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TUMOR NECROSIS FACTOR ENHANCES REPLICATION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) *IN VITRO*

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The effect of tumor necrosis factor (TNF) on the replication of human immunodeficiency virus type 1 (HIV-1) was investigated in several T4 lymphocyte cell lines. TNF markedly enhanced the cytopathogenicity of HIV-1, virion-associated reverse transcriptase (RT) activity in the cell culture supernatant, and viral antigen expression in MOLT-4 cells as early as 3 days after HIV-1 infection. A slight increase in RT activity was also observed in the supernatant of H9 cell cultures exposed to TNF. However, TNF did not increase either RT activity in MT-4 cell supernatants or viral antigen expression in HUT-78 cells. Thus, TNF is able to stimulate the replication of HIV-1 in *de novo* infected T4 cells although not all T4 cells seem to be sensitive to this stimulatory effect.

Acquired immune deficiency syndrome (AIDS) is a pandemic immunosuppressive disease caused by the depletion of helper T-lymphocytes. The causative agent, human immunodeficiency virus type 1 (HIV-1), is a lentivirus belonging to the family of the retroviridae, and various compounds have been reported to inhibit the replication of HIV-1 *in vitro* (1). Recently, cytokines such as granulocyte/macrophage colony-stimulating factor (GM-CSF) and recombinant interferon-γ (IFN-γ) were shown to exert a selective inhibitory effect on the replication of HIV-1 (2,3). Tumor necrosis factor (TNF), whether TNF-α or TNF-β (lymphotoxin), can also be considered as a cytokine. It selectively kills tumor cells *in vitro* and *in vivo* (4). TNF is also inhibitory to the replication of several viruses (5), and, when combined with IFN-γ, TNF markedly potentiates the inhibitory effect of IFN-γ on HIV-1 (6). On the other hand, Matsuyama *et al.* (7) reported that natural cytokines including TNF may enhance the production of HIV-1 particles from chronically infected T lymphocyte cells. Folks *et al.* (8) showed that cytokines other than TNF up-regulated the expression of HIV-1 in chronically infected promonocyte cells. Therefore, it seemed of interest to examine whether TNF inhi-

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bits or enhances the replication of HIV-1. We have explored the effect of TNF on the *de novo* infection of various T4 lymphocyte cell lines by HIV-1 and found that TNF is capable of enhancing the replication of HIV-1 in at least some T4 cell lines.

MATERIALS AND METHODS

The origin of the T4 lymphocyte cell lines, MOLT-4 (clone 8), H9, HUT-78 and MT-4 has been described elsewhere (9,10). The cells were cultured and maintained in RPMI-1640 medium supplemented with 10 % fetal calf serum, 100 U/ml penicilin G, and 100 μg/ml streptomycin (cell culture medium). HIV-1 was obtained from the cell culture supernatant of MOLT-4/HTLV-III_B cells, as previously described (11).

Recombinant TNF-α was obtained from Genzyme, Boston, MA., and stored at -70°C until used.

The effect of TNF on HIV-1 replication was evaluated by three different parameters : (i) virus-induced cytopathogenicity measured by trypan-blue exclusion, (ii) virion-associated reverse transcriptase (RT) activity in the cell culture supernatant, and (iii) viral antigen expression. For the first method, MOLT-4 cells (1×10^4 cells/well) were cultured in 96-well microtiter trays in the absence or presence of TNF and infected with HIV-1 at a multiplicity of infection (MOI) of 0.1. After 3 days of incubation at 37°C, the number of viable cells was determined microscopically in an hemacytometer by trypan blue exclusion. For the second method, MOLT-4, H9, and MT-4 cells were infected with HIV-1 and cultured in the absence or presence of TNF. After several days of incubation, the culture supernatant was collected, submitted to ultracentrifugation, and the pellet was examined for RT activity, as previously described (12). For the third method, MOLT-4 and HUT-78 were infected with HIV-1 and cultured for several days in the absence or presence of TNF. Then the cells were collected and examined for viral antigen expression by indirect immunofluorescence, using polyclonal antibody as probe. In each assay, the cytotoxicity of TNF was assessed based on the viability of mock-infected cells.

RESULTS

When MOLT-4 cells were infected with HIV-1 and cultured in the presence of TNF (10 U/ml), syncytium (multinuclear giant cell) formation was observed at day 3 (Fig. 1A). Such giant cells were not detected if the HIV-1-infected cells had been cultured in the absence of TNF (Fig. 1B). When the viability of virus- or mock-infected MOLT-4 cells, that had been incubated in the presence or absence of TNF (Fig. 2), was evaluated, TNF appeared to cause a dose-dependent decrease in the viability of the virus-infected cells. At a concentration of 10 U/ml, TNF reduced the viability of HIV-1-infected MOLT-4 cells by 67 % (Fig. 2). TNF did not affect the viability of mock-infected MOLT-4 cells even at concentrations up to 1000 U/ml (Fig. 2). These results suggest that TNF enhances the cytopathogenicity of HIV-1 in MOLT-4 cells.

The effect of TNF on HIV-1 replication was futher evaluated by monitoring the virion-associated RT activity in the supernatant of

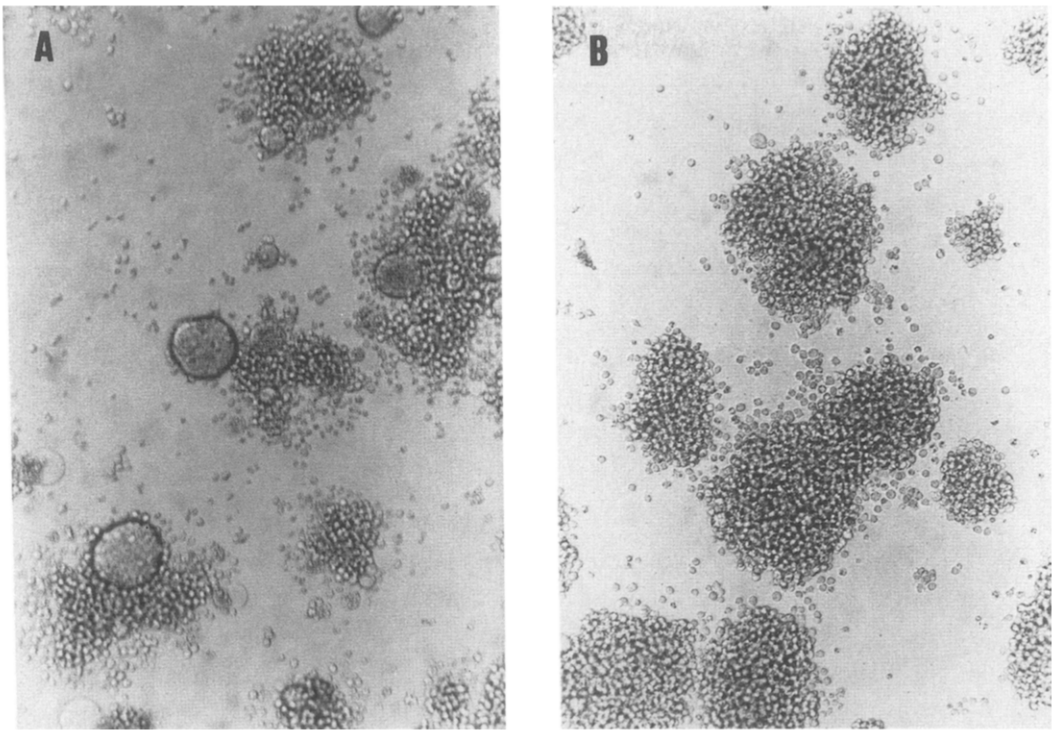
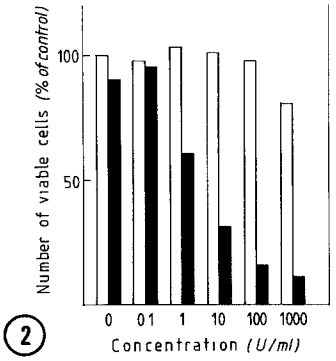


Figure 1. MOLT-4 cells following infection with HIV-1 and incubation for 3 days in the presence (A) or absence (B) of TNF (10 U/ml). Giant cell formation is observed in panel A, whereas no such giant cell formation is apparent from panel B.

the cell cultures. MOLT-4 cells were infected with HIV-1 and incubated in the presence of varying concentrations of TNF. The cell culture supernatant was collected at the 3rd day after infection and



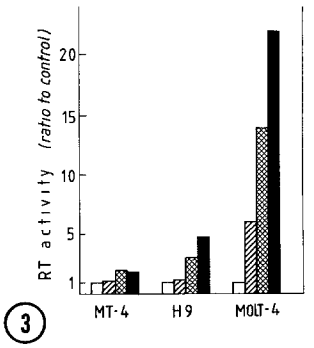


Figure 2. Effect of TNF on viability of HIV-1- and mock-infected MOLT-4 cells. Viability of HIV-1-infected (■) and mock-infected (□) MOLT-4 cells was measured by trypan blue exclusion at day 3 after infection.

Figure 3. Effect of TNF on virion-associated reverse transcriptase (RT) activity in the cell culture supernatant of HIV-1-infected MOLT-4, H9, and MT-4 cell cultures. The virus-infected cells were incubated in the presence of TNF at a concentration of 0 (□), 1 (▨), 10 (▩), or 100 (■) U/ml. RT activity was measured at day 3 (MOLT-4 and MT-4) or day 5 (H9) by a previously established method (12).

TABLE 1. Effect of TNF on viral antigen expression in HIV-1-infected MOLT-4 and HUT-78 cell cultures

Cell line	Incubation day	Number of viral antigen-positive cells (%)[a]			
		Concentration (U/ml)			
		0	1	10	100
MOLT-4	2	1.3	9.0	9.3	13.7
	3	3.0	16.0	36.5	49.0
	4	9.4	66.7	64.0	61.9
HUT-78	12	27.1	26.2	28.5	28.8

[a]Based on > 500 cells per cell culture.

examined for RT activity. TNF effected a dose-dependent increase in RT activity, amounting to 22-, 14- and 6-fold the control value following exposure to TNF at 100, 10 and 1 U/ml, respectively (Fig. 3). A 5-fold increase of RT activity was also noted with HIV-1-infected H9 cells which had been exposed to TNF (100 U/ml) for 5 days. However, no significant increase in RT activity was observed in the supernatant of HIV-1-infected MT-4 cells treated with TNF (Fig. 3).

Next we examined viral antigen expression in HIV-1-infected MOLT-4 and HUT-78 cells which had been incubated in the presence of various concentrations of TNF. As shown in Table 1, TNF markedly enhanced HIV-1 antigen expression in MOLT-4 cells. This enhancement was already apparent from the 2nd day. After 4 days of incubation in the presence of TNF (1, 10 or 100 U/ml) the percentage of viral antigen-positive cells rose from < 10 % to > 60 %. In contrast, the viral antigen expression in HUT-78 cells was not enhanced by TNF even at concentration up to 100 U/ml, following an incubation period of 12 days (Table 1).

DISCUSSION

As could be followed by different parameters (cytopathogenicity, RT activity in the cell culture supernatant, and viral antigen expression), HIV-1 replication in MOLT-4 cells was markedly enhanced when the infected cells were exposed to TNF. TNF clearly decreased the viability of the cells (Fig. 2) and increased viral antigen expression (Table 1). These findings were confirmed with TNF preparations from different sources (data not shown).

The marked increase in RT activity in the cell culture supernatant (Fig. 3) indicates that the release of virus particles from HIV-1-

infected MOLT-4 cells is stimulated in the presence of TNF. The increased release of virus particles may result from a selective killing effect of TNF on the HIV-1-infected cells [since TNF is a cytokine that can actually kill some cells (4)], or it may reflect an increased virus replication and thus increased production of new virus progeny particles. The data obtained for viral antigen expression (Table 1) support the latter alternative, that is an enhancing effect of TNF on virus replication *per se*.

When an inhibitor of phospholipase A2, a potential mediator of the selective killing effect of TNF on tumor cells, was added to the MOLT-4 cell culture together with TNF, phospholipase A2 did not affect the enhancement of HIV-1 replication by TNF (data not shown). On the other hand, dextran sulfate, which is a potent and selective inhibitor of HIV-1 replication (10), completely blocked the effect of TNF on HIV-1-infected MOLT-4 cells at a concentration of 20 μg/ml (data not shown). Thus, TNF may be assumed to directly interfere with the HIV replicative cycle. At which step it precisely interacts remains to be elucidated.

Another important question that remains to be resolved is why the effect of TNF on HIV-1 replication varies from one cell line to another. While HIV-1 antigen expression in MOLT-4 cells was markedly stimulated by TNF at a concentration of 1 U/ml, viral antigen expression in HUT-78 cells was not affected by TNF even at a concentration of 100 U/ml (Table 1). The latter finding is consistent with the observations of Wong *et al.* (6), who found little change in HIV-1 p24 antigen expression in HUT-78 cell cultures exposed to TNF.

Also, MT-4 cells proved refractory to the enhancing effect of TNF on virus particle release [monitored by RT activity in the cell culture supernatant (Fig. 3)]. MT-4 is a T4 lymphoblastoid cell line carrying human T-lymphotropic virus type 1 (HTLV-1) (13). MT-2, which represents another T4 lymphoblastoid cell line carrying HTLV-1, has been recently shown to release a lymphotoxin (TNF-β)-related cytotoxic factor in the supernatant of its culture (14). If such a substance is also produced by MT-4 cells, it may be expected to affect HIV-1 replication in the same way as shown here with TNF-α in MOLT-4 cells. This may then explain why HIV-1 replicates so rapidly in MT-4 cells and why the exogenous supply of TNF no longer affects virus growth in MT-4 cells as it is already optimally regulated by the endogenous TNF. Cytokines, such as GM-CSF, are considered as therapeutic modalities to augment host defense against opportunistic infections in AIDS (15). The fact that these cytokines are able to stimulate HIV-1 production, as has been shown in the present study

with TNF in lymphocytes and in other studies with GM-CSF and IFN-γ in macrophages (16), justifies extreme caution in the clinical use of these agents.

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