Effect of a glucosidase inhibitor on the bioactivity and immunoreactivity of human immunodeficiency virus type 1 envelope glycoprotein

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Apparently conflicting results have been reported regarding the role of env glycoprotein glycans in human immunodeficiency virus type 1 (HIV-1) infectivity and cytopathogenicity. Whereas we have shown that enzymic removal of carbohydrates from mature envelope glycoproteins has only limited effect on the ability of HIV-1 to bind to CD4 and to infect target cells, sugar analogues that interfere with the glycosylation process of the nascent molecule markedly reduce virus infectivity. Here we have investigated the effect of a glucosidase inhibitor, 1-deoxynojirimycin (dNM), on the bioactivity and immunoreactivity of precursor gp160 produced by recombinant vaccinia virus-infected BHK-21 cells (rgp160). dNM (4 mM) did not affect the amount of rgp160 recovered nor its secretion from the cells. As described by other authors the effect of dNM was incomplete, resulting in the production of rgp160, the glycosylation of which was heterogeneous with respect to apparent M, distribution and to sensitivity to endoglycosidase H and endoglycosidase F, all the species being susceptible to N-glycanase. A major reduction of the binding to CD4+ cells was noted with rgp 160 produced by dNM-treated cells using a quantitative indirect immunofluorescence assay and labelling with polyclonal human anti-HIV IgG. Similarly, dNM treatment altered the accessibility to murine monoclonal antibody 110-4 of the exposed V3 loop of HIV-1 gp120 by at least 10-fold, as determined by either ELISA capture assay or immunoaffinity purification. Such bioactivity and conformation modifications, which result from the abnormal folding of the nascent glycoprotein due to aberrant glycosylation, may account for the impaired HIV-1 infectivity elicited by dNM.

Introduction

The biological functions of human immunodeficiency virus type 1 (HIV-1) env glycoprotein glycans remain elusive although there is evidence to indicate that glycans are likely to play a major role in the HIV-1 life cycle: (i) carbohydrate moieties represent 50% of outer membrane glycoprotein (gp120) M, (Matthews et al., 1987; Fenouillet et al., 1989, 1990), (ii) N-glycosylation sites of the precursor gp160 sequence are highly conserved among the different primate immunodeficiency viruses (Alizon et al., 1986; Chakrabarti et al., 1987) and (iii) the carbohydrate composition of recombinant gp120 (rgp120) produced in different mammalian cell lines and of viral gp120 (vgp120) is similar (Geyer et al., 1987; Mizuochi et al., 1988; Fenouillet et al., 1989).

It is mostly the possible role of glycans in the interaction of HIV-1 with target cells, namely the binding of gp120 to the CD4 cell membrane receptor, the gp41-mediated fusion of the virus envelope and cell membrane, or syncytium formation (McCune et al., 1989; Freed et al., 1990; Klatzmann et al., 1990), that has been investigated so far. The results have been conflicting (Lifson et al., 1986; Gruters et al., 1987; Matthews et al., 1987; Robinson et al., 1987; Karpas et al., 1988; Montefiori et al., 1988; Fennie & Lasky, 1989; Fenouillet et al., 1989; Hansen et al., 1989; Kozarsky et al., 1989; Pal et al., 1989a). For example, different lectins can block HIV-1 infection in vitro (Lifson et al., 1986; Robinson et al., 1987; Hansen et al., 1989), but this simply results from steric hindrance (Anderson et al., 1991; L. Gattegno, A. Ramdani, T. Jouault, L. Saffar & J. C. Gluckman, unpublished results). Our own findings indicate that enzymic removal of glycans from mature rgp160 or rgp120, or from vgp120, does not significantly reduce the ability to bind to CD4 presented either in soluble form or at the cell membrane (Fenouillet et al., 1989, 1990). Moreover, when envelope glycoproteins of virions are completely deglycosylated, HIV-1 still binds to CD4+ cells with an affinity sufficient to result in infection, indicating that glycans of mature gp120/gp41 play but a limited role in HIV infectivity (Fenouillet et al., 1990). In contrast, Gruters et al. (1987) and others (Robinson et al., 1987; Karpas et al., 1988; Montefiori et
al., 1988; Kozarsky et al., 1989; Pal et al., 1989a) have demonstrated that sugar analogues which inhibit the processing of carbohydrate chains by interfering with the action of glucosidases or mannosidase I markedly reduce virus infectivity and syncytium formation. The apparent discrepancy between the latter and our results may be explained by the hypothesis that, after translation, glycans present on the mature envelope glycoproteins are not of paramount importance for HIV-1 infectivity, whereas abnormal glycosylation can affect the cellular routing, processing and folding of the glycoproteins, resulting in altered bioactivity.

As glycosylation is important for the bioactivity and/or immunoreactivity of glycoproteins (Olden et al., 1985; Fenouillet et al., 1986; Davis et al., 1990) and to test this hypothesis, we have compared the bioactivity and conformation of normal and glycosylation inhibitor-treated rgp160, as assessed respectively by its binding to CD4 and its immunoreactivity. We have used 1-deoxynojirimycin (dNM) (Elbein, 1987), a glucose analogue that inhibits by approximately 50 to 75% the removal of glucose residues from Glc3Man9GlcNAc2 moieties (Glc, glucose; Man, mannose; GlcNAc, N-acetyl glucosamine) by α-glucosidasides in the rough endoplasmic reticulum (RER) just after transfer of this glycan precursor to the nascent protein backbone (Kornfeld & Kornfeld, 1985). Thus, rgp160 produced in dNM-treated cells comprises a high number of mannosylated Glc3Man9GlcNAc2 species that cannot be further modified by glycosidases and glycosyltransferases, hence its aberrant glycosylation pattern.

Methods

Production of recombinant envelope glycoproteins. Cells of the BHK-21 line were cultured at 37°C in a humidified atmosphere of 5% CO2 in Glasgow medium (Gibco-BRL) with 5% foetal calf serum (FCS) in the presence of absence of 2 or 4 mM-dNM (a gift of R. Gruters and H. Ploegh, Red Cross Blood Transfusion Service, University of Amsterdam, The Netherlands). The culture was followed for 3 weeks to observe possible effects of dNM on cell viability and growth; cells were then infected with either a control or a recombinant vaccinia virus (0-1 plaque-forming units/ml) as described by Kieny et al. (1988). In the thymidine kinase gene of the latter the env coding sequence was replaced with the gene encoding the vaccinia virus TK (all human src gene). The recombinant virus, designated VSV30, was used as a specificity control for the binding of rgp160 to CD4 (Klatmann et al., 1990). In some experiments of the murine monoclonal antibody (MAb) 110-4 (Genetic Systems). After three washes with PBS, pH 7.4, 0.5% casein, 0.5% Tween 20, peroxidase-labelled anti-mouse or anti-human IgG antibodies (Amersham) were added at 1:25. After 2 h at 20°C and three more washes, staining was performed using diaminobenzidine in PBS with 1% H2O2. The results obtained with the different volumes of sample tested were compared with those obtained with a known reference rgp160 (batch ICC14; Pasteur-Mérieux Vaccins et Sérum, Marnes-la-Coquette, France).

Carbohydrate composition of the glycoproteins. Envelope glycoproteins were treated with endoglycosidases from Boehringer-Mannheim as previously described (Fenouillet et al., 1989, 1990). Briefly, 2 μg of rgp160 was mixed for 24 h at 37°C with 500 milliunits (mU) of endoglycosidase F–N-glycanase mixture (Endo FNG) which cleaves all glycan moieties, or with 500 mU of N-glycanase and endoglycosidase F (Endo F) which cleaves high-mannose and non-fucosylated biantennary chains, or with 5 mU of endoglycosidase H (Endo H) which cleaves high-mannose structures (Tarentino et al., 1989). No proteolysis was observed. Control rgp160 was mock-treated in the same manner. The bacterial rgp120 was treated with Endo FNG only.

Flow cytometry analysis. Increasing amounts of the different glycoprotein samples were incubated with 5 × 105 cells of the CEM 13 subclone (Fenouillet et al., 1989, 1990) in 100 μl of PBS, 0.5% bovine serum albumin (BSA), 0.05% NaCl, for 2 h at 20°C. This temperature allowed glycoprotein binding to the cells but prevented endoglycosidase activity when the enzymes were used (as discussed by Fenouillet et al., 1990). Staining was performed with a pool of polyclonal purified human anti-HIV-1 IgG from different patients and sheep anti-human IgG antibodies. Membrane fluorescence was measured using a fluorescence-activated cell sorter analyser (Becton-Dickinson).

Quantification of recombinant glycoproteins. The amount of rgp160 or rgp120 obtained in the different systems was measured as follows. Five, 2.5 and 1 μl of the different concentrations were blotted on a nitrocellulose filter and adjusted to the same 10 μl final volume of PBS, pH 7.4. After saturation, the nitrocellulose filter was incubated at 20°C for 2 h with a 100-fold dilution of human serum from an HIV-seropositive individual devoid of antibodies against vaccinia virus or, in some experiments, of the murine monoclonal antibody (MAb) 110-4 (Genetic Systems). After three washes with PBS, pH 7.4, 0.5% casein, 0.5% Tween 20, peroxidase-labelled anti-mouse or anti-human IgG antibodies (Amersham) were added at 1:25. After 2 h at 20°C and three more washes, staining was performed using diaminobenzidine in PBS with 1% H2O2. The results obtained with the different volumes of sample tested were compared with those obtained with a known reference rgp160 (batch ICC14; Pasteur-Mérieux Vaccins et Sérum, Marnes-la-Coquette, France).

Immunoreactivity of gp160

(i) ELISA. Microwell plates (Polyisorb; Nunc) were coated with 100 μl of MAb 110-4 diluted 1:100 in PBS. After 2 h at 20°C, the plates were washed with PBS, 0.05% Tween, and increasing amounts of concentrated env glycoproteins diluted in PBS were added and incubated overnight at 4°C. Endo FNG-deglycosylated rgp160 samples were also examined. After three washes with PBS, 0.05% Tween, purified polyclonal human anti-HIV-1 IgG antibodies (1:300 dilution; Amersham) were added for 2 h at 4°C. After four washes, substrate was added and absorbance at 450 nm was measured after 30 min.

(ii) Immunoaffinity purification. One mg of MAb 110-4 was incubated overnight at 4°C with 1 g of activated CNBr-Sepharose CL4B (Pharmacia) following the manufacturer’s instructions. One μg of rgp160 obtained from cells with or without dNM treatment was added for 10 h at 4°C, as described by Fenouillet et al. (1989). After washing, bound glycoproteins were eluted using 0.5 M-formic acid, 150 mM-
NaCl, and the pH was immediately raised to 7 using ammonium acetate (Fenouil et al., 1990). After lyophilization, 10 μl H₂O was added, and dot blot quantification of the glycoprotein present in the different eluates was performed with the anti-HIV-1 polyclonal human IgG or with MAb 110-4, revealed with appropriate anti-IgG antibodies. Results were compared with those of a standard curve obtained with the known reference rgp160 from batch ICC14.

Results

Characterization of rgp160 produced by dNM-treated cells (d⁺rgp160)

Concentrations of rgp160 from dNM-treated cells (d⁺rgp160) and of rgp160 from untreated cells (d⁻rgp160) were measured by a dot blot assay using a polyclonal anti-HIV human serum (Fig. 1a). The amounts of d⁺rgp160 and d⁻rgp160 (Fig. 1b) present in the concentrated supernatant were the same, 10 ng/ml approximately, as was the concentration of baculovirus rgp160 (Fig. 1c); bacterial rgp120 (Fig. 1d) was twice as concentrated; d⁻rgp160 and d⁺rgp160 also exhibited a similar immunoreactivity in dot blot assays using either MAb 110-4 as antibody (Fig. 1d) or heat-denatured rgp160 samples as antigens (data not shown), or in the Western blot (Fig. 2). No reactivity was noted when, as a control, 5 µl of vaccinia virus-infected cell supernatant was dotted on nitrocellulose, or when any step of the assay was omitted.

We next examined whether the carbohydrate structure of d⁺rgp160 was altered. As expected, d⁻rgp160 presented a glycosylation pattern that was identical to that of rgp160 from reference batch ICC14 when examined by endoglycosidase analysis (Fig. 2a, b). Approximately 50% of glycans were removed by Endo H or Endo F, and they were thus high-mannose or biantennary species; the remaining carbohydrates were composed of fucosylated or multiantennary structures that were resistant to these enzymes but sensitive to Endo FNG, which cleaves all glycans from the polypeptide backbone (Tarentino et al., 1989). The glycosylation pattern of d⁺rgp160 (Fig. 2c) was different from that of d⁻rgp160: in the absence of endoglycosidase treatment, the band that corresponded to d⁺rgp160 was spread between 170K and 140K, which is compatible with a family of molecules having highly heterogeneous glycosylation. The effect of dNM appeared to be incomplete when used at 2 mM (data not shown) as well as at 4 mM in that Endo H and Endo F treatment of d⁺rgp160 resulted in a smear corresponding to between 160K and 90K, indicating heterogeneity with respect to sensitivity to the enzymes. However, d⁺rgp160 was more susceptible to Endo H and Endo F and, thus, had more high-mannose species than d⁻rgp160.

Baculovirus rgp160, which is known to express glycans of the high-mannose type, had an Mᵣ of 140K. Both Endo H and Endo F treatments resulted only in a reduction to 110K, whereas Endo FNG led to a completely deglycosylated molecule of 90K (Fig. 2d). As expected, non-glycosylated bacterial rgp120 displayed a 60K band that was not affected by Endo FNG (Fig. 2e). In line with our previous findings (Fenouil et al., 1989, 1990), the efficiency of endoglycosidase treatment was independent of the presence of SDS (0-02%) and/or Triton X-100 (1%) (data not shown).
Both d-rgp160 and baculovirus rgp160 bound to the membrane of CD4+ cells to the same extent as rgp160 from batch ICC14, whereas the binding of d+rgp160 was strongly reduced; bacterial rgp120 did not bind at all (Fig. 3b).

Immunoreactivity of d+rgp160 as compared with d-rgp160

Proper processing of carbohydrates may direct the three-dimensional structure of glycoproteins and, therefore, influences their conformation and antigenicity (Alexander & Elder, 1984; Olden et al., 1985; Fenouillet et al., 1986; Davis et al., 1990). Whether this was the case for rgp160 was investigated by two approaches based on the recognition by MAb 110-4 of an epitope (amino acids 308 to 328) in the third variable domain (the V3 loop) of gp120. This MAb has comparable reactivity to native and enzymically deglycosylated d-rgp160 (Fenouillet et al., 1989), as well as to d-rgp160 and d+rgp160 blotted on a nitrocellulose filter (Fig. 4c, row E).

First, an ELISA was used, in which rgp160 was captured by MAb 110-4 and subsequently revealed with the pool of polyclonal human anti-HIV IgG known to recognize d-rgp160 and d+rgp160 to the same extent. Both baculovirus rgp160 and ICC14 rgp160 were similarly recognized in this test (Fig. 4a). However, 10-fold more d+rgp160 than d-rgp160 was required to obtain the same absorbance reading, but only a twofold reduction of immunoreactivity was noted when comparing Endo FNG-deglycosylated d-rgp160 with its untreated counterpart (Fig. 4a, b). In contrast, removal of glycans from d+rgp160 almost completely abolished binding to 110-4 MAb (Fig. 4b).

In the next series of experiments, 1 μg of d+rgp160 or d-rgp160 in solution was incubated overnight with 110-4-Sepharose complexes. After elution, bound rgp160 was blotted onto nitrocellulose, and again reacted with either human anti-HIV IgG, or MAb 110-4, in comparison with different known amounts of gp160 from batch ICC14 (Fig. 4c, row A). As controls, it was shown that Endo FNG present in the medium did not influence staining in the test (Fig. 4c, rows D and E), that supernatant obtained from acid elution of 110-4-Sepharose complexes in the absence of rgp160 could not be labelled by anti-human or anti-mouse IgG antibodies (Fig. 4c, rows D and E) and, thus, did not contain detectable amounts of MAb 110-4, and that 500 ng of deglycosylated d-rgp160 or d+rgp160 directly blotted on the filter were recognized to the same extent by the human or the mouse anti-gp160 antibodies (Fig. 4c, rows D and E). Here again, the ratio between soluble d-rgp160 and d+rgp160 retained by and eluted from 110-4-Sepharose was in the order of 10 (Fig. 4c, rows B and
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Fig. 4. Comparison of the immunoreactivity of rgp160 from different sources. (a and b) ELISA. Wells coated with MAb 110-4 were incubated with (a) ICC14 rgp160 (○), baculovirus rgp160 (●), d+rgp160 (●) or d-rgp160 (□) with the respective deglycosylated counterparts of d+rgp160 (d+rgp160 CHO-, (●)) and d-rgp160 (d-rgp160 CHO- (□)). The glycosylated d+rgp160 and d-rgp160 are represented by closed symbols, (●) and (□), respectively. (c) Immunoaffinity purification. Row A, decreasing amounts of rgp160 batch ICC14 were quantified in a dot blot assay using anti-HIV-1 human IgG. Positions: 1, 1000 ng of rgp160; 2, 500 ng; 3, 250 ng; 4, 120 ng; 5, 60 ng; 6, 30 ng; 7, 15 ng. Rows B and C represent d+rgp160 and d-rgp160, respectively, eluted from 110-4-Sepharose (incubated with 1 μg of d+rgp160 or d-rgp160 respectively). Positions 1 and 3, undiluted samples; positions 2 and 4, twofold diluted samples untreated (2) or treated (4) with Endo FNG before incubation with Sepharose. Row D. Position 1, eluate from 110-4-Sepharose not preincubated with C). As in the ELISA, Endo FNG-treated d+rgp160 was not retained by 110-4-Sepharose complexes in contrast to similarly treated d-rgp160 (Fig. 4c, row C).

Discussion

Our recent results (Fenouillet et al., 1989, 1990) indicate that, after translation and protein biosynthesis, carbohydrates present on mature env glycoproteins do not play a major role in HIV-1 infectivity. However, virus infectivity clearly depends on proper processing of carbohydrates of nascent glycoproteins (Gruters et al., 1987; Robinson et al., 1987; Karpas et al., 1988; Montefiori et al., 1988; Pal et al., 1989a), most probably because at early stages glycosylation can affect the cellular routing, processing and three-dimensional structure of these proteins.

To examine the role which sugar processing plays in the production of infectious HIV-1, we have investigated the modifications to rgp160 induced by an inhibitor of the biosynthesis and earlyprocessing of glycans, dNM. Such post-translational modifications, which occur in the RER and in the Golgi apparatus, may profoundly affect the proper synthesis, transport and secretion of glycoproteins. For example, the retention of Glc residues distorts routing in the RER; treatment with dNM inhibits formation of Sindbis virus particles from BHK cells (Elbein et al., 1987); and improperly glycosylated G protein of vesicular stomatitis virus is inefficiently transported to the plasma membrane (Gibson et al., 1981). With respect to HIV-1, virion formation is not affected by glucose trimming inhibitors such as dNM or castanospermine, as demonstrated by Gruters et al. (1987; and personal communication) and other authors (Karpas et al., 1988; Montefiori et al., 1988; Pal et al., 1989a), although these compounds as well as tunicamycin (which inhibits the biosynthesis of glycan precursor) or 1-deoxymannojirimycin (an inhibitor of mannosidase I) impair HIV-1 infectivity. Drugs that act on enzymes involved in later stages of the carbohydrate processing pathway have no observed effect (Gruters et al., 1987; Karpas et al., 1988; Montefiori et al., 1988).

Inhibition by dNM appears partial even at high concentrations such as 2 or 4 mM. This may explain the
low drug toxicity that is usually observed for cells, because only glycoproteins expressing a high number of glycans and/or the glycans of which are involved in folding are potentially affected by dNM treatment. But as rgpl60 contains more than 30 glycosylation sites (Geyer et al., 1988; Mizuochi et al., 1988; Fenouillet et al., 1989), the incomplete inhibition of α-glucosidases caused by dNM may still result in a sufficient number of aberrantly glycosylated glycans to lead to improper folding and perturb the bioactivity of the molecule. Under these conditions, dNM could be considered to be a rather specific potential antiviral agent. With this possibility in mind, we examined d+rgpl60 produced in high yield from eukaryotic cells using a concentration protocol that avoided any affinity step [antibody (Fenouillet et al., 1990) or lectin] so as to obtain an unselected population of molecules.

Under these conditions, dNM affected neither cell viability nor rgpl60 production, and inhibition of glucose trimming did not alter rgpl60 cell production and secretion (data not shown). However, d+rgpl60 displayed a wider range of M, than d-rgpl60 in Western blot, an indication of the heterogeneity in glycosylation of the molecules obtained from dNM-treated cells. Also endoglycosidase analysis of d+rgpl60 showed that some species were resistant to Endo H, even when they were more heterogeneous and in less significant amounts than was d-rgpl60. Such resistant species indicate that glycans other than high-mannose chains were present, which confirms the partial effect of dNM. A similar pattern was noted with Endo F, which cleaves also biantennary structures in addition to high-mannose chains. Endo FNG completely deglycosylated d+rgpl60 as d-rgpl60, even in the absence of denaturing agents, showing that possible modifications of conformation resulting from dNM treatment did not alter the accessibility of glycosylation sites to the enzyme. Two other recombinant glycoproteins were also investigated as controls: baculovirus-expressed rgpl60 was chosen as representative of a high-mannose-type glycosylated molecule (Morikawa et al., 1990b), and bacterial rgpl20 as a non-glycosylated molecule (Morikawa et al., 1990a). Baculovirus rgpl60 appeared more sensitive than d+rgpl60 to Endo H or to Endo F, but was not completely deglycosylated by Endo H as expected. This may be explained by the presence of some high-mannose-type glycans that may be resistant to the enzyme (Jarvis & Summers, 1989). However, using rgpl20 produced in a drosophila cell line, we could obtain complete deglycosylation of the molecule by Endo H, indicating efficacy of the enzyme (data not shown).

As described for other glycoproteins, abnormal glycosylation can affect the conformation of env glycoproteins, which may result in altered bioactivity. Concerning HIV-1 glycoproteins, Fennie & Lasky (1989) have shown that neither dNM nor deoxymannojirimycin impair the CD4-binding activity of rgpl120 produced in mammalian cells, indicating correct functional conformation; somewhat surprisingly, however, Endo H-mediated removal of high-mannose species from normal mature rgpl120 reduced its attachment to CD4, which is at variance with our previous results (Fenouillet et al., 1989, 1990). But in these experiments 1 mM-dNM was applied for only 1 h, the CD4-binding assay used was not quantitative and Endo H treatment was conducted on preformed CD4-gp120 complexes, and, thus, CD4-binding bioactive species of rgpl120 might have been selected. A similar conclusion was drawn from another study in which gp120-CD4 interaction was examined using a qualitative radioimmunoprecipitation assay (Pal et al., 1989b). For these reasons, we investigated this point using a quantitative CD4-binding assay already described (Fenouillet et al., 1989, 1990) and the whole heterogeneous rgpl60 population produced by dNM-treated cells. Under these conditions significant reduction of d+rgpl60 binding to CD4+ cells was observed. That this was not simply due to an excess of high-mannose species present on d+rgpl60 may be inferred by the observation that highly mannosylated baculovirus rgpl60 (Morikawa et al., 1990b) bound perfectly well to the cells. Thus, d+rgpl60 behaved almost like non-glycosylated bacterial gp120 with respect to CD4-binding, which argues that in both cases the bioactive conformation of the molecule was profoundly affected for related reasons. However, and although it is well known that proteins found in E. coli periplasm possess disulphide bonds (Skerra & Pluckthun, 1988), other mechanisms that result in incorrect folding may disturb a protein’s functional conformation in E. coli.

Because the major neutralization epitope of HIV-1 is located on the V3 loop, it is thought that this exposed region of gp120 must play an essential role in the post-CD4-binding events that lead to virus entry into its targets cells (Meloen et al., 1989). We used MAb 110-4, which recognizes a sequential epitope of the HIV-1 LAV-BRU V3 loop, to examine another possible modification of the molecule conformation which could be of relevance for its biological activity. In both assays used, the avidity of MAb 110-4 for d+rgpl60 in solution was reduced by at least 10-fold as compared with d-rgpl60, which was not the case when rgpl60 was ‘distorted after binding’ onto a nitrocellulose membrane, an observation that has been reported for other molecules (Parry et al., 1990). This reduced reactivity was certainly not due to steric hindrance by highly glycosylated mannosyl-type glycan species because Endo FNG treatment did not increase but rather profoundly reduced d+rgpl60 binding to MAb 110-4 as
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compared with d-rgp160. Moreover, baculovirus rgp160, which contains only high-mannose-type glycans, binds to 110-4 MAb to the same extent as ICC14 rgp160 does. Therefore, dNM treatment dramatically alters the conformation and accessibility of the V3 loop to antibodies, the recognized epitope being then partially masked for recognition due to the incorrect folding of the molecule.

Taken together, our results indicate that dNM profoundly affects the glycosylation and conformation of rgp160. This leads to the loss of rgp160 bioactivity, determined in terms of CD4-binding capability, and to modification of a region, the V3 loop of gp120, which certainly plays an important role in HIV-1 post-binding events. Modifications to the accessibility of the V3 loop and the conformation of the CD4 binding site together may completely prevent HIV-1 infectivity, in a synergistic manner similarly to MAbs against V3 and the putative CD4 binding site, which together neutralize virus as recently discussed (Tilley et al., 1991). Although other possible mechanisms such as impaired cleavage of gp160 into gp41 and gp120 have not been ruled out in this study, the described modifications alone may account for the impaired HIV-1 infectivity elicited by the drug.

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