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EFFECT OF CULTURE SUPERNATANT OF MT-2 CELLS ON HUMAN IMMUNODEFICIENCY VIRUS-PRODUCING CELLS, MOLT-4/$HIV_{HTLV\text{-}IIIB}$ CELLS

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The effect of culture supernatant of MT-2 cells on human immunodeficiency virus (HIV)-producing cells, MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells, was examined. As compared to the effect on MOLT-4 cells, parent cells not infected with HIV, a selective cytotoxic/cytostatic effect on MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells was observed 4 days after treatment with up to 640-fold-diluted MT-2 supernatant. Furthermore, under similar conditions, a 2- to 6-fold increase in the number of HIV particles was detected in the culture of MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells 6 hr after treatment. Complete blocking of these effects by anti-lymphotoxin monoclonal antibody, but not by anti-tumor necrosis factor antibody, indicates that these effects of MT-2 supernatant on MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells are attributable to a lymphotoxin-related cytotoxic factor.

Key words: AIDS — HIV — MT-2 cells — Cytotoxic factor

Human immunodeficiency virus (HIV) is a causative agent of acquired immunodeficiency syndrome (AIDS). Previously, we have reported that human T-lymphotropic virus type I (HTLV-I)-carrying cells are highly susceptible to HIV infection and are readily destroyed.[1] This aberrant pattern of HIV infection was originally attributed to the number of CD4 molecules on HTLV-I-carrying T-cells and not to the function of the pX gene of HTLV-I, because the expression of pX gene of HTLV-I did not affect the susceptibility of HTLV-I-carrying cells to HIV infection.[2] Furthermore, our recent study using cloned MOLT-4 cells[3] suggested that some cellular factor(s) other than the number of CD4 molecules is closely associated with the mechanism of cellular destruction by HIV infection.[4] Here, we describe an intriguing finding that culture supernatant of MT-2 cells,[5] an HTLV-I-carrying cell line, contains a large amount of a factor which specifically affects $HIV_{HTLV\text{-}IIIB}$-producing MOLT-4 cells (MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells).[4]

MT-2 cells, MOLT-4 cells and MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells used in this study were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (complete medium) at 37° in a CO_2 incubator. Supernatant of MT-2 cells (MT-2 sup) was obtained from a cell culture 4 days after adjusting the density of the cells to 30×10^4/ml with complete medium. This supernatant was filtered through a novel porous membrane filter which was recently developed for the removal of viruses such as hepatitis B virus, HIV and HTLV-I (Asahi Chemical Indust., Tokyo).[6] This membrane is made of regenerated cellulose membrane having various mean pore sizes, and scarcely absorbs protein molecules. The mean pore size of the membrane used in this experiment was 30 nm.

First, we examined the effect of MT-2 sup on cell growth of MOLT-4 cells and MOLT-4/HIV cells. MT-2 sup was serially diluted 4-fold from 1:5 to 1:5120 with complete medium and added to equal volumes of cell cultures which had been washed once with complete medium just before treatment. The final concentration of the cells was 3×10^5/ml. As shown in Fig. 1A and Fig. 2A, at 4 days after treatment only a slight growth inhibition was observed on MOLT-4 cells even at the concentration of 1:10, while on MOLT-4/HIV cells, remarkable growth inhibition and cell killing were observed at 4 days after treatment when cells were treated with MT-2 sup

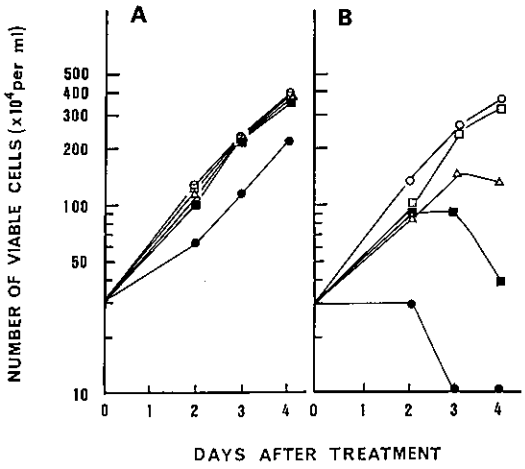

Fig. 1. Kinetics of the effect of MT-2 sup on growth of MOLT-4 cell (A) and MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells (B). Cells were treated with MT-2 sup at dilutions of 10-fold (●), 40-fold (■), 160-fold (△) and 640-fold (□) or were not treated (○). The number of cells was determined by a trypan blue dye exclusion method at the indicated times.

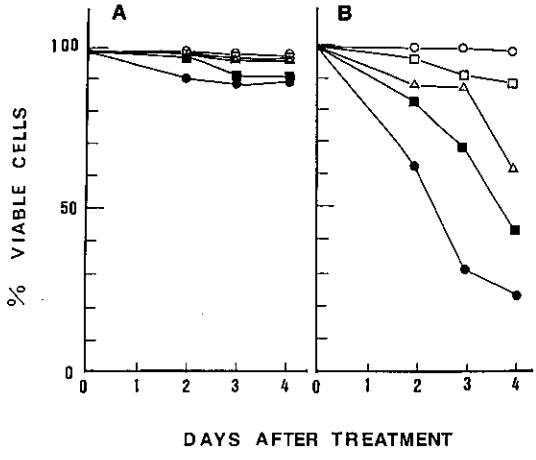

Fig. 2. Kinetics of the effect of MT-2 sup on cell viability of MOLT-4 cells (A) and MOLT-4/HIV cells (B). Symbols are the same as in Fig. 1.

at a concentration of more than 1:640 (Fig. 1B, Fig. 2B).

Next, we proceeded to determine whether MT-2 sup could not only inhibit the growth of HIV-infected cells but also inhibit the production of HIV from MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells.

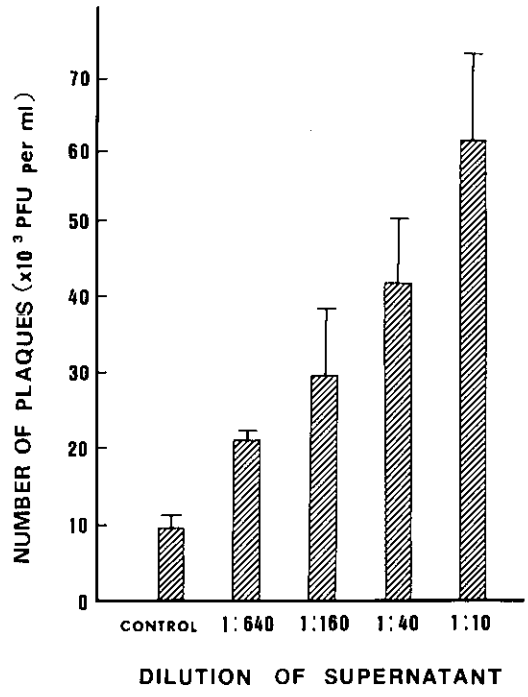

Fig. 3. Effect of MT-2 sup on HIV production. To evaluate the effect of MT-2 sup on HIV production, a plaque-forming assay was performed by using filtered culture supernatant of MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells treated for 6 hr with various concentrations of MT-2 sup. Experiments were carried out in triplicate. Each number represents the mean ±standard deviation.

For determining the number of infectious particles, a plaque-forming assay using MT-4 cells was performed.[7] Initially, we measured the level of infectious HIV particles released from MOLT-4/$HIV_{HTLV\text{-}IIIB}$ cells 2 days after treatment. However, in spite of the decreased number of cells in treated cultures, it was found that the number of HIV particles was approximately the same as that in the untreated cells (data not shown). Moreover, as shown in Fig. 3, the number of HIV particles released into the culture medium of MOLT-4/HIV 6 hr after treatment with MT-2 sup was increased as compared to the untreated control. Treatment with MT-2 sup at the concentrations of 1:10, 1:40, 1:160 and 1:640 resulted in the numbers of HIV particles of 63.0, 42.0, 29.3 and 21.0×10^3 PFU/ml, respectively. No treatment resulted in 9.7×10^3

PFU/ml. This indicated that MT-2 cells also produced a factor which enhanced the replication of HIV, or that a factor produced by MT-2 cells had enhanced HIV particle production, thus resulting in cell death. It has been reported that HTLV-I-infected cell lines produce various kinds of factors. Among HTLV-I cell lines, MT-2 cells produce a large amount of a cytotoxic factor which resembles tumor necrosis factor (TNF) or lymphotoxin (LT) in the killing of murine L_{929} cells.[8] Furthermore, TNF and LT can preferentially kill virus-infected cells.[9,10] Thus, we considered that at least the selective killing effect of MT-2 sup was attributable to the effect of a TNF-related cytotoxic factor. For determining the cytotoxic activity of MT-2 sup of our preparation, we measured the cytopathic effect of MT-2 sup against murine LM cells, a subline of L_{929} cells, as described by Nakano *et al.*[11] The cytotoxic activity of our preparation reached a value of 360 U/ml, which was equivalent to 86 ng/ml of that of recombinant TNF (r-TNF, Dainippon Pharmaceutical, Tokyo). The availability of anti-TNF specific monoclonal antibody (3-D-6, Hayashibara Biochemical, Okayama, 1.39×10^4 of our laboratory neutralizing unit/ml) and anti-LT specific monoclonal antibody (anti-TNFβ-I, Hayashibara Biochemical, 1.39×10^4 of our laboratory neutralizing unit/ml) prompted us to determine whether the effects of MT-2 sup could be neutralized by the antibody.

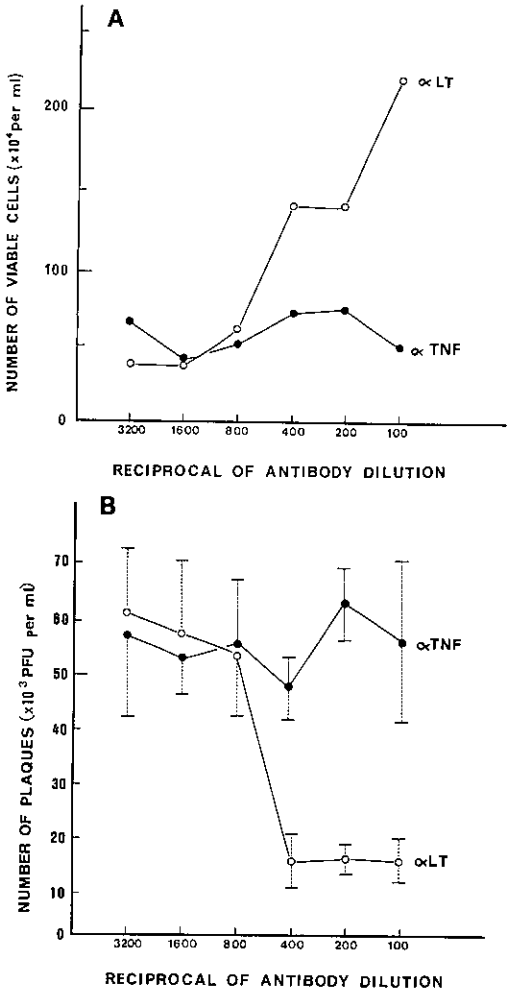

Fig. 4. Neutralization by anti-LT monoclonal antibody or anti-TNF monoclonal antibody of the effects of MT-2 sup on cell growth (A) and virus production (B). (A) One-half of the medium was changed on day 2, and an equal volume of complete medium containing a similar concentration of antibody-treated sup was added. The number of cells was counted on day 4 after treatment. (B) Each number represents the mean ± standard deviation. Experiments were carried out in triplicate.

For the neutralization experiment, 10-fold-diluted MT-2 sup was used. The antibodies used were diluted twice from 50-fold to 1600-fold, then added to an equal volume of MT-2 sup and incubated at 37° for 4 hr. As shown in Fig. 4A and Fig. 4B, both effects of MT-2 sup on MOLT-4/HIV cells were completely neutralized by anti-LT antibody, but not by anti-TNF antibody. From this finding, we concluded that the effects of MT-2 sup such as cytotoxicity on HIV-infected cells and enhancement of HIV replication were attributable to LT or a molecule immunologically closely related to LT.

This is the first report that a cytotoxic factor contributes to the killing of HIV-infected cells as well as enhancement of HIV replication. Further studies are required to establish the precise mechanism of the enhancement of HIV production by LT leading to cellular death.

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