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The prevention of cell adhesion and the cell-to-cell spread of HIV-1 in vitro by the α-glucosidase 1 inhibitor, 6-*O*-butanoyl castanospermine (MDL 28574)

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Abstract

The intercellular adhesion molecule (ICAM-1, CD54) and its counter receptor, the integrin leukocyte function associated antigen 1 (LFA-1, CD11a/CD18), have important roles in the immune response. These include guiding leukocytes to sites of inflammation (Issekutz and Issekutz, 1992), enhancement of antigen presentation (Moy and Brian, 1992) and potentiation of cytotoxic cell function (Umehara et al., 1992; Sanchez-Madrid et al., 1982). In addition to these activities LFA-1 and ICAM-1 are implicated in the cell-to-cell transmission of human immunodeficiency virus (HIV-1) since antibodies to CD18, CD54 or synthetic peptide analogs of ICAM-1 antagonise the formation of virus-induced syncytia (Fecondo et al., 1993; Gruber et al., 1991; Hildreth and Orentas, 1989; Valentin et al., 1990). The α-glucosidase 1 inhibitor 6-*O*-butanoyl castanospermine (MDL 28574) has antiviral activity for HIV which is manifested by a decrease in syncytia as well as the production of virus with altered gp120 and a reduced infectivity (Taylor et al., 1991).

Previously, it has been shown that the α-glucose 1 inhibitor (MDL 28574) treatment of human leukocytes in vitro or mouse lymphocytes in vivo affects the detection of LFA-1 but not domain 1 of CD4 nor several other CD markers (Bridges et al., submitted for publication). Here, we demonstrate that pre-treatment of HIV-permissive $CD4^+$ cells with MDL 28574 substantially reduces their capacity to bind with cells chronically infected with HIV-1 which results in reduced

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virus production. Indirectly, this suggests that compounds of this type, which alter both virus ligands and host cell adhesion molecules, may be of especial benefit in the treatment of viral infections.

Keywords: HIV; LFA-1; 6-*O*-Butanoyl castanospermine

HIV infection, and growth in its principal host cells T lymphocytes and mononuclear phagocytes, is initiated by the attachment of virus envelope gp120 to host cell CD4 (Dalgleish et al., 1984; Klatzmann et al., 1984) followed by membrane fusion mediated by gp41 (Dedera and Ratner, 1991). Virus growth also follows the fusion of infected cells with uninfected cells to form multinucleate giant cells (syncytia). Syncytia formation engendered by conventional gp120/CD4 interaction is promoted by LFA-1/ICAM-1 binding (Gruber et al., 1991; Golding et al., 1992; Busso et al., 1991). Treatment of HIV infected cells with the glycosylation inhibitor 6-*O*-butanoyl castanospermine results in the production of defective HIV (Taylor et al., 1994) and also, due to its action on the glycoprotein processing pathway of host cells, alters CD18 on leukocytes (Taylor et al., 1994; Bridges et al., 1994). In view of the latter finding it was possible that MDL 28574 treatment could abrogate the cell-to-cell spread of HIV.

To determine whether MDL 28574 treatment of permissive cells could alter the cell-to-cell transmission of HIV-1, a method that would separate the indicator cells from the bulk of infected cells was required. The dual expression of CD4/CD8 on JM cells offered the means for their separation by anti-CD8 magnetic beads since chronically infected H9 cells do not possess CD8. The validity of the cell-to-cell virus transmission assay was first examined with antibodies known to alter the formation of HIV-1-induced syncytia: Washed H9.HIV-1HXB2 (2E6) were mixed with an equal quantity of JM cells in a total volume of 400 μl phenol red free Hank's balanced salt solution (HBSS). Transmission was allowed to occur by incubating the mixtures for 1 h at RT, with occasional gentle agitation, in presence of various monoclonal antibodies at 20 μg/ml (Dako, Becton-Dickinson) (Ortho OKT4a at 5 μg/ml). Untreated control preparations contained sodium azide equivalent to the preservative concentration in antibody mixtures. JM cells were then removed by adding 50 μl anti-CD8 magnetic bead suspension (Dynabeads M-450 CD8), washed three times in HBSS and placed in conventional culture conditions in the absence of antibodies. Negative controls consisted of bead separations of H9.HIV-1HXB2 or JM cells alone which did not produce p24 antigen.

Table 1
Virus production after exposure of JM cells to H9.HIV-1HXB2 cells in the presence of monoclonal antibodies

Treatment	p24 (pg/ml)	Percent control
No treatment	3.8	–
CD2	4.7E4	> 100
CD3	2.8E4	73.6
CD4 (OKT4a)	8.6E3	22.6
CD18	7.0E3	18.4
CD54	1.1E4	28.9

Table 2
Virus production by JM cells pre-treated with MDL 28574 and subsequently exposed to chronically infected H9.HIV-1HXB2 cells [a]

MDL 28574 (μg/ml)	p24 (pg/ml) ± S.D.	Percent control
0	3.3E4 ± 3818	–
1	2.0E4 ± 1909	61
0	8.7E3 ± 1414	26
100	1E3 ± 141	3

[a] JM cells were grown in the presence of the drug for 7 days with compound replenishment at 48 and 96 h. Resulting cells were then placed in the cell-to-cell assay, separated and cultured for 7 days in the absence of drug. The efficiency of JM cell separation by magnetic beads was not altered by drug treatment.

After 3 days, supernatants were harvested for HIV p24 antigen estimations. As expected, antibodies to CD54, CD18 or CD4 suppressed the formation of syncytia in mixtures of H9.HIV-1HXB2 and JM cells (data not shown) and also reduced the production of p24 in anti-CD8 bead separated JM cell cultures (Table 1) (The reduction by CD3 antibody was not reproducible). Similarly, MDL 28574 pre-treatment of JM cells reduced the subsequent production of p24 in bead isolated cell preparations (Table 2) and altered the detection of CD18 (Fig. 1).

To find out whether p24 production in the untreated bead separated cell population was solely from cell-to-cell HIV infection of JM cells after brief contact with infected cells, or also due to H9.HIV-1HXB2 carry-over by cell conjugate or syncytium formation, bead separated preparations were analysed by flow cytometry. JM cells and H9.HIV-1HXB2 cells were fluorescently labelled with PKH2 (green, FL1) and PKH26 (red, FL2) dyes respectively according to the manufacturer's recommendations (Sigma). Resulting cells were mixed in the cell-to-cell assay as described above. JM cells were then

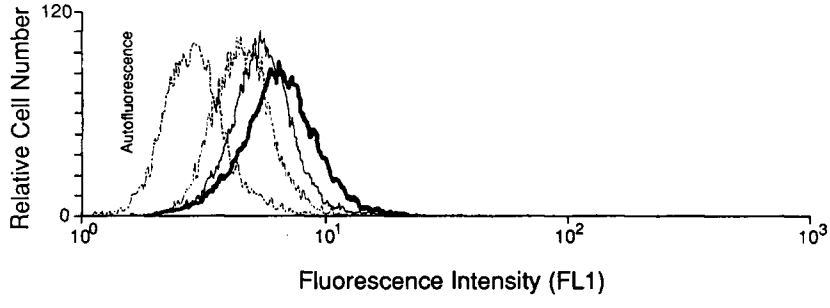

Fig. 1. Reduction of CD18 detection in JM cells exposed to MDL 28574. Untreated (bold continuous line); MDL 28574 1 μg/ml (continuous line); MDL 28574 10 μg/ml (dashed line); MDL 28574 100 μg/ml (overlaid with previous concentration, removed for clarity); autofluorescence (dashed-dot line). JM cells were grown for 3 days in the presence of MDL 28574 (0, 1, 10 and 100 μg/ml) and directly stained with anti-CD18 monoclonal antibody. The fluorescence data was logarithmically acquired and a minimum of 1E4 events were analysed with Lysis 2 V1.1 software using linearly converted values for histogram statistics. MDL 28574-treated JM cells show a lower mean fluorescence than untreated cells. Alteration in CD18 detection occurred at all concentrations with a peak change at 10 μg/ml.

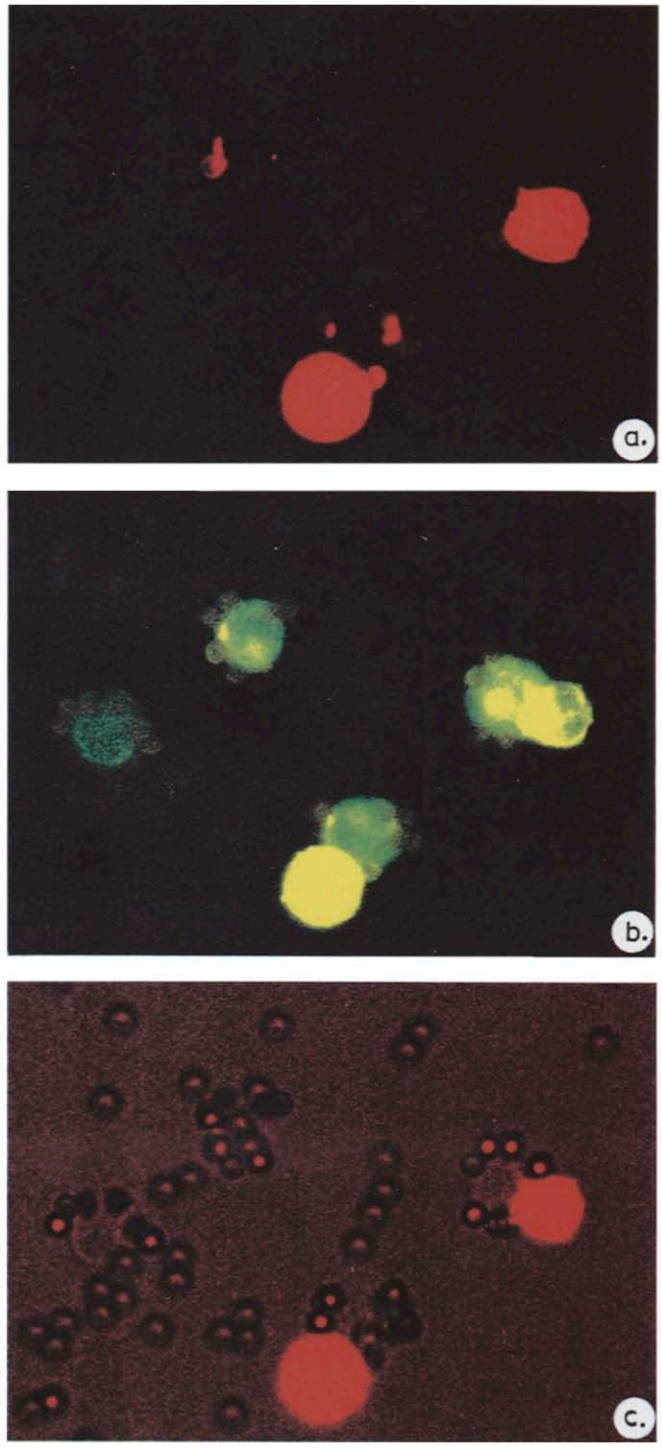
a.
b.
c.

Table 3
The effect of MDL 28574 pre-treatment of JM cells and monoclonal antibody to CD18 on the adhesion of H9.HIV1HXB2 cells and JM cells disclosed by flow cytometry [a]

Treatment	H9-HXB2 (UL events-FL2)	JM		Conjugates		
		Percent	(LR events-FL1)	Percent	(UR events-FL1 + FL2)	Percent
JM alone	3	0.06	4663	99.94	0	0
MDL28574 (100 μg/ml)	109	2.26	4599	95.59	103	2.14
Anti-CD18 (20 μg/ml)	49	0.99	4840	97.99	50	1.01
Untreated	254	5.17	4424	90.1	232	4.72

[a] Quadrant statistics reveal that a significant number of chronically infected H9 cells are firmly attached to JM cells in untreated cell populations. These events appear in the upper right (UR) quadrant as cell conjugates. More loosely attached H9.HIV-1 cells, which have presumably become detached during washing, appear in the upper left (UL) quadrant. Both anti-CD18 monoclonal antibody and MDL 28574 pre-treatment of JM cells reduces the number of conjugates and loose chronically infected cells.

separated by magnetic beads, washed and fixed (isotonic 4% Formalin-PBS/18 h). The fluorescence of these preparations was logarithmically acquired on a FACScan flow cytometer and a minimum of 5000 events were analysed with Lysis 2 V1.1 software using quadrant statistics (Becton Dickinson). Electronic compensation was performed by mixing the individual fixed cell populations and the cursor positioned to include > 98% of each cell population in its respective upper left or lower right quadrant. Analysis of untreated control preparations reveals that there were both free H9.HIV-1HXB2 cells as well as conjugates formed between JM cells and the chronically infected H9 cells (seen as events in the upper quadrants). This indicates that the assay measures the ability of infected H9 cells to form firm contacts with JM cells as well as looser ones. Microscopically, double positive events in the upper right quadrant were clusters of H9.HIV-1HXB2 cells and bead coated JM cells (Fig. 2). No syncytia were observed at this time (1 h). In a representative experiment the percentage of H9.HIV-1HXB2 events for untreated, anti-CD18 and MDL 28574 were 9.9, 2.0, and 4.4 respectively, representing decreases of 80% and 55% for the treated preparations (Table 3). Clearly, antibody to CD18 and to a lesser extent MDL 28574 pre-treatment of indicator JM cells inhibit the attachment of H9.HIV-1HXB2 cells. Separate experiments confirmed a dose-dependent effect of MDL 28574 on the adhesion between chronically infected H9.HIV-1HXB2 and JM cells (Table 4).

Fig. 2. Adhesion between chronically infected H9.HIV-1HXB2 cells and JM cells. Conjugates of H9.HIV-1 and JM cells are clearly identifiable in these serially filtered photomicrographs. (a) (red channel fluorescence) Stained H9.HIV-1 cells; (b) (green channel fluorescence) JM cells in a cell conjugate with chronically infected H9 cells; (c) (red channel fluorescence mixed with white light) beads coating JM cells can be more clearly seen. There is spectral overlap of the two dyes in the green channel of the microscope. H9.HIV-1 and JM cells were pre-stained as described in the text. The resulting cell preparations were mixed in the cell-to-cell spread assay and examined by fluorescence microscopy.

Table 4
The dose-dependent effect of MDL 28574 pre-treatment of JM cells and their adhesion with H9.HIV-1HXB2 cells disclosed by flow cytometry [a]

MDL 28574 Concentration (μg/ml)	H9-HXB2 (UL events-FL2)	JM		Conjugates		
		Percent	(LR events-FL1)	Percent	(UR events-FL1 + FL2)	Percent
100	38	0.39	9608	98.48	110	1.13
10	54	0.56	9416	97.38	199	2.06
1	155	1.63	8948	93.9	426	4.47
Untreated	153	1.6	8988	94.62	358	3.76

[a] Data from 10000 events.

The ability of antibodies or MDL 28574 to prevent the production of p24 in positively selected bead preparations is attributable not only to prevention of conjugate formation, and presumably fusion, but also infers a reduced efficiency of viral transmission arising from transient contacts. It is suggested that compounds such as MDL 28574 which affect glycosylation patterns of cell surface molecules involved in adhesion in turn alter the strength of cell-to-cell contacts. With regard to MDL 28574 it has previously been shown that treatment of human lymphocytes in vitro or mouse lymphocytes in vivo leads to an alteration of LFA-1 detection by monoclonal antibodies (Bridges et al., 1994). Cell-to-cell contact, not cell-free virus infection, appears to be the principal route of HIV-1 spread in vitro (Kiernan et al., 1990) and this mode, coupled to increased CD11/CD18 expression (Palmer and Hamblin, 1993), may also dominate in vivo. Also, in terms of cell-free virus infection it is possible that other host cell molecules incidentally present in the virus envelope, and which facilitate virus attachment (Schols et al., 1992), can be altered by MDL 28574 treatment. Compounds, exemplified by MDL 28574, which can offer antiviral mechanisms acting on both virus and host cell may be of particular benefit in the interdiction of cell-to-cell spread in vivo and also provide a measure of protection against heterologous drug-resistant virus strains (Larder et al., 1989). Moreover, the extra dimension to treatment afforded by the prevention of cell-to-cell spread could be of crucial value when this type of compound is used in drug combinations, as even established therapy may fail to influence this mode of HIV growth (Gupta et al., 1993).

References

Bridges, C.G., Taylor, D.L., Kang, M.S., Brennan, T.M. and Tyms, A.S. (1994) The α-glucosidase 1 inhibitor 6-*O*-Butanoyl Castanospermine (MDL 28574) affects the detection of LFA-1(CD18/CD11a) by monoclonal antibodies. Submitted for publication.

Busso, M., Thornthwaite, J. and Resnick, L. (1991) HIV-induced syncytium formation requires the formation of conjugates between vrus-infected and uninfected T cells in vitro. AIDS 5, 1425–1432.

Dalgleish, A.G., Beverley, P.C.L., Clapham, P.R., Crawford, D.H., Greaves, M.F. and Weiss, R.A. (1984) The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature 312, 767–768.

Dedera, D. and Ratner, L. (1991) Demonstration of two distinct cytopathic effects with syncytium formation-defective Human Immunodeficiency Virus type 1 mutants. J. Virol. 65, 6129–6136.

Fecondo, J.V., Pavuk, N.C., Silburn, K.A., Read, D.M.Y., Mansell, A.S., Boyd, A.W. and McPhee, D.A. (1993) Synthetic peptide analogs of intercellular adhesion molecule 1 (ICAM-1) inhibit HIV-1 replication in MT-2 cells. AIDS Res. Hum. Retroviruses 9, 733–740.

Golding, H., Dimtrov, D.S. and Blumenthal, R. (1992) LFA-1 adhesion molecules are not involved in the early stages of HIV-1 *env*-mediated cell membrane fusion. AIDS Res. Hum. Retroviruses 8, 1593–1598.

Gruber, M.F., Webb, D.S.A., Gerrard, T.L., Mostowski, H.S., Vujcic, L. and Golding, H. (1991) Re-evaluation of the involvement of the adhesion molecules ICAM-1/LFA-1 in syncytia formation of HIV-1-infected subclones of a CEM T-cell leukemic line. AIDS Res. Hum. Retroviruses 7, 45–53.

Gupta, P., Balachandran, R., Ho, M., Enrico, A. and Rinaldo, C. (1993) Cell-to-cell transmission of human immunodeficiency virus type 1 in the presence of azidothymidine and neutralizing antibody. J. Virol. 63, 2361–2365.

Hildreth, J.E.K. and Orentas, R.J. (1989) Involvement of a leukocyte adhesion receptor (LFA-1) in HIV-induced syncytium formation. Science 244, 1075–1078.

Issekutz, A.C. and Issekutz, T.B. (1992) The contribution of LFA-1 (CD11a/CD18) and MAC-1 (CD11b/CD18) to the in vivo migration of polmorphonuclear leucocytes to inflammatory reactions in the rat. Immunology 76, 655–661.

Kiernan, R., Marshall, J., Bowers, R., Doherty, R.R. and McPhee, D.A. (1990) Kinetics of HIV-1 replication and intracellular accumulation of particles in HTLV-1 transformed cells. AIDS Res. Hum. Retroviruses 6, 743–752.

Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J. and Montagnier, L. (1984) T-lymphocyte T4 molecule behaves as the receptor for human retrovirus. Nature 312, 767–768.

Larder, B.A., Darby, G. and Richman, D.D. (1989) HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science 243, 1731–1734.

Moy, V.T. and Brian, A.A. (1992) Signalling by lymphocyte function-associated antigen 1 (LFA-1) in B cells: enhanced antigen presentation after stimulation through LFA-1. J. Exp. Med. 175, 1–7.

Palmer, S. and Hamblin, A.S. (1993) Increased CD11/CD18 expression on the peripheral blood leukocytes of patients with HIV disease: relationship to disease severity. Clin. Exp. Immunol. 93, 344–345.

Sanchez-Madrid, F., Krensky, A.M., Ware, C.F., Robins, E., Strominger, J.L., Burakoff, S.J. and Springer, T.A. (1982) Three distinct antigens associated with human T-lymphocyte mediated cytolysis: LFA-1, LFA-2, LFA-3. Proc. Natl. Acad. Sci. USA 79, 7489

Schols, D., Pauwels, R., Desmyter, J. and De Clerq, E. (1992) Presence of Class II histocompatability DR proteins on the envelope of Human Immunodeficiency Virus demonstrated by FACS analysis. Virology 189, 374–376.

Taylor, D.L., Sunkara, P., Liu, P.S., Kang, M.S., Bowlin, T.L. and Tyms, A.S. (1991) 6-*O*-butanoyl castanospermine (MDL28574) inhibits glycoprotein processing and growth of HIVs. AIDS 5, 693–698.

Taylor, D.L., Kang, M.S., Brennan, T.M., Bridges, C.G., Sunkara, P. and Tyms, A.S. (1994) Inhibition of α-glucosidase 1 of the glycoprotein processing enzymes by 6-*O*-butanoyl castanospermine (MDL28574) and its consequences in HIV infected and uninfected T cells. Submitted for publication.

Umehara, H., Takashima, A., Minami, Y. and Bloom, E.T. (1992) Signal transduction via phosphorylated adhesion molecule, LFA-1b (CD18), is increased by culture of natural killer cells with IL-2 in the generation of lymphokine-activated killer cells. Int. Immunol. 5, 19–27.

Valentin, A., Lundin, K., Patarroyo, M. and Asjo, B. (1990) The leukocyte adhesion glycoprotein CD18 participates in HIV-1-induced syncytia formation in monocytoid and T-cells. J. Immunol. 144, 934–937.