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α-(1-3)- and α-(1-6)-D-Mannose-Specific Plant Lectins Are Markedly Inhibitory to Human Immunodeficiency Virus and Cytomegalovirus Infections In Vitro

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The α-(1-3)-D-mannose- and α-(1-6)-D-mannose-specific agglutinins (lectins) from *Galanthus nivalis*, *Hippeastrum hybrid*, *Narcissus pseudonarcissus*, and *Listera ovata* inhibited infection of MT-4 cells by human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) and simian immunodeficiency virus at concentrations comparable to the concentrations at which dextran sulfate (molecular weight, 5,000 [DS-5000]) inhibits these viruses (50% effective concentration, 0.2 to 0.6 μg/ml). Unlike DS-5000, however, the plant lectins did not inhibit the replication of other enveloped viruses, except for human cytomegalovirus (50% effective concentration, 0.9 to 1.6 μg/ml). The plant lectins suppressed syncytium formation between persistently HIV-1- or HIV-2-infected HUT-78 cells and uninfected MOLT-4 (clone 8) cells at concentrations that were 5- to 10-fold lower than that required for DS-5000. Unlike DS-5000, however, the plant lectins did not inhibit HIV-1 binding to CD4$^+$ cells. Combination of the plant lectins with DS-5000 led to a potent synergistic inhibition of HIV-1-induced cytopathogenicity in MT-4 cells and syncytium formation between HIV-infected HUT-78 cells and MOLT-4 cells. Our data suggest that α-(1-3)-D- and α-(1-6)-D-mannose-specific plant lectins interfere with an event in the HIV replicative cycle that is subsequent to the attachment of the virions to the cells (i.e., the fusion process).

Human immunodeficiency virus (HIV) is tropic for T lymphocytes, monocytes and macrophages, and other cell types that express the differentiation antigen CD4 (OKT4A/Leu-3a) on the cell surface (8, 14). Both the binding of HIV to its target cells and the fusion (syncytium formation) between HIV-infected and noninfected T4 cells require a specific interaction between the viral envelope glycoprotein gp120 and the cell surface CD4 molecule (8, 16, 18). In particular, the carbohydrate portion of gp120 is involved in the gp120-CD4 interaction (15, 17). Compounds that interact with either CD4 or gp120 should be able to block virus adsorption, virus-mediated cell fusion, infection, and cytopathogenicity of the virus.

Various substances have been shown to inhibit HIV infection through inhibition of the binding of HIV to its target cell. Among the class of anionic compounds, the carboxylated triphenylmethane dye aurintricarboxylic acid and sulfonated compounds such as suramin and Evans blue have proved effective in inhibiting HIV replication (3, 4, 20) and HIV binding to the cells (24, 25). For the class of sulfated polysaccharides (i.e., heparin, dextran sulfate, and pentosan polysulfate), the inhibitory effect of HIV replication can be attributed solely to inhibition of virus adsorption (1, 19, 24). Sulfated polysaccharides interact specifically with the viral gp120 (26).

Another class of compounds that interferes with HIV gp120 is D-mannose-specific lectins (12, 13, 15, 21, 23). Using a panel of lectins with different binding specificities, Lifson and coworkers (15) revealed that mannose-containing moieties of the gp120 molecules must be involved in the interactions with the cellular CD4 receptor (15). Also, Müller et al. (21) reported that the D-mannose-specific lectin from *Gerardia savaglia* inhibits HIV-1 infection of H9 cells and the associated syncytium formation via binding of the lectin to the carbohydrate moieties of gp120 (21). Later, Hansen et al. (13) reported that blockage of at least four different glycan structures present on gp120 inhibit CD4-gp120-induced cell fusion (13). In the present study we demonstrated that D-mannose-specific plant lectins achieve their anti-HIV activity by a mechanism that is clearly different from that of the sulfated polysaccharides.

MATERIALS AND METHODS

Compounds. The following lectins were used in our studies: *Galanthus nivalis* agglutinin (GNA) (snowdrop; family *Amaryllidaceae*), *Hippeastrum hybrid* agglutinin (HHA) (amaryllis; family *Amaryllidaceae*), *Narcissus pseudonarcissus* agglutinin (NPA) (daffodil; family *Amaryllidaceae*), and *Listera ovata* agglutinin (LOA) (twayblade; family *Orchidaceae*). GNA, HHA, and NPA were isolated from bulbs of snowdrop, amaryllis, and daffodil, respectively, as described by Van Damme and colleagues (27, 28, 30, 31). LOA was isolated from leaves of twayblade essentially as described previously (29). Purified preparations of the lectins were obtained by affinity chromatography with mannose immobilized on Sepharose 4B, hydrophobic interaction chromatography on phenyl Sepharose, and ion-exchange chromatography on Q fast flow (Pharmacia-LKB, Uppsala, Sweden). GNA and LOA have high affinities for α-(1-3)-D-mannose oligomers, NPA has a high affinity for α-(1-6)-D-mannose oligomers, and HHA has a high affinity for both α-(1-3)- and α-(1-6)-D-mannose oligomers. Dextran sulfate (molecular weight, 5,000 [DS-5000] or 10,000 [DS-10000]),

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pentosan polysulfate, and 3'-azido-2',3'-dideoxythymidine were obtained from Sigma Chemical Co. (St. Louis, Mo.), and 9-(1,3-dihydroxy-2-propoxymethyl)guanine was from Syntex (Palo Alto, Calif.). 2',3'-Dideoxyinosine was a gift from J. C. Martin (Bristol Myers, Wallingford, Conn.).

Viruses. HIV type 1 (HIV-1) (strain HTLV-III_B) was originally obtained from the culture supernatant of the persistently HIV-infected H9 cell line (H9/HTLV-III_B) (22) and was kindly provided by R. C. Gallo (National Cancer Institute, Bethesda, Md.). Virus stocks were prepared from the supernatants of HIV-1-infected MT-4 cells. HIV type 2 (HIV-2) (strain LAV-2) (7) was a gift from L. Montagnier (Pasteur Institute, Paris, France), and virus stocks were prepared from the supernatants of HIV-2-infected MT-4 cells. Simian immunodeficiency virus (SIV) (strain SIV_{Mac251}) was originally isolated by Daniel et al. (9) and was obtained from C. Bruck (Smith Kline-Rit, Rixensart, Belgium). Moloney murine sarcoma virus (MSV) was prepared from tumors induced following intramuscular inoculation of 3-day-old NMRI mice with MSV, as described previously (11). Simian AIDS-related virus (SRV) was provided by L. J. Lewenstine (California Primate Center, Davis) and was harvested from the culture supernatant of SRV-infected Raji cells. Human cytomegalovirus (CMV) (strains AD169 and Davis) was obtained from the American Type Culture Collection (Rockville, Md.) and was kindly provided by S. Michelson (Pasteur Institute). The origins of the other viruses (i.e., herpes simplex virus types 1 and 2, vaccinia virus, vesicular stomatitis virus, poliovirus type 1, coxsackievirus type B4, Sindbis virus, parainfluenzavirus type 3, Semliki forest virus, and reovirus type 1) have been described elsewhere (10).

Antiretrovirus assays. The methodology of the anti-HIV assays has been described previously (2). Briefly, MT-4 cells (4.5×10^5 cells per ml) were suspended in fresh culture medium and infected with HIV-1, HIV-2, or SIV at 100 $CCID_{50}$ (1 $CCID_{50}$ being the dose infective for 50% of the cell cultures) per ml of cell suspension. Then, 100 μl of the infected cell suspension was transferred to microplate wells, mixed with 100 μl of the appropriate dilutions of the test compounds (i.e., final concentrations of 200, 40, 8, 1.6, 0.32, and 0.062 μg/ml), and further incubated at 37°C. In the experiments in which the plant lectin LOA was combined with DS-5000, 2',3'-dideoxyinosine, or the plant lectin HHA or in which DS-5000 was combined with pentosan polysulfate, 50 μl of an appropriate concentration of either test compound was added to the infected cell suspension. After 5 days, the number of viable cells was determined in a blood cell-counting chamber by trypan blue staining for both virus-infected and mock-infected cell cultures. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration corresponded to the compound concentrations required to reduce by 50% the number of viable cells in the virus-infected and mock-infected cell cultures, respectively.

The effects of the test compounds on SRV-induced giant cell formation in Raji cells, HIV-1-induced giant cell formation in human T-lymphocyte CEM cells, and MSV-induced transformation of murine C3H/3T3 cells were examined on days 12, 4, and 6 postinfection, respectively, as described previously (5, 6).

Anti-CMV assay. Confluent human embryonic lung cells were infected with human CMV (strain AD169 or Davis). The virus was diluted in cell culture medium containing 2% fetal bovine serum and different concentrations of the plant lectins. After a 2-h incubation period at 37°C, the virus was removed and fresh medium containing the different drug concentrations was added to the cells. After 7 days of incubation, a virus-induced cytopathic effect was monitored microscopically following ethanol fixation and Giemsa staining.

Antiviral assays. The antiviral assays, other than antiretrovirus and anti-CMV assays, were based on an inhibition of virus-induced cytopathogenicity in HeLa cells (for vesicular stomatitis virus, poliovirus type 1, and coxsackievirus), Vero cells (for Sindbis virus, parainfluenzavirus type 3, Semliki forest virus, coxsackievirus type B4, and reovirus type 1), or primary rabbit kidney cells (for HSV-1, HSV-2, vaccinia virus, and vesicular stomatitis virus) following previously established procedures (10).

Cocultivation assays. Persistently HIV-1- or HIV-2-infected HUT-78 cells (designated HUT-78/HIV-1 and HUT-78/HIV-2, respectively) were washed to remove free virus from the culture medium, and 5×10^4 cells (50 μl) were transferred to 96-well microtiter plates (Sterilin). Then, 5×10^4 MOLT-4 (clone 8) cells (50 μl) and an appropriate concentration of test compound (100 μl) were added to each well. The mixed cells were cultured at 37°C in a CO_2-controlled atmosphere. The first syncytium arose after about 3 h of cocultivation. After 16 to 20 h, marked syncytium formation was noted, and syncytia were counted under a microscope.

Interference of plant lectins with binding of anti-CD4 (OKT4A) MAb to $CD4^+$ cells. The methodology to determine the effect of plant lectins on the binding of anti-CD4 (OKT4A) monoclonal antibody (MAb) on $CD4^+$ MT-4 cells has been described previously (1).

Interference of plant lectins with binding of anti-gp120 MAb to HUT-78/HIV-1 cells. HUT-78/HIV-1 cells (2×10^6/ml of RPMI 1640 culture medium) were washed and incubated with DS-5000 (25 μg/ml) or plant lectins (100 μg/ml) for 15 or 60 min. The cell cultures were then incubated with anti-gp120 MAb (NEA 9284) (E. I. du Pont de Nemours) for 45 min at 20°C, washed twice with cell culture medium, incubated with rabbit anti-mouse immunoglobulin G (IgG)–$F(ab')_2$–fluorescein isothiocyanate (Dako) for 45 min at 20°C, washed twice in phosphate-buffered saline, resuspended in 0.5 ml of 0.37% paraformaldehyde in phosphate-buffered saline, and analyzed with a fluorescence-activated cell sorter (FACSTAR; Becton-Dickinson).

Interference of plant lectins with binding of HIV-1 particles to $CD4^+$ cells. HIV-1 was incubated with appropriate concentrations of the plant lectins or DS-5000 for 20 s or 2 h prior to the addition of the virus suspension to MT-4 cells. The cell cultures were then incubated for 30 min at 37°C and washed twice with phosphate-buffered saline to remove unbound virus. Then, a high-titer polyclonal antibody derived from a patient with AIDS-related complex was added; and after another 30 min of incubation at room temperature, the cells were washed twice with phosphate-buffered saline, treated with fluorescein isothiocyanate-conjugated $F(ab')_2$ fragments of rabbit anti-human immunoglobulin antibody (Dako) for 30 min at room temperature, and assayed by fluorescence-activated cell sorter analysis.

Effect of delayed treatment of HIV-1-infected MT-4 cells or cocultures of HUT-78/HIV-1 cells with MOLT-4 cells with the test compounds. Test compounds (i.e., LOA and DS-5000) were added to HIV-infected MT-4 cells at the time of infection (0 h), or 45 min, 90 min, 2 h and 15 min, 4 h, or 8 h after infection. The viral input was 10,000 $CCID_{50}$/ml and the compound concentration was 20-fold the EC_{50} for inhibiting viral cytopathogenicity in MT-4 cells (i.e., 5 μg/ml). The number of viable cells was determined on day 4, and

TABLE 1. Antiretrovirus activities of plant lectins

Compound	EC_{50} (μg/ml)[a]					
	HIV-1 (MT-4)	HIV-2 (MT-4)	SIV (MT-4)	HIV (CEM)	SRV (Raji)	MSV (C3H/3T3)
GNA	0.4 ± 0.2	0.3 ± 0.2	0.8 ± 0.1	0.6 ± 0.2	100 ± 42	>100
HHA	0.4 ± 0.4	0.4 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	40 ± 14	32 ± 29
LOA	0.3 ± 0.3	0.1 ± 0.03	0.7 ± 0.2	0.4 ± 0.1	0.7 ± 0.2	8.9 ± 9.1
NPA	0.6 ± 0.4	0.6 ± 0.2	0.5 ± 0.3	0.7 ± 0.4	≥200	60 ± 56
DS-5000				0.4	≥200	14
DS-10000	0.4		1.2			

[a] EC_{50}s of HIV-1-, HIV-2-, or SIV-induced cytopathogenicity in MT-4 cells; HIV-1-induced giant cell formation in CEM cells; SRV-induced giant cell formation in Raji cells; or MSV-induced transformation of murine C3H/3T3 cells were determined. Data are the means ± standard deviations of at least three independent experiments.

HIV antigen expression on the virus-infected cell cultures was assayed by fluorescence-activated cell sorter analysis, as described above.

HUT-78/HIV-1 cells were cocultured with MOLT-4 cells, and LOA (25, 10, 4, 2.5, or 1 μg/ml) or DS-5000 (250, 100, 40, 25, or 10 μg/ml) was added at the time of infection (0 min) or 30, 60, 90, 120, 150, or 180 min after cocultivation. The first giant cells appeared at 3 h postinfection. Syncytium formation was recorded at 3, 6, 11, and 20 h postcocultivation.

RESULTS

Antiviral activity of plant lectins. Plant lectins were evaluated for their inhibitory effects against HIV-1-, HIV-2-, and SIV-induced cytopathogenicity in MT-4 cells, HIV-1-induced giant cell formation in CEM cells, SRV-induced giant cell formation in Raji cells, and MSV-induced transformation of murine C3H/3T3 cells (Table 1). At concentrations ranging from 0.2 to 0.8 μg/ml, the lectins GNA, HHA, LOA, and NPA inhibited virus-induced cytopathogenicity in MT-4 and CEM cells by 50%. Their EC_{50}s were similar to that of DS-5000 or DS-10000. Except for LOA, the lectins proved to be much less inhibitory to SRV-induced giant cell formation in Raji cells than they were to HIV or SIV infection in MT-4 or CEM cells. LOA also proved to be the most effective plant lectin against MSV, although it was 10- to 40-fold less active against MSV than it was against HIV, SIV, or SRV (Table 1).

When evaluated for their inhibitory effects on the replication of viruses other than retroviruses, the lectins proved to be markedly inhibitory to CMV replication in human embryonic lung cells (Table 2) but not HSV-1, HSV-2, vaccinia virus, or vesicular stomatitis virus in primary rabbit kidney cells; Sindbis virus, coxsackievirus type B4, parainfluenzavirus type 3, Semliki forest virus, or reovirus type 1 in Vero cells; and vesicular stomatitis virus, poliovirus type 1, or coxsackievirus type B4 in HeLa cells (data not shown). The anti-CMV activities of the lectins were determined against two different CMV strains (AD169 and Davis). The EC_{50}s of the lectins for CMV ranged from 0.9 to 1.6 μg/ml, which was comparable to the EC_{50} of 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG; ganciclovir) (Table 2).

Inhibition of syncytium formation between HUT-78/HIV and MOLT-4 (clone 8) cells by plant lectins. All the plant lectins, and in particular LOA, proved to be markedly inhibitory to giant cell formation. At concentrations as low as 0.7 to 4 μg/ml, syncytium formation was inhibited by 50% (Table 3). For DS-5000, a 10-fold higher concentration was required to afford a similar inhibitory effect on giant cell formation. Complete inhibition of giant cells was achieved by DS-5000 only at a concentration of 100 μg/ml, while LOA and HHA completely suppressed syncytium formation at concentrations of 2 and 10 μg/ml, respectively (data not shown). Unlike the plant lectins and DS-5000, the glycosylation inhibitors tunicamycin, castanospermine, and 2-deoxynojirimycin had no inhibitory effect on giant cell formation when evaluated under the same experimental conditions (data not shown).

Effect of preincubation of HUT-78/HIV-1 cells or MOLT-4 (clone 8) cells with plant lectins on syncytium formation. Preincubation of HUT-78/HIV-1 or MOLT-4 cells for 2 h with LOA, HHA, or DS-5000 before cocultivation did not result in an increase in the inhibitory effect of the test compounds on syncytium formation. LOA, HHA, and DS-5000 completely inhibited the appearance of giant cells at

TABLE 2. Activities of plant lectins against human CMV replication in HEL cells

Compound	EC_{50} (μg/ml)[a]		MTC (μg/ml)[a,b]
	Strain AD169	Strain Davis	
GNA	0.90 ± 0.07	1.6 ± 0.6	>100
HHA	0.87 ± 0.28	1.5 ± 0.24	100
LOA	1.1 ± 0.02	0.89 ± 0.38	>20
NPA	1.2 ± 0.3	1.6 ± 0.6	>100
DHPG[c]	0.80 ± 0.3	0.77 ± 0.04	>100

[a] Data are means of at least two to three independent experiments.
[b] MTC, Minimal cytotoxic concentration, or the concentration required to cause a microscopically detectable alteration of cell morphology.
[c] DHPG, 9-(1,3-Dihydroxy-2-propoxymethyl)guanine.

TABLE 3. Inhibitory effects of plant lectins on syncytium formation between HUT-78/HIV-1 or HUT-78/HIV-2 cells and uninfected MOLT-4 (clone 8) cells

Compound	EC_{50} (μg/ml)[a]	
	HUT-78/HIV-1 + MOLT-4	HUT-78/HIV-2 + MOLT-4
GNA	2.7 ± 1.2	3.0 ± 1.4
HHA	2.0 ± 0.0	2.0 ± 0.0
LOA	0.7 ± 0.3	1.0 ± 0.0
NPA	3.3 ± 1.2	4.0 ± 0.0
DS-5000	25	25–30

[a] Data are means ± standard deviations for three (HUT-78/HIV-1) or two (HUT-78/HIV-2) independent experiments.

TABLE 4. Inhibitory effects of plant lectins on the binding of anti-gp120 MAb (NEA 9284) to HUT-78/HIV-1-infected cells[a]

Compound	Concn (μg/ml)	% Inhibition of anti-gp120 (NEA 9284) MAb binding at the following incubation times:	
		15 min	60 min
GNA	100	47	72
HHA	100	45	80
LOA	100	62	90
NPA	100	12	56
DS-5000	25	99	100

[a] The threshold of positivity for the green fluorescence intensity was arbitrarily established on the basis of the control sample of uninfected HUT-78 cells incubated with MAb and rabbit anti-mouse IgG–F(ab')$_2$–fluorescein isothiocyanate. The percent inhibition of MAb binding was calculated according to the following formula: $100 \times [1 - (C_x - C_0)/(C - C_0)]$, where C_0 is the percentage of fluorescent cells in uninfected HUT-78 cell cultures, C is the percentage of fluorescent cells in HUT-78/HIV-1 cells, and C_x is the percentage of fluorescent cells in HUT-78/HIV-1 cells exposed to the test compounds at concentration x.

concentrations of 2, 10, and 100 μg/ml, respectively, irrespective of the preincubation of either cell line with the test compounds (data not shown).

Interference of plant lectins with binding of OKT4A MAb to MT-4 cells and anti-gp120 MAb to HUT-78/HIV-1 cells. OKT4A and anti-gp120 are MAbs against CD4 and gp120, respectively. No inhibition of OKT4A MAb binding to MT-4 cells was observed with the plant lectins at a concentration of 100 μg/ml (data not shown). However, the plant lectins were found to be inhibitory to anti-gp120 MAb binding to HUT-78/HIV-1 cells. When the HUT-78/HIV-1 cells were preincubated with the plant lectins (at 100 μg/ml) for 15 min, a slight (NPA) to moderate (HHA, GNA, LOA) inhibition of anti-gp120 MAb binding was observed (Table 4). Upon longer incubation times of the HUT-78/HIV-1 cells with the test compounds (i.e., 60 min), the plant lectins more strongly inhibited anti-gp120 MAb binding. However, their interference with anti-gp120 MAb binding was less pronounced than was that of DS-5000.

Influence of plant lectins on binding of HIV-1. MT-4 cells that adsorbed HIV-1 particles could be readily distinguished from MT-4 cells that were not exposed to HIV-1 by an indirect immunofluorescence technique with (polyclonal) anti-HIV-1 antibodies and flow cytometric analysis (Fig. 1A). When the virus particles were preincubated with the test compounds and then exposed to MT-4 cells in the continued presence of the test compounds, striking differences were noted between DS-5000 and the plant lectins. At 25 μg/ml, DS-5000 almost completely blocked virus adsorption (Fig. 1B). In contrast, the plant lectins slightly affected virus adsorption only at a concentration as high as 100 μg/ml (Fig. 1C and D). At 25 μg/ml, LOA and HHA had no effect whatsoever on virus binding (data not shown). Preincubation of the virus for 20 s (data not shown) or 2 h (Fig. 1) gave identical results. When DS-5000 at 25 μg/ml was mixed with LOA at 100 μg/ml, complete inhibition of virus binding to the cells was again observed (data not shown), suggesting that the plant lectins do not interfere with either the inhibitory effect of DS-5000 on the virus adsorption process or any of the stages involved in the analysis of the cells by flow cytometry.

Effect of delayed treatment of HIV-1-infected MT-4 cells and cocultures of HUT-78/HIV-1 and MOLT-4 cells by LOA. At a concentration of 5 μg/ml, that is, at a 20-fold higher dose than the EC_{50}, no cytopathogenicity was visible and viral antigen expression was significantly inhibited by both LOA and DS-5000 when added at the time of infection (0 min)

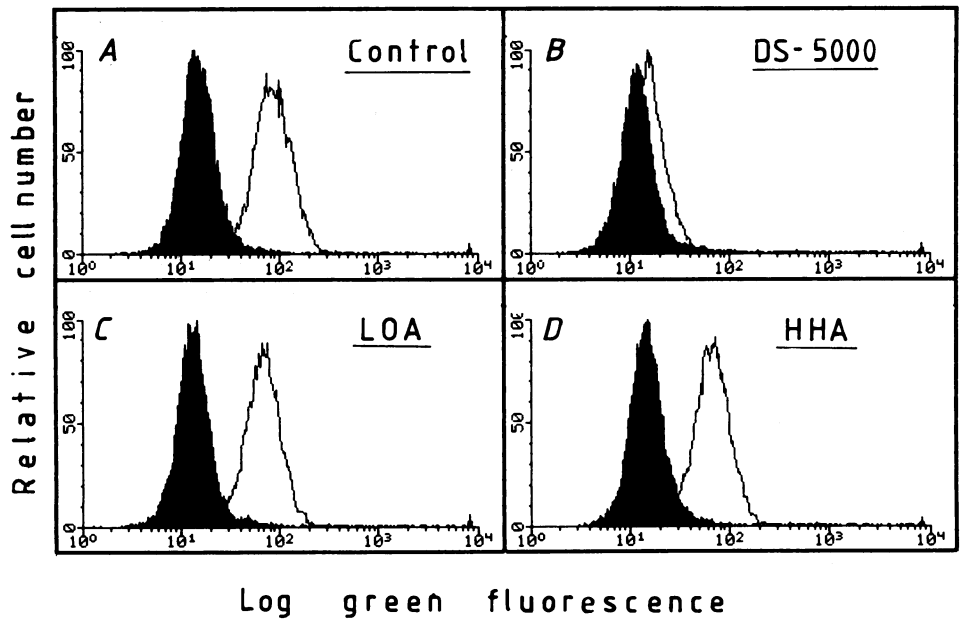

FIG. 1. Effect of test compounds on HIV-1 binding to MT-4 cells. The solid histograms represent cellular fluorescence resulting from nonspecific binding of HIV-1 antibody to MT-4 cells (which were not exposed to HIV-1 virions) in the absence of test compound (A) or in the presence of DS-5000 (25 μg/ml) (B), LOA (100 μg/ml) (C), or HHA (100 μg/ml) (D). The open histograms represent cellular fluorescence resulting from specific binding of HIV-1 antibody to MT-4 cells (which adsorbed HIV-1 virions) in the absence of test compound (A) or in the presence of DS-5000 (25 μg/ml) (B), LOA (100 μg/ml) (C), or HHA (100 μg/ml) (D).

TABLE 5. Effect of delayed treatment of HIV-1-infected MT-4 cells by LOA

Test compound[a]	% HIV-1 antigen-positive cells[b] at the following times of addition of test compound postinfection:					
	0 min	45 min	90 min	150 min	4 h	8 h
LOA	2.9	4.7	5.3	6.8	10	7.4
DS-5000	1.7	12	22	45	59	73

[a] The test compounds were used at concentrations of 5 μg/ml.
[b] The control value was 78. Data are means of two separate experiments.

(Table 5). However, delayed treatment of HIV-1-infected MT-4 cells resulted in a slight appearance of cytopathogenicity and a marked breakthrough of viral antigen expression for DS-5000 but not LOA. When DS-5000 was added as late as 8 h postinfection, virtually no inhibition of viral antigen expression was observed. Under the same experimental conditions, LOA still inhibited viral antigen expression by 90.5%.

When added at the time of cocultivation, LOA afforded a complete inhibition of giant cell formation at a 62-fold lower concentration than that required by DS-5000 (i.e., 4 and 250 μg/ml, respectively). Delay of addition of the test compounds at their protective doses for only 30 or 60 min resulted in the appearance of a limited number of giant cells at 3 h postcocultivation (~7.5 to 10% of the control value) (data not shown). A delay of 90 min or longer resulted in a marked breakthrough of syncytium formation. These data suggest that both DS-5000 and LOA interfere with giant cell formation at a very early event in the interaction between HUT-78/HIV and MOLT/4 cells.

Synergistic antiviral activity of LOA and DS-5000. The following combinations were investigated for their inhibitory effects on HIV-1 replication in MT-4 cells: LOA with DS-5000, LOA with HHA, LOA with 2′,3′-dideoxyinosine, and DS-5000 with pentosan polysulfate. Additive antiviral effects were found when LOA was combined with HHA or DS-5000 was combined with pentosan polysulfate, and additive to subsynergistic antiviral effects were noted when LOA was combined with 2′,3′-dideoxyinosine (data not shown). However, marked synergism in anti-HIV-1 activity was observed when LOA was combined with DS-5000 (Fig. 2). When LOA was combined with DS-5000 at concentrations at which both compounds afforded about 10% inhibition of virus replication, complete protection against the HIV-1-induced cytopathogenicity in MT-4 cells was achieved. A similar synergistic inhibitory action on giant cell formation between HUT-78/HIV-1 cells and MOLT-4 cells was obtained with the combination of LOA and pentosan polysulfate and the combination of HHA and DS-5000 (data not shown).

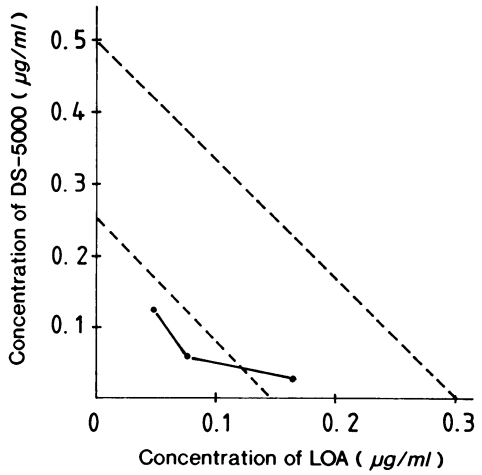

FIG. 2. Isobologram representation of the combined inhibitory effects of LOA and DS-5000 on HIV-1-induced cytopathogenicity in MT-4 cells. Broken lines represent the unity lines for the fractional inhibitory concentrations, which were equal to 1 and 0.5, respectively. The data correspond to the average values obtained from three independent experiments.

DISCUSSION

Results of our studies indicate that the α-(1-3)- and α-(1-6)-D-mannose-specific plant lectins are potent inhibitors of HIV replication in vitro. They also suppress giant cell formation between HUT-78/HIV-1 cells and uninfected MOLT-4 cells. Our data also suggest that the inhibitory effect of the plant lectins on HIV-induced cytopathogenicity and syncytium formation is due to an interaction with an early stage of the virus replicative cycle. In this respect, the action of the plant lectins is reminiscent of those of sulfated polysaccharides (i.e., dextran sulfate), which have been shown to block virus adsorption by shielding gp120 (26). It has been suggested (21) that plant lectins act like sulfated polysaccharides, that is, by inhibiting virus binding to the $CD4^+$ target cells. However, our observations make it clear that the plant lectins behave differently from the sulfated polysaccharides and should thus be considered a novel class of anti-HIV compounds with a unique mechanism of action.

First, unlike the sulfated polysaccharides, which are active against a broad spectrum of enveloped viruses (2), plant lectins are only inhibitory to HIV and CMV. Second, although the sulfated polysaccharides (i.e., DS-5000) and the plant lectins are equally inhibitory to HIV-1-induced cytopathogenicity, plant lectins are far more inhibitory to HIV-induced giant cell formation than DS-5000 is. Third, in contrast to what should be expected from our syncytium inhibition data, plant lectins interfered less efficiently with anti-gp120 MAb binding to HUT-78/HIV-1 cells than did DS-5000. Incubation with DS-5000 at 25 μg/ml for a period of as little as 20 s sufficed to inhibit anti-gp120 MAb binding by 99%. In contrast, for plant lectins a fourfold higher concentration was required, as was preincubation with the cells for at least 1 to 2 h to afford 90% inhibition. These data indicate that the plant lectins react only slowly with the gp120 molecules expressed on HIV-1-infected cells, do not demonstrate high affinity for gp120, or both. A fourth difference between the sulfated polysaccharides and the plant lectins is their differential behavior with regard to their influence on HIV binding to $CD4^+$ cells. While DS-5000 is a very efficient inhibitor of HIV-1 binding to $CD4^+$ (i.e., MT-4) cells, the plant lectins have virtually no effect on virion binding even at a concentration of 100 μg/ml, that is, at a 250- to 300-fold higher concentration than their EC_{50} for inhibition of HIV-1 replication. The fifth observation that points to a differential mechanism of action of sulfated polysaccharides and plant lectins is their unusually strong synergistic activity against HIV. Indeed, while combinations of two different plant lectins (i.e., LOA and HHA) or two sulfated polysaccharides (i.e., DS-5000 and pentosan polysulfate) resulted only in an additive antiviral effect, combination of LOA (or HHA) with DS-5000 (or pentosan polysulfate) proved strongly synergistic (Fig. 2). The sixth difference between sulfated polysac-

charides and plant lectins was the marked protection of HIV-1-infected cells when plant lectins were added at 4 to 8 h postinfection. At 4 to 8 h postinfection, virtually all virus particles were adsorbed to the cells, and the addition of DS-5000 at this stage was unable to prevent virus infection. However, at 4 to 8 h postinfection only a limited amount of the virus particles that were adsorbed to the cells penetrated into the cells, and thus, plant lectins may be expected to block virus infection even if they are added at 4 to 8 h after infection, if they act at a postadsorption step. In fact, we were unable to detect by electron microscopy any virus particles inside MT-4 cells which were infected with HIV-1 and subsequently treated with LOA (data not shown).

Taking into account the potent inhibitory effect of the plant lectins on syncytium formation together with their lack of inhibitory effect on the virus adsorption to the cells, we postulate that they interfere with the fusion process. Such targeted action at the fusion of the viral envelope with the cell plasma membrane may also be accompanied by a steric hindrance or conformational change of gp120, and thus reduced binding of anti-gp120 MAb to gp120$^+$ cells, as seems to happen in the presence of the plant lectins.

In conclusion, our data point to D-mannose-specific plant lectins as a unique class of potent and selective anti-HIV and anti-CMV agents. They exhibit a unique mechanism of antiviral action that is different from that of any other class of antiviral compounds. They are potent inhibitors of the HIV-induced giant cell formation and are assumed to interact directly with the virus-cell membrane fusion process.

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