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Is CD4 Sufficient for HIV Entry? Cell Surface Molecules Involved in HIV Infection

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Is CD4 sufficient for HIV entry? Cell surface molecules involved in HIV infection

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SUMMARY

HIV-1, HIV-2 and SIV each bind to CD4 as the first step in virus entry. However, alternative receptors may also be used. HIV-1 binds to glycolipids with terminal galactosylceramide residues on neural cells; opsonized virus binds to Fc receptors; HIV-2 can infect certain CD4-negative cells. Further receptors may also play a role in CD4-mediated infection, including cell adhesion molecules and possibly cell surface proteinases. After binding to CD4, immunodeficiency viruses require secondary molecules to effect fusion between the virus envelope and the cell membrane; these accessory requirements differ between HIV-1, HIV-2 and SIV.

1. INTRODUCTION

Cell surface glycoproteins, important in varied immunological processes are known to act as receptors for several viruses. The major group of non-enveloped rhinoviruses utilize the adhesion molecule ICAM-1 (Greve *et al.* 1989; Staunton *et al.* 1989), whereas the Herpes virus and Epstein-Barr virus (EBV) infect B-cells after binding to complement receptor 2 (CR2) (Weis *et al.* 1988), and the primate lentiviruses, human and simian immunodeficiency viruses (HIV and SIV) use the CD4 antigen (Dalgleish *et al.* 1984; Klatzman *et al.* 1984; Sattentau *et al.* 1988).

Binding of HIV to CD4 results in conformational changes in the virion glycoproteins (Sattentau & Moore 1991) and in CD4 (Walker *et al.* 1992). Structural alterations in virion gp120 and gp41 are probably essential to induce a short hydrophobic sequence (fusion domain) at the N-terminal of gp41 to insert into the lipid membrane at the cell surface. This insertion initiates virion and cell membrane fusion. Thus HIV is doubly dependent on CD4 for cell entry; first to attach to the cell surface and then for events leading to fusion.

This article reviews whether cell surface molecules other than CD4 are required for or can influence HIV entry into cells.

2. IS CD4 SUFFICIENT FOR ENTRY?

Evidence presented by Maddon *et al.* (1986) seven years ago suggested there were additional requirements to CD4 for HIV entry. Expression of CD4 on human but not mouse cells conferred susceptibility to HIV entry. This result suggested that there might be a factor or second component to the receptor, expressed on human cells but absent on mouse cells. If this is the case, then hybrid or heterokaryon cells made from mouse and human cells should permit HIV entry if positive for human CD4. Studies with such mouse–human hybrids initially gave ambiguous results. Weiner *et al.* (1991) were able to infect several hybrids and suggested that additional factors needed for HIV entry mapped to chromosome 13, 14, 20 or 21. In contrast, Tersmette *et al.* (1989) were unable to detect HIV-1 entry in several hybrids, one of which appeared to contain all human chromosomes. As well as spontaneously losing human chromosomes, its possible that mouse–human hybrids stop expressing human genes. Thus Weiner's positive results may be more meaningful than Tersmette's negative data. Short-term cultures of animal-human heterokaryons prepared by Drajic *et al.* (1992) and Broder *et al.* (1993) have provided more convincing evidence. Drajic used virons bearing HIV envelope glycoproteins and containing an MLV based genome constructed to include an expressible β-galactosidase gene. Entry was detected by adding a colour-forming galactosidase substrate to fixed virus treated cells. HIV-resistant CD4+ mouse 3T3 cells and CD4- human HeLa cells were fused by polyethylene glycol and resulting heterokaryons became sensitive to HIV-1 entry. Similar evidence has also been provided by Hoxie *et al.* (1988) who described a hybridoma cell line (CEMX174) sensitive to infection by certain SIV strains, but prepared from resistant parents; CD4+ CEM T− and CD4− B721.174 B-cells. Thus several reports present evidence that an additional factor can be provided to render resistant CD4-expressing cells permissive for HIV and SIV entry.

3. A SERINE PROTEASE IS A CANDIDATE SECOND COMPONENT

A serine protease, tryptase TL_2 has been implicated as a candidate second component. Tryptase TL_2 is a membrane bound serine esterase (Hattori *et al.* 1989)

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Table 1. *Susceptibility of CD4 expressing mammalian cell lines to HIV and SIV entry*

CD4+ cell lines		entry into CD4-positive cell lines by HIV-1	HIV-2	SIV
non-primate				
mouse	3T3	–	–	–
rat	XC	–	–	–
	HSN	–	–	–
rabbit	SIRC	–	+	+
cat	CCC	–	+	+
mink	Mv-1-lu	–	+	+
primate				
rhesus	LLCMK2	–	+	+
	FRhK	–	+	+
African green monkey	BGM	–	+	+
human	U87	–	+	+
	RD	+	+	–
	HeLa	+	+	–

which has recently been purified from human T cells and shown to specifically interact with the V3 loop of HIV-1 gp120 (Kido *et al.* 1991). A conserved tryptic cleavage site on V3 can be shown to be cut by proteases *in vitro* (Clements *et al.* 1991; Schulz *et al.* 1993), and is thought to be the target for tryptase TL_2. Convincing proof that tryptase TL_2 is required for HIV-1 entry awaits cloning and construction of vectors for its expression. It will be interesting to ascertain whether transfection and expression of tryptase TL_2 will render resistant CD4+ cell types (e.g. U87/CD4, see below) sensitive to HIV-1 entry.

4. DIFFERENT REQUIREMENTS FOR DIFFERENT VIRUS TYPES

We expressed human CD4 on a panel of CD4-negative human and non-human cell types. Although all cells expressing CD4 bound recombinant gp120, only CD4+ human cells were susceptible to HIV-1 infection (table 1) (Clapham *et al.* 1991). Each cell type was tested for sensitivity to infection by vesicular stomatitis virus (VSV) pseudotypes of HIV. VSV pseudotypes are mixed virions containing the nucleocapsid of VSV but bearing the envelope glycoproteins of HIV. They require a functional HIV receptor for entry, however the highly cytopathic VSV genome directs replication and a lytic plaque forms during overnight incubation. VSV pseudotypes thus provide a plaque assay specific for HIV entry. Only CD4+ human cells that permitted HIV-1 replication, were sensitive to VSV pseudotype infection. This result confirmed that there was a cell surface restraint to HIV-1 entry on CD4+ non-human cell types. In contrast, many CD4+ non-human cells were sensitive to HIV-2 and SIV entry. Cells derived from rhesus monkey, african green monkey, cat, mink and rabbits (but not rat or mouse) supported HIV-2 and SIV induced fusion and at least some replication. These results indicate that HIV-2 and SIV have different requirements for entry compared with HIV-1 (table 1)

Table 2. *HIV and SIV induced fusion of CD4-positive human cell lines*

virus	strain	fusion of CD4+ cell line U87	RD	MOLT4
HIV-1	RF	–	+ + +	+ + +
	IIIB	–	+ + +	+ + +
	NY5	–	+ + +	+ + +
	SF-2	–	+ + +	+ + +
	CBL-4	–	+ + +	+ + +
	Z39	–	+ + +	+ + +
	Z84	–	+ + +	+ + +
	Z129	–	+ + +	+ + +
	NDK	–	+ + +	+ + +
	U455	–	+ + +	+ + +
HIV-2	$LAV\text{-}2_{ROD}$	+ + +	+ + +	+ + +
	SBL6669	+	+ + +	+ + +
	CBL-20	+	+ + +	+ + +
	CBL-21	+	+ + +	+ + +
	CBL-22	+ + +	+ + +	+ + +
	CBL-23	+ + +	+ + +	+ + +
SIV	MAC	+ + +	–	+ + +
	SM	+ + +	–	+ + +
	AGM	+	–	+ + +

(Clapham *et al.* 1991; A. McKnight, unpublished observations). We observed similar differences on a human glioma cell line, U87. CD4+ U87 cells restricted entry by HIV-1 strains (also reported by Chesebro *et al.* 1990), but were permissive for HIV-2 and SIV fusion (table 2 and figure 1) and replication. Several groups (including ourselves) have observed that SIV strains (SM, MAC and AGM) fail to infect or fuse certain CD4+ human cell lines that are fully permissive to HIV-1 and HIV-2 (Hoxie *et al.* 1988; Koenig *et al.* 1989). Table 2 and figure 1 show that although CD4+ human RD rhabdomyosarcoma cells were sensitive to fusion by all HIV-1 and HIV-2 strains tested they resisted each SIV isolate tested. This lack of cell fusion seems to indicate an entry restraint in our system, however one study using chimeric SIV/HIV-1 viruses showed that sequences in the 5′ half of the genome and not envelope sequences determined the lack of infection by SIV_{AGM} in the human T cell line, MT-4 (Shibata & Adachi 1992).

Although these experiments rely on the lack of entry on CD4+ cells to imply extra requirements, they suggest that HIV-1, HIV-2 and SIV laboratory strains use distinct cell surface molecules during entry of human cells but after binding CD4.

5 IMPORTANCE OF CELL SURFACE EVENTS IN DETERMINING TROPISMS *IN VIVO*

Several reports have mapped determinants of tropism to gp120 envelope sequences of HIV-1. Some reports show that gp120 envelope sequences that include the V3 loop can determine macrophage or T cell tropism (Cann *et al.* 1992; O'Brien *et al.* 1990; Hwang *et al.* 1991; Shioda *et al.* 1991; Westerfelt *et al.* 1991, 1992; Bergeron *et al.* 1992) and Takeuchi *et al.* (1991) demonstrated that a single point mutation in V3 conferred the capacity of a Japanese isolate to infect

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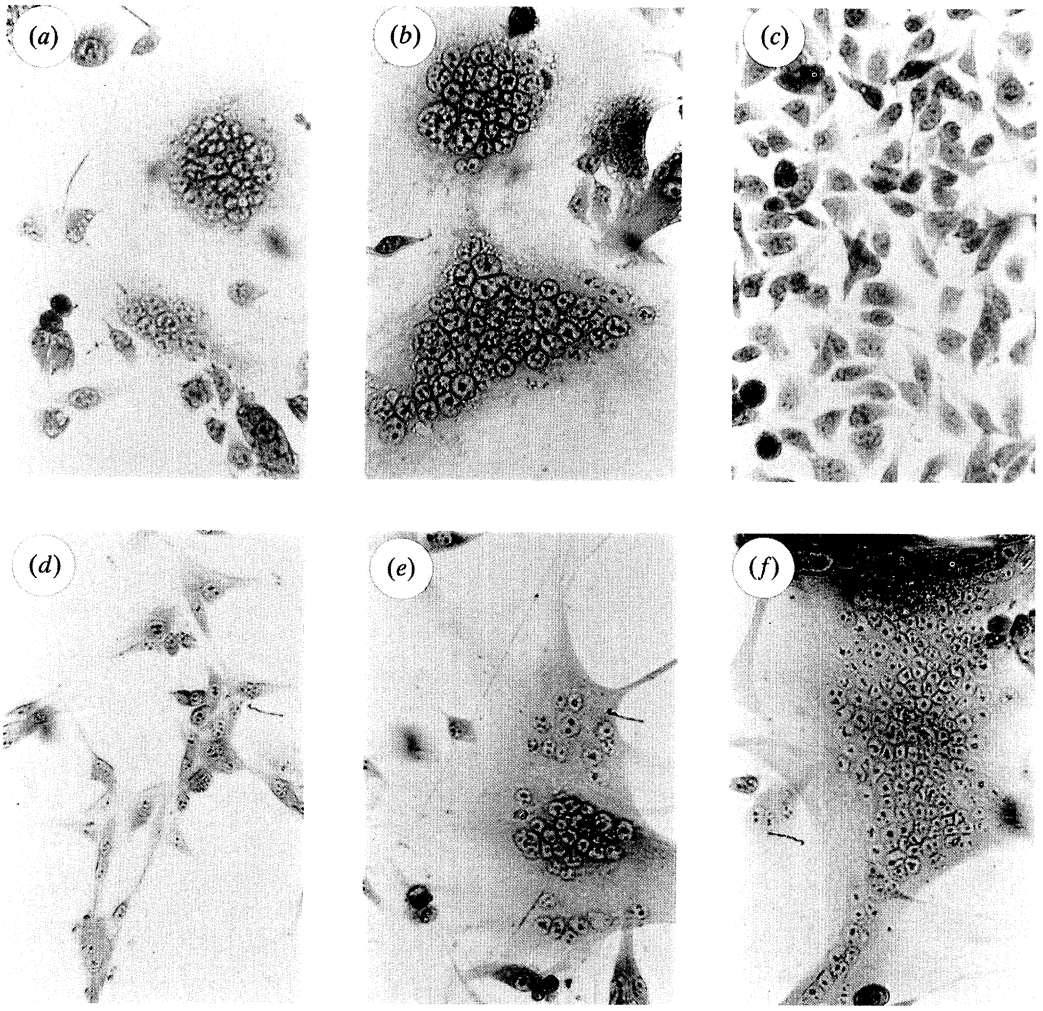

Figure 1. Cell fusion induced by HIV-1/RF (*a*,*d*), HIV-2/LAV-2$_{ROD}$ (*b*,*e*), or SIV$_{MAC}$ (*c*,*f*) producer cell lines co-cultivated overnight with either RD/CD4 (*a*–*c*) or U87/CD4 (*d*–*f*).

CD4+ brain cells. Other studies suggest that envelope sequences other than V3 can affect tropism (Cordonnier *et al.* 1989; Bergeron *et al.* 1992; Westerfelt *et al.* 1992; Boyd *et al.* 1993). For instance, Westerfelt *et al.*(1992) implicated three amino acid changes in V1 or V2 to affect macrophage infection and Boyd *et al.* (1993) showed that a single amino acid change in V1 expanded the T cell tropism of a primary strain to two established CD4+ T cell lines. Since sequences in V1, V2 and V3 are not part of the CD4 binding domain, and assuming they don't influence post fusion events, these findings also suggest that gp120 interacts with specific cell surface molecules other than CD4 to determine tropism.

It is difficult to estimate to what extent cell surface events determine tropism, but it does not seem absolute. Cheng-Meyer *et al.* (1991) reported that *tat* sequences determined the capacity of an HIV-1 strain to replicate in Hut78 cells and Fenyo *et al.* (1988) showed that many primary HIV-1 strains replicated in Jurkat cells if transfected recombinant *tat* was expressed. Recently, Potash *et al.* (1992) found that the capacity of a range of primary isolates to fuse with macrophages did not correlate with infectivity. Polymerase chain reaction (PCR) analysis of reverse transcription suggested an early post-fusion restriction for several isolates. We have also noted that several T cell line tropic viruses that are unable to replicate in primary macrophage cultures, are competent for fusion in syncytium inducing assays (figure 2). PCR analysis of reverse transcripts in this system suggests an early post-fusion restriction in replication (G. Simmons, unpublished results). However, differences in envelope-mediated cell-to-cell fusion and virion-to-cell fusion could explain our results.

6. ALTERNATIVE RECEPTORS FOR HIV?

Few viruses have been described that can use more than one receptor to attach to cells. One example is mouse hepatitis virus (MHV), a coronavirus which attaches to several members of a family of related carcinoembryonic antigens (CEA) (Yokomori & Lai 1992; Dveksler *et al.* 1993), but probably to the same binding site.

Many groups have reported that HIV-1 strains can infect several human cell types that do not express CD4 (reviewed by Clapham 1991). Generally, this type of infection is extremely inefficient, requiring a large dose of input virus to achieve infection of only a small number of cells.

Harouse *et al.* (1991) demonstrated that galactocereboside acts as a receptor for HIV-1 infection of neuronal cell types and Yahi *et al.* (1992) showed the

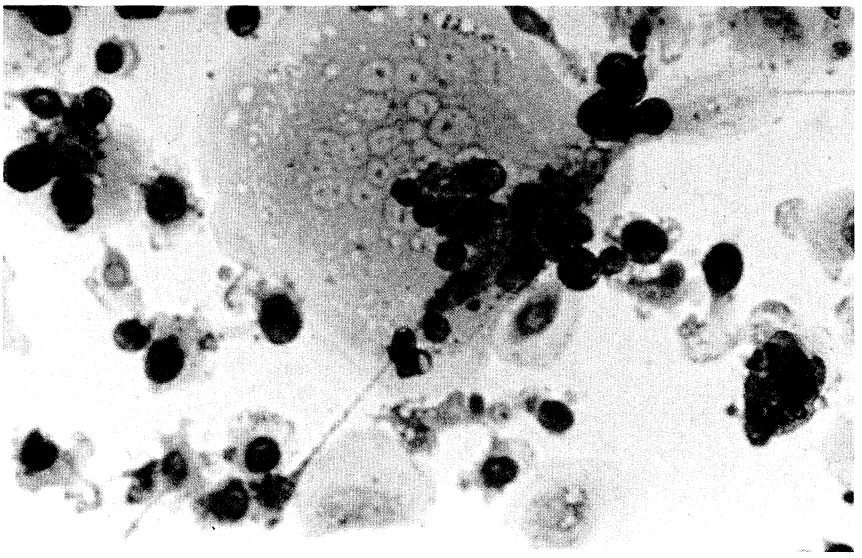

Figure 2. Primary macrophages fused after overnight co-cultivation with H9 cells producing T cell line tropic HIV/RF.

same (or a related) molecule is a receptor for infection of the colon carcinoma cell line HT-29. Curtis *et al.* (1992) were able to clone a gene encoding a membrane-associated C-type lectin that bound to gp120 to high affinity. This molecule however, did not serve as a receptor for infection when expressed on CD4-negative HeLa cells.

The double requirement of CD4 for HIV cell attachment and subsequent conformational alterations leading to fusion events may limit the probability that CD4-independent variants will emerge. Thus, although gp120 can efficiently bind to other cell surface molecules, e.g. to galactocereboside and to Curtis' C-type lectin, it is unlikely that either of these molecules can induce the structural alterations in the virion glycoproteins needed for fusion.

Although it is possible that CD4-independent HIV-1 strains exist *in vivo*, there is little evidence that this type of entry plays a role in pathogenesis. *In situ* hybridization and immunochemistry suggest that infected cells in the peripheral blood, lymph, skin, gut, lung and brain are restricted to cells likely to express CD4. These cells include T lymphocytes, monocytes, macrophages, dendritic and microglial cells (see review by Clapham 1991).

In contrast to HIV-1, we have described HIV-2 strains that infect a restricted number of CD4-negative human cell types (Clapham *et al.* 1992). These HIV-2 isolates were capable of inducing cell to cell fusion in RD rhabdomyosarcoma cells as well as Daudi and Raji B-cell lines. Although only some HIV-2 strains efficiently induced syncytia in RD or Daudi cells, soluble CD4 surprisingly enhanced cell fusion and even induced syncytium-negative viruses to fuse. The cell surface molecules contributing to this process are at present obscure, but are likely to involve at least one alternative receptor. We do not know if HIV-2 variants with an increased capacity to infect cells lacking CD4 exist *in vivo*. Such variants would be expected to infect an expanded range of cells, but few studies have yet been done to examine cell types infected by HIV-2 in the tissues of infected individuals. We are currently investigating whether primary HIV-2 isolates can be made that propagate in appropriate CD4-negative cells.

7. ALTERNATIVE RECEPTORS AND CD4-DEPENDENT ENTRY

It is conceivable that an accessory cell surface molecule required during CD4-dependent infection could also act as a CD4-independent receptor. In this situation an HIV strain would acquire the capacity to bind directly to the accessory molecule, bypassing CD4 entirely. If this was the case for the HIV-2 alternative receptor, then CD4-negative cells that resist HIV-2 entry, might be expected also to resist infection when CD4 is expressed. It has not been possible to test this premise on human cells as cells that lack CD4, permit low efficiency HIV-2 infection. As already discussed however, many non-human cells transfected with and expressing human CD4 are permissive to HIV-2 entry and replication (table 1). These cells must express all accessory cell surface molecules required for HIV-2 entry. The CD4-negative parental counterparts to these cells are completely resistant to all HIV-2 strains tested, indicating (at least for these non-human cells) that accessory molecules do not act as alternative receptors.

8. INVOLVEMENT OF ADHESION MOLECULES IN HIV-1 INFECTION AND FUSION

Several studies have shown that monoclonal antibodies (mAbs) to the β-chain of LFA-1 inhibit HIV-1 syncytia formation (Busso *et al.* 1991; Hildreth *et al.* 1989; Pantaleo *et al.* 1991; Vermot-Desroches *et al.* 1991). Vermot-Desroches *et al.* (1991) showed that inhibition was epitope dependent and that antibody interaction with LFA-1 expressed on the CD4+ target cell was crucial for inhibition. The ligand for LFA-1 in HIV-1 induced syncytia is not clear,

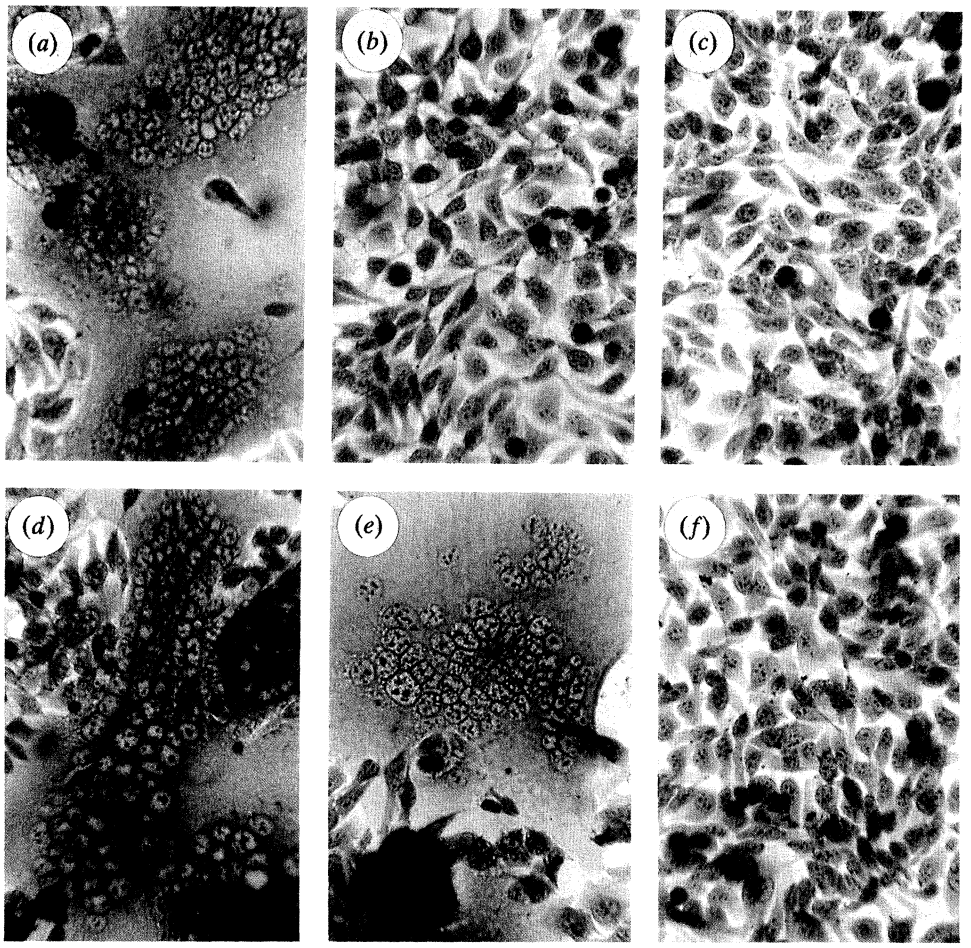

Figure 3. HIV-2 induced fusion of CD4-negative RD cells. H9 cells producing HIV were cocultivated overnight with RD cells in the presence (*d–f*) or absence (*a–c*) of 10 μg ml^{-1} soluble CD4. The LAV-2B variant of HIV-2 induced fusion with or without sCD4 (*a*,*d*). HIV-2/CBL-20 fusion was sCD4 dependent (*b*,*e*). HIV-1 did not fuse (*c*, *f*).

although two groups have reported weak inhibition with ICAM-1 antibodies (Vermot-Desroches *et al.* 1991; Gruber *et al.* 1991). Thus it is likely that LFA-1 directed cell conjugates must first form before efficient HIV-1 induced cell to cell fusion can occur. There is also controversy over whether LFA-1 mAbs inhibit HIV-1 infection by cell free virus. Kalter *et al.* (1991) and Hansen *et al.* (1991) both reported inhibition of cell free HIV-1 infection by LFA-1 mAbs; Kalter *et al.* on macrophages and Hansen *et al.* on T cells. Pantaleo *et al.* (1991), however showed that T-lymphocytes from patients deficient for LFA-1 were efficiently infected by HIV-1 but failed to form syncytia.

9. CONCLUSIONS

Although HIVs and SIVs use CD4 to attach to cells, there are clearly other cell surface molecules that can influence entry into cells. It is likely that at least one molecule additional to CD4 is essential before the virion and cellular membranes can fuse. The precise nature and function of this molecule is at present unknown. Other cell surface molecules can act as alternative receptors, either for attachment only (e.g. Curtis' lectin-like receptor) or for complete entry (e.g. our alternative HIV-2 receptor). The extent of influence that these alternative receptors have on CD4-dependent entry is not known. Receptors for ligands that also bind HIV virions may also affect attachment. Examples include CR2, the receptor for C1q that specifically interacts with a conserved region on gp41 (Ebenbichler *et al.* 1991), as well as Fc receptors that bind antibody–virus conjugates. Both these events have been shown to enhance HIV-1 infectivity.

Thus, an array of cell surface molecules have been described that can influence HIV or SIV entry. Full definition and evaluation of their contribution to natural infection still awaits cloning and identification of molecules implicated in biological assays.

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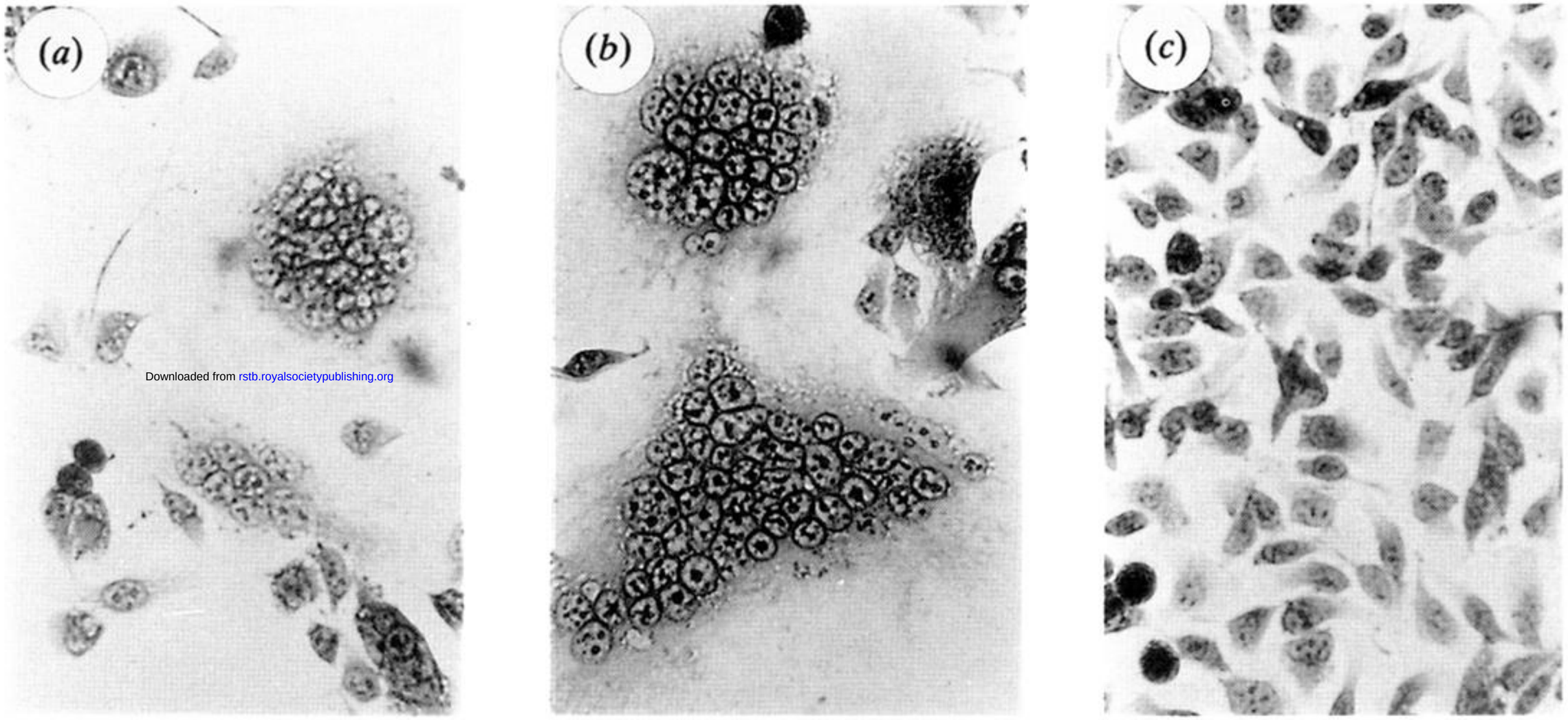
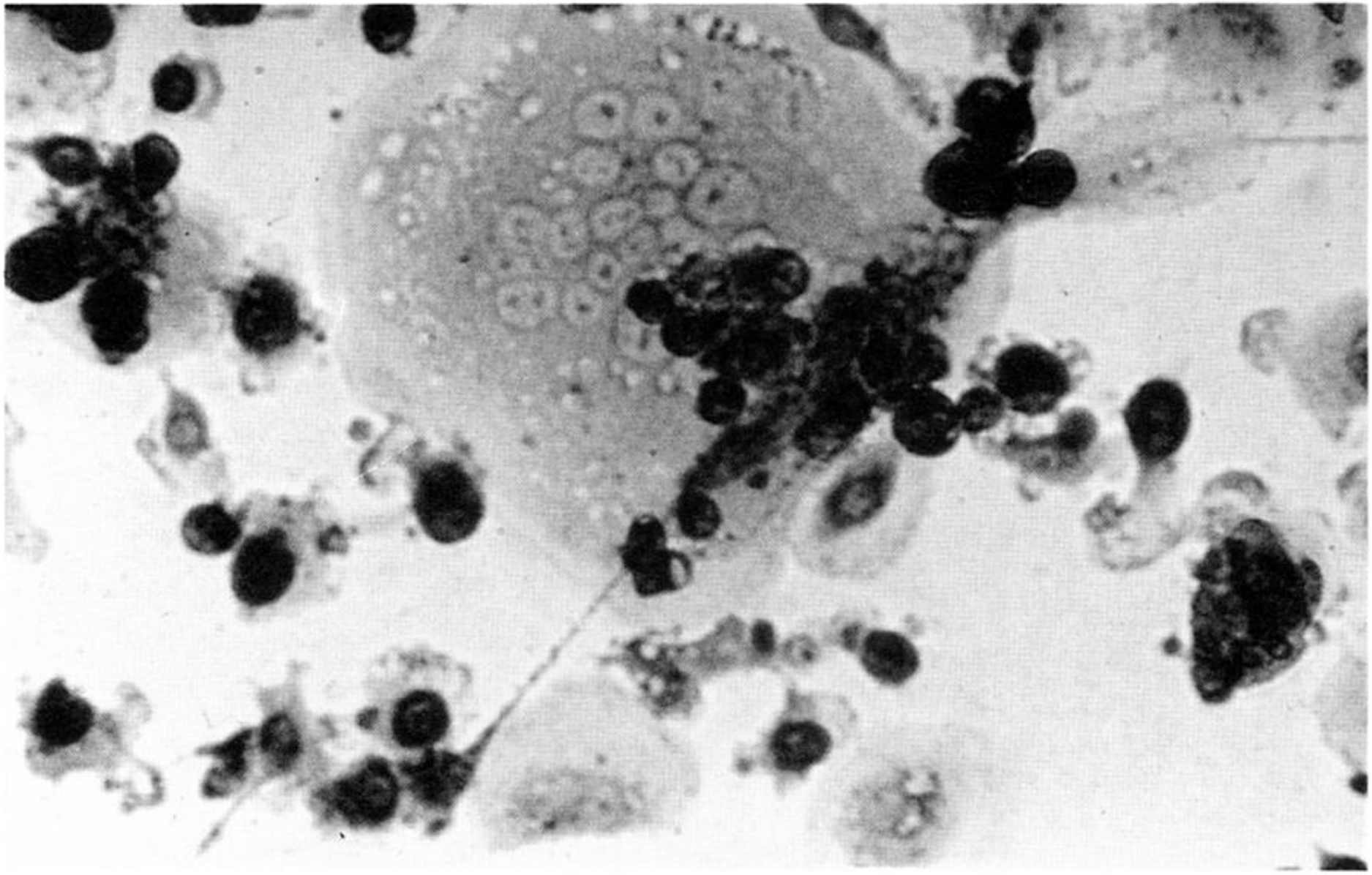
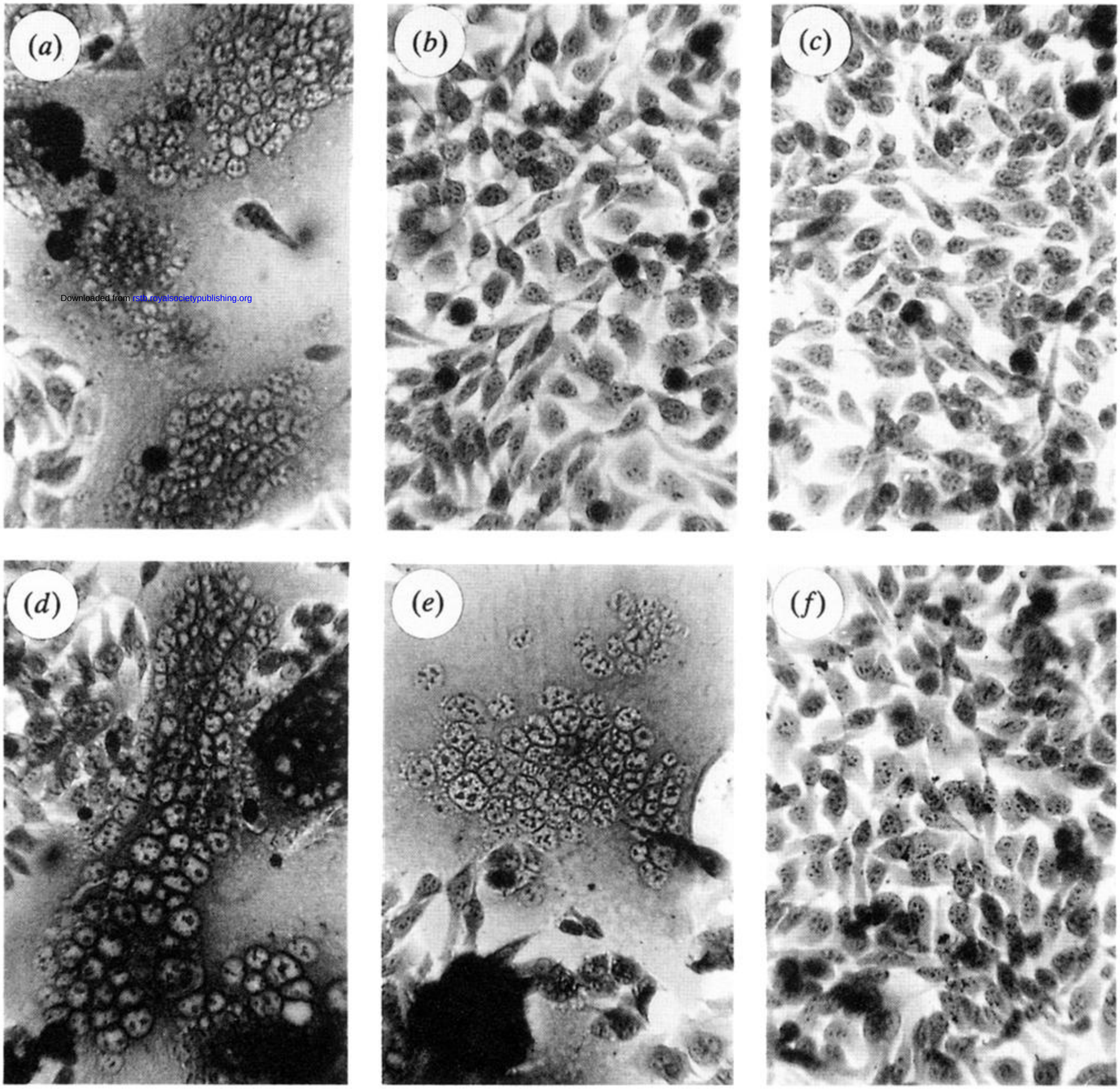
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



gure 1. Cell fusion induced by HIV-1/RF (*a*,*d*), HIV-2/LAV-2$_{ROD}$ (*b*,*e*), or SIV$_{MAC}$ (*c*,*f*) producer cell lines co-cultivated overnight with either RD/CD4 (*a*–*c*) or U87/CD4 (*d*–*f*).



gure 2. Primary macrophages fused after overnight co-cultivation with H9 cells producing T cell line tropic HIV/RF.



gure 3. HIV-2 induced fusion of CD4-negative RD cells. H9 cells producing HIV were cocultivated overnight th RD cells in the presence (*d–f*) or absence (*a–c*) of 10 μg ml^{-1} soluble CD4. The LAV-2B variant of HIV-2 duced fusion with or without sCD4 (*a,d*). HIV-2/CBL-20 fusion was sCD4 dependent (*b,e*). HIV-1 did not se (*c, f*).