1-1-1995

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Journal of General Virology (1995), **76**, 669–679. *Printed in Great Britain*

A monoclonal antibody to the gp120–CD4 complex has differential effects on HIV-induced syncytium formation and viral infectivity

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A murine monoclonal antibody (MAb F-91-55) raised against the complex of soluble CD4 and human immunodeficiency virus type 1 (HIV-1) gp120 had previously been found to inhibit syncytium formation without inhibiting the interaction of CD4 with gp120, and its binding site was localized within the first two domains (D1/D2) of CD4. We investigated whether this antibody inhibited the infectivity of HIV-1 in the $CD4^+$ T cell lines A3.01, Sup-T1 and H9. We also examined the effect of the antibody on syncytium formation between these cells and chronically infected H9 cells. Syncytium formation was found to depend critically on the incubation medium used. The effect of the MAb on HIV-1 infectivity was very limited with A3.01 and Sup-T1 cells, although it inhibited syncytium formation between A3.01 or Sup-T1 and chronically infected H9 cells. In contrast, the MAb inhibited significantly the infectivity of HIV-1 in H9 cells, but it also inhibited syncytium formation between H9 and chronically infected H9 cells to a greater extent than in the case of the other cell lines. Our results indicate that cellular systems used for syncytium assays differ in their susceptibility to inhibitory antibodies. In the A3.01 and Sup-T1 cell systems, the differences in the ability of the MAb to block viral entry or syncytium formation raise the possibility that the mechanisms of interaction of gp120/gp41 with cell membrane CD4 may be different in cell–cell and virus–cell membrane fusion.

Introduction

Human immunodeficiency virus type 1 (HIV-1) predominantly utilizes the T cell surface antigen CD4 as a receptor for infection of susceptible cells (reviewed in Levy, 1993). Both the fusion of the viral and target $CD4^+$ cell membranes and the fusion of HIV-infected cells with $CD4^+$ cells is a pH-independent process, and is preceded by the binding of the viral envelope glycoprotein gp120 to CD4 (Lifson *et al.*, 1986; Sodroski *et al.*, 1986; Sinangil *et al.*, 1988; Stein & Engleman, 1991; Levy, 1993). The mechanism of this fusion reaction is an area of active investigation. Binding of gp120 to CD4 is thought to induce conformational changes in both molecules (Moore *et al.*, 1991; Sattentau & Moore, 1991; Eiden & Lifson, 1992) resulting in the interaction of the fusion domain at the N terminus of gp41 with the target cell membrane, leading to membrane fusion and the release of the viral nucleocapsid into the cytosol. Although the mechanisms of virus–cell and cell–cell fusion are generally presumed to be similar, recent studies have indicated that there are significant differences between them (Callebaut *et al.*, 1994; Camerini & Seed, 1990; Kalter *et al.*, 1991; Pantaleo *et al.*, 1991*a*, *b*; Sato *et al.*, 1992).

The high-affinity binding site for gp120 on CD4 has been identified by mutagenesis, antibody-blocking and crystallographic techniques as comprising the complementarity determining region 2 (CDR2)-like region and supporting β-strands of the D1 domain of CD4 (reviewed in Klasse *et al.*, 1993). Antibodies to this region block gp120 and virus binding to $CD4^+$ cells, HIV infectivity of $CD4^+$ cells and HIV-1 mediated syncytium formation (McDougal *et al.*, 1986; Sodroski *et al.*, 1986; Peterson & Seed, 1988). In addition to acting as a receptor for virus binding, CD4 may also be involved in the subsequent membrane fusion step (Camerini & Seed, 1990; Hasanuma *et al.*, 1992; Poulin *et al.*, 1991; Truneh *et al.*, 1991). The CDR3-like region of CD4 (amino acids 81–102) (Sattentau *et al.*, 1989) is thought to be involved in HIV-mediated, CD4-dependent membrane fusion, independent of its role in binding to gp120 (Choe & Sodroski, 1992; Lifson *et al.*, 1988; Ohki *et al.*, 1990;

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Rausch *et al.*, 1992; Camerini & Seed, 1990). Certain monoclonal antibodies (MAbs) directed against the non-gp120 binding epitopes of CD4 were reported to block HIV infection and syncytium formation essentially without inhibiting CD4–gp120 binding. These include a MAb (L71) to the CDR3-loop (Truneh *et al.*, 1991), a MAb to the pre-CDR3-related region, amino acid residues 72–84 (Ohki *et al.*, 1992), a MAb (5A8) to the D2 domain (Burkly *et al.*, 1992; Moore *et al.*, 1992) and MAbs (Q425, Q428) to the D3 domain (Healey *et al.*, 1990). In contrast, some CD4 monoclonal antibodies have differing effects on HIV entry and cell–cell fusion. For example, an anti-CDR3 MAb, 13B8-2, inhibits both recombinant gp120 binding to cell surface CD4 (or sCD4) and HIV-induced cell–cell fusion, but not the binding of viral particles to cell-surface CD4 or virus entry into $CD4^+$ cells (Corbeau *et al.*, 1993). MAbs (5D4, 7C2) to the D3/D4 domains of CD4 inhibit HIV-1 infection but have no effect on CD4–gp120 binding and HIV-1-induced syncytium formation (Hasanuma *et al.*, 1992).

Another antibody raised against the complex of soluble CD4 and gp120 blocks syncytium formation between CEM cells chronically infected with HIV-1_{IIIB} (or HIV-1_{RF}) and uninfected Molt 4 cells, without inhibiting the interaction of CD4 with gp120 (Celada *et al.*, 1990). The binding site of MAb F-91-55 (MAb 55) has been localized within the first two domains (D1/D2) of CD4. The antibody exhibits greater binding to the gp120 complex of CD4 than to CD4 itself, suggesting that it recognizes a gp120-dependent conformational change in CD4. However, whether the antibody also inhibits viral infectivity has not been investigated (Celada *et al.*, 1990). Nevertheless, it has been assumed that MAb 55 inhibits viral infectivity as well as syncytium formation (Moore *et al.*, 1992; Gershoni *et al.*, 1993; Golding *et al.*, 1993; Klasse *et al.*, 1993). In view of the differential effects of several antibodies on these two processes, we have examined the ability of MAb 55 to inhibit HIV-1 infection of the $CD4^+$ T cell lines A3.01, Sup-T1 and H9, as well as its effect on syncytium formation between these three cell lines and chronically HIV-1-infected H9 cells.

Methods

Compounds and antibodies. Murine monoclonal antibody (MAb F-91-55, referred to as MAb 55), was raised in mice immunized with the soluble CD4–gp120 complex, as described by Celada *et al.* (1990), and was used either as a purified MAb or ascites fluid containing the MAb (ascites-55). Mouse immunoglobulin IgG1 was obtained from Becton-Dickinson; recombinant sCD4 was provided by Genentech. Phosphate-buffered saline containing 0·1 g/l $CaCl_2$, 0·1 g/l $MgCl_2.6H_2O$, 0·2 g/l KCl, 0·2 g/l KH_2PO_4, 8·0 g/l NaCl and 2·16 g/l $Na_2HPO_4.7H_2O$, designated PBS, was obtained from the UCSF Cell Culture Facility. Phosphate-buffered saline containing 10 mM-sodium/potassium phosphate and 0·9% NaCl, designated PBS Ca,Mg-free or PBS (CMF), was prepared from packets obtained from Zymed Laboratories and filter-sterilized.

Cells and viruses. A3.01 cells were obtained from T. Folks (Centers for Disease Control, Atlanta, Georgia, USA). Sup-T1 cells from J. Hoxie and H9 cells from R. C. Gallo were provided by the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH, Bethesda, Maryland, USA). H9/HTLV-IIIB cells were kindly provided by J. Mills (San Francisco General Hospital). H9 cells infected with HIV-1_{IIIB}, at 4 ng viral p24 per 1×10^6 cells, m.o.i. ≈ 0·0002, were designated as H9/HIV-1_{IIIB} and used 31 days post-infection. Viral p24 antigen was determined in cell-free supernatant by the antigen capture assay as described previously (Konopka *et al.*, 1990). All cell lines were maintained at 37 °C, under 5% CO_2 in Medium A consisting of RPMI 1640 medium (Irvine Scientific) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), penicillin (50 units/ml), streptomycin (50 μg/ml) and L-glutamine (2 mM). The cells were split 1:6 and fresh medium was added every 3 to 4 days. HIV-$1_{LAV\text{-}LAI}$ (formerly the LAV-BRU strain) (Wain-Hobson *et al.*, 1991), initially provided by F. Barré-Sinoussi (Pasteur Institute, Paris, France), was propagated in human lymphoblastoid leukemia (CEM) cells, and purified as described by Larsen *et al.* (1990) and stored at −70 °C. The purified HIV-1 was propagated in A3.01 cells and designated HIV-1_{BRU}. The supernatant of chronically infected H9/HTLV-IIIB cells was used as a source of the HIV-1 strain HIV-1_{IIIB}. The culture supernatants were harvested at times of peak p24 production, filtered through Millipore filters of 0·22 μm pore diameter, and stored in 1 ml aliquots at −80 °C. The reverse transcriptase activity of the virus stock solutions was $2{\cdot}5 \times 10^6$ and $3{\cdot}9 \times 10^6$ c.p.m./ml for HIV-1_{BRU} and HIV-1_{IIIB}, respectively, as detected by the method of Hoffman *et al.* (1985). The ELISA-reactive p24 protein yielded 2·6 μg/ml for HIV-1_{BRU} and 0·3 μg/ml for HIV-1_{IIIB}.

The tissue culture infectious dose, 50% endpoint ($TCID_{50}$), was determined as described by Johnson & Byington (1990). The infectious titre per 100 ng p24 antigen was $4{\cdot}6 \times 10^5$ $TCID_{50}$ (both on A3.01 and H9 cells) for HIV-1_{BRU}, and $4{\cdot}8 \times 10^3$ $TCID_{50}$ (on H9 cells) for HIV-1_{IIIB}.

Assays for CD4-dependent HIV-1-induced cell fusion. Syncytium formation was evaluated by inverted phase contrast microscopy at 25 × magnification, as described by Lifson (1993). After 16–20 h of co-cultivation, syncytium formation had proceeded to its full extent and cell fusion was typically extensive enough to warrant a 3+ score. A score of 4+ was assigned to numerous large syncytia with a low number of viable cells and 5+ to numerous syncytia and no viable cells. Diluents alone served as a neutral control; sCD4, known to block HIV-1-induced syncytium formation, served as a positive control for inhibition of cell fusion. Since sCD4 inhibits a process different from that inhibited by MAb55, the sCD4 concentration dependence of the inhibition of syncytia was not determined in our studies.

HIV infection of cells. Cells (2×10^7/ml) were exposed to HIV-1 for 2 h at 37 °C, washed three times to remove unbound virus, resuspended at $0{\cdot}5 \times 10^6$ or 1×10^6 cells/ml in Medium A and cultured in 24-well culture plates (2 ml per well). Viral inocula were standardized by their p24 content and cultures were infected at a concentration of 2 or 4 ng p24 per 1×10^6 cells. Where indicated, cells were infected in the presence of MAb 55, then washed three times and cultivated further in the absence of antibody. The MAb 55 was present *only* during the initial exposure of cells to the virus; therefore, the level of p24 antigen determined in the culture medium several days later is a measure of the effect of the antibody on the initial infection by cell-free virus, i.e. virus–cell fusion. Since the cells were infected at a very low m.o.i. and subsequently washed, the measured p24 values reflect *de novo* viral replication, and not any input virus. Control cells were treated similarly but not exposed to the virus. Every 2, 3 or 4 days, 1 ml of the supernatant was removed and fresh medium was added. p24 antigen in the harvested medium was

measured by ELISA (Konopka *et al.*, 1990). Cell viability was assessed visually both with and without the use of trypan blue. The viability of the cells was determined before each experiment, and only cultures with > 98% viability were used. In the infectivity experiments, viability was determined at times of p24 determination and ascertained to be comparable to controls.

Detection of CD4. The presence of cell surface CD4 was examined by direct immunofluorescence assay, using fluorescein isothiocyanate (FITC)-conjugated Leu3 antibody (Becton-Dickinson), with FITC-conjugated mouse IgG as a control. The fluorescence distribution was analysed with a fluorescence-activated cell sorter (FACScan; Becton-Dickinson).

Results

Inhibition of HIV-1-induced syncytium formation by MAb 55

We investigated the effect of MAb 55 on syncytium formation between chronically infected H9/HTLV-IIIB or H9/HIV-1_{IIIB} cells and three uninfected $CD4^+$ T cell lines, A3.01, Sup-T1 and H9. The relative efficacy of purified MAb and ascites-55 was compared in parallel experiments with Sup-T1 and A3.01 cells (Table 1). Both purified MAb and ascites-55 substantially inhibited syncytium formation, while no inhibitory effect was found for mouse IgG1 at 10 μg/ml. Complete inhibition was observed with purified MAb at 10 μg/ml and ascites at 1:200 or 1:100 dilution, with both cell lines. Sup-T1 cells were less sensitive to the anti-fusion effect of MAb than A3.01 cells; for example, at 1 μg MAb/ml or 1:500 dilution of ascites-55, complete inhibition of syncytium formation was observed with A3.01 cells while some cell–cell fusion occurred with Sup-T1 cells (Table 1).

To characterize the inhibitory effect of MAb 55 on cell–cell fusion under the conditions used in the infectivity studies, we performed the assay in complete or serum-free medium and compared that with results obtained in PBS and PBS (CMF). H9/HTLV-IIIB cells formed syncytia with Sup-T1 cells under all these conditions (Fig. 1). Ascites-55 blocked syncytium formation to a much higher extent in serum-free than in complete medium. For example, almost complete inhibition was observed at a 1:100 dilution of ascites-55 in serum-free medium, while significant cell–cell fusion occurred in complete medium. Although syncytium formation was less efficient in PBS (CMF) (score 3+) than in PBS (4+), the inhibitory activity of ascites-55 was comparable (Fig. 1). In contrast, syncytium formation between H9/HTLV-IIIB and H9 cells was very inefficient (data not shown). However, the more recently developed H9/HIV-1_{IIIB} cells were capable of forming syncytia with H9 cells at an efficiency comparable to that with Sup-T1 cells in complete medium (score 5+), and at a somewhat lower efficiency in serum-free medium (score 3+) (Fig. 2). Syncytium formation between H9 and H9/HIV-1_{IIIB} cells was more sensitive to the inhibitory effect of MAb than that between Sup-T1 and H9/HTLV-IIIB cells (Figs 3, 4*a* and 5*a*). Interestingly, fusion between H9 and H9/HIV-1_{IIIB} cells was also more sensitive to the assay conditions than was fusion between H9/HTLV-IIIB and A3.01 or Sup-T1 cells. For example, the efficiency of syncytium formation in the presence of PBS (score +) or PBS (CMF) (score ±) was very low (Fig. 2).

The effect of MAb 55 on HIV-1 infectivity depends on the cell type

We examined whether the inhibitory effect of MAb 55 on HIV-mediated cell–cell fusion was also exerted on virus–cell fusion. A3.01, Sup-T1 or H9 cells were infected with HIV-1_{BRU} or HIV-1_{IIIB} in the absence or presence of purified MAb 55 or ascites-55. FACS analysis indicated that 92%, 98% and 60% of A3.01, Sup-T1 and H9 cells, respectively, were CD4-positive. The background-subtracted mean fluorescence was 100, 140 and 40 (arbitrary units), for A3.01, Sup-T1 and H9 cells, respectively.

Surprisingly, in view of the dramatic inhibition of syncytia formation discussed above, the effect of MAb on HIV-1 infectivity in A3.01 and Sup-T1 cells was very limited. The antibody at 0·1 μg/ml did not affect viral p24 production in A3.01 cells. When A3.01 cells were infected in the presence of 1 μg MAb/ml, the production

Table 1. *Syncytium formation in the presence of purified MAb F-91-55 and ascites containing MAb-55**

Cell type	Purified MAb (μg/ml)			Ascites-55 (dilution)					IgG1 (10 μg/ml)	Control
	0·1	1	10	1:2000	1:1000	1:500	1:200	1:100		
Sup-T1	+	±	−	2+	+	±	−	−	3+	3+
A3.01	±	−	−	+	±	−	−	−	3+	3+

* Syncytia were scored 16–20 h after the addition of chronically infected H9/HTLV-IIIB cells to uninfected Sup-T1 or A3.01 cells. Appropriate dilutions of purified MAb 55 or ascites-55 were prepared in 200 μl PBS (CMF). MAb remained in the assay during the co-incubation period.

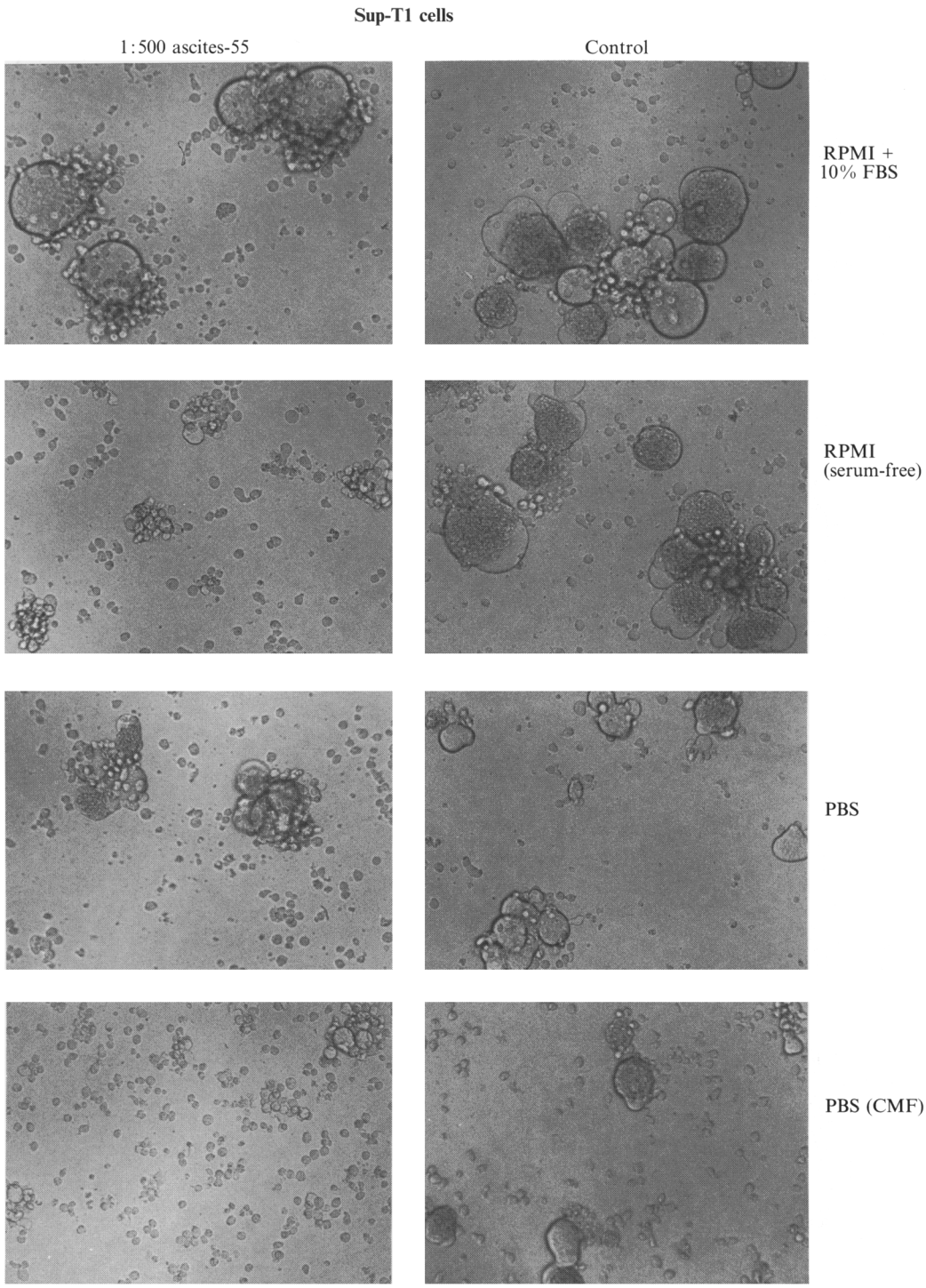

Fig. 1. Syncytium formation between Sup-T1 and H9/HTLV-IIIB cells under different conditions in the absence or presence of ascites-55 at a dilution of 1:500.

of p24 antigen in the culture supernatant was inhibited by 20% and 14·3%, on days 3 and 7 post-infection, respectively. At 10 μg/ml, the MAb reduced the infectivity of the virus by only 16%. The MAb inhibited the infectivity of the virus in Sup-T1 cells to a greater extent. The amount of p24 produced on day 3 was reduced by 20%, 31·1% and 44·4% at 0·1, 1·0 and 10 μg mAb/ml, respectively, and after 5 days the inhibition was 43·5%, 34·6% and 56·2%, respectively. The presence of mouse IgG1, at 10 μg/ml, during initial infection did not

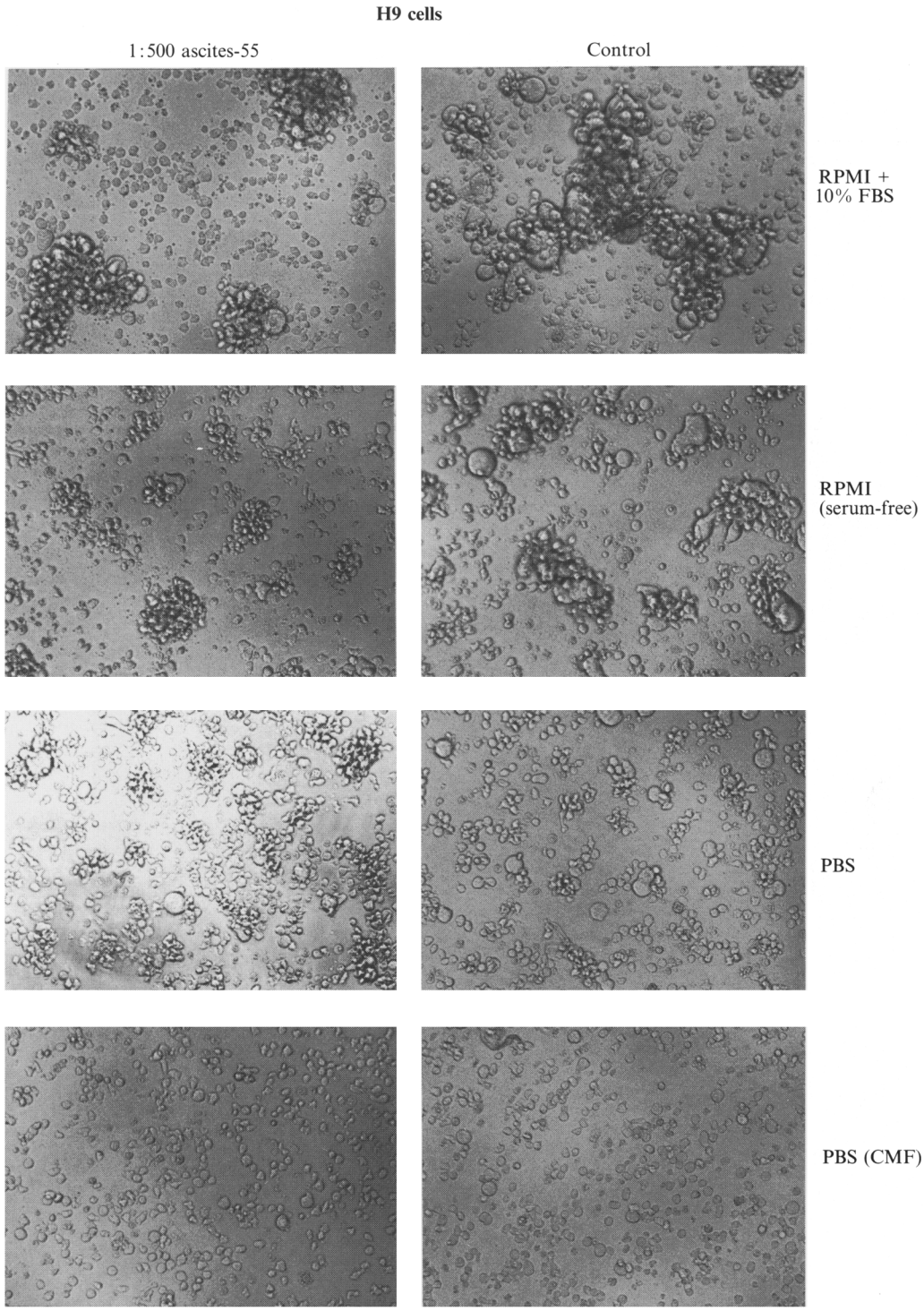

Fig. 2. Syncytium formation between H9 and H9/HIV-1_{IIIB} cells under different conditions in the absence or presence of ascites-55 at a dilution of 1:500.

affect virus production appreciably when compared to untreated A3.01 or Sup-T1 cells (data not shown).

We also determined virus production in A3.01 and Sup-T1 cells exposed to HIV-1_{BRU} in the presence of several dilutions of ascites-55. As with the purified MAb 55, ascites-55 reduced the p24 production with Sup-T1 cells to a greater extent than with A3.01 cells. For example, at a 1:100 dilution of ascites-55, 20% and

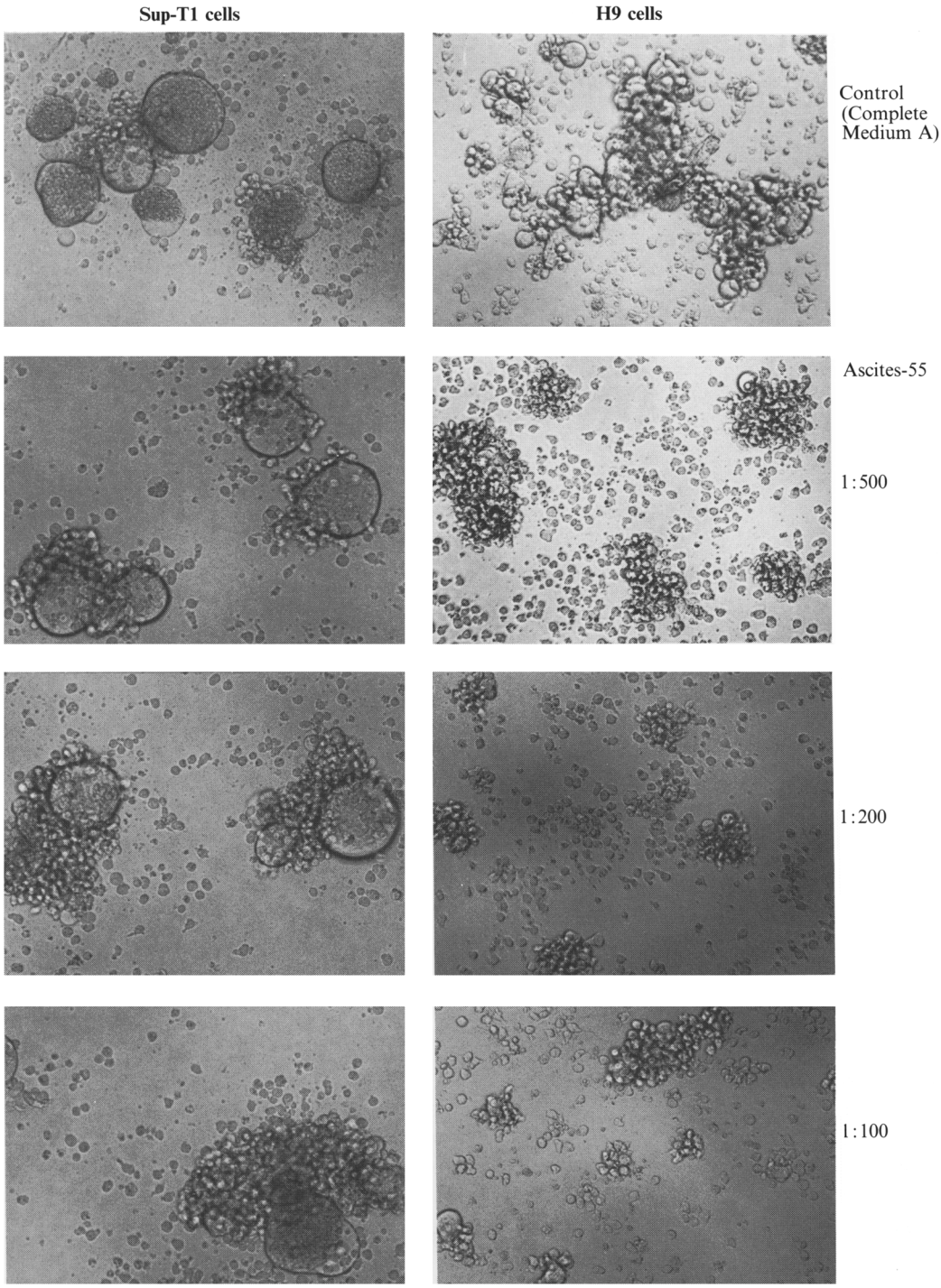

Fig. 3. Syncytium formation between Sup-T1 and H9/HTLV-IIIB or H9 and H9/HIV-1_{IIIB} cells in complete medium A in the absence and presence of ascites-55, at dilutions of 1:500, 1:200 and 1:100.

32·7% inhibition of p24 production was observed with A3.01 and Sup-T1 cells after 7 days, respectively.

Ascites-55 blocked syncytium formation between Sup-T1 and H9/HTLV-IIIB cells to a much higher extent in serum-free than in complete medium (Fig. 1). To test whether the absence of serum can increase the inhibitory effect of MAb on HIV-1 infectivity, p24 production was determined after the exposure of Sup-T1 cells to either HIV-1_{BRU} or HIV-1_{IIIB} in serum-free medium. Under these conditions ascites-55 did not inhibit *de novo*

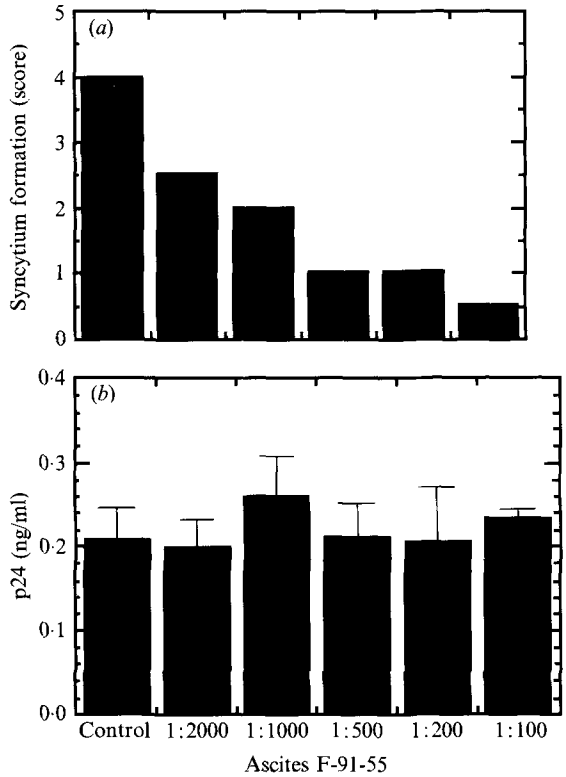

Fig. 4. Effect of ascites-55 on syncytium formation between Sup-T1 and H9/HTLV-IIIB (*a*) and HIV-1_{IIIB} replication in Sup-T1 cells (*b*). (*a*) Appropriate dilutions of ascites-55 in 200 µl of serum-free medium were added to the wells containing uninfected Sup-T1 cells and infected H9/HTLV-IIIB cells were added subsequently. After 16 h of co-cultivation in the continuous presence or absence of antibodies, syncytium formation was scored (score 1+ = 1). (*b*) Sup-T1 cells were exposed to HIV-1_{IIIB} at 2 ng p24 per 10^6 cells (at an m.o.i. of 0·0001) in serum-free medium in the presence of antibody as described in Methods. p24 antigen was determined in cell culture supernatant on day 3 post-infection. Data represent the mean ± standard deviation of p24 determinations in duplicate, in supernatants of two replicate wells ($n = 4$) or triplicate wells (control infections) ($n = 6$).

infection with either HIV-1_{IIIB} or HIV-1_{BRU} (Fig. 4*b* and data not shown). These results contrast sharply with the significant inhibition of syncytium formation (Fig. 4*a*).

Substantial differences were observed in the sensitivity of Sup-T1 and H9 cells to the inhibitory effect of MAb on syncytium formation with infected cells (Fig. 3). To test whether the infectivity of the virus might exhibit similar differences, we investigated the effect of MAb 55 on HIV-1 infection of H9 cells. When H9 cells were infected with HIV-1_{BRU} in the presence of 0·1 µg MAb/ml in complete medium, virus production was inhibited by 60% and 36%, on days 7 and 10 post-infection, respectively. At 1 and 10 µg/ml, MAb 55 reduced p24 production by 83·3% and 85·7%, respectively, on day 10 post-infection. A significant inhibitory effect was also observed when H9 cells were infected with the HIV-1_{BRU} isolate at a concentration of 13 ng p24 per 10^6 cells (m.o.i. = 0·06) in the presence of ascites-55 in serum-free medium. Ascites-55, diluted 1:100, reduced p24 production by 73% and 66·3%, on days 4 and 7 post-infection, respectively. The MAb inhibited the infectivity of HIV-1_{IIIB} in H9 cells to an even greater extent (Fig. 5*b* and data not shown). Virus production was reduced by 87%, 91% and 89%, on days 10, 14 and 17 post-infection, respectively, with a 1:100 dilution of ascites-55. Thus, under the same conditions, ascites-55 inhibited significantly the infectivity of HIV-1_{IIIB} in H9 cells (Fig. 5*b*) without affecting its infectivity in Sup-T1 cells (Fig. 4*b*).

Discussion

Our results on HIV-1-induced syncytium formation demonstrate that (i) it is influenced by the composition of the medium; (ii) significant differences exist among various uninfected and infected T cells in supporting cell–cell fusion; and (iii) the different T cell lines differ in their sensitivity to the inhibitory effect of MAb on HIV-induced syncytium formation. The molecular factors in the medium responsible for the different extents of syncytium formation are not known. The difference in the antibody sensitivity between the cell lines may be

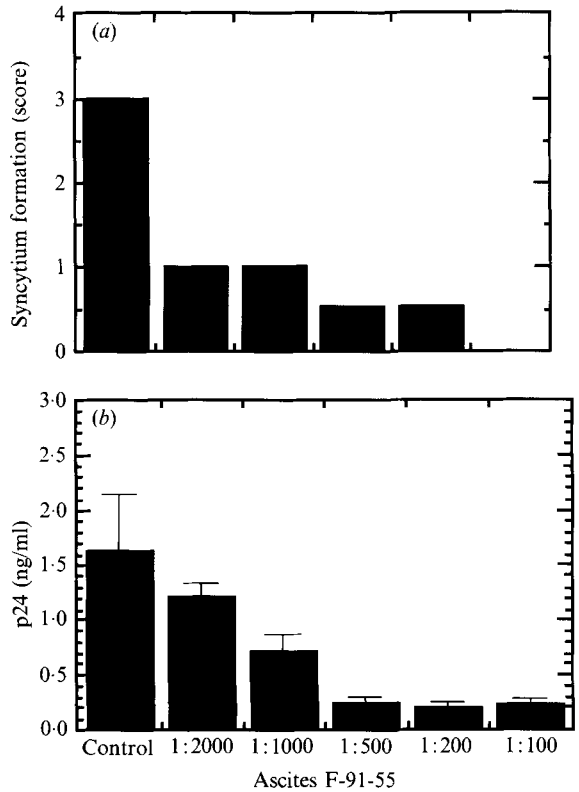

Fig. 5. Effect of ascites-55 on syncytium formation between H9 and H9/HIV-1_{IIIB} (*a*) and HIV-1_{IIIB} replication in H9 cells (*b*). (*a*) Appropriate dilutions of ascites-55 in 200 µl of serum-free medium were added to the wells containing uninfected H9 cells and infected H9/HIV-1_{IIIB} cells were added subsequently. After 16 h of co-cultivation in the continuous presence or absence of antibodies, syncytium formation was scored (score 1 + = 1). (*b*) H9 cells were exposed to HIV-1_{IIIB} at 4 ng p24 per 10^6 cells (at an m.o.i. of 0·0002), in serum-free medium in the presence of antibody as described in Methods. p24 antigen was determined in cell culture supernatant on day 10 post-infection. Data represent the mean ± standard deviation of p24 determinations in duplicate, in supernatants of two replicate wells ($n = 4$) or triplicate wells (control infections) ($n = 6$).

related to the difference in the intrinsic susceptibility of the cells to HIV-induced fusion. For example, H9 cells appear to be less prone to extensive syncytium formation compared to Sup-T1 cells, and the inhibition of cell–cell fusion by MAb 55 in this system is more pronounced. The lower syncytium-supporting ability of H9 cells observed here may be due to the lower level of CD4 expression on the cells compared to Sup-T1 cells, the lower percentage of $CD4^+$ cells in the cultures, and perhaps the lower fusogenic efficiency of H9 cells independent of the CD4 level (Lu *et al.*, 1992). Kabat *et al.* (1994) have found that the level of CD4 present on the surface of susceptible $CD4^+$ cells is not the sole determinant of virus entry or syncytium formation. The differences in syncytium formation observed under the different environmental conditions utilized in our studies are in agreement with the observation that Ca^{2+} are required for HIV-induced cell fusion but not for binding of sCD4 to gp120/gp41-expressing cells (Dimitrov *et al.*, 1993). Our observation that various T cells differ in their ability to support HIV-induced syncytium formation under different conditions suggest that the 'fusogenic properties' of *both* uninfected $CD4^+$ and infected cells influence syncytium formation. For example, De Jong *et al.* (1992) reported that A3.01 cells do not support syncytium formation, despite a high level of viral replication, while in our studies A3.01 cells could easily form syncytia with chronically infected H9 cells.

While our results on three different cell lines have confirmed previous observations that MAb 55 inhibits syncytium formation (Celada *et al.*, 1990), they indicate that the antibody is not particularly inhibitory to HIV infectivity in Sup-T1 and A3.01 cells. This MAb is thought to prevent a post-binding event necessary for membrane fusion without inhibiting the interaction of CD4 with gp120. One question raised by our findings is why virus–cell fusion should be less affected by the antibody than cell–cell fusion in the A3.01 and Sup-T1 cell systems. It has been reported that a mutation (517A) in the N terminus of HIV-1 gp41 markedly affects cytopathicity, with only minimal effects on virus replication (Kowalski *et al.*, 1991). Similarly, virus entry is

disrupted less than syncytium formation by changes in HIV-1 gp41 residues 566 to 596 (Cao *et al.*, 1993). Mutations in the N terminus of HIV-2 gp41 which increase the charge and the polarity and/or decrease the hydrophobicity of the fusion domain severely reduce the capacity of the virus to induce syncytia, but only slightly affect virus infectivity (Steffy *et al.*, 1992). It is also possible that viral gp120/gp41 can interact more effectively than cellular gp120/gp41 with accessory molecules in the cell membrane that may be involved in the membrane fusion reaction. Syncytium formation does not occur in LFA-1$^-$ CD4$^+$ T lymphocytes despite efficient HIV infection (Pantaleo *et al.*, 1991*a*, *b*). In addition, HIV-1 isolates differ in their capacity to induce syncytia *in vitro* (Tersmette *et al.*, 1989). Clearly, assays for syncytia formation should not be used as sole indicators of HIV infection.

Another question raised by our findings is why MAb 55 inhibits HIV-1 infection in H9 cells more effectively than in A3.01 or Sup-T1 cells. One possibility is that the interaction of HIV-1 with H9 cell membranes leading to virus–cell membrane fusion and nucleocapsid delivery into the cytoplasm has more stringent molecular requirements than its interaction with Sup-T1 cells. Thus, MAb 55 can more readily interfere with the proper orientation or alignment of HIV-1 envelope and cell surface molecules necessary for the membrane fusion reaction, subsequent to binding to CD4. Indeed, we have found that the extent of viral nucleocapsid delivery into H9 cells within 30 min at 37 °C is 5·5-fold less than that into Sup-T1 cells, although the extent of membrane lipid mixing is comparable (L. Stamatatos, S. Nir & N. Düzgüneş, unpublished data). This observation suggests that the membrane reorganization leading to complete membrane fusion (with nucleocapsid delivery) is less efficient in the case of H9 cells than Sup-T1 cells. Thus, the presence of the antibody interferes effectively with an already inefficient process in H9 cells, while it cannot interfere with an efficient fusion reaction in the case of Sup-T1 cells. Similarly, the cell type used markedly affects the anti-HIV activity of polyanionic compounds and glycosylation inhibitors (Schols *et al.*, 1992).

Recently, Gershoni *et al.* (1993) have described several MAbs against the CD4/gp120 complex, some of which inhibit cell–cell fusion to different degrees; however, their effect on HIV infection was not reported. Moore *et al.* (1992) have shown that *preincubation* of sCD4 with MAb 55 substantially reduces binding of sCD4 to HIV-1_{IIIB}-infected H9 cells and, as a result, inhibits sCD4-induced gp120 shedding. In contrast, using recombinant proteins, Celada *et al.* (1990) have shown that MAb 55 does not inhibit sCD4–gp120 binding when all the components are *co-incubated*. Thus, the results reported by Moore *et al.* (1992) suggest that the formation of the sCD4–MAb complex is necessary for the inhibition of sCD4–gp120 binding, and emphasize the importance of the experimental conditions in the interaction of these molecules.

Investigations on the regions of CD4 other than the gp120 binding site involved in HIV-1 envelope-mediated membrane fusion have provided contradictory data (Lifson *et al.*, 1988; Camerini & Seed, 1990; Ohki *et al.*, 1990; Rausch *et al.*, 1992; Corbeau *et al.*, 1993; Broder & Berger, 1993). The role of the anti-CDR3 MAb 13B8-2, specific for sequences surrounding amino acid 87, is also controversial. According to Rey *et al.* (1991) and Corbeau *et al.* (1993), MAb 13B8-2 blocks both the binding of gp120 to sCD4 and HIV-induced syncytium formation, but has varying degrees of effect on virus binding to cell-surface CD4 and HIV-1 infectivity. Our observations that MAb 55 was more effective in blocking syncytium formation than HIV-1 infectivity are in general agreement with those of Rey *et al.* (1991) and Corbeau *et al.* (1993) with MAb 13B8-2. Curiously, Dalgleish *et al.* (1984) presented data (in their Table 3) indicating that all the anti-CD4 MAbs they used inhibited both HIV-1-induced syncytium formation and pseudotype infection, while they noted in the text that pseudotype inhibition was not always achieved with some MAbs.

Interestingly, MAbs 5D4 and 7C2 to the D3/D4 domains of CD4 inhibit HIV-1 infection without inhibiting HIV-1 induced syncytium formation (Hasanuma *et al.*, 1992), thus exerting the opposite differential effect compared to MAb 55. Similarly, the inhibition of infection without impairment of virus binding and syncytium formation was reported with a plant lectin, jacalin, which binds to the carbohydrates located on the D3/D4 domains of CD4 (Corbeau *et al.*, 1994; Favero *et al.*, 1993). Thus, it becomes evident that although both virus–cell and cell–cell fusion require the specific interaction of gp120/gp41 with CD4, there are other characteristics of CD4, aside from its gp120 binding, which may play critical roles in post-binding events.

Our studies have been performed with laboratory isolates of HIV-1. In view of the differences between such strains and primary isolates with respect to syncytium induction, tropism, CD4-dependence and susceptibility to inhibition by sCD4 (Daar *et al.*, 1990; Levy, 1993; Kabat *et al.*, 1994), it will be useful in future studies to examine the effect of MAb 55 on infection of peripheral blood mononuclear cells by primary isolates and on syncytium formation in these cells. Finally, the observation of Benkirane *et al.* (1993) that the MAb 13B8-2 against the CDR3-like region of CD4 inhibits viral replication in previously HIV-infected CEM cells leads to the question of whether MAb 55 will have a similar effect in the same or other infected cell lines.

We thank Mr Harold Legg for the reverse transcriptase assay and Dr Dan Stites for flow cytometry. This work was supported by grants from the National Institute of Allergy and Infectious Diseases (AI-25534 and AI-32399; N. Düzgünes), the California Universitywide AIDS Research Program (R91 UP002; N. Düzgünes) and the Aaron Diamond Foundation (F. Celada).

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(*Received 22 July 1994; Accepted 24 October 1994*)