Clin Exp Immunol 1994; 96:187–192

Alveolar macrophages from subjects infected with HIV-1 express macrophage inflammatory protein-1α (MIP-1α): contribution to the CD8$^+$ alveolitis

M. DENIS & E. GHADIRIAN* *Pulmonary Research Unit, Faculty of Medicine, University of Sherbrooke, Sherbrooke, and* **Montreal General Hospital Research Institute, Montreal, Canada*

(Accepted for publication 3 February 1994)

SUMMARY

We examined the synthesis and release of MIP-1α in alveolar macrophages obtained from normal subjects or subjects infected with HIV-1, at different stages of the disease. HIV-1-infected subjects in groups II, III and IV all had significant interstitial pneumonitis, featuring a significant infiltration of CD8$^+$ lymphocytes in the bronchoalveolar lavage. Alveolar macrophages from HIV-1-infected subjects were shown to express significant levels of MIP-1α via immunohistochemistry, both spontaneously and in response to lipopolysaccharide (LPS), whereas cells from normal subjects expressed very low levels of the cytokine. Supernatants of alveolar macrophages from HIV-1-infected subjects exerted strong chemotactic activity for purified activated blood CD8$^+$ T lymphocytes, which was strongly inhibited by neutralizing MIP-1α. Studies of patients with HIV-1 infection at different stages of the disease showed that MIP-1α secretion increased as viral infection developed. There was a significant positive correlation between MIP-1α secretion and the CD8$^+$ alveolitis in HIV-1-infected subjects. Infection of alveolar macrophages *in vitro* with three distinct strains of HIV-1 which replicated profusely in macrophages did not induce the expression of MIP-1α. Collectively, these data suggest that HIV-1 infection *in vivo* induces MIP-1α expression and release in alveolar macrophages, and this appears to contribute significantly to the alveolar lymphocytosis seen in HIV-1-infected subjects.

Keywords HIV-1 MIP-1α alveolar macrophages CD8$^+$ alveolitis

INTRODUCTION

The hallmark of infections with HIV-1 is a progressive decline in the number of CD4$^+$ T lymphocytes accompanied by an enhanced susceptibility to some forms of neoplasia and opportunistic infections [1,2]. Before the appearance of these devastating symptoms, a number of immunological abnormalities occur, which include T or B cell derangements, a skewing of the immune response towards a Th2 response, activation of macrophages at the mucosal surfaces, and other evidence of an up-regulation of some immune compartments [1,3,4]. Many HIV-1-infected subjects develop a significant lymphocytic alveolitis, comprised mostly of CD8$^+$ T lymphocytes [5–8]. This is accompanied by enhanced cytokine release by alveolar macrophages (i.e. tumour necrosis factor-alpha (TNF-α), granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-1) [9–12].

The lung is an important organ in the expression of HIV-induced pathophysiology, and many HIV-1-infected subjects develop significant impairment of lung function. Recent data have shown the frequent isolation of HIV-1 from the lungs and alveolar cells of AIDS patients, and HIV-1-specific T cells are found in the bronchoalveolar lavage (BAL) of HIV-1-infected subjects [6,13,14].

MIP-1α is a newly described cytokine, which is a heparin binding protein, originally identified by its capacity to cause a localized inflammatory reaction in footpads of mice [15,16]. MIP-1 and MIP-2 represent two families of structurally related proteins, and are part of the proinflammatory supergene 'intercrine' cytokine family [17]. MIP-1α/MIP-1β have been shown to exert a variety of important effects, including modulation of macrophage function, pyrogenic activity *in vivo*, as well as T cell adhesion and chemotaxis [18–23].

We hypothesized, given the up-regulation of the activation status of mucosal macrophages and the lymphocytic alveolitis in AIDS subjects, that MIP-1α could be synthesized and released by alveolar macrophages from HIV-1-infected subjects.

Correspondence: Michel Denis, Pulmonary Research Unit, Faculty of Medicine, University of Sherbrooke, 3001 12th Avenue N., Sherbrooke, Canada, J1H 5N4.

SUBJECTS AND METHODS

Subjects

Eight male HIV-1$^-$ subjects were studied as controls (average age 36 ± 7 years). They did not belong to any of the recognized

Table 1. Characteristics of subjects

Group	Circulatory CD4+ T cells (cells/mm^3)	Total BAL cells ($\times 10^3$/ml lavage)	Macrophages (%)	PMN (%)	Lymphocytes (%)	CD8 (%)	CD4 (%)
Normal	841 ± 91^a	130 ± 13^a	89 ± 3^a	1 ± 1^a	6 ± 3^a	—	—
HIV+ (group II)	532 ± 58^b	240 ± 36^b	71 ± 12^b	2 ± 1^a	21 ± 3^b	53 ± 11	13 ± 6
HIV+ (group III)	193 ± 81^c	312 ± 60^b	64 ± 17^b	3 ± 2^a	30 ± 11^b	67 ± 15	12 ± 9
HIV+ (group IV)	81 ± 24^d	413 ± 101^c	56 ± 20^b	8 ± 6^b	34 ± 12^b	69 ± 14	13 ± 6

Groups identified by different letters are significantly different from each other (by ANOVA).
BAL, Bronchoalveolar lavage; PMN, polymorphonuclear neutrophils.

risk groups, and had a normal physical examination and chest x-ray. Nine HIV-1-infected subjects in group II (Centres for Disease Control (Atlanta, GA) classification) were studied; they were all asymptomatic, and had no detectable microbial or non-HIV viral infections in their BAL. Seven of these subjects were taking 3′-azido-3′-deoxythymidine (AZT) at the time of study (mean duration of treatment $6 \pm 1{\cdot}4$ months). Group III subjects ($n = 11$) had generalized lymphadenopathy with no other concurrent infection or illness than HIV; 10 were receiving AZT (treated over 12 ± 3 months). Eleven subjects were in subgroup IVC, i.e. HIV-1-infected patients with either *Pneumocystis carinii* or *Candida* infections; all these subjects had been receiving AZT (treated over 16 ± 6 months). Other characteristics of the subjects are shown in Table 1. This protocol was approved by our local ethics committee for guidelines on clinical research. HIV-1-infected subjects in groups III and IV were undergoing BAL for diagnostic procedures. Subjects in group II were all recruited as part of a longitudinal study on pulmonary involvement of HIV-1 infection. All patients read and signed informed consent forms approving the procedure, and group II patients understood that the BAL would not modify their therapy in any way.

Bronchoalveolar lavage

BAL was performed after local anaesthesia. A total of 250 ml of saline solution was injected via bronchoscopy, with vacuum aspiration after each aliquot. The BAL fluid was filtered with gauze and cells washed with Neuman-Tytell medium (GIBCO, Grand Island, NY). A differential count of cells was performed by examining DiffQuik (Baxter, McGaw Park, IL) stained cytospins, and CD4+ and CD8+ T lymphocytes were identified on cytospins using a peroxidase-labelled MoAb-based kit from Becton Dickinson (Mountain View, CA). Alveolar macrophages were purified by rosetting T cells with neuraminidase (Sigma, St Louis, MO), followed by passage on Ficoll–Hypaque (Sigma). The resulting cell population was more than 98% macrophages, based on esterase staining; viability was more than 98%, judging from trypan blue exclusion.

Alveolar macrophage stimulation

Macrophages were cultured at 5×10^5 cells/ml in Neuman-Tytell medium (GIBCO) supplemented with 2 mM glutamine and antibiotics, in polystyrene tubes (Costar, Oxnard, CA). Cells were either left untreated or were stimulated with 5 μg/ml of bacterial lipopolysaccharide (LPS from *Esherichia coli* O111:B4; Sigma). After overnight incubation, supernatants were centrifuged and stored at −70°C.

Production of anti-MIP-1α antibodies

New Zealand White rabbits (Charles River Inc., St-Constant, Canada) were immunized with a total of 5 mg of recombinant MIP-1α (R&D Systems, Minneapolis, MN) emulsified in Freund's complete adjuvant (FCA; Difco, Detroit, MI). The IgG fraction of the serum was purified over a protein A column (Immunocorp, Montreal, Canada) and used in sandwich ELISA and neutralization procedures (see below). One milligram of rabbit anti-MIP-1α neutralized 0·2 mg of MIP-1α in the T cell chemotaxis assay described below. In control experiments, we found that up to 1 mg of antibody failed to bind to or neutralize IL-8, IL-6, MIP-1β, MIP-2, interferon-gamma (IFN-γ) and IL-2.

MIP-1α ELISA

MIP-1α was quantified by a sandwich ELISA. Goat anti-MIP-1α antibody (50 ng/well; Genzyme, Boston, MA) was applied to each well of a flat-bottomed 96-well microtitre plate (Costar) for 16 h at 4°C. Plates were blocked with 2% bovine serum albumin (BSA) in PBS for 90 min at 37°C. Plates were rinsed four times with wash buffer, and samples or recombinant MIP-1α were applied, followed by a 1 h incubation at 37°C. Plates were washed four times, followed by addition of 150 ng/well of biotinylated rabbit anti-MIP-1α and then incubated for 30 min at 37°C. Plates were washed and a 1:1000 dilution of streptavidin-peroxidase (BioRad, Richmond, CA) applied to each well. Plates were washed and a chromogen substrate (BioRad) added. Plates were read at 490 nm after a 15-min incubation.

Immunohistochemistry

Cells were deposited on a glass slide with a Cytospin. After drying, slides were fixed with 4% paraformaldehyde in PBS and then with ice-cold acetone. Endogenous peroxidase was quenched by incubating the slides for 30 min in absolute methanol and 3% H_2O_2. Slides were then blocked with a 1:50 dilution of goat serum for 30 min at 37°C, then treated for 2 h at 37°C with a 1:1000 dilution of rabbit anti-human MIP-1α serum or the same dilution of preimmune rabbit IgG. After incubation, preparations were rinsed with PBS, overlaid with biotinylated goat anti-rabbit IgG (1:200; Pierce, Bedford, IL) for 20 min and rinsed. Slides were then treated with streptavidin-peroxidase for 15 min at 37°C; after rinsing, they were overlaid with 10 ml of 3-amino-9-ethylcarbazole 40 mg/ml in N,N-dimethylformamide

(Sigma) and treated with 3% H_2O_2 in 0·1 M sodium acetate for 15 min at 37°C to allow colour development. After rinsing, slides were counterstained with haematoxylin. In competitive inhibition studies to demonstrate antibody specificity, immunostaining for human MIP-1α showed 100% inhibition by addition of exogenous MIP-1α.

Isolation of CD8⁺ T lymphocytes

Mononuclear cells were obtained by centrifuging peripheral blood cells over Ficoll–Hypaque (Sigma). Monocytes were depleted by multiple adherence steps on plastic Petri dishes (Falcon no. 3007). T cells were positively selected for by rosetting with sheep erythrocytes. Pure CD8⁺ T lymphocytes were obtained by using a complement (Low-Tox, Cedarlane)-dependent killing technique of the CD4⁺ subset in the presence of an anti-CD4 MoAb (Becton Dickinson). Briefly, 5×10^7 T lymphocytes were incubated with a 1:100 dilution of anti-CD4 MoAb with a 1:10 dilution of Low-Tox rabbit complement for 1 h at 37°C. Resulting viable cells were obtained by centrifugation on Ficoll–Hypaque, with over 97% being pure CD8⁺ T cells. Purified CD4⁺ T lymphocytes were obtained by a CD8⁺ T lymphocyte depletion, using a similar method.

In selected experiments, cells were activated by incubation on anti-CD3-coated plastic dishes for 16 h at 37°C. Briefly, 10^7 cells were cultured in a Petri dish (no. 3071, Costar) which had been coated with 15 μg/ml of a CD3 MoAb (Orthoimmune, Sandoz, Basel, Switzerland). This anti-CD3 MoAb activates T cells, judging from cell proliferation and lymphokine synthesis (data not shown and manufacturer's data). Cells were then harvested and used in the chemotaxis assay.

Chemotactic activity

The chemotaxis of lymphocytes was studied by a method suggested by Pilaro *et al.* [24]. Briefly, chemotaxis was assessed in a 48-well microchemotaxis chamber. Autoclaved, polyvinyl pyrrolidine-free polycarbonate filters (Nucleopore, Cabin John, MD) were coated with 1 mg/cm² of fibronectin (Sigma) in deionized water for 45 min at 37°C. Filters were removed, air-dried for 45 min and used immediately. Thirty microlitres of the chemoattractant or control reagents were placed into the wells and the upper chamber set into place; 50 μl of freshly isolated, enriched T lymphocytes (see below) in RPMI 1640 containing 0·5% BSA and 10 mM HEPES buffer pH 7·2 were added to each upper chamber. Following incubation for 3–5 h at 37°C, the filters containing the adhered, migrated lymphocytes were removed, fixed and stained with DiffQuik for enumeration. Chemotaxis was measured as the number of cells that had migrated through the filter in 10 oil immersion fields (× 1000). Experiments were repeated three to five times with similar results. The data are presented as the average of six samples ± s.e.m. from one representative experiment.

In vitro *infections with HIV*

After establishment of alveolar macrophage cultures, cells were infected with 50 μl of HIV-1/ADA isolate ($7{\cdot}5 \times 10^6$ reverse transcriptase activity (ct/min per ml) and 900 ng/ml p24 Ag; $TCID_{50}$ (tissue culture infective dose) 10^4/ml; a kind gift of Dr H. Gendelman, Walter Reed Army Institute of Research, Bethesda, MD). This viral preparation was found to be mycoplasma-free (Gen-Probe Inc., San Diego, CA). Supernatants were obtained at indicated intervals, and levels of reverse transcriptase measured. Briefly, supernatants were centrifuged for 5 min at 500 *g* and viruses lysed by 0·1% Triton X-100 (Sigma). Aliquots (10 μl) were added to 40 μl of a mixture with poly (rA), oligo (dT), $MgCl_2$, dithiothreitol and ^{3}H-dTTP (Amersham, Arlington Heights, IL). After 1 h at 37°C, the reaction mixture was spotted onto DE81 filter paper (Whatman) and allowed to dry at room temperature. Filters were washed in 5% Na_2HPO_4, water and 70% ethanol, and the filters processed by scintillation counting.

Statistical analysis

Differences between groups were determined by a two-way analysis of variance (ANOVA) using the Statview program. If a significant F value was obtained ($P < 0{\cdot}05$), relevant comparisons between groups were made by Student's t-test. Statistical significance for all comparisons was set at $P < 0{\cdot}05$.

RESULTS

Characteristics of subject groups

The characteristics of the subjects are shown in Table 1; all HIV-1-infected subjects had a significant alveolitis, with an increased number of macrophages and lymphocytes. As reported by others, the lymphocytic alveolitis was predominantly of the CD8⁺ subset, with more than 50% of the lymphocytes being CD8⁺. Patients not undergoing AZT treatment did have an alveolitis very similar to treated subjects.

Chemotaxis for CD8⁺ T lymphocytes

Supernatants from control and from HIV-1-infected alveolar macrophages (spontaneous and LPS-induced) were obtained and their ability to attract anti-CD3-stimulated CD8⁺ T lymphocytes was assessed. Figure 1 shows that supernatants of control alveolar macrophages attracted low levels of CD8⁺ T lymphocytes. Supernatants from alveolar macrophages of HIV-1-infected subjects were shown to attract CD8⁺ T lymphocytes, mostly via the generation of MIP-1α, as shown in neutralization studies. Normal rabbit globulin had no effect on CD8⁺ T lymphocyte chemotaxis. As an additional control, the anti-MIP-1α IgG had no effect on the migration of CD8⁺ T cells to the cytokine RANTES (data not shown). In separate control experiments, supernatants from cells of all subjects were applied in equal amounts on both sides of the filter; this condition abrogated T cell migration by 95% in all cases, ruling out an effect based on random cell migration.

MIP-1α expression and release by alveolar macrophages from controls and from HIV-1-infected subjects

Figure 2 shows results of immunohistochemistry; alveolar macrophages from HIV-1-infected subjects expressed high levels of MIP-1α, whereas cells from control subjects expressed very low or undetectable levels of MIP-1α. Virtually 100% of alveolar macrophages from HIV-1-infected subjects in groups III and IV expressed MIP-1α (Fig. 3). Figure 4 shows antigenic MIP-1α secretion in HIV-1-infected subjects at different stages of the disease; cells from HIV-1-infected subjects showed very high MIP-1α release, both spontaneously and in response to LPS. MIP-1α release in HIV-1-infected subjects was also shown to correlate strongly with the intensity of the CD8⁺ alveolitis (Fig. 5).

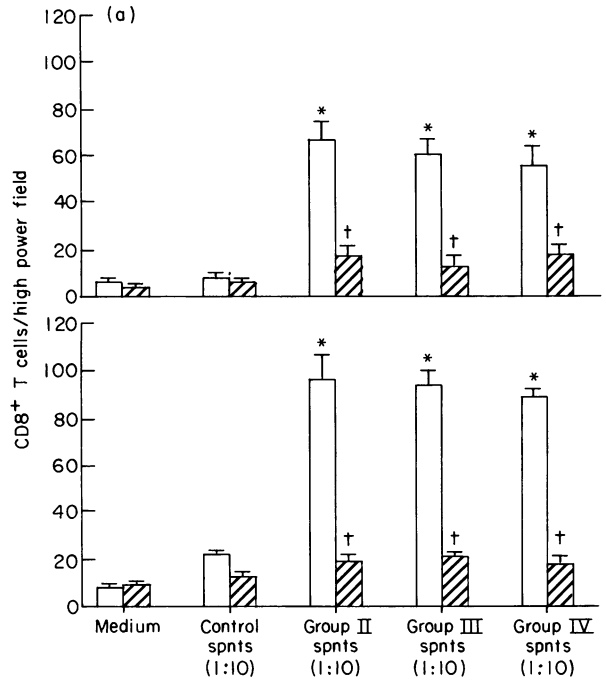

Fig. 1. Activated $CD8^+$ T lymphocyte chemotaxis in response to macrophage supernatants. Anti-CD3-stimulated $CD8^+$ T cells were obtained as described in Subjects and Methods. Supernatants (spnts) from normal subjects ($n=9$), group II HIV-1-infected subjects ($n=11$), group III ($n=9$) and group IV ($n=11$) were tested with rabbit immunoglobulin or anti-MIP-Iα. (a) Spontaneous production of chemotactic factors. (b) Lipopolysaccharide (LPS)-induced production. Results are means ± s.e.m.; each subject was tested in triplicate. Results of one experiment, repeated twice with identical results. $*P<0{\cdot}05$ *versus* control supernatants (ANOVA); $\dagger P<0{\cdot}05$ *versus* rabbit IgG (ANOVA). □, Rabbit IgG; ■, anti-MIP-1α.

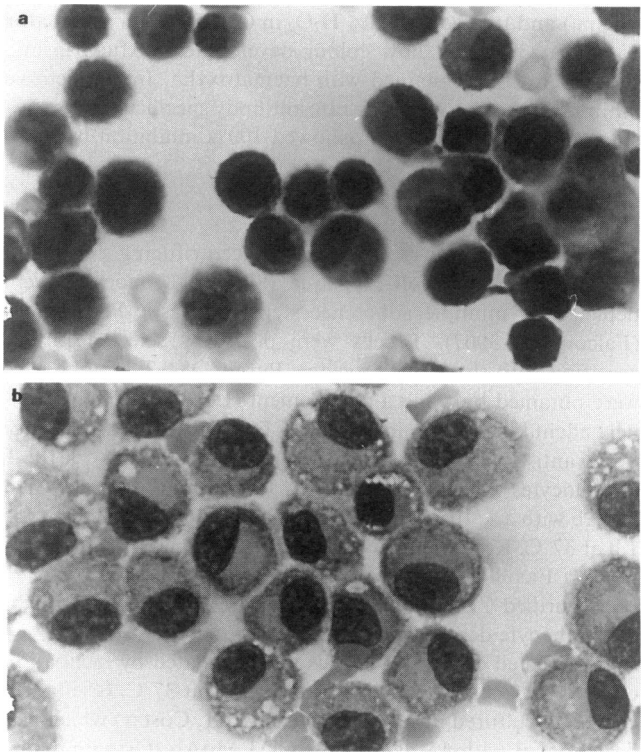

Fig. 2. Expression of MIP-1α by alveolar macrophages from normal subjects and HIV-1-infected subjects shown by immunohistochemistry. Cells from an AIDS patient (a) and from a normal subject (b).

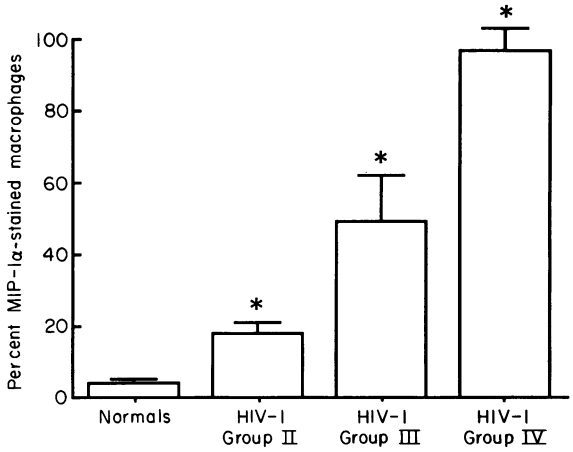

Fig. 3. Expression of MIP-1α by immunohistochemistry in alveolar macrophages from the various groups. Results are means ± s.e.m.; numbers of subjects in each group as in Fig. 1. $*P<0{\cdot}05$ *versus* normals (ANOVA).

HIV-1 infection does not directly stimulate MIP-1α release by alveolar macrophages

In a last set of experiments, alveolar macrophages were obtained and cells were infected with a rapidly replicating strain of HIV-1 (at day 20 after infection: > 10 000 ct/min of reverse transcriptase/μl of supernatant). Supernatants from these cells were obtained after 2 weeks, and MIP-1α levels measured by ELISA. Such supernatants contained very low levels of MIP-1α (131 ± 72 pg/10^6 cells, whereas uninfected cells had 96 ± 31 pg/10^6 cells; mean ± s.e.m. of seven samples). Similar cells pulsed with 1 μg/ml of LPS released 5431 ± 230 pg/10^6 cells of MIP-1α. This amount of MIP-1α was equivalent to that released by uninfected cells (6038 ± 531 pg/10^6 cells). Similar data were obtained with two other donors. Two other macrophage-tropic HIV-1 strains were used to infect alveolar macrophages, and neither of these two strains induced a significant release of MIP-1α by alveolar macrophages (data not shown).

DISCUSSION

Our data suggest that alveolar macrophages from subjects infected with HIV-1 synthesize high levels of the newly described cytokine MIP-1α. MIP-1α appears to be one of the earliest factors secreted after macrophage activation [17]. MIP-1 is composed of two related proteins, MIP-1α and MIP-1β, which have distinct DNA sequences, and code for highly homologous proteins whose hydrophobicities are similar [22]. MIP-1 elicits a localized inflammatory response when infused *in vivo*, and it activates neutrophils and monocytes [15]. Infusion of MIP-1 in rabbits elicited a fever, which was not blocked by cyclooxygenase inhibitors [25]. These data point to an important role for MIP-1 as an early mediator of inflammation and host resistance.

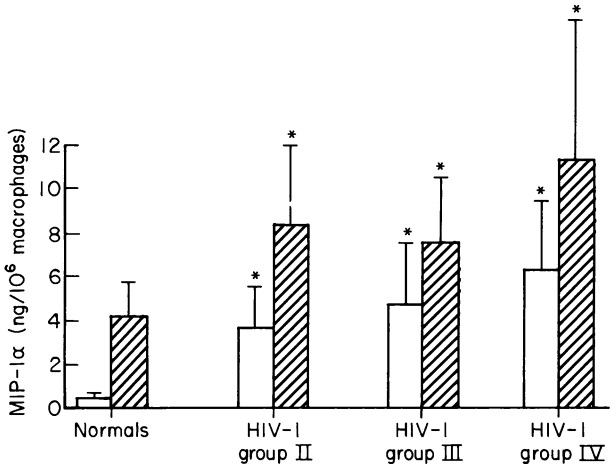

Fig. 4. MIP-1α release by alveolar macrophages from normal subjects or HIV-1-infected subjects. MIP-1α release was quantified by ELISA, as described in Subjects and Methods. Each subject was tested in triplicate. $*P < 0{\cdot}05$ *versus* normals (ANOVA). □, Untreated; ■, lipopolysaccharide (1 μg/ml).

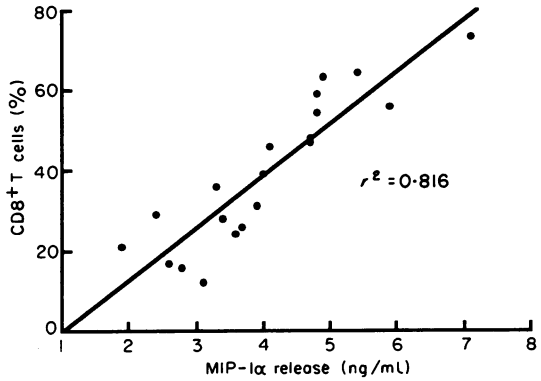

Fig. 5. Correlation between CD8⁺ T cell alveolitis and MIP-1α release in HIV-1-infected subjects.

HIV-1 infection is characterized by a dysregulation of a variety of immune parameters even before there is evidence for enhanced susceptibility to neoplasia and opportunistic pathogens [1,3,4]. Mucosal surfaces appear to be important early targets of immune dysfunction, including an enhanced release by alveolar macrophages of cytokines such as IL-6, GM-CSF and TNF-α [11,12]. *In vitro* data have suggested that elaboration of such cytokines (particularly TNF-α) might hasten the progression of viral disease, by enhancing viral replication in monocytic or lymphocytic cells [26]. Moreover, the exuberant production of cytokines such as TNF-α may contribute significantly to the pathology of the final stages of AIDS, and TNF-α neutralization in the final stages of AIDS is a promising therapy [27].

MIP-1α may contribute to the lymphocytic alveolitis in HIV-1-infected subjects by its chemotactic activity towards activated CD8⁺ T cells. Indeed, MIP-1α is strongly chemotactic for CD8⁺ T cells, whereas MIP-1β has the same effect on CD4⁺ T cells [28,29]. Recent data have shown that CD8⁺ T cells may provide substantial antiviral activity, via the production of a soluble factor which blocks HIV-1 replication in infected CD4⁺ T lymphocytes [30,31]. Studies have shown that CD8⁺ T lymphocytes can kill cells expressing HIV-1 proteins [32,33]. These cytotoxic CD8⁺ cells are present in large numbers during the asymptomatic period, but their activity declines with progression of the disease [33]. However, an infiltration with activated CD8⁺ T lymphocytes may contribute to tissue pathology via 'bystander' cytotoxicity towards uninfected cells or enhanced lymphokine production (reviewed in [1]).

Direct infection of macrophages with HIV-1 did not induce any significant production of MIP-1α, in general agreement with other findings which have suggested that HIV-1 infection of macrophages induces a very modest modulation of macrophage monokine release ([34] and references therein). In the case of HIV-1-infected subjects in group IV, the cytokine up-regulation may result from exposure to opportunistic pathogens. Other groups have shown an elevated cytokine production and activation status of alveolar macrophages from HIV-1-infected asymptomatic subjects [3,11,12]; this has been ascribed to a general up-regulation of the immune system, perhaps related to circulating IFN-γ in HIV-1-infected patients [3,35]. With regard to the impact of MIP-1α release on resistance to opportunistic pathogens, a recent study has shown a role for MIP-1α in the formation of granulomas against *Schistosoma mansonii* [18]; the impact of MIP-1α on resistance to other infectious agents remains unclear. It remains to be seen whether MIP-1α plays a role in resistance to AIDS-associated opportunistic pathogens.

Our data suggest a role for MIP-1α in the CD8⁺ lymphocytic alveolitis which occurs in symptomatic and asymptomatic HIV-1-infected subjects. Once the impact of this CD8⁺ lymphocytic alveolitis on AIDS progression is clarified (protective *versus* tissue damaging), opportunities to slow disease progression, whether viral or microbial, by modulating MIP-1α release may become apparent.

ACKNOWLEDGMENTS

We thank E. Shavers and N. Carrier for expert technical assistance, M. Bédard for work on the data and manuscript, and Dr E. Smilis for help with the BAL samples. We also thank Dr M. G. Bergeron (Université Laval, Canada) for guidance on samples from AIDS patients. This study was supported in part by grants from the Medical Research Council/National Health Research and Development Program Joint Committee on AIDS and from the 'Fonds de la recherche en santé du Québec' (FRSQ). M.D. is an FRSQ scholar.

REFERENCES

1 Levy JA. Pathogenesis of human immunodeficiency virus infection. Microbiol Rev 1993; **57**:183–289.

2 Centers for Disease Control, Atlanta, USA. Classification system for human T-lymphotropic virus type III/lymphadenopathy-associated virus infections. Ann Intern Med 1986; **105**:234–7.

3 Buhl R, Jaffe MA, Holroyd KJ *et al.* Activation of alveolar macrophages in asymptomatic HIV-1 infected individuals. J Immunol 1993; **150**:1019–28.

4 Clerici M, Hakim FT, Venzon DJ, Blatt S, Hendrix CW, Wynn TA, Shearer GM. Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals. J Clin Invest 1993; **91**:759–65.

5 Guillon JM, Autran B, Denis M, Fouret P, Plata F, Mayaud CM, Akoun GM. Human immunodeficiency virus-related lymphocytic alveolitis. Chest 1988; **94**:1264–9.

6 Autran B, Mayaud CM, Raphael M *et al.* Evidence for a cytotoxic T-lymphocyte alveolitis in human immunodeficiency virus-infected patients. AIDS 1988; **2**:179–85.

7 Itescu S, Dalton J, Zhang HZ, Winchester R. Tissue infiltration in a CD8 lymphocytosis syndrome associated with human immunodeficiency virus-1 infection has the phenotypic appearance of an antigenically driven response. J Clin Invest 1993; **91**:2216–25.

8 Itescu S, Brancato LJ, Buxbaum J *et al.* A diffuse infiltrative CD8 lymphocytosis syndrome in HIV infection: a host immune response, associated with HLA-DR5. Ann Intern Med 1990; **112**:3–10.

9 Meltzer MS, Skillman DS, Gomatos PJ. Role of mononuclear phagocytes in the pathogenesis of human immunodeficiency virus infection. Ann Rev Immunol 1990; **8**:169–94.

10 Gendelman HE, Orenstein JM, Baca LM, Weiser B, Burger H, Meltzer MS. The macrophage in the persistence and pathogenesis of HIV infection. AIDS 1989; **3**:475–95.

11 Israël-Biet D, Cadvanel J, Beldjord K, Andrieu JM, Jeffrey A, Even P. Tumor necrosis factor production in HIV-seropositive subjects. J Immunol 1991; **147**:490–4.

12 Agostini C, Trentin L, Zambello R *et al.* Release of granulocyte-macrophage colony stimulating factor by alveolar macrophages in the lungs of HIV-1 infected patients. J Immunol 1992; **149**:3379–85.

13 Salahuddin SZ, Rose RM, Groopman JE, Markham PD, Gallo RC. Human T lymphotropic virus type III infection of human alveolar macrophages. Blood 1986; **68**:281–7.

14 Pearce TE, Nowakowski M, Eden E *et al.* Uniform detection of HIV-1 in alveolar macrophages of pediatric but not adult AIDS patients. J Leukocyte Biol 1993; **53**:722–6.

15 Davatelis G, Tekamp-Olson P, Wolpe SD *et al.* Cloning and characterization of a cDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokinetic properties. J Exp Med 1988; **167**:1939–44.

16 Wolpe SD, Davatelis G, Sherry B *et al.* Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. J Exp Med 1988; **167**:570–81.

17 Oppenheim JJ, Zachariae COC, Mukaido N, Matsushima K. Properties of the novel proinflammatory supergene 'intercrine' cytokine family. Annu Rev Immunol 1991; **9**:617–43.

18 Lukacs NW, Kunkel SL, Strieter RM, Warmington R, Chensue SW. The role of macrophage inflammatory protein Ia in *Schistosoma mansonii* egg-induced granulomatous inflammation. J Exp Med 1993; **177**:1551–9.

19 Wolpe SD, Cerami A. Macrophage inflammatory proteins 1 and 2; members of a novel superfamily of cytokines. FASEB J 1989; **3**:2565–73.

20 Tekamp-Olson P, Gallegos C, Bauer D *et al.* Cloning and characterization of cDNAs for murine macrophage inflammatory protein 2 and its human homologue. J Exp Med 1990; **172**:911–9.

21 Wolpe SD, Sherry B, Juers D, Davatelis G, Yurt RW, Cerami A. Identification and characterization of macrophage inflammatory protein 2. Proc Natl Acad Sci USA 1989; **86**:612–6.

22 Sherry B, Tekamp-Olson P, Gallegos C *et al.* Resolution of the two components of macrophage inflammatory protein 1 and cloning and characterization of one of these components, macrophage inflammatory protein 1 beta. J Exp Med 1988; **168**:2251–9.

23 Standiford TJ, Rolfe MW, Kunkel SL *et al.* Macrophage inflammatory protein-1α expression in interstitial lung disease. J Immunol 1993; **151**:2852–63.

24 Pilaro AM, Sayers TJ, McCormick KL, Reynolds CW, Wiltrout RW. An improved *in vitro* assay to quantitate chemotaxis of rat peripheral blood large granular lymphocytes. J Immunol Methods 1990; **135**:213–23.

25 Davatelis G, Wolpe SD, Sherry B, Dayer JM, Chicheportiche R, Cerami A. Macrophage inflammatory protein-1; a prostaglandin-independent endogenous pyrogen. Science 1989; **243**:1066–8.

26 Osborn L, Kunkel SL, Nabel GJ. Tumor necrosis factor alpha and interleukin-1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κB. Proc Natl Acad Sci USA 1989; **86**:2336–40.

27 Dezube BJ, Pardee AB, Chapman B *et al.* Pentoxifylline decreases tumor necrosis factor expression and serum triglycerides in people with AIDS. J Acquir Immune Defic Syndr 1993; **5**:1099–104.

28 Schall TJ, Bacon K, Camp RDR, Kaspari JW, Goeddel DV. Human macrophage inflammatory protein alpha (MIP-1α) and MIP-1β chemokines attract distinct populations of lymphocytes. J Exp Med 1993; **177**:1821–5.

29 Taub DD, Conlon K, Lloyd AR, Oppenheim JJ, Kelvin DJ. Preferential migration of anticated $CD4^+$ and $CD8^+$ T cells in response to MIP-α and MIP-1β. Science 1993; **260**:355–8.

30 Walker CM, Moody DJ, Stites DP, Levy JA. $CD8^+$ lymphocytes can control HIV infection *in vitro* by suppressing virus replication. Science 1986; **234**:1563–6.

31 Brinchmann JE, Graudernack G, Vartdal F. $CD8^+$ cells inhibit HIV replication in naturally infected $CD4^+$ T cells; evidence for a soluble inhibitor. J Immunol 1990; **144**:2961–6.

32 Nixon DF, McMichael AJ. Cytotoxic T cell recognition of HIV proteins and peptides. AIDS 1991; **5**:1049–59.

33 Gotch F, Rowland-Jones S, Nixon D. Longitudinal study of HIV *gag*-specific cytotoxic T lymphocyte responses over time in several patients. In: Racz P, Letvin NL, Gluckmann JC, eds. Cytotoxic T cells in HIV and other retroviral infections. Basel: S. Karger, 1992:60–65.

34 Molina JM, Scadden DT, Amirault C, Woon A, Vannier E, Dinarello CA, Groopman JE. Human immunodeficiency virus does not induce interleukin-1, interleukin-6 or tumor necrosis factor in mononuclear cells. J Virol 1990; **64**:2901–6.

35 Griffin DE, McArthur JC, Cornblath DR. Neopterin and interferon gamma in serum and cerebrospinal fluid of patients with HIV-associated neurologic disease. Neurology 1991; **41**:69–75.