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Immunology

Anti-Fas monoclonal antibody is cytocidal to human immunodeficiency virus-infected cells without augmenting viral replication

(mouse monoclonal antibody/tumor necrosis factor)

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ABSTRACT A cytotoxic monoclonal antibody (anti-Fas mAb) against the 200-kDa cell surface Fas antigen, which is associated with the tumor necrosis factor (TNF) receptor, was examined for its *in vitro* activity on human immunodeficiency virus (HIV)-infected cells. It was found that both TNF and anti-Fas mAb selectively killed the chronically HIV-infected cells. Uninfected cells were less sensitive to the antibody than those infected with HIV. When the cells were cultured in the presence of anti-Fas mAb immediately after the HIV infection, the spread of HIV-infected cells was suppressed by the antibody. TNF augmented both the synthesis of HIV-specific mRNA in HIV-infected cells and formation of multinucleated giant cells. In contrast, the anti-Fas mAb did not augment HIV replication or enhance the HIV-induced formation of syncytia. The results indicated that anti-Fas mAb mimicks the cytocidal action of TNF but does not augment HIV replication.

Tumor necrosis factor (TNF) is a monokine that was initially described as a tumoricidal agent (1) and has been implicated as an effector molecule in several types of cell-mediated cytolysis (2–4). TNF also has pleiotropic effects on various types of cells (5–7) and possesses anti-viral activities (8–10). Furthermore, TNF is a mediator of several inflammatory states and is a primary mediator in endotoxic shock, whereas the systemic release of TNF is important in the development of septic shock and hemorrhagic tissue necrosis. Elevated serum TNF levels have been reported in patients with certain types of cancer (11–13) and have been reported to occur during the progression of AIDS (14, 15), a virulent disease caused by the human retrovirus termed human immunodeficiency virus (HIV) (16–18). It has been suggested that TNF is possibly linked to the cachexia of AIDS patients and may play an essential role as a cofactor that accelerates the progression of AIDS (19, 20). We (21–26) and others (27, 28) have shown that TNF is selectively cytotoxic to HIV-infected cells but augments HIV replication by inducing the cellular factor known as NF-κB. Our investigations of the cytocidal effects of TNF on HIV-infected cells have shown that protein kinase C inhibitors can augment its cytocidal activity without stimulating HIV replication (29). This finding suggested that the cytocidal activity of TNF could be separated from its effect of augmenting HIV replication.

We have prepared a mouse monoclonal antibody (mAb), anti-Fas mAb that has a cytocidal effect on human cells that are sensitive to the cytolytic activity of TNF (30). Anti-Fas mAb recognizes the Fas antigen, a 200-kDa human cell surface component that is associated with but different from the TNF receptor. Expression of the Fas antigen was shown to be down-regulated along with that of the TNF receptor when cells were treated with either TNF or anti-Fas mAb. The cytolytic activity of TNF and anti-Fas mAb was augmented by cotreatment of cells with interferon. Thus the cytolytic activity of TNF may be mediated by the Fas antigen and the anti-Fas mAb may not be able to exert other functions of TNF (30).

The present experiments were, therefore, undertaken to determine the effect of the anti-Fas mAb on HIV-infected cells and HIV replication. The results show that anti-Fas mAb is selectively cytolytic to the HIV-infected cells but does not augment HIV replication.

MATERIALS AND METHODS

Cells and Virus. The human T-cell lines MOLT-4, Jurkat, CCRF-CEM, and MT-4 and the promonocyte cell line U937 were maintained at 37°C in RPMI 1640 medium supplemented with penicillin (100 international units/ml), streptomycin (100 μg/ml), and 10% (vol/vol) heat-inactivated fetal bovine serum. Chronically infected cell lines were established by infection with the HTLV-IIIB strain of HIV-1 as reported (24). More than 90% of the cells of each chronically HIV-infected cell line expressed HIV antigen.

Quantitation of Multinucleated Giant Cell Formation. The extent of multinucleated giant cell formation between MOLT-4/HIV cells and MOLT-4 cells was quantitated as described (31). In brief, MOLT-4/HIV cells and MOLT-4 cells were mixed at a ratio of 1:9, and the mixtures (5×10^5 cells per ml) were incubated for 30 hr at 37°C. The cell profile was then monitored using a Cell Multisizer (Coulter), which measures the diameter of each cell and gives a histogram profile. The percentage of giant cells (cells larger than 20 μm in diameter) was calculated as follows: % giant cells = (number of cells >20 μm/number of cells >9 μm) × 100.

Assays for Augmentation of HIV Replication. Augmentation of HIV replication was assayed by dot-blot hybridization to determine the HIV-specific RNA content (24), by a plaque assay for the quantitation of the number of infective HIV particles (32), or by an HIV long terminal repeat (LTR)-directed chloramphenicol acetyltransferase (CAT) assay for the determination of the HIV LTR-directed transcriptional activity (33). For the CAT assay, 5 μg of plasmid (pH3LTRCAT) containing the HIV LTR linked to the 5′ end of the CAT gene (34) was transfected into 2×10^6 MOLT-4/HIV cells by the DEAE-dextran method (35). Twenty-four hours after transfection, TNF at 100, 25, or 1 ng/ml or

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Abbreviations: TNF, tumor necrosis factor; HIV, human immunodeficiency virus; mAb, monoclonal antibody; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase.
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anti-Fas mAb at 100, 25, or 1 ng/ml was added to the cells, which were then incubated for another 16 hr. Cells were suspended in 100 μl of 0.25 M Tris·HCl (pH 8.0), and a cell lysate was prepared by three cycles of freezing–thawing. The amount of the lysate used for the CAT assay was adjusted in terms of protein concentration.

Reagents. Mouse anti-Fas IgM (anti-Fas mAb) was purified by FPLC, as described (30), and stored at −20°C until used. Recombinant human TNF-α was generously supplied by D. Mizuno (Teikyo University). The specific activity of purified recombinant TNF was 4×10^6 units/mg (36). The HIV-specific probe employed in the present experiment was the *Sac* I fragment of pNK5.2, which covers almost the entire sequence of HIV (37). The β-actin-specific probe (38) was kindly supplied by T. Kakunaga (Osaka University). The [^{14}C]chloramphenicol used was purchased from New England Nuclear (CAT assay grade; NEN).

RESULTS

Cytocidal Activity of Anti-Fas mAb Toward HIV-Infected Cells. Chronically HIV-infected MOLT-4 (MOLT-4/HIV) cells and uninfected MOLT-4 cells were cultured in the presence of various concentrations of purified anti-Fas mAb. Cell viability was scored daily by the trypan blue dye exclusion method. As shown in Fig. 1, anti-Fas mAb was strongly cytocidal to MOLT-4/HIV cells but had little effect on MOLT-4 cells. After 3 days of culture with anti-Fas mAb (50 ng/ml), viability of MOLT-4/HIV cells dropped to 3%, whereas viability of MOLT-4 cells was 82%. Similar results were obtained with Jurkat/HIV, CCRF/CEM, and U937/HIV cells, although the sensitivity of HIV-infected cells to anti-Fas mAb varied somewhat depending on the cell line (Fig. 2). In contrast, the human T-cell leukemia virus I-infected human T-cell line MT-4, which is insensitive to the cytocidal action of TNF (24), was also insensitive to anti-Fas mAb; up to 100 ng/ml of the antibody failed to affect the viability of the cells. Normal mouse IgM was used as an isotype control and showed no effect on the growth of any cell line tested in this study (data not shown).

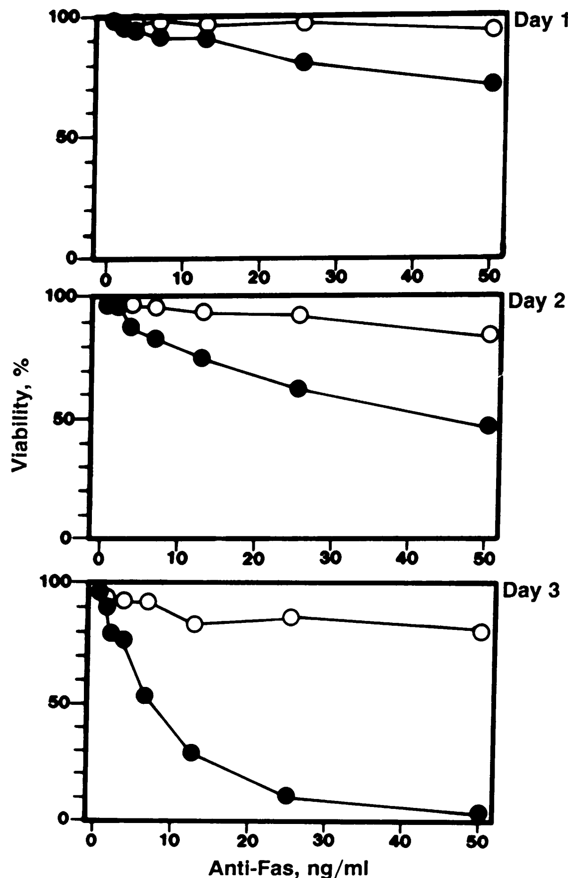

FIG. 1. Kinetics of the cytocidal effect of anti-Fas mAb on MOLT-4/HIV and MOLT-4 cells. MOLT-4/HIV (●) or MOLT-4 (○) cells (3×10^5 cells per ml) were cultured in the presence of various concentrations of anti-Fas mAb and cell viability was calculated daily. Each point is the average of duplicate determinations.

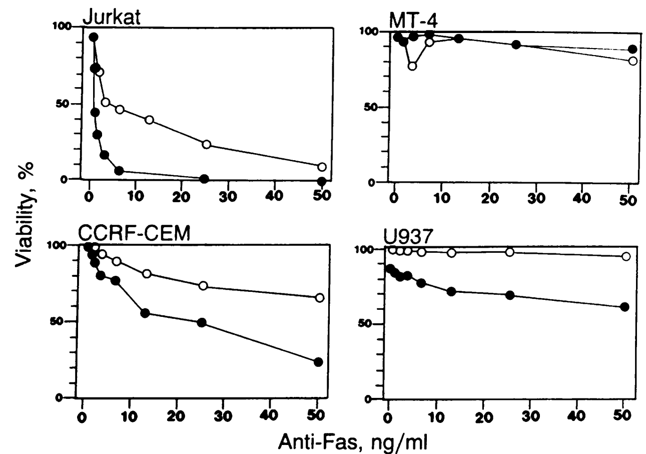

FIG. 2. Kinetics of the cytocidal activity of anti-Fas mAb on various cell lines chronically infected with HIV. Jurkat, CCRF-CEM, MT-4, and U937 cells (open circles) and their chronically HIV-infected counterparts (Jurkat/HIV, CCRF-CEM/HIV, MT-4/HIV, and U937/HIV; solid circles) were cultured in the presence of various concentrations of anti-Fas mAb for 3 days. Viability of the cells was determined by the trypan blue dye exclusion method. Averages of duplicate experiments are plotted.

Effect of Anti-Fas Antibody on Infection with HIV. MOLT-4 cells (3×10^5 cells per ml) were infected with HIV and cultured for 6 days in the presence of anti-Fas mAb at 100 ng/ml or 5 ng/ml. The percentage of HIV-infected cells was monitored daily by immunofluorescence using anti-HIV-positive human serum (Fig. 3). In the absence of anti-Fas mAb, 100% of the MOLT-4 cells became HIV positive on day 5 after infection. In contrast, only 12.5% and 31.9% of the

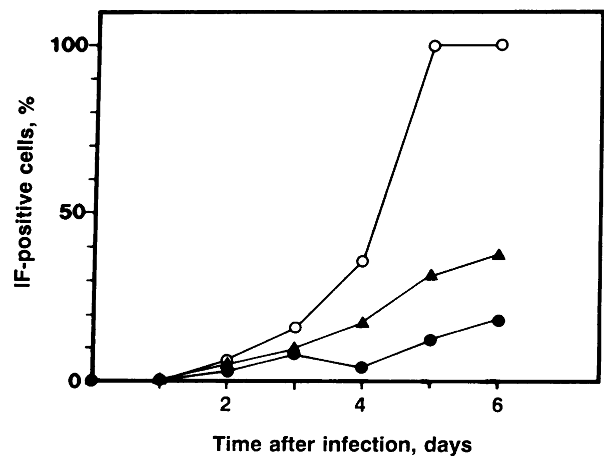

FIG. 3. Effect of anti-Fas on fresh infection with HIV. MOLT-4 cells (3×10^5 cells per ml) were infected with HIV (1 infectious virus particle per cell) and cultured in the presence of anti-Fas mAb at 100 ng/ml (●), 5 ng/ml (▲), or 0 ng/ml (○). The percentages of HIV antigen-positive cells were determined daily by immunofluorescence (IF) using seropositive human serum. A representative experiment of the three performed is shown in the figure.

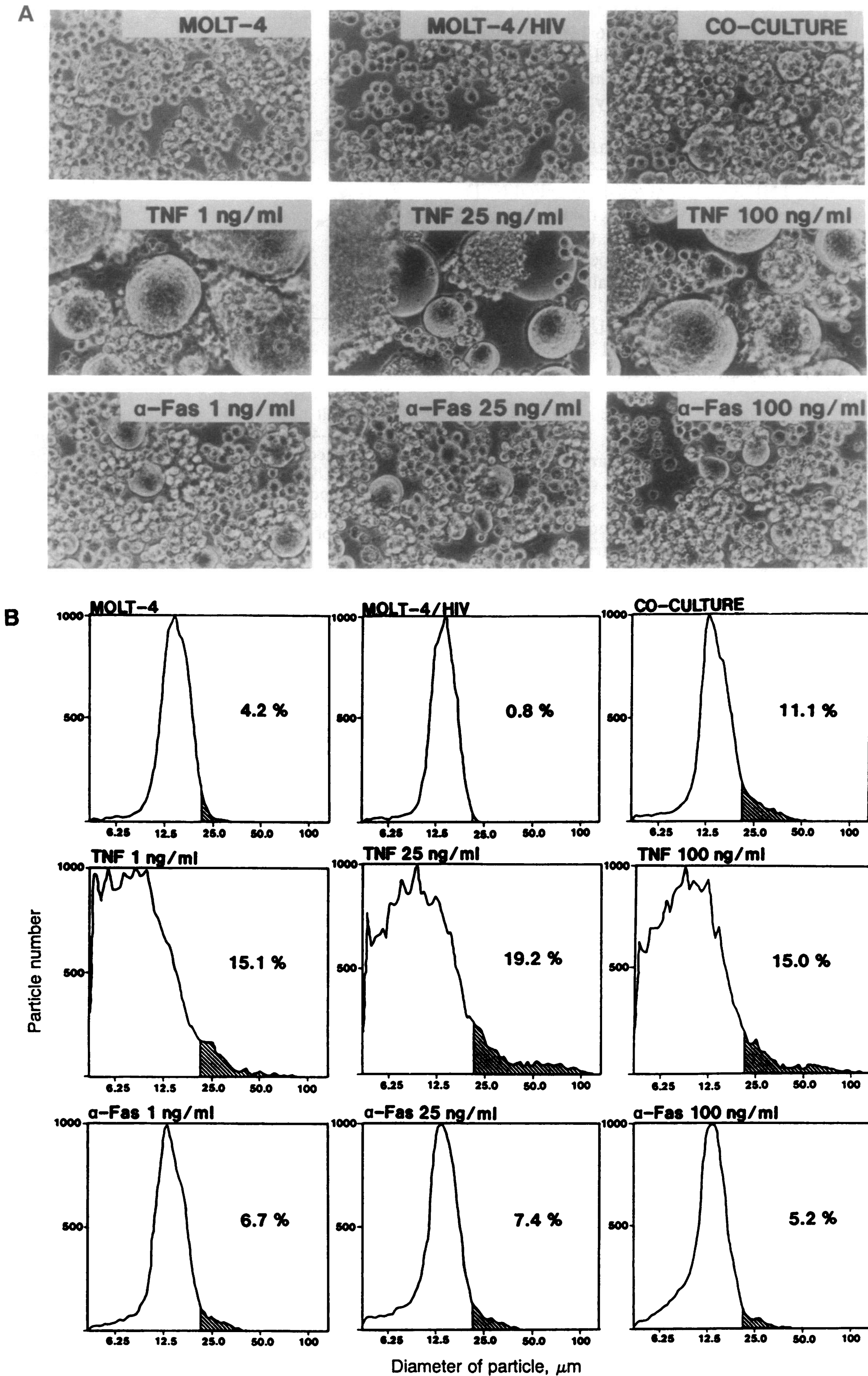

FIG. 4. Effect of anti-Fas mAb on HIV-induced giant-cell formation. Giant-cell formation between MOLT/HIV cells and MOLT-4 cells was assessed in the presence or absence of various concentrations (1 ng/ml, 25 ng/ml, or 100 ng/ml) of TNF or anti-Fas mAb (α-Fas). (A) Micrographs taken 30 hr after the start of mixed culture. (B) Giant-cell formation was quantitated by measuring the cell diameter profile using a Cell Multisizer.

cells became HIV positive in the presence of anti-Fas mAb at 100 ng/ml and 5 ng/ml, respectively.

The effect of anti-Fas mAb on multinucleated giant-cell formation was also examined by adding it at various concen-

trations to a mixed culture of MOLT-4/HIV and MOLT-4 cells at a ratio of 1:9. Anti-Fas mAb at up to 100 ng/ml had almost no enhancing effect on HIV-mediated giant-cell formation, whereas TNF at as little as 1 ng/ml augmented multinucleated giant-cell formation (Fig. 4). After 30 hr of culture, the percentage of the cells larger than 20 μm in diameter was increased to 15.1%, 19.9%, and 15.0% by TNF at 1 ng/ml, 25 ng/ml, and 100 ng/ml, respectively. On the other hand, for cells cultured with anti-Fas mAb at 1 ng, 25 ng, and 100 ng, the respective percentages were 6.7%, 7.4%, and 5.2%. Indeed, the percentages of giant cells in these cultures were lower than that in a control culture in which MOLT-4/HIV and MOLT-4 were cultured. Thus anti-Fas mAb tends to prevent giant-cell formation (Fig. 4*B*).

Effect of Anti-Fas mAb on HIV Gene Expression. MOLT-4/HIV, CEM/HIV, Jurkat/HIV, and MT-4/HIV cells (5×10^5 cells per ml) were cultured for 24 hr in medium containing anti-Fas mAb (10 ng/ml), TNF (10 ng/ml), or no additives. Total RNA was extracted from the cells and RNA from 1×10^5 cells was spotted onto nitrocellulose filters in a slot configuration. The filters were then hybridized with ^{32}P-labeled probes specific for either HIV or β-actin. As reported (24), TNF at 10 ng/ml augmented HIV-specific RNA synthesis in CEM/HIV, Jurkat/HIV, and MOLT-4/HIV cells but did not augment synthesis in MT-4/HIV cells, whereas anti-Fas mAb had no effect on the HIV-specific RNA content in any of the cells tested (Fig. 5). Anti-Fas mAb at concentrations of up to 100 ng failed to augment HIV-specific RNA synthesis (data not shown). Neither TNF nor anti-Fas mAb affected the synthesis of β-actin RNA.

To examine whether or not anti-Fas mAb failed to augment HIV replication at the level of transcription, the HIV LTR conjugated with the bacterial CAT gene was transfected into MOLT-4/HIV cells. From 24 hr after transfection, cells were incubated with either TNF or anti-Fas mAb for another 16 hr and then the CAT activity of cell lysates was examined. As shown in Fig. 6, the percentage conversion of chloramphenicol to its acetylated form was only 0.4% in HIV-LTR-CAT transfected MOLT-4/HIV cells. Treatment with TNF (100, 25, or 1 ng/ml) stimulated the CAT activity to 97.3, 88.9, and 7.8%, respectively, whereas anti-Fas mAb did not cause any augmentation of HIV replication as measured at the level of transcription.

We also examined whether or not anti-Fas mAb enhanced the HIV production by chronically HIV-infected cells. Supernatants from cultures of MOLT-4/HIV cells grown with TNF (10 ng/ml), anti-Fas mAb (10 ng/ml), or no additives were assessed for the number of infectious HIV particles by the plaque assay method. It was found that anti-Fas mAb did not augment HIV production (Table 1).

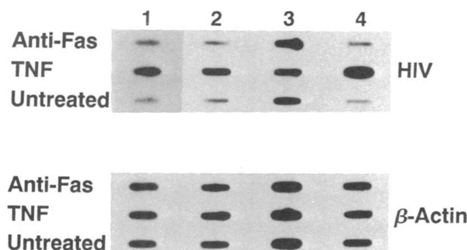

FIG. 5. RNA dot-blot analysis of anti-Fas mAb- or TNF-treated cell lines chronically infected with HIV. Chronically HIV-infected Jurkat (column 1), CCRF-CEM (column 2), MT-4 (column 3), and MOLT-4 (column 4) cells (5×10^5 cells per ml) were treated with anti-Fas mAb (10 ng/ml) or TNF (10 ng/ml) for 24 hr. Total RNA was then extracted and dot-blot hybridization was performed using HIV-specific or β-actin-specific probes, nick-translated with [^{32}P]dCTP.

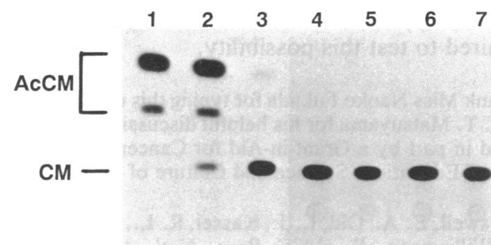

FIG. 6. Effect of anti-Fas mAb and TNF on HIV-LTR-directed CAT activity. The CAT reaction mixture was incubated for 10 min at 37°C. One of the two experiments performed is shown. Cultures contained the following additions. Columns: 1, TNF at 100 ng/ml; 2, TNF at 25 ng/ml; 3, TNF at 1 ng/ml; 4, anti-Fas mAb at 100 ng/ml; 5, anti-Fas mAb at 25 ng/ml; 6, anti-Fas mAb at 1 ng/ml; 7, untreated sample. AcCM, acetylated chloramphenicol; CM, chloramphenicol. The percentage of acetylation of chloramphenicol is as follows: Columns: 1, 97.3%; 2, 88.9%; 3, 7.8%; 4, 0.2%; 5, 0.3%; 6, 0.2%; 7, 0.4%.

DISCUSSION

In this study, we examined the effect of anti-Fas mAb on HIV-infected cells. The data in Figs. 1 and 2 clearly show that anti-Fas mAb selectively killed HIV-infected cells but did not augment the expression of HIV even at the level of transcription (Figs. 5 and 6 and Table 1). HIV-induced giant cell formation was found to be mediated by gp120, a glycosylated protein encoded by the viral *env* gene, and by CD4 molecules expressed on the cell surface (39). The mechanism of the enhancement of giant cell formation by TNF was attributed to an increase in the content of gp120 in HIV-infected cells (23). As anti-Fas mAb did not enhance HIV replication, the antibody did not augment HIV-induced giant cell formation; however, anti-Fas mAb was still cytolytic to HIV-infected cells and tended to reduce the number of multinucleated giant cells (Fig. 4).

Neither anti-Fas mAb nor TNF was cytolytic to MT-4/HIV cells (24). Human T-cell lines infected with human T-cell leukemia virus I produced large amounts of lymphotoxin (40). MT-4 is one of the well-known human T-cell leukemia virus I-infected cell lines that produce lymphotoxin as well as TNF (41). This finding explains why MT-4 is insensitive to both exogeniously added TNF and anti-Fas mAb.

We have reported (42) the effects of a combination of 3′-azido-3′-deoxythymidine (an anti-HIV substance) and TNF on peripheral blood monocytes obtained from AIDS and AIDS-related complex patients (42). Our findings suggested that combined treatment with 3′-azido-3′-deoxythymidine and TNF might suppress the spread of HIV in HIV-infected individuals. Since retroviruses integrate into host chromosomal DNA as proviruses, it is almost impossible to eliminate the viruses unless the infected cells are removed. TNF is the first agent known to selectively kill virus-infected cells and this observation leads to the possibility of administering TNF to HIV-infected individuals. Perhaps TNF could be administered to AIDS patients with anti-HIV substances such as 3′-azido-3′-deoxythymidine. Perhaps anti-Fas mAb may also

Table 1. Effect of anti-Fas antibody on HIV replication

Treatment	Infectious titer, pfu/ml
Anti-Fas (50 ng/ml)	$11 \pm 4.0 \times 10^3$
Anti-Fas (10 ng/ml)	$10 \pm 1.7 \times 10^3$
TNF-α (10 ng/ml)	$53 \pm 7.0 \times 10^3$
None	$12 \pm 4.2 \times 10^3$

Supernatants of 24-hr MOLT-4/HIV cell cultures were assayed for infectious HIV particles by plaque assay (22). pfu, plaque-forming units. Average values (±SD) of duplicate experiments are shown.

be clinically applicable in HIV infection but further studies are required to test this possibility.

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