JOURNAL OF VIROLOGY, June 1989, p. 2504–2509
0022-538X/89/062504-06$02.00/0

Vol. 63, No. 6

Cytocidal Effect of Tumor Necrosis Factor on Cells Chronically Infected with Human Immunodeficiency Virus (HIV): Enhancement of HIV Replication

TOSHIFUMI MATSUYAMA,[1] YOSHIAKI HAMAMOTO,[1] GEN-ICHIRO SOMA,[2] DEN'ICHI MIZUNO,[2] NAOKI YAMAMOTO,[1] AND NOBUYUKI KOBAYASHI[1]*

Department of Virology and Parasitology, Yamaguchi University, 1144 Kogushi, Ube, Yamaguchi 755,[1] and Biotechnology Research Center, Teikyo University, Sagamiko-cho, Tsukui-gun, Kanagawa 199-01,[2] Japan

Received 5 August 1988/Accepted 17 January 1989

Tumor necrosis factor (TNF), a monokine initially described as a tumoricidal agent, facilitated the replication of human immunodeficiency virus (HIV) in vitro. The viability of human T-cell line MOLT-4/HIV, chronically infected with HIV, was affected by the addition of a low dose (10 ng/ml) of recombinant TNF-α (rTNF-α), while uninfected MOLT-4 cells were resistant to treatment with rTNF-α at concentrations up to 1,000 ng/ml. A marked increase in the level of HIV-specific RNA was detected in MOLT-4/HIV cells as early as 1 h after exposure to rTNF-α and reached almost maximum level within 6 h. Production of HIV particles from MOLT-4/HIV was also increased at 6 h after treatment with rTNF-α. Nearly identical phenomena were observed in CCRF-CEM/HIV, Jurkat/HIV, and H9/HIV cells, although the sensitivity of these cell lines to rTNF-α varied. A human T-lymphotropic virus type 1-infected cell line, MT-4, was insensitive to treatment with rTNF-α.

It is well recognized that human immunodeficiency virus (HIV) is the primary causative agent of acquired immunodeficiency syndrome (AIDS) (1, 11, 26). However, during the long latency period between HIV infection and clinical progression of disease, HIV does not have full transcriptional activity, and the gene expression of HIV is greatly activated prior to clinical manifestation of AIDS (10, 13). Thus, it can be readily speculated that some cofactors which enhance the gene expression of HIV are associated with the progression of AIDS. A major immunological abnormality of AIDS is characterized as the selective depletion of $CD4^+$ lymphocytes, which are known as the target cells of HIV (30, 31). However, the number of cells infected with HIV is very small (1 of 100,000 or less) even in the peripheral blood lymphocytes (PBL) of AIDS patients (7). Thus, it is unknown whether mere activation of HIV genes could explain the mechanism of selective depletion of $CD4^+$ cells.

Several factors have been reported to activate HIV gene expression. Those include granulocyte-macrophage colony-stimulating factor (4), immunological stimuli (12, 40), and infection with viruses such as herpes simplex virus type 1 (19). We have also reported that a tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), stimulated the replication of HIV in chronically HIV-infected cells in vitro (6). The precise mechanisms of HIV gene activation by these factors have not been clearly demonstrated yet, however, and how one of these factors could act as the cofactor which accelerates the development of disease remains to be answered.

As a candidate for such cofactors, we previously reported that lymphotoxin (LT) enhanced HIV replication and was cytocidal to cells chronically infected with HIV (12a, 12b). The availability of purified recombinant tumor necrosis factor α (rTNF-α), a cytokine biologically related to LT, prompted us to investigate in more detail the mechanism of enhancement of HIV replication by TNF-α.

MATERIALS AND METHODS

Cells. The origins and characteristics of a human T-cell leukemia virus type I-positive T-cell line, MT-4 (18), and the negative T-cell lines CCRF-CEM (3), H9 (26), Jurkat (29), and MOLT-4 (14) and a promonocyte line, U937 (33), have been fully described elsewhere. Each chronically HIV-infected cell line was established by infection with human T-lymphotropic virus type IIIB at a multiplicity of infection of 0.002 in this laboratory and was greater than 90% positive for HIV antigens. All cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 μg/ml) at 37°C and subcultured every 4 days.

Cell survival. Each cell line was treated with various concentrations of rTNF-α for 6 days. On day 3 the number of viable cells was counted by the trypan blue dye exclusion method. The culture was then diluted 1:4 with fresh medium containing the same concentrations of rTNF-α as before. On day 6, the number of viable cells was counted again. In a certain experiment (see Fig. 1), MOLT-4/HIV cells were counted daily for 4 days without passage, and the percent cell viability was calculated. The relative cell growth was determined as follows: relative cell growth = (viable cell no. in the presence of rTNF-α)/(viable cell no. in the absence of rTNF-α).

rTNF-α and anti-TNF-α antibody. rTNF-α used in this study was purified from *Escherichia coli* carrying $TNF\text{-}S_{AM2}$ genes as described previously (32). The purity (>95%) of $rTNF\text{-}S_{AM2}$ was estimated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and contamination with lipopolysaccharide was less than 0.0002%. The cytotoxic assay results were confirmed by using L929 cells, and the specific activity was 4×10^6 U/mg.

* Corresponding author.

Anti-rTNF-α monoclonal antibody was kindly provided by Dainippon Pharmaceutical Co., Ltd., Osaka, Japan. For the neutralization experiment, 1 ng of rTNF-α per ml was preincubated with the anti-TNF-α antibody at 37°C for 4 h and then applied to MOLT-4/HIV cells for 6 h. Tenfold more antibody was used than was required to neutralize the cytotoxic activity of rTNF-α against murine L929 cells.

Dot blot hybridization. The analysis of HIV-specific mRNA by the dot blot technique was performed as described previously (37). Cells were harvested by low-speed centrifugation and washed twice with ice-cold phosphate-buffered saline. Then, 10^6 cells were suspended in 100 μl of ice-cold TE (10 mM Tris, 1 mM EDTA) containing 0.5% Nonidet P-40 and kept on ice for 15 min with vigorous shaking. The cell lysate was then centrifuged at 15,000 rpm in an Eppendorf microfuge for 15 min. The supernatant was mixed with 150 μl of formaldehyde and 150 μl of 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and incubated at 60°C for 15 min. The solutions were stored at −20°C until use. The resulting RNA solutions, corresponding to 10^5 cells, were immobilized in slot configuration on a nitrocellulose filter (BA85; Schleicher & Schuell, Inc.) and hybridized with ^{32}P-labeled probes. Hybridization was carried out at 42°C for 16 h in 5× SSPE (1× is 0.18 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA)–5× Denhardt solution (1× is 0.02% bovine serum albumin, 0.02% Ficoll [Pharmacia Fine Chemicals], 0.02% polyvinylpyrrolidone)–50% formamide–0.2% SDS–200 μg of heat-denatured salmon sperm DNA per ml. Washing of the filter was performed first with 2× SSPE–0.2% SDS and then with 0.1× SSPE–0.2% SDS at 50°C. The HIV-specific probe used in this study was the *Sac*I fragment of pNK5.2, which covers approximately 90% of the HIV-1 genome (39). The human β-actin-specific probe used was the 0.44-kilobase *Hin*fI fragment of bacteriophage λHa160, which was kindly supplied by T. Kakunaga (20). The specific activity of ^{32}P-labeled probes used in this study was about 4×10^8 dpm/μg of DNA.

RT assay. The number of MOLT-4/HIV cells was adjusted to 3×10^5 cells per ml, and they were cultured for 6 h in the presence of various concentrations of rTNF-α. At 6 h after treatment, 1 ml of culture fluid was harvested. The culture fluids were first centrifuged at 1,500 × *g* for 10 min to remove cell debris and then centrifuged at 100,000 rpm for 30 min in a Beckman TL100 benchtop ultracentrifuge. The resulting pellets were suspended in 50 μl of dilution buffer (10 mM potassium phosphate [pH 7.2], 2 mM dithiothreitol, 0.2% Triton X-100, 10% glycerol), and 10 μl was used for the reverse transcriptase (RT) assay as described previously (24).

RESULTS

Cytocidal effect of rTNF-α on MOLT-4/HIV cells. First, the cytotoxicity of rTNF-α on MOLT-4 cells and MOLT-4 cells chronically infected with HIV-1 (MOLT-4/HIV cells) was examined. Initially the number of cells was adjusted to 3×10^5 per ml in the medium with or without rTNF-α (0, 0.1, 10, and 1,000 ng/ml), and viability of the cells was scored on the days indicated in the figure legends. The viability of MOLT-4/HIV cells dropped dramatically with rTNF-α treatment at 10 and 1,000 ng/ml (Fig. 1A). On day 4, the viability of MOLT-4/HIV with rTNF-α at 10 and 1,000 ng/ml was 23 and 2%, respectively, whereas uninfected MOLT-4 cells were not severely affected by rTNF-α treatment, and the viability at 1,000 ng/ml on day 4 was 89% (Fig. 1B).

Enhancement of HIV production by rTNF-α. As previously reported, the cytocidal effect of LT-like activity in the MT-2 cell supernatant was associated with the stimulation of virus production (12a, 12b). The supernatant fluids of MOLT-4/HIV cells treated with rTNF-α for 6 h were concentrated by ultracentrifugation, and the resulting pellets were examined for RT activity (Table 1). The increase in RT activity was detected even at a concentration of 0.1 ng/ml (5.5 times higher than in the control) and at a concentration of 10 ng/ml, as much as 15.6 times more RT activity was observed. Neutralization of these effects by TNF-specific monoclonal antibody (Table 1, experiment B) suggested that other impurities in the rTNF-α preparation could be excluded in the present experiment.

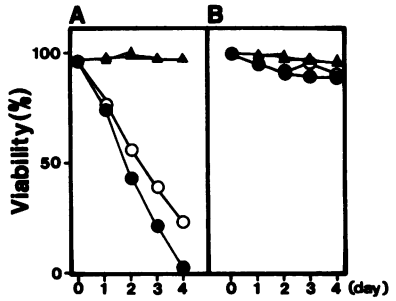

FIG. 1. Kinetic study of the viability of MOLT-4/HIV and MOLT-4 cells after treatment with rTNF-α. On the days indicated, viable cells were counted by the trypan blue dye exclusion method. rTNF-α was used at 0 (△), 0.1 (▲), 10 (○), and 1,000 (●) ng/ml. (A) MOLT-4/HIV cells; (B) MOLT-4 cells.

The level of HIV-1 mRNA determined by dot hybridization of cytoplasmic extracts of rTNF-α-treated MOLT-4/HIV cells was then studied. As shown in Fig. 2A, the level of HIV-specific mRNA increased after only 1 h of treatment with rTNF-α at concentrations of 10 and 1,000 ng/ml. The amount increased to nearly eight times that in the untreated control when the densities of X-ray film were measured by laser beam densitometer (data not shown). At 6 h after rTNF-α treatment, a slight increase in HIV mRNA was detected even at a concentration of 0.1 ng/ml, and at 1,000 ng/ml the level of enhancement was over 30-fold. On the other hand, the level of β-actin mRNA, one of the representative housekeeping genes, was not significantly enhanced by the rTNF-α treatment even at a concentration of 1,000 ng/ml. Previous reports suggested that the cytocidal

TABLE 1. RT activity in the supernatant of rTNF-α-treated MOLT-4/HIV cells[a]

Expt	Treatment	RT activity (mean cpm incorporated ± SD)
A	rTNF-α (ng/ml)	
	1,000	28,466 ± 3,394
	10	26,595 ± 5,120
	0.1	9,328 ± 429
	0	1,702 ± 166
B	rTNF	11,546 ± 1,351
	rTNF + anti-TNF	1,586 ± 270
	Anti-TNF	2,372 ± 163
	None	1,932 ± 414

[a] In experiment A, MOLT-4/HIV cells were adjusted to 3×10^5 cells per ml and cultured for 6 h in the presence of various concentrations of rTNF-α. Incorporation of [^{3}H]TTP by 10-μl samples, prepared as described in Materials and Methods, is shown. In experiment B, the neutralization experiments were done as described in Materials and Methods. RT activity of the culture fluids was assayed as in experiment A. Experiments were performed in triplicate.

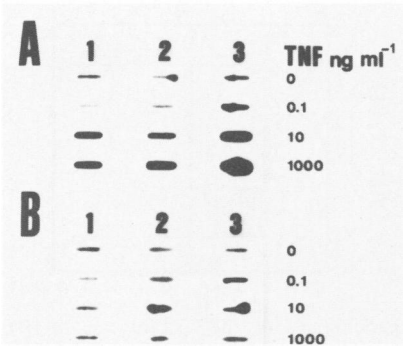

FIG. 2. Quantitation of HIV RNA in rTNF-α-treated MOLT-4/HIV cells. MOLT-4/HIV cells (3×10^5/ml) were cultured at 37°C for 1, 4, and 6 h in the presence of rTNF-α (0, 0.1, 10, or 1,000 ng/ml). Cytoplasmic RNA was extracted, immobilized on a nitrocellulose filter, and hybridized with ^{32}P-labeled probes as described in Materials and Methods. (A) HIV-specific probe; (B) human β-actin-specific probe. Lane 1, 1 h after treatment; lane 2, 4 h after treatment; lane 3, 6 h after treatment.

effect of TNF-α did not require de novo protein synthesis (7a). Thus, whether the cytocidal effect of rTNF-α could be detected in cycloheximide (CHX)-treated cells was examined. CHX (40 μg/ml) was added to MOLT-4/HIV cells 30 min prior to the addition of rTNF-α, total RNA was extracted 2 h after the addition of rTNF-α, and the level of HIV RNA was examined. Although CHX itself slightly enhanced the level of HIV mRNA, overall enhancement of HIV RNA by rTNF-α was not influenced by CHX treatment (data not shown).

Effect of rTNF-α on various T-cell lines chronically infected with HIV. Whether the cytocidal effect of rTNF-α was limited only to MOLT-4/HIV cells was examined next. Human T-cell lines chronically infected with HIV (Jurkat/HIV, H9/HIV, CCRF-CEM/HIV [CEM/HIV], MT-4/HIV) and a human monocyte cell line (U937/HIV) were examined for their sensitivity to rTNF-α. Jurkat/HIV, MOLT-4/HIV, H9/HIV, CEM/HIV, U937/HIV, and the respective uninfected control cell lines were cultured in the presence of various concentrations of rTNF-α (0, 0.1, 1, 10, and 100 ng/ml), and the number of viable cells on days 3 and 6 was counted. Figure 3 demonstrates the relative cell growth of rTNF-α-treated and untreated cells on days 3 and 6. On day 3, Jurkat/HIV, H9/HIV, and CEM/HIV cells showed sensitivity to rTNF-α treatment at concentrations of 10 and 100 ng/ml, and their uninfected counterparts were less influenced by rTNF-α. However, the extent of sensitivity to rTNF-α was not as significant as in MOLT-4/HIV cells. The growth of MT-4/HIV and MT-4 cells was not affected by rTNF-α even at 1,000 ng/ml. On the other hand, in the case of U937, which is known to be sensitive to rTNF-α (25), the cells became rather resistant to TNF treatment when chronically infected with HIV. On day 6, the phenomena observed on day 3 became more prominent. Thus, growth of not only MOLT-4/HIV but also Jurkat/HIV, H9/HIV, and CEM/HIV cells was affected at a certain concentration of rTNF-α. However, growth of MT-4/HIV cells was not affected by rTNF-α. The effect of rTNF-α was not only cytostatic but also cytocidal, and viabilities of CEM/HIV, Jurkat/HIV, and MOLT/HIV cells on day 6 with rTNF-α at 10 ng/ml were 84, 43, and <0.1%, respectively, whereas values for CEM, Jurkat, and MOLT-4 cells were 92, 73, and 95%, respectively. The elevation of HIV RNA in rTNF-α-treated cells

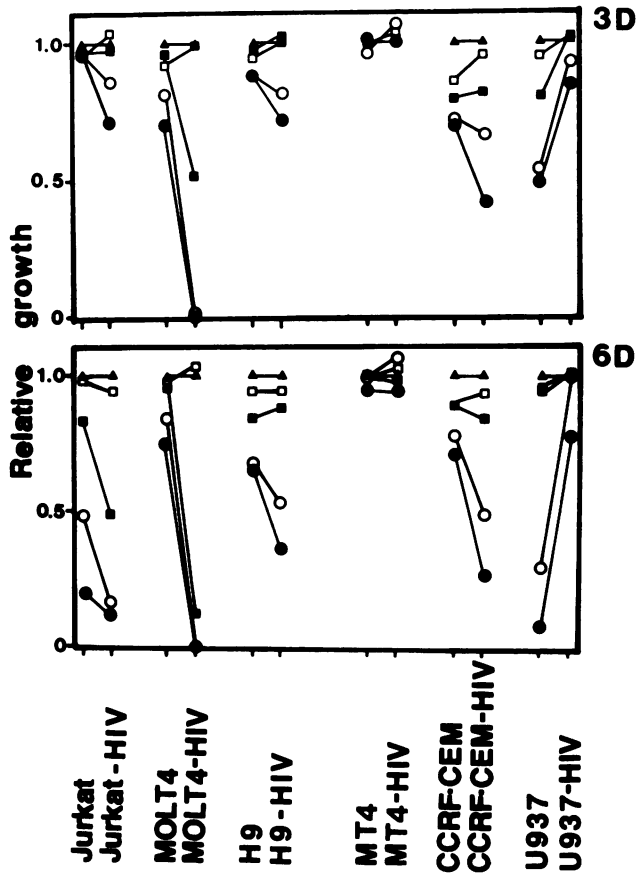

FIG. 3. Cytocidal effect of rTNF-α on chronically HIV-infected human cell lines. Jurkat/HIV, MOLT-4/HIV, H9/HIV, MT-4/HIV, CEM/HIV, and U937/HIV cells and their uninfected counterparts were cultured in the presence of various concentrations of rTNF-α. On days 3 and 6 after culture, the number of viable cells was measured. The relative cell numbers compared with untreated cells were calculated as described in Materials and Methods. The concentrations of rTNF-α used were 0 (▲), 0.1 (□), 1 (■), 10 (○), and 1,000 (●) ng/ml.

was also measured (Fig. 4A). Cells chronically infected with HIV were treated with 5 ng of rTNF-α per ml for 6 h, and cytoplasmic RNA was examined for HIV-specific RNA. Elevation of HIV RNA was detected not only in MOLT-4/HIV but also in Jurkat/HIV and CEM/HIV cells, while in MT-4/HIV, H9/HIV, and U937/HIV cells elevation of HIV RNA was not detected. The expression of the β-actin gene was not enhanced in all of the cells examined (Fig. 4B).

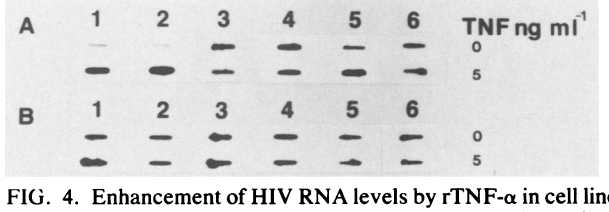

FIG. 4. Enhancement of HIV RNA levels by rTNF-α in cell lines chronically infected with HIV. Jurkat/HIV (lane 1), MOLT-4/HIV (lane 2), H9/HIV (lane 3), MT-4/HIV (lane 4), CEM/HIV (lane 5), and U937/HIV (lane 6) cells were treated with 5 ng of rTNF-α per ml for 6 h; cytoplasmic RNA was extracted, and dot hybridization was performed as described in Materials and Methods. ^{32}P-labeled probes used were specific to (A) HIV and (B) human β-actin.

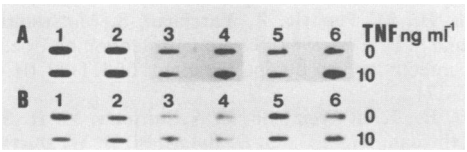

FIG. 5. Effect of long-term exposure to rTNF-α on enhancement of HIV RNA levels in chronically HIV-infected human T-cell lines. Cytoplasmic RNA was extracted 3 and 6 days after culture in the presence of rTNF-α, and dot hybridization was performed with ^{32}P-labeled probes specific to (A) HIV or (B) human β-actin. Cell lines used were MT-4/HIV (lane 1, day 3; lane 2, day 6), U937/HIV (lane 3, day 3; lane 4, day 6), and H9/HIV (lane 5, day 3; lane 6, day 6).

However, when H9/HIV and U937 were exposed to rTNF-α for longer periods (3 and 6 days), the level of HIV-specific RNA was increased (Fig. 5A). Under identical conditions, enhancement of HIV-specific RNA contents in MT-4/HIV cells was not observed.

DISCUSSION

The onset of AIDS can be summarized as a depletion of CD4$^+$ cells by HIV infection (30, 31), resulting in a host lacking an immune system to protect itself from opportunistic infection. Upon infection with HIV, human T cells are destroyed in vitro. Thus, a direct cytocidal effect of HIV accounts for part of the depletion of CD4$^+$ cells in vivo, although it is not certain whether HIV is directly responsible for the entire depletion of CD4$^+$ cells in vivo. The molecular mechanism responsible for the reduction of CD4$^+$ cells is poorly understood. However, accumulated evidence revealed that only a portion of lymphoid cells express HIV at any one time in AIDS patients (7). Thus, it is not feasible to postulate that HIV alone is responsible for the depletion of CD4$^+$ cells, and a cofactor was naturally postulated to act with HIV in the manifestation of AIDS. A role for such cofactors, especially LT, has been postulated (23, 27, 28).

The data described here indicate that TNF-α, biologically related to LT, preferentially kills chronically HIV-infected cells and enhances HIV replication. LT (recently, LT was called TNF-β and TNF was called TNF-α) has been implicated as an effector molecule in various types of cell-mediated lysis (8, 23, 35). If TNFs and related cytokines are involved in the action of the immune system in HIV-infected individuals, it is possible that TNF could turn against the host, resulting in the progression of AIDS. TNF-β could be produced by T cells, and TNF-α could be produced by macrophages upon various stimulations, including infection with another virus (2). Thus, HIV carriers, when infected with other virus or immunologically stimulated, could begin to produce TNFs. These TNFs could then attack HIV-infected T cells and preferentially destroy cells, which could lead to depletion of CD4$^+$ cells and cause immunodeficiency. When HIV-infected T cells are destroyed, they produce large amounts of HIV progeny, which could also lead to HIV antigenemia. If our postulation on the mechanism of the development of AIDS is correct, many symptoms associated with AIDS, for example, fever of unknown cause, hypergammaglobulinemia, central nervous system disturbance, and cachexia, could also be explained as effects of TNFs. Recently, it was reported that the level of TNFs in the serum of AIDS and AIDS-related complex patients was significantly higher than in normal healthy persons (9). It was also reported that when the PBL of AIDS patients were cultured in vitro, they produce much higher levels of TNFs than the PBL of normal persons (27). These results support our postulation as a reasonable explanation for the mechanism of AIDS development.

The mechanism of enhancement of HIV replication by TNFs has not yet been determined. We have previously reported that a tumor promoter, TPA, enhances HIV replication concomitantly with lysis of chronically HIV-infected cells (6). Thus, TNFs may act like TPA on HIV-infected cells. However, the effect of enhancement by TNFs takes place in less than 1 h, as described in this study, which is much faster than in the case of TPA. Whether TNF-α itself reacts with the HIV genome directly or acts via an unknown factor is not clear. Recently we found that activation of HIV replication by TNFs is associated with the activity of the *tat* gene of HIV (T. Okamoto, T. Matsuyama, S. Mori, Y. Hamamoto, N. Kobayashi, S. F. Josephs, F. Wong-Staal, and K. Shimotohno, AIDS Res. Hum. Retroviruses, in press). We transfected the HIV-LTR-CAT plasmid into cells and examined the chloramphenicol acetyltransferase activity in response to TNF; we found that the activity was only enhanced by TNF when cells were cotransfected with the *tat* gene.

Recently Wong et al. reported that TNF together with gamma interferon inhibited HIV infection (38). Their observations do not coincide with our present data. The difference might come from different cell lines used; however, our experiment with cotreatment with interferon gamma and TNF failed to reproduce their experiment, and addition of interferon gamma gave almost identical results as with TNF alone (data not shown). Based on the experimental data of Wong and her colleagues, a phase I trial of TNFs and interferon gamma was started with AIDS patients in the United States. However, our data suggest that mere application of TNFs to HIV-infected individuals might accelerate the development of AIDS. However, studies with TNFs suggested the possible application of TNFs to AIDS, since this is the first compound found which could eliminate HIV-infected cells. Many compounds have been reported to inhibit HIV infection (5, 16, 17, 21, 22, 34). Azidothymidine inhibits HIV infection by inhibiting viral RT activity, and dextran sulfate inhibits HIV infection by interfering with the binding of HIV to the cell membrane (15). These compounds, however, are not effective once HIV has integrated into host chromosomal DNA. Thus, radical treatment of AIDS is completed only when cells infected with HIV are totally eliminated. If TNFs are used for AIDS patients together with another anti-HIV drug such as azidothymidine and dextran sulfate, they could eliminate HIV-infected cells and at the same time prevent new HIV infection via the anti-HIV drugs. If the enhancement of HIV replication by TNFs is separated from its cytocidal activity, a single application of TNFs could also be effective for AIDS patients. In any case, further precise studies of the function of TNFs should be done before TNFs are used for AIDS patients for therapeutic purposes.

ACKNOWLEDGMENTS

We thank N. Fukuda for typing the manuscript.

This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture and the Ministry of Health and Welfare of Japan, and grants from the Uehara Memorial Foundation, the Mochida Memorial Foundation, and the Ichiro Kanehara Foundation.

LITERATURE CITED

1. **Barré-Sinoussi, F., J.C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, and C. Axler-Blin.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220**:868–871.
2. **Beutler, B., and A. Cerami.** 1987. Cachectin: more than a tumor necrosis factor. N. Engl. J. Med. **316**:379–385.
3. **Foley, G. E., H. Lazarus, S. Farber, B. G. Uzman, B. A. Boone, and R. E. McCarthy.** 1965. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer **18**:522–529.
4. **Folks, T. M., J. Justement, A. Kinter, C. A. Dinarello, and A. S. Fauci.** 1987. Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. Science **238**:800–802.
5. **Hamamoto, Y., H. Nakashima, T. Matsui, A. Matsuda, T. Ueda, and N. Yamamoto.** 1987. Inhibitory effect of 2′,3′-didehydro-2′,3′-dideoxynucleosides on infectivity, cytopathic effects, and replication of human immunodeficiency virus. Antimicrob. Agents Chemother. **31**:907–910.
6. **Harada, S., Y. Koyanagi, H. Nakashima, N. Kobayashi, and N. Yamamoto.** 1986. Tumor promoter, TPA, enhances replication of HTLV-III/LAV. Virology **154**:249–258.
7. **Harper, M. E., M. L. Marselle, R. C. Gallo, and F. Wong-Staal.** 1986. Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by in situ hybridization. Proc. Natl. Acad. Sci. USA **83**:772–776.

7a. **Kirstein, M., and C. Baglioni.** 1986. Tumor necrosis factor induces synthesis of proteins in human fibroblasts. J. Biol. Chem. **261**:9565–9567.

8. **Kondo, L. L., W. Rosenau, and D. W. Wara.** 1981. Role of lymphotoxin in antibody-dependent cell-mediated cytotoxicity (ADCC). J. Immunol. **126**:1131–1133.
9. **Lahdevirta, J., C. P. J. Maury, A. M. Teppo, and H. Repo.** 1988. Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. Am. J. Med. **85**:289–291.
10. **Levy, J. A.** 1988. Mysteries of HIV: challenges for therapy and prevention. Nature (London) **333**:519–522.
11. **Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Landis, and J. M. Shimabukuro.** 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science **225**: 840–842.
12. **McDougal, J. S., A. Mawle, S. P. Cort, J. K. A. Nicholson, G. D. Cross, J. A. Scheppler-Campbell, D. Hicks, and J. Sligh.** 1985. Cellular tropism of the human retrovirus HTLV-III/LAV. I. Role of T cell activation and expression of the T4 antigen. J. Immunol. **135**:3151–3162.

12a. **Matsuyama, T., Y. Hamamoto, S. Kobayashi, M. Kurimoto, J. Minowada, N. Kobayashi, and N. Yamamoto.** 1988. Enhancement of human immunodeficiency virus production by natural lymphotoxin. Med. Microbiol. Immunol. **177**:181–187.

12b. **Matsuyama, T., Y. Hamamoto, T. Yoshida, Y. Kido, S. Kobayashi, N. Kobayashi, and N. Yamamoto.** 1988. Effect of culture supernatant of MT-2 cells on human immunodeficiency virus-producing cells, MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells. Jpn. J. Cancer Res. **79**:156–159.

13. **Melbye, M., R. J. Bigger, P. Ebbesen, C. Neuland, J. Goedert, V. Faber, I. Lorenzen, P. Skinhoj, R. C. Gallo, and W. A. Blattner.** 1986. Long-term seropositivity for human T-lymphotropic virus type III in homosexual men without the acquired immunodeficiency syndrome: development of immunologic and clinical abnormalities. Ann. Intern. Med. **104**:496–500.
14. **Minowada, J., T. Ohnuma, and G. E. Moore.** 1972. Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus-derived lymphocytes. J. Natl. Cancer Inst. **49**:891–895.
15. **Mitsuya, H., D. J. Looney, S. Kuno, R. Ueno, F. Wong-Staal, and S. Broder.** 1988. Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to $CD4^+$ cells. Science **240**:646–649.
16. **Mitsuya, H., M. Popovic, R. Yarchoan, S. Matsushita, R. C. Gallo, and S. Broder.** 1984. Suramin protection of T cell in vitro against infectivity and cytopathic effect of HTLV-III. Science **226**:172–174.
17. **Mitsuya, H., K. J. Weinhold, P. A. Furman, M. H. St. Clair, S. N. Lehrman, R. C. Gallo, D. Bolognesi, D. W. Barry, and S. Broder.** 1985. 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Proc. Natl. Acad. Sci. USA **82**:7096–7100.
18. **Miyoshi, I., H. Taguchi, I. Kubonishi, S. Yoshimoto, Y. Ohtsuki, Y. Shiraishi, and T. Akagi.** 1982. Type C virus-producing cell lines derived from adult T cell leukemia. Gann Monogr. **28**: 219–228.
19. **Mosca, J. D., D. P. Bednarik, N. B. K. Raj, C. A. Rosen, J. G. Sodroski, W. A. Haseltine, and P. M. Pitha.** 1987. Herpes simplex virus type 1 can reactivate transcription of latent human immunodeficiency virus. Nature (London) **325**:67–70.
20. **Nakajima-Iijima, S., H. Hamada, P. Reddy, and T. Kakunaga.** 1985. Molecular structure of the human cytoplasmic β-actin gene: interspecies homology of sequences in the introns. Proc. Natl. Acad. Sci. USA **82**:6133–6137.
21. **Nakashima, H., Y. Kido, N. Kobayashi, Y. Motoki, M. Neushul, and N. Yamamoto.** 1987. Antiretroviral activity in a marine red alga: reverse transcriptase inhibition by an aqueous extract of *Schizyimenia pacifica*. J. Cancer Res. Clin. Oncol. **113**:413–416.
22. **Nakashima, H., O. Yoshida, T. S. Tochikura, T. Yoshida, T. Mimura, Y. Kido, Y. Motoki, Y. Kaneko, T. Uryu, and N. Yamamoto.** 1987. Sulfation of polysaccharides generates potent and selective inhibitors of human immunodeficiency virus infection and replication in vitro. Jpn. J. Cancer Res. (Gann) **78**: 1164–1168.
23. **Old, L. J.** 1987. Tumor necrosis factor (TNF). Science **230**: 630–632.
24. **Ortaldo, J. R., R. Winkler-Pickett, A. C. Morgan, C. Woodhouse, R. Kantor, and C. W. Reynolds.** 1987. Analysis of rat natural killer cytotoxic factor (NKCF) produced by rat NK cell lines and the production of a murine monoclonal antibody that neutralizes NKCF. J. Immunol. **139**:3159–3165.
25. **Poiesz, B. J., F. W. Ruscetti, A. F. Gazdar, P. A. Bunn, J. D. Minna, and R. C. Gallo.** 1980. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc. Natl. Acad. Sci. USA **77**:7415–7419.
26. **Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo.** 1984. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science **224**:497–500.
27. **Ratner, L., S. H. Polmar, N. Paul, and N. Ruddle.** 1987. Cytotoxic factors secreted by cells infected by human immunodeficiency virus type I. AIDS Res. Hum. Retroviruses **3**: 147–155.
28. **Ruddle, N. H.** 1986. Lymphotoxin production in AIDS. Immunol. Today **7**:8–9.
29. **Schneider, U., H. U. Schwenk, and G. Bornkamm.** 1977. Characterization of EBV genome-negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. Int. J. Cancer **19**:621–626.
30. **Schroff, R. W., M. S. Gottlieb, H. E. Prince, L. L. Chai, and J. L. Fahey.** 1983. Immunological studies of homosexual men with immunodeficiency and Kaposi's sarcoma. Clin. Immunol. Immunopathol. **27**:300–314.
31. **Seligmann, M., L. Chess, J. L. Fahey, A. S. Fauci, P. J. Lachmann, J. L'Age-Stehr, J. Ngu, A. J. Pinching, F. S. Rosen, T. J. Spira, and J. Wybran.** 1984. AIDS—an immunologic reevaluation. N. Engl. J. Med. **311**:1286–1292.
32. **Soma, G., Y. Tsuji, Y. Tanabe, K. Noguchi, N. Kitahara-Tanabe, T. Gatanaga, H. Inagawa, M. Kawakami, and D. Mizuno.** 1988. Biological activities of novel recombinant tumor necrosis factor having N-terminal amino acid sequences derived from cytotoxic factors produced by THP-1 cells. J. Biol. Response Mod. **7**:587–595.

33. **Sundstrom, C., and K. Nilsson.** 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int. J. Cancer **17**:565–577.
34. **Tochikura, T. S., H. Nakashima, A. Tanabe, and N. Yamamoto.** 1988. Human immunodeficiency virus (HIV)-induced cell fusion: quantification and its application for the simple and rapid screening of anti-HIV substances in vitro. Virology **164**:542–546.
35. **Ware, C. F., and G. A. Granger.** 1981. Mechanisms of lymphocyte-mediated cytotoxicity. III. Characterization of the mechanism of inhibition of the human alloimmune lymphocyte-mediated reaction by polyspecific anti-lymphotoxin sera in vitro. J. Immunol. **126**:1934–1940.
36. **Weitzen, M. L., R. S. Yamamoto, and G. A. Granger.** 1983. Identification of human lymphocyte-derived lymphotoxins with binding and cell-lytic activity on NK-sensitive cell lines in vitro. Cell. Immunol. **77**:30–41.
37. **White, B. A., and F. C. Bancroft.** 1982. Cytoplasmic dot hybridization. Simple analysis of relative mRNA levels in multiple small cell or tissue samples. J. Biol. Chem. **257**: 8569–8572.
38. **Wong, G. H. W., J. F. Krowka, D. P. Stites, and D. V. Goeddel.** 1988. In vitro anti-human immunodeficiency virus activities of tumor necrosis factor-α and interferon-γ. J. Immunol. **140**: 120–124.
39. **Yoshiyama, H., N. Kobayashi, T. Matsui, H. Nakashima, T. Kajii, K. Yamato, S. Kotani, I. Miyoshi, and N. Yamamoto.** 1987. Transmission and genetic shift of human immunodeficiency virus (HIV) in vivo. Mol. Biol. Med. **4**:385–396.
40. **Zagury, D., J. Bernard, R. Leonard, R. Cheynier, M. Feldman, P. S. Sarin, and R. C. Gallo.** 1985. Long-term cultures of HTLV-III-infected T cells: a model of cytopathology of T-cell depletion in AIDS. Science **231**:850–853.