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55- and 75-Kilodalton Tumor Necrosis Factor Receptors Mediate Distinct Actions in Regard to Human Immunodeficiency Virus Type 1 Replication in Primary Human Macrophages

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We report in this study that repeated tumor necrosis factor alpha (TNF-α) pretreatment, starting before and continued after infection by human immunodeficiency virus type 1 (HIV-1), inhibits replication of the monocytotropic Ada strain in primary tissue culture-differentiated macrophages (TCDM), as assessed by sixfold lower levels of reverse transcriptase (RT) activity than that in untreated cells and absence of syncytium formation in TCDM cultures. In order to determine the pathways involved in inhibition of HIV-1 replication in primary TCDM pretreated with TNF-α, we tested TNF-α mutants T55 and T75, which recognize either the 55-kDa (TNF-R1) or the 75-kDa (TNF-R2) TNF receptor, respectively. Pretreatment of TCDM with the T75 mutant decreased the RT activity compared with that in untreated infected control cells fivefold and almost totally inhibited syncytium formation. In contrast, when TCDM were pretreated with the T55 mutant alone, syncytia were observed and RT activity was decreased about one-half. These results suggest that the inhibition of HIV-1 replication in TCDM pretreated with TNF-α might be mediated mainly through the 75-kDa TNF receptor (TNF-R2) rather than through the 55-kDa receptor (TNF-R1). Inhibition of HIV-1 replication in TCDM was observed with both T75 mutant pretreatment and posttreatment, starting at 1 h or 3 days after infection, whereas posttreatment with the T55 mutant, but not pretreatment, stimulated HIV-1 growth in primary TCDM. Both pre- and posttreatment with TNF-α inhibited HIV-1 replication in primary TCDM. The stimulation of HIV-1 replication by TNF-α in a chronically infected promonocytic cell line, U1, which contains two copies of integrated provirus, was mediated through the 55-kDa TNF-R1 alone and not through the 75-kDa TNF-R2. These results demonstrate that the 55-kDa TNF-R1 is involved in postintegration stimulation of HIV-1 while the 75-kDa TNF-R2 is involved in the inhibition of an early step of the viral life cycle in primary human TCDM.

Unbalanced cytokine production is considered to be a key component leading to the progressive immunosuppression observed in AIDS. Parallel to the TH1/TH2 cytokine switch (4), chronic activation of the immune system might account for the increased levels of proinflammatory cytokines, especially tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) detected in plasma and tissues of AIDS patients (12). In vitro, TNF-α is secreted by primary macrophages infected in culture by human immunodeficiency virus type 1 (HIV-1) or treated with envelope glycoprotein gp120 and by HIV-infected monocytes isolated from patients (16, 21, 34). In vivo, high levels of TNF-α detected in plasma and tissues might contribute to the anorexia, cachexia, and fever observed in AIDS patients (25).

TNF-α has been reported as stimulating HIV-1 replication through induction of nuclear factor κB (NF-κB) and further activation of the long terminal repeat in chronically infected T-cell and promonocytic cell lines which both contain integrated HIV DNA (9, 39). In contrast, contradictory results have been reported after TNF-α treatment of primary macrophages infected by HIV-1 in vitro, with the treatment either enhancing, inhibiting, or having no effect on viral expression (17, 22, 33). These apparently contradictory results could be resolved by determining the role of each TNF receptor (TNF-R) in regard to HIV-1 replication in primary tissue culture-differentiated macrophages (TCDM). TNF-α acts through binding to two TNF-Rs of 55 (TNF-R1) and 75 (TNF-R2) kDa, which display no homology between their intracellular domains, suggesting that they utilize separate signaling pathways (53). TNF-R1 mediates cytotoxicity, fibroblast proliferation, resistance to chlamydial infection, synthesis of prostaglandin E_2, and stimulation of NF-κB (10, 11, 49). By contrast, TNF-R2 has been implicated in proliferation of a murine cytotoxic T-cell line and thymocytes, induction of granulocyte/macrophage colony-stimulating factor secretion, inhibition of early hematopoiesis, and TNF-dependent proliferation of human mononuclear cells (14, 19, 52, 54).

TNF-Rs belong to a receptor superfamily including among others low-affinity nerve growth factor receptor, CD30, CD40, and Fas antigen. Increased concentrations of soluble TNF-R in serum have been found in HIV-infected individuals, and the levels correlate with disease progression and degree of immunodeficiency (20, 45). Recently, it has been reported that an increase of the serum-soluble form of CD30 in the early phase of HIV-1 infection is an independent predictor of progression to AIDS (41). Moreover in vivo, an increased proportion of $CD8^+$ $CD30^+$ T cells could be detected in the blood of AIDS patients during progression of the disease and in vitro stimulation of surface CD30 increased HIV-1 replication (31, 41, 46). These data underline the potential role of the receptors belonging to the TNF-R superfamily in the pathogenesis of AIDS in addition to the contribution of increased levels of TNF-α in plasma and tissues to metabolic dysfunctions.

We reported previously that 75-kDa TNF-R2, but not 55-

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kDa TNF-R1, is implicated in the inhibition of HIV-1 entry into primary TCDM by TNF-α (17). Here we determine the overall relevance of the TNF-R-dependent modulation of HIV-1 entry into primary TCDM during a full course of acute infection and show that 55-kDa TNF-R1 and 75-kDa TNF-R2 display opposite actions in regard to HIV-1 replication in primary TCDM. TNF-R1 is involved in stimulation of HIV-1 replication probably following proviral integration; in contrast, TNF-R2 mediates inhibition of viral replication by targeting an early step of the viral cycle, the viral entry.

Inhibition of HIV-1 replication in TNF-α-pretreated TCDM is mediated through the 75-kDa TNF-R2 and not through the 55-kDa TNF-R1. TNF-α acts on target cells through two independent receptors, TNF-R1 and TNF-R2. In order to determine the receptor(s) involved in inhibition of HIV-1 replication in TCDM, we pretreated 3-day-old TCDM isolated as reported previously (17) with TNF-α and TNF-α mutants specific for either TNF-R1 or TNF-R2 before infection with HIV-1_{Ada} (multiplicity of infection [MOI] of 0.10). Pretreatment consisted of addition of TNF-α every day before infection, at the time of infection, and every 2 days afterwards. Macrophage-tropic viral stock HIV-1_{Ada} (H. E. Gendelman, AIDS Reagent Program, National Institute of Allergy and Infectious Diseases) was grown and titrated in TCDM as described elsewhere (17). TNF-α mutants (kindly provided by W. Lesslauer, Hoffmann-La Roche, Basel, Switzerland) R32W-S86T and D143N-A145R, termed T55 and T75, are specific for human TNF-R1 and TNF-R2, respectively. T55, R32W-S86T (arginine at position 32 replaced by tryptophan and serine at position 86 replaced by threonine), has more than 5,000-fold decreased affinity for TNF-R2 compared with wild-type TNF-α but only a 2.2-fold reduction in binding to TNF-R1 (28). T55 elicits a full cytotoxic response in human KYM-1 cells indistinguishable from that of wild-type TNF-α (28). T75, D143N-A145R (aspartic acid at position 143 replaced by asparagine and alanine at position 145 replaced by arginine), has more than 2,500-fold decreased affinity for TNF-R1 but has 10 times lower binding affinity for TNF-R2 compared with wild-type TNF-α (28). In our experiments, we used TNF-α and T55 and T75 mutants at 20, 20, and 200 ng/ml, respectively. Inhibition of HIV-1 replication in primary TCDM was observed with TNF-α, T75 mutant, and both T55 and T75 together as assessed by decreased reverse transcriptase (RT) levels versus those in untreated control TCDM (Fig. 1). At day 14 postinfection, TCDM pretreated with TNF-α, the T75 mutant, or both mutants together showed an eightfold decreased RT activity versus that in untreated infected TCDM. At day 21 postinfection, the strongest inhibitory effect on HIV-1 replication in TCDM was observed with TNF-α, with RT levels decreased up to 20-fold, while the T75 mutant and both T55 and T75 mutants together showed 5- and 4-fold decreased levels, respectively, versus untreated infected TCDM. At both 14 and 21 days postinfection, T55 pretreatment inhibited approximately one-half RT activity versus that in untreated infected TCDM. In agreement with the RT data, observation of TCDM cultures by phase-contrast microscopy confirmed the above results (Fig. 2). Human recombinant TNF-α, the T75 mutant, and both mutants together inhibited syncytium formation (Fig. 2B, D, and E). More than 95% of syncytium formation was inhibited by TNF-α pretreatment at day 14 postinfection (MOI, 0.10) (data not shown). The T75 mutant and both mutants mixed together yielded 10-fold fewer syncytia compared with untreated HIV-1-infected TCDM (data not shown), while the T55 mutant decreased syncytium formation only partially (Fig. 2C and data not shown).

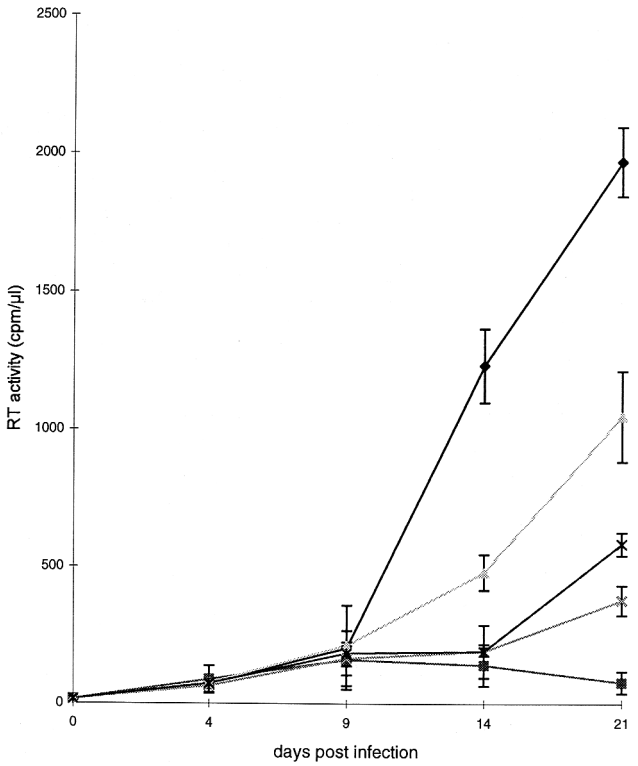

FIG. 1. Decreased RT activity in culture supernatants of TNF-α-pretreated TCDM infected with HIV-1_{Ada} is mediated through the 75-kDa TNF-R2. RT activity was detected in supernatants of monocyte-derived TCDM isolated from peripheral blood and cultivated in RPMI medium supplemented with 10% human serum (17). Pretreatment consisted of addition of cytokine every day before infection, at the time of infection, and every 2 days afterwards. RT activity was measured as previously described (17) in culture supernatants of 3-day-old TCDM untreated (◆) or pretreated for 72 h with 20 ng of TNF-α per ml (■), 20 ng of T55 mutant per ml (△), 200 ng of T75 mutant per ml (✕), or both mutants together (×) and then infected with HIV-1_{Ada} (MOI, 0.10). These results are representative of three independent experiments. The means and standard deviations are shown.

Pretreatment with T75 mutant inhibits, whereas posttreatment with T55 mutant stimulates, HIV-1 replication in primary TCDM. HIV-1_{Ada}-infected TCDM (MOI, 0.10) were kept untreated, were pretreated starting at day 3 before infection, or were posttreated with 20 ng of TNF-α per ml, 20 ng of T55 per ml, and 200 ng of T75 mutant per ml, alone or together. Posttreatment consisted of addition of the cytokines starting at 1 h or 3 days postinfection and every 2 days afterwards. After pre- or posttreatment of HIV-infected TCDM with TNF-α, almost no syncytia could be observed (data not shown) and sixfold lower RT activity levels were observed in these cultures compared with those in untreated infected control TCDM at day 14 postinfection (Fig. 3). To specify the role of each TNF-R in regard to inhibition or stimulation of HIV-1 replication, we pre- or posttreated as described above primary human TCDM with either TNF-α or T55 and T75 mutants, alone or together. In contrast to T55 pretreatment, which inhibits about one-half RT activity, delayed T55 posttreatment starting at day 3 postinfection, but not at 1 h after infection, stimulated HIV-1 growth in primary human TCDM as assessed by 50% increased RT activity (Fig. 3) and enhanced syncytium formation (data not shown), versus that observed in untreated infected TCDM. These data suggest that 55-kDa TNF-R1 might be involved in stimulation of HIV-1 replication in primary TCDM, probably by targeting a late step—after

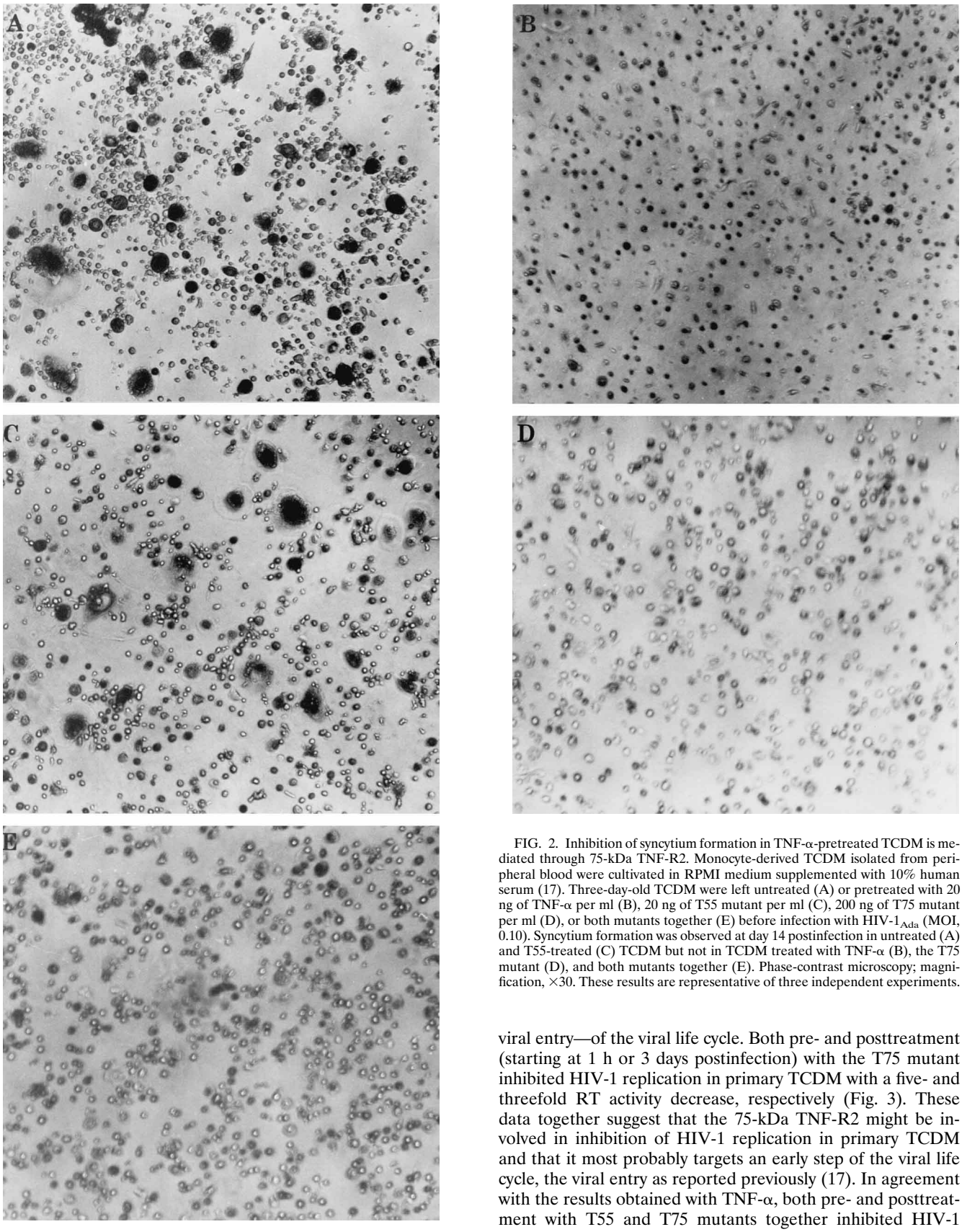

FIG. 2. Inhibition of syncytium formation in TNF-α-pretreated TCDM is mediated through 75-kDa TNF-R2. Monocyte-derived TCDM isolated from peripheral blood were cultivated in RPMI medium supplemented with 10% human serum (17). Three-day-old TCDM were left untreated (A) or pretreated with 20 ng of TNF-α per ml (B), 20 ng of T55 mutant per ml (C), 200 ng of T75 mutant per ml (D), or both mutants together (E) before infection with HIV-1_{Ada} (MOI, 0.10). Syncytium formation was observed at day 14 postinfection in untreated (A) and T55-treated (C) TCDM but not in TCDM treated with TNF-α (B), the T75 mutant (D), and both mutants together (E). Phase-contrast microscopy; magnification, ×30. These results are representative of three independent experiments.

viral entry—of the viral life cycle. Both pre- and posttreatment (starting at 1 h or 3 days postinfection) with the T75 mutant inhibited HIV-1 replication in primary TCDM with a five- and threefold RT activity decrease, respectively (Fig. 3). These data together suggest that the 75-kDa TNF-R2 might be involved in inhibition of HIV-1 replication in primary TCDM and that it most probably targets an early step of the viral life cycle, the viral entry as reported previously (17). In agreement with the results obtained with TNF-α, both pre- and posttreatment with T55 and T75 mutants together inhibited HIV-1 growth in primary TCDM, but to a lesser extent than that with TNF-α (Fig. 3).

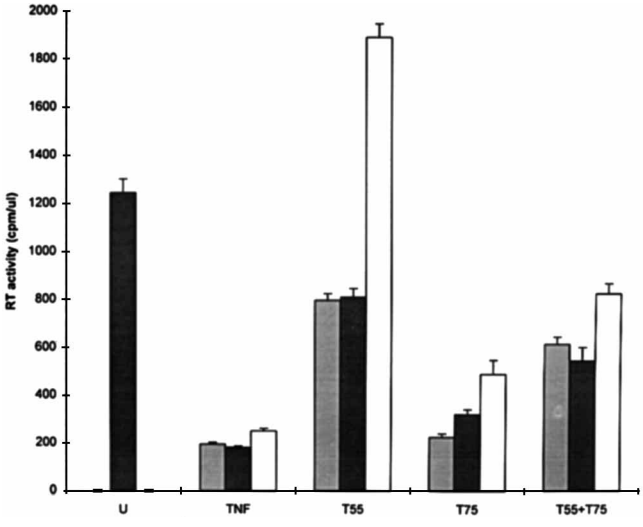

FIG. 3. Effects of pre- and posttreatment with TNF-α and both TNF-α mutants on HIV-1 replication in primary TCDM. RT activity was detected in supernatants of monocyte-derived TCDM isolated from peripheral blood and cultivated in RPMI medium supplemented with 10% human serum (17). Three-day-old TCDM were left untreated (U), pretreated for 72 h before infection (▨), or posttreated at 1 h (■) or 3 days (□) after infection with HIV-1_{Ada} (MOI, 0.10) with 20 ng of TNF-α per ml, 20 ng of T55 mutant per ml, 200 ng of T75 mutant per ml, or 20 ng of T55 and 200 ng of T75 mutant together per ml. At day 14 postinfection, the RT activity was determined in culture supernatants as previously described (17). These results are representative of three independent experiments. The means and standard deviations are shown.

Fifty-five-kilodalton TNF-R1, but not 75-kDa TNF-R2, is involved in stimulation of HIV-1 replication in the chronically infected U1 promonocytic cell line. In order to determine the respective role of each TNF-R in regard to the stages of the viral life cycle following proviral integration, we tested TNF-α and T55 and T75 mutants alone or together for effects on HIV-1 replication in the chronically infected promonocytic cell line U1. The promonocytic cell line U1, derived from cells surviving acute infection of the U937 cell line (13), contains two integrated HIV copies per cell. Cells were resuspended at 2×10^5 per ml in RPMI 1640 medium (Biowhittaker, Walkersville, Md.) supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (Gibco-BRL, Gaithersburg, Md.) and treated as follows: 20 ng of TNF-α (Sigma, Poole, United Kingdom) per ml, 20 ng of T55 mutant per ml, 200 ng of T75 mutant per ml, both mutants mixed together, 20 ng of heat-inactivated (70°C for 30 min) TNF-α per ml, or 20 ng of TNF-α per ml mixed with 2.3 μg of anti-human TNF-α monoclonal antibody (MAb) cA2 (kindly provided by M. Feldmann, Kennedy Institute of Rheumatology, London, United Kingdom) (50) per ml as described previously (17). After incubation for 48 h at 37°C in 7% CO_2–93% air, supernatants were harvested and tested for RT activity. In agreement with previous reports (42, 44), TNF-α stimulated HIV-1 replication in U1 cells 20-fold. HIV-1 stimulation with TNF-α could be totally inhibited by the anti-human TNF-α MAb cA2 or by heat inactivation of TNF-α at 70°C for 30 min. In contrast to the T75 mutant, which did not modify HIV-1 replication in U1 cells, the T55 mutant stimulated viral replication with RT activity levels similar to those of TNF-α-treated U1 cells. The mixture of T55 and T75 mutants yielded RT activity levels almost similar to those of TNF-α-treated U1 cells (Fig. 4).

The evidence presented here clearly documents distinct actions mediated through 55-kDa TNF-R1 and 75-kDa TNF-R2 in regard to HIV-1 replication in human primary TCDM. Inhibition of HIV-1 replication in primary TCDM by TNF-α is mediated through the 75-kDa TNF-R2 by inhibiting an early stage in the viral life cycle, that of entry as reported previously (17). In contrast, TNF-R1 stimulates a later stage in the viral life cycle, probably following proviral integration in primary TCDM. Postintegration TNF-R1-mediated stimulation of HIV-1 is in agreement with the following data: (a) the stimulation of HIV-1 replication in primary TCDM is observed only after T55 posttreatment and not after pretreatment, and (b) the stimulation of HIV-1 replication by TNF-α in the promonocytic cell line U1—which contains two integrated copies of HIV—is mediated by TNF-R1 but not by TNF-R2 alone.

We have shown previously that TNF-α can inhibit HIV-1 replication in primary TCDM and that HIV-1 replication can be restored by an anti-human TNF-α mouse-human chimeric MAb (17). The time of addition of TNF-α in regard to HIV input (pre- or posttreatment) did not modify the observed HIV-1 inhibition, and this is in contrast with other cytokines such as gamma interferon and transforming growth factor β, which display opposite effects on HIV-1 replication in primary TCDM depending on the time of cytokine addition (23, 26, 43). In our previous study (17), TNF-α treatment of primary phytohemagglutinin-activated peripheral blood lymphocytes did not inhibit HIV-1 growth, suggesting that inhibition of HIV-1 growth by TNF-α is macrophage specific as reported previously for TH2 cytokines (35, 36). The inhibition of HIV-1 replication in TNF-α-treated TCDM was highly significant and reproducible with sixfold lower RT activity and absence of syncytium formation compared with untreated infected control cells. The inhibition of viral growth was not due to a toxic effect of TNF-α on the primary TCDM, shown by identical cell survival in untreated and TNF-α-treated TCDM as measured by performing a colorimetric reaction (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide [MTT]) based on the capacity of mitochondrial dehydrogenase of living cells to reduce

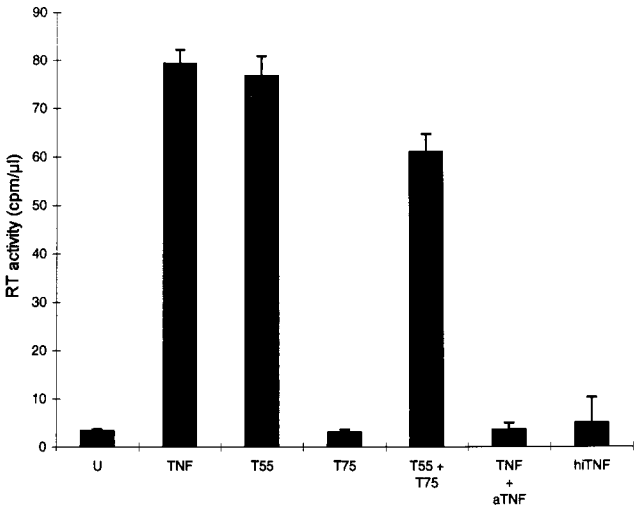

FIG. 4. Effects of TNF-α and both TNF-α mutants on HIV-1 replication in the chronically infected promonocytic cell line U1. U1 cells were plated in 96-well plates at 2×10^5 cells/ml in RPMI 1640 medium supplemented with 10% (vol/vol) heat-inactivated fetal calf serum. The cells were left untreated (U) or treated with 20 ng of TNF-α per ml, 20 ng of T55 mutant per ml, 200 ng of T75 mutant per ml, 20 ng of T55 and 200 ng of T75 mutant together per ml, 20 ng of TNF-α mixed with 2.3 μg of anti-human TNF-α MAb cA2 per ml, or 20 ng of heat-inactivated (70°C for 30 min) TNF-α per ml. Culture supernatants were harvested after 48 h of incubation and tested for RT activity as previously reported (17). These results are representative of three independent experiments. The means and standard deviations are shown.

MTT to formazan (data not shown) (40). As lipopolysaccharide has been reported to inhibit HIV-1 growth in primary human TCDM (22), we tested TNF-α and T55 and T75 mutant stocks for the presence of endotoxin; all were found to be free of endotoxin (data not shown). Our stock of TNF-α was biologically active as demonstrated by stimulation of HIV replication in the promonocytic U1 cell line in agreement with previous reports (42, 44).

In order to determine the TNF-R(s) involved in inhibition of HIV-1 replication in primary TCDM, we tested two human TNF-α mutants, T55 and T75, which recognize with high selectivity either the 55-kDa or the 75-kDa TNF-R, respectively. These TNF-Rs trigger distinct intracellular pathways and therefore might be helpful in understanding TNF-α effects on HIV-1 replication in TCDM. Our results shows that TNF-R2 is involved in inhibition of HIV-1 replication in primary TCDM and that the viral inhibition is twofold greater after T75 pretreatment than after T75 posttreatment. These results suggest that TNF-α through TNF-R2 could target an early step of the virus life cycle. This is in agreement with our previous observation that pretreatment with TNF-α delays and inhibits the appearance of proviral long terminal repeat in primary TCDM and that inhibition of HIV-1 entry into primary TCDM treated with TNF-α is mediated through TNF-R2, not through TNF-R1, and is independent of surface CD4 expression (15, 17, 21). As TNF-R2 mediates inhibition of viral entry into TCDM, this could explain the absence of action of the T75 mutant in the U1 promonocytic cell line, which contains two integrated copies of HIV and does not allow the study of the early stages of infection.

The proviral activation observed in chronically HIV-infected promonocytic and lymphoid cell lines is mediated through NF-κB, which has been shown to be activated through TNF-R1 (24, 30). A soluble TNF-R1 has been reported to block the HIV-1 transactivation via NF-κB induced by TNF-α in both U1 promonocytic and ACH-2 lymphocytic cell lines (18). In agreement with previous observations (2), our data show that TNF-α stimulates HIV-1 replication in the U1 cell line through TNF-R1 and not TNF-R2. Taken together, these findings suggest that in chronically infected cell lines TNF-R1 could mediate the HIV-1 transactivation by TNF-α through NF-κB. Interestingly, our results demonstrate that distinct actions, either inhibitory or stimulatory, could be mediated through TNF-R1 in primary TCDM depending on the time of receptor stimulation in regard to HIV input. The T55 mutant stimulated HIV-1 replication in primary TCDM only after posttreatment starting at day 3 postinfection, not after pretreatment. In contrast to TNF-R2-mediated HIV-1 inhibition, TNF-R1-mediated stimulation of HIV-1 in TCDM could involve a later stage of the viral life cycle, after proviral integration. We demonstrated that the T55 mutant through TNF-R1 does not modulate HIV-1 entry into primary TCDM, and therefore, the cellular target of the T55 mutant might be subsequent to (and including) reverse transcription (17). Reverse transcription in primary TCDM infected with HIV-1 monocytotropic strains is slower than in lymphoid cell lines and takes 48 h to be completed (6, 37, 38). Posttreatment with the T55 mutant starting at day 3 postinfection, after probable completion of reverse transcription and proviral integration, which have not occurred by 1 h after infection, may therefore stimulate transcription of the provirus in primary TCDM. Our results suggest that, as observed for the chronically infected promonocytic cell line U1 and as reported previously (2), TNF-R1 could stimulate HIV-1 growth in primary TCDM through activation of NF-κB and subsequent proviral transcription. The moderate inhibition of HIV-1 replication observed in TCDM pretreated with the T55 mutant does not seem to be mediated at the level of viral entry in agreement with similar amounts of viral genomic RNA detected in both T55-pretreated and untreated infected control TCDM (17). NF-κB and I-κB are tightly autoregulated; therefore, the partial inhibitory effect of T55 pretreatment on HIV replication might be explained by increased cytoplasmic levels of I-κB following repeated activation of TNF-R1 before infection (27, 51). Increased cytoplasmic levels of I-κB could inhibit HIV replication either by a posttranscriptional mechanism negatively regulating Rev function (55) or by making the TCDM refractory to HIV induction through NF-κB (32).

Both pre- and posttreatment with T55 and T75 mutants mixed together inhibited HIV-1 replication in primary TCDM as observed with the wild-type TNF-α. This suggests that in our culture system TNF-α action was mediated predominantly through TNF-R2 rather than TNF-R1. Interestingly, both HIV-1 infection and addition of exogenous TNF-α have been reported to selectively increase cell surface TNF-R2 expression in the promyelocytic cell line OM-10.1 (2); selective upregulation of cell surface TNF-R2 in HIV-1-infected primary TCDM might account for the inhibition of viral growth observed after TNF-α treatment. Among proinflammatory cytokines, IL-1 has been reported to stimulate HIV-1 replication through activation of NF-κB or by an independent mechanism (39, 42), although, in contrast to TNF-α, it does not inhibit HIV-1 entry into primary TCDM (17). In the chronically HIV-infected promonocytic U1 cell line, IL-1 upregulates HIV-1 replication through the cell surface IL-1 receptor type 1, but not through the IL-1 receptor type 2 (42). Thus, several proinflammatory cytokine cell surface receptors could mediate distinct actions in regard to HIV replication both in the promonocytic cell line U1 and in primary human TCDM.

The β chemokines, macrophage inflammatory proteins 1α and 1β and RANTES, are released by $CD8^+$ T lymphocytes and have been reported to inhibit HIV-1 replication (5). Recently, the β chemokine receptors have been shown to act as fusion cofactors for macrophage-tropic HIV-1 strains (1, 3, 7, 8), and β chemokines could be released from TNF-α-treated primary human monocytes/macrophages (48). We have shown that TNF-α through TNF-R2 is involved in inhibition of HIV-1 replication in primary TCDM by blocking viral entry (17). The monocytotropic Ada strain has been reported to enter into the cells by using essentially CC-CKR5 as a cofactor (3, 47). So far, a modulation of CC-CKR5 by TNF-α has not been reported, although it has been shown that cytokines such as IL-2 could upregulate the expression of CC-CKR1 and CC-CKR2 in T lymphocytes (29). We cannot exclude that TNF-α through modulation of CC-CKR5 could modulate $HIV\text{-}1_{Ada}$ entry and subsequent replication in primary TCDM. In regard to the fact that β chemokines and TNF-α act together in immune regulation and might also synergize in anti-HIV defense, the respective roles of TNF-R2 and β chemokine receptors in regard to inhibition of HIV-1 growth in primary TCDM need further investigation.

In conclusion, we have demonstrated distinct roles of 55- and 75-kDa TNF-Rs in regard to HIV-1 replication in primary human macrophages. TNF-R1 mediates stimulation of HIV-1 replication at a postintegration level both in the promonocytic U1 cell line and in primary human TCDM. In contrast, TNF-R2 inhibits HIV-1 replication in primary TCDM by targeting an early stage of the viral life cycle, that of entry. A better understanding of distinct TNF-R pathways in HIV-1-infected primary human TCDM should lead to the development of selective new therapeutic approaches taking into account both TNF-α and its cell surface and soluble receptors.

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