Modulation of the Effector Function of Human Macrophages for *Histoplasma capsulatum* by HIV-1

Role of the Envelope Glycoprotein gp120

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Abstract

We have demonstrated that monocyte-derived macrophages (Mϕ) from HIV+ individuals are deficient in their capacity to phagocytose *Histoplasma capsulatum* (Hc) yeasts, and are more permissive for the intracellular growth of Hc. To determine whether these defects in Mϕ function were caused by HIV infection of the Mϕ and/or by pathological events associated with HIV infection, cultured normal human Mϕ were infected with the HIV-1_{BaL} strain. Virus production, quantified by reverse transcriptase activity and p24 antigen, was evident on day 8 after infection and peaked on day 16. On days 12, 16, and 20 after infection, HIV-1–infected Mϕ were deficient in their capacity to recognize and bind Hc yeasts compared with control Mϕ, and also were more permissive for the intracellular growth of Hc. Culture of normal Mϕ with the envelope glycoprotein gp120 inhibited phagocytosis of Hc yeasts by Mϕ in a concentration-dependent manner, but did not cause more rapid intracellular growth of Hc. Normal Mϕ cultured in the serum of HIV+ individuals with impaired Mϕ function subsequently were deficient in their capacity to phagocytose Hc yeasts, and were more permissive for the intracellular growth of yeasts compared with Mϕ cultured in normal serum. Conversely, culture of normal Mϕ in the serum of HIV+ patients with normal Mϕ function did not affect the interaction of Hc yeasts with Mϕ. Moreover, when Mϕ from HIV+ individuals that were initially defective in host defense against Hc were cultured in normal HIV− serum, normal Mϕ function was demonstrated. Adsorption of gp120 from the serum of two HIV+ patients removed the capacity of the serum to cause a Mϕ defect in phagocytosis of Hc, but had no effect on the capacity of the serum to cause accelerated intracellular growth. These data demonstrate that observed defects in Mϕ interaction with Hc yeasts may be caused by gp120 and other, as yet unknown serum component(s) probably released into serum by HIV-infected cells. (*J. Clin. Invest.* 1997. 100:1465–1474.) Key words: monocytes • histoplasmosis • phagocytosis • AIDS • retrovirus

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Introduction

Histoplasma capsulatum (Hc)[1] is a dimorphic fungal pathogen of worldwide importance that causes a broad spectrum of disease activity. In the United States, Hc is endemic to the Ohio and Mississippi River Valleys, and it has been estimated that 500,000 new infections occur each year (1). Although the course of infection is mild in most immunocompetent individuals, Hc may produce progressive disseminated infections in individuals immunocompromised by hematologic malignancies (2–5) and cytotoxic therapy (6–8). More importantly, disseminated histoplasmosis is seen with increasing frequency (5–25% of patients) as a complication of AIDS, particularly in the Ohio and Mississippi River Valleys where Hc is endemic (9–12). Furthermore, AIDS patients with disseminated histoplasmosis tend to have a high rate of relapse even after apparently successful therapy with amphotericin B (9).

Monocyte/macrophages (Mϕ) are the final effector cells in mediating the clearance of many of the opportunistic pathogens that infect HIV+ individuals. In addition, in vivo, Mϕ serve as an important reservoir for viral persistence and dissemination (13–15). Thus, a critical concern for the host is the functional capacity of Mϕ from HIV+ individuals to perform their normal functions in host defense, particularly with respect to their capacity to destroy opportunistic pathogens. The susceptibility of HIV+ patients to histoplasmosis may be related to intrinsic defects in the function of monocyte/Mϕ, or related to the generalized impairment of cell-mediated immunity.

We have demonstrated previously that Mϕ from HIV+ patients exhibit two functional defects in their ability to interact with Hc yeasts. First, Mϕ were profoundly deficient in their capacity to recognize and bind Hc yeasts. Second, Mϕ were more permissive for the intracellular growth of Hc (16). It was not clear from these initial studies whether the defects in Mϕ function were caused by HIV-1 infection of Mϕ and/or by other pathological events associated with HIV infection. Unlike CD4+ T lymphocytes, which are the major reservoir of HIV-1, $< 1\%$ of monocytes and only $\sim 10\%$ of tissue Mϕ are infected with HIV-1 (14, 17–19), implying that factors other than direct infection with HIV-1 may be involved in Mϕ dysfunction against Hc or other opportunistic pathogens of AIDS patients.

The purpose of this study was to determine if HIV-1 infection alone and/or exposure of normal Mϕ to components of HIV-1 was sufficient to cause Mϕ dysfunction with respect to host defense against Hc. Our results demonstrate that not only does infection with HIV-1 impair Mϕ interaction with Hc

1. *Abbreviations used in this paper:* ASI, association index; HBSA, HBSS containing 0.25% BSA; Hc, *Histoplasma capsulatum*; Mϕ, macrophage; RT, reverse transcriptase.

yeasts, but culture of normal Mϕ with HIV-1 viral products found in AIDS patients' sera is sufficient to induce abnormal Mϕ function.

Methods

HIV-1. The monocytotropic strain HIV-1_{BaL} was obtained from Dr. Roger Floyd (Clinical Virology Laboratory, University of Cincinnati Hospital). Virus stock was grown in PHA and IL-2–stimulated PBMC from normal human donors (18). Supernatant was harvested at the peak of infectivity (20–30 d after infection), and cellular debris was pelleted by centrifugation. Growth of the virus was quantified by reverse transcriptase (RT) activity (20) as follows. 10 μl of culture supernatant was added to 50 μl of a cocktail containing poly(A), oligo(dt) (Pharmacia Biotech, Piscataway, NJ), $MgCl_2$, Nonidet P-40, and [^{32}P]dTTP (Dupont/New England Nuclear Research Products, Boston, MA) and incubated for 24 h at 37°C. Then, 10 μl of the reaction mixture was spotted on DE81 paper (Whatman International, Maidstone, United Kingdom) and air dried. The filters were washed twice in saline citrate buffer (0.3 M NaCl/0.03 M sodium citrate), twice in 95% ethanol, and dried. The filters were cut and placed into scintillation vials, scintillation cocktail added, and radioactivity was quantified in a liquid scintillation spectrometer (model LS7000; Beckman Instruments, Fullerton, CA). Supernatant containing 8×10^6 cpm/ml was filtered though a 0.22-μm filter and stored at −80°C.

HIV reagents. Recombinant nonglycosylated gp120 and p24 antigen (HIV-1) expressed in a baculovirus system were purchased from Intracel Corp. (Cambridge, MA). Glycosylated rgp120 expressed in Chinese hamster ovary cells was obtained from the NIH AIDS Research and Reference Reagent Program (Bethesda, MD). This "natural" recombinant gp120 was engineered for use as an AIDS vaccine, and by all functional criteria tested is indistinguishable from monomeric gp120 shed from HIV-1 virions (21).

Hc yeasts. Hc strain G217B was maintained as described (22). Yeasts were grown in HMM medium (23) at 37°C in a water bath with orbital shaking at 150 rpm. After 48 h, log phase yeasts were harvested by centrifugation, washed three times in HBSS containing 0.25% BSA (HBSA), and resuspended to 30 ml in HBSA. Large aggregates were removed by centrifugation at 200 *g* for 5 min at 4°C. The top 2 ml was removed, and the single cell suspension obtained was standardized to 5×10^4/ml in RPMI 1640 containing 5% heat-inactivated FCS (GIBCO-BRL, Gaithersburg, MD) and 10 μg/ml gentamicin.

Single cell suspensions of yeasts also were heat-killed at 65°C for 1 h and standardized to 2×10^8/ml in PBS containing 0.05% azide. For the phagocytosis assay, heat-killed Hc yeasts (10^8/ml) were incubated in 0.01 mg/ml FITC (Sigma Chemical Co., St. Louis, MO) in 0.5 M carbonate-bicarbonate buffer, pH 9.5, for 15 min at 25°C. The yeasts were washed twice in HBSA and resuspended in HBSA to 10^7/ml (22).

Preparation of serum from HIV+ individuals and controls. Serum from HIV+ individuals was from our earlier study (16) and was a by-product of the cell isolation procedure. After centrifugation of citrate anticoagulated whole blood at 150 *g* for 20 min, platelet-rich plasma was removed, and serum was obtained by incubation with 20 μl/ml of 1 M $CaCl_2$ at 37°C for 30 min. Serum was separated from the fibrin clot and filtered though a 0.45-μm filter, and stored at −80°C until use. Serum from HIV− donors was prepared in an identical manner.

Monocyte isolation and culture. For infection with HIV-1, mononuclear cells were prepared from the peripheral blood of HIV-1 seronegative volunteers as described previously (22, 24, 25). After removal from the Ficoll-Hypaque interface, monocytes were further purified following the procedure described by Hassan et al. (26). PBMC were suspended at $2–4 \times 10^6$/ml in DMEM (GIBCO-BRL) containing 20% horse serum (GIBCO-BRL) and 15 ml was aliquoted in 75-cm^2 tissue culture flasks (Corning Glass Works, Corning, NY) coated with 2% gelatin. After incubation for 1 h at 37°C, nonadherent cells were removed, and adherent cells were washed four times with serum-free DMEM, and once with 10 ml of a 1:1 mixture of 10 mM EDTA in PBS and DMEM with 20% horse serum to remove loosely adherent lymphocytes. Adherent monocytes were detached by incubation for 10 min with 10 ml of the EDTA-DMEM horse serum media, washed once in DMEM, and suspended to 7×10^5/ml in DMEM/20% horse serum. 1-ml aliquots were added to each well of a 24-well tissue culture plate (Costar Corp., Cambridge, MA) containing 12-mm-diameter glass coverslips, and 0.1 ml of monocytes was added to the wells of 96-well tissue culture plates (Corning Glass Works). Monocytes were adhered for 2 h at 37°C in 5% CO_2/95% air, and then cultured in M199 (GIBCO-BRL) containing 10% autologous serum and 10 μg/ml gentamicin. Every 3–4 d, medium was removed and replaced with fresh medium.

Alternatively, Ficoll-Hypaque isolated mononuclear cells were washed in HBSS containing 20 mM Hepes and 10 μg/ml gentamicin (Hanks'/Hepes), and suspended to $3–4 \times 10^6$/ml in Hanks'/Hepes containing 0.1% autologous serum. 1-ml aliquots were added to each well of a 24-well tissue culture plate containing 12-mm-diameter glass coverslips, and 0.1 ml of monocytes was added to the wells of 96-well tissue culture plates. After 1 h of incubation at 37°C, the adherent monocytes were washed vigorously with Hanks'/Hepes to remove the lymphocytes, and then were cultured at 37°C in 5% CO_2/95% air in M199 containing 10% serum and 10 μg/ml gentamicin. On day 3 or 4, the medium was replaced, and the monocyte-derived Mϕ were tested for functional activity on day 7.

HIV-1 infection of monocyte-derived Mϕ. After 7–10 d of culture, Mϕ were incubated with 0.25 ml/well (24-well plates) or 0.05 ml/well (96-well plates) of HIV-1 containing 8×10^6 cpm/ml RT activity. After 3 h of incubation at 37°C, the inoculum was removed and the Mϕ were washed four times with serum-free M199 to remove unadsorbed virus. For each experiment, the final wash supernatant was tested for viral p24 antigen and RT activity, and found to be free of virus. Mϕ then were cultured in M199 containing 10% autologous serum for up to 20 d. Approximately 50% of the culture medium was replaced with fresh medium every 4 d. Samples of culture medium were saved and tested for viral p24 antigen and RT activity. p24 antigen was quantified in culture supernatants using a commercially available ELISA kit (Abbott Laboratories, North Chicago, IL).

Limiting dilution assay to quantify the percentage of Mϕ infected with HIV. 16 d after infection Mϕ were detached from 24-well plates by incubating them for 30 min at 37°C with trypsin/EDTA solution (Sigma Chemical Co.) containing 500 U/ml porcine trypsin and 180 μg/ml of EDTA in PBS. Mϕ were centrifuged at 1,200 rpm and washed twice with RPMI containing 20% heat-inactivated FCS. Five-fold dilutions of HIV-infected Mϕ ranging from 2.5 Mϕ/well to 4×10^4 Mϕ/well were cocultured with 10^6 PHA-stimulated (1–3 d) normal PBMC in RPMI containing 20% heat-inactivated FCS, 5% IL-2, 250 U/ml penicillin, 250 μg/ml streptomycin, and 2 mM glutamine in 24-well plates for 7 d at 37°C. After 1 wk of culture, one-half of the medium was replaced with fresh medium containing 5×10^5 PHA-stimulated PBMC and cultured for another 7 d. Then, culture supernatant was removed and tested for RT activity.

Phagocytosis assay. Phagocytosis of Hc yeasts by HIV-1–infected or uninfected Mϕ was quantified as described previously (22). At various times after infection with HIV-1, infected and control Mϕ were incubated with 1 ml of FITC-labeled heat-killed Hc yeasts (10^7/ml) for 30 min at 37°C. Then, Mϕ were washed twice with HBSA to remove nonadherent yeasts. Trypan blue (1 mg/ml in PBS) was added for 15 min at 25°C to quench the fluorescence of bound but uningested organisms. The monolayers then were washed twice with HBSA and fixed at 4°C in 1% paraformaldehyde. Coverslips were mounted cell-side down onto microscopic slides in 90% glycerol in PBS, and phagocytosis was quantified by phase-contrast and fluorescence microscopy. 100 Mϕ were counted per coverslip, and the number of yeasts ingested or bound but uningested were enumerated. Results are expressed as the association index (ASI, the total number of bound plus ingested yeasts per 100 Mϕ).

Quantitation of intracellular growth of Hc yeasts in Mϕ. Intracellular growth of Hc yeasts in HIV-1–infected and control Mϕ was quantified by the incorporation of [^{3}H]leucine (24, 25). Mϕ cultured in 96-well plates were washed with RPMI and incubated with 5×10^3 viable yeasts. After incubation for 24 h at 37°C, the plates were centrifuged, and the supernatants were carefully aspirated though a 27-gauge needle. 50 μl (1.0 μCi) of [^{3}H]leucine (specific activity, 153 Ci/nmol; Dupont/New England Nuclear) in sterile water and 5 μl of a 10× yeast nitrogen broth (Difco Laboratories, Detroit, MI) were added to each well. After further incubation for 24 h at 37°C, 50 μl of L-leucine (10 mg/ml) and 50 μl of sodium hypochlorite were added to each well. The contents of the wells were harvested onto glass fiber filters using an automated harvester (Skatron, Sterling, VA). The filters were placed into scintillation vials, scintillation cocktail was added, and the vials were counted in a liquid scintillation spectrometer (model LS7000; Beckman Instruments). The results are expressed as the cpm incorporated by Hc yeasts in Mϕ infected with HIV-1 compared with uninfected controls.

Adsorption of gp120 from the sera of HIV+ individuals. Hybridoma cells producing anti-gp120 mAb were a generous gift from Suzanne Epstein (National Institutes of Health). After recloning in culture, 2×10^7 hybridoma cells were injected intraperitoneally into pristane primed female balb/c mice. IgG was purified from ascites fluid by caprylic acid and ammonium sulfate precipitation (27). Contaminating albumin was removed by molecular sieve chromatography on a Sephadex G100 column. Fractions containing anti-gp120 mAb were detected by double diffusion in agar using a rabbit anti–mouse IgG antibody. Fractions containing mAb were pooled and concentrated on an Amicon YM30 filter, and protein concentration was determined at 280 nm using an extinction coefficient of 1.4. The mAb was pure as determined by SDS-PAGE and staining with Coomassie blue. Goat anti–human IgG was obtained from Organon-Teknika (West Chester, PA).

Anti-gp120 mAb and anti–human IgG antibody were dialyzed against 0.2 M citrate buffer, pH 6.5, at 4°C overnight, and coupled to cyanogen bromide–activated Sepharose 4B (Pharmacia Biotech) according to the manufacturer's instructions. The affinity columns were arranged in tandem and sera from two HIV+ patients that had previously been found to have abnormal Mϕ function were run over the columns to remove immune-complexed gp120 as well as free gp120. Serum from an HIV− individual prepared in an identical manner served as the control. Sera were concentrated to their original volume and stored at 4°C until use.

Statistical analysis. Statistical analysis of the data was performed using Sigma Stat (Jandel Scientific, San Rafael, CA). Depending on the data, either the standard *t* test or the Mann-Whitney rank sum test was used for comparison of two groups. Results were considered significant at P values of < 0.05.

Results

Kinetics of HIV-1 replication in human Mϕ. Monocytes from normal HIV− individuals were cultured for 10 d and then infected with the HIV-1_{BaL} strain. Fig. 1 shows the time course of virus production in HIV-1–infected Mϕ as quantified by RT activity and p24 antigen concentration. No virus was detected until 8 d after infection, after which virus production increased though day 16, and then slightly declined by day 20. By day 16, 80% of Mϕ were infected with HIV-1 as determined by limiting dilution assay. The viability of HIV-1–infected Mϕ at day 20 after infection was > 95% as determined by trypan blue dye exclusion. In addition, HIV-1 infection did not cause a loss of Mϕ from the monolayers, nor were any cytopathic effects observed.

HIV-1–infected Mϕ are deficient in their capacity to recognize and bind Hc yeasts. The effect of HIV-1 infection on the phagocytosis of heat-killed, unopsonized, FITC-labeled Hc yeasts was quantified in Mϕ from nine individual donors. Phagocytosis was quantified beginning at 8 d after infection since no viral growth was detected earlier. At 8 d after infection, there was no significant difference in the ASI between

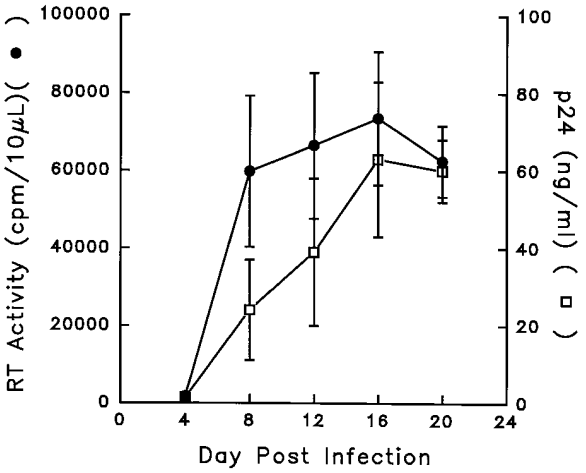

Figure 1. Kinetics of HIV-1 replication in human monocyte-derived Mϕ. 7–10-d cultured Mϕ were infected with HIV-1 containing 8×10^6 cpm/ml RT activity. After 3 h of incubation at 37°C, the inoculum was removed and the Mϕ were washed with M199 to remove nonadsorbed virus. Mϕ then were cultured in M199 containing 10% autologous serum for 20 d. Samples of culture medium were removed on the days shown and tested for viral p24 antigen and RT activity as described in Methods. The data shown are the mean±SEM from four individual donors.

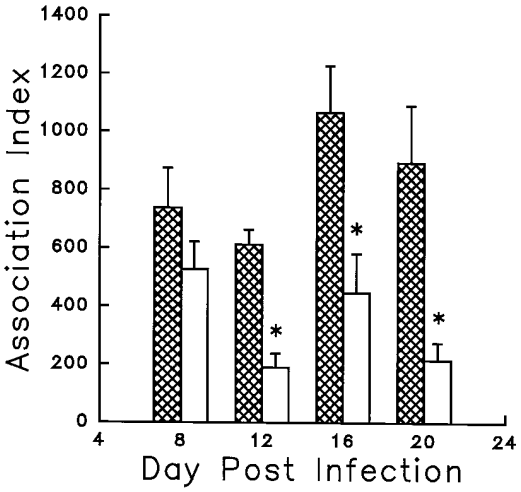

Figure 2. Phagocytosis of Hc yeasts by HIV-infected Mϕ at various times after infection. At the times shown, Mϕ were incubated with 1 ml FITC-labeled heat-killed Hc yeasts (10^7/ml) for 30 min at 37°C. Nonadherent yeasts were removed by washing, and the Mϕ then were incubated with trypan blue for 15 min to quench the fluorescence of bound but uningested yeasts. The monolayers were washed twice more and then fixed at 4°C in 1% paraformaldehyde. Phagocytosis was quantified by phase-contrast and fluorescence microscopy as described in Methods. Results are expressed as the ASI, that is, the total number of bound plus ingested yeasts per 100 Mϕ. The data are the mean±SEM of nine individual experiments. *$P \leq 0.01$ compared with HIV− control Mϕ. *Hatched bars*, HIV− control Mϕ; *white bars*, HIV+ Mϕ.

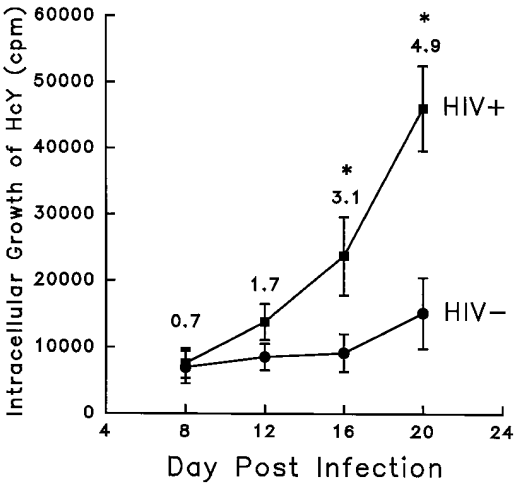

Figure 3. Intracellular growth of Hc yeasts in HIV-infected Mϕ at various times after infection. At the times shown, Mϕ cultured in 96-well plates were washed with RPMI and incubated with 5×10^3 viable Hc yeasts for 24 h at 37°C. The plates then were processed as described in Methods. The results are expressed as the cpm incorporated by Hc yeasts in Mϕ infected with HIV-1 compared with uninfected Mϕ. The data are the mean±SEM of six individual experiments. *$P \leq 0.01$ compared with HIV− Mϕ.

control and HIV-1–infected Mϕ (Fig. 2). However, at 12, 16, and 20 d after infection, the ASI of HIV-1–infected Mϕ was significantly decreased compared with controls ($P \leq 0.01$). As found previously (16), the percentage of attached yeasts that were ingested was the same for both HIV-1–infected and normal Mϕ (data not shown). Thus, Mϕ infected with HIV-1 in vitro were defective in their capacity to recognize and bind Hc yeasts but ingestion of bound yeasts was normal.

HIV-1–infected Mϕ are more permissive for the intracellular growth of Hc than uninfected Mϕ. We next sought to determine if HIV-1 infection also caused Mϕ to become more permissive for the intracellular growth of Hc yeasts. At day 8 after infection, the intracellular growth of Hc yeasts in HIV-1–infected and control Mϕ was identical (Fig. 3). However, at 12, 16, and 20 d after infection, yeasts multiplied more rapidly in HIV-1–infected Mϕ as evidenced by 1.7-, 3.1-, and 4.9-fold increases, respectively, in cpm compared with control Mϕ.

HIV-1 gp120 impairs Mϕ phagocytosis of Hc yeasts. Studies with other microorganisms have demonstrated that the envelope glycoprotein gp120 can modulate Mϕ phagocytosis and antimicrobial activity through binding to Mϕ CD4 receptors (28, 29). Therefore, we sought to determine if gp120 might impair Mϕ interaction with Hc. Mϕ from normal donors were cultured for 7 d, and varying concentrations of nonglycosylated rgp120 were added for the final 2 d of culture. Then, the Mϕ were tested for their capacity to bind and ingest Hc yeasts. Coculture of Mϕ with rgp120 resulted in a concentration-dependent inhibition of the ASI (Fig. 4 *A*). At 400 ng/ml, the ASI was inhibited by 50%, but this was not statistically significant. Concentrations of rgp120 up to 1 μg/ml did not cause further inhibition of phagocytosis (data not shown). In addition, incubation of Mϕ with rgp120 for the entire 7-d culture period caused no additional decrease in the ASI (data not shown).

However, when Mϕ were cocultured for the last 2 d with varying concentrations of glycosylated rgp120, phagocytosis of Hc yeasts was decreased by 79% with as little as 10 ng/ml of gp120 (Fig. 4 *B*). Higher concentrations of gp120 did not further decrease Mϕ function (data not shown). Thus, glycosylated rgp120 inhibited Mϕ function at considerably lower concentrations and to a greater extent than nonglycosylated rgp120. Culture of Mϕ with the HIV-1 core protein p24 had no effect on Mϕ binding of Hc (data not shown).

In contrast to its effects on Mϕ phagocytosis of Hc yeasts, neither glycosylated nor nonglycosylated rgp120 induced accelerated intracellular growth of Hc (Table I). p24 antigen, at a concentration up to 1 μg/ml also did not cause an increase in the rate of intracellular growth of Hc yeasts (data not shown).

In vitro differentiation of monocytes from normal HIV− individuals in serum from HIV+ patients induces defects in Mϕ interactions with Hc. In our previous study demonstrating abnormal function of Mϕ from HIV+ individuals (16), mono-

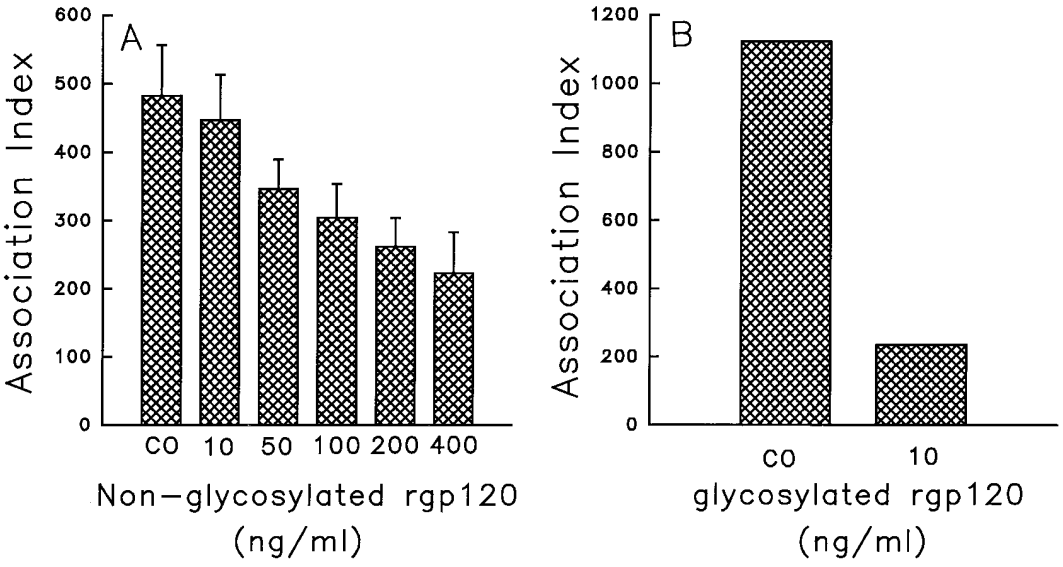

Figure 4. Inhibition of Mϕ phagocytosis of Hc yeasts after culture of Mϕ for 48 h with recombinant or native gp120. Freshly isolated monocytes from normal HIV− donors were cultured for 5 d. Then, varying concentrations of recombinant or native gp120 were added for the last 2 d of culture. Mϕ then were tested for their capacity to bind and ingest yeasts as described in the legend to Fig. 2. The data are the mean±SEM of five experiments with rgp120 and the mean of two experiments with native gp120.

Table I. gp120 Does Not Induce Mϕ to Become More Permissive for the Intracellular Growth of Hc Yeasts

Concentration	cpm*	
	Nonglycosylated rgp120	Glycosylated rgp120
ng/ml		
Control	4848±871	10699±4392
10	4903±939	7642±2691
100	5284±620	10543±4037
1000	5661±422	10720±4206

Freshly isolated monocytes from normal HIV− donors were cultured for 5 d, and then varying concentrations of glycosylated or nonglycosylated rgp120 were added for the last 2 d of culture. Mϕ then were incubated with 5×10^3 viable Hc yeasts for 24 h at 37°C, and intracellular growth was quantified as described in Methods. *Mean±SEM ($n = 4$).

cytes from patients and controls were cultured in autologous serum to avoid possible effects on Mϕ function induced by culture in heterologous sera. As gp120 is present in the serum of HIV+ patients at concentrations ranging from 1 to 100 ng/ml (30), we considered the possibility that the defects observed in Mϕ function in the earlier study (16) and in the present experiments might be induced by viral products secreted into the serum of HIV+ individuals.

Therefore, normal monocytes were allowed to differentiate in vitro in medium containing 10% autologous serum, 10% heterologous sera from normal HIV− donors, and in 10% sera from 13 HIV+ patients. After 7 d in culture, the monocyte-derived Mϕ were tested for their capacity to interact with Hc yeasts. For the phagocytosis experiments, the sera from the HIV+ patients were divided into two groups based on the interaction of their Mϕ with Hc yeasts as observed previously (16). Group 1 consisted of sera from seven HIV+ patients whose CD4+ T cell counts were < 200 and their Mϕ were defective in recognizing and binding Hc. Group 2 consisted of sera from six HIV+ patients whose CD4+ T cell counts were > 200, and whose Mϕ recognized and bound Hc as efficiently as control Mϕ.

First, there was no significant difference between the ASI of normal Mϕ cultured in autologous vs. heterologous normal HIV− sera (Fig. 5, *A* and *B*). However, the mean ASI of Mϕ cultured in six of seven patients' sera from group 1 were significantly ($P \leq 0.01$) decreased compared with the ASI of control Mϕ (Fig. 5 *A*). Likewise, the mean ASI of normal Mϕ cultured in five of six patients' sera from group 2 were not significantly different from control Mϕ (Fig. 5 *B*).

Next, we determined the effect of culturing normal HIV− monocytes in the presence of HIV+ patient sera on the intracellular growth of Hc yeasts. Sera from 13 HIV+ individuals were studied. Sera 1–9 (Fig. 6, *Perm. Pts.*) were from HIV+ patients whose Mϕ permitted accelerated intracellular growth of Hc yeasts (16), and sera 10–13 (Fig. 6, *NP Pts.*) were from HIV+ patients whose Mϕ previously demonstrated normal intracellular growth of Hc (16). There was no difference in the intracellular growth of yeasts in Mϕ cultured in autologous versus heterologous normal HIV− sera (Fig. 6, *Controls*). However, the mean cpm from eight of nine patients' sera in the permissive group were significantly ($P < 0.05$) higher than

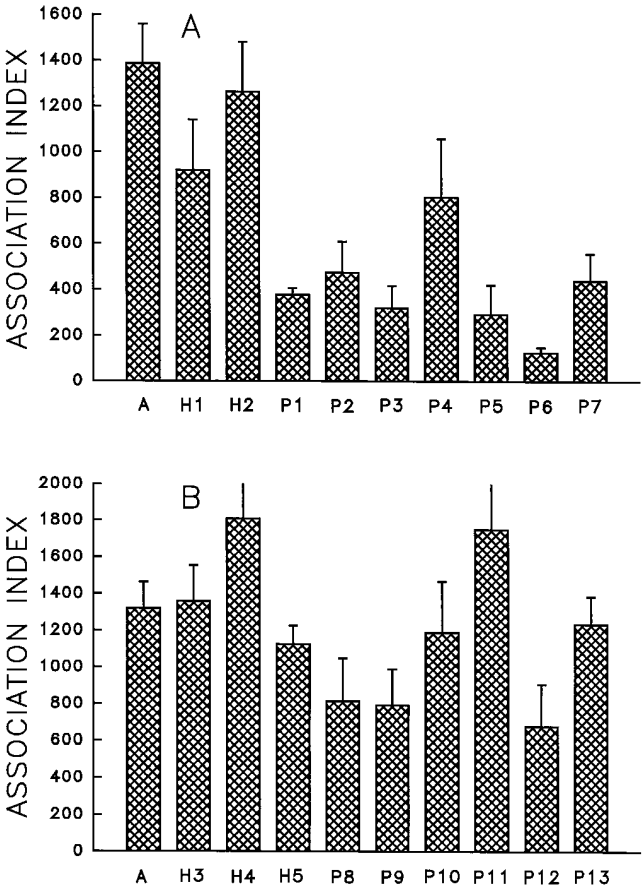

Figure 5. Culture of normal HIV− monocytes in serum from HIV+ individuals inhibits Mϕ phagocytosis of Hc yeasts. Freshly isolated monocytes from normal HIV− donors were cultured in media containing 10% autologous serum, 10% heterologous serum from HIV− donors, or 10% heterologous serum from HIV+ individuals. After 7 d of culture, phagocytosis of Hc yeasts was quantified as described in the legend to Fig. 2. *A* is sera from HIV+ patients who demonstrated a defect in phagocytosis in the earlier study (16). The ASI of all patients' sera except patient 4 was significantly ($P \leq 0.01$) decreased compared with the autologous serum control. *B* is sera from HIV+ patients who exhibited normal phagocytosis of Hc yeasts in the previous study (16). Only the ASI of patient 12 was significantly ($P = 0.037$) different from the autologous serum control. The data are the mean±SEM of five individual experiments in *A* and six experiments in *B*. *A*, Autologous serum; *H*, heterologous serum; *P*, HIV+ patient serum.

the mean for the control group. Likewise, the mean cpm of three of four patients' sera in the nonpermissive group were not significantly different from the mean of the controls.

Mϕ from HIV+ patients cultured in normal serum demonstrate normal interactions with Hc yeasts. As culture of normal Mϕ in HIV+ patient serum induced abnormal Mϕ function, we sought to determine if Mϕ from HIV+ individuals might demonstrate normal function if cultured in serum from HIV− persons. Monocytes from HIV+ patients previously found to be defective in recognition of yeasts, and more permissive for the intracellular growth of Hc, were cultured in their own serum and heterologous serum from an HIV− individual. Simultaneously, monocytes from the HIV− individual

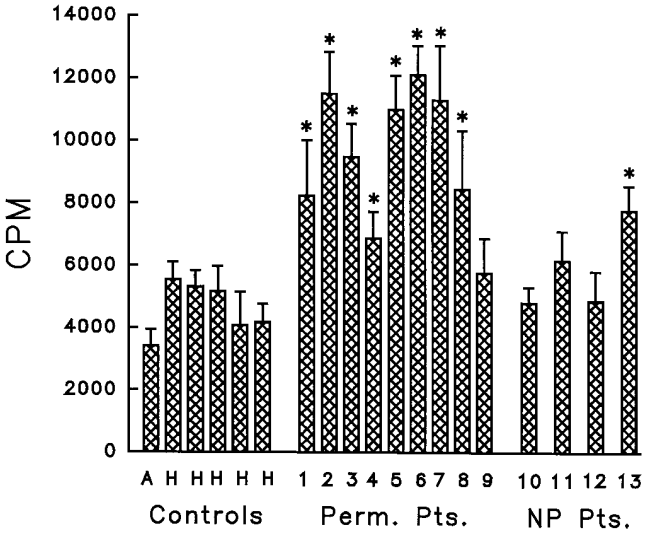

Figure 6. Culture of normal HIV− monocytes in serum from HIV+ individuals accelerates the rate of intracellular growth of Hc yeasts in Mϕ. Freshly isolated monocytes from normal HIV− donors were cultured in media containing 10% autologous serum, 10% heterologous serum from HIV− donors, or 10% heterologous serum from HIV+ individuals. After 7 d of culture, the intracellular growth of Hc yeasts was quantified as described in the legend to Fig. 3. *A*, Autologous serum; *H*, heterologous serum; *Perm. Pts.* (permissive patients), serum from HIV+ individuals whose Mϕ permitted accelerated replication of Hc yeasts in a previous study (16); *NP Pts.* (nonpermissive patients), serum from HIV+ individuals whose Mϕ demonstrated normal intracellular growth of Hc yeasts previously (16). The data are the mean±SEM of four individual experiments. $*P < 0.05$ compared with the mean of the controls.

also were cultured in both sera. After 7 d of culture, the monocyte-derived Mϕ were evaluated for their phagocytic and fungistatic activity against Hc yeasts. As found previously, Mϕ from HIV+ patients cultured in autologous serum were defective in binding Hc yeasts compared with normal Mϕ cultured in normal serum (Fig. 7). In contrast, HIV+ Mϕ cultured in normal serum demonstrated a normal capacity to bind and ingest Hc yeasts. As before, the ASI of normal Mϕ cultured in HIV+ patients sera was decreased (Fig. 7). Likewise, normal intracellular growth of Hc yeasts was observed when monocytes from either HIV+ patients or HIV− individuals were cultured in normal HIV− serum, and more rapid intracellular growth was evident in Mϕ derived from HIV+ or HIV− individuals when the monocytes were cultured in HIV+ patient serum (Fig. 8).

Adsorption of gp120 from HIV+ patient serum restores the capacity of Mϕ to bind and ingest Hc yeasts. The above data suggest that the ability of HIV+ patient serum to ablate the capacity of normal Mϕ to recognize and bind Hc yeasts may be mediated gp120. To further test this hypothesis, gp120 was removed from the sera of two HIV+ individuals by adsorption on affinity columns containing mAb to gp120 and polyclonal antibody to human IgG. The latter column was included to insure the removal of gp120 in immune complexes. Serum from an HIV− donor was prepared in a similar manner. Monocytes from an HIV− donor then were cultured in autologous serum, and heterologous serum and both HIV+ sera before and after adsorption on the affinity columns. Binding and ingestion of Hc yeasts and the intracellular growth of Hc then was quantified after 7 d in culture.

The ASI of Mϕ cultured in autologous serum, heterologous serum, and adsorbed heterologous serum was identical (Fig. 9). Addition of 400 ng/ml of rgp120 to adsorbed heterologous serum decreased the ASI by 67%. As before, the ASI of normal Mϕ cultured in HIV+ patient serum was greatly decreased. However, the ASI of Mϕ cultured in adsorbed HIV+ patient sera was restored to normal. Furthermore, readdition

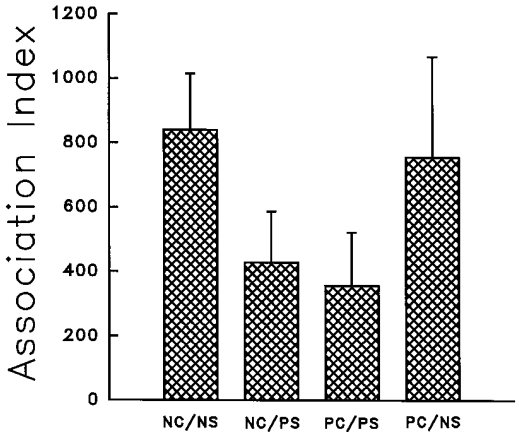

Figure 7. Culture of monocytes from HIV+ individuals in normal serum results in normal Mϕ phagocytosis of Hc yeasts. Freshly isolated monocytes from HIV+ and HIV− donors were cultured in media containing 10% autologous serum or 10% heterologous serum. After 7 d of culture, phagocytosis of Hc yeasts was quantified as described in the legend to Fig. 2. The data are the mean±SEM of thee individual experiments. *NC*, normal (HIV−) cells; *NS*, normal (HIV−) serum; *PC*, patient (HIV+) cells; *PS*, patient (HIV+) serum.

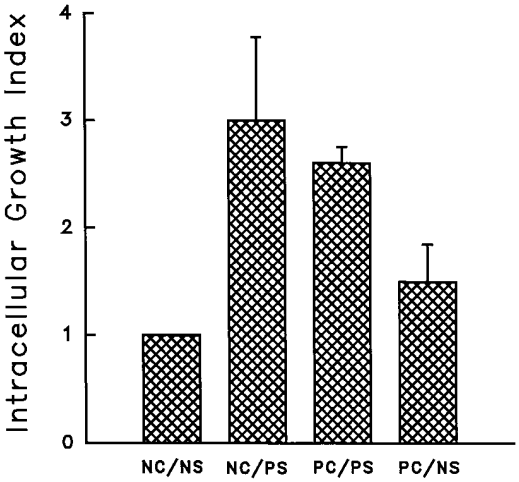

Figure 8. Culture of monocytes from HIV+ individuals in HIV− serum results in normal intracellular growth of Hc in Mϕ. Freshly isolated monocytes from HIV+ and HIV− donors were cultured in media containing 10% autologous serum or 10% heterologous serum. After 7 d of culture, the intracellular growth of Hc yeasts was quantified as described in the legend to Fig. 3. Because of the wide variation in cpm in the controls (HIV− monocytes and serum), the data are presented as an index in which the controls are arbitrarily designated as 1. Thus, the numbers in the index represent the fold increase in cpm compared with the controls. The data are the mean±SEM of four individual experiments. Abbreviations are the same as for Fig. 7.

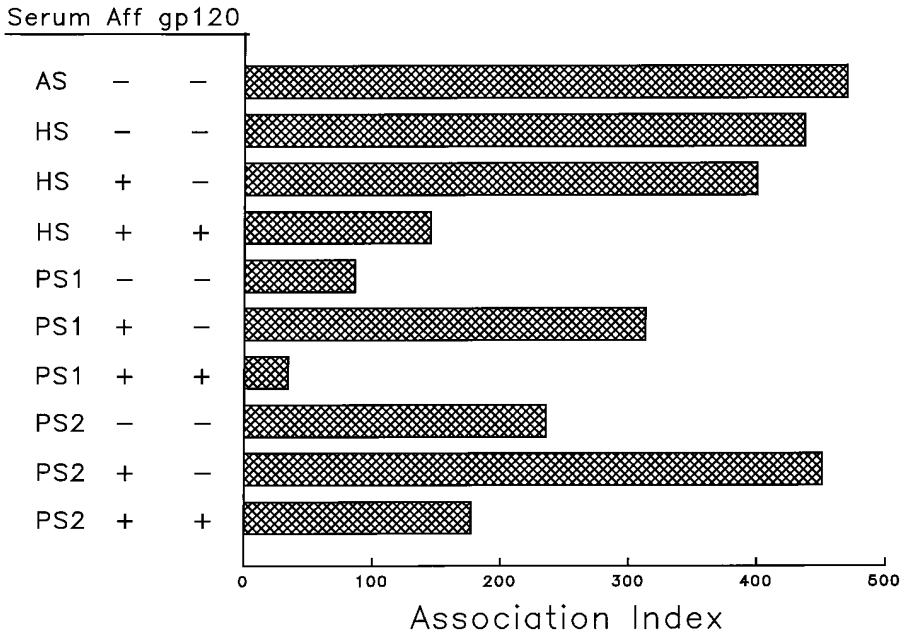

Figure 9. Adsorption of gp120 from HIV+ serum restores the capacity of Mϕ to phagocytose Hc yeasts. Sera from two HIV+ individuals and one HIV− individual were run over two affinity columns arranged in tandem containing anti-gp120 mAb and goat anti–human IgG, respectively. The sera then were concentrated to their original volume. Freshly isolated normal HIV− monocytes were cultured in autologous serum, heterologous serum, and HIV+ sera before and after adsorption on the affinity columns. After 7 d of culture, phagocytosis of Hc yeasts was quantified as described in the legend to Fig. 2. The data are the mean from two experiments with monocytes from different donors.

of gp120 to adsorbed HIV+ patient sera decreased the ASI to levels lower than with untreated HIV+ patient serum (Fig. 9). In contrast, removal of gp120 from HIV+ patient serum did not effect the intracellular growth of Hc yeasts. Regardless of treatment, the cpm obtained from Mϕ cultured in HIV+ patient serum was fourfold higher than the cpm obtained when Mϕ were cultured in autologous or heterologous serum (Table II).

Discussion

The human immunodeficiency virus causes immune dysfunction, predominantly through a selective depletion of CD4+ T lymphocytes, that are an essential component of the cell-mediated immune response. This loss of CD4+ T cells is associated with an increased susceptibility to multiple opportunistic infections including *Pneumocystis carinii*, *Toxoplasma gondii*, *Mycobacterium-avium-intracellulare*, *Cryptococcus neoformans*, *Candida albicans*, and *H. capsulatum* (31–33).

Table II. Removal of gp120 from HIV+ Serum Does Not Effect the Intracellular Growth of Hc Yeasts in Mϕ

Serum source	cpm*
AS	4994
HS	4064
HS + Aff Col	4174
HS + Aff Col + gp120	5648
PT	16398
PT + Aff Col	17191
PT + Aff Col + gp120	13756

Sera from an HIV+ individual and an HIV− individual were run over two affinity columns arranged in tandem containing anti-gp120 mAb and goat anti–human IgG, respectively. The sera then were concentrated to their original volume. Freshly isolated normal HIV− monocytes were cultured in autologous serum, heterologous serum, and HIV+ serum before and after adsorption on the affinity columns. In some wells containing adsorbed serum, rgp120 (400 ng/ml) was added back to the medium. After 7 d of culture, the intracellular growth of Hc yeasts was quantified as described in Methods. *AS*, Autologous serum; *HS*, heterologous serum; *PT*, patient (HIV+) serum. *Mean of two experiments with different donors.

In contrast, the role of mononuclear phagocytes in the pathogenesis of HIV is less clear. Monocyte/Mϕ are critical components of host defense and are important in the killing of microbial pathogens, presentation of antigen, and in the release of diverse bioactive cytokines in the normally intact immune system. Normal Mϕ function is required for the clearance and killing of many infectious agents including pathogens frequently seen in patients with AIDS. Because of their resistance to the cytopathic effects of HIV, Mϕ may serve as a reservoir for HIV and as a cellular mediator of HIV transmission (13–15). Thus, a critical concern for the host is the functional integrity of HIV-infected Mϕ to perform their normal functions in host defense, particularly with respect to their capacity to destroy opportunistic pathogens.

With respect to host defense against Hc, we hypothesized that HIV infection of Mϕ might induce intrinsic defects that would modulate Mϕ interaction with Hc yeasts. Thus, there might be a defect in phagocytosis of Hc yeasts. This could be a defect in recognition of the yeasts, or in ingestion of the yeasts after binding to the Mϕ surface. Second, HIV-infected Mϕ could become more permissive for the intracellular growth of Hc yeasts than normal Mϕ. Third, HIV-infected Mϕ may not become activated by cytokines to inhibit the intracellular growth of Hc yeasts. None of these hypotheses were mutually exclusive.

These hypotheses were tested in a previous study (16), in which peripheral blood monocytes from HIV+ and HIV− individuals were cultured for 7 d in medium containing autologous serum, and then the monocyte-derived Mϕ were studied for their interaction with Hc yeasts. The results of these experiments demonstrated that Mϕ from HIV+ individuals were defective in their capacity to recognize and bind Hc yeasts, and were more permissive for the intracellular growth of yeasts

compared with Mϕ from HIV− individuals. Since < 1% of monocytes and only ∼ 10% of tissue Mϕ are infected with HIV-1 (14, 17–19), it was unclear from this study whether infection with HIV alone was sufficient to cause these impairments in Mϕ function. Furthermore, since we had cultured monocytes from both HIV+ and HIV− individuals in their own serum, it was not clear from these experiments whether or not viral products found in HIV+ serum influenced the results.

Therefore, this study was initiated to determine if HIV infection alone and/or components of HIV-1 were sufficient to induce Mϕ dysfunction with respect to host defense against Hc. The results presented herein clearly demonstrate that HIV-1 infection of normal human Mϕ impairs their capacity to recognize and bind Hc yeasts, and causes accelerated intracellular replication of Hc. Mϕ dysfunction was first detected at 12 d after infection, and continued through day 20. These results complement other reports that in vitro infection of Mϕ with HIV-1 is associated with defective phagocytosis of *C. albicans* (34) and heat-killed *T. gondii* (35).

Hc yeasts bind to Mϕ via CD11a/CD18 (LFA-1), CD11b/CD18 (CR3), CD11c/CD18 (P150,95) family of adhesion promoting glycoproteins (22, 36). Thus, decreased binding may be caused by a loss of individual CD18 receptors; a loss in the capacity of one or more of the receptors to recognize the ligand(s) on the surface of Hc yeasts; or one or more of the CD18 receptors may have lost its ability to mobilize within the plasma membrane, thereby reducing the overall efficiency of CD18 receptors in mediating the attachment of Hc (36).

Studies of monocytes (37–40) and alveolar Mϕ (39) from HIV+ individuals have reported normal levels of CD11b, and CD11c has been found to be both decreased (40) and increased (37, 41) on monocytes from AIDS patients. Monocytes from asymptomatic HIV+ individuals have been found to have increased levels of CD11a, CD11b, and CD11c (41, 42). Others have demonstrated decreased levels of CD11a on monocytes and alveolar Mϕ from AIDS patients (40). In contrast, in vitro infection of monocyte-derived Mϕ with HIV-1 caused downregulation of CD11b (34, 43). In addition, it was demonstrated that in vivo there is impaired CR3-mediated clearance of ^{51}Cr-labeled iC3b-coated autologous erythrocytes (44). These investigators also found normal levels of CD11b on HIV+ patient monocytes, and, therefore, suggested that there is an impairment of Mϕ phagocytosis. Since our studies used HIV-infected monocyte-derived Mϕ, further studies of these cells are required to determine any putative defects in the expression or function of CD18 receptors that might account for the profound decrease in Mϕ binding of Hc yeasts.

Also, in concordance with the present report, in vitro infection of Mϕ with HIV-1 has been shown to impair the capacity of Mϕ to restrict the intracellular growth of *M. avium* (45, 46), *T. gondii* (35), *Leishmania major* (13), and *Canada pseudotropicalis* (47). Cameron et al. (48) reported decreased anticryptococcal activity by human peritoneal Mϕ and monocyte-derived Mϕ, but not alveolar Mϕ, infected with HIV for at least 14 d. However, no deficiency in fungistasis against *C. neoformans* was found in Mϕ infected with HIV for < 7 d. Likewise, Nottet et al. (49) observed normal killing of opsonized *Escherichia coli* and *C. albicans* by normal Mϕ infected with HIV for only 5–6 d. These results and our own suggest that some threshold level of viral replication may be required to inhibit human Mϕ antimicrobial activity in vitro.

In addition to direct viral infection of Mϕ, culture of Mϕ with the envelope glycoprotein gp120, but not p24 antigen, impaired the capacity of Mϕ to recognize and bind Hc yeasts, but did not cause Mϕ to become more permissive for the intracellular growth of Hc. Furthermore, glycosylated rgp120 was considerably more effective than nonglycosylated rgp120, and caused 90% inhibition of binding at a concentration of 10 ng/ml. These observations are in agreement with other investigators (50, 51) who have demonstrated that the capacity of gp120 to stimulate human mononuclear cells to secrete cytokines depends upon its primary protein structure, as well as posttranslational modification. Thus, glycosylated rgp120 stimulated monocytes to release cytokines, whereas nonglycosylated rgp120 expressed in a baculovirus system failed to stimulate monokine production (51). Thus, our data provide further confirmation for the apparent importance of posttranslational modification in the biological activity of this glycoprotein.

In contrast to our results, other investigators have found that gp120 modulates both the phagocytic and fungistatic properties of Mϕ against *C. neoformans* (28) and *M. avium* (29). Wagner et al. (28) reported that culture of human alveolar Mϕ with rgp120 for 60 min decreased Mϕ fungistatic activity against *C. neoformans*, and decreased the ingestion of, but not the attachment of *C. neoformans* yeasts. Likewise, Shiratsuchi et al. (29) found that culture of human monocytes with gp120 for 2 d inhibited the phagocytosis of *M. avium*, and enhanced intracellular replication. Although the exact mechanism(s) is unknown, both reports demonstrated that the effects of gp120 were mediated, in part, through binding of gp120 to Mϕ CD4 (28, 29). In agreement with the present study, the latter group also found that pretreatment with p24 did not affect monocyte interactions with *M. avium*.

As the concentration of gp120 in serum ranges from 1 to 100 ng/ml (30), there clearly is sufficient gp120 in HIV+ sera to induce the observed defect in Mϕ function. Indeed, culture of normal HIV− monocytes into Mϕ in the presence of HIV+ serum resulted in decreased binding of Hc to Mϕ, and induced the Mϕ to become more permissive for the intracellular growth of Hc. Conversely, culture of monocytes from HIV+ patients in the presence of normal HIV− serum resulted in normal Mϕ function. These data demonstrate that observed defects in Mϕ function with respect to host defense against Hc do not require active infection of the Mϕ by HIV-1, but merely exposure to components of the virus. This conclusion is further supported by the fact that adsorption of gp120 from HIV+ serum before culture with normal HIV− monocytes completely abrogates the ability of the HIV+ serum to decrease the capacity of Mϕ to recognize and bind Hc yeasts. Thus, gp120 is probably the sole factor in HIV+ serum that induces the phagocytic defect in normal HIV− Mϕ.

Although the mechanism of dissemination of Hc yeasts from the lung is not completely understood, it has been presumed to occur within blood-borne Mϕ. However, both patient studies (52, 53) and studies with CD4 lymphocyte–depleted mice (54) have reported an abundance of extracellular as well as intracellular organisms in the lung. As dissemination of Hc yeasts almost always occurs in AIDS patients, these findings suggest that extracellular Hc may play a role in the pathogenesis of disease in immunocompromised hosts. Hc yeasts bind avidly to the extracellular matrix protein laminin, and it has been proposed that this binding is an important first step in the capacity of the yeasts to recognize and traverse the alveo-

lar basement membrane to disseminate via the circulation (54). In AIDS patients with high levels of free gp120, the inhibition of the uptake of Hc yeasts by Mϕ may increase the number of yeasts that disseminate extracellularly by this proposed route. Furthermore, these extracellular yeasts might escape elimination by cytokine-activated Mϕ further exacerbating the disease state.

The factor(s) in HIV+ serum that cause accelerated growth of Hc yeasts in Mϕ is unknown. However, we have determined that the factor(s) is resistant to heating at 56°C for 1 h. Also, as previous studies from our laboratory have demonstrated the importance of iron for the intracellular survival and replication of Hc yeasts in Mϕ (25, 55), we determined the total iron, total iron binding capacity, and percent saturation in 39 sera from our original study (16). 26 of the sera came from patients with normal Mϕ function, and 13 of the sera were from patients whose Mϕ demonstrated accelerated intracellular growth of Hc yeasts. For all three of the iron parameters quantified, there were no significant differences between the two groups of patients.

We hypothesize that the factor(s) that cause accelerated replication of Hc yeasts in Mϕ may be viral components that are released into serum by HIV-infected cells or, possibly, chemokines or cytokines that are produced as part of the pathophysiologic events that occur during HIV infection of humans. Current experiments are directed toward identifying these factor(s).

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