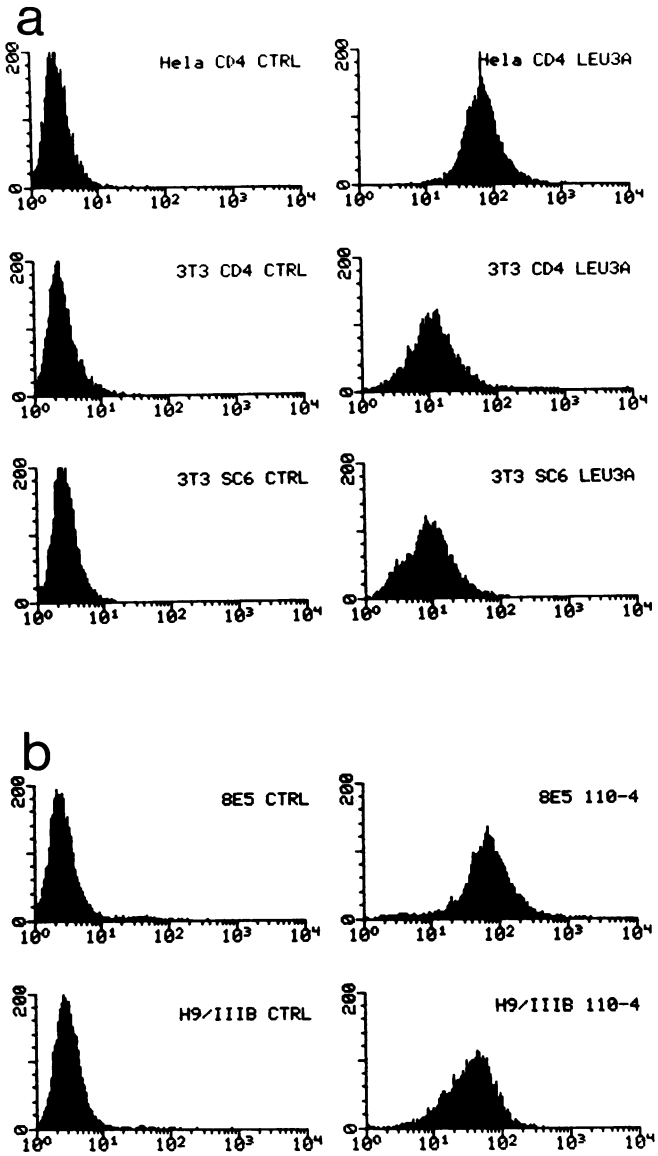

FIG. 1. CD4 (a) and HIV envelope (b) expression. Cell type and MAb used for staining are indicated for each FACS profile. CD4 was detected with MAb Leu3A (a, right panels). HIV envelope was detected with anti-gp120 MAb 110-4 (b, right panels). A mouse immunoglobulin G1 MAb was used as a control (left panels). 3T3 SC6 refers to the LTR-*lacZ* derivative of 3T3-CD4.

Heterokaryons. A 1:1 ratio of the two cell types to be fused was used. A total of 10^6 cells were plated on a 35-mm-diameter six-well petri plate to obtain a subconfluent monolayer the next day. For PEG-induced cell fusion, medium was aspirated and the cells were overlaid with 1 ml of a prewarmed 50% (wt/vol) PEG 6000 (Merck) solution in DMEM. After 1 min, the PEG solution was extensively washed off (four times) with phosphate-buffered saline (PBS), culture medium was added, and the plates were returned to 37°C. Heterokaryons started to form 2 to 3 h after PEG treatment and remained viable for several days.

Syncytium formation and detection. About 2×10^6 HIV-envelope expressing 8E5 or H9/IIIB cells, as indicated, were cocultured overnight with the target cells or heterokaryons (in which case they were added 2 h after PEG treatment). The next day, the plate was washed with PBS, and the cells were fixed in 0.5% glutaraldehyde (Sigma) for 5 min at room temperature, washed twice in PBS, and incubated in 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) buffer (34) at 37°C for 2 h. Incubations were stopped by two washes in PBS. For experiments reported in the tables, all blue foci visible under a 40×-magnifying stereomicroscope, regardless of their size and morphology, were counted.

RESULTS

Histochemical assay for the detection of cell fusion events. Our purpose was to test the ability of murine/human heterokaryons to undergo CD4/HIV envelope-mediated membrane fusion. PEG treatment disrupts the architecture of the cell monolayer and generates multinucleated cellular structures. It is therefore not possible to rely on morphological changes to detect fusion events between heterokaryons and effector cells. Cells chronically infected with HIV such as H9/IIIB cells (32) can be conveniently used as effectors in syncytium formation assays. These cells also express the HIV-1 transactivator, Tat, and we have taken advantage of the tight control that this protein exerts on the HIV LTR (11). Our assay is based on the induction of expression of a reporter gene placed under the control of the HIV-1 LTR in the heterokaryon after it has undergone membrane fusion with a *tat*-expressing effector cell.

PEG fusion of a cell line containing an HIV LTR-driven chloramphenicol acetyltransferase (CAT) gene with another cell line expressing *tat* results in an overall increase in CAT activity (15, 22). The use of an LTR-*lacZ* reporter allowed us to detect transactivation in situ by a histochemical X-Gal assay. PEG treatment of cocultures of HeLa-LTRlacZ and *tat*-expressing cell lines results in a dramatic increase in *lacZ* expression, indicated by the detection of intensely blue-stained foci after short X-Gal incubations (Fig. 2a). In the absence of PEG treatment, no blue-stained cells are detected (Fig. 2b), which indicates that coculture of HeLa-LTRlacZ and *tat*-expressing cells, even at high densities, is not sufficient to induce *lacZ* expression. Transactivation by cell-cell contact was recently proposed to explain the increase in CAT activity seen in cocultures of HeLa-LTRCAT and *tat*-expressing cells (20). We did not observe such a phenomenon with LTR-*lacZ* reporter cell lines, possibly because of a lower sensitivity of this enzymatic assay or because of a position effect on the reporter construct that may modulate its response to *tat* transactivation. Also, the CAT assay is performed on extracts, and the possible contribution of spontaneous fusion events of LTR-CAT and *tat*-expressing cells cannot be ruled out.

To show that transactivation takes place when cells fuse following an HIV envelope-CD4 interaction, we used CD4-positive HeLa-LTRlacZ H12 cells in which *lacZ* expression is highly induced by HIV-1 infection (5). When a subconfluent monolayer of H12 cells was cocultured overnight with H9/IIIB cells, most syncytia were stained in a 2-h X-Gal assay (Fig. 2c), indicating a high level of *lacZ* expression consistent with *tat* transactivation of the HIV LTR. The assay was dependent on the presence of CD4 and HIV envelope, since no blue foci could be detected on cocultures of H12 cells and Jurkat-tat cells (35), which are HIV envelope-negative (Fig. 2f) or when CD4-negative HeLa-LTR-lacZ cells were used as targets with H9/IIIB cells (see below).

To show that LTR-*lacZ* transactivation after cell-cell fusion was possible in the absence of HIV replication, we used the cloned human lymphoblastoid 8E5 cell line carrying a single reverse transcriptase-defective HIV-1 provirus (16). With the 8E5 effector cells, the formation of syncytia on HeLa-CD4 cells was less efficient than with the H9/IIIB cells, but in both cases, the X-Gal staining revealed intensely blue foci too small to be unambiguously detected by direct light microscopy or conventional histological techniques (Fig. 2c and e). The X-Gal assay allowed discrimination of these fusion events from bubblelike structures often present in monolayers of HeLa cells. These small blue foci probably correspond to the initial fusion event of an envelope-expressing cell and a target CD4 cell. Similar images have been observed after membrane labelling with fluorescent dyes and shown to form before or even in the absence of large multinucleated syncytia (14). Our histochemical assay was therefore independent of HIV replication and could detect cell-cell fusion events not seen by classical syncytium formation assays, both being prerequisites for such experiments with heterokaryons.

Envelope-mediated fusion with CD4-expressing heterokaryons. In the first series of experiments, we used a CD4-negative HeLa-LTRlacZ clone designated Z24 and characterized by a null *lacZ* background, even in overnight X-Gal incubations, and a dramatic *lacZ* induction upon introduction of *tat* (5). As expected, Z24 cells did not form syncytia when cocultured with HIV envelope-expressing cells, and the X-Gal staining remained negative (not shown). When subconfluent cocultures of Z24 and HeLa-CD4 cells were treated with PEG, heterokaryons started to form 2 to 3 h later, and 10 to 30% of the cells were incorporated in multinucleated structures by 16 to 18 h when the cells were fixed for the X-Gal assay. No further morphological change could be observed when effector cells expressing HIV-1 *tat* and *env* (either H9/IIIB or 8E5) had been added to the plates 2 h after PEG treatment and left in contact overnight (see Materials and Methods). However, numerous blue foci were readily detected by a 2-h X-Gal assay (Fig. 3a and b). When CD4-negative HeLa/Z24 heterokaryons were tested, or when envelope-negative Jurkat-tat cells were used as effectors, no heterokaryon (or isolated cell) was stained blue (Fig. 3c and d). This result further demonstrated the requirement for an envelope-CD4 interaction for the induction of *lacZ* expression in our assay and showed that PEG was efficiently removed from the plate, any remaining traces being insufficient to mediate fusion with effector cells.

Complementation of 3T3-CD4 cells in human/murine heterokaryons. PEG fusions were performed on cocultures of HeLa-LTRlacZ (Z24) and murine cells (3T3 or 3T3-CD4). Blue foci were detected when cocultures of Z24 and 3T3-CD4 cells, but not Z24 and 3T3 cells, were PEG treated and put in contact of the 8E5 or H9/IIIB effector cells (Fig. 3e). The results of several independent experiments are summarized in Table 1. Relatively little variation is observed in the number of blue foci between experiments. H9/IIIB cells were more efficient as effector cells than were 8E5 cells. Background *lacZ* activity was always null in the absence of a CD4-positive fusion partner or envelope-expressing effector. The fusion-resistant phenotype of murine CD4 cells appears to be complemented by phenotypical mixing with human cells.

To limit the risk of inactivation of genes expressed in the parental cell lines, heterokaryons were tested early after

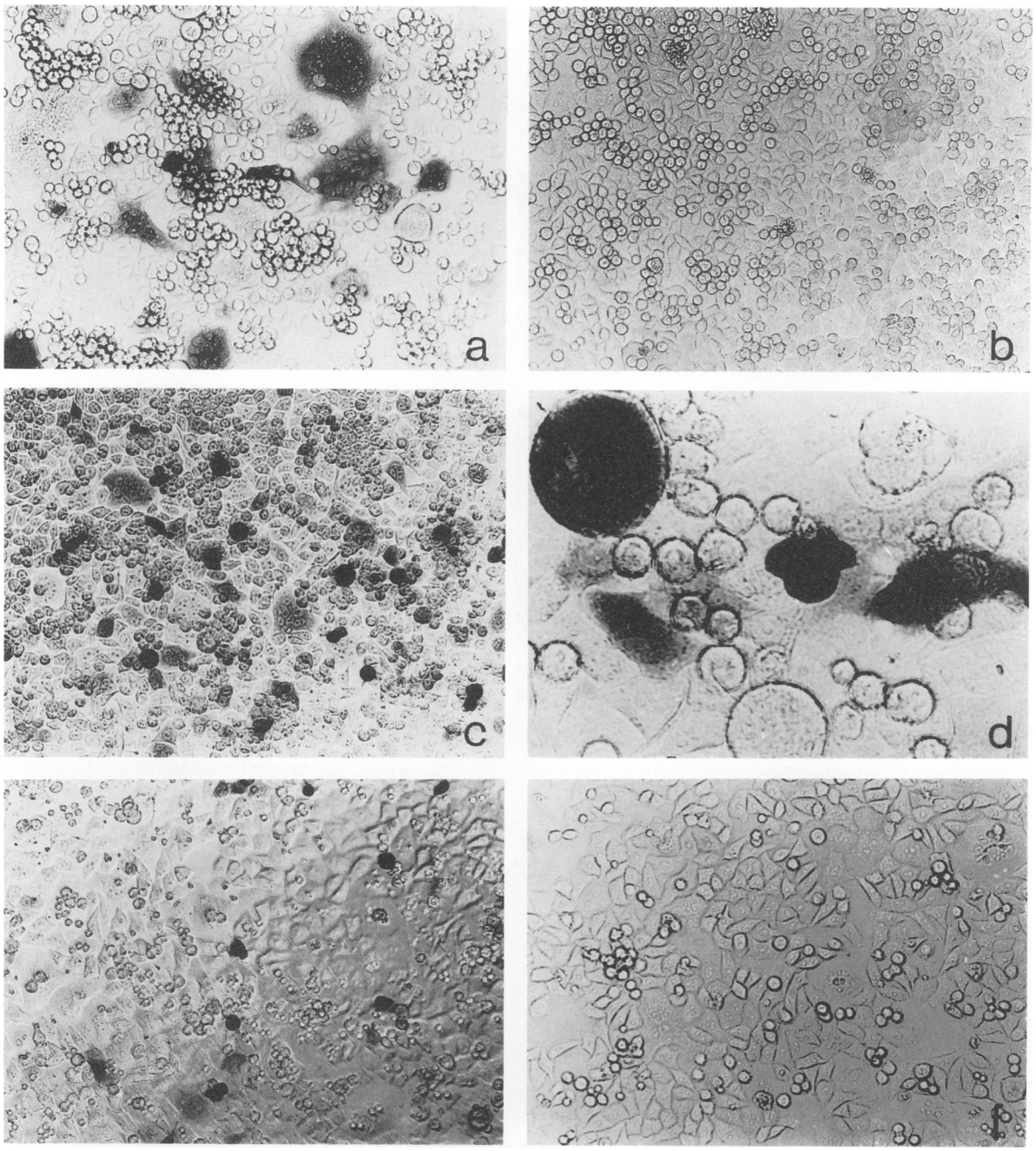

FIG. 2. *tat* transactivation of an HIV-1 LTR-*lacZ* reporter gene after cell-cell fusion. HeLa-LTRlacZ cells (a and b) or the CD4-positive derivative (c to f) were cocultured with HeLa-tat (a and b), HIV envelope-expressing H9/IIIB (c and d) or 8E5 (e), or envelope-negative Jurkat-tat (f) cells. In panel a, PEG fusion was performed on a subconfluent coculture as described in Materials and Methods, and cells were left overnight to be fixed and stained together with the non-PEG-treated control (b). In all other cases, subconfluent monolayers of HeLa-CD4-LTRlacZ cells were cocultured overnight with *tat*-expressing effector cells and fixed. The X-Gal incubations were for 2 h. Plates were photographed under ×250 magnification except for panel d (×1,000).

their formation; effector cells were added 2 h after PEG treatment and left in contact overnight (14 to 18 h). The type of assay that we have used did not allow us to perform a precise kinetic analysis of complementation or to study the effect of protein synthesis inhibitors. However, we observed envelope/CD4-mediated fusion when effector cells were put in contact with human/murine heterokaryons 24 h after their formation (not shown), which indicates that complementation is not a transient membrane phenomenon.

These experiments showed that CD4 is required for heterokaryons to undergo envelope-mediated fusion with effector cells but did not rule out an artifact of the PEG treatment. To perform a negative control in the absence of human cells, we designed the SC6 murine target cells by introducing the

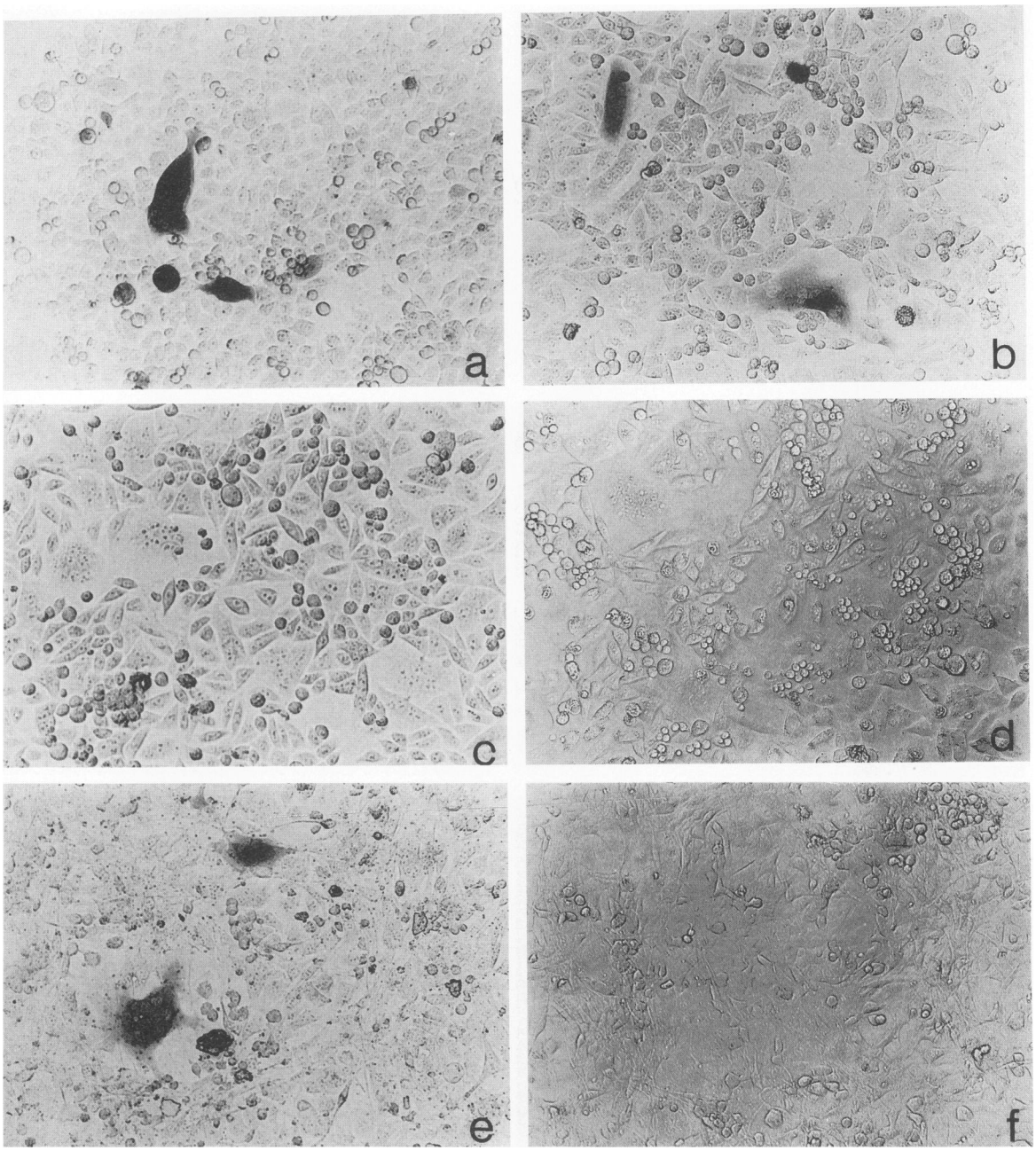

FIG. 3. Detection of membrane fusion between CD4-positive heterokaryons and HIV envelope-expressing cells. Subconfluent cocultures of HeLa-LTRlacZ and HeLa-CD4 (a, b, and d), HeLa (c), or 3T3-CD4 (e and f) cells were treated with PEG as described in Materials and Methods. *tat*-expressing HIV-1 envelope-positive H9/IIIB (a, c, and e) or 8E5 (b) or envelope-negative Jurkat-tat (d and f) cells were added 2 h after PEG fusion and cocultured overnight. X-Gal incubations were for 2 h. Plates were photographed under ×250 magnification.

LTR-*lacZ* reporter in 3T3-CD4 cells. The SC6 cells used in this series of experiments express the same level of CD4 as do the parental 3T3-CD4 cells (Fig. 1a), and *lacZ* expression is induced by PEG fusion with HeLa cells expressing *tat* (Fig. 4a). The efficiency of *tat* transactivation is extremely low in murine cells (4, 11) but seemed comparable in human cells and human/murine heterokaryons, as shown by PEG fusion of HeLa-tat cells with HeLa-LTRlacZ (Fig. 2a) and 3T3-LTRlacZ (Fig. 4a) cells. This point had already been established by PEG fusion experiments between a human LTR-CAT cell line and either murine or human cells expressing *tat* (15). We did not observe evidence for CD4/HIV envelope-mediated fusion after PEG treatment of cocultures of 3T3-CD4-LTRlacZ (SC6) cells and either murine 3T3 or simian COS7 cells. Blue-stained foci were readily detected in parallel experiments in which the murine SC6 cells were cocultured to form heterokaryons with human HeLa cells (Fig. 4b; Table 2). This series of experiments confirmed that human HeLa cells could complement murine 3T3 cells for CD4/envelope-mediated fusion. A few weakly blue cells

TABLE 1. Heterokaryons between HeLa-LTRlacZ (Z24) cells and human or murine cells

No. of blue-stained foci[a] with fusion partner:				Effector cells	HIV envelope
HeLa	HeLa-CD4	3T3	3T3-CD4		
0	620 ± 80	0	39 ± 7	8E5	+
0	954 ± 203	0	68 ± 6	H9/IIIB	+
ND	0	ND	0	Jurkat-tat	−

[a] Number of foci per 35-mm plate, after a 2-h X-Gal incubation, indicating fusion events with *tat*-expressing effector cells. Each number is the average of four independent experiments. ND, not done.

were occasionally observed, but this background was clearly independent of PEG treatment or the presence of *tat*-expressing effector cells (Table 2).

The negative results obtained with the 3T3/COS7 heterokaryons further ruled out an artifactual role of the PEG treatment. Indeed, the efficiency of *tat* transactivation is comparable in COS7 and HeLa cells (4, 11), and the sensitivity of the assay should be the same in murine/simian and murine/human heterokaryons. COS7 cells are derived from the African green monkey cell line CV-1, previously found to be resistant to CD4/gp120-induced membrane fusion (3). COS7 cells were therefore not expected to complement 3T3-CD4 cells for HIV envelope-mediated fusion. To confirm this point and rule out a dominant-negative effect, we generated a *tat*-inducible COS7-LTRlacZ clone, Z25 (Fig. 4d). COS7-Z25/3T3-CD4 heterokaryons could not engage fusion with H9/IIIB, while COS7-Z25/HeLa-CD4 heterokaryons could (Fig. 4e and f). In the context of a heterokaryon with human HeLa cells, neither COS7 nor 3T3 cells appeared to express a dominant-acting inhibitor able to block membrane fusion induced by the HIV envelope-CD4 interaction.

DISCUSSION

We have shown that the inability of murine 3T3-CD4 cells to undergo HIV envelope-mediated membrane fusion can be complemented in human/murine heterokaryons. These events were detected by a sensitive assay based on *tat* transactivation of a reporter *lacZ* gene by cell-cell fusion. In a large series of experiments, this complementation was a constant phenomenon. However, we always observed fewer fusion events with HIV-infected cells for interspecific human/murine or human/simian heterokaryons than for intraspecific CD4-positive human heterokaryons (Tables 1 and 2). We must remain cautious with such comparisons, since different cell lines may not respond in the same way to PEG treatment, in terms of both efficiency of phenotypic mixing and sensitivity to the toxic effects. Different explanations could account for the higher number of blue-stained human/human heterokaryons.

The assay could be less sensitive in the case of human/murine heterokaryons because *tat* transactivation of the HIV LTR is inefficient in murine cells (4, 11). However, human/murine heterokaryons seem to provide a relatively favorable environment for *tat* transactivation. Also, *tat* efficiency is equivalent in COS7 and HeLa cells, and the assay should have the same sensitivity in human/simian and human/human heterokaryons. It can be envisioned that the effect of a fusion inhibitor expressed in murine or simian cells is partially relieved in the context of a human/murine heterokaryons. The simplest explanation is that in human/human heterokaryons in which one of the fusion partners is a HeLa-CD4, the membrane is immediately able to engage fusion with the HIV envelope-expressing cells. In human/murine or human/simian heterokaryons, diffusion of diluted human factors probably is needed to allow fusion with effector cells. The time needed for the complementation process could explain the lesser efficiency of the assay.

Human cellular factors other than CD4 seem necessary for the HIV envelope to exert its fusogenic activity after receptor binding. The identification of these factors could allow novel antiviral approaches, and in this respect, murine or other CD4-positive cells resistant to fusion represent a valuable material. Candidate molecules have already been proposed. The LFA-1 integrin was implicated in the HIV-induced formation of syncytia (21, 42), but it does not directly control the envelope-mediated fusion process since LFA-1-negative cell lines, such as HeLa-CD4, can readily be infected by HIV (3). Some protease inhibitors were shown to reduce HIV infectivity, and it was proposed by analogy with other enveloped viruses that the fusogenic activity of the HIV envelope was activated by proteolytic cleavage (19). More recently, cleavage of the V3 domain of recombinant gp120 was observed in the hamster cell line CHO (9). However, the same CHO cells expressing CD4 could not be infected by HIV (7). If V3 cleavage has a role in the HIV life cycle, it does not seem sufficient to reveal the fusogenic activity of the envelope.

The complementation of murine cells for CD4/envelope-mediated fusion in the context of human/murine heterokaryons is in apparent contradiction with previously published studies on human-murine somatic hybrids. Tersmette et al. (40) have tested four hybrids made between a human CD4 cell line and a murine cell line and found them to be resistant to HIV-1 infection. In their study, one hybrid carried all human chromosomes but was tested only by direct infection. For the other hybrids, infection with VSV (HIV) pseudotypes or a syncytium formation assay was used. These techniques, which are independent of HIV replication and certainly more sensitive in the case of rodent cells, still gave negative results. However, chromosomes 15, 18, 19, and 20 were not present in any of the three hybrids tested. Also, if several chromosomes were necessary for complementation, their combination was unlikely to be present in such a small set of hybrids.

Ashorn et al. (3) have expressed human CD4 in a larger and previously characterized collection of rodent-human somatic hybrids. No CD4-positive hybrid was found able to form syncytia with cells expressing the HIV envelope precursor, gp160. The assay relied on the detection of cell fusion by direct light microscopy, and syncytia had to form within a few hours of coculture, before cells were killed by the vaccinia virus used to express CD4 and gp160. Under these conditions, the assay could have been not sensitive enough to detect complementation of rodent cells by human factors. Indeed, we observed in our assay that HIV-infected cells fused much less efficiently with human/murine heterokaryons than with human CD4 cells or even human/human heterokaryons.

Besides the issue of assay sensitivity, the major problem with experiments performed with interspecific hybrids is the possible loss, mutation, or inactivation of the human genetic material thought to be present after karyotype analysis. For example, a large study of rodent-human hybrids has yielded good evidence that human chromosome 17 carries the gene encoding the human T-cell leukemia virus (HTLV) receptor (38). Nonetheless, two hybrids could not be infected by VSV

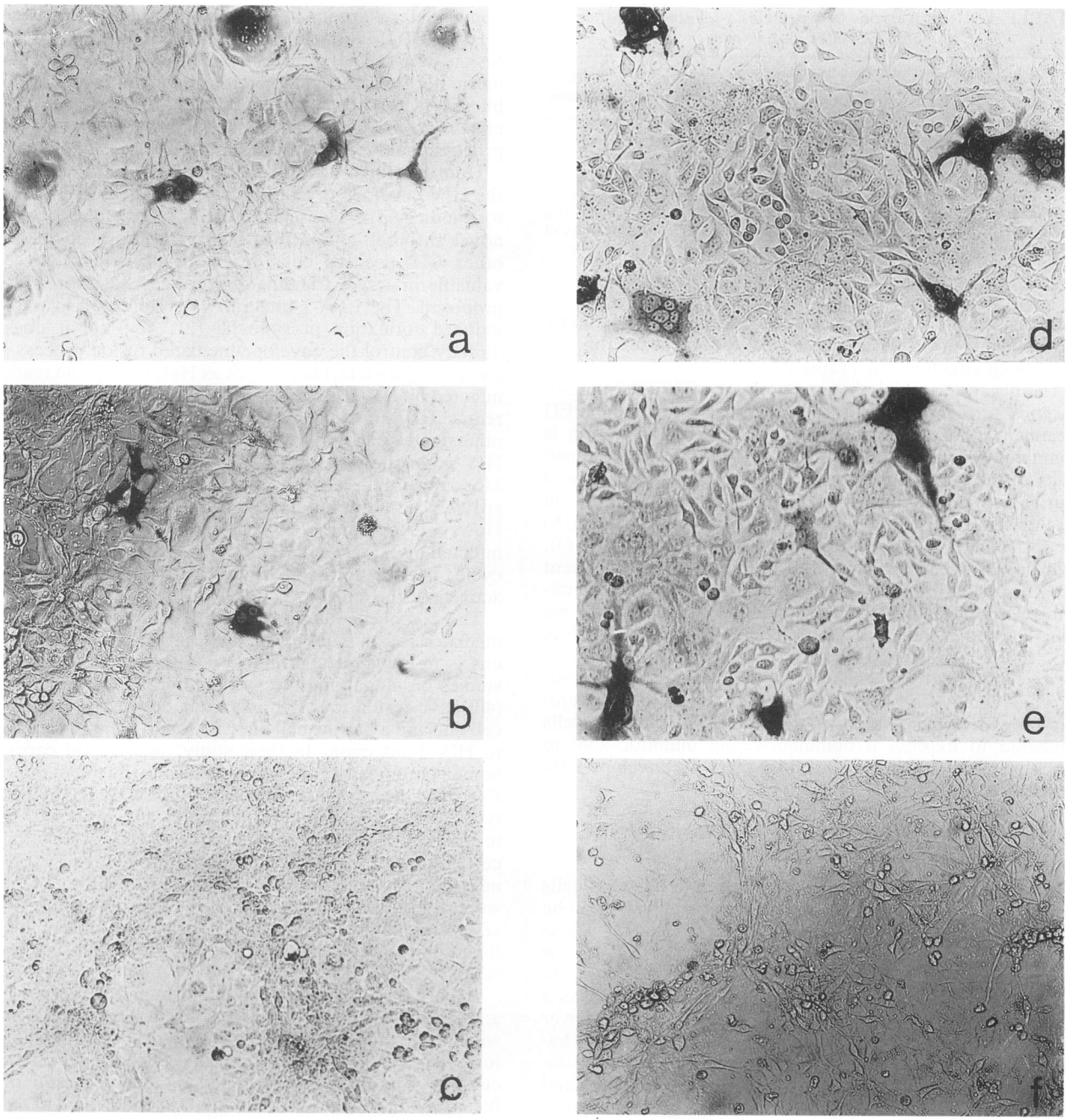

FIG. 4. Detection of membrane fusion between human/murine or human/simian heterokaryons and HIV envelope-expressing cells. 3T3-CD4-LTRlacZ cells were cocultured with HeLa-tat (a), HeLa (b), or COS7 (c) cells. COS7-LTRlacZ cells were cocultured with HeLa-tat (d), HeLa-CD4 (e), or 3T3-CD4 (f) cells. Subconfluent cocultures were treated with PEG (see Materials and Methods). In panels b, c, e, and f, H9/IIIB effector cells were added 2 h after and cocultured overnight. X-Gal incubations were for 2 h. Plates were photographed under ×250 magnification.

(HTLV) pseudotypes, despite the presence of chromosome 17, evidenced by karyotype analysis and expression of specific surface markers (38). In the case of HIV entry, we think that too small a number of rodent-human hybrids has been studied to allow definitive conclusions to be drawn. Our histochemical assay is rapid and sensitive, and it could allow faster and more reliable screening of collections of somatic cell hybrids; they would not have to be manipulated for CD4 expression, but only PEG fused to 3T3-CD4-LTRlacZ cells. In fact, hybrids could be tested before the tedious process of establishing them as genetically stable cell lines, and only those able to complement murine CD4 cells would be recloned and analyzed for their human chromosomal content.

Heterokaryons made between murine 3T3-CD4 and simian COS7 cells could not fuse to HIV envelope-expressing cells. Since COS7 and 3T3 cells did not express a dominant-acting inhibitor of fusion (in the context of heterokaryons

TABLE 2. Heterokaryons between 3T3-CD4-LTRlacZ or COS7-LTRlacZ and human, murine, or simian cells

LTR-*lacZ* cells	No. of blue-stained foci[a] with fusion partner:					Effector cells	HIV envelope
	HeLa	HeLa-CD4	3T3	3T3-CD4	COS7		
3T3-CD4 (SC6)	ND	ND	ND	ND	ND	8E5	+
	46 ± 4	ND	1 ± 1	ND	2 ± 1	H9/IIIB	+
	1 ± 1	ND	2 ± 1	ND	1 ± 1	Jurkat-tat	−
COS7 (Z25)	0	19 ± 1	0	0	ND	8E5	+
	0	39 ± 5	0	0	ND	H9/IIIB	+
	ND	0	ND	0	ND	Jurkat-tat	−

[a] Number of foci per 35-mm plate, after a 2-h X-Gal incubation, indicating fusion events with *tat*-expressing effector cells. Each number is the average of four independent experiments. ND, not done.

with HeLa cells), we conclude that these simian and rodent cell lines lack some common human factor(s) necessary for the HIV envelope fusogenic activity. However, different genetic defects could cause the same fusion-resistant phenotype, and it will be interesting to perform similar complementation experiments with other cell lines resistant to HIV entry, in particular with the two human cell lines, U87 and SCL1 (6).

The histochemical assay that we have developed detects CD4/HIV envelope membrane fusion events not seen by classical syncytium formation assays and can be performed in the absence of viral replication. It could be standardized and used to screen drugs or antibodies for their ability to interfere with the gp120-CD4 interaction and the subsequent membrane fusion event.

ACKNOWLEDGMENTS

We thank Olivier Danos for support of this work and comments on the manuscript, and we thank Robin Weiss for the gift of HeLa-CD4 cells. We are indebted to René Olivier for assistance with the FACS analysis and to Olivier Bayonne for photographs.

This work was supported in part by the Agence Nationale de Recherches sur le SIDA.

REFERENCES

1. **Adra, C. N., P. H. Boer, and M. W. McBurney.** 1987. Cloning and expression of the mouse pgk-1 gene and the nucleotide sequence of its promoter. Gene **60**:65–74.
2. **An, G., K. Hidaka, and L. Siminovitch.** 1982. Expression of bacterial β-galactosidase in animal cells. Mol. Cell. Biol. **2**:1628–1632.
3. **Ashorn, P. A., E. A. Berger, and B. Moss.** 1990. Human immunodeficiency virus envelope glycoprotein CD4-mediated fusion of non-primate cells with human cells. J. Virol. **64**:2149–2156.
4. **Barry, P. A., E. Pratt-Lowe, R. E. Unger, and P. A. Luciw.** 1991. Cellular factors regulate transactivation of human immunodeficiency virus type 1. J. Virol. **65**:1392–1399.
5. **Charneau, P., M. Alizon, and F. Clavel.** 1992. A second origin of DNA plus-strand synthesis is required for optimal human immunodeficiency virus replication. J. Virol. **66**:2814–2820.
6. **Chesebro, B., R. Buller, J. Portis, and K. Wehrly.** 1990. Failure of human immunodeficiency virus entry and infection in CD4-positive human brain and skin cells. J. Virol. **64**:215–221.
7. **Clapham, P. R., D. Blanc, and R. A. Weiss.** 1991. Specific cell surface requirements for the infection of CD4-positive cells by human immunodeficiency virus types 1 and 2 and by simian immunodeficiency virus. Virology **181**:703–715.
8. **Clavel, F., and J. M. Orenstein.** 1990. A mutant of human immunodeficiency virus with reduced RNA packaging and abnormal particle morphology. J. Virol. **64**:5230–5234.
9. **Clements, G. J., M. J. Price-Jones, P. E. Stephens, C. Sutton, T. F. Schulz, P. R. Clapham, J. A. McKeating, M. O. McClure, S. Thomson, M. Marsh, J. Kay, R. A. Weiss, and J. P. Moore.** 1991. The V3 loop of the HIV-1 and HIV-2 surface glycoproteins contain proteolytic cleavage sites: a possible function in viral fusion? AIDS Res. Hum. Retroviruses **7**:3–16.
10. **Coffin, J. M.** 1991. Retroviridae and their replication, p. 645–708. *In* B. N. Fields (ed.), Fundamental virology, 2nd ed. Raven Press, New York.
11. **Cullen, B. R.** 1986. Trans-activation of human immunodeficiency virus occurs via a bimodal mechanism. Cell **46**:973–982.
12. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312**:763–766.
13. **Danos, O., and R. Mulligan.** 1988. Safe and efficient generation of recombinant retroviruses with ecotropic and amphotropic host ranges. Proc. Natl. Acad. Sci. USA **85**:6460–6464.
14. **Dimitrov, D. S., H. Golding, and R. Blumenthal.** 1991. Initial stages of HIV-1 envelope glycoprotein-mediated cell fusion monitored by a new assay based on redistribution of fluorescent dyes. AIDS Res. Hum. Retroviruses **7**:799–805.
15. **Drysdale, C. M., and G. N. Pavlakis.** 1991. Rapid activation and subsequent down-regulation of the human immunodeficiency virus type 1 promoter in the presence of *tat*: possible mechanisms contributing to latency. J. Virol. **65**:3044–3051.
16. **Folks, T. M., D. Powell, M. Lightfoote, S. Koenig, A. S. Fauci, S. Benn, A. Rabson, D. Daugherty, H. E. Gendelmann, M. D. Hoggan, S. Venkatesan, and M. A. Martin.** 1986. Biological and biochemical characterization of a cloned Leu-3A(−) cell line surviving infection with the AIDS retrovirus. J. Exp. Med. **164**:280–290.
17. **Gritz, L., and J. Davies.** 1983. Plasmid-encoded hygromycin-B resistance: the sequence of hygromycin-B-phosphotransferase and its expression in *E. coli* and *S. cerevisiae*. Gene **25**:179–188.
18. **Hartman, S. C., and R. C. Mulligan.** 1988. Two dominant-acting selection markers for gene transfer studies in mammalian cells. Proc. Natl. Acad. Sci. USA **85**:8047–8051.
19. **Hattori, T., A. Koito, K. Takatsuki, H. Kido, and N. Katunuma.** 1989. Involvement of tryptase-related protease(s) in human immunodeficiency type 1 infection. FEBS Lett. **248**:48–52.
20. **Helland, D. E., J. L. Welles, A. Caputo, and W. A. Haseltine.** 1991. Transcellular transactivation by the human immunodeficiency virus type 1 *tat* protein. J. Virol. **65**:4547–4549.
21. **Hildreth, J. E. K., and R. J. Orentas.** 1989. Involvement of a leukocyte adhesion receptor (LFA-1) in HIV-induced syncytium formation. Science **244**:1075–1079.
22. **Jeang, K. T., P. R. Shank, A. B. Rabson, and A. Kumar.** 1988. Synthesis of functional human immunodeficiency virus *tat* protein in baculovirus as determined by a cell-cell fusion assay. J. Virol. **62**:3874–3878.
23. **Kennet, R. H.** 1979. Cell fusion, p. 345–359. *In* W. B. Jakoby and I. H. Pastan (ed.), Cell culture. Academic Press, San Diego, Calif.
24. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guétard, T. Hercend, J.-C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature (London) **312**:767–768.
25. **Lifson, J. D., M. B. Feinberg, J. R. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moss, F. Wong-Staal, K. S. Steimer, and E. G. Engleman.** 1986. Induction of CD4-depen-

dent cell fusion by the HTLVIII/LAV envelope glycoprotein. Nature (London) **323:**725–728.

26. **Linsley, P. S., J. A. Ledbetter, E. Kinney-Thomas, and S. L. Hu.** 1988. Effects of anti-gp120 monoclonal antibodies on CD4 receptor binding by the *env* protein of human immunodeficiency virus type 1. J. Virol. **62:**3695–3702.
27. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47:**333–348.
28. **Maddon, P. J., J. S. McDougal, P. R. Clapham, A. G. Dalgleish, S. Jamal, R. A. Weiss, and R. Axel.** 1988. HIV infection does not require endocytosis of its receptor, CD4. Cell **54:**865–874.
29. **McClure, M. O., M. Marsh, and R. A. Weiss.** 1988. Human immunodeficiency virus infection of CD4-bearing cells occurs by a pH-independent mechanism. EMBO J. **7:**513–518.
30. **McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Mawle, and J. K. A. Nicholson.** 1986. Binding of HTLVIII/LAV to $T4^+$ T cells by a complex of the 110K viral protein and the T4 molecule. Science **231:**382–385.
31. **Peden, K., M. Emerman, and L. Montagnier.** 1991. Changes in growth properties on passage in tissue culture of viruses derived from infectious molecular clones of HIV-1_{LAI}, HIV-1_{MAL}, and HIV-1_{ELI}. Virology **185:**661–672.
32. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224:**497–500.
33. **Ringertz, N. R., and R. E. Savage.** 1976. Cell hybrids. Academic Press, London.
34. **Rocancourt, D., C. Bonnerot, H. Jouin, M. Emerman, and J. F. Nicolas.** 1990. Activation of a beta-galactosidase recombinant provirus: application to titration of human immunodeficiency virus (HIV) and HIV-infected cells. J. Virol. **64:**2660–2668.
35. **Rosen, C. A., J. G. Sodroski, K. Campbell, and W. A. Haseltine.** 1986. Construction of recombinant murine retroviruses that express the human T-cell leukemia virus type II and human T-cell lymphotropic virus type III *trans* activator genes. J. Virol. **57:**379–384.
36. **Simonsen, C. C., and A. D. Levinson.** 1983. Isolation and expression of an altered mouse dihydrofolate reductase cDNA. Proc. Natl. Acad. Sci. USA **80:**2495–2499.
37. **Sodroski, J., W. C. Goh, C. Rosen, K. Campbell, and W. Haseltine.** 1986. Role of the HTLVIII/LAV envelope in syncytium formation and cytopathogenicity. Nature (London) **322:** 470–474.
38. **Sommerfelt, M. A., B. P. Williams, P. R. Clapham, E. Solomon, P. N. Goodfellow, and R. A. Weiss.** 1988. Human T-cell leukemia viruses use a receptor determined by human chromosome 17. Science **242:**1557–1559.
39. **Stein, B. S., S. D. Gowda, J. D. Lifson, R. C. Penhallow, K. G. Bensch, and E. G. Engleman.** 1987. pH-independent HIV entry into CD4-positive T cells via envelope fusion to the plasma membrane. Cell **49:**659–668.
40. **Tersmette, M., J. J. M. Van Dongen, P. R. Clapham, R. E. Y. DeGoede, I. L. M. Wolvers-Terrero, A. Geurts van Kessel, J. G. Huisman, R. A. Weiss, and F. Miedema.** 1989. Human immunodeficiency virus infection studied in CD4-expressing human-murine T-cell hybrids. Virology **168:**267–273.
41. **Trono, D., M. B. Feinberg, and D. Baltimore.** 1989. HIV-1 gag mutants can dominantly interfere with the replication of the wild-type virus. Cell **59:**113–120.
42. **Valentin, A., K. Lundin, M. Patarroyo, and B. Asjoe.** 1990. The leukocyte adhesion glycoprotein CD18 participates in HIV-1 induced syncytia formation in monocytoid and T-cells. J. Immunol. **144:**934–937.
43. **Wain-Hobson, S., P. Sonigo, O. Danos, S. Cole, and M. Alizon.** 1985. Nucleotide sequence of the AIDS virus, LAV. Cell **40:**9–17.