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Connor, R I.; Dinces, N B.; Howell, A L.; Romet-Lemonne, J L.; Pasquali, J L.; and Fanger, M W., "Fc Receptors for IgG (Fc gamma Rs) on Human Monocytes and Macrophages Are Not Infectivity Receptors for Human Immunodeficiency Virus Type 1 (HIV-1): Studies Using Bispecific Antibodies to Target HIV-1 to Various Myeloid Cell Surface Molecules, including the Fc Gamma R." (1991). *Dartmouth Scholarship*. 1068.

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Fc receptors for IgG (FcγRs) on human monocytes and macrophages are not infectivity receptors for human immunodeficiency virus type 1 (HIV-1): Studies using bispecific antibodies to target HIV-1 to various myeloid cell surface molecules, including the FcγR

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Communicated by Alfred Nisonoff, July 15, 1991 (received for review May 15, 1991)

ABSTRACT FcγRs (FcγRI, FcγRII, and FcγRIII) are highly expressed on human mononuclear phagocytes and function in the clearance of immune complexes and opsonized pathogens. We have examined the role of FcγR in mediating antibody-dependent clearance of HIV-1 by human monocytes and monocyte-derived macrophages by using bispecific antibodies (BsAbs) to independently target the virus to FcγRI, FcγRII, or FcγRIII. Virus production was markedly reduced in monocytes cultured with strain HIV-1_{IIIB} opsonized with BsAbs that target the virus to either FcγRI or FcγRII compared to monocytes cultured with virus in the absence of BsAbs or in the presence of BsAbs that target the virus to non-FcγR surface antigens (CD33 and HLA-A,B,C). These results were confirmed using the monotropic isolate HIV-1_{JRFL}. Interaction of HIV-1_{JRFL} with FcγRI or FcγRII on human monocytes and FcγRI, FcγRII, or FcγRIII on monocyte-derived macrophages resulted in markedly reduced levels of virus production in these cultures. Moreover, HIV-1 infection of monocytes and monocyte-derived macrophages was completely blocked by anti-CD4 monoclonal antibodies, indicating that interaction with CD4 is required for infectivity even under conditions of antibody-mediated binding of HIV-1 to FcγR. Thus, we propose that highly opsonized HIV-1 initiates high-affinity multivalent interactions with FcγR that trigger endocytosis and intracellular degradation of the antibody-virus complex. At lower levels of antibody opsonization, there are too few interactions with FcγR to initiate endocytosis and intracellular degradation of the antibody-virus complex, but there are enough interactions to stabilize the virus at the cell surface, allowing antibody-dependent enhancement of HIV-1 infection through high-affinity CD4 interactions. However, our results suggest that interaction of highly opsonized HIV-1 with FcγRs through BsAbs may reduce viral infectivity through FcγR-mediated cytotoxic mechanisms and, therefore, that BsAbs offer promise as therapeutic reagents in HIV-1 infections.

Infection of monocytes/macrophages with HIV-1 occurs through the direct interaction of the viral envelope glycoprotein gp120 with the CD4 receptor (1). Several studies have suggested that infectivity may also occur through the interaction of antibody-opsonized HIV-1 with one or more of the FcγRs on mononuclear phagocytes (2–6), termed antibody-dependent enhancement (ADE). In contrast, others have reported that infection of human macrophages in the presence of enhancing antibodies (7, 8) and HIV-antibody complexes (2) is dependent on gp120–CD4 binding (7–9). These findings suggest that several critical factors may be involved in determining HIV infectivity mediated through interactions

involving the FcγR, including the target cell population, the source and isolate of HIV-1, the specificity and neutralizing capacity of anti-HIV-1 antibodies, and the class of FcγR. The present study used bispecific antibodies (BsAbs) composed of Fab fragments of an anti-FcγR monoclonal antibody (mAb) covalently linked to a Fab anti-HIV mAb to selectively target HIV-1 to specific FcγRs, allowing direct independent analysis of the role of FcγRI, FcγRII, and FcγRIII in infection. HIV-1 complexed with BsAb served as a model for antibody opsonization and was used to evaluate the interaction with FcγRs on selected target cells.

Our findings indicate that interaction with FcγRI and FcγRII on monocytes and FcγRI, FcγRII, and FcγRIII on macrophages decreases infection with HIV-1 under conditions consistent with high antibody opsonization. Interactions with HLA class I or CD33 antigen did not decrease infection with HIV-1. Moreover, infection was completely blocked by pretreatment of the cells with anti-CD4 mAb, indicating that viral interaction with CD4 is required for infectivity, even under conditions of antibody-mediated binding to FcγR.

MATERIALS AND METHODS

Effector Cells. Monocytes were purified from normal volunteers as described (10).

HIV Preparations. Strain HIV-1_{JRFL} (11) was cultured in phytohemagglutinin-stimulated peripheral blood mononuclear cells and titered by end-point dilution to determine the 50% tissue-culture infectious dose (TCID₅₀). Strain HIV-1_{IIIB} was obtained from cultures of infected H9 cells and was similarly titered.

Antibodies and Antibody Fragments. The derivation and properties of the mouse mAbs specific to each of the FcγR have been reported. mAb 32.2 (IgG1) recognizes FcγRI (12), mAb IV.3 (IgG2b) recognizes FcγRII (13, 14), and mAb 3G8 (IgG1) recognizes FcγRIII (15). The mouse mAb 251 (IgG1) specific for CD33 (16) and mAb W6/32 (IgG2a), which recognizes a common epitope of HLA-A, B, C, were used as controls. mAb gpIII₂₃, a mouse IgG1, recognizes an epitope comprising residues 302 to 324 in the V3 region of HIV glycoprotein gp120 (17). mAb gpI_{11,2}, a mouse IgG1 (17),

Abbreviations: FcγR, Fc receptor for IgG; HIV-1, human immunodeficiency virus type 1; M/M, monocyte-derived macrophage; BsAb, bispecific antibody; mAb, monoclonal antibody; ADE, antibody-dependent enhancement; TCID₅₀, 50% tissue-culture infectious dose; TNB, thiobis(2-nitrobenzoic acid); SATA, *N*-succinimidyl-S-acetylthioacetate.

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recognizes a 23-residue epitope at the highly conserved gp120 N-terminal region.

IgG mAbs were produced in hollow-fiber cartridges (Amicon) and purified by HPLC on a DEAE 5PQ column (Waters). mAbs gpI_{11,2} and W6/32 were purified from ascites fluid by similar methods. mAb gpIII₂₃ was purified from ascites fluid by gel filtration and affinity chromatography using a Bio-Sil TSK 250 HPLC column (Bio-Rad) and Protein A-agarose (Sigma). F(ab')₂ fragments of the IgG1 and IgG2a mAbs were made by pepsin digestion (18) and purified by gel filtration using a TSK 3000 HPLC column (TosoHaas, Philadelphia, PA). Fab fragments of the IgG2b mAb IV.3 were obtained using immobilized papain and separated using protein A (Pierce).

BsAb Formation. F(ab')₂ fragments of mAbs 32.2, 3G8, 251, and W6/32 were reduced to Fab' fragments by 10 mM mercaptoethylamine hydrochloride (Sigma) under nitrogen (30 min, 37°C). 5,5'-Dithiobis(2-nitrobenzoic acid) (Sigma) was added to 20 mM, and the mixture was incubated under nitrogen (3 hr, 25°C). The Fab-thiobis(2-nitrobenzoic acid) (TNB) was isolated on a TSK 3000 column. F(ab')₂ fragments of mAbs gpIII₂₃ and gpI_{11,2} were reduced to Fab'-SH with 10 mM mercaptoethylamine and the Fab' fragments were isolated using a Sephadex G-25 column (Pharmacia). Equimolar amounts of the appropriate Fab-TNB and Fab'-SH antibodies were mixed under nitrogen and incubated at 22°C for 18 hr. The Fab × Fab BsAb was purified from the mixture by HPLC gel filtration chromatography on a TSK 3000 column and sterilized by 0.2-μm filtration. The protein concentration of each conjugate was determined by BCA assay using a Micro BCA kit (Pierce).

To prepare the gpI_{11,2} Fab × IV.3 Fab conjugate, gpI_{11,2} F(ab')₂ was reduced to Fab' using mercaptoethylamine, and TNB groups were introduced. IV.3 Fab was treated with *N*-succinimidyl-*S*-acetylthioacetate (SATA) in the presence of 1 mM EDTA, yielding two or three SATA groups per Fab fragment. Excess SATA was removed on Sephadex G-25. IV.3 Fab-SATA was then mixed with a 1.5 molar excess of gpI_{11,2} Fab'-TNB (1.5 mol of TNB per mol of SATA) under nitrogen and incubated at 22°C for 18 hr. The Fab × Fab cross-linked conjugate was purified as described above and sterilized by 0.2-μm filtration.

Reactivity of BsAb. Binding of the anti-gp120 end of the BsAb was demonstrated by ELISA. Recombinant gp120 (American Biotechnologies, Columbia, MD) was added to a 96-well plate at 0.2 μg per well in phosphate-buffered saline (PBS). After incubation at 37°C for 4 hr, washing, and blocking with bovine serum albumin [5% (wt/vol)], antibody stocks (100 μl per well) at concentrations from 10 ng/ml to 10 μg/ml were added in PBS/1% bovine serum albumin (PBA), and the plate was incubated 2 hr at 37°C. After thorough washing, goat anti-mouse immunoglobulin conjugated to alkaline phosphatase diluted 1:250 was added (75 μl per well). After an 18-hr incubation at 22°C, the plate was washed thoroughly and developed using *p*-nitrophenyl phosphate disodium (Sigma) (60 μl per well from a stock of 2 mg/ml).

The binding of the effector cell end of the BsAb was verified by flow cytometry. Approximately 1 × 10⁶ buffy-coat cells from normal peripheral blood were incubated for 90 min at 4°C with BsAb or control antibody (10 ng/ml to 100 μg/ml) in presence of human IgG (4 mg/ml), washed three times in PBA, and treated for a further 90 min with fluorescein isothiocyanate-conjugated goat anti-mouse IgG/F(ab')₂ (Caltag, South San Francisco, CA). After three washes, the cells were fixed in 1% paraformaldehyde. The percent of positively stained cells and the mean fluorescence intensity values were determined using an Ortho 50H cytofluorograph.

Radiolabeled Virus Preparation. HIV-1_{IIIB} (5 × 10⁴ TCID₅₀) was inoculated into cultures of phytohemagglutinin-stimulated peripheral blood mononuclear cells, and after

72–96 hr, supernatants from these cultures were added to fresh phytohemagglutinin-stimulated peripheral blood mononuclear cells. After 24 hr, the cells were washed and labeled with [³⁵S]methionine and [³⁵S]cysteine (DuPont) in methionine- and cysteine-free medium for 24 hr. Supernatants from the labeled cultures were precleared at 3000 × *g* at 4°C for 20 min and centrifuged at 100,000 × *g* for 60 min at 4°C, and radioactivity in the concentrated virus pellets was measured in a liquid scintillation counter.

Binding of Labeled HIV-1 to Human Monocyte FcγRs. [³⁵S]HIV-1 (150 × 10³ cpm) was incubated for 15 min at 37°C with BsAb (10 μg/ml) or with medium alone. The individual mixtures were cooled for 15 min on ice and added to 1 × 10⁶ monocytes. To control for the binding of [³⁵S]HIV-1 to CD4, monocytes were pretreated for 1 hr at 4°C with the anti-CD4 mAb Leu3a (Becton Dickinson) at saturating concentrations. Binding of [³⁵S]HIV-1 in the presence or absence of BsAb was carried out at 4°C for 1 hr. The cells were then washed extensively to remove unbound virus and lysed with 0.5 ml of 1% SDS, and the amount of cell-bound HIV-1 was determined by scintillation counting.

Infectivity Assays. Anti-FcγR × anti-HIV or control BsAbs were incubated for 30 min at 37°C with 50 TCID₅₀ of either HIV-1_{IIIB} or HIV-1_{JRFL}. Monocytes were then incubated with these virus-antibody preparations at 37°C for 4 hr, washed twice, and cultured in 24-well plates at 1 × 10⁶ cells per well. Samples of culture supernatants were taken on day 0 and fresh medium was added on day 3 of culture. HIV-1 production was determined at regular intervals by measuring p24 antigen levels in culture supernatants (Abbott). In parallel experiments, monocytes from the same donor were similarly studied after pretreatment for 1 hr at 4°C with mAb Leu3a to block CD4 prior to infection. Cultures of Leu3a-treated and control monocytes were evaluated for HIV-1 production at paired time points, and the data are expressed as pg of p24 based on p24 standards.

Monocyte-Derived Macrophages. Freshly isolated monocytes were cultured for 7 days in RPMI 1640 medium containing 10% (vol/vol) fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and 200 mM L-glutamine, yielding >99% monocyte-derived macrophages (M/Ms) as determined by adherence, histologic staining, and indirect immunofluorescent staining for expression of FcγRI, FcγRII, FcγRIII, and CD14. Prior to infection, macrophages were treated for 1 hr at 4°C with medium alone or with Leu3a (12.5 μg/ml) to block CD4. Anti-FcγR × anti-HIV or control BsAbs were incubated for 30 min at 37°C with 50 TCID₅₀ of HIV-1_{JRFL}, and these preparations were incubated with control or Leu3a-treated M/Ms for 4 hr at 37°C, washed twice, and cultured in 24-well plates at 1 × 10⁶ cells per well. HIV-1 production was then measured on the days shown.

RESULTS

Binding of BsAbs to Monocytes. As reported (3), FcγRI and FcγRII are highly expressed on monocytes and M/Ms, whereas FcγRIII is expressed at low levels on monocytes and high levels on M/Ms. BsAbs containing Fab fragments of mAbs 32.2, IV.3, and 3G8 linked to Fab anti-HIV-1 mAb (gpIII₂₃ or gpI_{11,2}) reacted with monocytes to the same extent as the unconjugated Fab anti-FcγR mAbs, confirming both the specificity and binding capacity of these BsAbs (Table 1).

The ability of BsAbs to link radiolabeled virus to monocytes was then examined. To prevent binding of HIV-1 directly to CD4, these studies were performed in the presence of saturating concentrations of Leu3a, an anti-CD4 mAb. High levels of HIV-1 binding to monocytes were observed with BsAbs that directed HIV-1 to FcγRI, FcγRII, CD33, or HLA-A,B,C; whereas low levels of binding were seen using BsAbs that directed the virus to FcγRIII (Fig. 1). These

Table 1. BsAb binding to monocyte Fc γ R

Receptor	% positive cells			
	mAb	Fab	mAb \times Fab gpIII ₂₃	mAb \times Fab gpI _{11,2}
Fc γ RI	32.2	98	97	96
Fc γ RII	IV.3	98	99	99
Fc γ RIII	3G8	20	27	26
HLA-A,B,C	W6/32	100	100	100
CD33	251	99	99	98

Fab fragments of mAbs to Fc γ RI (32.2), Fc γ RII (IV.3), Fc γ RIII (3G8), HLA-A,B,C (W6/32), and CD33 (251) and BsAbs consisting of these Fab fragments coupled to Fab fragments of anti-gp120 mAb (gpIII₂₃ or gpI_{11,2}) were evaluated for binding to human monocytes using flow cytometry. Results shown are from a representative experiment.

results are consistent with the pattern of Fc γ R, CD33, and HLA class I expression on monocytes (3) and confirm the ability of BsAbs to mediate interaction between HIV-1 and monocyte Fc γ R.

Infection of Human Monocytes with HIV-1_{IIIB} in the Presence of BsAbs. BsAbs containing Fab fragments of mAb gpIII₂₃ were initially used to selectively target HIV-1 to Fc γ RI, Fc γ RII, Fc γ RIII, or CD33. Control cultures infected with HIV-1_{IIIB} produced p24, which was first detected on day 16 of culture (Fig. 2a). However, monocytes infected in the presence of BsAbs that target HIV-1 to Fc γ RI, Fc γ RII, or Fc γ RIII showed very little p24 production throughout the culture period. Virus production from monocytes infected in the presence of anti-CD33 \times Fab gpIII₂₃ BsAbs was similar to, or greater than, that seen in control cultures (Fig. 2a). Pretreatment with Leu3a completely blocked virus production in cultures infected in the absence or in the presence of any of the BsAbs (Fig. 2b), suggesting that interaction with CD4 was necessary for infection.

Effect of BsAb on Fc γ R-Mediated Infection with a Monotropic HIV-1 Isolate. That HIV-1 infection of monocytes may be inhibited by interaction of antibody-coated virus with surface Fc γ R was further examined using the monotropic isolate HIV-1_{JRFL} (11). For all subsequent experiments,

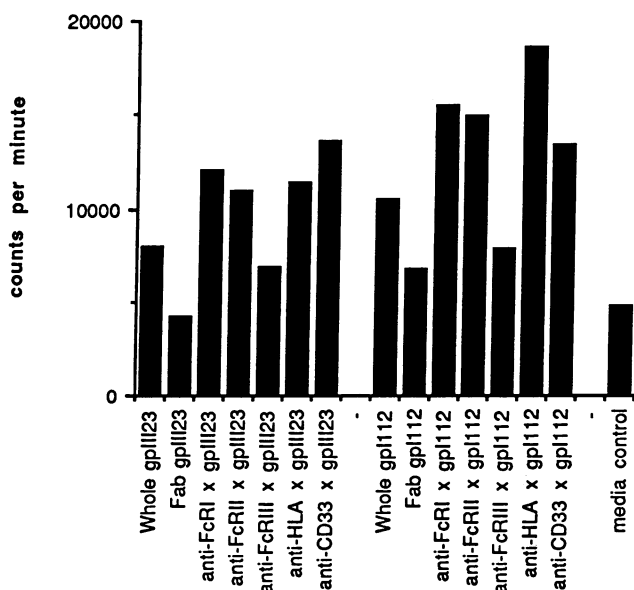


FIG. 1. Binding of HIV-1 to monocytes mediated by BsAbs. BsAb-opsonized and ³⁵S-labeled HIV-1_{IIIB} was incubated with monocytes to measure binding of opsonized virus. Binding was measured in the presence of mAb Leu3a to block interaction of virus with CD4. The BsAbs used are indicated.

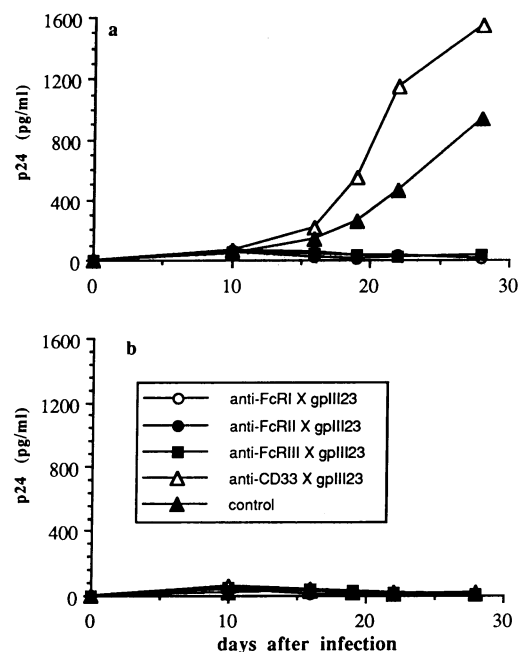


FIG. 2. Infection of human monocytes mediated through interaction with Fc γ Rs. Monocytes were incubated with HIV-1_{IIIB} in the presence of anti-Fc γ R \times gpIII₂₃ (20 μ g/ml) or control BsAbs containing gpIII₂₃ (20 μ g/ml) (a) or were pretreated with mAb Leu3a prior to incubation with BsAbs (b). HIV-1 production was determined by measuring p24 antigen in culture supernatants at designated time points. The BsAbs used are indicated.

BsAbs were constructed using Fab fragments of gpI_{11,2}, which binds a conserved region of gp120, covalently linked to Fab fragments of anti-Fc γ R or control mAbs. Compared with monocytes incubated with HIV-1_{JRFL} in the absence of BsAb or in the presence of BsAbs that target the virus to CD33 or HLA-A,B,C (Fig. 3a), significantly less p24 was measured in monocyte cultures incubated in the presence of BsAbs (20 μ g/ml) that target HIV-1 to either Fc γ RI or to Fc γ RII. When lower concentrations of anti-Fc γ R \times anti-HIV-1 BsAbs were used (0.4 and 2 μ g/ml), p24 production was comparable to that seen in control cultures. Infectivity in the presence of all BsAbs was completely blocked by Leu3a (Fig. 3b), supporting our previous observation that binding of opsonized HIV-1 to Fc γ R or other surface molecules is not sufficient for infectivity in the absence of CD4 interaction.

Fc γ RIII-Mediated Infection of M/Ms. Variable decreases in p24 levels were measured in independent experiments after virus interaction with Fc γ RIII on monocytes (Table 2) and may relate to the low levels of Fc γ RIII expressed by peripheral blood monocytes. Therefore, we examined the effect of HIV-1 interaction with Fc γ RI, Fc γ RII, or Fc γ RIII on M/Ms, since these cells have significant Fc γ RIII expression (19). Virus production from M/Ms infected with HIV-1_{IIIB} was observed in control cultures and in cultures infected in the presence of anti-HLA-A,B,C \times anti-HIV-1 BsAbs (Fig. 4a). Much lower levels of p24 were measured in M/M cultures infected in the presence of anti-Fc γ R \times anti-HIV-1 BsAbs and, of particular interest, in the presence of BsAbs that target to Fc γ RIII. This finding was confirmed in similar experiments using the monotropic isolate HIV-1_{JRFL} (data not shown). In all cases, infection with either HIV-1_{IIIB} (Fig. 4b) or HIV-1_{JRFL} (data not shown) was completely blocked by pretreatment of the cells with Leu3a.

DISCUSSION

Fc γ Rs expressed on human myeloid cells function primarily as cytotoxic trigger molecules in the extracellular lysis of

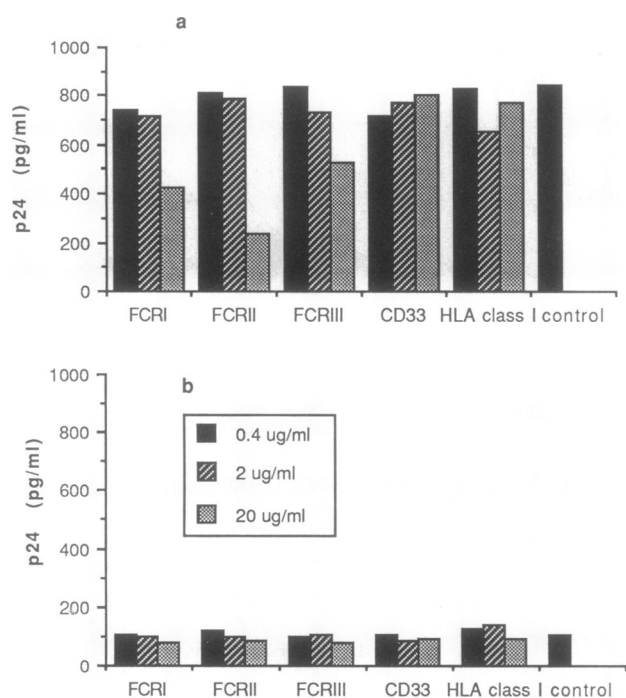


FIG. 3. Fc γ R-mediated infection of human monocytes with HIV-1_{JRFL}. Monocytes were incubated with the monotropic isolate HIV-1_{JRFL} opsonized with BsAbs made using mAb gpI_{11,2}. Monocytes were incubated with virus in the absence (a) or presence (b) of Leu3a. Virus production was determined by measuring p24 antigen in culture supernatants on day 7. Bars indicate amount of p24 produced after treatment with different BsAbs.

antibody-coated targets and the uptake and degradation of immune complexes and opsonized pathogens (3). In virus infections, including HIV-1 (20, 21), increasing anti-viral antibody titers are usually associated with a decline in the viral burden. However, the process of Fc γ R-mediated binding can also be exploited by certain pathogens to facilitate entry into Fc γ R-bearing cells. ADE of infection has been described for a number of viruses including HIV-1 (22). ADE of HIV-1 infection is thought to occur through interaction of HIV-1 with complement-fixing antibodies and subsequent binding to cell surface complement receptors (23, 24) or through interaction of HIV-1 with Fc γ R in the presence of subneutralizing concentrations of HIV-1 antibody (4, 5, 7–9).

The present studies were undertaken to systematically evaluate whether antibody-mediated binding to each class of Fc γ R on human monocytes/macrophages results in differential infection by HIV-1. BsAbs allowed independent analysis of the role of each Fc γ R in mediating infectivity. Although significantly increased levels of virus binding occurred in the presence of anti-Fc γ R, anti-HLA, and anti-

Table 2. Fc γ RIII-mediated infectivity using monocytes from various donors

Donor	p24, pg/ml			
	Control	0.4	2.0	20
1	>1000	788	608	446
2	>1000	>1000	966	712
3	662	731	656	646
4	701	832	711	322
5	810	763	333	154

Monocytes from five donors were incubated with HIV-1_{JRFL} in the absence (control) or presence of Fab 3G8 \times Fab gpI_{11,2} BsAbs at 20, 2, or 0.4 μ g/ml, which target HIV-1 to Fc γ RIII. Levels of p24 antigen in culture supernatants of infected monocytes were determined on day 7 after infection.

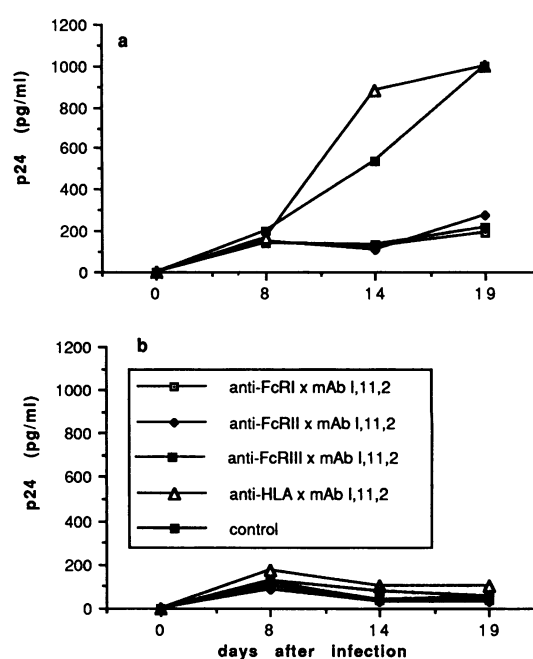


FIG. 4. Fc γ R-mediated infection of M/Ms. M/Ms were incubated with BsAb-opsonized HIV-1_{JRFL} in the absence (a) or presence (b) of Leu3a. Levels of p24 antigen in the supernatants were determined at the times indicated. The BsAbs used are indicated.

CD33 BsAbs (when compared to Fab fragments of these mAbs alone), infection of monocytes with HIV-1_{JRFL} occurred only in the presence of BsAbs that target non-Fc γ R surface antigens. Thus, interaction of opsonized virus with Fc γ R on human monocytes appeared to inhibit infection with HIV-1. Interaction of HIV-1 with other surface antigens either had no effect on infectivity or resulted in ADE.

We also examined Fc γ R-mediated infectivity using the monotropic isolate HIV-1_{JRFL} and utilized BsAb-containing Fab fragments of another anti-HIV-1 mAb, gpI_{11,2}. Although the Fab fragment of this mAb caused partial inhibition of subsequent p24 production, more appropriate controls were used including both anti-CD33 \times anti-HIV-1 and anti-HLA class I \times anti-HIV-1 BsAbs, neither of which inhibited HIV-1_{JRFL} infectivity. Consistent with our findings for HIV-1_{JRFL}, targeting of HIV-1_{JRFL} to Fc γ RI and Fc γ RII by using BsAbs (20 μ g/ml) caused a significant decrease in p24 production.

Since one study (5) has reported that Fc γ RIII on human macrophages plays a key role in ADE of HIV-1 infection and that infection was independent of interaction with CD4, we evaluated the role of Fc γ RIII in infection using M/Ms that express high levels of this receptor. HIV-1 directly targeted to Fc γ RIII using BsAbs (Fab 3G8 \times Fab gpIII₂₃ or Fab 3G8 \times Fab gpI_{11,2}) resulted in decreased HIV-1 infection. Moreover, this infection was completely blocked by Leu3a, indicating that, under these conditions, CD4 is required for infection even in the presence of antibodies. Compelling evidence from several recent studies (7–9) supports this conclusion. Another report (6), also suggesting that HIV-1 infectivity may occur through a CD4-independent FcR-mediated pathway, involved human fibroblasts coinfecting with cytomegalovirus. These cells express cytomegalovirus-encoded Fc receptors that are distinct from human Fc γ R, making further interpretation of those results difficult.

Interestingly, although HIV-1 production from both monocytes and M/Ms infected by HIV-1_{JRFL} was decreased by anti-FcR \times anti-HIV-1 BsAbs (20 μ g/ml), at lower concentrations, HIV-1 production was the same as in the controls. Moreover, in other studies using these same BsAbs, but at lower concentrations (0.1–0.001 μ g/ml), and the monocyte-

like cell line U937, ADE of HIV-1 infection was observed**. Although care should be taken in drawing parallels between these two studies since the tropism and replication kinetics of HIV-1_{IIIB} are markedly different in U937 cells when compared to primary monocyte/macrophages, these findings suggest that the degree of antibody opsonization is a critical factor in determining the outcome of the HIV-1-FcγR interaction and that binding to either FcγRI or FcγRII can result in lower infectivity. Our present data are directly relevant to, but not inconsistent with, observations of ADE of HIV-1 infection. In particular, we propose that HIV-1 coated with sufficient antibody will initiate high-affinity multivalent interactions with FcγRs that effectively trigger endocytosis and intracellular degradation of the antibody-virus complex. This interpretation may, in fact, explain the recent observation that increasing anti-HIV-1 titers in patients undergoing primary infection usually relate to decreased viral burden (20, 21). At lower levels of antibody opsonization, there will be too little interaction with FcγR to initiate FcγR-mediated endocytosis and intracellular degradation of the receptor-virus complex but enough to enhance stability of the virus at the cell surface, allowing infectivity to proceed through high-affinity CD4 interactions. Bolognesi (25) has described such a mechanism as a means of ADE of HIV-1 infection in monocytes. This could explain, in part, why low concentrations of antibody are needed for ADE of HIV-1 infection *in vitro*. Consistent with this possibility, the results of our study and those of others indicate that CD4 interaction is essential for infectivity, even under conditions of antibody-mediated FcγR binding.

That the magnitude of ADE of HIV infection is far less than with Dengue virus (25) may reflect differences in factors that govern viral entry, including post-binding fusion events. As with other enveloped viruses, internalization of Dengue virus through FcγR-mediated endocytosis may allow the virus to enter the cytoplasm through pH-dependent fusion with endosomal membranes. By contrast, our studies suggest that HIV-1 internalized through similar mechanisms may be unable to establish infection. This is consistent with several studies that indicate that HIV-1 entry occurs by direct fusion with the plasma membrane of the target cell in a manner that is pH-independent and does not require endocytosis of the CD4 receptor (26, 27).

The likelihood that high levels of anti-HIV-1 antibody reduce viral infectivity through both direct and indirect mechanisms is supported by observations from studies involving vaccine trials where high titers of anti-HIV-1 antibody provide some protection against live virus. Also, as indicated above, increased anti-HIV-1 antibody is related to decreased HIV-1 titer *in vivo* (20, 21). Furthermore, CD16⁺ cells from HIV-1-seropositive individuals have been shown to mediate killing of gp120-expressing targets, indicating that antibody in conjunction with Fc receptors on effector cells may play an important role in host defense (28).

In summary, CD4 interaction with HIV-1 appears essential for infectivity, even under conditions of antibody-mediated FcγR binding. Moreover, interaction of HIV-1 with FcγR expressed on human monocytes and macrophages through BsAbs may, under certain conditions, reduce viral infectivity through FcγR-mediated killing mechanisms. Although further studies are needed to define more clearly the conditions

that permit FcγRs to function as cytotoxic trigger molecules for HIV-1, our findings suggest that BsAbs directing interaction of HIV-1 with FcγRs offer a potentially promising therapeutic approach *in vivo*.

We thank Dr. David Ho for use of research facilities and Dr. Irvin Chen who provided HIV-1_{JRFL}. This work was supported in part by National Institutes of Health Grants AI19053, AI29375, and CA44794.

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**Vkobayashi, K., Takeda, A., Fanger, M. W. & Ennis, F. A., Seventh International Conference on AIDS, June 1991, Florence, Italy, abstr. MA 1008.