TNF-α Inhibits HIV-1 Replication in Peripheral Blood Monocytes and Alveolar Macrophages by Inducing the Production of RANTES and Decreasing C-C Chemokine Receptor 5 (CCR5) Expression

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TNF-α Inhibits HIV-1 Replication in Peripheral Blood Monocytes and Alveolar Macrophages by Inducing the Production of RANTES and Decreasing C-C Chemokine Receptor 5 (CCR5) Expression

Brian R. Lane,*‡ David M. Markovitz,*‡ Nina L. Woodford,§ Rosemary Rochford,§ Robert M. Strieter,† and Michael J. Coffey‡

The pathogenesis of HIV-1 infection is influenced by the immunoregulatory responses of the host. Macrophages present in the lymphoid tissue are susceptible to infection with HIV-1, but are relatively resistant to its cytopathic effects and serve as a reservoir for the virus during the course of disease. Previous investigators have demonstrated that increased serum levels of TNF-α contribute to the clinical symptoms of AIDS and that TNF-α stimulates the production of HIV-1 in chronically infected lymphocytic and monocytic cell lines by increasing HIV-1 gene expression. Although previous studies have suggested that TNF-α may increase HIV-1 infection of primary human mononuclear cells, some recent studies have indicated that TNF-α suppresses HIV-1 infection of macrophages. We now demonstrate that TNF-α suppresses HIV-1 replication in freshly infected peripheral blood monocytes (PBM) and alveolar macrophages (AM) in a dose-dependent manner. As TNF-α has been shown to increase the production of C-C chemokine receptor (CCR5)-binding chemokines under certain circumstances, we hypothesized that TNF-α inhibits HIV-1 replication by increasing the expression of these HIV-suppressive factors. We now show that TNF-α treatment of PBM and AM increases the production of the C-C chemokine, RANTES. Immunodepletion of RANTES alone or in combination with macrophage inflammatory protein-1α and -1β block the ability of TNF-α to suppress viral replication in PBM and AM. In addition, we found that TNF-α treatment reduces CCR5 expression on PBM and AM. These findings suggest that TNF-α plays a significant role in inhibiting monocytopathic strains of HIV-1 by two distinct, but complementary, mechanisms.

Mononuclear phagocytes are susceptible to infection with HIV-1, but are relatively resistant to its cytopathic effects (1, 2). Macrophages present in the peripheral lymph nodes, lung, and gut-associated lymphoid tissue therefore may serve as a reservoir for the virus during clinical latency (2, 3). Although responsible for the production of only a small percentage of the viral load present in the infected host, macrophages may serve a necessary role in producing virus that can infect other target cells (4–6). Following infection with HIV-1, macrophages release several immunoregulatory and inflammatory factors, including TNF-α, IL-1, and IL-6 (7). The production of these cytokines by infected and uninfected immune cells, in turn, influences viral proliferation and disease associated with HIV-1 infection.

High levels of TNF-α have been detected in the plasma and tissues of individuals infected with HIV, and TNF-α may contribute to anorexia, cachexia, and fever in individuals with AIDS (8). TNF-α is secreted by primary macrophages infected by HIV-1 or treated with the HIV-1 envelope protein gp120 in vitro (9–11). TNF-α is an inflammatory cytokine that can lead to either cellular activation or programmed cell death by triggering different signaling pathways (12). TNF-α has long been known to stimulate HIV-1 replication through induction of NF-κB and activation of transcription from the HIV-1 long terminal repeat in chronically infected T cell and promonocytic cell lines (13–16). Although previous studies have indicated that TNF-α stimulates HIV-1 replication in cultured PBMC (17, 18), recent studies have found no correlation between levels of TNF-α and HIV-1 replication in lymphoid tissue (19). Further, one group has demonstrated that TNF-α suppresses HIV-1 production in peripheral blood monocytes (PBMs) (20, 21). These studies indicate that TNF-α may have distinct effects on latently infected cells and cells encountering virus for the first time.

Although TNF-α has widely been considered an activator of HIV-1 replication, TNF-α has also been shown to stimulate the production of several factors that are protective against HIV. For example, in lymphocytic cells, expression of RANTES, which is a potent suppressor of HIV-1 entry and hence replication (22, 23), is increased following TNF-α treatment (24). In addition, the production of MIP-1α and MIP-1β is stimulated by TNF-α in human...
fetal microglial cells (25). RANTES, MIP-1α, and MIP-1β, members of the C-C chemokine family, are the ligands for the C-C chemokine receptor CCR5, which serves as a coreceptor for viral entry by most primary isolates of HIV-1 (26–29). A genetic mutation in CCR5 (CCR5Δ32) prevents surface expression of the receptor (30), and subjects homozygous for CCR5Δ32 are highly resistant to infection with HIV (31, 32). Cocchi et al. first demonstrated that the CCR5 ligands, RANTES, MIP-1α, and MIP-1β, prevent infection by monocytotropic CCR5 using (R5) isolates of HIV-1 (33). We have shown RANTES alone to be the primary inhibitor of HIV-1 R5 isolates in both PBM and alveolar macrophages (AM) (22).

We hypothesized that TNF-α, in contrast to its effect on latently infected cell lines, would decrease viral entry and replication in mononuclear phagocytes by preventing the interaction between HIV-1 and CCR5. We show here, first, that TNF-α decreases HIV-1 replication in both freshly infected PBM and AM. This is due to a stimulation of CCR5 ligand production. Immunodepletion of RANTES alone or in combination with MIP-1α and MIP-1β reversed the suppression of viral replication caused by TNF-α. In addition, stimulation with TNF-α decreased the expression of CCR5 by a post-transcriptional mechanism. Therefore, in primary human PBM and AM, the dominant biological effect of TNF-α is to suppress HIV-1 replication by two distinct, but complementary, mechanisms.

Materials and Methods

Isolation of PBM

PBM were harvested and isolated as described previously (22). Briefly, heparinized venous blood was collected from healthy volunteers, and mononuclear cells were separated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation. PBM were purified by adherence to plastic for 1 h at 37°C at a concentration of 5 × 10⁶/ml. Cells were >90% PBM as determined by Diff-Quik analysis. PBM were cultured in DMEM and 10% FBS for 1–9 days before infection with HIV-1. Cellular proliferation, viability, and activation were assayed by using an MTT-based colorimetric assay according to the manufacturer’s instructions (Boehringer Mannheim, Mannheim, Germany). Cellular viability and death were measured by trypan blue exclusion, and apoptosis was assayed by measuring CPP32 (casepase-3, Yama) activity present in cell lysates using the ApoAlert CPP32 Assay Kit (Clontech, Palo Alto, CA).

Isolation of AM

AM were harvested as described previously (22). Briefly, AM were collected by bronchoalveolar lavage of nonsmoking healthy volunteers without lung disease or HIV infection. The recovered BAL fluid was centrifuged, and the cells were resuspended in DMEM and 10% FBS. AM were purified by plastic adherence for 1 h at 37°C at a concentration of 5 × 10⁶/ml. Cells were >90% pure, as determined by Diff-Quik staining. AM were cultured 1–2 days before infection.

HIV-1 infection of PBM and AM

The laboratory monocytotropic strain HIV-1baLyt obtained from the National Institutes of Health AIDS Research and Reference Reagent Program, was used to infect PBM or AM at a multiplicity of infection of about 0.06 (3 × 10⁴ RT counts/10⁵ cells). After infection overnight in the presence of virus, cells were washed, and fresh medium was added. A portion of the medium (25%) was removed and replaced twice weekly. Cytokines were added as described in the figure legends.

Cytokine reagents

TNF-α (Life Technologies, Grand Island, NY; R&D Systems, Minneapolis, MN) was added at concentrations ranging from 5–500 ng/ml to the C-C chemokines RANTES, MIP-1α, and MIP-1β and, as a control, nonimmune goat serum (R&D Systems) were added at a concentration of 25 μg/ml.

HIV-1 replication

Viral replication was assayed at several time points following infection by measuring the RT activity present in supernatants from HIV-1-infected cells, as previously described using a poly(A)-oligo(dt) template primer (34). Briefly, 32P-labeled deoxythymidine triphosphate incorporated in DNA was bound to DE81 paper (Whatman, Clifton, NJ) and was quantitated for 60 min on a Betascope radioisotope imaging system. Peak RT activity typically occurred 7–10 days postinfection of PBM and 2–4 wk after infection of AM. There was no difference in HIV infectivity of monocyte-derived macrophages and AM.

Cytokine ELISAs

Extracellular immunoreactive RANTES, MIP-1α, MIP-1β, or TNF-α were quantified using a modification of a double-ligand method as previously described (35). Briefly, flat-bottom, 96-well microtiter plates (Nunc Immuno-Plate I 96-F, Copenhagen, Denmark) were coated with 50 μl/well of rabbit anti-RANTES, MIP-1α, MIP-1β, or TNF-α Abs purified from immune rabbit serum by a protein A-Sepharose column (1 μg/ml in 0.6 M NaCl, 0.26 M H₃BO₃, and 0.08 M NaOH, pH 9.6) for 16 h at 4°C and then washed with PBS (pH 7.5) and 0.05% Tween-20 (wash buffer). Nonspecific binding sites were blocked with 2% BSA in PBS (200 μl), and the plates were incubated for 90 min at 37°C. Plates were rinsed four times with wash buffer and cell-free supernatants (alone and diluted 1/10 in wash buffer) were added, followed by incubation for 1 h at 37°C. Plates were washed four times, followed by the addition of 50 μl/well of biotinylated anti-murine Abs (3.5 μg/ml in PBS (pH 7.5), 0.05% Tween-20, and 2% FBS), and then incubated for 30 min at 37°C. Plates were washed four times, streptavidin-peroxidase conjugate (Bio-Rad, Richmond, CA) was added, and the plates were incubated for 30 min at 37°C. Plates were then washed four times, and chromogen substrate (Bio-Rad) was added. The plates were then incubated at room temperature to the desired extinction, and the reaction was terminated with 50 μl/well of 3 M H₂SO₄ solution. Plates were read at 490 nm in an ELISA reader. Standards were 0.5 log dilutions of recombinant human RANTES, MIP-1α, MIP-1β, or TNF-α (R&D Systems) from 1 pg/ml to 100 ng/ml. This ELISA method consistently detected cytokine levels >50 pg/ml.

RNase protection assay (RPA)

RNA was extracted from PBM and AM with TRizol according to the manufacturer’s instructions (Life Technologies). Yeast transfer RNA (10 μg) was added as carrier to samples before isopropanol precipitation. RNA was then analyzed by RPA using the hCK-5 and hCR-5 multiprobe template sets (RiboQuant, Pharmingen, San Diego, CA) as previously described (36). Linearized templates were used for T7-directed synthesis of 32P-labeled riboprobes. The probes were hybridized in excess with target RNA samples and then digested with RNase T1 and RNase A to remove unhybridized probe and mRNA. The protected probes were purified and electrophoresed on a 5% PAGE. Bands were visualized by autoradiography (XAR film, Eastman Kodak, Rochester, NY) and quantified using the series 400 PhosphorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). The volume measurements of the PhosphorImager signals were normalized for the number of radiolabeled uridines present in each riboprobe (RANTES, 82 uridines; MIP-1α, 52; MIP-1β, 73; CCR5, 60; L32, 48). To account for variability in total RNA present in each sample, the amount of RNA is presented as a percentage of the amount of the internal housekeeping control L32 band.

Flow cytometry for CCR5

Cell staining was performed using one of three mAbs to human CCR5: 5C7 (LeukoSite, Cambridge, MA), 2D7 (LeukoSite), and 45531.111 (R&D Systems; LeukoSite, Cambridge, MA), 2D7 (LeukoSite), and 45531.111 (R&D Systems) for 30 min at 4°C. Cells were washed with PBS (pH 7.5), 0.05% Tween-20, and 2% FBS), and then incubated for 90 min at 37°C. Plates were rinsed four times, streptavidin-peroxidase conjugate (Bio-Rad, Richmond, CA) was added, and the plates were incubated for 30 min at 37°C. Plates were washed four times, and chromogen substrate (Bio-Rad) was added. The plates were then incubated at room temperature to the desired extinction, and the reaction was terminated with 50 μl/well of 3 M H₂SO₄ solution. Plates were read at 490 nm in an ELISA reader. Standards were 0.5 log dilutions of recombinant human RANTES, MIP-1α, MIP-1β, or TNF-α (R&D Systems) from 1 pg/ml to 100 ng/ml. This ELISA method consistently detected cytokine levels >50 pg/ml.
Previous studies have demonstrated that TNF-α decreases HIV-1 replication in PBM, but did not resolve the mechanism by which TNF-α acts (20, 21). To determine the effect of TNF-α on HIV-1 replication in freshly infected mononuclear phagocytes, we infected primary human PBM and AM in the presence or the absence of TNF-α (Fig. 1). PBM were readily infectable with HIV-1 after culture for as little as 24 h, but freshly isolated PBM were not infected consistently in our hands, while AM were infectable directly after isolation (data not shown). TNF-α significantly suppressed RT activity in HIV-1-infected PBM (Fig. 1a) and AM (Fig. 1b). HIV-1 replication was suppressed when TNF-α was added twice weekly throughout the course of infection, 24 h before and after infection, or only 24 h before infection (data not shown). Furthermore, viral replication was decreased by treatment with TNF-α in a dose-dependent fashion (Fig. 2). The ability of TNF-α to decrease viral replication was similar in PBM cultured between 1–9 days before infection (data not shown). Coupled with the evidence that TNF-α suppresses HIV-1 replication in AM, these data suggest that the effect of TNF-α on infection of mononuclear phagocytes is independent of monocytic differentiation to macrophages.

As TNF-α may act either as an initiator of programmed cell death or as a cellular activator, we examined the effect of TNF-α on cellular viability, apoptosis, and cellular activation. Cells were >95% viable as determined by trypan blue exclusion, and there was no significant difference in the percentage of viable cells following TNF-α treatment (data not shown). Because stimulation with TNF-α can initiate a cascade of apoptotic proteases (caspases) in some cells, we assessed the amount of CPP32 (caspase-3, Yama) activity present in PBM. There were very low levels of CPP32 activity in both unstimulated and TNF-α-stimulated PBM compared with the amount present in a positive control, polymorphonuclear cells cultured for 24 h (Table I), indicating that TNF-α is not responsible for an increase in apoptosis in PBM. In addition, TNF-α treatment of PBM and AM did not result in significant differences in cell viability as determined by measurement of the metabolism of MTT to a formazan dye (Table I). These data demonstrate that the antiviral effect seen with TNF-α treatment is not due to an increase in cell death or a decrease in cell viability.

### Table I. Apoptosis and cellular viability of PBM following stimulation with TNF-α

<table>
<thead>
<tr>
<th>Apoptotic Protease (CPP32) Activity (units)</th>
<th>MTT Assay (absorbance (A570–A650) ± SD)</th>
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<tbody>
<tr>
<td>Background</td>
<td>9.0</td>
</tr>
<tr>
<td>No stimulation</td>
<td>17.4</td>
</tr>
<tr>
<td>TNF-α (5 ng/ml)</td>
<td>20.3</td>
</tr>
<tr>
<td>TNF-α (50 ng/ml)</td>
<td>19.0</td>
</tr>
<tr>
<td>PMN</td>
<td>457</td>
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* Assays were performed using 10⁷ PBM (or PMN). Data represent the mean of duplicate samples for the apoptotic protease (CPP32) activity assay and quadruplicate samples for the cellular viability assay (MTT assay).
TNF-α increases C-C chemokine production in PBM and AM

RANTES, MIP-1α, and MIP-1β can suppress replication of monocytotropic HIV isolates by decreasing viral entry while binding to CCR5 (23, 33). We have previously shown that RANTES decreases viral replication in PBM and AM (22). Therefore, we determined whether the addition of RANTES in combination with TNF-α would further decrease viral replication. Simultaneous addition of RANTES and TNF-α did not result in further suppression of RT activity (data not shown), suggesting that these factors may suppress viral replication by acting through the same pathway.

Since the literature implicates chemokines and chemokine receptors in HIV entry, we chose to examine the effect of TNF-α on the expression of RANTES, MIP-1α, and MIP-1β in control and HIV-1-infected PBM and AM. Expression of C-C chemokines increased dramatically after a single treatment with TNF-α (Fig. 3A). RANTES levels were significantly elevated (74-fold), as were MIP-1β levels (40-fold), with a trend toward an increase in MIP-1α. TNF-α treatment of AM resulted in significant increases in
antigenic RANTES, MIP-1α, and MIP-1β (Fig. 3B). Addition of Abs that neutralize RANTES, MIP-1α, or MIP-1β to TNF-α-treated cells reduced the amount of C-C chemokines to below the limit of detection for ELISA (data not shown). In HIV-1-infected PBM, there was a significant elevation in the level of antigenic RANTES and a trend toward an increase in MIP-1α and MIP-1β levels in TNF-α-treated cells compared with those in untreated cells (Fig. 4A). Similarly, following TNF-α treatment of HIV-1-infected AM, RANTES and MIP-1α expressions were significantly increased, with a trend toward an increase in MIP-1β levels (Fig. 4B).

Because TNF-α is known to stimulate chemokine expression by a NF-κB-dependent increase in transcription, we next studied the effect of TNF-α on C-C chemokine mRNA levels in PBM following treatment with TNF-α, using a quantitative RPA (Fig. 5).

TNF-α increased the amount of RANTES, MIP-1α, and MIP-1β mRNA by factors of 2.9, 2.5, and 2.2, respectively. Identical increases in CCR5 ligand mRNA was observed in both uninfected and HIV-1-infected PBM.

Anti-RANTES Abs block the TNF-α-mediated suppression of HIV-1 replication

Next we wanted to determine whether the increase in C-C chemokine production induced by TNF-α treatment was responsible for the decrease in HIV-1 replication caused by TNF-α. The addition of a neutralizing Ab to RANTES to either PBM (Fig. 6A) or AM (Fig. 6B) completely reversed the TNF-α-mediated suppression of HIV-1 replication. Addition of neutralizing Abs to either MIP-1α or MIP-1β alone partially blocked this effect (Fig. 7A), but did not further increase HIV-1 replication above the level seen with Abs to RANTES alone (Fig. 7B). HIV-1 replication in control cultures

FIGURE 5. Stimulation with TNF-α increases levels of C-C chemokine mRNA. PBM (3 × 10^5) were cultured for 2 days before infection and stimulation on day 0. Supernatants (25%) were collected, and fresh medium was added along with TNF-α (50 ng/ml) on days 1, 4, and 7. Cells were harvested on day 10, and total cellular RNA was extracted for analysis by RPA. A, Autoradiogram of RNA from uninfected PBM left untreated (Control; C) or treated with 50 ng/ml TNF-α (TNF), and from PBM infected with HIV-1 and left untreated (HIV) or treated with 50 ng/ml TNF-α (HIV + TNF). B, The signals present in the RANTES, MIP-1α, and MIP-1β bands of the dried gel were standardized to enable comparison with one another and are presented as a percentage of the signal in the internal control band (L32). This experiment is representative of three independent experiments.

FIGURE 6. Immunodepletion of RANTES completely blocks the TNF-α-mediated suppression of HIV-1 replication in PBM and AM. HIV-1-infected PBM (A) or AM (B) were either left untreated (Control) or treated with 50 ng/ml TNF-α alone or in combination with an Ab that neutralizes RANTES (25 μg/ml). RT activity was assayed between 10 and 13 days (A) or 4 wk (B) after infection. Each bar represents the mean of multiple wells from three independent experiments ± SEM. Statistical significance was determined by ANOVA between the TNF-α-treated cells and each of the other two treatment conditions.
was not altered by the addition of Abs to RANTES, MIP-1α, MIP-1β, or nonimmune goat serum (Fig. 7B).

**TNF-α decreases surface expression of CCR5**

To examine whether TNF-α regulated chemokine receptor expression as well as chemokine expression, we analyzed the surface expression of CCR5 in PBM by flow cytometry. CCR5 expression increased during the incubation following adherence to plastic (data not shown). After 24 h, CCR5 expression was greatly reduced in TNF-α-treated PBM compared with that in the untreated controls (Fig. 8A). TNF-α stimulation also decreased surface expression of CCR5 on AM (data not shown), but did not affect the expression of CCR5 on PBL (Fig. 8B). Similar results were seen using Abs to three different epitopes of CCR5 (data not shown). Incubation of TNF-α-treated PBM with Abs to the CCR5 ligands did not reverse the decrease in CCR5 surface expression, indicating that TNF-α may alter CCR5 expression by a mechanism other than ligand-mediated endocytosis (Fig. 9). TNF-α can therefore decrease the amount of CCR5 available for HIV-1 binding to PBM and AM.

We next determined by RPA whether the TNF-α-mediated reduction in CCR5 was also observed at the RNA level. We observed an almost 6-fold increase in CCR5 mRNA following HIV-1 infection of either PBM or AM, which was independent of TNF-α treatment (Fig. 10). After incubation for 24 h in the presence of TNF-α, mRNA levels of CCR5 were unchanged in both PBM and AM (Fig. 10). Therefore, we conclude that the decrease in CCR5 surface expression following treatment of PBM and AM with TNF-α is not mediated at the RNA level.

**Discussion**

TNF-α plays an important role in HIV-1 disease and has been associated with some of the clinical symptoms of AIDS (8). Furthermore, TNF-α has been shown to act in a positive feedback loop on HIV-1 replication, e.g., HIV-1 infection of monocytic cells increases TNF-α production, and TNF-α, in turn, further increases HIV-1 replication (9, 13–16). Most of the studies implicating TNF-α as an activator of HIV-1 involved cell lines containing stably integrated HIV-1 genomes (13–15). These studies have demonstrated the effect of TNF-α on latently infected cells, but...
have not examined the action of TNF-α on freshly infected cells. Recent studies have called into question the role of TNF-α as an exclusively positive regulator of HIV-1 replication (19–21). We present data that TNF-α does not stimulate, but rather suppresses, HIV-1 replication in primary human mononuclear phagocytes. The mechanism of suppression of HIV-1 replication by TNF-α is 1) by increasing the expression of the CCR5 ligand RANTES, and 2) by decreasing the surface expression of the HIV-1 receptor CCR5.

Treatment of both PBM and AM with TNF-α resulted in a highly reproducible and dose-dependent reduction in HIV-1 replication. The reduction in viral replication was observed following treatment with TNF-α before or within 24 h of infection or throughout the course of the experiment. This effect was not due to either cytotoxicity or induction of apoptosis by TNF-α, which is consistent with other reports that TNF-α protects PBM from apoptosis via NF-κB activation (37, 38). In other experiments we saw no significant change in the activity of the caspase CPP32 in response to TNF-α, again indicating that the suppression of HIV-1 replication in PBM was not due to an induction of apoptosis by TNF-α.

Several studies have shown that TNF-α induces the production of RANTES, MIP-1α, and MIP-1β in various cell types (24, 25). We and others have demonstrated that RANTES inhibits viral replication by competing with HIV-1 for binding to CCR5 on mononuclear phagocytes (22, 23). Therefore, we examined the hypothesis that the antiviral effect of TNF-α was mediated through the elaboration of C-C chemokines. Treatment of control and HIV-infected PBM and AM with TNF-α resulted in an increase in the synthesis of the CCR5 ligand RANTES. There was a trend toward an increase in MIP-1α and MIP-1β, but it did not reach statistical significance. To determine whether increased C-C chemokine production was essential for the suppression of HIV-1 replication by TNF-α, we demonstrated that the addition of neutralizing Abs to C-C chemokines reversed the suppression of HIV-1 replication by TNF-α. Notably, immunodepletion of all three CCR5 ligands or RANTES alone in TNF-α-stimulated cultures was sufficient to block the effect of TNF-α in PBM and AM, while immunodepletion of MIP-1α or MIP-1β alone only partially reversed this effect. Therefore, the suppression of HIV-1 replication by TNF-α appears to be mediated by stimulation of the C-C chemokine RANTES.
endocytosis. A recent study demonstrates that TNF-α decreases surface expression of chemokine receptors by stimulating the release of matrix metalloproteinases that degrade chemokine receptors on PBM (39). Further studies are being conducted to determine the precise mechanism responsible for the decreased expression of CCR5. Nonetheless, these data indicate that in addition to increasing the ligands for CCR5 (e.g., RANTES), which can compete with HIV-1 for binding to CCR5, stimulation with TNF-α decreases the availability of CCR5 itself to serve as an entry coreceptor for HIV-1.

Our findings suggest that the effect of TNF-α on HIV-1 replication depends on the timing of exposure of mononuclear phagocytes to this cytokine. Endogenous TNF-α released by infected macrophages may protect uninfected monocytes and macrophages from infection with HIV-1. However, upon integration of the virus into the cellular genome, the continual production of TNF-α may enhance HIV-1 replication. From the clinical standpoint, these findings may explain why treatment with agents that suppress TNF-α levels in patients with AIDS, e.g., pentoxifylline, have had little impact on serum p24 Ag levels despite the fact that they inhibit replication in cells infected with HIV-1 in vitro (40). Pentoxifylline may undermine a natural block to viral entry into uninfected macrophages, namely the production of TNF-α by these cells. Genetic evidence suggests that high levels of TNF-α may actually slow the course of HIV-1 disease. Possession of the TNF-α c2 microsatellite allele has been correlated with high levels of TNF-α production and slower progression of disease in patients infected with HIV-1 (41). By contrast, other investigators have described a correlation between persistently raised TNF-α levels and failure of antiretroviral therapy despite an early adequate virologic response (42). This discrepancy between the findings of this study and the above observations could be explained by raised TNF-α levels being a marker of persistent low level, undetectable viral replication, because HIV replication stimulates enhanced TNF-α levels. These clinical and genetic studies suggest that, in vivo, TNF-α does not stimulate HIV-1 replication and are consistent with our findings that TNF-α inhibits HIV-1 replication in primary human macrophages.

In summary, we have demonstrated that stimulation of PBM and AM with TNF-α inhibited HIV-1 replication by increasing the expression of RANTES. Furthermore, immunodepletion of RANTES restored viral replication in these same cells. In addition, CCR5 expression was decreased in TNF-α-stimulated cells. Therapies aimed at the suppression of TNF-α in HIV disease may not slow the progression of disease and may, in fact, increase the susceptibility of macrophages to HIV-1 infection. Conversely, TNF-α-enhancing therapies may be able to prevent the spread of HIV-1 within the body if implemented soon after infection is first detected.

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