CYTOKINE-INDUCED INTERLEUKIN-8 PRODUCTION IS DEPRESSED IN CHRONIC AS OPPOSED TO ACUTE HUMAN IMMUNODEFICIENCY VIRUS 1 INFECTION OF PROMONOCYTIC CELLS

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Summary. – Spontaneous secretion of interleukin 8 (IL-8) was higher in latently infected U1 cells than in acutely infected or uninfected parental U937 cells. However, the induction of IL-8 by various cytokines (IL-1α, TNF-α, IL-6, TNF-β, GM-CSF, IFN-γ) was significantly reduced in U1 cells. Cytokine modulation of IL-8 production in U937 cells acutely infected with a T cell-tropic strain (IIIB) or monocytotropic strain (ADA) of human immunodeficiency virus 1 (HIV-1) (HIV-1_{IIIB} and HIV-1_{ADA}) was variable and showed strain-specific differences. The obtained results showed that the *in vitro* induction of IL-8 is impaired in promonocytic cells latently infected with HIV-1 and is differently modulated under acute conditions of infection depending on the IL-8 inducing cytokine and on the infecting virus strain.

Key words: interleukin-8; HIV-1; promonocytic cells; chronic and acute infection

In asymptomatic HIV-1 infection, infected cells in the periphery are believed to be non-productively infected and persistent and latent infections are established in several tissues. Up to 20% of the lymphocytes in lymph nodes of asymptomatic HIV-1-infected patients harbor HIV-1 proviral DNA, with very few cells (0.1–0.001% or less) actively replicating virus (Embretson *et al.*, 1993b; Harper *et al.*, 1986). In patients with acquired immunodeficiency syndrome (AIDS) there is an increase in the proportion of lymphocytes and monocytes (up to 80%) in lymphoid tissue that harbor proviral DNA (Embretson *et al.*, 1993a,b). However, it has been estimated that less than 1% of virions are derived from latently infected cells (Perelson *et al.*, 1996). In contrast to HIV-1 infection of $CD4^+$ T cells which is cytolytic, the cells of the monocyte/macrophage (M/M) lineage survive infection and so represent a major reservoir of the virus thereby allowing its persistence and dissemination. As for $CD4^+$ T cells, monocytes also require activation in order to support a productive HIV-1 replication.

Monocytes are potent producers of IL-8 (Matsushima *et al.*, 1988; Schröder *et al.*, 1987), a member of the C-X-C or α-chemokine subfamily of chemokines (Baggiolini *et al.*, 1994), which acts as a chemoattractant for polymorphonuclear neutrophils and T lymphocytes and mediates many phagocyte functions essential to the resolution of primary or secondary bacterial and fungal infections. A role for IL-8 in HIV-1 pathogenesis has been suggested on the basis of significantly elevated levels of IL-8 in the peripheral circulation of HIV-1-infected individuals (Matsumoto *et al.*, 1993; Meddows-Taylor *et al.*, 1999; Thea *et al.*, 1996).

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Abbreviations: AIDS = acquired immunodeficiency syndrome; AM = alveolar macrophage; ELISA = enzyme-linked immunosorbent assay; GM-CSF = granulocyte macrophage-colony stimulating factor; HIV-1 = human immunodeficiency virus 1; HIV_{ADA} = ADA strain of HIV-1; HIV_{IIIB} = IIIB strain of HIV-1; HIV_{SF2} = SF2 strain of HIV-1; IL-1α, IL-1β, IL-6, and IL-8 = interleukins 1α, 1β, 6, and 8; M/M = monocyte/macrophage; PBMC = peripheral blood mononuclear cell; PBS = phosphate-buffered saline; SI = stimulation index; TNFα, TNFβ = tumor necrosis factors α, β

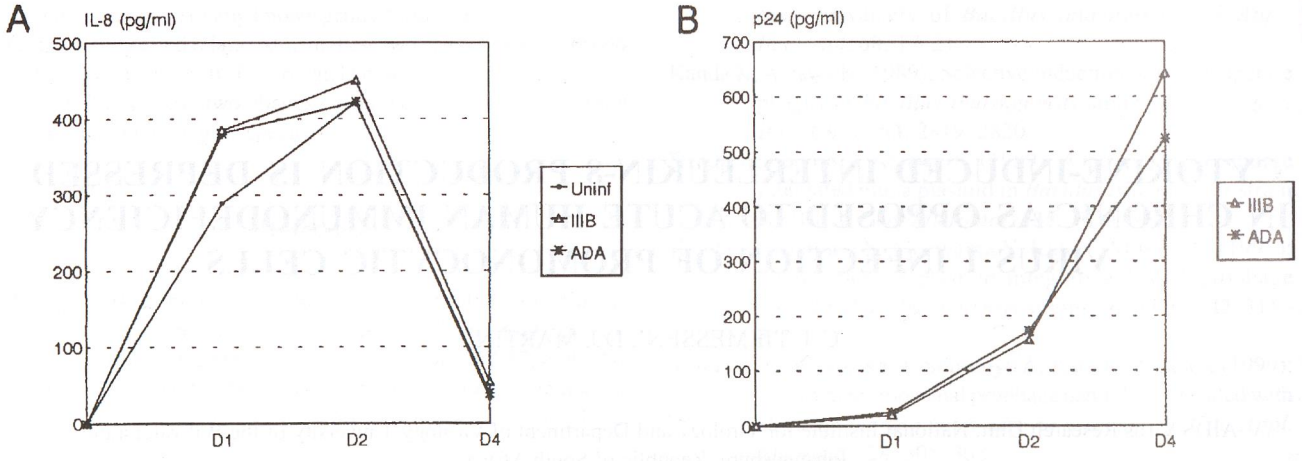

Fig. 1

Time course of IL-8 (A) and p24 (B) protein production by U937 cells

U937 cells at a concentration of 10^6 cells/ml were infected for 1 hr at 37°C with HIV-1_{IIIB} (final concentration 3 ng p24/ml and 6 x 10^6 RNA copies/ml) and HIV-1_{ADA} (final concentration 6 ng p24/ml and 6 x 10^6 RNA copies/ml). The cells were washed 3 times with PBS, adjusted to 2 x 10^5 cells/ml in RPMI-1640 medium supplemented with 10 mmol/l Hepes, 100 µg/ml penicillin, 100 µg/ml streptomycin, and 10% (v/v) of fetal calf serum and incubated for the indicated times. Levels of p24 and IL-8 were estimated by ELISA (Coulter Corp., Hialeah, USA and Biotrak™, Amersham, UK, respectively). The results shown are averages from 3 independent experiments (standard error values in all experiments were below 15% of the means).

What the consequences of latent as opposed to acute HIV-1 infection of M/M are with respect to cytokine production in response to exogenous stimuli is largely not known. A number of studies have found an altered cytokine production *ex vivo* in stimulated and non-stimulated peripheral blood mononuclear cells (PBMCs) and alveolar macrophages (AMs) from HIV-1-infected individuals (Chehimi *et al.*, 1994; Denis and Ghadirian, 1994; Israel-Biet *et al.*, 1991). In addition, direct effects of HIV-1 infection (Esser *et al.*, 1991; Tsai *et al.*, 1991) and of HIV-1 proteins (Borghi *et al.*, 1995; Merrill *et al.*, 1989) on cytokine production in monocytes have been described. As many essential functions of neutrophils and the trafficking of T lymphocytes are dependent on adequate secretion of chemokines such as IL-8 by stimulated M/M, we thought it important to question whether the pool of latently as opposed to acutely infected monocytes in HIV-1 infection could contribute to dysregulation of this chemokine. In order to gain some insight into this question we employed the promonocytic U1 cell model of HIV-1 latency and its parental uninfected cell line, designated U937, for acute infection with HIV-1.

The production of IL-8 has been reported to be induced by HIV-1 infection of M/M (Esser *et al.*, 1991; Tsai *et al.*, 1991). As promonocytic U937 cells represent an immature stage of monocyte differentiation it was important first to prove whether IL-8 could be induced in these cells by HIV-1 infection. U937 cells were infected with HIV_{IIIB} and HIV_{ADA} at an input multiplicity that resulted in comparable growth kinetics. Fig. 1 shows a comparison of IL-8 and p24 antigen secreted by U937 cells into the culture medium over a 4 day period. IL-8 production was maximal at day 2 in all cultures returning to undetectable levels at day 4, with HIV-1_{IIIB} and HIV-1_{ADA} p24 levels continuing to rise in infected cultures over this time period. Acute infection with either strain of HIV-1 induced IL-8 production in U937 cells only marginally but consistently above (1.3–1.4-fold) that spontaneously produced by uninfected cells within the first 24 hrs of culture. Corresponding p24 levels at day 1 were below detectable levels indicating that the small increase was due to some early event in HIV-1 replication not dependent on progeny virion production. Furthermore, the infection with heat-inactivated virus showed IL-8 levels comparable to that of uninfected U937 cultures, indicating that the increase in IL-8 production, owing to infection with viable HIV-1, occurred at a post-entry stage. This was further supported by the inability of U937 cells to produce IL-8 in response to recombinant HIV-1_{SF2} envelope protein gp120 (500 ng/ml) (data not shown).

As the effects on IL-8 production by HIV-1 infection were best detected in promonocytic cells within the first 24 hrs of culture this time point was chosen for further experiments. U937 and U1 cells (2 x 10^5/ml) were incubated in the presence of a number of cytokines known to enhance IL-8 production and the stimulation index (SI) calculated for each cytokine (Fig. 2). SI values, defined as the degree of IL-8 induction above background, were lower in U1 cells as compared to uninfected U937 cells for all cytokines tested (except for interleukin 1β (IL-1β)). This difference was also

found after a phorbol 12-myristate 13-acetate treatment of both cell types (data not shown). In contrast, U1 cells in the absence of IL-8 inducing cytokines spontaneously secreted 13-fold more IL-8 into the culture medium as compared to uninfected U937 cells.

Having found that cytokine-induced IL-8 secretion in cells chronically infected with HIV-1 is substantially reduced as compared to their uninfected counterparts, we next questioned the effects of acute HIV-1 infection on cytokine-induced IL-8 production. As for uninfected U937 cells, acutely infected U937 cells, whether infected with HIV-1_{IIIB} or HIV-1_{ADA}, produced higher levels of IL-8 than chronically infected U1 cells for all cytokines tested except IL-1β (Fig. 3). IL-8 responses to acute infection with either virus strain relative to that for the uninfected control could be grouped into four patterns of modulation by cytokines. TNFα-decreased IL-8 production in acutely infected U937 cells (Fig. 3A). In the presence of IL-6, the infection with HIV-1_{IIIB} further increased IL-8 production, while the infection with HIV-1_{ADA} resulted in no change (Fig. 3B). GM-CSF and TNFβ-substantially enhanced IL-8 production, with GM-CSF showing a greater synergy with HIV-1_{ADA} (Fig. 3C). HIV-1 infection did not appreciably alter IL-8 secretion in the presence of IFN-λ or IL-1β (Fig. 3D). The modulation of IL-8 production in U937 cells by HIV-1 infection in the presence of IL-8 inducing cytokines was therefore dependent on the inducing cytokine and, in the case of GM-CSF and IL-6, on the infecting virus strain. GM-CSF showed a greater synergy with HIV-1_{ADA} while the reverse situation occurred in the presence of IL-6 where it synergized with HIV-1_{IIIB} in enhanced production of IL-8. Therefore there were definite virus strain-specific differences in IL-8 modulation by some cytokines.

Cytokines used in this study for IL-8 induction belong to those known to induce HIV-1 expression in U1 cells (Vicenzi and Poli, 1994). NF-IL6 binding sites have been shown to be essential for IL-8 gene activation (Mukaida *et al.*, 1990). An inducible transcription factor, NF-κB, a heterodimeric complex of two subunits, a 50 K subunit or p50 (NF-KB1) and a 65 K subunit or p65 (RelA) (Baeuerle, 1991) supports HIV-1 LTR-driven transcription. In contrast, it has been found that the κB-regulated expression of the IL-8 gene is regulated by RelA homodimers (Kunsch and Rosen, 1993). Despite these differences, it is reasonable to suggest that the activation either of HIV-1 LTR or IL-8 gene promoter would involve competition for common nuclear transcription factors, the extent of which may be dependent on the signal transduction pathways utilized by different cytokines. For example, IL-1β, unlike TNF-α, has been shown to induce HIV-1 expression independent of the activation of NF-κB and utilizes type I IL-1 cell surface receptors (Poli *et al.*, 1994). This may provide an explanation why IL-1β specifically showed no defective IL-8 secretion in U1 cells as compared to uninfected or acutely infected U937 cells. This suggests that the HIV-1 induced from latency by IL-1β did not interfere with its concomitant induction of IL-8 secretion as seen with other cytokines. Whatever the mechanisms, the overall consequence of chronic infection of promonocytic cells on IL-8 production was a diminished ability to respond to most cytokine agonists. These results support those of another study utilizing the same cell model which demonstrated that IFN-α could rescue IL-8 secretion in latently infected promonocytic cells (Ohashi *et al.*, 1994).

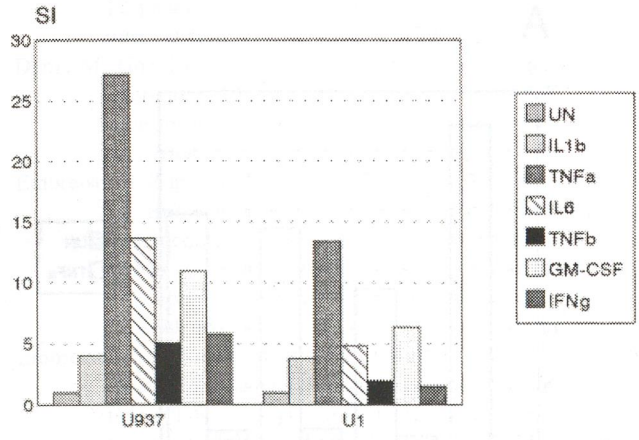

Fig. 2
Comparison of IL-8 induction by various cytokines in uninfected U937 cells and U1 cells chronically infected with HIV-1
U937 and U1 cells (2 x 10^5 cells/ml) were either untreated or treated with the following cytokines: 2.5 ng/ml TNF-α, 500 U/ml IL1-β, 100 U/ml IL-6, 25 ng/ml TNF-β, 100 U/ml GM-CSF and 100 U/ml IFN-γ (Boehringer Mannheim, Germany). The supernatants were collected after 24 hrs of incubation at 37°C and their IL-8 content was determined by Biotrak™ ELISA. SI for IL-8 expression were calculated as antigen levels obtained in cytokine-treated cultures divided by levels of antigen obtained in untreated cultures. Stimulation index (SI) values for each of the U937 and U1 untreated controls was therefore equivalent to one (signifying no stimulation above background IL-8 levels). The results shown are from one representative experiment. Altogether 3 independent experiments were performed (standard error values in all experiments were below 15% of the means).

The increased spontaneous secretion and the reduced ability of U1 cells to be induced to secrete IL-8 in response to cytokines is reminiscent of reports of enhanced spontaneous release (Denis and Ghadirian, 1994; Israel-biet *et al.*, 1991; Meddows-Taylor *et al.*, 1999) and a diminished capacity of HIV-1 patient PBMCs and AMs to produce a number of cytokines when stimulated *in vitro* (Chehimi *et al.*, 1994; Meddows-Taylor *et al.*, 1999). Our results show that M/M harboring HIV-1 in a latent form could contribute to dysregulation of IL-8 *in vivo* (i) by spontaneously producing IL-8 in the absence of any overt stimulus, the cell perhaps existing in an already primed state, or (ii) by presenting a diminished response when challenged with an IL-8 inducing stimulus.

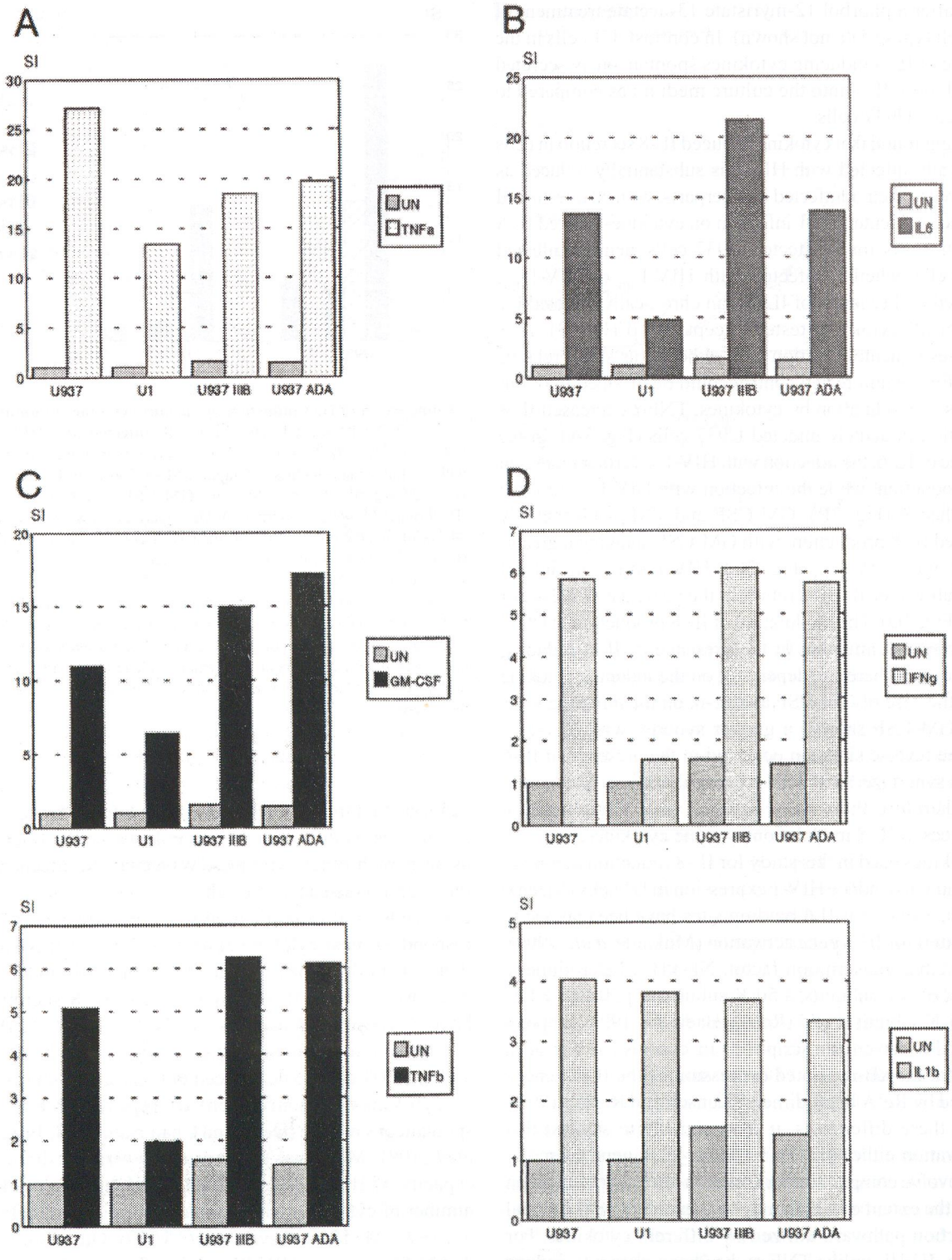

Fig. 3

Patterns of IL-8 production in acutely HIV-1-infected promonocytic U937 cells in the presence of various cytokines

U937 cells, uninfected or infected with HIV_{IIIB} or HIV_{ADA}, were treated with the indicated cytokines at the concentrations described in Fig. 2, and the supernatants were harvested after 24 hrs of incubation and assayed for IL-8 content by ELISA. The four patterns of IL-8 modulation are represented by cytokines TNF-α (A), IL-6 (B), GM-CSF and TNF-β (C), and IFN-γ and IL-1β (D). Corresponding results obtained with latently infected U1 cells are included for comparison. All the results are expressed in SI values.

The dysregulated production of chemokines could lead to altered infiltration of leukocytes into areas of inflammation, affecting both the composition and number of infiltrating leukocytes. As a result, there may be inappropriate or absent cell priming by the chemokines for various effector activities or, alternatively, altered primary agonist-dependent phagocyte or T cell functions. Altered cell trafficking owing to IL-8 dysregulation in HIV-1 infection could have important implications with respect to the role of neutrophils in bacterial and fungal infections, and in dissemination of virus by altered trafficking of infected T lymphocytes. In this regard we have recently shown that both IL-8 receptors (CXCR-1 and CXCR-2) are down-regulated on neutrophils and lymphocytes of HIV-1-infected patients and that their neutrophils have an impaired ability to migrate in response to IL-8 (Meddows-Taylor *et al.*, 1998). This may be a consequence of altered regulation of IL-8 in HIV-1-infected individuals. This *in vitro* study emphasizes the importance of being aware that, even when viral loads in HIV-1 patients receiving anti-retroviral treatment may be below detection, HIV-1 harbored as latent genomes in cells may still contribute to immune dysregulation in these patients. Further studies using the *in vitro* U937/U1 cell model may provide additional insights into how the state of HIV-1 production/non-production may modulate cytokine production in response to immune agonists.

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