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Mechanism of inhibitory effect of dextran sulfate and heparin on human T-cell lymphotropic virus type I (HTLV-I)-induced syncytium formation in vitro: role of cell-to-cell contact

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Summary

Cell-to-cell contact is usually essential for syncytium formation by HTLV-I-infected cell lines. The present study was undertaken to determine the inhibitory effect of polyanionic compounds, dextran sulfate and heparin, on HTLV-I-induced syncytium formation, as demonstrated by the fusion of HTLV-I-infected cells with target cells. These two compounds almost completely blocked syncytium formation in the early phase of the reaction at a concentration of 125 μg/ml, but dextran, as a control, did not inhibit it at concentrations up to 625 μg/ml. 50% inhibition of syncytium formation was detected at a concentration of 2 μg/ml of dextran sulfate 5000, 3 μg/ml of dextran sulfate 8000 and 8 μg/ml of heparin. The binding of radiolabeled HTLV-I-infected cells (HCT-1) to the target cells was inhibited by addition of dextran sulfate and heparin, and the inhibitory effects were concentration-dependent. No marked changes were detected in the expression of adhesion molecules on the virus-infected cells and target cells, and in the expression of envelope proteins on the virus-infected cells after exposing them to the polyanionic compounds. These results suggest that the blocking of cell-to-cell contact by polyanionic compounds, probably independent of surface adhesion molecules, is important for their inhibitory effect on HTLV-I-induced syncytium formation.

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Human T-cell lymphotropic virus type I (HTLV-I); Syncytium formation; Dextran sulfate; Heparin; Cell fusion; Adhesion

Introduction

It has recently been reported that the sulfated polysaccharides dextran sulfate and heparin are efficient inhibitors of human immunodeficiency virus type 1 (HIV-1) replication in vitro (Ueno and Kuno, 1987; Ito et al., 1987; Baba et al., 1988a, 1988b; Mitsuya et al., 1988). These polyanionic compounds are thought to inhibit HIV-1 replication mainly by preventing the attachment of the virion to the receptor molecule (CD4) (Baba et al., 1988a; Mitsuya et al., 1988; Schols et al., 1989).

HTLV-I is also one of the human C-type retroviruses like HIV-1, and is causally related to two different kinds of diseases, adult T-cell leukemia/lymphoma (ATLL) (Poiesz et al., 1980; Yoshida et al., 1982) and chronic myelopathy in south western Japan (HTLV-I-associated myelopathy:HAM) (Osame et al., 1986) and in the tropics (Tropical spastic paraparesis:TSP) (Gessain et al., 1985; Rodgers-Johnson et al., 1985). Recently, several clinical syndromes associated with HTLV-I infection involving the joints, respiratory tract, and muscles were reported as chronic inflammatory arthropathy associated with HTLV-I (Nishioka et al., 1989; Iwakura et al., 1991; Sakai et al., 1993), HTLV-I-associated bronchopneumonopathy (Maruyama et al., 1988), and HTLV-I polymyositis (Wiley et al., 1989). The manner of transmission of this virus is not fully clarified, but clearly different from HIV-1. HTLV-I transmission has proved successful when co-cultivation of target cells with HTLV-I-producing cells is employed (Miyoshi et al., 1981; Yamamoto et al., 1982; Popovic et al., 1983), while the transmission of HIV-1 can occur by cell-free infection. Co-cultivation also induces syncytium formation of some target cell lines, such as human osteosarcoma cell line (HOS), rat sarcoma cell line (XC) (Nagy et al., 1983; Weiss et al., 1985). Viral envelope proteins on the HTLV-I-infected cell surface were shown to play a critical role in HTLV-I-induced syncytium formation – anti-HTLV-I envelope antibodies from HTLV-I-infected individuals block the HTLV-I-induced syncytium formation - (Nagy et al., 1983; Hoshino et al., 1983; Kiyokawa et al., 1984).

In this study, we show that the fusion of HTLV-I-infected cells and target cells contribute to syncytium formation, and that a syncytium formation assay could be a model of HTLV-I transmission in vitro. Furthermore, we tested the effects of dextran sulfate and heparin on HTLV-I-induced syncytium formation in vitro and found that they are inhibitory, especially in the early phase of the phenomenon.

Materials and Methods

Cell lines. HCT-1, an interleukin 2-dependent, OKT4-positive T cell line established from the cerebrospinal fluid obtained from a patient with HTLV-I-associated myelopathy (Nakamura et al., 1989), was grown in RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum (FBS, GIBCO, Grand Island, NY), antibiotics (100 units of penicillin per ml and 100 μg of streptomycin per ml), glutamic acid (GIBCO) and 100 units/ml IL-2 (Shionogi, Osaka, Japan). XC, rat sarcoma cell line with the Rous sarcoma virus was obtained from the American Type Culture Collection. The XC cell line was cultured in plastic plates (Falcon 3003, Becton Dickinson, CA) in RPMI 1640 medium containing 10% FBS and antibiotics.

Sera. Sera were obtained from two patients with HAM and one healthy subject. Sera from two patients with ATLL were provided by Dr. Amenomori (Atomic Disease Institute, Nagasaki University, Nagasaki, Japan). These sera were tested for the presence of anti-HTLV-I antibodies by an enzyme-linked immunosorbent assay (ELISA; Eitest-ATL kit, Eisai, Tokyo, Japan), a particle agglutination assay (Serodia-ATL kit, Fujirebio, Tokyo, Japan), and by Western blot analysis using HTLV-I antigens derived from the MT-2 cell line.

Compounds. Dextran (molecular weight, MW, approximately 10200), dextran sulfate (MW approximately 5000 or 8000), sodium heparin (refined from porcine intestinal mucosa) and polybrene were purchased from Sigma Chemical Company (St. Louis, USA).

Monoclonal antibodies. Monoclonal antibodies reactive with human very late antigen 4, VLA-4 (8F2), VLA-5 (2H6), and CD29 (4B4) were kindly provided by Dr. Morimoto (Dana-Faber Cancer Institute, Boston, MA). Monoclonal antibodies to lymphocyte function-associated antigen 1α, LFA-1α (CD11a), LFA-1β (CD18), and intercellular adhesion molecule 1, ICAM-1 (CD54), were purchased from Cosmo Bio Company (Tokyo, Japan), Leu-44 (CD44) from Becton Dickinson Immunocytometry Systems (San Jose, CA), and T11 (CD2) from Coulter Immunology (Hialeah, FL), and HTLV-I env (reactive with HTLV-I envelope protein, gp46 and envelope precursor, gp63) from EPITOPE (Beavenon, OR). Affinity isolated goat Fab'_2 anti-mouse immunoglobulins, gamma and light chains, human Ig adsorbed fluorescein conjugate was purchased from TAGO (Burlingame, CA). Mouse anti-rat monoclonal antibodies; anti-ICAM-1 (1A29), anti-LFA-1α (WT.1), and anti-LFA-1β (WT.3) were purchased from Seikagaku Corporation (Tokyo, Japan).

Immunostaining. Immunohistochemical staining was done by the avidin-biotin-immunoperoxidase method and the indirect immunofluorescent method. The cells were cultured in chamber slides (Nunc, North Aurovo Road, Naperville, IL). After incubation, the cells were fixed with 4%

paraformaldehyde (Wako, Osaka, Japan) solution for 10 min at 4°C. The cells were stained by the labeled-streptavidin biotin method using HISTOFINE staining kit (Nichirei, Tokyo, Japan). After blocking with non-immune goat IgG, the cells were stained with the following: mouse anti-human monoclonal antibodies; anti-human HLA-DR (Becton Dickinson, San Jose, CA), GIN 14 (reactive with HTLV-I core protein p19 and p28, Fujirebio), or mouse anti-rat monoclonal antibody; anti-ICAM-1 (1A29) for 1 h in room temperature, followed by biotinylated goat anti-mouse IgG, and then horseradish peroxidase (HRP)-labeled streptavidin. The HRP sites were visualized by using 3,3'-diamino-benzidine as the hydrogen donor. These cells were counterstained with hematoxylin. For control staining, mouse anti-human insulin antibody (Nichirei) was used.

Syncytium formation assay. XC cells were harvested by trypsin EDTA, washed three times by phosphate-buffered saline (PBS) and resuspended in RPMI 1640 with 10% FBS and antibiotics at a concentration of 1×10^5 cells/ml. XC cells were incubated in 24-well flat-bottomed plates at a final volume of 1 ml/well. After 24 h, the medium was removed and 1×10^5 cells/ml HCT-1 cells in RPMI 1640 with 10% FBS and antibiotics were added to each well at a final volume of 1 ml/well. After 16 h, the medium was removed from the XC cells and the cells were washed once by PBS and stained with methanol including 0.5% methylene blue and 0.125% basic fuchsin. Syncytia were counted in three fields at random by phase inverted microscope (50 ×) and the sum of three fields scored as syncytium numbers. In other experiments, XC cells were cultured with HCT-1 cells in a Millicell (Millipore Products Division, Bedford, MA) equipped with the transparent, 0.4 μm pore membrane. In this system, XC cells were cultured without contact in HCT-1 cells, while having the same medium.

Syncytium inhibition assay. When HCT-1 cells were added to XC cells that were incubated previously, several concentrations of compounds were added to each well at a final volume of 1 ml/well at the same time. After 16 h, medium was removed, washed once by PBS and stained.

Cell viability test. XC cells were incubated in 24-well flat-bottomed plates at a concentration of 1×10^5 cells/well. After 24 h, the medium was removed and RPMI 1640 containing 10% FBS and antibiotics with or without several concentrations of compounds was added to each well at a final volume of 1 ml/well. After 16 h, the number of viable cells was determined microscopically in an hemacytometer by trypan blue exclusion. HCT-1 cells were incubated in 24-well flat-bottomed plates at a concentration of 1×10^5 cells/well in the same medium and the number of viable cells was determined after 16 h.

Adhesion assay. We next examined the adhesion of HCT-1 cells to XC cells. The assay methods have been described elsewhere (Kawakami et al., 1991;

Ichinose et al., 1992). Briefly, XC cells (2×10^4/well) were cultured in quadruplicate in 96-well flat-bottom microtiter plates for 24 h. HCT-1 cells were radiolabeled with $Na_2{}^{51}CrO_4$ (Amersham International, Amersham, UK) at 37°C for 1.5 h, with occasional shaking, then washed 3 times with RPMI 1640 containing 5% FBS, and resuspended at 5×10^5/ml in RPMI 1640 supplemented with 10% FBS. Chromium-labeled HCT-1 cells (1×10^5/0.2 ml) were dispensed into the XC cell monolayer, and the mixture was incubated for 2 h at 37°C in a humidified atmosphere of 5% CO_2 in air. To remove the nonattached HCT-1 cells, the microtiter plates were washed 5 times. The adherent HCT-1 cells were lysed by the addition of 200 μl of 1% Triton-X (Wako, Osaka, Japan). 100 μl of each supernatant was harvested after centrifugation. The residual radioactivity was measured by a gamma counter.

Analysis of adhesion molecules or envelope proteins by a flow cytometer. HCT-1 cells and XC cells were harvested and separated to fisher tube at a concentration of 5×10^5 cells/ml. Each tube was centrifuged once and 5 μl of human normal immunoglobulin (Midori Jyuji, Osaka, Japan) were added to the pellet for blocking Fc receptor on cells and incubated at 37°C for 20 min. Each tube was washed twice by PBS containing 1% FBS and each monoclonal antibody was added and incubated at 4°C for 30 min (class matched mouse IgG was added to the control tube.). After two washes, FITC labeled goat anti-mouse IgG was added to each tube and incubated at 4°C for 30 min. Each tube of HCT-1 cells and XC cells was analyzed by a flow cytometer (FACScan, Becton Dickinson) after washing twice.

Statistical analysis. Statistical analysis was performed using Student's *t*-test.

Results

Time-course of HTLV-I syncytium formation

After XC cells and HCT-1 cells were co-cultivated, a few syncytia appeared after 2 h and the number of syncytia increased until 8 h (Fig. 1). The time-course of syncytia is shown in Fig. 2. The number of syncytia reached a plateau after 8 h.

Sera from HAM and ATLL patients inhibit HTLV-I syncytium formation

The titer of anti-HTLV-I antibodies including the antibodies against envelope proteins were analyzed by the particle agglutination assay. Two sera from HAM patients had a titer of 2^{15}, two sera from ATLL patients had a titer of 2^{10} and 2^8, respectively. Two sera from HAM patients completely inhibited HTLV-I-induced syncytium formation at a 1:80 dilution and two sera from ATLL patients completely inhibited it at a 1:20 and 1:10 dilution, respectively, while serum from healthy subject had no inhibitory effect (data not shown).

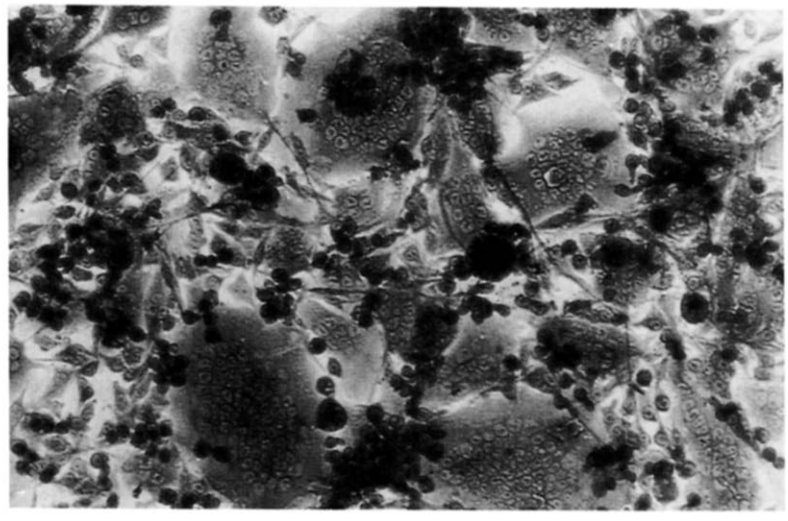


Fig. 1. Syncytia at 8 h after co-cultivation. This photograph shows the stained syncytia at 8 h, observed by phase inverted microscope (at original magnification × 50).

This result strongly suggest that HTLV-I itself is required for this syncytium formation.

Necessity of cell contact for HTLV-I syncytium formation

Instead of HCT-1 cells, we used 100%, 50% or 10% supernatant of HCT-1 cells culture medium containing HTLV-I particles, or HCT-1 cells that were included in Millicell device for blocking contact between the two cells. The syncytia were not formed by co-cultivation of target cells with virion or HTLV-I-infected cells that were separated from them by the device (data not shown). This study demonstrated that cell-to-cell contact was needed for HTLV-I-induced syncytium formation.

Immunohistochemical analysis of syncytia

Syncytia were stained by the avidin-biotin-immunoperoxidase method. Syncytia reacted positively with monoclonal antibodies against HTLV-I gag proteins (GIN 14) (Fig. 3a) and human class II molecules (anti-DR) (Fig. 3b), and with monoclonal antibodies against rat ICAM-1 molecules (Fig. 3c). Before co-cultivation, HTLV-I gag proteins and class II molecules were present in HTLV-I-infected cells (HCT-1) but not in target cells (XC), and rat ICAM-1 molecules were expressed on XC cells but not on HCT-1 cells. As these three molecules were expressed in syncytia, we conclude that syncytia were formed by fusion of both cells.

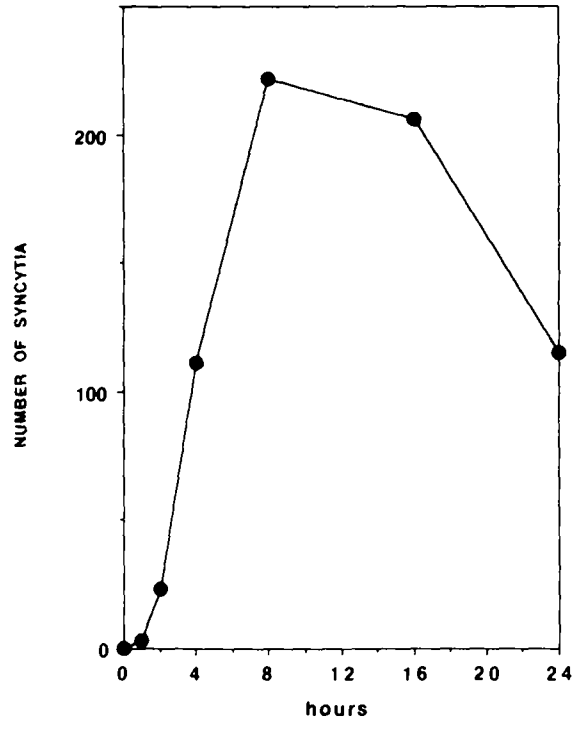


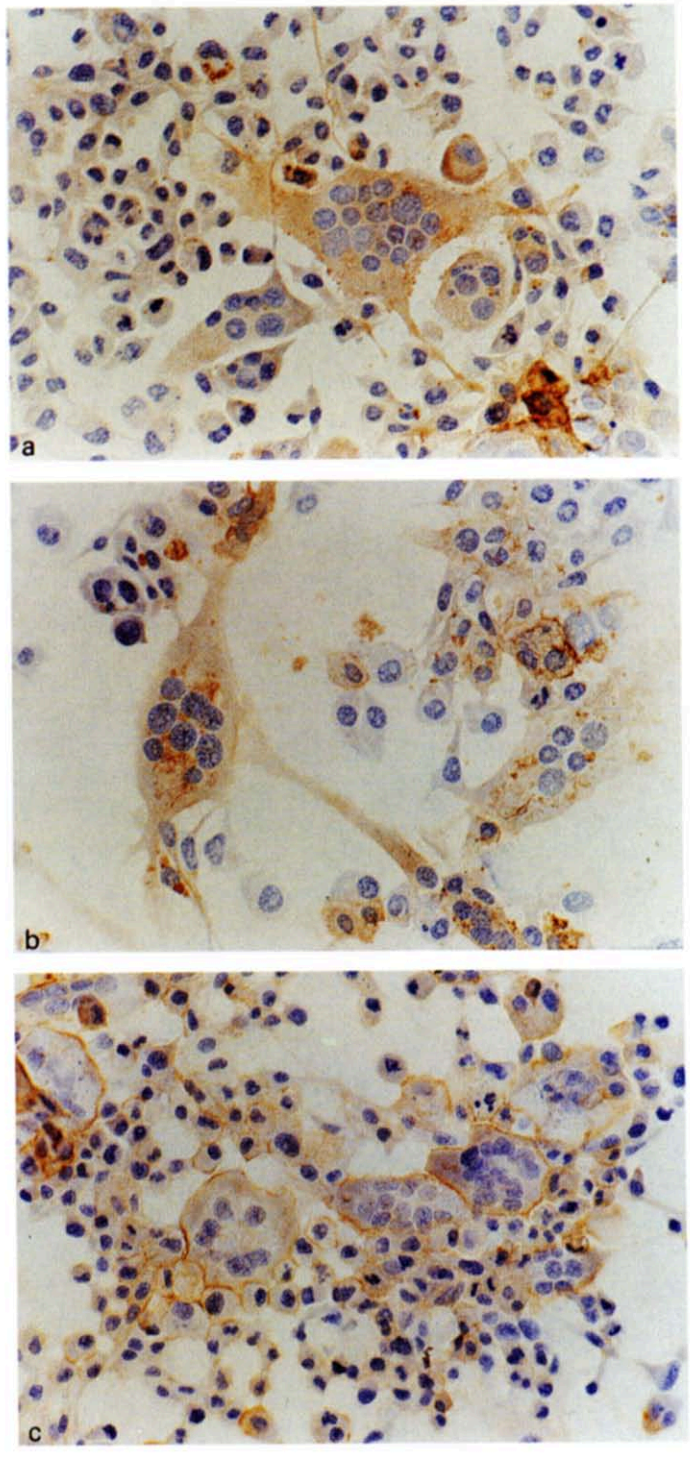
Fig. 2. Time-course of syncytium formation. After HCT-1 cells were added to XC adherent cells, syncytia were fixed and stained at 1 h, 2 h, 4 h, 8 h, 16 h, 24 h and the number of syncytia was counted by phase-inverted microscope.

Anti-HTLV-I activity by dextran sulfate and heparin

We evaluated the inhibitory effect of dextran sulfate and heparin by using this HTLV-I-induced syncytium formation system. Dextran sulfate (MW 5000 or 8000) and heparin inhibited syncytium formation at the concentrations above 1 μg/ml, and 5 μg/ml, respectively, and almost completely blocked it at 125 μg/ml (Fig. 4). On the other hand, dextran (MW 10200) did not inhibit syncytium formation at concentrations up to 625 μg/ml. Cytotoxicity of the compounds for HCT-1 cells or XC cells was measured by trypan blue exclusion. No cytotoxicity was noted at concentration up to 625 μg/ml dextran sulfate 5000 or 8000, and 625 μg/ml heparin in both cells (data not shown). Fig. 4 shows that dextran sulfate 5000 or 8000 and heparin inhibited syncytium formation at a concentration of 1 μg/ml and up.

Inhibitory effect of dextran sulfate and heparin at an early phase of HTLV-I syncytium formation

To find out at what phase dextran sulfate and heparin interact with syncytium formation, we added them to each well at different times. When XC


a
b
c

←
Fig. 3. Immunohistochemical staining of syncytia. Syncytia reacted positively with monoclonal antibodies against HTLV-I gag proteins (a) and human class II molecules (b) and with monoclonal antibodies against rat ICAM-1 molecules (c). Original magnification: × 100.

cells and HCT-1 cells were mixed for co-cultivation, we added the compound to the first well (Time 0). After 30 min, 1 h, 2 h, 3 h, 6 h, and 12 h of co-cultivation, the compound was added to each successive well. Each well was stained after 16 h. The inhibitory effect of dextran sulfate 5000 appeared in the wells that contained each compound during the first 2 h of co-cultivation, whereas this substance did not inhibit syncytium formation when it was added to the well after 2 h of co-cultivation (data not shown). This indicates that dextran sulfate and heparin have an effect only at an early phase of HTLV-I syncytium formation.

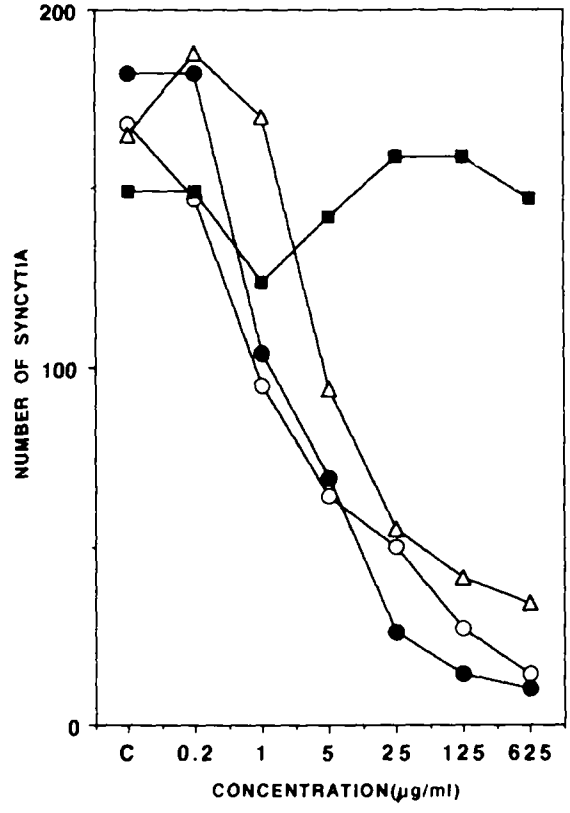


Fig. 4. Anti-HTLV-I activity of dextran sulfate and heparin. When HCT-1 cells were added to XC adherent cells, several concentrations of compounds, dextran sulfate 5000 (●), 8000 (○), heparin (△) and dextran 10 200 (■), were added to each well at the same time. After 16 h of co-cultivation, syncytia were fixed and stained.

Inhibition of cell-to-cell adhesion by dextran sulfate and heparin

In attempt to determine the mechanism of action of dextran sulfate and heparin on HTLV-I-induced syncytium formation, cell adhesion was examined. Chromium-labeled HCT-1 cells (HTLV-I-infected cells) were incubated with adherent XC cells for 2 h. After washing 5 times, residual radioactivity of HCT-1 cells bound to XC cells were counted in a gamma counter. About 20 to 30% of radiolabeled HCT-1 cells bound to XC cells in the absence of these compounds. Dextran sulfate 5000 and heparin inhibited the binding between the two cells at a concentration of 25 μg/ml and 5 μg/ml, respectively. As the concentration of the two compounds was increased, binding of the two cells was decreased in a concentration-dependent manner. However, dextran had no

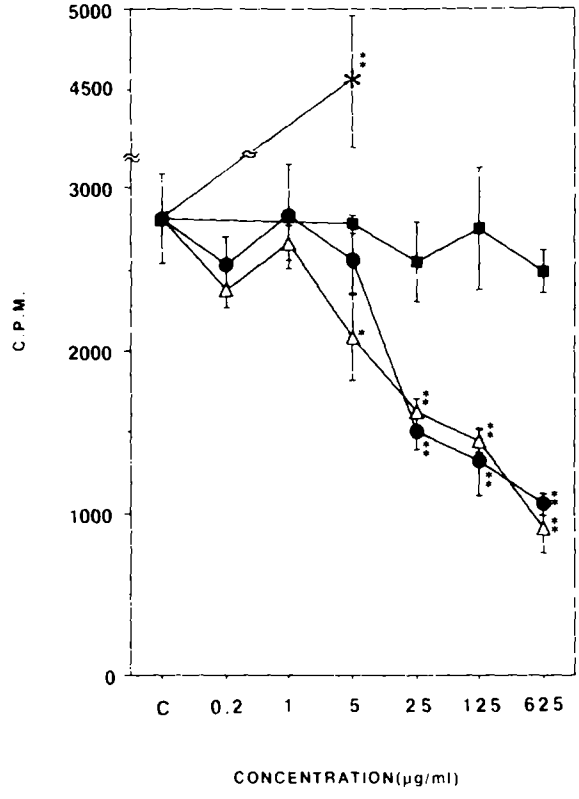


Fig. 5. Inhibition of cell-to-cell adhesion by dextran sulfate and heparin. XC cells (2×10^4/well) were cultured in 96-well flat-bottom microtiter plates for 24 h. Chromium-labeled HCT-1 cells (1×10^5/0.2 ml) were dispensed into the XC cell monolayer, and the mixture was incubated in the absence or presence of substances, dextran sulfate 5000 (●), heparin (△), dextran 10200 (■), and polybrene (*) for 2 h. To remove the nonattached HCT-1 cells, the plates were washed 5 times. The adherent HCT-1 cells were lysed by the addition of 1% Triton-X. Each supernatant was harvested after centrifugation. The residual radioactivity was measured by a gamma counter. One representative experiment out of three is shown. The data represent the means of quadruplicate cultures ± S.D. Statistical analysis is based on P values ($^{*}P<0.05$, $^{**}P<0.01$).

inhibitory effect and polybrene augmented adhesion (Fig. 5).

Influence of expression of adhesion molecules on HTLV-I producing cells by dextran sulfate and heparin

As dextran sulfate and heparin inhibited adhesion between HTLV-I-infected cells and target cells, we analyzed the influence of these two compounds on expression of adhesion molecules on the two cells. Before treatment, HCT-1 cells expressed several adhesion molecules, such as LFA-1α, LFA-1β, VLA-4, VLA-5, CD29, ICAM-1, CD44 and CD2, whereas XC cells expressed ICAM-1 molecules. After treatment for 2 h, we examined the changes of expression of adhesion molecules on both cells by a flow cytometer. Dextran sulfate 5000 (250 μg/ml) and heparin (250 μg/ml) did not alter the expression of adhesion molecules on both cells, nor did dextran (250 μg/ml) or polybrene (5 μg/ml) (data not shown).

Influence of expression of envelope proteins on HTLV-I producing cells by dextran sulfate and heparin

Envelope protein is known to be essential for HTLV-I-induced syncytium formation. If envelope protein is masked by dextran sulfate or heparin as a consequence of binding between envelope protein and these two compounds, syncytium formation should be inhibited. To assess these possibility, we analyzed the influence of these two compounds on expression of envelope proteins on HCT-1 cells by a flow cytometer. HCT-1 cells expressed envelope protein, gp46 on the surface. After treatment for 2 h by dextran sulfate 5000 (250 μg/ml) or heparin (250 μg/ml), the expression of gp46 was not changed (data not shown). These results ascertain that the inhibitory effects of the two compounds on syncytium formation is not caused by the decrement of envelope proteins on HTLV-I-producing cells.

Discussion

The cell-to-cell interaction between HTLV-I-infected cells and certain target cells leads to syncytium formation in vitro (Nagy et al., 1983; Hoshino et al., 1983; Hayami et al., 1984). Also a close cell-to-cell interaction between HTLV-I-producing cells and target cells is usually essential for transmission of the virus in vitro and in vivo (Miyoshi et al., 1981; Yamamoto et al., 1982; Popovic et al., 1983), although rare cases of persistent infection by cell-free preparations of HTLV-I have been reported (Clapham et al., 1983; Hoxie et al., 1984). It is not exactly known whether these two phenomena are based on the same mechanism. However, there are conditions common to these two phenomena, i.e, only HTLV-I-infected cells that actually release the virus can induce these two phenomena and anti-envelope protein antibodies can inhibit them (Nagy et al., 1983; Kiyokawa et al., 1984; Weiss et al., 1985). Natural transmission of the virus is believed to depend on the transfer of infected cells and subsequent

fusion with receptor-bearing cells in a new host. The nuclei of the HTLV-I-induced syncytia were homogeneous and appeared to be derived mainly from the indicator cells.

In the present study, we detected both the human class II MHC (HLA-DR) gene products and the viral structural gene products derived from virus-infected cells in the syncytia as well as rat ICAM-1 molecules expressed on the indicator cells. We conclude that the fusion of the virus-infected cells with indicator rat XC cells contributed to syncytium formation in this system and that the syncytium induction assay, used in this study, could be served as an in vitro model of HTLV-I transmission.

HTLV-I-producing cells can induce syncytia in many cell types derived from diverse mammalian species (Nagy et al., 1983; Weiss et al., 1985). Although a cell surface receptor on these cells for the virus has not been identified, the viral envelope glycoproteins expressed on HTLV-I-infected cells are primarily responsible for the binding to the target cells and subsequent syncytium formation (Kiyokawa et al., 1984; Weiss et al., 1985). The precursor envelope protein, gp61, is proteolytically cleaved into gp46 and gp21 and expressed on the cell surface (Hattori et al., 1984; Lee et al., 1984; Schneider et al., 1984). Pique et al. (1990) induced 33 random mutations along the HTLV-I envelope gene and observed that the mutations affecting cleavage of gp61 completely abrogated syncytium formation. In this virus, as well as other retroviruses, cleavage of the immature glycoprotein is required for the exposition of its fusion domain, probably the N-terminal hydrophobic region of gp21 (Nagy et al., 1984). The other major external envelope glycoprotein gp46, like gp120 of HIV-1, is considered to be the viral cell attachment protein and the neutralizing antibodies in HTLV-I-infected individuals are directed against it. Tanaka et al. (1991) made monoclonal antibodies against gp46 in rat and revealed that one of them (LAT-27 clone), that reacted with HTLV-I-envelope peptide 190-199, could neutralize the infectivity of the virus. In the previous studies, we synthesized nine peptides, including peptide 190-199, of HTLV-I envelope proteins and analyzed epitopes recognized by T-cells and antibodies in patients' sera (Kurata et al., 1989; Ida et al., 1991). We also tested inhibitory activities of these envelope peptides on the HTLV-I-induced syncytium formation to determine the regions of envelope protein responsible for the attachment to target cells. None of nine peptides, including the immunodominant T-cell sites and B-cell sites identified in our previous studies, inhibited the HTLV-I-induced syncytium formation (data not shown). Thus, we could not identify cell attachment regions of the envelope proteins in this study.

The sulfated polysaccharides dextran sulfate and heparin have proved to be potent inhibitors of human immunodeficiency virus type 1 (HIV-1) in vitro (Ueno and Kuno, 1987; Ito et al., 1987; Baba et al., 1988a, 1988b; Mitsuya et al., 1988). Although these agents have been shown in some studies to inhibit binding of HIV-1 to CD4 molecules on target cells (Mitsuya et al., 1988; Schols et al., 1989), there is some controversy (Lederman et al., 1989; Parish et al., 1990; Callahan et al., 1991) as to their exact mode of action. We investigated

their inhibitory effects on HTLV-I, using an HTLV-I-induced syncytium formation assay. Dextran sulfate and heparin inhibited syncytium formation, particularly when the compounds were added in the early phase of the assay. These results suggest that the compounds probably inhibit the attachment of the viral protein of the infected cells to the (as yet unidentified) receptors, on the indicator cells. To investigate this possibility further, we tested the inhibitory effect of these compounds on cell-to-cell attachment. As shown in Fig. 5, attachment of radiolabeled HTLV-I-infected cells to target cell was efficiently inhibited by these two compounds, while polybrene, an activator of viral replication, enhanced the attachment. Although the mechanism of viral transmission is different between HTLV-I and HIV-1, the polyanionic compounds, dextran sulfate and heparin, were effective in suppressing transmission of both viruses in vitro.

Syncytium formation induced by HIV-1-infected cells is inhibited by monoclonal antibodies against CD4, the HIV-1 receptor (Dalgleish et al., 1984; Klatzmann et al., 1984), and also by monoclonal antibodies against LFA-1 or CD18, the common subunit of LFA-1 and other leukocyte cell adhesion molecules (Hildreth and Orentas, 1989; Valentin et al., 1990). The HTLV-I-infected HCT-1 cell line, used as inducer of syncytia in this study, is an activated T-cell line and expresses several adhesion molecules, such as LFA-1α, LFA-1β, VLA-4, VLA-5, CD29, ICAM-1, CD44 and CD2 on its surface. We tested the effects of the polyanionic compounds on the expression of cell adhesion molecules on HCT-1 cells and XC cells by an indirect immunofluorescent technique and a flow cytometry. No marked changes in the expression of these adhesion molecules were detected (data not shown). Furthermore, we analyzed the inhibitory effects of mouse monoclonal antibodies against human LFA-1α, LFA-1β, and CD29 (4B4) and mouse monoclonal antibodies against rat ICAM-1 on syncytium formation (data not shown). No inhibitory effect was observed in this system, although some roles of the adhesion molecules, LFA-1α, LFA-1β, and ICAM-1, has been shown in other HTLV-I-induced syncytium assays (Fukudome et al., 1992).

In this study, we showed that the HTLV-I-induced syncytia included the fusion of the virus infected cells with target cells and a syncytium formation assay could be a good tool for analyzing the mechanism of HTLV-I transmission. Two polyanionic compounds, dextran sulfate and heparin, were shown to inhibit HTLV-I-induced syncytium formation in the early phase of the reaction, probably the process of cell attachment. These compounds were suggested by De Clercq as potential anti-HIV-1 agents because of their putative effect on the virus adsorption process (De Clercq, 1986; Ito et al., 1987; Baba et al., 1988a, 1988b, 1990; Schols et al., 1989).

Unfortunately, following oral administration, extremely high doses of dextran sulfate and heparin might be needed for reaching the concentrations at which these compounds inhibit HTLV-I-induced syncytium formation in vivo (Abrams et al., 1989; Lorentsen et al., 1989; Dryjski et al., 1989; Hartman et al., 1990; Flexner et al., 1991). There is no evidence that HTLV-I-induced

syncytium formation plays an important role in the progression of HTLV-I-related diseases. However, our present study indicate that these polyanionic compounds may be positive candidates for protecting HTLV-I transmission.

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