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Role of protein N-glycosylation in pathogenesis of human immunodeficiency virus type 1

(glycoprotein processing/acquired immunodeficiency syndrome)

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ABSTRACT **Human immunodeficiency virus type 1 (HIV-1), the retrovirus responsible for acquired immunodeficiency syndrome (AIDS), contains two heavily glycosylated envelope proteins, gp120 and gp41, which mediate attachment of virions to glycosylated cell surface receptor molecules (CD4 antigens) and appear to be responsible for syncytium formation and associated cytopathic effects of this virus. A comprehensive study of the effects of N-linked glycoprotein processing inhibitors on HIV-1 replication, infectivity, cytopathicity, target-cell infectibility, syncytium formation, and gp120 electrophoretic mobility was conducted to assess the importance of protein glycosylation in the pathogenesis of HIV-1 *in vitro*. The electrophoretic mobility of gp120 was decreased when gp120 was synthesized in the presence of castanospermine or 1-deoxynojirimycin (inhibitors of glucosidase I), increased when gp120 was synthesized in the presence of 1-deoxymannojirimycin (mannosidase I) or swainsonine (mannosidase II), and unaffected when gp120 was synthesized in the presence of bromoconduritol (glucosidase II). Inhibition by tunicamycin (lipid-linked oligosaccharide precursor synthesis), castanospermine, 1-deoxynojirimycin, and 1-deoxymannojirimycin attenuated HIV-1 infectivity and blocked HIV-1-induced syncytium formation and cytopathicity, whereas bromoconduritol and swainsonine failed to have such effects. None of the inhibitors interfered with virus replication in acutely infected cells or affected the ability of target cells to form syncytia with untreated HIV-1-infected cells. These results demonstrate that protein N-glycosylation is critical to the pathogenesis of HIV-1 at the levels of viral infectivity and cytopathicity but not at the level of virus replication or of host-cell infectibility.**

Human immunodeficiency virus type 1 (HIV-1) is a highly variable D-type retrovirus identified as the etiologic agent of acquired immunodeficiency syndrome (AIDS) (1–3). Encoded by the *env* gene of this virus are two heavily glycosylated proteins located in the viral membrane. These proteins are translated as a precursor, gp160, which is subsequently cleaved to the amino-terminal-derived, outer-membrane gp120 and the carboxyl-terminal-derived, transmembrane gp41 (4, 5). Several features of HIV-1 pathogenesis have been attributed to these glycoproteins. One is the tropism that HIV-1 exhibits for cells expressing the CD4 surface antigen, which acts as receptor for the virus (6, 7), and where binding involves gp120–CD4 interactions (8). Major targets for HIV-1 infection are the helper/inducer subset of T lymphocytes (9) and cells of monocyte/macrophage lineage (10–12).

Another feature of HIV-1 *env*-mediated pathogenesis is cytopathicity that appears to be mediated by syncytium formation (13, 14). Syncytium formation is common to many cytopathic viral infections and is thought to involve an interaction of virus-encoded proteins in the cell membrane with receptor proteins on the surface of adjacent cells (6, 7, 15, 16). Results of transfection experiments with expression vectors containing the HIV-1 *env* gene suggest a central role for gp120 in HIV-1-induced syncytium formation and related cytopathic effects (CPEs) (17). The gp41 also appears to be essential for the post-binding fusion reaction (18). A role for gp120–CD4 interactions in syncytium formation and cytopathicity is suggested by the observations (*i*) that anti-CD4 antibodies and *in vitro* site-directed mutations in gp120 block these activities (6, 7, 18) and (*ii*) that anti-gp120 antibodies neutralize HIV-1 (19). As a result of the cellular tropism and cytopathicity of HIV-1, helper T lymphocytes are depleted and the consequential immune dysfunctions associated with AIDS ensue (20). HIV-1-induced CPEs are not observed in monocyte-derived cell lines, in contrast to the marked cytopathicity observed in most T-lymphocyte-derived cell lines (1–3, 10–12). Thus, cells of monocyte/macrophage lineage may serve as an *in vivo* HIV-1 reservoir and may be responsible for viral dissemination across the blood/brain barrier in cases of AIDS-associated acute encephalopathy (10–12, 21).

In this report, we describe the effects of various N-linked glycoprotein processing inhibitors on HIV-1 replication, infectivity, cytopathicity, target-cell infectibility, syncytium formation, and gp120 electrophoretic mobility in order to assess the role of protein glycosylation in HIV-1 pathogenesis *in vitro*.

MATERIALS AND METHODS

Cells and Virus. T-lymphoblastoid cell lines H9 (obtained from R. C. Gallo, National Cancer Institute), CEM (obtained from J. C. Chermann and L. Montagnier, Pasteur Institute), and MT-2 (obtained from D. Richman, Veterans Administration Medical Center, University of California, San Diego) and the monocyte/macrophage continuous cell line U-937 (American Type Culture Collection, CRL 1593) were grown and maintained at 37°C in RPMI-1640 containing 12% heat-inactivated fetal bovine serum and 50 μg of gentamicin per ml. The HIV-1 isolate HTLV-III$_B$ (1) was obtained from conditioned culture fluids of H9/HTLV-III$_B$ cultures. Conditioned fluids were clarified of cells by low-speed centrifugation followed by 0.45-μm filtration. Virus was quantitated by reverse transcriptase (RT) activity and by 50% tissue culture infectious dose (TCID$_{50}$) values obtained by end-point microtitration on MT-2 cells (22).

Inhibitors. The N-glycosylation inhibitors tunicamycin (Tu), castanospermine (Csp), 1-deoxynojirimycin (dNM),

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Abbreviations: HIV-1, human immunodeficiency virus type 1; AIDS, acquired immunodeficiency syndrome; Tu, tunicamycin; Csp, castanospermine; dNM, 1-deoxynojirimycin; Bcu, bromoconduritol; dMM, 1-deoxymannojirimycin; Sw, swainsonine; RT, reverse transcriptase; IFA, indirect immunofluorescence assay; CPE, cytopathic effect; TCID$_{50}$, 50% tissue culture infectious dose.

bromoconduritol (Bcu), and 1-deoxymannojirimycin (dMM) were purchased from Boehringer Mannheim. Swainsonine (Sw) was provided by H. Broquist (Vanderbilt University). The activity of Sw was routinely checked by jackbean α-mannosidase assays (23) and its purity was confirmed by ^{1}H NMR and mass spectrometry.

Infection Assays. Infection in flask cultures was monitored by indirect immunofluorescence assay (IFA) for viral antigen synthesis (24) and by RT activity in culture fluids for virus production (25). For infection, 10-ml cell suspensions were challenged with 2 ml of conditioned culture fluid. After 5 hr, unadsorbed virus was removed by two washes in growth medium. Cultures were then incubated in 20 ml of growth medium for the remainder of the incubation period.

Anti-HIV-1 activities were quantitated by microtiter infection assays using MT-2 cells as targets (22). CPE was quantitated by vital dye (neutral red) uptake of poly(L-lysine)-adhered cells as a measure of viable cells for end point of infection. Percent protection is defined by the difference in absorbance between test wells and virus control wells divided by the difference in absorbance between cell control and virus control wells.

Syncytium Inhibition Assays. The ability of N-glycosylation inhibitors to block HIV-1-induced cell fusion and resulting CPE was examined in 96-well microdilution plates. Cultures of H9/HTLV-III$_B$ cells were washed twice in growth medium and then incubated in the presence or absence of inhibitors for 2 days with one change of the growth medium after 1 day. MT-2 cells were then mixed with these cells at a 4:1 ratio (4×10^5 MT-2 cells plus 10^5 H9/HTLV-III$_B$ cells) in triplicate in a 225-μl total volume per well of a 96-well microdilution plate. Syncytia were scored after 24 hr by microscopic examination (×100 magnification). Percent viability was determined after 24 hr by vital dye uptake of poly(L-lysine)-adhered cells and syncytia as described above. The range for percent protection was defined by the difference between the average of eight wells containing a mixture of 4×10^5 untreated MT-2 cells and 10^5 untreated H9 cells as a noncytopathic control and the average of eight wells containing a mixture of 4×10^5 untreated MT-2 cells and 10^5 untreated H9/HTLV-III$_B$ cells as a full CPE control. A full CPE produced 70–80% lysis of cells after 24 hr.

NaDodSO$_4$/PAGE of Radiolabeled gp120. Cultures of washed H9/HTLV-III$_B$ cells were incubated for 48 hr, in the presence or absence of inhibitors, with 1 mCi of α-D-[2-^{3}H]mannose (20–30 Ci/mmol, ICN; 1 Ci = 37 GBq) per 10 ml. The culture fluids were then clarified of cells by low-speed centrifugation and filtration through 0.45-μm cellulose acetate filters. Virus was collected by centrifugation at 18,000 rpm (JA-20 rotor; Beckman) for 3.5 hr at 20°C and washed once in 5 ml of phosphate-buffered saline (PBS: 137 mM NaCl/10 mM phosphate, pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and the final viral pellet was suspended in 200 μl of PBS with 1 mM PMSF. Samples (60 μl) of viral suspensions were then added to NaDodSO$_4$/PAGE sample buffer (20 μl) and electrophoresed under reducing conditions in 7.5% polyacrylamide, and labeled proteins were detected by fluorography (24).

RESULTS AND DISCUSSION

Inhibitors of Eukaryotic N-Glycosylation Alter the Electrophoretic Mobility of HIV-1 gp120. The biochemical steps governing N-linked glycoprotein processing have been reviewed (26–28). N-glycosylation is initiated by the transfer of preformed oligosaccharide from an oligosaccharide-diphosphodolichol intermediate to an asparagine residue of the nascent peptide chain through N-glycosidic bonds. Protein N-glycosylation can be prevented by the antibiotic Tu, which blocks the synthesis of the lipid-linked oligosaccharide precursor (29). Potential glycosylation sites occur at peptide sequences containing Asn-Xaa-Thr or Asn-Xaa-Ser, where Xaa is any amino acid other than proline or aspartic acid. Asparagine-linked oligosaccharides are then subjected to a series of processing steps leading to the formation of mature glycoproteins.

Cellular and viral membrane glycoproteins consist of two major types of N-linked oligosaccharides: (*i*) high-mannose-type [(Man)$_{5-9}$-(GlcNAc)$_2$-Asn] and (*ii*) complex-type {[(±NeuAc)-Gal-GlcNAc]$_{2-4}$-(Man)$_3$-[(GlcNAc)$_2$-(±Fuc)]-Asn} (27). The HIV-1 gp120 contains high-mannose-type, hybrid-type, and fucosylated, partially sialylated mono-, bi-, tri-, and tetraantennary complex-type oligosaccharides (30, 47). Several inhibitors of specific reactions in the N-linked glycoprotein processing pathway have recently become available, including Csp and dNM as inhibitors of glucosidase I, Bcu as an inhibitor of glucosidase II, dMM as an inhibitor of mannosidase I, and Sw as an inhibitor of mannosidase II. All of these inhibitors have a common structural resemblance to the transition-state intermediate in the specific reaction they inhibit, thereby blocking the conversion of high-mannose-type glycoproteins to complex-type glycoproteins (26–28). With the exception of Bcu, each of these inhibitors was found to significantly affect the apparent molecular mass of HIV-1 gp120 produced under their influence (Fig. 1). Synthesis of gp120 in the presence of the glucosidase I inhibitor Csp or dNM resulted in an increased molecular mass (125 kDa). In contrast, synthesis of gp120 in the presence of the mannosidase I inhibitor dMM or the mannosidase II inhibitor Sw resulted in a decreased molecular mass (110 kDa). As expected, very little gp120 labeled with [^{3}H]mannose in the presence of Tu was visible; this represents incomplete inhibition by Tu with resultant normal processing yielding gp120 of molecular mass equivalent to the control (lane C).

While the minor differences seen between Bcu, dMM, dNM, Sw, and their respective controls were even less obvious in other experiments, the greatly diminished band observed with Csp relative to the control was unexpected and was a reproducible phenomenon. Since Csp causes the accumulation of high-mannose-type glycoproteins (26–28), a reduction in mannose incorporation would not seem to be responsible. We suspect, therefore, that synthesis in the presence of Csp lessened the affinity of gp120 for gp41, thus reducing the amount of gp120 bound to virus. This is supported by evidence that gp120 is not covalently bound to gp41 by disulfide bonds (31) and that gp120 is readily shed from virus particles *in vitro* (32). Furthermore, *in vitro* mutagenesis has identified several regions in the gp120 and gp41 molecules that function as contact points between these two glycoproteins (18); mutation of an N-glycosylation site in this region of gp120 [residue 280, numbered according to Ratner *et al.* (33)] has been shown to attenuate HIV-1 infectivity without affecting the synthesis, processing, and stability of gp120 and gp41 or the binding of gp120 to the CD4 molecule (34). Reductions in virus production or gp120

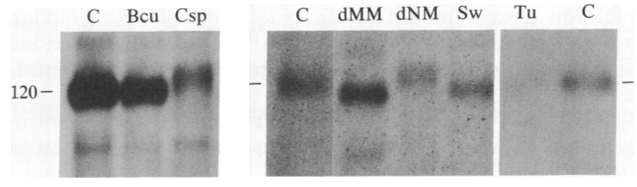

FIG. 1. [^{3}H]Mannose-labeled HIV-1 gp120 produced in the presence or absence of glycosylation inhibitors. Equal-density H9/HTLV-III$_B$ cultures in 20 ml of growth medium were treated with no inhibitor (control, lanes C), 0.25 mM Bcu, 1 mM Csp, 1 mM dMM, 1 mM dNM, 1 mM Sw, or 0.05 μg of Tu per ml.

(gp125) synthesis do not account for our observed loss of gp120 in the presence of Csp, since virus-associated RT activity in H9/HTLV-III$_B$ cultures was not reduced (Table 1) and since cell-derived gp120 (gp125) and immunoprecipitated gp120 (gp125) in cell-free culture supernatants of Csp-treated H9/HTLV-III$_B$ cultures have been shown to be unaffected (35).

The apparent decrease in molecular mass from gp120 to gp110 in the presence of dMM or Sw is in agreement with the known ability of these inhibitors to block the formation of high molecular weight, complex-type oligosaccharides (26–28). A decrease of 10 kDa is well within reason, since nearly 50% of the gp120 molecular mass is in the form of carbohydrate as determined electrophoretically after enzymatic removal of carbohydrate (36) and as estimated from the amino acid sequence of the protein backbone (4). However, it is also possible that altered proteolytic processing of the gp160 precursor was responsible for these changes.

The increase in apparent molecular mass of gp120 synthesized in the presence of Csp or dNM is more difficult to explain. Altered proteolytic processing of the gp160 precursor such that a larger form of gp120 was generated with a corresponding smaller form of gp41 is unlikely, since on occasions when both gp120 and gp41 have been observed, both demonstrated increased apparent molecular size (data not shown). Further, Walker *et al.* (35) found that cell-derived gp160 precursor has an increased apparent molecular size on NaDodSO$_4$/PAGE when synthesized in the presence of Csp. A more likely explanation is that the carbohydrate moieties on gp160 that accumulate in the presence of Csp and dNM are larger than fully processed moieties. Biochemical characterization of these carbohydrates is needed.

The absence of an effect of Bcu on the electrophoretic mobility of gp120 (Fig. 1) must be viewed with caution since Bcu is highly unstable. However, the cell toxicity observed (Fig. 2) indicates that Bcu was present in sufficient amounts long enough to have physiological effects on exposed cultures. The lack of antiviral activity with Bcu (Fig. 2) suggests that glucosidase II activity could have been circumvented by an alternative mechanism of glucose removal. A similar phenomenon has been observed in a glucosidase II-deficient mouse lymphoma cell line where oligosaccharide processing continued to result in complex-type carbohydrate formation (37). Further, an enzymatic activity that hydrolyzes the glucosyl-$\alpha(1\rightarrow3)$-mannose bond of N-linked oligosaccharides and that is insensitive to Bcu has been identified (38, 39).

Viral Infectivity as a Function of Protein Glycosylation. Any effect of glycoprotein processing inhibitors against HIV-1 *in vitro* could come in one or more of several forms, including the inhibition of virus production, the synthesis of defective viral particles, or the synthesis of altered cell surface HIV-1 receptor molecules. Initial identification of inhibitors having anti-HIV-1 activity by any of these mechanisms was made by a microtiter infection assay at low multiplicity of infection, thus allowing progeny virions synthesized in the presence of inhibitors to be responsible for virus-induced CPE while permitting changes in HIV-1 receptor molecules to potentially account for differences in CPE as well. Results of these infection assays identified Tu, Csp, and dNM as having anti-HIV-1 activity, whereas Bcu, dMM, and Sw had none (Fig. 2). Optimal concentrations of the inhibitors demonstrating anti-HIV-1 activity were 0.031–0.062 μg/ml for Tu and 0.25–1.0 mM for Csp and dNM. These optimal concentrations had little or no toxic effect on the MT-2 cells used as targets for infections (Fig. 2).

One approach to characterizing the anti-HIV-1 effect of these inhibitors was to examine their effects on virus production and infectivity (Table 1). None of the inhibitors was found to inhibit virus production, since RT values never fell significantly (within 10% error, which is generally our range

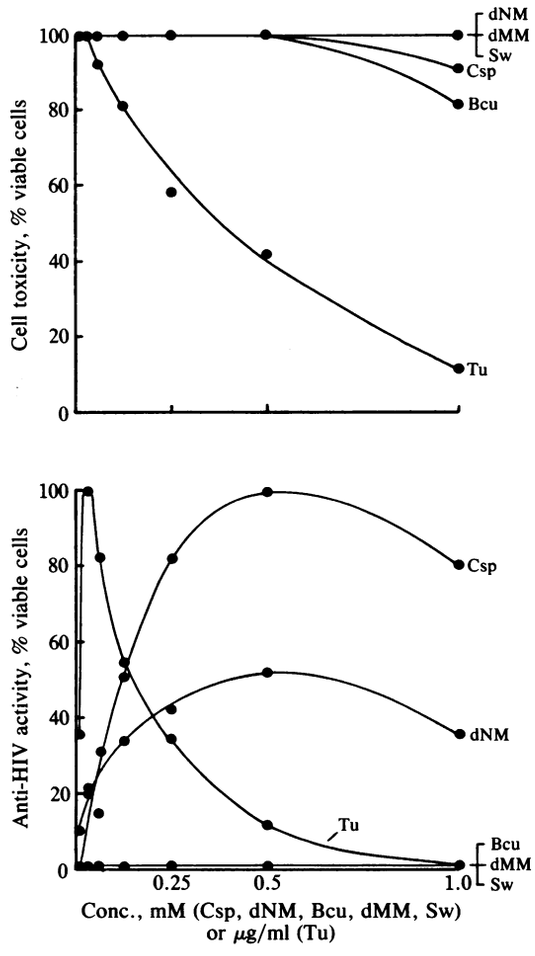

FIG. 2. Toxicity and anti-HIV-1 activity of glycoprotein processing inhibitors in MT-2 cell microtiter assays. Virus was added at a multiplicity of infection of 0.1. Cell toxicity assays were performed as described for microtiter infection assays except that virus was omitted.

of variability between samples) below the control culture values. It was interesting that RT values were significantly higher for Csp, dNM, and Tu than for the control. This was apparently due to a delay in cytopathicity and therefore a

Table 1. Effect of glycoprotein processing inhibitors on infectious viral yields

Inhibitor	RT activity, cpm × 10^{-3} per ml	Infectious unit per cpm of RT activity
Csp (0.25 mM)	2354	0.01
dNM (0.5 mM)	1328	0.16
Bcu (0.25 mM)	915	0.24
dMM (0.5 mM)	756	0.032
Sw (0.5 mM)	766	0.28
Tu (0.05 μg/ml)	2134	0.0038
None	830	0.26

Cultures of MT-2 cells were challenged with the HIV-1 isolate HTLV-III$_B$ (H9) at a multiplicity of infection >1. Virus was allowed to adsorb for 5 hr and then was removed by washing in growth medium. The cells were then incubated in the presence or absence of inhibitor as indicated for 4 days with one change of the growth medium (± inhibitor) after 2 days. All cells were positive at this time for HIV-1 antigen synthesis as judged by IFA. Conditioned culture fluids were made cell-free by low-speed centrifugation followed by 0.45-μm filtration and then were assayed for RT activity and infectious viral units.

prolonged synthesis of virus particles since, in the remaining cultures, the majority of cells had lysed as a result of infection by day 4. $TCID_{50}$ assays revealed that HIV-1 infectivity was attenuated when virus was synthesized in MT-2 cells in the presence of 0.25 mM Csp, 0.5 mM dNM, 0.5 mM dMM, or 0.05 μg of Tu per ml: the ratios of infectious units to RT activity (cpm) were 0.01, 0.16, 0.032, and 0.0038, respectively, relative to a control value of 0.26. Values of 0.24 and 0.28 for Bcu (0.25 mM) and Sw (0.5 mM), respectively, indicated that these inhibitors had no effect on HIV-1 infectivity. Since N-glycosylation appears to be critical for full HIV-1 infectivity, it is not surprising that Tu, which inhibits overall glycosylation, was more effective at attenuating HIV-1 than inhibitors of carbohydrate-trimming enzymes. An unexpected result was that dNM was much less effective than Csp, although both inhibit glucosidase I. This apparent discrepancy may be explained by differences in the stability or metabolism of Csp and dNM in culture. Alternatively, isozymes of glucosidase I could exist that exhibit different inhibition profiles for Csp than for dNM. These differences may also be explained by other sites of action of each inhibitor. For example, dNM has been reported to inhibit glycosylation (40) and to block the formation of the lipid-linked oligosaccharide precursor Glc_3-Man_9-$(GlcNAc)_2$-*PP*-dolichol (41).

Our results with Csp and dNM in MT-2 cells are consistent with the results of others using the HIV-1 isolates $HTLV\text{-}III_B$ and CLB-32 made in H9 cells and using H9 and phytohemagglutinin-stimulated peripheral blood lymphocytes as targets (35, 42, 43). Since Gruters *et al.* (42) failed to identify the anti-HIV-1 activity that we observed for the mannosidase I inhibitor dMM, the effect of producer and target cell lines on this anti-HIV-1 activity was characterized further. dMM at 0.062–1.0 mM had no effect on virus production as judged by RT activity in culture fluids; however, the infectivity of HIV-1 was dramatically attenuated in a dose-dependent manner to a maximum reduction factor of 120–130 at 0.5–1.0 mM dMM (Table 2). This effect was much greater than that observed for $HTLV\text{-}III_B$ produced in MT-2 cells, where the optimal concentration of dMM reduced infectivity by a factor of only 8 (Table 1). These results suggest that isozyme forms or other cellular factors may influence the effectiveness of inhibitors of carbohydrate-trimming enzymes and may vary between different host cells. These differences were investigated further by using virus made in the presence or absence of dMM in two producer cell lines ($H9/HTLV\text{-}III_B$ and $U\text{-}937/HTLV\text{-}III_B$) to infect four different target cell lines (H9, CEM, MT-2, and U-937). Although the previous set of data (Table 2) suggested quantitative differences between dMM responses in different cell types, no qualitative differences were identified (Table 3). Therefore, the anti-HIV-1 activity of dMM, although demonstrating quantitative variability, is common to many HIV-1-permissive cell types.

Although dMM caused the production of attenuated virus in $H9/HTLV\text{-}III_B$ and $U\text{-}937/HTLV\text{-}III_B$ cells as determined with H9, CEM, MT-2, or U-937 cells as targets (Tables 2 and 3), it never demonstrated anti-HIV-1 activity in the MT-2 cell microtiter infection assay (Fig. 2). We later found that if MT-2 cells were preincubated in the presence of dMM for 2 days prior to virus challenge, progeny virions synthesized in the presence of dMM were not attenuated. However, progeny virions synthesized in the presence of dMM were attenuated if there was no preincubation with dMM (data not shown). We conclude, therefore, that MT-2 cells have a compensatory mechanism making them resistant to the antiviral effects of dMM after an initial exposure period. The nature of the compensatory mechanism is unknown.

Table 2. Dose-dependent effect of dMM on HIV-1 infectivity

dMM, mM	RT activity, cpm × 10^{-3} per ml	Infectious unit per cpm of RT activity
0	1611	0.097
0.062	1458	0.021
0.125	1532	0.020
0.25	1624	0.0038
0.5	1755	0.0007
1.0	1482	0.0008

Equal-density cultures of washed $H9/HTLV\text{-}III_B$ cells were incubated in the presence or absence of various concentrations of dMM for 2 days with one change of the medium and fresh inhibitor after 1 day.

Table 3. Anti-HIV-1 activity of dMM in different cell types

	% IFA-positive cells							
	H9		CEM		MT-2		U-937	
Source of virus	−	+	−	+	−	+	−	+
$H9/HTLV\text{-}III_B$	100	10	27	5	NT	NT	50	1
$U\text{-}937/HTLV\text{-}III_B$	100	10	65	<1	50	2	50	2

Virus was obtained from the indicated chronically infected cultures after cells had been washed in growth medium and incubated for 2 days in the presence (+) or absence (−) of 0.5 mM dMM. Conditioned culture fluids were made cell-free by low-speed centrifugation and filtration (0.45-μm pore size) and then used for virus challenge. IFA to detect HIV-1 antigen synthesis was performed every 2 days after virus challenge. Data represent day 6 for H9 cells, day 10 for CEM cells, day 2 for MT-2 cells, and day 8 for U-937 cells. NT, not tested.

HIV-1-Induced Syncytium Formation and Cytopathicity. When $H9/HTLV\text{-}III_B$ cells that had been incubated for 2 days in the presence of Csp, dNM, dMM, or Tu were mixed with untreated MT-2 cells, syncytium formation and cytopathicity were greatly reduced (Fig. 3). In contrast, only small reductions were observed for Bcu and Sw in these experiments. These results suggest that HIV-1-induced syncytium formation and its associated CPE are dependent on proper glycoprotein processing involving the activity of glucosidase I and mannosidase I but not the activity of glucosidase II or mannosidase II. In reciprocal experiments where untreated $H9/HTLV\text{-}III_B$ cells were mixed with MT-2

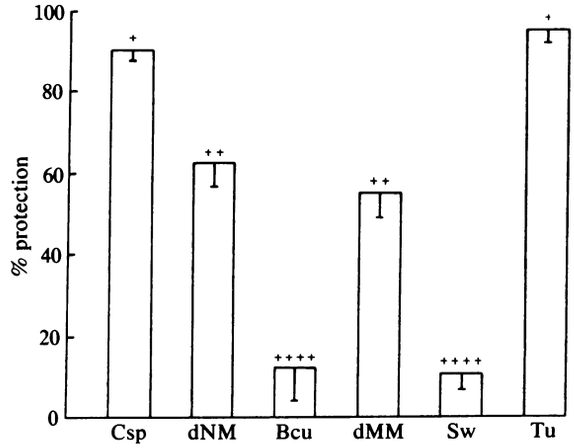

FIG. 3. Effect of glycoprotein processing inhibitors on HIV-1 induced syncytium formation and cytopathicity. Cultures of washed $H9/HTLV\text{-}III_B$ cells were incubated in the presence or absence of inhibitors for 2 days with one change of the growth medium after 1 day. Syncytia were scored after 24 hr by microscopic examination (×100 magnification): +, <1% of cells involved in syncytium formation; ++++, >90%. Data represent the mixture of inhibitor-treated $H9/HTLV\text{-}III_B$ cells with untreated MT-2 cells. Csp, dNM, Bcu, dMM, and Sw were used at 0.5 mM; Tu was used at 0.05 μg/ml. These concentrations were maintained after uninfected and infected cells were mixed.

cells preincubated for 2 days in the presence or absence of inhibitors, no attenuation of syncytium formation or cytopathicity was observed (data not shown); therefore, proper glycosylation of HIV-1 target-cell proteins is apparently not required for HIV-1-induced syncytium formation and cytopathicity. Similarly, with untreated MT-2 and H9/HTLV-III_B cells, the addition of inhibitor at the time of mixing had no effect on syncytium formation and cytopathicity (data not shown). Thus, the ability of glycoprotein processing inhibitors to block H9/HTLV-III_B-mediated syncytium formation and cytopathicity (Fig. 3) was not merely due to the physical presence of inhibitor during incubation of the mixed cells.

Conclusions. The results reported here demonstrate that the full pathogenic potential of HIV-1 *in vitro* is realized only if viral envelope proteins are N-glycosylated and the carbohydrate moieties are subsequently processed correctly. The pathogenic mechanisms affected by protein glycosylation include virus infectivity and cytopathicity, whereas mechanisms apparently unaffected include virus replication and target-cell infectibility. Once HIV-1 envelope proteins are glycosylated, the carbohydrate moieties must be processed by actions that include the activities of glucosidase I and mannosidase I in order to elicit a full pathogenic response. In contrast, the processing activities of glucosidase II and mannosidase II appear to be dispensable. These results are consistent with studies utilizing lectins (44, 45) and endoglycosidase F (36), which have yielded evidence suggesting a major functional role for surface carbohydrate in HIV-1 pathogenesis (44, 45). In view of these findings, inhibitors of glycoprotein processing that act at the levels of lipid-linked oligosaccharide precursor synthesis, glucosidase I activity, and mannosidase I activity may have potential as anti-HIV-1 therapeutic agents. Since carbohydrates can act as antigenic determinants of glycoproteins (46), future investigations may find an equally important role for protein glycosylation in the host immune response to this virus as well.

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