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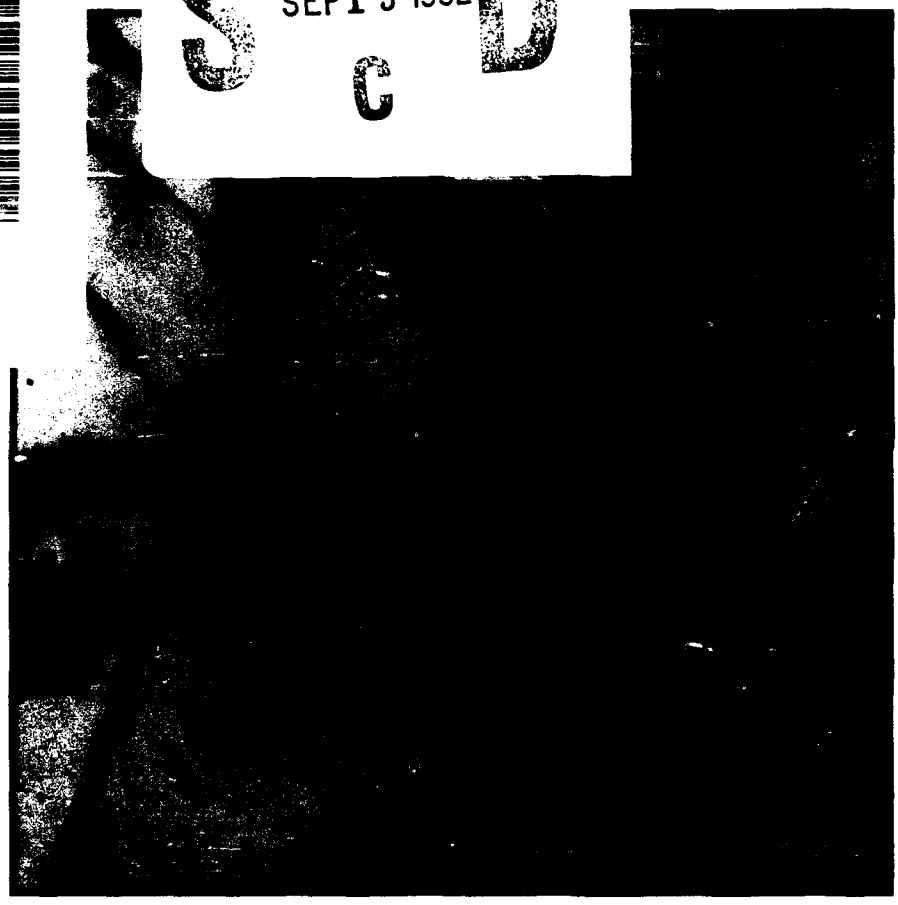

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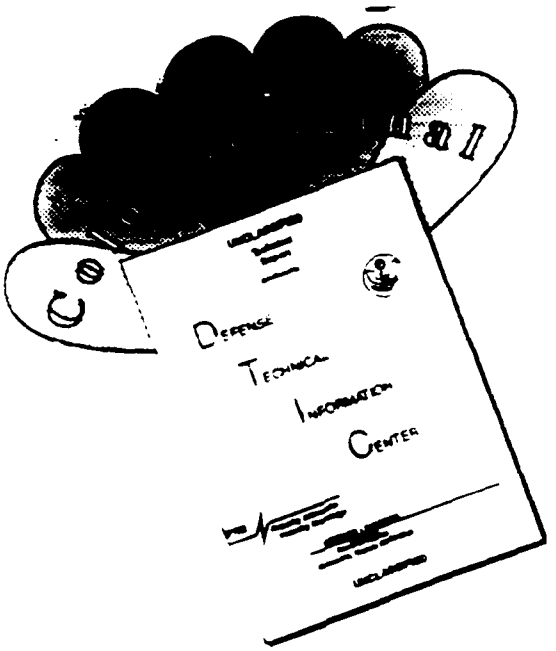

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BRIEF COMMUNICATION

Interactions between HIV-infected monocytes and the extracellular matrix: HIV-infected monocytes secrete neutral metalloproteases that degrade basement membrane protein matrices

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Abstract: The frequency of human immunodeficiency virus (HIV)-infected monocytes that spread on a model basement membrane was about twofold greater than that of an equal number of uninfected control cells through the initial 12 to 18 h of culture. By 24 h, virtually all HIV-infected and uninfected control cells spread on the basement membrane gel. The frequency of spread cells in the uninfected control population was < 10% of total cells by 12 days. In contrast, 30 to 40% of HIV-infected monocytes remained spread through this time interval and formed a dense interdigitated network of cell processes on and into the gel matrix. Invasion of the basement membrane matrix by HIV-infected monocytes suggested increased secretion of proteases able to digest the gel. Indeed, levels of neutral protease activity in culture fluids from HIV-infected monocytes were significantly higher than those from equal numbers of uninfected control cells. High levels of protease activity in culture fluids of HIV-infected monocytes required productive virus infection and were not observed with cells exposed to T cell-tropic HIV isolates. The predominant protease activity in these cultures was a 92-kd neutral metallogelatinase. HIV-induced changes in monocyte metalloprotease activity may be important for extravasation of infected cells to tissue or for the development of AIDS-associated neuropathology, carcinogenesis, and opportunistic infection. *J. Leukoc. Biol.* 52: 244–248; 1992.

Key Words: *basement membrane • extracellular matrix • metalloprotease • HIV-1*

The tissue distribution of infected macrophages in patients with human immunodeficiency virus (HIV) disease is not homogeneous: macrophages of the central nervous system, lung, and lymph nodes are infected at high frequency (10 to 50% of cells in affected tissue); perivascular and parenchymal macrophages of liver and other organs are infected only rarely if at all [1–6]. Factors that control trafficking and localization of HIV-infected monocytes to tissue or susceptibility of tissue macrophages to HIV infection are poorly understood. Tissue macrophages can certainly be infected by HIV from the fluid phase. Cell tropism of HIV is controlled by the amino acid sequence of the V3 loop, the site against which most neutralizing antibodies are directed. Most of the virus found in blood and tissues of HIV-infected patients is macrophage tropic. Indeed, T cell-tropic HIV isolates are statistically rare (<0.4%) but become more prevalent as disease progresses [7]. However, several studies now suggest that cell-cell spread of HIV may be more rapid or efficient than infection from the fluid phase and consequently more important to the initiation and progression of HIV disease [8].

Blood monocytes are infected with HIV at low frequency. In a recent survey, the frequency of infected cells in all blood leukocytes was about 0.01%. HIV DNA was detected in 95% of purified CD4+ T cells and 62% of monocyte populations [9]. The circulating half-life of human blood monocytes is estimated to be about 0.5 to 3 days [10]. Extravasation of blood monocytes is a complex multistep reaction that involves adhesion to vascular endothelium through specific integrin receptors and migration through the basement membrane into tissue, where these cells then differentiate into macrophages. Tissue macrophages may survive for months to years. Indeed, certain resident tissue macrophage populations proliferate at low levels and sustain their numbers in the steady state [11].

Any change in the manner by which HIV-infected monocytes interact with vascular endothelium, the basement membrane proteins, or the extracellular matrix could markedly influence their localization and extravasation into tissues. We previously reported that HIV-infected macrophages adhere to and spread on extracellular matrix proteins more efficiently than do equal numbers of uninfected control cells. Furthermore, the extent of virus replication and of HIV-associated cytopathic effects in monocytes also increases with culture on certain extracellular matrix proteins [12]. In this report, we show that the capacity of HIV-infected monocytes to spread on, digest, and invade a model basement membrane was significantly greater than that of uninfected control cells.

Equal numbers of HIV-infected and control monocytes were cultured on a matrix of basement membrane proteins in gel form (Matrigel) through 2 weeks. The major components of Matrigel in decreasing order are laminin, collagen

Abbreviations: Ag, antigen; HIV, human immunodeficiency virus; IFN-α, interferon-α; IL-1β, interleukin-1β; MCSF, macrophage colony-stimulating factor; TNF-α, tumor necrosis factor α.

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IV, heparan sulfate proteoglycans, and entactin. Levels of bacterial endotoxins in these preparations were < 0.25 ng/ml by *Limulus* amebocyte lysate assay (Collaborative Biomedical Products, Bedford, MA). During the initial 24 h of culture, the percentage of HIV-infected monocytes that spread on Matrigel was about twofold greater than that of uninfected control cells: 22 ± 5% versus 10 ± 2% at 6 h, 81 ± 11% versus 40 ± 2% at 12 h, and 100 ± 0% versus 72 ± 12% at 18 h (**Fig. 1**, inset). By 24 h, virtually all HIV infected and uninfected control monocytes were spread. This magnitude of difference in the frequency of spread cells between HIV-infected and uninfected control monocytes on Matrigel was highly reproducible and evident in each of three separate experiments.

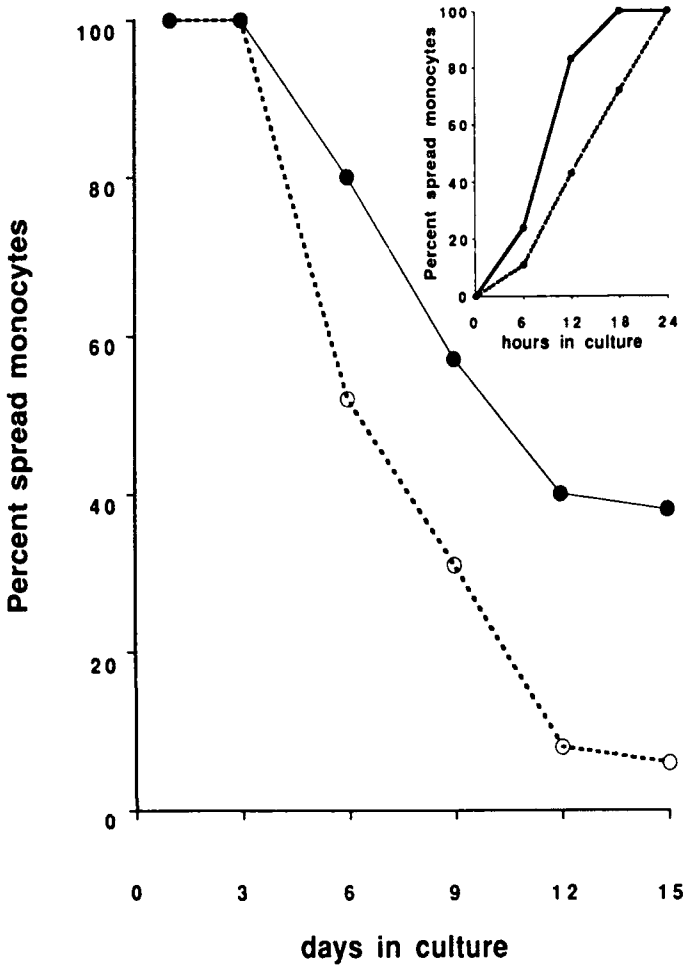

Fig. 1. Interaction of HIV-infected and uninfected control monocytes with basement membrane protein matrix. Monocytes recovered from peripheral blood mononuclear cells of HIV- and hepatitis B-seronegative donors after leukapheresis were purified (> 98% monocytes) by countercurrent centrifugal elutriation and cultured in Teflon flasks at a density of 2×10^6 cells/ml in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) with 10% heat-inactivated AB$^+$ human serum, 50 μg/ml gentamycin, and 1000 U/ml highly purified human recombinant macrophage colony-stimulating factor (rMCSF) (lot FAP-809, Cetus Corporation, Emeryville, CA). All culture reagents were negative for endotoxin contamination. At 5 to 7 days of culture, monocytes were exposed to HIV-1_{ADA}, a monocytotropic virus originally isolated and passaged in monocytes, at a multiplicity of infection of 0.05 infectious virus/target cell [14]. All viral stocks were free of mycoplasma contamination (Gen-probe II, Gen-probe Inc., San Diego, CA). Three days after infection, HIV-infected (filled circles, solid lines) and uninfected control (open circles, dashed lines) monocytes were washed three times, resuspended in serum-free macrophage medium (SFM-macrophage medium, Gibco) with rMCSF, and cultured at 2×10^4 monocytes/plastic culture well (Costar^{24}, Costar, Cambridge, MA) on a mixture of basement membrane proteins in gel form (Matrigel, Collaborative Biomedical Products, Bedford, MA). The mean number of spread and total cells was determined by microscopic examination of three separate 1.4-mm^2 fields in duplicate cultures [12]. Data presented are representative of results in at least three replicate experiments.

With time in culture, the number and extent of spread of uninfected control monocytes decreased so that by 12 days less than 11 ± 4% of cells remained spread. It is important to note that this loss of spreading by uninfected control monocytes on basement membrane matrix was not related to cell viability. The number of viable cells (trypan blue dye exclusion) remained constant in these cultures through 2 weeks. The pattern of cell spreading with HIV-infected monocytes was quite different from that of uninfected control cells. The percentage of spread HIV-infected monocytes also decreased with time in culture, but a large and distinct subpopulation of cells (39 ± 13% of total cells) maintained a spread morphology through 2 weeks. Indeed, the spread HIV-infected monocytes at 2 weeks in culture formed a dense interdigitated network of cell processes on and into the basement membrane matrix (**Fig. 2b**). Careful analysis of the HIV-infected monocytes by phase microscopy showed obvious degradation of the Matrigel around the multinucleated giant cells characteristic of HIV infection (data not shown) [1]. Furthermore, numerous monocyte cell bodies and processes were clearly evident below the plane of focus on the gel surface and extended to the underlying plastic surface of the culture well (about 0.5 to 1 mm). At least 10-fold more HIV-infected monocytes than uninfected control cells reached the underlying plastic surface in the 2-week culture interval (data not shown).

The relationship between extent of HIV infection and numbers of cells spread on Matrigel was examined by quantitation of cells that expressed p24 antigen (Ag) by flow cytometry. All cells were recovered from Matrigel (Collaborative Biomedical Products) after treatment with a bacterial neutral protease (Dispase, Collaborative Biomedical Products) and examined for p24 Ag expression by monoclonal antibody (phycoerythrin-conjugated anti-p24 HIV core protein, Coulter Source, Marietta, GA) and flow cytometry. The frequency of monocytes that expressed p24 Ag on Matrigel increased with time in culture (17% at 4 days, 31% at 7 days, and 33% at 10 days). At 12 days after HIV infection, 37% of total monocytes expressed p24 Ag. This increase was coincident with a similar increase in levels of virion-associated reverse transcriptase activity in the culture fluids and exactly matched the frequency of spread monocytes at this time point (39% at 12 days). In a previous study, we showed that most monocytes able to spread on extracellular matrix proteins during HIV infection were productively infected by virus as quantified by indirect immunofluorescence with pooled sera from HIV-infected patients [12]. These data together strongly suggest (but do not prove) that most of the monocytes spread on and in the Matrigel were also productively infected with HIV.

The markedly increased capacity of HIV-infected monocytes to spread on, digest, and enter into the basement membrane matrix suggested that these cells released high levels of protease activities not present with the uninfected control cells. This possibility was directly examined by analysis of culture fluids from equal numbers of HIV-infected and uninfected control monocytes for neutral metalloprotease activities by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 1 mg/ml gelatin and the divalent cations Zn^{2+} and Ca^{2+} at pH 7.6 [13]. Culture fluids from HIV-1_{ADA}-infected monocytes were rich in a 92-kd gelatinase present at much lower levels in culture fluids from

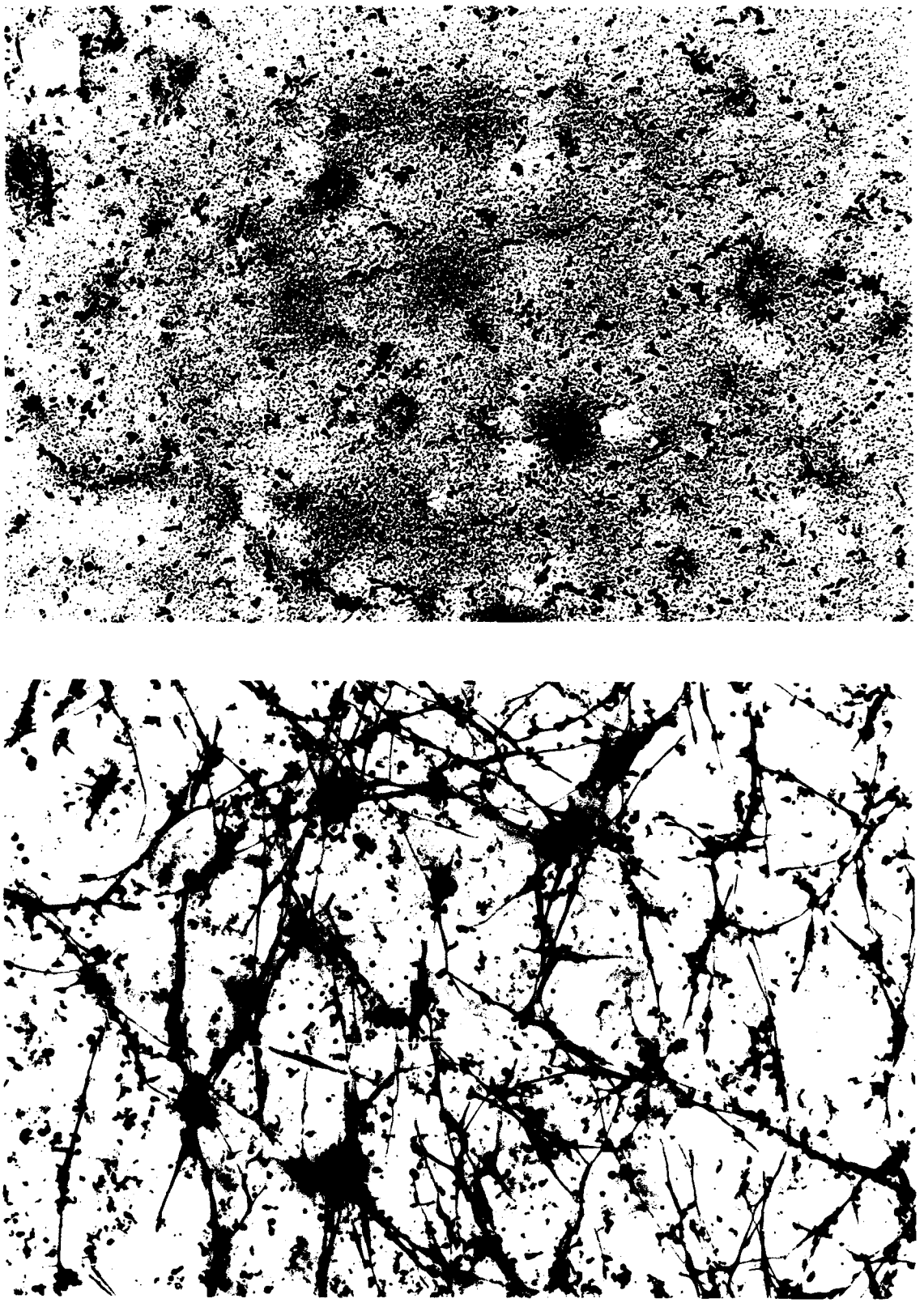

Fig. 2. Morphology of HIV-infected and uninfected control monocytes on basement membrane protein matrix. Equal numbers of HIV-infected and uninfected control monocytes were added to Matrigel in plastic culture wells. After 15 days, cells were fixed in 0.5% paraformaldehyde, Wright stained, and microscopically examined. (a) Uninfected control monocytes, (b) HIV infected cells. × 200.

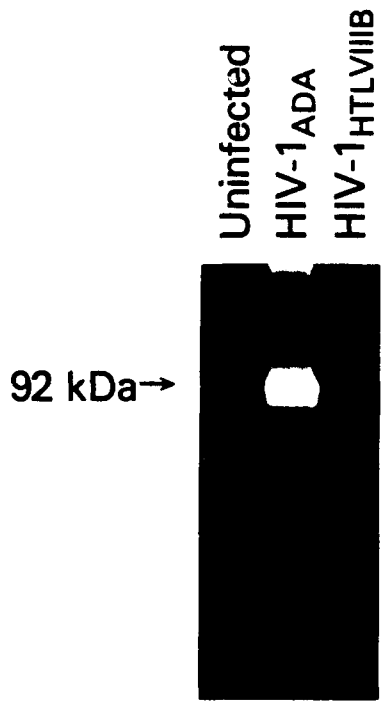

Fig. 3. Neutral metalloprotease activity in culture fluids of HIV-infected and uninfected control monocytes. Equal numbers (1×10^7 cells/plastic culture well) of uninfected control monocytes and cells infected with HIV-1_{ADA} or HIV-$1_{HTLVIIIB}$ 12 days previously were cultured in serum-free medium for 18 h. Cell-free culture fluids without added reducing agents were examined for neutral metallogelatinase activity after electrophoresis on a 10% polyacrylamide gel with 1 mg/ml gelatin (Novex Corporation, San Diego, CA). Gels were immersed in pH 7.6 buffer with $ZnCl_2$ and $CaCl_2$ for 2 h at 37°C, then stained with Coomassie Brilliant Blue, and destained with methanol-acetic acid. Gelatinase activity was estimated by the band with enzymatic activity indicated by negative staining in the gels [13]. Data presented are representative of results in at least three replicate experiments.

uninfected control cells or cells exposed to HIV-$1_{HTLVIIIB}$, a T cell-tropic HIV isolate that does not replicate in monocytes [14] (**Fig. 3**). All protease activities in both HIV-infected and uninfected control monocytes were completely inhibited by addition of 10 mM ethylenediaminetetraacetic acid. The marked difference in 92-kd neutral metallogelatinase levels in culture fluids of monocytes exposed to HIV-1_{ADA} and HIV-$1_{HTLVIIIB}$ is of special interest. The gp120 of HIV-$1_{HTLVIIIB}$ binds to cell surface determinants of monocytes including CD4 [15]. Transmission electron microscopic examination of monocytes exposed to HIV-$1_{HTLVIIIB}$ also shows viral particle uptake [16]. But this T cell-tropic HIV-1 strain replicates poorly or not at all in monocytes [17]. Whatever signals initiate the increased neutral metalloprotease activity in culture fluids of HIV- infected monocytes must require reactions beyond gp120-CD4 interactions and internalization of virus. Furthermore, we and others showed that HIV infection of monocytes does not induce the mRNA or activity of interleukin-1β (IL- 1β), IL-6, tumor necrosis factor α (TNF-α), or interferon-α (IFN-α) [18, 19]. Thus these particular cytokines are unlikely participants in the changes reported here.

Blood monocytes and tissue macrophages are potentially able to secrete an assortment of neutral proteases with capacity to degrade the extracellular matrix. Such proteases range from granule-associated serine proteases of monocytes to the neutral metalloproteases of tissue macrophages (interstitial collagenase, 92- and 72-kd type IV/type V collagenase, and stromelysin) [20, 21]. The secretion profile for these macrophage-derived proteases changes with cell differentiation, inflammation, and exposure to bacterial endotoxins and certain cytokines (IFN-γ, IL-4) [20–23]. The neutral metalloproteases of macrophages participate to a major extent in tissue remodeling, wound healing, tumor invasion, and inflammation. That the secretion of the 92-kd metallogelatinase of macrophages was markedly increased by productive HIV infection was unexpected but has obvious implications for the progression of HIV infection and the development of HIV-associated neuropathology, carcinogenesis, and opportunistic infection.

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