Brief Definitive Report

COMPLEMENT RECEPTOR 2 MEDIATES ENHANCEMENT OF HUMAN IMMUNODEFICIENCY VIRUS 1 INFECTION IN EPSTEIN-BARR VIRUS-CARRYING B CELLS

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The human immunodeficiency virus (HIV) has a marked tropism for human T lymphocytes expressing the CD4 glycoprotein. This molecule has been identified as the major cellular receptor for the HIV envelope protein gp120 (reviewed in reference 1). However, several other cells, such as dendritic cells, glial cells, monocytes/macrophages, and myeloid progenitor cells, which all express low levels of the CD4 molecule, can also be infected by HIV (2–5). Recent reports have suggested that the CD4 molecule may not be the only binding site permitting virus entry. In fact, HIV infection of brain and muscle cells could not be prevented by soluble CD4 or mAbs against CD4 (6). Infection of $CD4^-$ human fibroblastoid cells has also been demonstrated (7).

A large proportion of sera from HIV-infected subjects have been shown to enhance HIV infection in vitro via an antibody-dependent enhancement (ADE) mechanism; this suggests that both Fc and complement receptor (CR) might be involved in the internalization of HIV-1 in some cases. Fc receptor enhancement of HIV infectivity has been reported in several cell types (8, 9); however, there is only one report describing a role for CR in enhancement of HIV infectivity in a T cell line expressing unusually high levels of CD4 and CR2 (10). With the knowledge that EBV-transformed B cells are often $CD4^+$ and express high levels of CR2, which is the receptor for EBV, we have studied the involvement of this latter molecule in the enhancement of HIV infectivity. Our results demonstrate a very significant enhancement of HIV-1 infection in these cells by a mechanism involving CR2. Furthermore, the enhancement is strictly dependent on the expression of CD4.

Materials and Methods

Establishment of EBV-carrying B Cell Lines and Sera. PBMC, isolated from each of two healthy donors, were infected with the B95-8 strain of EBV, as previously described (11). Serum samples

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were obtained from three HIV-1-infected individuals. Two of these patients were asymptomatic and one fulfilled the criteria for AIDS of the Centers for Disease Control (12). Additional sera from healthy sero-negative donors were used as controls. Sera were heat inactivated (HI) for 30 min at 56°C before use. Lyophilized human complement-containing serum from Sigma Chemical Co. (St. Louis, MO) was used as a source of human complement and was reconstituted immediately before use.

Viruses. The HIV-III$_B$ strain of HIV-1 was harvested from culture fluids of the chronically infected H-9 T lymphoid cell line, kindly supplied by Dr. R. C. Gallo (National Institutes of Health, Bethesda, MD) (13). A clinical variant of HIV-1, recently isolated in our laboratory by a coculture assay (14), was also included in the study. Titers of these HIV-1 preparations were determined by plaque assay using MT-4 cells (HTLV-I-carrying cells) as targets (15).

Immunofluorescence and Progeny Virus Production. The presence of HIV-1 antigens was detected by an indirect immunofluorescence assay. Toward this end, mouse mAbs specific for the viral core protein p24 (kindly supplied by Dr. R. C. Gallo) were used in conjunction with a FITC-conjugated goat anti-mouse Ig (Cappel Laboratories, Cochranville, PA). Infectious titers ($TCID_{50}$) of progeny virus production in culture supernatant fluids were calculated using the Kärber formula, on the basis of viral cytopathicity for MT-4 cells.

Flow Cytometry. Mouse mAbs used in these studies were anti-Leu-3a (anti-CD4) (Becton Dickinson Immunocytometry Systems, Mountain View, CA) and OKB7 (anti-CR2) (Ortho Diagnostics Systems Inc., Westwood, MA). EBV-positive B cells were first incubated with the appropriate mAb, washed, and incubated with FITC-conjugated goat anti-mouse Ig. The fluorescence data of 10,000 cells were analyzed using a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems). Nonspecific staining was determined using nonbinding isotype-matched mAbs.

Complement-mediated ADE of HIV-1 Infection. 200 μl of culture fluid containing HIV-1 (10^5 $TCID_{50}$ for HIV-III$_B$, and 10^4 $TCID_{50}$ for the clinical isolate) was mixed with an equal volume of HI HIV-1 antibody-positive serum, supplemented with 1:20 human complement-containing serum, and incubated for 1 h at 37°C. Then, 5×10^5 EBV-carrying B cells were added to each sample and incubated for 2 h at 37°C. The cells were washed twice and resuspended in 500 μl of fresh medium before seeding. Samples were harvested periodically and assayed for viral infection. Controls were performed using sera from HIV-1 antibody-negative individuals.

Results and Discussion

The role of complement-mediated antibody-dependent enhancement (CM-ADE) of HIV infection was studied in two independently derived EBV-carrying B cell lines. These two cell lines (A and B) expressed variable levels of both CR2 (Fig. 1, *A* and *C*) and CD4 (Fig. 1, *B* and *D*), as shown by flow cytometry histograms. Although these two cell lines also expressed Fc receptors, we could not detect any ADE of HIV-1 infectivity using sera from each of three seropositive individuals (Fig. 2).

The two B cell lines were infected with HIV-III$_B$ in the presence of the above-mentioned HIV-1-positive sera and a constant amount of human complement (1:20). The results of Fig. 2 *A* indicate that a significant increase (2–5-fold) in the number of cells expressing HIV-1 p24 antigen was observed in cell line A, after 8 d of exposure to the virus. Cell line B, which expressed low levels of CR2 and CD4, was almost nonsusceptible to HIV-1 infection (Fig. 2 *B*). However, when these cells were incubated with HIV-III$_B$, together with the above-mentioned sera and complement, a 5–10-fold increase in the percentage of cells that expressed p24 was observed.

To confirm that this phenomenon was not restricted to HIV-III$_B$, the same experiments were performed on cell line A with a clinical isolate of HIV-1. As shown in Fig. 2 *C*, a similar enhancement effect was observed in the CM-ADE assay.

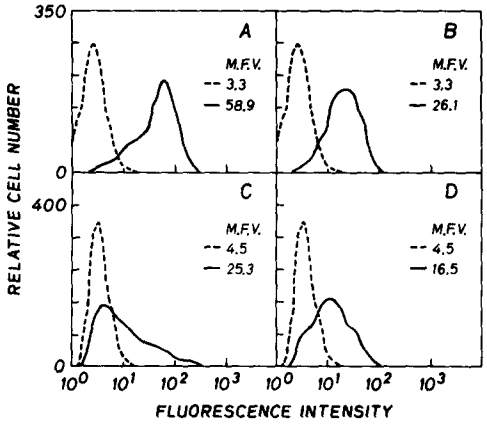

FIGURE 1. Flow cytometry analysis of two EBV-transformed B cell lines, showing surface expression of CR2 and CD4. EBV-positive cell lines A (*A* and *B*) and B (*C* and *D*) were first incubated with the murine mAbs OKB7 (*A* and *C*, *solid lines*) and anti-Leu-3a (*B* and *D*, *solid lines*), washed, and subsequently exposed to FITC-conjugated goat anti-mouse Ig. The fluorescence of these cells was analyzed using a FACScan flow cytometer. The dashed line represents background fluorescence. M.F.V., mean fluorescence value.

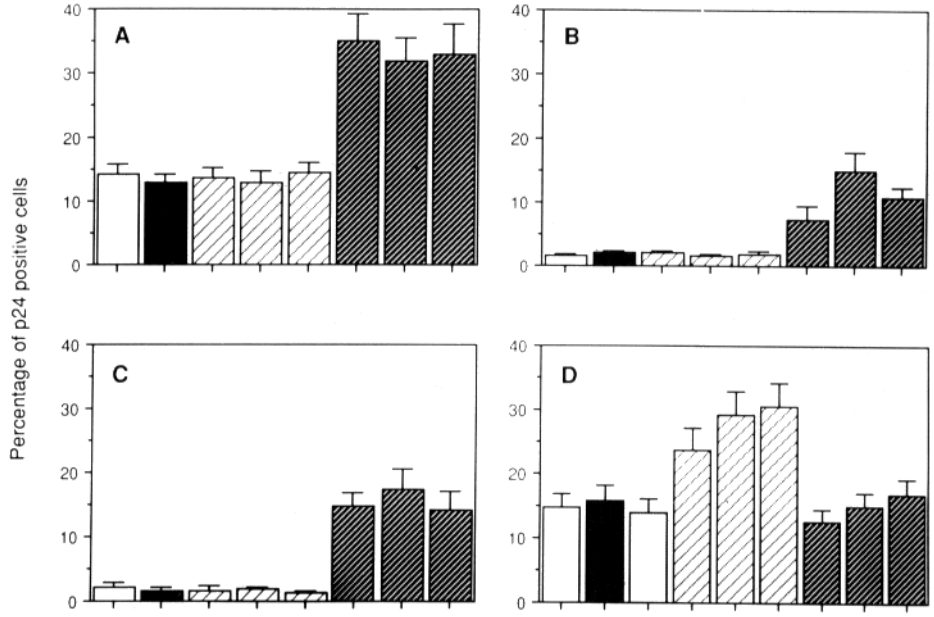

FIGURE 2. Complement-mediated ADE of HIV-1 infection in EBV-transformed B cell lines. (*A–C*) EBV-positive B cell lines were infected with HIV-1 alone (□), HIV-1 plus human complement (■), or HIV-1 plus a 10^{-4} dilution of serum 1, 2, and 3 in the absence (▨) or presence (▩) of human complement (1:20). Samples were harvested at regular intervals and assayed for p24 antigen by indirect immunofluorescence. (*A*) Percentage of p24-positive cells 8 d after infection of cell line A with HIV-III_B. (*B*) Percentage of p24-positive cells 12 d after infection of cell line B with HIV-III_B. (*C*) Percentage of p24-positive cells 12 d after infection of cell line A with a clinical isolate of HIV-1. (*D*) Results obtained with EBV-positive B cell line A was infected with HIV-1 alone (□), HIV-1 plus human complement (■), HIV-1 plus cobra venom factor (▩), or HIV-1 plus a 10^{-4} dilution of serum 1, 2, and 3 supplemented with human complement (1:20) and pretreated in the absence (▨) or presence (▩) of cobra venom factor. The percentage of p24-positive cells at 8 d after infection is shown. The data are the means ± SEM of determinations carried out with three replicate samples.

We also investigated whether the CM-ADE of HIV-1 infection would result in increased progeny virus production. Table I shows that the yields of infectious virus in culture supernatants were increased by 3–10-fold when cells were infected in the presence of any of three samples of HIV-1-positive sera and human complement. It has been reported that HIV-1-infected EBV-positive B cells can be isolated from the PBMC of patients with AIDS-related complex (ARC) and AIDS (16). Our results support the notion that EBV-transformed B cells, which are noteworthy for their high expression of CR2 and which can also express CD4, may participate in the pathogenesis of disease by releasing more infectious viral particles after HIV-1 infection through the CM-ADE mechanism.

Experiments were performed to determine what the effect of complement depletion would be on the observed CM-ADE of HIV-1 infection. Fig. 2 *D* indicates that cobra venom factor, which is known to cause the inactivation of C3, inhibited the CM-ADE of HIV-1 infection when included in the assay.

We next carried out a series of experiments to identify the molecules involved in the CM-ADE of HIV-1 infection. The involvement of CD4 in the CM-ADE mechanism was clearly demonstrated by the observation that anti-Leu-3a, a mAb specific for the gp120 binding site of CD4, totally abrogated HIV-1 infection in the presence of HIV-1-positive sera and complement (Fig. 3 *A*). This inhibition occurred at concentrations as low as 25 ng/ml, confirming that CD4 is the primary binding site for HIV-1. Fig. 3 *B* shows that mAb OKB7, which is specific for the CR2, inhibited the increase in p24-positive cells in a dose-dependent fashion. Complete inhibition of the CM-ADE was observed when 10 μg/ml of OKB7 was used. Control experiments were performed using D1-12, a mAb specific to MHC class II molecules. This mAb did not inhibit HIV-1 infection.

These results indicate that CD4 and CR2 synergize for the entry of HIV-1 into EBV-transformed B cells. This synergy might be relatively important in cells that express low levels of CD4 and/or where the fusion-mediated entry of HIV-1 is inefficient (e.g., cell line B). In our model, the CR2 structure would permit HIV-1 infection through viral endocytosis after initial binding to CD4. It is relevant that CR2 has previously been reported to mediate the internalization of EBV via an endocytic pathway (17).

TABLE I

Presence of Progeny Virus in Cell-free Culture fluids of HIV-1-infected Cells

HIV-1 + serum dilution	Serum sample 1	2	3
		$TCID_{50} \times 10^5/ml$	
0*	8.95 ± 1.7	5.04 ± 1.2	8.95 ± 1.8
10^{-3}	28.32 ± 3.4	50.36 ± 4.5	28.31 ± 2.8
10^{-4}	15.92 ± 2.1	28.31 ± 1.9	15.92 ± 2.4

Titers of infectious virus were determined in culture fluids 8 d after HIV-1 infection ($HIV\text{-}III_B$) of an EBV-transformed B cell line (cell line A) in the presence of sera from HIV-1-infected subjects and human complement, as described in Materials and Methods.

* HIV-1 alone.

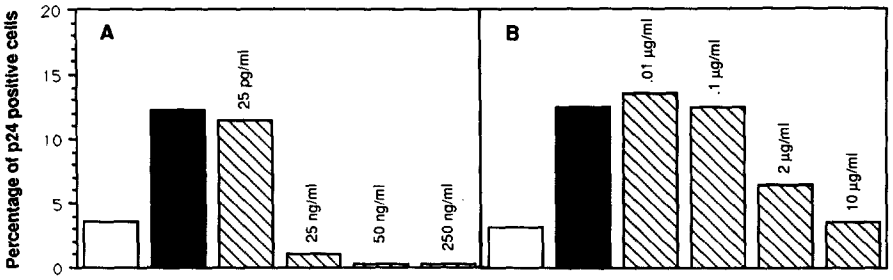

FIGURE 3. CR2 and CD4 are required for complement-mediated ADE of HIV-1 infection in EBV-carrying B cells lines. EBV-positive B cells (cell line B) were incubated for 2 h at 37°C with varying dilutions of anti-Leu-3A (*A*) or OKB7 (*B*) (▨). These cells were then washed, and infected with HIV-III_B in the presence of 10^{-4} dilution of serum 1, supplemented with human complement (1:20). Controls consisted of EBV-carrying B cells, nontreated with the above mentioned mAbs, infected with HIV-III_B alone (□), or HIV-1 plus a 10^{-4} dilution of serum 1 in the presence of human complement (1:20) (■). The data represent the percentage of p24-positive cells 12 d after viral infection.

The phenomenon of CM-ADE of HIV-1 infection observed in EBV-carrying B cells thus involves each the CD4 receptor, the CR2, and complement. This enhancement of HIV-1 infection was detected using sera from each of two asymptomatic individuals and one patient with AIDS. Our results suggest that HIV-1 may be able to more efficiently infect EBV-carrying B cells in vivo through a CD4-CR2 receptor complex. This may allow for circumvention of the effects of neutralizing antibodies.

It is further relevant that, in contrast to normal B cells, most EBV-transformed B cell lines express cell surface CD4 to some degree (18). Indeed, the ability of HIV-1 to infect such cells can be blocked by anti-CD4 antibodies, regardless of the extent of cell surface CD4 representation. This issue may have important clinical relevance, given that the numbers of EBV-transformed B cells are elevated in patients with AIDS and, accordingly, could represent an important target for HIV-1 in such individuals (18). Moreover, EBV-carrying B cells are relatively resistant to the cytopathic effects of HIV-1, in comparison with T lymphocytes (18).

Summary

Although the CD4 glycoprotein is the primary receptor for HIV-1, recent reports have suggested that other molecules might be involved in the enhancement of HIV-1 infection. We investigated the possible role of the complement receptor 2 in enhancement of HIV-1 infection in $CD4^+$ EBV-containing B cells by infecting such cells in the presence of sera from HIV sero-positive donors, with or without added human complement. A marked increase in production of viral p24 and infectious progeny virus was observed only when infection had been carried out in the presence of human complement. The addition of mAb to the human complement receptor 2 completely inhibited this enhancement. This mechanism was CD4 dependent, suggesting a cooperative effect between these two ligands in the potentiation of viral entry.

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