Infection of human macrophages with an endogenous tumour necrosis factor-α (TNF-α)-independent human immunodeficiency virus type 1 isolate is unresponsive to the TNF-α synthesis inhibitor RP 55778

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Monocyte-derived macrophages (MDM) were demonstrated to be susceptible to productive infection by the monocytotropic human immunodeficiency virus type 1 (HIV-1) strain HIV-1/Ba-L and by three primary HIV-1 isolates, HIV-1/DAS, HIV-1/PAR and HIV-1/THI. Production of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-1β was monitored between days 3 and 26 after MDM infection. TNF-α and IL-6 were detected in cell culture supernatants from days 16 to 21 following HIV-1/DAS, HIV-1/PAR and HIV-1/Ba-L infection, at the time of high viral replication. IL-1β was not found at the same time points. TNF-α mRNA expression occurred around the peak of both TNF-α levels and supernatant RT activities. In HIV-1/THI-infected macrophage cultures no endogenously produced TNF-α was observed, despite high levels of HIV-1 in MDM. This result demonstrates that a primary isolate may replicate independently of TNF-α in MDM. To investigate the relationship between TNF-α and viral replication we used a TNF-α synthesis inhibitor, RP 55778. Treatment throughout the course of cell culture resulted in a significant decrease in both TNF-α levels and viral production in HIV-1/DAS-, HIV-1/PAR- and HIV-1/Ba-L-infected MDM cultures. This phenomenon is reversed by adding recombinant human TNF-α to the RP 55778-treated cell cultures from day 14 post-infection. No effect of RP 55778 was observed in MDM cultures infected with the primary isolate HIV-1/THI, whose replication is independent of TNF-α production and therefore remained unchanged after RP 55778 treatment. We conclude that the clinical value of such a drug is directly dependent on the ability of the HIV-1 strains involved to induce TNF-α production at the time of viral replication.

Introduction

Human immunodeficiency virus (HIV) (Barré-Sinoussi et al., 1983) infection of monocytes and macrophages plays an important role in the pathogenesis of AIDS (Ho et al., 1987). It has been established that monocytes and macrophages are infected in vivo and can be infected in vitro (Ho et al., 1986; Nicholson et al., 1986; Gartner et al., 1986a; Gendelman et al., 1988; Potts et al., 1990; Popovic & Gartner, 1987). These cells may act as a reservoir of infection (McElrath et al., 1989) allowing virus persistence and they may also behave as a vehicle for the dissemination of HIV into tissues including the brain (Koenig et al., 1986; Plata et al., 1987; Armstrong & Horne, 1984; Rappersberger et al., 1988; Gartner et al., 1986b). Various functional abnormalities of monocytes and macrophages have been reported in patients with HIV infection, including abnormal secretion of macrophage-derived cytokines. Tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-1β are pleiotropic inflammatory cytokines, produced mostly by activated macrophages. They can mediate a wide variety of biological and immunomodulatory effects (Le & Vilcek, 1987; Dinarello, 1989; Kishimoto, 1989; Beutler & Cerami, 1989).

High levels of circulating TNF-α are observed in the sera of AIDS patients (Lähdevirta et al., 1988), and peripheral blood monocytes from such patients have been reported to secrete high levels of TNF-α spontaneously (Wright et al., 1988). Furthermore, IL-6 and IL-1β are detected in the cerebrospinal fluid of HIV-1-infected patients (Gallo et al., 1989). Therefore, the secretion of high levels of cytokines during disease might be partly responsible for the clinical symptoms observed in HIV infections such as cachexia and fever (Cerami & Beutler, 1988). However, others (Cox et al., 1990) have reported that lipopolysaccharide-stimulated bronchoalveolar macrophages from patients at various stages of
HIV-1 infection have significantly lower TNF-α levels in AIDS patients than in healthy HIV-1-seronegative individuals, asymptomatic HIV-1 carriers or in patients with AIDS-related complex.

TNF-α, IL-6 and IL-1β have been shown to enhance HIV expression in different cell systems. For example, stimulation of U1 (a chronically HIV-1-infected promonocytic cell line; Folks et al., 1988) and ACH2 (a chronically HIV-1-infected T lymphocytic cell line; Folks et al., 1985) cells with phorbol myristate acetate (PMA) results in the induction of TNF-α mRNA and the secretion of endogenous TNF-α, which is able to mediate an autocrine mechanism of HIV induction (Fauci, 1988; Poli et al., 1990a). In addition, IL-6 induces HIV expression in U1 cells and synergizes with TNF-α in this effect (Poli et al., 1990b). In the same way, acutely infected primary monocyte-derived macrophage (MDM) cultures exposed to various concentrations of TNF-α and IL-6 showed an increase in HIV replication (Poli et al., 1990b).

Several in vitro experiments have been conducted to assess TNF-α, IL-6 and IL-1 production in human MDM after HIV-1 infection. Increases in IL-6 mRNA synthesis and IL-6 secretion are observed soon after monocyte/macrophage infection with infectious or inactivated HIV-1 (Nakajima et al., 1989). Recently, we demonstrated an increase in TNF-α synthesis and TNF-α gene expression at times of increased virus replication in HIV-1-infected peripheral blood monocyte/macrophage culture supernatants (Le Naour et al., 1992).

Fazely et al. (1991) have demonstrated that pentoxifylline, a molecule that blocks TNF-α production in lipopolysaccharide-treated macrophages (Srieter et al., 1988), induced a significant decrease in HIV-1 replication in infected human peripheral blood mononuclear cells. These observations raise the possibility that inhibition or blocking of TNF-α secretion during HIV-1 production might be partly responsible for the decrease in HIV replication. Likewise, Weissman et al. (1993) demonstrated using the promonocytic cell line U1 that RP 55778, a potent TNF-α synthesis inhibitor (Floch et al., 1989), decreases TNF-α secretion induced by PMA treatment and reduces HIV production by up to 95% compared with control cells. We report here the kinetics of TNF-α secretion and TNF-α gene expression between days 3 and 26 post-infection (p.i.) of human MDM by three primary HIV-1 isolates. Infections were carried out in the presence or absence of RP 55778 to determine the relationship between TNF-α production and virus replication in MDM at different times during in vitro infection and also the potential of RP 55778 to inhibit virus production in primary cell cultures.

Methods

Reagents. The MAX1 monoclonal antibody (MAb), which specifically recognizes differentiated macrophages (Andreasen et al., 1986), was a gift of R. Andreasen (Freiburg, Germany). The anti-CD64 MAb (anti-FcyRI 22.2) was provided by Medarex. This MAb exhibits specific high-affinity binding to FcyRI (CD64) on human mononuclear phagocytes (Fanger et al., 1989). The anti-CD3, anti-CD4 and anti-CD11b (a chain of the CR3, C3b receptor) MAbs were purchased from Becton Dickinson. Human recombinant TNF-α (hrTNF-α) was purchased from BASF/Knoll.

Isolation and culture of human monocyte/macrophages. Peripheral blood mononuclear cells (PBMC) were obtained from healthy donors (seronegative for human T cell leukaemia virus, hepatitis B virus, hepatitis C virus and HIV) after centrifugation of heparinized venous blood over Ficoll–Hypaque gradients. Monocytes were isolated from human PBMC by countercurrent centrifugal elutriation using a Beckman J2-21/ME induction drive centrifuge and a JE-B6 rotor (Beckman Instruments). Freshly elutriated monocytes (20 x 10⁶) were cultured in 75 cm² plastic flasks (Becton Dickinson) containing 20 ml RPMI-1640 medium supplemented with 10% inactivated fetal calf serum (FCS; Boehringer Mannheim), 1% penicillin/streptomycin/ neomycin (Gibco/BRL) and 20 mM-L-glutamine. Macrophages were obtained by 7 day culture differentiation of monocytes. All culture materials used were endotoxin-free, as detected by the Limulus polyphemus amoebocyte lysate assay (limit of detection of endotoxin, 25 to 50 pg/ml; Sigma). Seven days after isolation, cells were extensively washed to remove non-adherent cells. The presence of the membrane antigens CD64, CD4, CD11b and MAX1 was investigated by flow cytometry of elutriated human monocytes and MDM.

Virus strains and virus replication detection. HIV-1 strain DAS (HIV-1/DAS) which replicates efficiently in macrophage cultures (Mabondzo et al., 1992) and HIV-1/PAR were obtained from patients who developed acute and regressive encephalopathy concomitant with primary infection (Boussin et al., 1987; Chermann, 1990). The primary isolate HIV-1/THI was obtained from an HIV-seropositive patient at the time of acute and regressive meningo-encephalopathy (abnormal reflexes, subtle cognitive changes, ataxia, pyramidal dysfunction) without opportunistic infections. HIV-1/Ba-L was a generous gift of Dr A.-M. Aubertin (Strasbourg, France). This strain was initially obtained from the broncho-alveolar fluid of a seropositive patient (Gartner et al., 1986a). This virus replicates well in macrophage cultures. All viruses were grown to high titre in phytohaemagglutinin-stimulated, cord-blood mononuclear cells. The cell-free supernatant was centrifuged at 40000 r.p.m. (SW-41 rotor; Beckman Instruments) for 2 h, resuspended in PBS, and stored at ~ 80 °C. Virus stocks were titrated on cord-blood lymphocytes by endpoint dilution TCID₅₀ assay (Harada et al., 1985). All infections were carried out using endotoxin-free HIV-1 and mock preparations. Reverse transcriptase (RT) activity assays were performed as previously described (Rey et al., 1984). RT activity values were expressed as picomoles of [³H]TTP incorporated per hour at 37 °C by 1 ml of culture supernatant (pmol/h/ml).

General design of the experiments. Seven days after isolation, MDM were washed three times in PBS and were infected with 10⁴ TCID₅₀/10⁶ cells of HIV-1/DAS, HIV-1/PAR, HIV-1/THI or HIV-1/Ba-L (see above) in 7 ml RPMI 1640. Twenty-four h p.i., MDM were washed to remove excess virus, placed in 15 ml fresh medium and were treated or...
not treated with RP 55778. Mock infection was run in parallel using culture supernatants of uninfected phytohaemagglutinin-stimulated cord-blood mononuclear cells. RP 55778 was added every 2 or 3 days and cultures were maintained in fresh medium supplemented with 10% FCS during the time course of HIV-1 infection. Infected MDM culture supernatants were sampled every 2 or 3 days for cytokine level determination and virus replication measurement by HIV RT assay (Rey et al., 1984). Concomitant with supernatant harvesting, cells were washed, scraped off the culture flasks, lysed and total cellular RNA was extracted by the phenol–chloroform method (Chomczynski & Sacchi, 1987) for Northern blot analysis. In two independent experiments, 600 pg/ml of hrTNF-α was added to the culture supernatants of RP 55778-treated MDM 14 days after infection and maintained until the culture was stopped. Every 2 or 3 days after hrTNF-α treatment, culture supernatants were sampled for RT activity evaluation.

Cytokine assays. The presence of TNF-α, IL-6 and IL-1β in the cell culture supernatants was measured by means of quantitative ELISAs (Quantikine; R and D Systems). The detection limits of the ELISAs for human TNF-α, IL-6 and IL-1β are 4.8, 3.5 and 4.5 pg/ml, respectively (manufacturer’s information). The ELISA kits do not display cross-reactivity with other cytokines. The occurrence of TNF-α secretion was also confirmed by a biological activity test using actinomycin D-treated L-929 cells as described by Fish & Gifford (1983).

RNA analysis. Total cellular RNA was prepared using standard methods as previously described (Chomczynski & Sacchi, 1987). Briefly, MDM were lysed in guanidinium isothiocyanate solution and total RNA was extracted by the phenol–chloroform method; the RNA was then precipitated in isopropanol at −20°C for 2 h and washed twice in 75% ethanol. The RNA preparations were resuspended in a 5 mm-EDTA, 0.1% diethylpyrocarbonate solution. RNA samples were quantified spectrophotometrically by A₂₆₀ measurement and RNA integrity was monitored by electrophoresis. For Northern blot analysis, 5 μg of total RNA was loaded per lane, separated in a formaldehyde–1:5% agarose gel and blotted overnight onto a nylon filter (Schleicher & Schuell) using 20 × SSC buffer and fixed by exposure to u.v. light for 5 min. Filters were prehybridized for 18 h at 42°C in a mixture containing 50% (v/v) formamide, Denhardt’s solution, 20 × SSPE, 20% SDS and 20 μg/ml of salmon sperm DNA. They were then hybridized for 18 h at 42°C with the same solution as above containing a nick-translation ³²P-labelled cDNA probe for TNF-α (2 × 10⁶ to 4 × 10⁷ c.p.m./μg DNA). This TNF-α probe consists of a cDNA clone of human TNF-α in PAPI53 and was kindly provided by Professor W. Fiers (Ghent, Belgium). After hybridization, the filters were extensively washed for 15 min at room temperature in 2 × SSC/0.1% SDS, followed by 15 min at room temperature in 1 × SSC/0.1% SDS and then 20 min at 55°C in 0.1 × SSC/0.1% SDS. Transcripts were visualized by exposure of hybridized filters to X-ray film at −80°C. In all experiments, signal intensity was normalized by densitometric analysis using a probe for constitutively expressed β-actin. The β-actin nick-translation cDNA probe was provided by Dr S. Alonso (Paris, France) and was used as an internal control (Alonso et al., 1986).

Fig. 1. Time course of TNF-α, IL-6 and RT activities in long term HIV-1/Ba-L- (a), HIV-1/DAS- (b), HIV-1/PAR- (c) and HIV-1/THI-infected (d) MDM cultures. O, TNF-α production in HIV-1-infected MDM culture supernatants; ▲, IL-6 production in HIV-1-infected MDM culture supernatants; △, TNF-α production in mock-infected MDM culture supernatants; ■, IL-6 production in mock-infected MDM culture supernatants; ○, RT activity in HIV-1-infected MDM culture supernatants. Data are presented as a single representative experiment out of three independent experiments. Each point related to cytokine production is plotted as mean ± S.D. of three samples.
**Flow cytometry.** Immunophenotyping of freshly elutriated human monocytes and MDM was carried out by standard fluorescence-activated cell sorter (FACS) analysis (Becton Dickinson). Briefly, $5 \times 10^5$ cells in 50 µl of PBS supplemented with 2% FCS and 0.2% sodium azide were mixed with 0.25 µg of anti-CD4, 0.5 µg anti-CD3, 0.5 µg anti-CD11b, 10 µg anti-CD64, or 1 µg anti-MAX1 MAb at 4 °C for 30 min. After incubation, the cells were washed twice in supplemented PBS and stained with 10 µl of fluorescein isothiocyanate-labelled goat (F(ab')2 anti-mouse IgG for 30 min at 4 °C. The cells were then washed twice, fixed in 1% paraformaldehyde in PBS, and analysed by flow cytometry.

**Results**

**Characterization of membrane antigens of HIV-1 infected MDM and the time course of virus production**

The expression of the cell surface antigens CD3, CD4, CD64, CD11b and MAX1 was investigated in freshly isolated monocytes and 7-day-old MDM by flow cytometry analysis. Data were expressed as mean fluorescence intensity (MFI). A few hours after isolation of monocytes by countercurrent elutriation, MFI for background autofluorescence and for the membrane antigens CD3, CD4, CD64, CD11b, MAX1 were 5, 8, 22, 24, 208 and 21 respectively [MFI values (log scale) of stained cells were rounded to the nearest integer]. On day 7 after isolation, the levels of the same cell markers were investigated. MFI for the negative controls for CD3, CD4, CD64, CD11b and MAX1 were 5, 8, 22, 24, 208 and 21 respectively [MFI values (log scale) of stained cells were rounded to the nearest integer]. The results show that monocytes expressed high levels of the CD11b marker and markedly lower amounts of CD4, CD64 and MAX1 antigens. Seven days after isolation the monocytes have differentiated into macrophages and exhibit a significant increase in MAX1 antigen, whereas CD4 expression decreases. These results are in accord with previous data (Andreesen et al., 1986; Kazazi et al., 1989; Perno et al., 1990).

Because residual CD4+ T cells may be present in the monocyte population, even when centrifugal elutriation is used, all the following results were obtained after infection of 7-day-old MDM.

We have investigated the capacity of HIV-1 strain Ba-L and three primary HIV-1 isolates (HIV-1/DAS, HIV-1/PAR and HIV-1/THI) to replicate in 7-day-old adherent MDM. Culture supernatants of infected cells were collected at 2 to 3 day intervals over a period of 26 days and all the supernatants from each experiment were assayed at the same time for RT activity. Under these experimental conditions all four HIV strains replicated efficiently in infected MDM (Fig. 1). High levels of RT activity were detected in culture supernatants of infected MDM by day 9 p.i. and reached a maximum between 16
HIV-1 and anti-TNF-α agent RP 55778

Cytokine production during infection of MDM with primary HIV-1 isolates

TNF-α, IL-6 and IL-1β production was assayed in the culture supernatants of HIV-1-infected MDM using HIV-1/Ba-L and the three primary isolates. Fig. 1 illustrates the capacity of HIV-1/Ba-L, HIV-1/DAS and HIV-1/PAR to induce significant secretion of TNF-α and IL-6 between 16 and 21 days after MDM infection. During these experiments, bursts of TNF-α and IL-6 biological activity took place at times of high viral replication and then rapidly decreased about 5 day after these peaks. However, one primary isolate, HIV-1/THI, was unable to induce any TNF-α or IL-6 production in culture supernatants of infected MDM in spite of high viral replication. When MDM were exposed to mock virus preparations, no TNF-α or IL-6 secretion was detectable in culture supernatants between days 2 and 26 p.i. (Fig. 1). In contrast, no significant effect of virus or mock infecting preparations on the production of IL-1β was detected (Fig. 2). In addition, in all experiments, HIV-1-infected MDM were harvested between 14 to 26 days p.i. to analyse TNF-α gene expression around the peak of RT activity. The results are shown in Fig. 3. Except for the primary isolate HIV-1/THI, TNF-α mRNA production is highly stimulated by the infectious HIV-1/DAS, HIV-1/PAR and HIV-1/Ba-L virus strains at the peak of both biological TNF-α and supernatant RT activities. TNF-α mRNA was not observed in uninfected macrophage cultures. Despite the lack of TNF-α mRNA at 15 days p.i. with HIV-1/PAR, we detected TNF-α protein in the cell culture supernatant at this time. This may be explained by a high translation activity of levels of TNF-α mRNA below the detection limit of our assay.

Effects of RP 55778 on TNF-α production and HIV-1 infection

RP 55778 has been shown to protect mice from lipopolysaccharide-induced septic shock and to inhibit TNF production from murine peritoneal macrophages treated in vitro with thioglycollate (Floch et al., 1989). The ability of RP 55778 to block TNF production was investigated in MDM cultures infected with the four strains of HIV-1. Twenty-four h p.i., cells were extensively washed and treated or mock-treated with RP 55778 at a concentration of 3 × 10⁻⁴ M. Fig. 4 illustrates both the reduced TNF-α production and HIV replication and 22 days p.i. No c.p.e., defined as the detection of syncytia, was observed in HIV-1-infected MDM cultures and no virus-induced cell death was detected by trypan blue exclusion assay.

Fig. 4. Time course of TNF-α levels and RT activities in MDM cultures infected with different HIV-1 isolates [HIV-1/Ba-L, (a); HIV-1/DAS, (b); HIV-1/PAR, (c)] and treated with RP 55778. ○, TNF-α production in HIV-1-infected MDM culture supernatants; □, TNF-α production in culture supernatants of HIV-1-infected and RP 55778-treated MDM; ▽, RT activity in HIV-1-infected and RP 55778-treated MDM; □, RT activity in culture supernatants of HIV-1-infected and RP 55778-treated MDM. Data are presented as a single representative experiment out of three independent experiments. Each point related to cytokine production is presented as mean ± s.d. of three samples.
in MDM treated with RP 55778: treatment of HIV-1/Ba-L-, HIV-1/DAS- and HIV-1/PAR-infected MDM with RP 55778 resulted in significantly reduced virus production (up to 80% reduction of RT activity for HIV-1/Ba-L infection) concomitant with the decrease in TNF-α secretion. No cytotoxicity of RP 55778 was observed in either infected and treated cultures, or in mock-infected and treated cultures. Furthermore, RP 55778 treatment of infected mononuclear phagocytes did not significantly inhibit IL-6 production (Fig. 5). In contrast, no TNF-α or IL-6 production was detected in HIV-1/THI-infected cultures as previously described and, remarkably, no inhibition of RT activity was observed using RP 55778 (Fig. 6). This last result therefore shows that the decrease in viral replication observed in HIV-1/Ba-L-, HIV-1/DAS- and HIV-1/PAR-infected cells is probably due to inhibition of TNF-α and not to a direct effect of RP 55778 on virus production. Northern blot analysis showed that TNF-α mRNA induced by HIV-1 at the time of elevated RT activity was not abolished by RP 55778 (Fig. 3). Hence RP 55778 treatment of infected MDM may affect TNF-α gene expression at the post-transcriptional level. Normalization of signal intensity by densitometric analysis using a probe for constitutively expressed β-actin showed that TNF-α mRNA is produced at equivalent levels in RP 55778-treated and untreated MDM cultures (data not shown).

Dose–response effects of RP 55778 on cytokine production and HIV infection are shown in Fig. 7. RP 55778 decreases the production of TNF-α and the replication of HIV-1/DAS in MDM in a dose-dependent manner. At concentrations of $3 \times 10^{-4}$ M and $10^{-4}$ M, a maximum effect on TNF-α production was achieved, and HIV-1/DAS replication was decreased by about 90% as measured by RT activity in culture supernatants. These concentrations are similar to those found to be
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Fig. 7. Effect of RP 55778 on TNF-α production (a) and RT activity (b) in HIV-1/DAS-infected MDM cultures; dose-response curves. ○, HIV-1/DAS-infected MDM cultures; △, HIV-1/DAS-infected MDM cultures treated with 3 x 10^-4 M-RP 55778; ■, HIV-1/DAS-infected MDM cultures treated with 10^-4 M-RP 55778; □, HIV-1/DAS-infected MDM cultures treated with 3 x 10^-5 M-RP 55778; ●, HIV-1/DAS-infected MDM cultures treated with 10^-5 M-RP 55778; ▲, mock-infected MDM cultures. Data are presented as a single representative experiment out of two independent experiments. Each point related to TNF-α production is presented as mean ± s.D. of three samples.

Addition of hrTNF-α to culture supernatants of HIV-1-infected and RP 55778-treated MDM

Since it appeared that endogenous TNF-α was partly responsible for virus production in HIV-1-infected MDM, the effect of hrTNF-α on virus replication in RP 55778-treated infected MDM was explored. Fourteen days after infection of MDM treated with RP 55778 rhTNF-α (600 pg/ml) was added. Fig. 8 shows that in HIV-1/PAR-, HIV-1/DAS- and HIV-1/Ba-L-infected and RP 55778-treated MDM an increase in RT activity was detected in culture supernatants following rhTNF-α addition. However, under the same experimental conditions the virus strain HIV-1/THI appeared to be unresponsive to exogenous TNF-α treatment (Fig. 8).

Discussion

HIV-1-infected monocytes/macrophages participate in the immunopathology of HIV infection. Because mononuclear phagocytes express many of their regulatory functions through inflammatory cytokine secretion, we have studied the in vitro relationship between TNF-α production and virus replication during infection of mononuclear phagocyte cells by primary HIV-1 isolates. In the first set of experiments, 7-day old MDM were examined for their susceptibility to infection by the monocytotropic strain HIV-1/Ba-L and by three primary HIV-1 isolates, HIV-1/DAS, HIV-1/PAR and HIV-1/THI. All isolates were able to infect MDM cultures. Progeny virions were reproducibly detected 16 to 21 days p.i.

In a previous report we demonstrated TNF-α synthesis and gene expression at the time when RT activity peaks in MDM cultures infected with a lymphotropic strain HIV-1/LAI (Le Naour et al., 1992). In this study, using HIV-1/Ba-L and the clinical isolates HIV-1/DAS and HIV-1/PAR we confirmed that HIV MDM infection results in TNF-α secretion during the high proviral DNA transcription phase, i.e. 16 to 21 days p.i. In the same experimental conditions, IL-6 but not IL-1β production was detected. To explain our results, we have put forward the hypothesis that TNF-α may be responsible for triggering virus production. Such a correlation agrees with the data provided by several other authors who have demonstrated that TNF-α induces HIV production in different cell lines (Folks et al., 1989; Osborn et al., 1989). The mechanism by which TNF-α influences HIV provirus transcription is still not clear. HIV induction by PMA (Nabel & Baltimore, 1987) and TNF-α (Duh et al., 1989; Osborn et al., 1989; Griffin et al., 1989) has been correlated with increased binding of the transcriptional factor NF-κB to the HIV long terminal repeat (LTR).

However, no TNF-α or IL-6 production was observed in MDM cultures infected with the primary isolate HIV-1/THI despite high viral replication. Hence TNF-α secretion appears not to be strictly associated with significant virus production. We conclude that endogenous production of TNF-α or IL-6 is not responsible in all macrophage-tropic strains for maintenance of HIV-1 production. To rule out the possibility of cytokine contamination of our cultures we used virus and mock preparations containing less than 25 pg/ml endotoxin. To determine precisely the relationship between cytokine
production and virus replication, we have postulated that events which inhibit the expression of the TNF-α gene and the secretion of TNF-α may also decrease HIV replication. In a recent report, Weissman et al. (1993) have observed that a 30 min pretreatment of the chronically infected promonocytic U1 cell line with a TNF-α synthesis inhibitor, RP 55778, inhibits TNF-α-dependent expression of HIV-1. Furthermore, U1 cells pretreated for 24 h with RP 55778 and then stimulated with PMA secreted substantially less TNF-α than did cells stimulated with PMA in the absence of RP 55778. We therefore tested the ability of RP 55778 to block both TNF-α and HIV production in infected MDM cultures. Treatment of infected MDM cultures with RP 55778 suppresses TNF-α induction and strongly decreases HIV production. No similar decrease in the level of TNF-α transcripts was observed, suggesting that RP 55778 regulates TNF-α gene expression at the post-transcriptional level. This last observation is concordant with that of Weissman et al. (1993) who described no significant change in the amount of HIV RNA and TNF-α mRNA in U1 cells treated with RP 55778. In one independent experiment we have verified that when RP 55778 was removed from infected cultures 24 h after treatment, TNF-α secretion and virus production were restored (data not shown). The degree of inhibition of TNF-α production in infected cell cultures therefore depends on the duration of treatment. On the other hand, RP 55778 treatment of infected MDM results in a minor decrease in the level of IL-6 in the cell culture supernatants. Thus, IL-6 production seems not to be related to virus production. In the same experimental conditions, evaluation of RT activity in culture supernatants of RP 55778-treated and HIV-1/THI-infected MDM shows that this isolate is refractory to this treatment.

The addition of RP 55778 to infected MDM cultures resulted in a dose-dependent decrease in virus production. Nevertheless, virus production is not entirely abolished by the presence of RP 55778: baseline viral replication is reproducibly observed in all infected cultures treated with RP 55778. Another hypothesis may

Fig. 8. Effect of the addition of hrTNF-α to MDM cultures infected with different HIV-1 isolates [HIV-1/Ba-L, (a); HIV-1/DAS, (b); HIV-1/PAR, (c) HIV-1/THI, (d)] and treated with RP 55778. Twenty-four h p.i., MDM were washed, and treated with RP 55778 at the concentration of 3×10^-4 M. RP 55778 was maintained throughout culture. hrTNF-α (600 pg/ml) was added 14 days after MDM infection (i.e. 13 days after RP 55778 treatment). ○, RT activity in HIV-1-infected MDM cultures; ●, RT activity in MDM cultures infected with HIV-1 and treated with RP 55778; [], RT activity in MDM cultures infected with HIV-1 and treated with hrTNF-α in the presence of RP 55778. Data are presented as a single representative experiment out of two independent experiments.
therefore have to be considered to explain the correlation between TNF-α production and viral replication, namely that HIV could itself induce the secretion of TNF-α. The increase of TNF-α gene expression and TNF-α release might therefore be due to activation of the TNF-α promoter by HIV-1 regulatory gene products. This hypothesis is supported by Buonaguro et al. (1992) who demonstrated that the viral trans-activator Tat activates the expression of TNF in acutely and chronically infected T cells. On the other hand, binding sites for NF-κB are present in the regulatory regions of several cytokine genes including those for TNF and IL-6 but not IL-1 (Leonardo & Baltimore, 1989). This difference may be responsible for the absence of IL-1 protein in HIV-1-infected cultures.

Treatment of RP 55778-treated, virus-infected MDM cultures with hTNF-α at 14 days p.i. resulted in an increased level of virus production, except for those infected with the strain HIV-1/THI. This last result is very important indicating that viral gene transcription may be independent of the binding of the transcriptional factor NF-κB to the HIV-1/THI LTR.

Macrophages differ from T cells in a number of aspects that may affect the anti-HIV activity of different agents. Unfortunately, few drugs exhibit anti-HIV activities in the monocyte/macrophage. Our study demonstrated the ability of a TNF-α synthesis inhibitor, RP 55778, to decrease HIV replication by inhibition of TNF-α synthesis during HIV-1 infection of MDM. This is a further promising in vitro result. However, our results suggest that pathogenic HIV-1 strains may be divided in two types, (i) those in which virus replication is concomitant with TNF-α production, and therefore would be responsive to RP 55778 treatment, and (ii) those in which replication is independent of TNF-α secretion and is therefore totally unresponsive to a TNF-α synthesis inhibitor. The clinical value of this novel class of therapeutic agents for lentivirus infections is dependent on the ability of the primary virus strain to induce endogenous TNF-α during virus production.

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