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# Two Receptors Are Required for Antibody-Dependent Enhancement of Human Immunodeficiency Virus Type 1 Infection: CD4 and Fc<sub>γ</sub>R

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**Evidence of antibody-dependent enhancement of human immunodeficiency virus type 1 (HIV-1) infection via Fc receptor (FcR) was published previously (A. Takeda, C. U. Tuazon, and F. A. Ennis, *Science* 242:580-583, 1988). To define the entry mechanism of HIV-1 complexed with anti-HIV-1 antibody, we attempted to determine the receptor molecules responsible for mediating enhancement of HIV-1 infection of monocytic cells. Monoclonal antibodies to FcRI for immunoglobulin G substantially blocked antibody-dependent enhancement of HIV-1 infection. Furthermore, we demonstrate a requirement for the CD4 molecule in antibody-enhanced HIV-1 infection via FcR. Soluble CD4 prevented infection by HIV-1 antibody-treated virus, and enhancement of infection of virus-antibody complexes was abrogated by a monoclonal antibody to CD4 (anti-Leu3a antibody). Treatment of human macrophages with an anti-CD4 antibody also inhibited antibody-enhanced HIV-1 infection of macrophages, supporting our contention that antibody-dependent enhancement of HIV-1 infection via FcR requires CD4 interaction with the virus glycoprotein.**

Infection with human immunodeficiency virus type 1 (HIV-1) may induce a humoral immune response that facilitates infection rather than protecting the host. Recent studies have shown the presence of antibodies which enhance infection in the sera of HIV-1-infected individuals (10, 20, 25). These antibodies may contribute to the pathogenesis of the infection, and they are a concern in regard to the development of HIV vaccines because vaccination may induce such enhancing antibodies. The antibody-dependent enhancement of HIV-1 infection is mediated by complement receptors (20) or by Fc receptors (FcRs) for immunoglobulin G (IgG), as we demonstrated using an FcR-bearing cell line (25). The evidence for the latter is as follows. Heat-inactivated sera from HIV-1 antibody-positive individuals or an IgG fraction from the sera at subneutralizing concentrations enhanced infection of a monocytic cell line, U937. This enhancement of infection was blocked by treatment of the cells with heat-aggregated gamma globulin. The F(ab')<sub>2</sub> fraction showed only neutralizing activity, indicating the requirement of the Fc portion of IgG for the enhancement of infection. Compatible results were also reported by Jouault et al. and Matsuda et al., who used radiolabeled HIV-1 envelope protein (11, 16). There are, however, three distinct classes of FcRs for IgG on human myeloid cells (2). IgG FcRI (Fc<sub>γ</sub>RI) is the only FcR that binds to monovalent IgG (human IgG1 and IgG3) with high affinity and is expressed on monocytes and macrophages. Fc<sub>γ</sub>R<sub>II</sub> is a low-affinity receptor for monovalent human IgG1 and IgG3 and appears to be specific for immune complexes. This receptor is expressed on a wide range of human cells, such as monocytes, macrophages, granulocytes, B lymphocytes, and platelets. Fc<sub>γ</sub>R<sub>III</sub> also has low affinity for monomeric IgG and is expressed on macrophages, granulocytes, natural killer cells, and some T lymphocytes. It is important to identify the relevant class of FcR to define the entry mechanism of antibody-complexed HIV-1 into cells. Although it is recognized that HIV-1

infection is initiated by a specific interaction of viral envelope protein with the cell surface receptor CD4 (4, 12), some glioma cell lines, rhabdomyosarcoma cells, and fibroblasts appear to be infected with HIV-1 by a CD4-independent mechanism (3, 8, 26). It is possible that molecules other than CD4 are responsible for HIV-1 entry, and FcR may also be a receptor for HIV-1. Therefore, we attempted to determine the molecules which are responsible for mediating antibody-dependent enhancement of HIV-1 infection via FcRs. Our results indicate that antibody-dependent enhancement of HIV-1 infection proceeds via Fc<sub>γ</sub>RI and requires virus interaction with the CD4 molecule.

## MATERIALS AND METHODS

**Cells and virus.** The human histiocytic lymphoma-derived cell line U937, which has monocyte characteristics, was obtained from the American Type Culture Collection, Rockville, Md., and was used for the infection enhancement assay. The T-lymphoid cell line CEM was used in indicated experiments. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (GIBCO, Grand Island, N.Y.), 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 2 mM L-glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml.

HIV-1, HTLV-III<sub>B</sub> strain, was propagated in H9 cells. The HTLV-III<sub>B</sub>-infected H9 cells were grown in RPMI 1640 medium supplemented with 20% fetal bovine serum and antibiotics. Culture supernatants were harvested and titrated on C8166 cells by a 50% tissue culture infectious dose (TCID<sub>50</sub>) assay.

**Reagents.** A recombinant, soluble form of CD4 protein (sCD4) (6) was kindly provided by Keith C. Deen (Smith-Kline and Beecham Laboratories, King of Prussia, Pa.). A monoclonal antibody (MAb) (anti-Leu3a) without NaN<sub>3</sub> was kindly supplied by Noel Warner (Becton Dickinson, Mountain View, Calif.). MAb 197 (mouse IgG2a) to Fc<sub>γ</sub>RI (7) and MAb IV.3 (mouse IgG2b) to Fc<sub>γ</sub>R<sub>II</sub> (15) were kindly provided by Michael W. Fanger (Dartmouth Medical School, Hanover, N.H.). Both MAbs act as ligands for the Fc-

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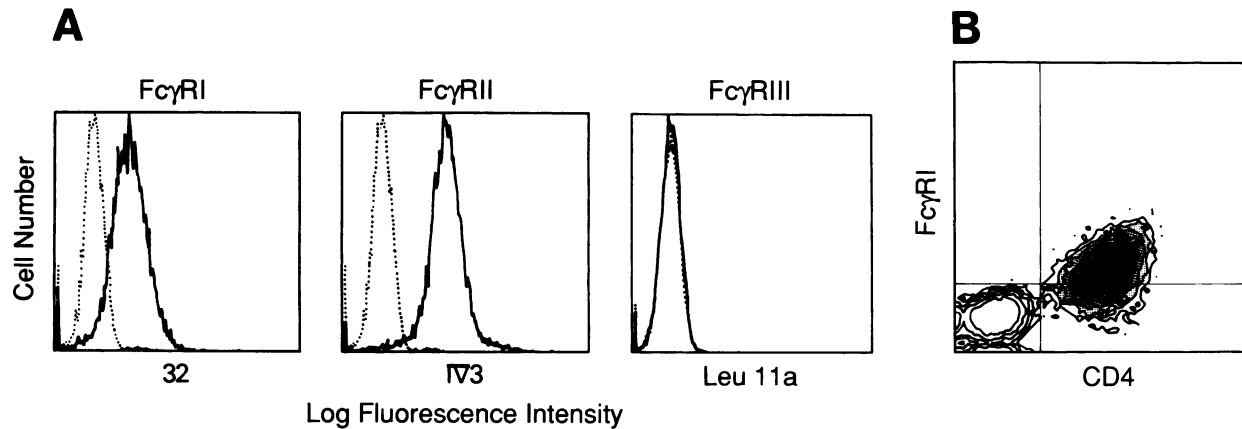


FIG. 1. Immunofluorescence flow cytometry analysis of  $Fc\gamma RI$ ,  $Fc\gamma RII$ ,  $Fc\gamma RIII$ , and CD4 expression in U937 cells with MABs 32.2, IV.3, anti-Leu11a, and anti-Leu3a, respectively. (A) U937 cells were stained for  $Fc\gamma RI$ ,  $Fc\gamma RII$ , and  $Fc\gamma RIII$  as described in Materials and Methods. Dotted lines depict negative controls treated with the second antibody alone (for MABs 32.2 and IV.3) or control mouse IgG1 (for MAB anti-Leu11a). (B) U937 cells were stained for dual parameter analysis of  $Fc\gamma RI$  and CD4.

binding domain. MAB 32.2 (mouse IgG1), which binds to  $Fc\gamma RI$  at a site distinct from the ligand-binding site, was also provided by M. W. Fanger and used for detection of  $Fc\gamma RI$  (1). The MABs anti-Leu11a (Becton Dickinson), anti-Leu3a (Becton Dickinson), and Mo2 (Coulter, Hialeah, Fla.) were used for staining  $Fc\gamma RIII$ , CD4, and CD14 (a monocyte/macrophage phenotype), respectively.

**Analysis of surface antigens on cells.** Cells ( $10^6$ ) in 20  $\mu l$  of phosphate-buffered saline containing 0.1%  $NaN_3$  were mixed at 4°C for 1 h with 20  $\mu l$  of MAB 32.2 or MAB IV.3 (60  $\mu g/ml$ ) plus 20  $\mu l$  of normal human gamma globulin (12 mg/ml; used to block Fc region-specific binding of MAB). The cells were then washed three times, stained with fluorescein isothiocyanate-conjugated (FITC) goat F(ab')<sub>2</sub> anti-mouse IgG at 4°C for 1 h, washed three times, and analyzed with a flow cytometer (FACS 440; Becton Dickinson). The cells were also stained with FITC anti-Leu11a, FITC anti-Leu3a, and FITC Mo2 as described elsewhere. Isotype-specific control antibodies were run with each sample. For dual parameter staining of FcRs and CD4, the cells were stained first with FITC anti-FcR antibodies and then with phycoerythrin-conjugated anti-Leu3a antibodies.

**Preparation of IgG from sera.** IgG was fractionated from HIV-1 antibody-positive sera on a sucrose density gradient, examined for contamination by radial immunodiffusion (Cooper Biomedical, Malvern, Pa.), and quantitated by nephelometry with antibodies to Fc (Beckman, Brea, Calif.). The purity was also determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

**Infection enhancement assay.** The assay was performed as previously described (25). Briefly, purified IgG from an HIV-1 antibody-positive serum was serially diluted in phosphate-buffered saline at serum dilution equivalents from  $10^{-1}$  through  $10^{-6}$ , and each of these dilutions (1 ml) was mixed with an HIV-1 inoculum containing  $10^6$  TCID<sub>50</sub>. The serum dilution equivalent was based on the original concentration of IgG in the serum. These mixtures were incubated for 30 min at 4°C and then incubated for 2 h at 37°C with  $10^6$  U937 cells. A multiplicity of infection (MOI) of 0.01 was used unless otherwise stated. After infection, cells were washed three times and cultured with fresh medium in 25-cm<sup>2</sup> plastic flasks (Costar, Cambridge, Mass.). On day 3, 5 ml of the same medium was added, and on day 5, 5 ml of medium was replaced. Supernatants and cells were har-

vested separately on day 7. Infection of U937 cells was monitored by assays of the p24 antigen content in culture supernatants with an HIV-1 capture enzyme-linked immunosorbent assay (Du Pont, Wilmington, Del.) (17) and by detection of cytoplasmic HIV-1 antigens in U937 cells in an indirect immunofluorescence microscopy assay; a 1:200 dilution of a human serum specimen with antibodies to HIV-1 was used as the first antibody, and a FITC F(ab')<sub>2</sub> fragment of goat antibody to human IgG (Organon Teknika, Cappel, Malvern, Pa.) was used as the second antibody. As we have reported previously, the enhancement of infection determined by p24 production is also characterized by an increase in the number of HIV-1 antigen-positive cells (25).

**Isolation and culture of macrophages.** Human monocytes were isolated by countercurrent centrifugal elutriation of the mononuclear-cell fraction of peripheral blood cells from normal donors. Cells in the fraction which contained >85% monocytes, as judged by cell morphology on Wright-Giemsa-stained cytosmears, were cultured as monolayers of adherent cells in 48-well plates (Cluster 48; Costar) in AIM-V medium (GIBCO) supplemented with 1,000 U of granulocyte-macrophage colony-stimulating factor (Amgen, Thousand Oaks, Calif.) per ml. On day 11, nonadherent cells were removed by gentle washing, and monolayers were pretreated with 5 U of gamma interferon (Collaborative Research, Bedford, Mass.) for 3 days. At that point, macrophages were checked for cell markers by flow cytometry, and the percentages of cells positive for CD14 (Mo2),  $Fc\gamma RI$  (MAB 32.2),  $Fc\gamma RII$  (MAB IV.3),  $Fc\gamma RIII$  (anti-Leu11), and CD4 (anti-Leu3a) were 99.3, 99.6, 99.5, 82.1, and 14.6, respectively. On day 14, they were infected with HIV-1 in the absence or presence of anti-HIV-1 antibody.

**Statistical analysis.** Statistical significance was determined by analysis of variance and the Student-Newman-Keuls procedure of multiple comparisons.

## RESULTS

**Effect of MABs to  $Fc\gamma RI$  and  $Fc\gamma RII$  on infection enhancement in the presence of antibodies to HIV-1.** As illustrated in Fig. 1A, the human monocytic cell line U937 bears two classes of FcRs,  $Fc\gamma RI$  and  $Fc\gamma RII$ , but does not express  $Fc\gamma RIII$ ; in this respect it is similar to monocytes (15). To identify the class of FcR which is responsible for antibody-

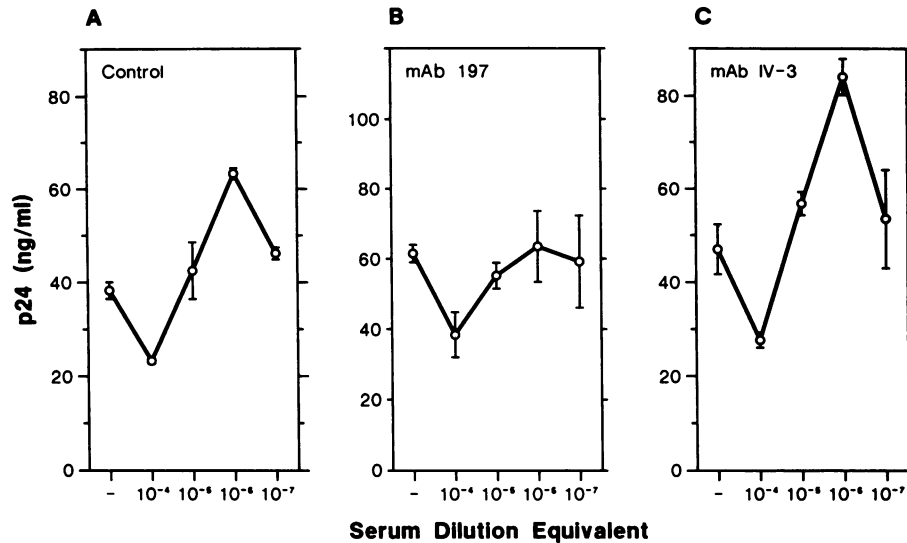


FIG. 2. Effect of MAbs to Fc<sub>γ</sub>RI and Fc<sub>γ</sub>RII on infection enhancement in the presence of antibodies to HIV-1. U937 cells (10<sup>6</sup>) were untreated (A) or pretreated with MAb 197 to Fc<sub>γ</sub>RI (B) or MAb IV-3 to Fc<sub>γ</sub>RII (C) at 5 μg/ml for 30 min at 4°C and then incubated for 2 h at 37°C with 10<sup>4</sup> TCID<sub>50</sub> of HIV-1 (MOI, 0.01) or HIV-1 complexed with various concentrations of purified IgG from an HIV-1 antibody-positive serum in the presence of each MAb at 5 μg/ml. The MAbs at this concentration saturate the binding sites of Fc<sub>γ</sub>Rs (7). After infection, the cells were washed and cultured. Supernatants were harvested on day 7 for determination of p24. Data are presented as the means ± standard deviations of p24 values from duplicate cultures. A repeated experiment showed similar results. The p24 yields from the culture of the cells which were infected in the presence of IgG antibody to HIV-1 diluted at the serum equivalent of 10<sup>-6</sup> were significantly higher than those in the absence of IgG ( $P < 0.001$ ) in treatment groups A and C but not in treatment group B.

dependent HIV-1 entry, we used the anti-FcR antibodies MAb 197 (mouse IgG2a) and MAb IV.3 (mouse IgG2b), which block Fc<sub>γ</sub>RI and Fc<sub>γ</sub>RII, respectively (7, 15). HIV-1 infection was significantly enhanced by the presence of IgG antibody to HIV-1 diluted to the serum equivalent of 10<sup>-6</sup> as compared with the absence of IgG ( $P < 0.001$ ) when cells were untreated or treated with MAb IV.3, but infection was not enhanced when cells were treated with MAb 197 (Fig. 2). Thus, treatment of U937 cells with MAb 197 to block Fc<sub>γ</sub>RI inhibited enhancement of infection in the presence of HIV-1 antibodies, whereas MAb IV.3, a ligand for Fc<sub>γ</sub>RII, did not block enhancement.

**Effect of sCD4 on enhancement of HIV-1 infection in the presence of antibodies to HIV-1.** The CD4 molecule has been identified as a crucial component of the cell surface receptor for HIV-1 (4, 12). All populations of U937 cells were found to express CD4 molecules (Fig. 1B). It was thus impossible to separate a CD4-negative cell population. To address the question of whether the infection pathway via FcR is independent of CD4, we used a recombinant soluble form of the CD4 molecule (sCD4). We first tested the capacity of sCD4 to block infection of U937 cells with HIV-1 (Fig. 3A). The results showed that the sCD4 preparation blocked the infectivity of HIV-1 for U937 cells. Three hundred nanograms of sCD4 was required to block the infectivity of an HIV-1 inoculum which possessed 10<sup>4</sup> TCID<sub>50</sub>. On the basis of these results, we examined the effect of sCD4 on enhancement of HIV-1 infection in the presence of antibodies to HIV-1 (Fig. 3B). HIV-1 infection was significantly enhanced by the presence of IgG antibody to HIV-1 diluted at serum equivalents of 10<sup>-4.5</sup> and 10<sup>-5</sup> as compared with the absence of IgG ( $P < 0.001$ ). In contrast to the enhancement of infection found in the absence of sCD4, preincubation with 1,500 ng of sCD4 completely inhibited infection not only by HIV-1 but also by HIV-1 complexed with antibodies. In this experiment, neither nonspecific inhibition of cell growth nor toxic

effects of sCD4 were observed in the cultures. When U937 cells were treated with 300 ng of sCD4, a low level of infection was detectable at the 10<sup>-5</sup> dilution of IgG antibodies, the optimal dilution of serum for demonstrating enhancement. The results of another experiment we performed also indicated that antibody-dependent enhancement of infection is completely blocked by a sufficient quantity of sCD4, regardless of whether antibodies are added before or after treatment with sCD4 (data not shown).

**Effect of anti-CD4 antibody on infection enhancement in the presence of antibodies to HIV-1.** In parallel experiments, we examined whether a monoclonal antibody to the CD4 antigen would block infection enhancement of monocytic cells (Table 1). The antibody we used was anti-Leu3a antibody because the recognition site on CD4 for gp120 is close to the Leu3a antigen epitope (21) and because this antibody has a murine IgG1 subclass that cannot be a ligand for FcR by itself (2). U937 cells were pretreated with anti-Leu3a antibody or an irrelevant murine IgG1 antibody at 5 μg/ml for 30 min at room temperature and then incubated for 2 h at 37°C with 10<sup>5</sup> TCID<sub>50</sub> of HIV-1 (MOI, 0.1) or HIV-1 complexed with various concentrations of IgG from an HIV-1 antibody-positive serum. These samples were further cultured in the continuous presence of each antibody at 5 μg/ml, and the supernatants and cells were harvested on day 7. HIV-1 infection of U937 cells was significantly enhanced by IgG antibody to HIV-1 diluted at serum equivalents of 10<sup>-3</sup> and 10<sup>-4</sup> as compared with the control, which was infected with HIV-1 alone, when cells were cultured in either the absence or the presence of an irrelevant murine IgG1 antibody ( $P < 0.05$ ). In contrast, the presence of anti-Leu3a antibody in the cultures abrogated the enhancement of infection; essentially no cells that were positive for HIV-1 cytoplasmic antigens were detected in these cultures (less than 0.1%). The virus titers contained in samples for cells exposed to HIV-1 alone and HIV-1 complexed with IgG at the 10<sup>-3</sup> dilution were 40

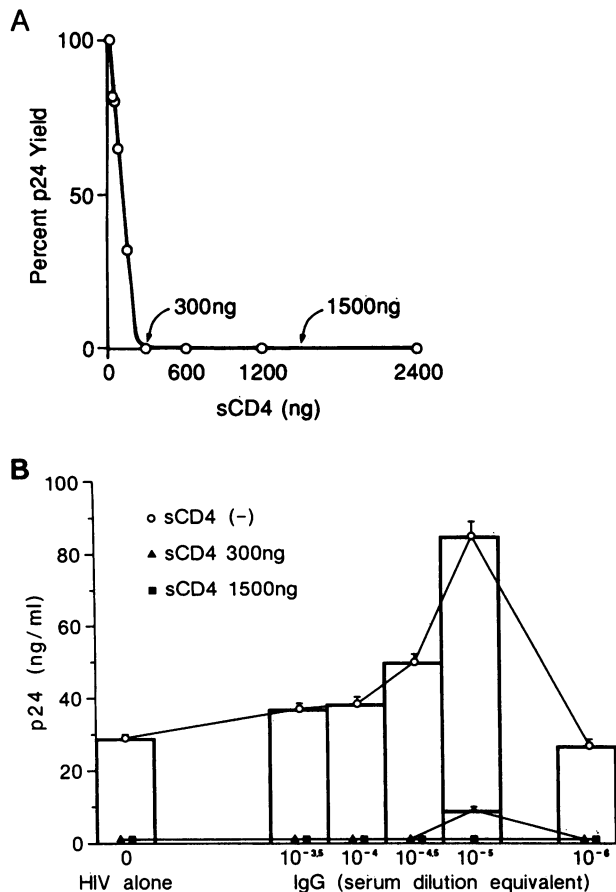


FIG. 3. Inhibition of infectivity of HIV-1 for U937 cells by sCD4. An HIV-1 inoculum containing  $10^4$  TCID<sub>50</sub> was preincubated with various concentrations of sCD4 for 30 min at 37°C and then incubated for 2 h at 37°C with  $10^6$  U937 cells. The infected cells were then washed and cultured with fresh media. The supernatants and cells were harvested separately on day 7. Each plot represents the means of duplicate samples. The p24 values from cultures not treated with sCD4 were calculated as 100% of virus yield. The cells which were exposed to the inoculum with 300 ng or more of sCD4 were negative for cytoplasmic HIV-1 antigens. (B) Effect of sCD4 on enhancement of HIV-1 infection in the presence of antibodies to HIV-1. The IgG from an HIV-1 antibody-positive serum was serially diluted and mixed with an HIV-1 inoculum containing  $10^4$  TCID<sub>50</sub>. These mixtures were untreated or treated with 300 or 1,500 ng of sCD4 for 30 min at 37°C and then incubated for 2 h at 37°C with  $10^6$  U937 cells (MOI, 0.01). After infection, the cells were washed and cultured. The supernatants were harvested on day 7. Data represent the means  $\pm$  standard deviations of two values. The p24 concentrations from cultures treated with 1,500 ng of sCD4 were undetectable (<0.001 ng/ml), and the cells from these cultures were negative for cytoplasmic HIV-1 antigen by indirect immunofluorescence.

and 160 TCID<sub>50</sub> of HIV-1, respectively, whereas the titers in all samples from cells treated with anti-Leu3a were less than 4 TCID<sub>50</sub>.

The results showed that treatment with anti-Leu3a antibody substantially reduced the level of virus yields from U937 cells as well as CEM cells that were exposed to HIV-1, indicating that infection of U937 cells with HIV-1 or antibody-complexed HIV-1 also requires interactions between HIV-1 and CD4 molecules. Moreover, anti-Leu3a antibody abrogated enhancement of infection with HIV-1 in the presence of antibodies to HIV-1, which indicated that cellular CD4 molecules are necessary for infection enhancement.

**Infection enhancement in cultured macrophages and the effect of anti-CD4 antibody.** To supplement our conclusions concerning a requirement for CD4 in FcR-mediated entry, we performed experiments with cultured human macrophages (Fig. 4). HIV-1 infection was significantly enhanced at subneutralizing concentrations of an IgG antibody to HIV-1, e.g., at a serum equivalent of  $10^{-6}$  ( $P < 0.001$ ). In contrast, treatment of cultured macrophages with anti-Leu3a antibody inhibited enhancement of HIV-1 infection, supporting the results obtained in U937 cells.

## DISCUSSION

This report shows convincing evidence that HIV-1 interaction with CD4 is required for infection by HIV-1 antibody complexes. First, antibody-dependent enhancement of HIV-1 infection was completely blocked by a sufficient quantity of sCD4. It may be asked whether effect of sCD4 may be due to nonspecific blocking of the binding of virus-antibody complexes to FcR by sCD4 on the basis of homology between CD4 and the Fc portion of IgG as part of the immunoglobulin gene superfamily. However, this possibility was ruled out because we found that sCD4 did not inhibit FcR-mediated infection enhancement in the dengue virus system (data not shown). Second, treatment of the target cells with anti-CD4 antibody abrogated enhancement of infection by HIV-1 in the presence of antibodies to HIV-1. This finding concurs with the results of our experiments with sCD4 and argues against the possibility that sCD4 blocks infection enhancement by inducing conformational changes of other sites, such as the fusion site on HIV-1 gp160. Additional experiments which we performed with cultured human macrophages, which possess Fc $\gamma$ RI, Fc $\gamma$ RII, and Fc $\gamma$ RIII, supplemented our conclusion concerning the requirement for CD4 in the entry of HIV-1 antibody complexes.

The studies reported here demonstrate that Fc $\gamma$ RI, a high-affinity FcR for IgG, is involved in antibody-dependent enhancement of HIV-1 infection. We have previously presented evidence that FcRs mediate enhanced entry of antibody-complexed HIV-1, using a monocytic cell line, U937 (25). We now report that a MAb to Fc $\gamma$ RI blocked enhancement of HIV-1 infection. Because of its high affinity ( $K_a = 10^8$  to  $10^9$  M<sup>-1</sup>) for IgG (2), Fc $\gamma$ RI may well attract antibody-complexed HIV-1 virions more efficiently than other Fc $\gamma$ Rs do. The results of our experiments, however, do not exclude a possible role of Fc $\gamma$ RII and Fc $\gamma$ RIII in the enhancement of HIV-1 infection. Although treatment with MAb to Fc $\gamma$ RII failed to block enhancement of infection, it is conceivable that binding of the monomeric ligand antibody IV.3 to Fc $\gamma$ RII may be overwhelmed by antibody-virus complexes which are potent ligands for Fc $\gamma$ RII. Distinct from Fc $\gamma$ RI, which attracts monomeric IgG, Fc $\gamma$ RII has low affinity for the monomeric ligand (2), and thus blocking of Fc $\gamma$ RII by MAb IV.3 may not be as stable as blocking of Fc $\gamma$ RI by MAb 197. There is some evidence from studies with flaviviruses that both a trypsin-sensitive Fc $\gamma$ R for IgG2a and a trypsin-resistant Fc $\gamma$ R for IgG2b, or Fc $\gamma$ RI and Fc $\gamma$ RII, can contribute to the phenomenon of infection enhancement (13, 14, 23). In this respect, we are currently conducting a more precise study to determine the role of Fc $\gamma$ RII in the enhancement of HIV-1 infection by employing heterocross-linked antibodies made by antibodies which are directed against envelope proteins of HIV-1 and against Fc $\gamma$ RII. We are also creating a cell line for the same objective which will express Fc $\gamma$ RII as well as CD4, but not Fc $\gamma$ RI or Fc $\gamma$ RII, by transfection of the CD4 gene into Fc $\gamma$ RII-bearing cell lines.

TABLE 1. Effect of anti-CD4 antibody on infection enhancement in the presence of antibodies to HIV-1

Cell line	IgG concn (serum dilution equivalent)	p24 concn (ng/ml) <sup>a</sup> with:		
		No treatment	Normal mouse IgG1 <sup>b</sup>	Anti-Leu3a <sup>b</sup>
U937	0 <sup>c</sup>	39.6 ± 2.3 (1.0)	26.0 ± 0.4 (1.0)	2.5 ± 0.1
	10 <sup>-1</sup>	1.1 ± 0.1 (0.03)	2.5 ± 0.1 (0.06)	1.4 ± 0.0
	10 <sup>-2</sup>	35.6 ± 0.1 (0.90)	22.1 ± 0.8 (0.85)	2.6 ± 0.2
	10 <sup>-3</sup>	79.1 ± 1.0 (2.00)	41.2 ± 0.2 (1.58)	2.6 ± 0.1
	10 <sup>-4</sup>	58.8 ± 2.3 (1.48)	40.0 ± 0.6 (1.54)	2.4 ± 0.1
	10 <sup>-5</sup>	ND <sup>d</sup>	ND	2.5 ± 0.0
	10 <sup>-6</sup>	41.7 ± 1.1 (1.05)	32.6 ± 1.2 (1.25)	2.6 ± 0.1
CEM	0 <sup>c</sup>	36.3 ± 0.5	24.4 ± 0.1	2.5 ± 0.1

<sup>a</sup> Results are expressed as the means ± standard deviations of duplicate cultures. The numbers in parentheses show the enhancement indices relative to the infection with HIV-1 alone.

<sup>b</sup> Anti-Leu3a (subclass IgG1) and irrelevant (normal mouse) IgG1 were used at concentrations of 5 μg/ml.

<sup>c</sup> HIV alone.

<sup>d</sup> ND, Not determined.

Recently, results compatible with ours were presented by Perno et al. who reported that enhanced infection of HIV-1 in fresh and cultured human monocytes/macrophages was blocked by inhibitors of gp120-CD4 binding, that is, sCD4 and OKT4A (19). On the other hand, there is a report which contrasts with our results. Homsy et al. have reported that in the presence of antibodies to HIV-1, Fc<sub>γ</sub>RIII mediates HIV-1 infection of cultured macrophages which possess Fc<sub>γ</sub>RI, Fc<sub>γ</sub>RII, and Fc<sub>γ</sub>RIII, and that this antibody-enhanced infection is not associated with CD4 (9). In that study, neither an antibody to Fc<sub>γ</sub>RI nor an antibody to Fc<sub>γ</sub>RII blocked enhancement. An antibody to CD4 (OKT4A) also did not prevent enhancement of HIV-1 infection, although inhibition by sCD4 was ambiguous. It is possible that the cells, the MABs used for blocking, or the sera studied may be responsible for these differences. Studies with MABs are complicated by the possibility that the

antibodies may nonspecifically alter viral infection through steric hindrance of other receptor molecules. Experiments using cells transfected with a gene for a single class of FcRs and for CD4 might further resolve this question.

With respect to our conclusion of the requirement for CD4, it is not certain at which stage of the entry process of the antibody-complexed virus the interaction between HIV-1 and CD4 molecules occurs. The binding of the virus-antibody complexes to Fc<sub>γ</sub>RI might facilitate viral entry by potentiating virus interaction with CD4 receptors on the cell surface or on the membrane of endosomes. Although the ordinary pathway of HIV-1 infection is still controversial, CD4 receptor-mediated endocytosis appears to be the mode of free virus infection (18) in addition to CD4-dependent entry via direct fusion between the viral and plasma membranes (24). The internalization of HIV-1 into endosomes may be promoted by FcR-mediated endocytosis of antibody-virus complexes, and after internalization HIV-1 may penetrate into the cytosol via intracellular fusion with the endosomal membrane as a result of binding with CD4 receptors on the endosomal membranes.

Our results provide an intriguing suggestion about the role of virus receptors in the antibody-dependent enhancement of infection by other viruses, in which antibody-mediated attachment of virus to the cell via FcRs has been considered to be sufficient to establish infection without participation of virus receptors (5, 22). Identification of the specific virus receptor molecules should allow a more precise understanding of the structural basis for the mechanism of infection enhancement in other virus systems. The ability of sCD4 to block infection enhancement *in vitro* may have important implications for preventing or reducing the development of HIV-1 infection which may be mediated by antibodies in patients infected with HIV-1.

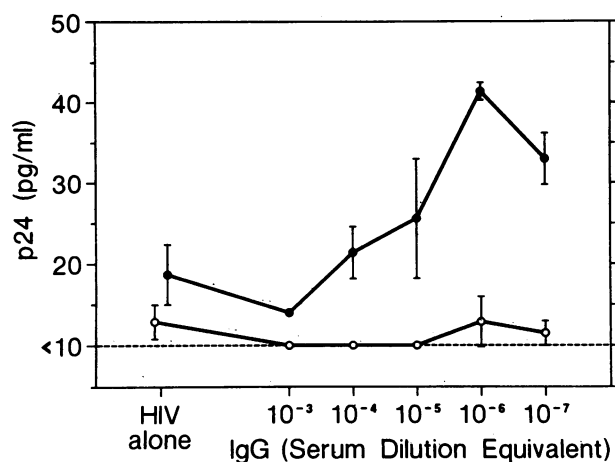


FIG. 4. Infection enhancement in cultured macrophages and the effect of anti-CD4 antibody. Adherent macrophages ( $5 \times 10^5$ ) were treated with anti-Leu3a antibody at 5 μg/ml (○) or left untreated (●) for 30 min at room temperature and then incubated for 2 h at 37°C with  $5 \times 10^4$  TCID<sub>50</sub> of HIV-1 (MOI, 0.1) or HIV-1 complexed with various concentrations of IgG from an HIV-1 antibody-positive serum in the absence or presence of anti-Leu3a antibody. The infected macrophages were gently washed three times and cultured with the same medium. Supernatants were harvested on day 6 for determination of p24. Each plot represents the means ± standard deviations of duplicate cultures.

#### ACKNOWLEDGMENTS

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