Relative Inefficiency of Soluble Recombinant DC4 for Inhibition of Infection by Monocyte-Tropic HIV in Monocytes and T Cells

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Abstract:

but not all HIV isolates infect macrophages. The molecular basis for this restrictive target cell tropism and the mechanisms by which HIV infects macrophages are not well understood: virus uptake by CD4-dependent and -independent pathways have both been proposed. Soluble rCD4 (sCD4) binds with high affinity to gp120, the envelope glycoprotein of HIV, and at relatively low concentrations (<100ng/ml) completely inhibits infection of many HIV strains in T cells or T cell lines. HTLV-IIIB infection of the H9 T cell line was completely inhibited by prior treatment of virus with 10ng/ml sCD4: no p24Ag or HIV-induced T cell syncytia were detected in cultures of H9 cells exposed to 1 x 10⁴ TCID₅₀ HTLV-IIIB in the presence of sCD4. Under identical conditions and at a 100-fold lower viral inoculum, 100 ng/ml sCD4 had little or no effect on infection of monocytes by an of six different HIV isolates by three different criteria: p24Ag release, virus-induced cytopathic effects, and the frequency of infected cells that express HIV-specific mRNA. At 10-to 100-fold higher concentrations of sCD4, however, infection was completely inhibited. Monoclonal anti-CD4 for inhibition of HIV infection in monocytes was a property of the virion, not the target cell: HIV isolates that infect both monocytes and T cells required similarly high levels of sCD4 (100 to 200 ng/ml) for inhibition of infection. These data suggest that the gp120 of progeny HIV derived from macrophages interacts with sCD4 differently than that of viruses derived from T cells. For both variants of HIV, however, the predominant mechanism of virus entry for infection is CD4-dependent.
RELATIVE INEFFICIENCY OF SOLUBLE RECOMBINANT CD4 FOR INHIBITION OF INFECTION BY MONOCYTE-TROPIC HIV IN MONOCYTES AND T CELLS

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Macrophages are major viral reservoirs in the brain, lungs, and lymph nodes of HIV-infected patients. But not all HIV isolates infect macrophages. The molecular basis for this restrictive target cell tropism and the mechanisms by which HIV infects macrophages are not well understood: virus uptake by CD4-dependent and -independent pathways have both been proposed. Soluble rCD4 (sCD4) binds with high affinity to gp120, the envelope glycoprotein of HIV, and at relatively low concentrations (<1 μg/ml) completely inhibits infection of many HIV strains in T cells or T cell lines. HTLV-IIIB infection of the H9 T cell line was completely inhibited by prior treatment of virus with 10 μg/ml sCD4: no p24 Ag or HIV-induced T cell syncytia were detected in cultures of H9 cells exposed to 1 × 10^4 TCID_50 HTLV-IIIB in the presence of sCD4. Under identical conditions and at a 100-fold lower viral inoculum, 10 μg/ml sCD4 had little or no effect on infection of monocytes by any of six different HIV isolates by three different criteria: p24 Ag release, virus-induced cytopathic effects, and the frequency of infected cells that express HIV-specific mRNA. At 10- to 100-fold higher concentrations of sCD4, however, infection was completely inhibited. Monoclonal anti-CD4 also prevented infection of these same viral isolates in monocytes. The relative inefficiency of sCD4 for inhibition of HIV infection in monocytes was a property of the virion, not the target cell: HIV isolates that infect both monocytes and T cells required similarly high levels of sCD4 (100 to 200 μg/ml) for inhibition of infection. These data suggest that the gp120 of progeny HIV derived from macrophages interacts with sCD4 differently than that of virions derived from T cells. For both variants of HIV, however, the predominant mechanism of virus entry for infection is CD4-dependent.

Interaction between the HIV envelope glycoprotein, gp120, and T cell plasma membrane CD4 is a necessary event for infection: antibodies to CD4 inhibit HIV infection of T cells (1-3); certain human cells not normally susceptible to HIV develop a productive infection after transfection with CD4 cDNA (4). sCD4 also binds with high affinity to gp120 (K_d ~ 10^-9 M) and at relatively low concentrations (1 μg/ml) inhibits the infection of many HIV strains in T cells or T cell lines (5-10). But T cells are not the only target for HIV in infected patients. Macrophages of brain, lymph nodes, and lung are major virus reservoirs (11-13). The mechanisms by which HIV infects macrophages are not well understood. Indeed, laboratory strains of HIV-1 passaged in T cells or T cell lines (such as HTLV-IIIB) and used for many of the preceding studies do not readily infect monocytes (14-17). In contrast, virus recovered from blood leukocytes of HIV-infected patients on mono or monocyte target cells from uninfected donors can be readily passed in other monocytes. For most seropositive patients, HIV isolates can be recovered from blood leukocytes in both the conventional T cell and monocyte cultures. Serial passage of these HIV clinical isolates into T lymphoblast and monocyte target cells documents a strong tropism of certain HIV for monocytes. HIV isolated in either T cells or monocytes can be serially passaged into cultures of PHA/IL-2-treated lymphoblasts. Analysis of such HIV-infected lymphoblasts by levels of p24 Ag and RT activity, in situ hybridization for HIV-specific mRNA, formation of cell syncytia during infection, down-modulation of T cell plasma membrane CD4, and transmission-electron microscopy (progeny virions budding at the plasma membrane only with no intracytoplasmic accumulation of viral particles) shows no qualitative or quantitative differences between T cell and monocyte-derived HIV isolates: these monocytic HIV grow equally well in monocytes and T cells (18). In marked contrast to the preceding results, viral isolates initially recovered from PHA/IL-2-treated lymphoblasts (T cell-tropic HIV) show little or no growth on monocytes by the criteria of virus-induced cytopathic effects, p24 Ag or RT activity levels, or infectious titer. Thus, HIV isolated in monocytes show dual tropism and infect monocytes and T cells equally; viruses isolated in PHA/IL-2-treated lymphoblasts replicate efficiently only in T cells (15-20).

1 Abbreviations used in this paper: sCD4, soluble rCD4; RT, reverse transcriptase; MCSF, macrophage-CSF; TCID_50, 50% tissue culture infective dose.
We show in this report that pretreatment of monocytes with mAb against CD4 completely prevented productive infection by monocyte-tropic HIV. However, under conditions in which sCD4 completely inhibited HTLV-IIIB infection of T cells, this recombinant molecule had little or no effect on the ability of six different monocyte-tropic HIV strains to infect macrophages. The apparent refractoriness of these monocyte-tropic HIV to inhibition by sCD4 was quantitative and evident with both monocyte and T cell targets. Under identical conditions and taking into account the amount of sCD4 used and the viral inoculum, there was at least a 10,000-fold difference in the efficacy of sCD4 for inhibition of monocyte-tropic HIV infection in monocytes vs that of HTLV-IIIB in T cells.

**Materials and Methods**

Isolation and culture of monocyte. T lymphoblast, or H9 target cells. Monocytes were recovered from PBMC of HIV and hepatitis B-seronegative donors after leukapheresis and purified by countercurrent centrifugation of mononuclear leukocyte-rich fractions of blood cells. Cell suspensions were >98% monocytes by criteria of cell morphology on Wright-stained cytocentrifuge, by granular peroxidase, and by nonspecific esterase. Monocytes were cultured as adherent cell monolayers (1.5 x 10^6 cells/6-mm tissue culture well) in 0.15 ml DMEM (formula 78-176A, Gibco, Grand Island, NY) supplemented with 10% heat-inactivated A" human serum, 50 mM gentamicin, and 1000 U/ml recombinant human MCSF (a generous gift from the Cetus Corporation, Emeryville, CA) [17]. The H9 T cell lymphoblast cell line (Dr. R. Gallo, contributor, AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases, Bethesda, MD) was cultured in RPMI 1640 medium (Gibco) with gentamicin, 15% FCS (Stevens, Inc., Logan, UT), and 10% partially purified human IL-2 (Advanced Biotechnologies, Inc., Columbia, MD, PBMC, isolated from peripheral blood by Ficoll-diatrizoate density gradient centrifugation, were cultured at 1 x 10^6 viable cells/ml in RPMI 1640 medium (Gibco) with 5 ug/ml PHA (Sigma Chemical Co., St. Louis, MO), 10% purified human IL-2 (Advanced Biotechnologies), and 15% heat-inactivated FCS (Stevens Systems).

HIV infection of monocyte and T cell targets. HTLV-IIIB/H9 (Dr. R. Gallo, contributor, AIDS Research and Reference Reagent Program, AIDS Program, NIAID, NIH), an HIV strain adapted to T cell multinucleated giant cells (16, 17, 21). The H9 T cell line used in these experiments contained >100-fold more infectious particles than the ADA inoculum used to infect monocytes. Thus, under identical conditions and taking into account both the amount of sCD4 used and the viral inoculum, there was at least a 10,000-fold difference in the efficacy of sCD4 for inhibition of HIV infection in T cells vs macrophages.

The inability of sCD4 to inhibit HIV infection of monocytes was confirmed by two other independent means of detecting virus infection. HIV induces a characteristic cytopathic effect in monocyte cultures: formation of multinucleated giant cells (16, 17, 21).

**Results and Discussion**

Initial studies confirmed previous reports that HTLV-IIIB infection of the H9 T cell line was completely inhibited by prior treatment of virus with 10 μg/ml sCD4 and with the same concentration of sCD4 continuously maintained in culture (Fig. 1). No p24 Ag was detected by ELISA in culture fluids of H9 cells exposed to 1 x 10^6 TCID_{50} HTLV-IIIB in the presence of sCD4 (or in replicate experiments with 1 x 10^6 TCID_{50} HTLV-IIIB). Whereas HTLV-IIIB-infected H9 cells showed about 215 syncytia/1 x 10^6 cells in triplicate cultures at 7 days, no syncytia were observed in replicate cultures treated with 10 μg/ml sCD4. In other experiments and as previously reported, HTLV-IIIB infection of H9 cells was completely inhibited by as little as 0.1 to 1 μg/ml sCD4 (5-10). Inhibition of infection by sCD4 was also evident with HTLV-IIIB and PHA/IL-2-treated lymphoblasts from PBMC: no p24 Ag was detected through 3 wk in culture fluids of PHA/IL-2-treated lymphoblasts exposed to 1 x 10^6 TCID_{50} HTLV-IIIB in the presence of 10 μg/ml sCD4: no HIV-induced syncytia were evident among the cells of these same sCD4-treated cultures. Similar results have been reported by others with at least five different HIV strains in both T cell targets. Under identical conditions and taking into account both the amount of sCD4 used and the viral inoculum, there was at least a 10,000-fold difference in the efficacy of sCD4 for inhibition of HIV infection in T cells vs macrophages.

Figure 1. Effect of sCD4 on HIV infection of H9 T cells and MCSF-treated monocytes. The H9 T cell line was cultured in RPMI 1640 medium with gentamicin, 15% FCS, 2 μg/ml Polybrene, and 10% partially purified human IL-2 at 1 x 10^6 cells/6-mm culture well. HTLV-IIIB was pretreated with 10 μg/ml sCD4 for 30 min at 37°C and added at 1 x 10^6 TCID_{50} to the H9 cells. PBMC purified to >98% monocytes were incubated as adherent monolayers at 1.5 x 10^6 cells/6-mm culture well in 0.15 ml DMEM with 10% human serum, and 1000 U/ml MCSF. At 7 to 10 days, monocytes were exposed to 1 x 10^6 TCID_{50} ADA, a monocyte-tropic HIV isolate pretreated with 10 μg/ml sCD4. The sCD4 was maintained at 10 μg/ml with both H9 and monocytes throughout the culture interval. All cultures were refed with fresh medium every 2 to 3 days. Levels of p24 Ag in culture fluids were determined by ELISA.
MONOCYTE-TROPIC HIV INEFFICIENTLY INHIBITED BY SOLUBLE rCD4

Figure 2. Effect of sCD4 on HIV-induced cytopathic effects in MCSF-treated monocytes. PBMC purified to >98% monocytes were incubated as adherent monolayers in medium with 10% human serum and 1000 U/ml MCSF. At 7 to 10 days, monocytes were exposed to 1 × 10^2 TCID₅₀ ADA pretreated with 10 μg/ml sCD4. The sCD4 was maintained at 10 μg/ml throughout the culture interval. Cultures were refed with fresh medium every 2 to 3 days. Photomicrographs of adherent cells fixed in methanol and Wright-stained 15 days after infection are at 200x original magnification. Left panel: uninfected MCSF-treated monocytes; middle panel: HIV-infected monocytes; right panel: HIV-infected monocytes with 10 μg/ml sCD4.

control monocytes without multinucleated giant cells in culture for 3 wk. Multinucleated giant cells were evident in about 20 to 40% of total cells in the HIV-infected cultures. There was no difference in the number or extent of these multinucleated giant cells in replicate cultures treated with 10 μg/ml sCD4. In similar experiments, about 27 ± 2% of total monocytes (mean ± sem of 200 cells in duplicate determinations) expressed HIV mRNA as detected by in situ hybridization with radiolabeled ssRNA probes at 17 days. The frequency of monocytes that express HIV mRNA in cultures with 10 μg/ml sCD4 was 31 ± 2%.

The inability of sCD4 to inhibit infection of HIV in monocytes was not limited to the ADA isolate. We examined six different HIV isolates that infect monocytes (strains ADA, 16, 24, 36, 37, and 38). In each case, the HIV inoculum was less than 0.001 monocyte infectious doses per cell. Results for four of these isolates (strains 16, 24, 36, and 38) are shown in Figure 3. In each instance, 10 μg/ml sCD4 had little or no effect on virus infection: levels of p24 Ag released into monocyte culture fluids at 3 wk with or without sCD4 were indistinguishable. In separate experiments, we obtained identical results when we used as an inoculum viral stock that had been pelleted after ultracentrifugation. Such washing after centrifugation would remove any soluble gp120 present in the virus stock that could potentially have interacted with the sCD4.

The preceding results showed that 10 μg/ml sCD4 had little or no effect on infection of monocytes by any of six different HIV isolates by three different criteria: p24 Ag release, virus-induced cytopathic effects, and the frequency of infected cells that express HIV-specific mRNA. The role of plasma membrane CD4 in the initiation of infection by HIV in monocytes was further examined by two different approaches by block these receptors: a)

competitive inhibition by excess HTLV-IIIB, an HIV strain that interacts with CD4 receptors in many different cell lines but fails to infect monocytes, and b) use of mAb that bind to epitopes on CD4 close to or at the site of binding for virus gp120. HTLV-IIIB does not grow in monocytes, but it does enter these cells and is present in
endocytic vacuoles shortly after adsorption (14). We exposed monocytes to HTLV-IIIB at an inoculum titer 1000- to 10,000-fold more than that used for any of the monocyte-tropic HIV isolates (Table I). The time course and ultimate levels of p24 Ag released into monocyte culture fluids after infection with any of the monocyte-tropic HIV isolates were unchanged by prior exposure of these cells to an excess of HTLV-IIIB. No p24 Ag was detected in culture fluids of monocytes exposed to HTLV-IIIB alone through 3 wk. Thus, if HTLV-IIIB occupied the CD4 receptors on the monocytes, such receptor occupancy did not affect the ability of monocyte-tropic HIV isolates to initiate a productive infection.

These observations raise the possibility of another receptor or mechanism of entry for HIV in monocytes independent of CD4. However, this possibility was made less likely by subsequent experiments with mAb directed against the gp120 binding site on CD4. Infection of monocytes by monocyte-tropic HIV isolates was completely abrogated by prior treatment of cells with the mAb, Leu 3a (Table I) or OKT4a (data not shown). As a control, equal amounts of mAb directed against HLA-DR had no effect.

These data document an apparent paradox. mAb against CD4 completely inhibited infection of any of three different monocyte-tropic HIV isolates in monocytes. This observation strongly suggests that the interaction between HIV and monocyte CD4 is a necessary event for the initiation of infection. Yet, sCD4 at levels 1000- to 10,000-fold higher than those required to inhibit HTLV-IIIB infection of T cells, fails to inhibit the infection of monocyte-tropic HIV isolates in monocytes. If CD4 is the receptor for HIV on monocytes, then the interaction of sCD4 with monocyte-tropic HIV may differ from that of T cell-tropic HIV (perhaps similar in concept to previously observed differences in efficacy of sCD4 for inhibition of HIV-1 and HIV-2 infection) (10). Furthermore, the interaction of sCD4 which lacks the transmembrane and cytoplasmic sequences of CD4 (6-9) with monocyte-tropic envelope "spikes", the morphologic representation of the radioimmunoprecipitation analysis. Characteristic envelope "spikes", the morphologic representation of gp120, are evident in the HIV released from infected T cells. The progeny virus of HIV-infected monocytes shows little or no "spikes" and are relatively bald (21). These observations document a substantial quantitative decrease in the amount of envelope in progeny virus released from infected monocytes and may explain differences in the interaction of sCD4 with the gp120 of monocyte and T cell-tropic HIV.

Monocyte-tropic HIV isolates preferentially infect monocytes, but will also infect T cells (18). With certain

### Table 1

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<tr>
<th>HIV Isolate</th>
<th>Release of p24 Ag (ng/ml) in cultures treated with Leu 3a anti-CD4</th>
<th>anti-HLA DR</th>
<th>HTLV-IIIB</th>
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<td>ADA</td>
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<tr>
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*PBMC purified to >98% monocytes were incubated as adherent monolayers in medium with 10% human serum, and 1000 U/ml MCF. At 7 to 10 days, monocytes were treated with dilutions of Leu 3a anti-CD4 or anti-HLA DR (Becton Dickinson Immunocytochemistry Systems, Mountain View, CA) or 1 x 10^3 TCID_{50} HTLV-IIIB. After 30-min treatment, monocytes were exposed to 1 x 10^3 TCID_{50} of three different monocyte-tropic HIV isolates. After virus adsorption, cultures were washed and refed with fresh medium every 2 to 3 days through 3 wk. Monocytes were continuously exposed to dilutions of mAb throughout the 3-wk interval. Levels of p24 Ag in culture fluids were determined by ELISA. The p24 Ag in monocyte cultures treated with HTLV-IIIB alone was <0.05 ng/ml through 3 wk. Results in Table I represent one of four replicate experiments.
monocyte-tropic HIV isolates this amphotropism can be truly equal. In contrast, HIV strains initially isolated in T cells and passed only in T cell targets do not infect monocytes. If the relative inefficiency of sCD4 for inhibition of HIV infection of monocytes is a property of the virus (and not the target cell), then this quality might also be expressed with T cell targets. Monocyte-tropic HIV isolates 36 and ADA also replicate in PHA/IL-2-treated lymphoblasts from PBMC. In contrast to previous observations with the HTLV-III B strain, sCD4 at 10 μg/ml failed to inhibit infection of either monocyte-tropic HIV isolate in T cells. Higher concentrations of sCD4, however, completely prevented infection. No p24 Ag was detected through 2 wk of culture with PHA/IL-2-treated lymphoblasts exposed to monocyte-tropic HIV isolates 36 or ADA pretreated with 625 μg/ml sCD4 (Table II). The concentration of sCD4 required to inhibit 50% maximum levels of p24 Ag in the HIV-infected PHA/IL-2-treated lymphoblast cultures was 180 μg/ml for HIV isolate 36 and 130 μg/ml for HIV isolate ADA. These data strongly suggest that the relative inefficiency of sCD4 for inhibition of monocyte-tropic HIV infection of monocytes or T cells is a property of the virion and not of the target cell. The preceding data is consistent with the hypothesis that HIV interactions with CD4 is an obligate reaction for infection of both T cells and monocytes. None of these observations, however, preclude another, CD4-independent route of infection. The frequency of monocytes that express plasma membrane CD4 may range from 5 to 90% (15, 17, 23–26). Expression of CD4 on infected macrophages in the central nervous system, the predominant target cell for HIV in this tissue, may be especially low (27). HIV may enter macrophages through phagocytosis (14), FcR-mediated endocytosis (26), or interaction with receptors for mannose-lysylated proteins (29). Several recent reports document at least one CD4-independent route of infection in susceptible T cell and monocyte targets (19, 28, 30, 31). Infection of T cells or monocytes with HIV was markedly enhanced (5- to 10-fold increase in RT activity in culture fluids) by sera from certain HIV-infected patients. Antibody-mediated enhancement in both monocytes and T cells was not inhibited by monoclonal anti-CD4 (Leu 3a) (19). In monocytes, antibody-mediated enhancement of HIV infection is inhibited by monoclonal anti-FcRII (the predominant Fc receptor in tissue macrophages and monocytes in culture, but absent on circulating blood monocytes) but not mAb directed against FcRI or FcRII (19). Antibody-mediated enhancement of HIV infection in the myeloid cell line U937, which lacks FcRII but does express FcRI and FcRII, was blocked by heat-aggregated IgG (28). In these studies, the infection pattern of U937 simulates more closely that of T cells rather than that of monocytes or macrophages. U937 is also a susceptible target cell for several T cell-tropic HIV, but not monocyte-tropic viruses (20). Interestingly, infection of U937 by HTLVIII-B or other T cell-tropic HIV is also blocked by relatively low concentrations of sCD4 (10).

By whatever mechanism, the preceding data convincingly shows that sCD4 is much less effective in blocking infection of HIV derived from infected monocytes than of virions derived from T cells. If our results obtained in cell culture systems have significance for patients, then macrophages that serve as reservoirs for HIV throughout the body and may ultimately be responsible for chronic central nervous system disease, may not be protected by sCD4 treatment.

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REFERENCES


