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Human Immunodeficiency Virus Type 1 Infection of Human Macrophages Modulates the Cytokine Response to *Pneumocystis carinii*

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The present studies examined production of the cytokines tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and IL-6 by human monocyte-derived macrophages exposed to *Pneumocystis carinii* in vitro and the impact of concurrent macrophage infection with human immunodeficiency virus type 1 (HIV-1) on these cytokine responses. Macrophages were infected with the HIV-1 BaL monocytotropic strain for 10 to 14 days and then exposed to *P. carinii*. At various times following *P. carinii* treatment, culture supernatants were harvested to assess the cytokine profile. Addition of *P. carinii* to HIV-uninfected macrophages resulted in augmented production of IL-6, TNF-α, and IL-1β protein. By contrast, in HIV-infected macrophages exposed to *P. carinii*, only the release of IL-6 was increased compared with that for HIV-uninfected macrophages, while the levels of TNF-α and IL-1β decreased. This altered response was confirmed at the molecular level for TNF-α mRNA. Preventing physical contact between *P. carinii* and macrophages by a membrane filter inhibited all cytokine release. Substituting *P. carinii* with a preparation of *P. carinii* 95- to 115-kDa major membrane glycoprotein A yielded a response similar to that obtained by addition of intact *P. carinii*. These results suggest that HIV-1 infection of human macrophages modulates cytokine responses to *P. carinii*.

Pneumocystis carinii pneumonia remains a serious complication in individuals infected with the human immunodeficiency virus type 1 (HIV-1) (9). Despite this circumstance, it is not completely understood how HIV-1 affects the susceptibility of the host to this pathogen. Insight into this process could provide valuable new information about potentially correctable defects in host defenses against *P. carinii* in AIDS. *P. carinii* is an extracellular organism which is dependent upon attachment to alveolar epithelial cells for growth and replication (42). A protective host response against this pathogen likely involves the participation of functional $CD4^+$ T lymphocytes and alveolar macrophages (15). In vitro, normal macrophages are capable of ingesting and digesting *P. carinii* (30, 41). Macrophages may also contribute to the control of *P. carinii* infection by secreting cytokines such as interleukin-1β (IL-1β) (7), which stimulates proinflammatory and immune processes of potential benefit in the host's response to infection, and tumor necrosis factor alpha (TNF-α), which may be directly toxic for *P. carinii* cysts (36). TNF-α has been shown to be spontaneously produced by alveolar macrophages obtained from individuals with AIDS, and elevated levels of this cytokine in bronchoalveolar lavage fluid are associated with reduced numbers of *P. carinii* in respiratory secretions (25, 36).

These findings suggest not only that cytokines are an important aspect of host defense against *P. carinii* but that the modulation of cytokine production by *P. carinii* may be abnormal in the AIDS setting. In the present studies, we evaluated the impact of HIV-1 infection of macrophages on their cytokine-generating capacity during in vitro challenge with *P. carinii*. The goal of these studies was to examine the possibility that the production of potentially protective cytokines following *P. carinii* exposure is altered in HIV-1-infected human macrophages.

MATERIALS AND METHODS

Cells. Human peripheral blood leukocytes were obtained by leukopheresis from healthy, HIV-1-seronegative donors (New England Deaconess Hospital Blood Bank). Following separation using Ficoll-Hypaque gradients (Pharmacia, Uppsala, Sweden), macrophages were isolated from the mononuclear cell preparation by adherence to plastic at 37°C and 5% CO_2. Mononuclear cells were cultured in Iscove's modified Dulbecco's medium (IMDM) (Hyclone Laboratories, Logan, Utah) with 2 mM L-glutamine (Sigma, St. Louis, Mo.) and 10% pooled human male serum (Sigma). Aliquots of 5×10^7, 1.2×10^7, or 0.4×10^7 mononuclear cells (30% monocytes, determined by differential counting prior to plating) were seeded into 75-cm^2 flasks, six-well tissue culture plates, or 24-well tissue culture plates (Costar), respectively. The nonadherent cells were removed after 48 h, and the adherent cells were cultured for an additional 5 to 7 days before HIV-1 infection. The purified adherent cell population consisted of >99% macrophages, as determined by nonspecific esterase staining (kit no. 91-A; Sigma). All media, culture additives, reagents, and buffers were endotoxin-free as assayed by the LAL method (Whitaker Bioproducts, Walkersville, Md.). The sensitivity of the assay was <1 pg of endotoxin per ml.

HIV-1 infection. The macrophage-tropic HIV-1 BaL strain (S. Gartner, M. Popovic, and R. Gallo, National Cancer Institute, Bethesda, Md.) was used for infection. Stock virus was prepared from culture supernatants of chronically infected primary macrophages from normal human donors, aliquoted, and frozen at −70°C until used. Briefly, macrophage culture supernatants were removed and replaced by 5, 1.2, and 0.4 ml

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of stock virus in 75-cm^2 flasks, 6-well plates, and 24-well plates, respectively. Following 4 h of incubation at 37°C and 5% CO_2, adherent macrophage cultures were washed two times with IMDM without serum, and IMDM with 5% human serum was added to cultures, which were maintained at 37°C and 5% CO_2. Macrophages were cultured for 10 to 14 days post-HIV-1-infection to ensure that they were chronically infected. p24 determinations were made to assess productive HIV-1 replication. It has been shown that HIV expression in macrophages reaches a plateau 7 to 10 days postinfection (21).

p24 determination. HIV-1 replication was assessed by monitoring cultures for cytopathic effect (multinuclear giant cell formation) and p24 antigen production measured with an enzyme immunoassay (Abbott Laboratories, No. Chicago, Ill.). Levels of p24 in culture supernatants ranged from 100 to 320 ng/ml, which correlated with a viral infectivity titer of 10^4 to 10^5 tissue culture infective doses per ml, indicating that a highly productive infection was established.

Isolation of *P. carinii*. Because of the lack of a consistent source of purified human-derived *P. carinii* (2, 29), organisms for these studies were derived from steroid-treated, immunosuppressed rats by the transtracheal inoculation method (2, 10). Virus- and pathogen-free, barrier-raised, 150- to 174-g Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, Ind.) were fed water containing dexamethasone (1.2 mg/liter) and tetracycline (500 mg/liter) ad libitum. Rats also received a normocaloric, low-protein (8%) rat diet (ICN Biomedicals, Costa Mesa, Calif.). After 7 days on this regimen, the rats were inoculated intratracheally with 10^6 to 10^7 freshly isolated *P. carinii* organisms (including up to 10% cyst forms as determined by Giemsa-stained smears). Inoculation was performed under direct tracheal visualization, using 0.1 ml of *P. carinii* in sterile phosphate-buffered saline (PBS) followed by 0.25 ml of air through a 22- or 23-gauge needle. Lungs were harvested under sterile conditions after 6 to 12 weeks. Impression smears were taken from the cut surface of each lung, and the lung was cultured for bacteria and fungi and chopped and homogenized in a Stomacher apparatus (Tekmar Co., Cincinnati, Ohio). The homogenate was briefly centrifuged at 1,800 × *g*, and the supernatant was diluted to 40 ml with Dulbecco's modified Eagle's medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and Fungizone (amphotericin B) (25 μg/ml). The supernatant was sequentially filtered through Nuclepore filters with pore sizes of 10, 8, and 5 μm twice, and the *P. carinii* organisms were collected by centrifugation for 15 min at 1,800 × *g*. The organisms were washed three times in Dulbecco's modified Eagle's medium and resuspended in a desired volume. Smears of 10 μl of resuspended organisms were spread over a 1-cm^2 premarked slide and stained with DiffQuik (Baxter, McGaw Park, Ill.) for counting of *P. carinii* nuclei and host cells. Five contiguous areas were counted in three smears from each preparation under oil immersion. *P. carinii* viability was estimated by fluorescein diacetate-propidium iodide staining (nonviable organisms stained red) (17, 24). All preparations were well dispersed and had greater than 85% viability. No intact nucleated cells were present after filtration. Contaminating red cells were lysed with ammonium chloride buffer, and the pellet was rewashed twice and resuspended in a desired volume. Preparations of *P. carinii* testing positive for the presence of lipopolysaccharide by *Limulus* assay (34) were discarded.

Additionally, to investigate the specificity of *P. carinii* in this system, control preparations were made from rat lungs not infected with *P. carinii*. These were harvested from the lungs of rats inoculated with 0.1 ml of PBS and receiving the same diet and immune suppression but treated with trimethoprim-sulfamethoxazole (200 mg of trimethoprim per liter and 1 g of sulfamethoxazole per liter). Control lungs were harvested and processed identically to *P. carinii*-infected lungs. Because of the lack of a human model, the rat model of *P. carinii* pneumonia has proven useful in the assessment of antibiotics and of diagnostic techniques (e.g., radionuclide imaging) for use with human patients (2, 10). Rat-derived *P. carinii* shares many antigenic moieties and monoclonal antibody-defined antigens with human-derived *P. carinii* (13). Histopathologically, the characteristics of infection are very similar to that seen in human infection (2, 10). Epidemiologically, both cellular and humoral immune responses in humans have been assessed using rat-derived *P. carinii* organisms as antigenic targets (13, 14).

Coinfection of cultures with *P. carinii*. Culture supernatants were removed and replaced by IMDM plus 5% human serum with or without *P. carinii* at an organism-to-adherent-cell ratio of 5 to 1. This ratio was found to be optimal in preliminary studies (24). At various time points ranging from 2 to 72 h after *P. carinii* infection, supernatants were removed, centrifuged at 800 × *g* to remove cellular debris, and then stored at −70°C until assayed for cytokine levels. Cell viability was determined by counting the number of adherent cells remaining in the wells or flasks at various times post-HIV-infection and *P. carinii* exposure under a microscope by trypan blue exclusion. The adherent cell monolayer was used for RNA extraction as described below.

To examine whether the *P. carinii* glycoprotein A (GP-A) (12) surface antigen was capable of substituting for the cytokine-inducing function of the whole organism, several cultures were treated with 20 μg of rat *P. carinii* GP-A per ml, prepared according to the method of Radding et al. (37) and generously provided by F. Richards, Yale University.

Filter studies. Cell culture insert filters (0.45-μm pore size; Cyclopore, Falcon 3090; Becton Dickinson, Lincoln Park, N.J.) were placed into six-well tissue culture plates containing adherent macrophages. These filter units contain an inert porous filter membrane suspended in a raised circular support sized to the culture plate; this insert allows the free exchange of medium over the cells and across the membrane while separating the organisms on the membrane from the cell monolayer on the culture plate. *P. carinii* organisms were cultured in IMDM plus 5% serum and added to the upper chamber in 3 ml at an organism-to-cell ratio of 5 to 1; another 3 ml of IMDM medium was added to the feeder cell layer under the insert.

Cytokine assays. All culture supernatants were treated with 1% β-propiolactone (Sigma) at 4°C overnight to inactivate HIV-1 (39). Control experiments in our laboratory and elsewhere indicated that this treatment does not influence the cytokine activities tested (39). Cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA) for TNF-α and IL-1β (R & D Systems, Minneapolis, Minn.) and for IL-6 (Genzyme, Cambridge, Mass.).

RNA analysis. After incubation of the cells under the various conditions outlined above, total RNA was extracted by using guanidine isothiocyanate and subsequent cesium chloride gradient centrifugation (38). The mRNAs were then fractionated by electrophoresis in formaldehyde-agar gels (38) and transferred onto Zetabind nitrocellulose paper (Kuno, Meriden, Conn.). Northern (RNA) blots were generated by hybridization with a random-primed ^{32}P-labelled probe TNF-α, a 1.0-kb *Hin*dIII-*Bam*HI fragment of TNF-α genomic DNA (L. Lin, Cetus Corp., Emeryville, Calif.). Results were quantitated by densitometry (E-C Apparatus Corp., St. Petersburg, Fla.). The densitometric values for total ethidium bro-

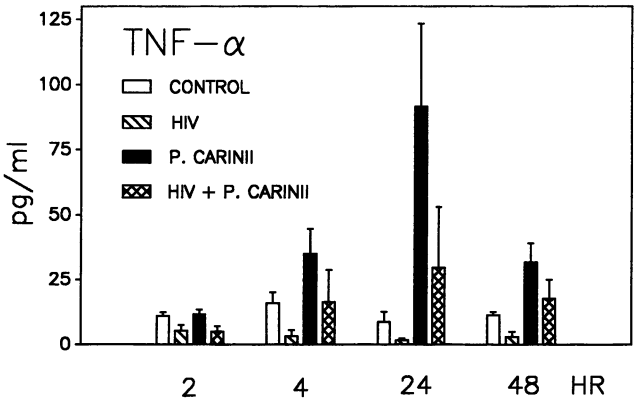

FIG. 1. Effect of *P. carinii* on production of TNF-α in HIV-uninfected and HIV-infected human macrophages. HIV-uninfected and HIV-infected macrophages (5.0×10^5 cells per ml) were incubated without *P. carinii* (control and HIV, respectively) or incubated with *P. carinii* at a *P. carinii*/macrophage ratio of 5 to 1 (*P. carinii* and HIV + *P. carinii*, respectively). The supernatants were harvested at the time points indicated on the abscissa and assayed in an ELISA. The results represent the means ± standard errors of three experiments using three different donors. From ANOVA, the main-effect *P* values for *P. carinii* compared with the control and HIV-infected compared with the control were $P = 0.0025$ and 0.0002, respectively. The value for HIV-*P. carinii* interaction was $P = 0.024$.

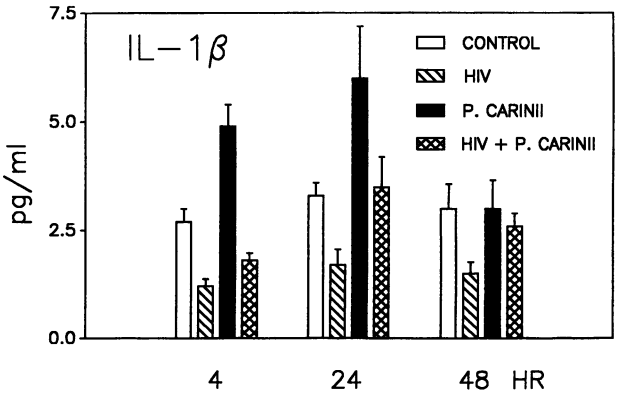

FIG. 2. Effect of *P. carinii* on production of IL-1β in HIV-noninfected and HIV-infected human macrophages. HIV-uninfected and HIV-infected macrophages (5×10^6 cells per ml) were incubated without *P. carinii* (control and HIV, respectively) or incubated with *P. carinii* at a *P. carinii*/macrophage ratio of 5 to 1 (*P. carinii* and HIV + *P. carinii*, respectively). The supernatants were harvested at the time points indicated on the abscissa and assayed in an ELISA. The results represent the means ± standard errors of the means of three experiments using three different donors. The ANOVA *P* values for time main effects, HIV main effects, and *P. carinii* main effects were all <0.0001.

mide-stained RNA of each sample before hybridization with the specific probe were used to correct for variations in sample size.

Statistical analysis. F tests in a repeated-measures analysis of variance (ANOVA) were performed to analyze the effects of time, HIV-1, and *P. carinii* on cytokine production. Since increased individual variation was observed in larger responses, values were weighted inversely to their level to account for this heteroscedasticity (8). All computations were done using the SAS system on a Sun Unix workstation.

RESULTS

HIV-1 infection alters *P. carinii*-dependent cytokine production. Incubation of HIV-uninfected macrophages with *P. carinii* (5:1, organisms to cells) induced the production of TNF-α, IL-1β, and IL-6. TNF-α and IL-1β production peaked at 24 h of incubation and fell by 48 h (Fig. 1 and 2). By contrast, IL-6 (Fig. 3) production was most pronounced at 24 to 48 h, consistent with earlier preliminary observations (19, 20).

When HIV-1-infected macrophages were exposed to *P. carinii*, TNF-α and IL-1β production were significantly decreased ($P = 0.024$ and 0.023, respectively) at all time points in comparison with the production of cytokines by HIV-uninfected cells exposed to *P. carinii* (Fig. 1 and 2). By contrast, the production of IL-6 was greatly enhanced ($P = 0.0035$), with the appearance of additive effects of HIV-1 and *P. carinii* (Fig. 3). In one experiment in which cells were incubated with *P. carinii* for 72 h, only low levels of TNF-α and IL-1β were detected (data not shown), regardless of whether the cells were infected with HIV-1. However, IL-6 values at 72 h were 370 pg/ml in *P. carinii*-treated HIV-1-noninfected cells and 540 pg/ml in *P. carinii*-treated HIV-1-infected cells. (Background values were 10 pg/ml in control cells and 50 pg/ml in HIV-1-infected cells.) These data suggest that HIV-1 infection of macrophages specifically modulates the *P. carinii*-dependent production of TNF-α, IL-1β, and IL-6 proteins.

To determine whether the deficiency of TNF-α production in *P. carinii*-challenged HIV-infected macrophages manifests itself at the transcriptional level, steady-state levels of TNF-α mRNA in HIV-1-infected and HIV-1-uninfected macrophages stimulated with *P. carinii* were measured. Figure 4 shows that TNF-α mRNA accumulation was downregulated in HIV-infected macrophages inoculated with *P. carinii* at 2 and 6 h. This finding indicates that HIV-1 infection of macrophages modulates TNF-α production at the transcriptional level.

To test the effect of varying the size of the *P. carinii*

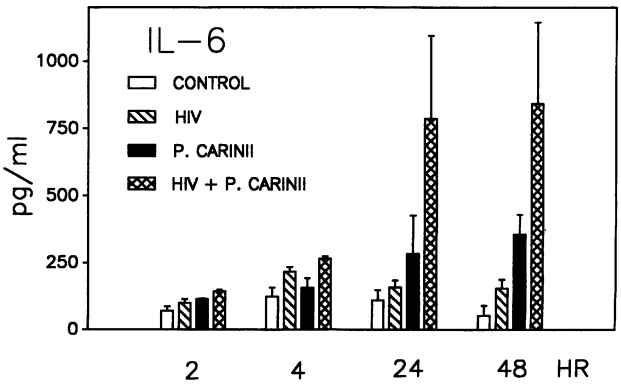

FIG. 3. Effect of *P. carinii* on production of IL-6 in HIV-uninfected and HIV-infected human macrophages. HIV-uninfected and HIV-infected macrophages (5.0×10^5 cells per ml) were incubated without *P. carinii* (control and HIV, respectively) or incubated with *P. carinii* at a *P. carinii*/macrophage ratio of 5 to 1 (*P. carinii* and HIV + *P. carinii*, respectively). The supernatants were harvested at the time points indicated on the abscissa and assayed in an ELISA. The results represent the means ± standard errors of three experiments using three different donors. From ANOVA, the main-effect *P* values for *P. carinii* and HIV were $P = 0.002$ and $P = 0.0035$, respectively. The value for HIV-*P. carinii* interaction was not significant.

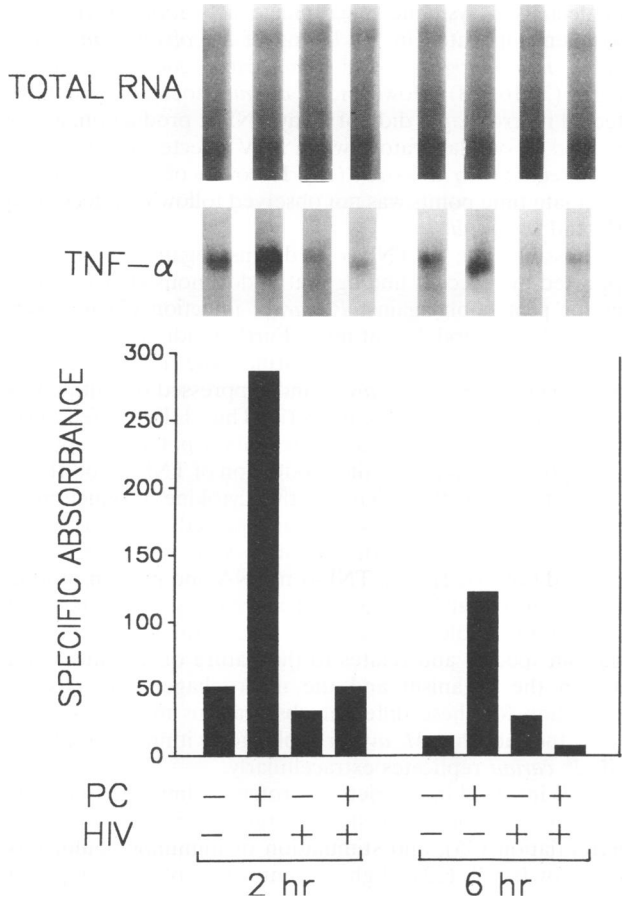

FIG. 4. Effect of *P. carinii* (five organisms per macrophage) on TNF-α mRNA accumulation by HIV-uninfected and -infected human macrophages. The ordinate shows the specific absorbance of the blots (lower panel) with the total RNA depicted (upper panel). The abscissa depicts the conditions of the incubation. Cells were harvested at 2 and 6 h, and the total RNA was isolated and analyzed by Northern blotting using a human TNF-α probe. The size of the TNF mRNA band is 1.9 kb.

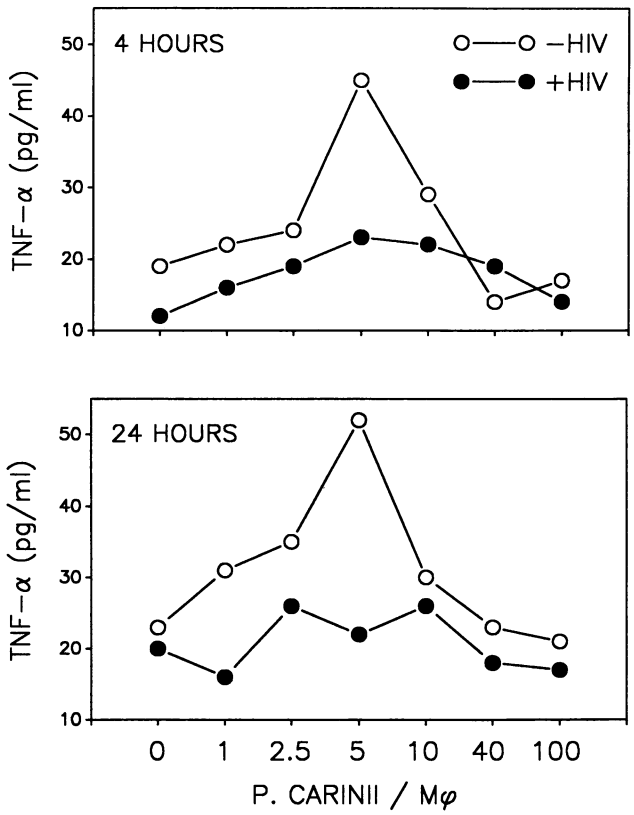

FIG. 5. Effect of different *P. carinii* inocula on production of TNF-α in HIV-1-infected and -uninfected macrophages (Mϕ). The ordinates show the amount of TNF-α produced, and the abscissa shows the *P. carinii* inocula tested. Representative results from two experiments using 4-h supernatants are shown in the upper panel, and representative results from two experiments using 24-h supernatants are shown in the lower panel.

inoculum on TNF-α release, adherent monolayers of HIV-1-uninfected and -infected macrophages were exposed to organisms at multiplicities of infection ranging from 1 to 1 to 100 to 1 (*P. carinii* organisms to macrophages). As is demonstrated in Fig. 5, at the ratio of one organism per cell, increased TNF-α production was not seen in comparison with that in noninoculated macrophages. However, at five organisms per cell, significant release of TNF-α was observed at 4 and 24 h after exposure to *P. carinii*. Further increasing the number of *P. carinii* organisms per cell greater than 5 to 1 did not induce higher levels of TNF-α. Additionally, at multiplicities greater than 5 to 1, macrophage monolayers appeared to be disrupted when assessed morphologically by inverse-phase microscopy (Fig. 5).

Surface glycoprotein component of *P. carinii* substitutes for intact *P. carinii* in induction of TNF-α, IL-1β, and IL-6. The major *P. carinii*-specific antigen on the surface of the microbial organism is the 95- to 115-kDa surface glycoprotein A (GP-A) (12, 24, 37). To examine whether GP-A alone mediates the *P. carinii*-specific modulation of cytokine expression, 20 μg of endotoxin-free GP-A per ml was incubated with 5×10^6 HIV-1-uninfected and HIV-1-infected macrophages per ml for 24 h and the production of TNF-α, IL-1β, and IL-6 was measured. Although the amounts were lower, the pattern of cytokine production was similar to that observed when macrophages were incubated with intact *P. carinii*. However, the increase in IL-1β production was not as high with GP-A in the absence of HIV-1 infection as with intact *P. carinii* (Fig. 6). This suggests that macrophage responses to *P. carinii* may be mediated, in part, by interaction with GP-A.

Requirement for physical contact between *P. carinii* and macrophages for cytokine effects. Because surface glycoproteins or other components derived from the preparation of *P. carinii* might be released into the supernatant and exert biological activity, experiments were performed to assess whether physical contact between *P. carinii* and the macrophage is necessary for the induction of cytokine production. When tissue culture inserts (0.45-μm-pore-size filters) were inserted into plates to provide physical separation between *P. carinii* and normal macrophages but to allow exchange of proteins and soluble products, enhanced TNF-α production was not observed (Table 1). Likewise, IL-6 production by HIV-1-infected and control macrophages exposed to *P. carinii* remained at background levels when filters were inserted (data not shown). These experiments indicate that cytokine induction by *P. carinii* requires physical contact between the macrophage and *P. carinii*.

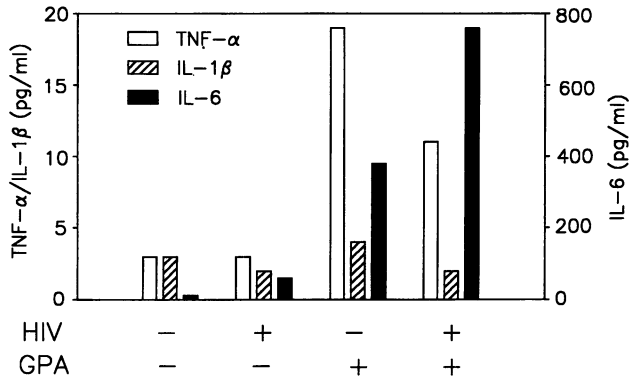

FIG. 6. Effect of *P. carinii* surface protein gp120 (GP-A) on production of TNF-α, IL-1β, and IL-6. HIV-infected macrophages (5×10^5 per ml) were incubated with 20 μg of GP-A for 24 h, and the amounts of TNF-α, IL-1β, and IL-6 were measured in an ELISA. The ordinates show the levels of the cytokines detected and the abscissa indicates the incubation conditions in a representative experiment of two conducted.

DISCUSSION

The central finding of this study is that HIV-1 infection of human-monocyte-derived macrophages, in the absence of T lymphocytes, specifically alters the cytokine response to *P. carinii*. Exposure of normal cells to *P. carinii* results in a significant increase of TNF-α protein levels at 24 h. Levels of IL-1β and IL-6 were also increased by exposure of macrophages to *P. carinii* at 24 h and persisted at 48 h. This response was also observed at the transcriptional level, since steady-state levels of TNF-α mRNA peak as early as 2 h after exposure to *P. carinii*. By contrast, exposure of HIV-1-infected macrophages to *P. carinii* reduced the secretion of both TNF-α and IL-1β protein induced by *P. carinii* but increased the release of IL-6.

These results have important implications for understanding the pathobiology of *P. carinii* in the AIDS setting. Production of the inflammatory cytokines TNF-α and IL-1β are key factors mediating the resistance of macrophages to microbial infection, thereby promoting host protection (1, 3, 5, 18, 22, 27, 28, 30–32, 40). TNF-α has been shown to cause killing of *P. carinii* (35) as well as activation of macrophages and other immune cells in vitro (36). Refractoriness of HIV-1-infected macrophages to the induction of TNF-α production by *P. carinii* could, therefore, result in reduced killing of *P. carinii* and decreased systemic macrophage activation. TNF-α has also been implicated in the killing of *Mycobacterium avium*, *Listeria monocytogenes*, and *Toxoplasma gondii* by macrophages (3, 16, 18). However, *P. carinii* inoculation of HIV-infected macrophages did not delay TNF-α production, as was observed in our laboratory when HIV-infected macrophages were infected with *M. avium* (33). Recovery of TNF-α production at late time points was not observed following infection by HIV and *P. carinii*.

TABLE 1. Effect of separating *P. carinii* from macrophages on production of TNF-α[a]

Macrophages	Amt (pg/ml) of TNF-α			
	4 h		24 h	
	−	+	−	+
Controls	11	8	6	0
Incubated with *P. carinii*	42	7	27	15
Prevented from direct contact with *P. carinii* by membrane	4	4	6	2

[a] Macrophages (1.2×10^7 cells per 3 ml) infected with HIV (+) or not infected with HIV (−) were cultured in six-well plates, and a Falcon Cyclospore membrane (0.45-μm pore size) was either placed on top of the cells or not. *P. carinii* was seeded at a *P. carinii*/macrophage ratio of 5 to 1 directly on top of the cells or on the membrane. After 4 and 24 h the supernatants were collected and analyzed for TNF-α in an ELISA.

A possible role of TNF-α in defense against *P. carinii* is supported by a recent finding that endogenous IL-1 is important for protection against *P. carinii* infection (7) in severe combined immunodeficient mice. Further, administration of a monoclonal antibody against murine type I IL-1 receptor impaired clearance of *P. carinii* and suppressed recruitment of immune effector cells to the lungs (7). Thus, HIV-1 infection of peripheral blood macrophages prevents a potentially protective response by inhibiting the production of TNF-α and IL-1β.

The effects of *P. carinii* on the cytokine production of HIV-infected macrophages were unexpected in light of previous studies (11, 33) in our laboratory in which *M. avium* increased both IL-1β and TNF-α mRNA and protein production by peripheral blood-derived macrophages infected with HIV-1. Our studies suggest that the cytokine response is organism specific and relates to the nature of the interaction between the organism and the macrophage. One possible explanation for these different observations may be, in part, due to the fact that *M. avium* replicates within macrophages while *P. carinii* replicates extracellularly.

IL-6 is involved in a variety of protective immune functions, including induction of T-cell proliferation (23), cytolytic T-cell differentiation (35), and stimulation of immunoglobulin production by B cells (23). High concentrations of IL-6 have been found in body fluids in a variety of diseases, including HIV-1 infections (4). The dramatic increase in IL-6 production by *P. carinii*-exposed HIV-infected macrophages would provide a signal for B-cell proliferation and B-cell differentiation into plasma cells. IL-6 is also a central tumor growth factor for malignant plasma cells and may contribute to the induction of B-cell lymphomas in AIDS patients. Enhanced IL-6 production may play a role in the development of the hypergammaglobulinemia seen in AIDS patients, which is due to polyclonal B-cell activation (26).

IL-6 levels were elevated in severe combined immunodeficient mice with *P. carinii* pneumonia compared with levels in *P. carinii*-free mice (6). In our system, *P. carinii* exposure produced increased amounts of IL-6. This may suggest that the most common nonviral opportunistic infection of AIDS, *P. carinii* pneumonia, may contribute significantly to the high levels of IL-6 observed in HIV patients. Other opportunistic pathogens in addition to *P. carinii* may also contribute to elevated levels of IL-6 in plasma.

The effects of *P. carinii* on macrophage cytokine release required cell-organism contact. The induction of TNF-α was blocked by the interposition of a porous filter between the organisms and the target macrophages. Furthermore, the major *P. carinii* surface antigen, GP-A, was able to partially mimic the effects of intact organisms, suggesting an important role for GP-A.

Our studies utilized human monocyte-derived macrophages and rat-derived *P. carinii*. Since rat-derived organisms share antigenic moieties with human-derived *P. carinii* and the histology of infection is similar to that seen in human disease (10, 13), this in vitro culture system is relevant to the human condition. Organisms from immunosuppressed rats have been

used successfully in a number of clinical studies that investigated cellular and humoral immunity to *P. carinii* (13, 14).

Other workers have examined the influence of *P. carinii* and HIV-1 on monocyte-derived cells. One study demonstrated elevated spontaneous TNF-α production by lung macrophages in AIDS patients with active *P. carinii* pneumonia but not in patients without infection (25). While high TNF-α protein production was associated with lower counts of *Pneumocystis* cysts in the bronchoalveolar lavage fluid (suggesting a therapeutic benefit of TNF on *P. carinii* infection), no assessment of the numbers of trophozoites (over 95% of organisms) was made. Technical differences and the absence of data on cytokine mRNA levels make direct comparison of these studies difficult; however, our observations of increased TNF-α and IL-1β production in response to *P. carinii* were performed at time points earlier than those in the previous study. No increase in TNF-α was seen in our studies at 72 h.

Our findings demonstrate that, in contrast to normal monocyte-derived macrophages, cells infected with HIV demonstrate an altered cytokine response following exposure to *P. carinii*. Furthermore, the nature of this response may potentially have adverse consequences on host defense. Despite the limitations of this system in extrapolating to AIDS, these studies contribute to the understanding of host susceptibility to *P. carinii* in AIDS.

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