Glycosylation inhibitors and neuraminidase enhance human immunodeficiency virus type 1 binding and neutralization by mannose-binding lectin

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Mannose-binding lectin (MBL), a C-type lectin component of the human innate immune system, binds to the gp120 envelope glycoprotein of human immunodeficiency virus type 1 (HIV-1). The objective of this study was to assess the effects of inhibitors of endoplasmic reticulum glucosidases and Golgi mannosidase as well as neuraminidase (NA) on the interaction between HIV and MBL. Production of HIV in the presence of the mannosidase I inhibitor deoxymannojirimycin (dMM) significantly enhanced binding of HIV to MBL and increased MBL neutralization of an M-tropic HIV primary isolate. In contrast, culturing HIV in the presence of α-glucosidase I and II inhibitors castanospermine and deoxynojirimycin only slightly affected virus binding and neutralization by MBL. Removal of sialic acid from HIV by NA also significantly enhanced virus binding and neutralization by MBL. Treatment of virus grown in the presence of dMM with endoglycosidase F1 substantially reduced binding to MBL, indicating that dMM increased MBL binding by increasing high-mannose carbohydrates on the virus. In contrast, endoglycosidase F1 did not decrease the MBL interaction with NA-treated virus, suggesting that NA exposed novel MBL binding sites. Treatment with dMM increased the immunocapture of HIV by monoclonal antibodies 2F5 and 2G12, indicating that altering the glycosylation of viral glycoproteins increases the accessibility or reactivity of some epitopes. This study shows that specific alterations of the N-linked carbohydrates on HIV gp120/gp41 can enhance MBL-mediated neutralization of virus by strengthening the interaction of HIV-1 with MBL.

INTRODUCTION

The gp120 glycoprotein of human immunodeficiency virus type 1 (HIV-1) initiates infection by interacting with CD4 on cells. While many studies have explored the feasibility of inducing antibodies to gp120 to inhibit HIV-1 replication, the efficacy of this approach in vivo is limited by the rapid mutation of the variable portions of the peptide backbone of gp120 and the dense glycosylation of gp120 that blocks antibody access to conserved regions of gp120 (Wyatt et al., 1998). In fact, approximately half of the molecular mass of gp120 consists of N-linked carbohydrates. For example, the gp120 of HIV-IIIB was found to contain 13 complex-type glycans and 11 high-mannose or hybrid-type glycans (Leonard et al., 1990).

Mannose-binding lectin (MBL) is a C-type lectin, present in human serum, that acts as an effector molecule of the innate immune system (Jack et al., 2001; Petersen et al., 2001). MBL binds to carbohydrates on microorganisms that express repetitive mannose and/or N-acetylglucosamine residues, such as Candida albicans, Salmonella typhimurium and Neisseria gonorrhoeae, resulting in opsonization and activation of the lectin complement pathway (van Emmerik et al., 1994; Neth et al., 2000). Several studies have shown that MBL also interacts with gp120 of HIV-1. For example, complement was activated following MBL binding to purified gp120 (Haurum et al., 1993), recombinant MBL bound to gp120 and gp160 purified from HIV-IIIB (Ohtani et al., 1999) and preincubation of HIV with MBL inhibited infection of a T cell line (Ezekowitz et al., 1989). Furthermore, HIV particles lacking gp120/gp41 do not bind MBL, indicating that carbohydrates on gp120 mediate the interaction between whole virus and MBL (Saifuddin et al., 2000). A recent study showed that MBL binds to HIV via high-mannose carbohydrates on gp120 (M. L. Hart, unpublished).

Several enzyme inhibitors are available that prevent the formation of complex and hybrid N-linked saccharides during glycoprotein processing in the endoplasmic reticulum (ER) and Golgi (reviewed in Sears & Wong, 1998). For example, castanospermine (Csp) and 1-deoxynojirimycin (dNM) inhibit α-glucosidase I and α-glucosidase II, respectively, in the ER while 1-deoxymannojirimycin (dMM)
inhibits mannosidase I in the Golgi. Treatment of cells with these inhibitors results in N-linked carbohydrates that lack sialic acid but have a relatively high content of mannose residues. Many of the inhibitors have been shown to decrease the infectious titre of HIV in vitro, possibly by inhibiting the correct folding of gp160 (Gruters et al., 1987; Jacob, 1995; Karpas et al., 1988; Mehta et al., 1998; Montefiori et al., 1988; Walker et al., 1987). Several of these drugs have been evaluated in vivo as treatments for conditions such as diabetes, cancer metastases and viral infections including HIV infection (Jacob, 1995).

While the high density of oligosaccharides on gp120 is thought to play a protective role by reducing the immunogenicity of gp120, it is possible that the glycans on gp120 could be used as a target in some antiviral strategies. The objective of the current study was to explore the effect of glycosylation inhibitors on the interaction between HIV and MBL, since alteration of virus carbohydrates in vitro could increase the amount of MBL-binding carbohydrates on the virus surface, resulting in more efficient clearance and/or neutralization of virus. Since one effect of these drugs is to prevent addition of sialic acid residues to gp120 as it is neutralized, we also tested the effect of treatment of virus with neuraminidase (NA) on binding and neutralization of HIV by MBL. Our results showed that both NA treatment and certain glycosylation inhibitors strengthen the interaction of HIV-1 with MBL and thereby enhance MBL-mediated neutralization of HIV-1.

**METHODS**

**Cells and viruses.** T lymphoblastic H9 cells were obtained from the AIDS Research and Reference Reagent Program (ARRRP), National Institutes of Health (NIH, Rockville, MD, USA), and grown in RPMI 1640 medium containing 10 % foetal bovine serum (FBS) and 50 μg gentamicin ml⁻¹ (all from BioWhittaker). Human liver fibroblast (HLF) cells, obtained from Toshishuki Kawasaki (Kyoto University, Japan), were grown in DMEM (BioWhittaker) supplemented with 10 % FBS and gentamicin.

The HIV-1MN (X4) virus obtained from AARRP (contributed by Robert Gallo) was produced in H9 cells, and primary isolates of HIV (HIVCP, HIVTH and HIVna) were grown in peripheral blood mononuclear cells (PBMCs) obtained from normal healthy donors, as previously described (Takefman et al., 1998). The HIVCP (X4) and HIVTH (RS) were previously isolated in our laboratory (Takefman et al., 1998) and HIVna (RS) was obtained from the ARRRP. Phytohaemagglutinin (PHA; Sigma)-stimulated PBMCs were infected with HIV primary isolates in the presence of 30 U recombinant interleukin-2 ml⁻¹ (obtained through the AARRP from Maurice Gately, Hoffman LaRoche). For up to 10 days after infection, culture supernatants were harvested and tested for virus production by p24 ELISA (AIDS Vaccine Program, Frederick, MD, USA). To produce virus in the presence of inhibitors, PBMCs were infected for 7 days and were washed and cultured for an additional 48 h in the presence or absence of 1 mM Csp, dMM or dNM. Similarly, HIV-1MN-infected H9 cells were also washed and cultured for 48 h in the presence or absence of the drugs.

**Antibodies.** Serum from an HIV-antibody-negative AB+ donor was heat-inactivated (56°C for 50 min) as a source of normal human serum (NHS). Serum samples from five HIV-seropositive individuals were heat-inactivated and pooled in equal volumes (HIVPS). Monoclonal antibodies 2FS, 2G12 and IgG1b12 were obtained through AARRP. 2FS and 2G12 were contributed by Hermann Katinger (Purtser et al., 1994; Trkola et al., 1996), and IgG1b12 was contributed by Dennis Burton and Carlos Barbas (Barbas et al., 1992). Polyclonal sheep anti-HIVgp120 antibody to the conserved C-terminal sequence (aa 497–511) of HIV-1 gp120 (Moore et al., 1989) was purchased from International Enzymes.

**Reagents.** Csp was purchased from ICN Biomedicals and dNM, dMM and Clostridium perfringens a2–3,6,8-NA were obtained from Sigma. Endoglycosidase F1 was from Prozyme.

**Mannose-binding lectin.** Recombinant vaccinia virus expressing the cDNA sequence for human MBL was used to produce recombinant MBL (rMBL) (Ma et al., 1997). Briefly, HLF cells were infected at an m.o.i. of 5 and supernatants were collected 48 h after infection. MBL was purified by passage over a mannan-Sepharose 4B column, as previously described (Kawasaki et al., 1983). The resulting purified MBL was >95 % pure by Coomassie blue staining and Western blots of reducing gels. Some MBL monomers (approximately 30 kDa) were observed on non-reducing gels, but the majority of the purified material was estimated to contain multimers between 90 and 400 kDa.

**Western blotting.** Virus (10 ng p24) was lysed in SDS sample buffer, separated by 7.5 % SDS-PAGE and transferred to nitrocellulose membranes. Western blotting was performed as previously described (Takefman et al., 1998) using 2-5 μg sheep anti-gp120 antibody ml⁻¹ and horseradish peroxidase-conjugated rabbit anti-sheep immunoglobulin (Biosource). Antibody binding to gp120 was detected with an enhanced chemiluminescence detection system (Amersham) followed by exposure to X-ray film (Fuji Photo Film Co.).

**Glycosidase treatment.** Virus (0.25 ml at 40 ng p24 ml⁻¹) was treated with 1 U endoglycosidase F1 ml⁻¹ for 24 h at 37°C in the presence of 0.5 mM PMSF (Sigma). Virus samples were then diluted to 500 μl with veronal-buffered saline (5 mM veronal, pH 7.5, 0.145 M NaCl) containing 10 mM CaCl₂ (VBS-Ca). HIVTH was treated with 0.1 U NA ml⁻¹ (Sigma) overnight.

**Binding of HIV-1 to MBL.** Ninety-six-well tissue culture plates (Costar) were coated with 100 μl 10 μg rMBL ml⁻¹ diluted in VBS-Ca (Saifuddin et al., 2000). After overnight incubation at room temperature, wells were blocked with 3 % BSA for 1 h, washed with VBS-Ca and then incubated for 4 h with 100 μl (500 pg p24) of HIVCP, HIVTH or HIVna, grown in the presence or absence of glycosylation inhibitors. The plates were washed, virus bound was lysed with 0.5 % Triton X-100 and p24 was measured by ELISA.

**MBL Neutralization of HIV.** The TCID₅₀ of viruses produced in the presence or absence of inhibitors was determined by limiting dilution. Briefly, PHA-stimulated PBMCs (2 × 10⁶ cells) were infected for 24 h at 37°C with 0.1 ml of serial tenfold dilutions (1 : 10 to 1 : 100,000) of virus produced in 96-well tissue culture plates (Costar). Cells were washed and resuspended in 200 μl culture medium containing IL-2 (20 U ml⁻¹). After 7 days, culture wells were scored as positive or negative for virus growth by p24 ELISA. To assess HIV neutralization mediated by MBL, 100 TCID₅₀ of virus grown in the presence or absence of glycosylation inhibitors was treated with 10 μg rMBL ml⁻¹ for 2 h at 37°C. Treated virus was then cultured with PHA-stimulated PBMCs for 24 h. Cells were washed and resuspended in 200 μl culture medium containing IL-2, and on day 7 supernatants were assayed for virus production by p24 ELISA.

**Immunocapture of HIV.** Virus immunocapture was performed as previously described (Takefman et al., 1998). Briefly, virus was...
adjusted to 100 ng p24 ml\(^{-1}\) and incubated on ice for 1 h with NHS or HIVPS at a 1 : 10 dilution or with 50 µg 2F5, 2G12 or IgG1b12 antibody ml\(^{-1}\). The virus–antibody mixtures were then ultracentrifuged over 20 % (w/v) sucrose in 50 mM Tris/HCl (pH 8.0) at 150 000 g for 1 h. Virus was resuspended in RPMI 1640 medium. Approximately 200 pg p24 of purified virus was incubated overnight at 4 °C with 50 µl Staphylococcus aureus cells expressing protein A (Pansorbin). Cells were then washed with PBS containing 1 % BSA and bound virus was lysed by treatment with 0.5 % Triton X-100 and p24 measured by ELISA.

**RESULTS**

**The effects of N-linked glycosylation inhibitors on MBL binding to HIV-1**

To determine whether production of virus in the presence of N-linked glycosylation inhibitors altered the glycosylation of gp120, virus preparations grown in the presence of 1 mM Csp, dNM or dMM were analysed by Western blotting. As shown in Fig. 1(A), gp120 from HIV\(\text{MN}\) produced in the presence of either Csp or dNM was slightly decreased in mobility. In contrast, production of virus in the presence of the mannosidase I inhibitor dMM markedly increased the mobility of gp120. Similar changes in gp120 mobility were observed for HIV primary isolates produced in PHA-stimulated PBMCs (not shown). These changes in gp120 electrophoretic mobility are similar to those observed in several other studies using these three inhibitors (Dedera et al., 1990; Gruters et al., 1987; Montefiori et al., 1988; Walker et al., 1987). Similar changes in mobility were observed with 0.5, 1, 2 and 4 of each inhibitor for both HIV primary isolates and HIV\(\text{MN}\) (not shown). Therefore, 1 mM of each inhibitor was used in all further experiments.

We hypothesized that culturing virus in the presence of either α-glucosidase or mannosidase inhibitors would affect binding of virus to MBL, since it would potentially increase the N-linked terminal mannose residues and decrease the terminal sialic acid residues exposed on gp120. To test this, virus preparations were added to wells of microtitre plates coated with MBL, and HIV captured by MBL was determined by measuring p24 core protein. In a previous study, we found that this capture method sensitively detected binding of both HIV primary isolates and cell-line adapted virus strains to MBL, and that HIV bound to the carbohydrate-recognition domain of MBL, since binding did not occur in the absence of calcium and was blocked by pre-incubation of MBL-coated wells with mannan (Saifuddin et al., 2000).

Production of HIV\(\text{TH}\) in the presence of dMM significantly increased virus binding to MBL while binding to BSA-coated wells was not affected (Fig. 1B). Binding of virus to
MBL was significantly enhanced for all three primary isolates in multiple experiments (X4 HIVGP, R5 HIVTH, \( P<0.0005 \), t-test; R5 HIVBa-L, \( P<0.05 \), t-test) when grown in the presence of dMM (Fig. 1C). In contrast, virus binding only slightly increased when grown in the presence of dNM, while Csp treatment did not significantly increase binding (Fig. 1C). None of the glycosylation inhibitors increased binding to BSA-coated wells (not shown). The inhibitor dMM also significantly increased the binding of HIVMN produced in H9 cells to MBL, while Csp and dNM did not (not shown).

To determine whether virus binding to MBL was mediated by high-mannose-type carbohydrates on the virus, HIVTH produced in the presence of dMM was treated with endoglycosidase F1 (eF1), an enzyme that preferentially cleaves N-linked high-mannose and hybrid oligosaccharides. MBL binding to HIVTH grown in the presence of dMM was substantially reduced when virus was treated with eF1, providing evidence that the increase in virus binding to MBL due to dMM was the result of an increase in the number of N-linked high-mannose or hybrid glycans on the virus surface (Fig. 2). Since the major difference between the structure of N-linked carbohydrates produced in the presence of either α-glucosidase and mannosidase I inhibitors is two to three terminal glucose residues, these data suggest that terminal glucose residues substantially inhibit the interaction of MBL with N-linked high-mannose glycans.

**The influence of glucosidase and mannosidase inhibitors on neutralization mediated by MBL**

Virus-infected cells were grown in the presence of glycosylation inhibitors, and supernatants containing virus were harvested. Viable cell numbers were not affected by the inhibitors and no significant inhibition of virus production was observed, since p24 values were similar in control-treated cultures and cultures with drug concentrations from 0.5 to 4 mM (not shown). However, HIVTH produced in PBMCs treated with 1 mM of each of the inhibitors showed an approximate 1 log reduction in infectious virus compared with virus produced from untreated cells (Fig. 3A). Similar reductions in virus titres from all three inhibitors were also observed for HIVGP and HIVBa-L (not shown).

The ability of MBL to neutralize HIV primary isolates produced in the presence of glycosylation inhibitors was assessed. MBL mediated low levels of neutralization of the three primary isolates (2–10%) when produced in the absence of glycosylation inhibitors (Fig. 3B). Production of virus in the presence of dMM resulted in a trend towards increased neutralization by MBL, since neutralization increased 2.5- to 6-fold depending on the virus strain. However, only the increase in neutralization of HIVTH
was significant at the $P<0.05$ level, while HIVBa-L showed near significant neutralization ($P<0.10$). In contrast, virus production in the presence of Csp or dNM did not significantly increase neutralization by MBL.

Since one effect of these three inhibitors is to prevent addition of sialic acid to N-linked carbohydrates, we also assessed the effect of NA removal of sialic acid on MBL-mediated neutralization and binding. To confirm that NA treatment removed sialic acid from gp120, a gp120 Western blot was performed. Treatment of virus with NA removed a substantial amount of sialic acid residues from gp120, as evidenced by a change in mobility to approximately 105 kDa (Fig. 4A). NA treatment significantly increased neutralization by MBL ($P<0.0005$, t-test) (Fig. 4B). The increase in MBL-mediated neutralization due to NA treatment corresponded to an approximate fourfold increase in binding of virus to MBL (Fig. 4C). Interestingly, eF1 did not significantly decrease the amount of NA-treated virus binding to MBL, suggesting that NA exposed MBL binding sites on the virus that were not N-linked high-mannose glycans (Fig. 4C).

**Effect of dMM on interaction with anti-HIV antibodies**

The results shown above indicate that changes in the glycosylation of HIV affect virus interaction with MBL. Experiments were also performed to determine whether the dMM-induced changes in carbohydrates on intact virus affected the immunoreactivity of anti-gp120 or anti-gp41 antibodies. Virus was grown in the presence or absence of dMM and assessed for binding to either several monoclonal antibodies or HIVPS. Production of HIV in the presence of dMM had no effect on the amount of virus bound by IgG1b12 anti-CD4 binding site monoclonal antibody or HIVPS (Fig. 5). However, treatment with dMM significantly increased virus immunocapture by the anti-gp41 2F5 monoclonal antibody (Muster *et al.*, 1993). A significant increase in immunocapture due to dMM was also seen for the 2G12 antibody, which recognizes a conformational, carbohydrate-dependent epitope in the C3–C4 region of gp120 (Trkola *et al.*, 1996). These results indicated that while production of virus in the presence of dMM increases virus interaction with MBL, alteration of carbohydrates on gp120/ gp41 by dMM can also enhance the immunoreactivity of HIV with some antiviral antibodies but not others.

**DISCUSSION**

This study has shown that alteration of carbohydrates on HIV can increase binding and neutralization of virus by MBL. Binding and neutralization by MBL were significantly increased when virus was produced in the presence of the mannosidase I inhibitor dMM or when virus was treated with NA. In contrast, MBL binding and neutralization were relatively unchanged when virus was produced in the presence of Csp and dNM, inhibitors of $\alpha$-glucosidase I and II, respectively. Treatment with the mannosidase I inhibitor results in N-linked glycans containing approximately nine mannose residues terminated with mannose while treatment with the glucosidase inhibitors yields similar glycans terminated with two or three glucose residues (Sears & Wong, 1998). Therefore, this study has shown that increasing
the high-mannose glycans on HIV increases virus neutralization by MBL. Furthermore, the data suggest that terminal glucose residues inhibit the interaction of MBL with N-linked glycans.

Treatment with NA also substantially increased virus neutralization and binding by MBL. Removal of sialic acid by NA has several potential effects on MBL binding, including reducing negative charge on the virus, reducing the size of the complex glycan and revealing galactose as a terminal sugar residue. However, the increase in virus neutralization by MBL was not likely to be due to a reduction of the negative charge or a decrease in the size of the glycans, since the α-glucosidase inhibitors would have had these same effects on the virus but had little effect on neutralization. Furthermore, in contrast to virus produced in the presence of dMM, eF1 treatment did not reduce the amount of NA-treated virus binding to MBL. Together, these data indicate that NA treatment increased neutralization by exposing new MBL binding sites on the virus that were distinct from N-linked high-mannose carbohydrates, although it is possible that the identity of the binding sites is unclear since it is reported that MBL binds well to terminal N-acetylgalactosamine, mannose, glucose and fucose residues, but binding is reduced or blocked by terminal galactose (Childs et al., 1989). However, although sparse, some terminal N-acetylgalactosamine residues are normally present on N-linked complex structures including gp120 (Malhotra et al., 1995; Scanlan et al., 2002), and it is possible that removing sialic acid provides better access of MBL to these residues.

While MBL is a serum protein, it reacts weakly with host high-mannose N-linked carbohydrates due to the relatively low density of these glycans and the low affinity of MBL monomers for sugars. In contrast, the multimeric nature of both native MBL and terminal MBL-binding sugar residues on microbes results in a high-avidity interaction. Also, the conformation of MBL suggests that it does not react with multiple terminal mannose residues within one N-linked high-mannose carbohydrate structure and therefore reactivity of MBL with one high-mannose carbohydrate structure is likely to be of low avidity (Weis et al., 1998). In the case of HIV gp120, there is a very high density of N-linked carbohydrates (about half being the high-mannose type), which most likely accounts for the interaction of HIV with MBL. Thus, MBL reacts strongly with HIV but does not bind to virus particles lacking gp120 (Saifuddin et al., 2000) and we observed in the current study that treatment of HIV with eF1 substantially reduced binding of virus to MBL. While treatment with the mannosidase I inhibitor would change host-cell protein complex glycosylation to the high-mannose type and could thus contribute to MBL binding to virus, we propose that the majority of the dMM-mediated increase in binding between HIV and MBL is most likely to be due to an increase in high-mannose carbohydrates on gp120, since dMM treatment would most likely double the density of MBL binding sites on gp120. Since the complex carbohydrates on gp120 are thought to be relatively exposed, their change to the high-mannose type may have an especially large effect on MBL binding (Moore et al., 1994; Wyatt et al., 1998).

Two glycosylation inhibitor drugs have been tested in HIV-infected people since early studies showed that some of these inhibitors had antiviral activity in vitro (Jacob, 1995). However, both in vivo trials used α-glucosidase inhibitors. According to our studies, this alteration of carbohydrates would probably not have substantially affected the interaction of virus with MBL in vivo. Mannosidase inhibitors have been used in vivo in trials to inhibit tumour metastasis (Jacob, 1995). Our data suggest that administration of mannosidase inhibitors during HIV infection could increase MBL-mediated antiviral effects such as neutralization, complement activation and virus clearance. These enhanced MBL effects could also change the way that virus is presented to the adaptive immune system by affecting virus uptake by antigen-presenting cells.
While the binding of MBL to virus was increased by dMM, virus binding to monoclonal antibodies 2F5 and 2G12 was also increased, although the virus interaction with pooled serum and monoclonal antibody IgG1b12 was unchanged. These results could be partially explained by studies showing that complex-type glycans have a more pronounced shielding effect on antibody binding and are located on more exposed regions of gp120 than high-mannose glycans (Back et al., 1994; Moore et al., 1994; Wyatt et al., 1998). Therefore, by inhibiting production of bulkier complex carbohydrates with dMM, virus is produced that binds at higher levels to some neutralizing antibodies. Alternatively, recent studies have shown that the 2G12 epitope is not only dependent on carbohydrates but also appears to be at least partially composed of mannose residues (Sanders et al., 2002; Scanlan et al., 2002). Therefore, it is possible that treatment with dMM increases the density of mannose residues on gp120 causing the increased binding of 2G12. Thus, alteration of carbohydrates can simultaneously affect the interaction of HIV with arms of both the innate and adaptive immune responses.

A study by Means & Desrosiers (2000) assessed the effect of glycosidases and glycosylation inhibitors on neutralization of simian immunodeficiency virus (SIV) by pooled sera from infected macaques. Treatment of two SIV strains with NA decreased neutralization sensitivity while other glycosidases did not have a consistent effect on sensitivity. Treatment of virus-producing cells with either the mannosidase inhibitor swainsonine or DANA, an inhibitor of sialic acid, also slightly decreased neutralization sensitivity. These results suggest that removal of sialic acid somehow increases the resistance of virus to antibody neutralization by the pooled sera, a somewhat unexpected result given that the bulk of complex glycans is thought to shield the envelope protein from antibody binding (Wyatt et al., 1998). The effect of NA and swainsonine in the SIV system are also interesting in light of our results with MBL neutralization of HIV, since we found that NA treatment of virus or dMM treatment of virus-producing cells increased MBL-mediated neutralization.

This is the first study to assess MBL-mediated effects on HIV after treatment of virus-producing cells with N-linked glycosylation inhibitors or treatment of virus with NA. The results show that, by altering the glycosylation of the envelope protein of HIV with dMM or NA, neutralization by and binding of MBL is increased. It is not known whether dMM or related compounds offer a realistic prospect of therapy for those infected with HIV, but this study suggests that further exploration of this possibility is warranted. Important issues to be considered before therapies are attempted include whether effective in vitro drug concentrations can be achieved and the resultant side effects. No clinical trials of dMM have yet been reported. A trial in HIV-infected people with the α-glucosidase inhibitor N-butyl-deoxyxojirimycin resulted in significant side effects including diarrhoea, flatulence, leukopenia and neutropenia (Tierney et al., 1995) and the concentration achieved in serum was slightly lower than the antiviral concentration predicted in vitro. Many of the glycosylation inhibitor drugs cause similar side effects (Jacob, 1995). N-onyl-deoxyxojirimycin, another α-glucosidase inhibitor, was shown to substantially reduce viraemia in hepadnavirus-infected woodchuck (Block et al., 1998). Also, the α-mannosidase I inhibitor swainsonine was given to cancer patients over 5 days and serum levels of the drug were achieved that were much higher than the 50 % in vitro inhibitory concentrations (Baptista et al., 1994). The above studies suggest that, while inhibitory concentrations of some of the glycosylation inhibitors could be achieved in vivo for treatments of short duration, long-term treatment with these types of drug, which is likely to be needed in the case of HIV-infected people, may be difficult due to their toxicity.

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REFERENCES


