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The α-Glucosidase Inhibitor *N*-Butyldeoxynojirimycin Inhibits Human Immunodeficiency Virus Entry at the Level of Post-CD4 Binding

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The α-glucosidase inhibitor *N*-butyldeoxynojirimycin (NB-DNJ) is a potent inhibitor of human immunodeficiency virus (HIV) replication and syncytium formation in vitro. However, the exact mechanism of action of NB-DNJ remains to be determined. In this study we have examined the impairment of HIV infectivity mediated by NB-DNJ. By two independent HIV entry assays [PCR-based HIV entry assay and entry of Cocal(HIV) pseudotypes], the reduction in infectivity was found to be due to an impairment of viral entry. No effect of NB-DNJ treatment was seen on the kinetics of the interaction between gp120 and CD4 (surface plasmon resonance; BIAcore) or on the binding of virus particles to H9 cells (using radiolabeled virions). We therefore conclude that a major mechanism of action of NB-DNJ as an inhibitor of HIV replication is the impairment of viral entry at the level of post-CD4 binding, due to an effect on viral envelope components.

The N-linked oligosaccharide processing inhibitor *N*-butyldeoxynojirimycin (NB-DNJ) is a glucose analog which inhibits α-glucosidases I and II in the endoplasmic reticulum (9, 35). Because of its inhibition of α-glucosidases I and II, treatment of cells with NB-DNJ would be expected to lead to the production of glycoproteins carrying only neutral glucosylated precursor oligosaccharides. The recent discovery of a Golgi-resident endomannosidase (19, 20, 27, 31), bypassing glucosidases in the endoplasmic reticulum, may, however, explain why some mature oligosaccharides are found on glycoproteins, even in the presence of high concentrations of α-glucosidase inhibitors (11, 13, 15, 35, 38).

α-Glucosidase inhibitors, such as DNJ, NB-DNJ, and castanospermine, are potent inhibitors of human immunodeficiency virus (HIV) replication and HIV-mediated syncytium formation in vitro (10, 14, 18, 33, 40). In contrast, inhibitors of the later stages in glycoprotein processing, such as deoxymannojirimycin and swainsonine, have no effect (14, 28). NB-DNJ is one of the most potent of these inhibitors. It exhibits very little cytotoxicity and is currently in clinical trials as an HIV therapeutic agent. However, the exact mechanism of action of NB-DNJ as an inhibitor of HIV replication still remains to be determined.

Antiviral concentrations of NB-DNJ are known to impair the processing of gp120-associated N-linked oligosaccharides, resulting in predominantly neutral glucosylated precursor *N*-glycans (17, 28, 33, 39). This change in glycan composition is likely to explain the decrease in processing of gp160 into gp41 and gp120 seen in HIV-infected cells treated with α-glucosidase inhibitors (8, 28, 40). No reduction in gp120 released from cells has, however, been observed (14, 17, 33, 40). Likewise, no reductions in the release of reverse transcriptase activity (RT) (23, 39, 40) or p24 antigen (39) from the cells after treatment have been observed. Treatment of cells does, however, cause a reduction in the amount of infectious virus released from the cells, indicating that a major mechanism of action of α-glucosidase inhibitors as inhibitors of HIV replication is a reduction in virus infectivity rather than virus output. Our aim in this study was to confirm this finding using refined assays and to investigate the impairment of HIV infectivity mediated by NB-DNJ, with emphasis on host cell binding and viral entry.

Validation of RT as a marker for virus release. RT has previously been used as a marker for virus output from cells treated with α-glucosidase inhibitors such as DNJ, NB-DNJ, and castanospermine (8, 23, 39, 40). However, its validity has never been tested, and previous findings showing no effect on virus output have been based on the assumption that treatment with α-glucosidase inhibitors did not adversely affect virion assembly, causing an increase in the release of non-virion-associated RT. Further more, validation of RT as a marker for the number of virus particles present in preparations of HIV grown in different concentrations of NB-DNJ was essential for standardization of virus particles in the binding and entry assays described below.

HIV type 1_{IIIB} (HIV-1_{IIIB}) was generated in the presence of different concentrations of NB-DNJ from an acute infection of H9 cells at a multiplicity of infection of 0.75. On day 4 of the infection the cells were divided into four flasks containing either 0, 50, 500, or 2,000 μM NB-DNJ. After 8 h and again 12 h later (day 5), the cells were pelleted and resuspended in medium containing the appropriate concentration of NB-DNJ. After a further 24 h (day 6), virus was harvested by pelleting the cells (Beckman GS-GR; 10 min, 2,000 rpm, 4°C) and the supernatant was filtered (0.22-μm pore size). Virus stocks were stored at −70°C prior to use.

By size exclusion chromatography on S-1000, as described elsewhere (22, 26), NB-DNJ was found to have no effect on the distribution of RT between virus particles and the fluid phase (data not shown). In accordance with previously published results (22, 26), with virus grown in the absence of NB-DNJ, approximately 85% of the RT released from the cells, under the conditions described above, was virus associated irrespec-

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tive of the presence of the different concentrations of NB-DNJ. The use of RT as a marker for virus particle production in the presence of NB-DNJ was therefore valid.

Effect of NB-DNJ on viral output and infectivity. Previous reports addressing the effect of α-glucosidase inhibitors on the output of HIV have all studied cumulative output over 3 to 5 days (8, 23, 39, 40), without taking into account potential effects of NB-DNJ on the cells and other stages in the viral life cycle. We wanted to investigate the effect of NB-DNJ on virus output and infectivity using a 24-h output assay (day 5 to day 6), as described above, involving only one round of replication. RT was used as a marker for the number of virus particles released. The 50% tissue culture infective dose, as determined by limiting dilution on C8166 cells, was used as a marker for the amount of infectious virus released. Treatment with NB-DNJ resulted in a small dose-dependent reduction in the number of virus particles released, amounting to 21% at 0.5 mM and 38% at 2 mM. However, a much greater dose-dependent reduction in the amount of infectious virus produced over the same time period was seen, amounting to 90% at 0.5 mM and 98% at 2 mM. These results confirm that the major effect of NB-DNJ on the virus produced is on relative infectivity (number of infectious virus particles produced divided by the number of virions produced) (100% [0 μM], 103% [50 μM], 13% [500 μM], and 4% [2,000 μM]) rather than on virus output. No significant HIV-mediated cytopathic effects were seen at day 6 of the infection, as determined by trypan blue dye exclusion (data not shown). Also, no cytopathic effects due to NB-DNJ were observed in the assay, and the presence of NB-DNJ was found to have no direct effect in the RT assay (data not shown).

Effect of NB-DNJ on the infectivity of Cocal(HIV) pseudotypes. The nature of the NB-DNJ-induced reduction in HIV infectivity was analyzed by an entry assay based on the entry of Cocal(HIV) pseudotypes carrying an HIV envelope and a Cocal virus core into HeLa T4 cells. The Cocal(HIV) pseudotypes enter cells via the HIV envelope and can be neutralized by antibodies to HIV-1 gp120. However, after entry the Cocal core is released into the cells, leading to plaque formation due to the cytolytic replication of Cocal virus. Wild-type HIV and HIV(Cocal) pseudotypes, produced along with the Cocal(HIV) pseudotypes, do not form plaques in these cells because of their HIV genome, and plaques formed by wild-type Cocal virions can be neutralized by anti-Cocal antiserum.

Cocal(HIV) pseudotypes grown in different concentrations of NB-DNJ were prepared essentially as described above, by superinfecting HIV-1_{IIIB}-infected cells at day 5 postinfection with the *Vesiculovirus* Cocal at a multiplicity of infection of 100. Pseudotype viruses were harvested 24 h later. The virus produced was titrated on HeLa T4 cells following preneutralization with either no antiserum, anti-Cocal antiserum, or anti-Cocal antiserum plus anti-gp120 antiserum, as described elsewhere (12, 37).

The result showed that NB-DNJ had no effect on the titers obtained from wild-type Cocal virions (Fig. 1, open bars). Taken together with the observation that NB-DNJ had only a minor effect on the overall output of wild-type HIV, this indicates that the output of Cocal(HIV) pseudotypes in the presence of different concentrations of NB-DNJ is likely to be similar. The number of plaques derived from Cocal(HIV) pseudotypes generated in the presence of NB-DNJ was, however, greatly reduced (Fig. 1, shaded bars), indicating that the ability of these pseudotypes to enter $CD4^+$ cells was greatly reduced after treatment with NB-DNJ. No plaques were seen in the presence of both anti-Cocal and anti-gp120 antisera.

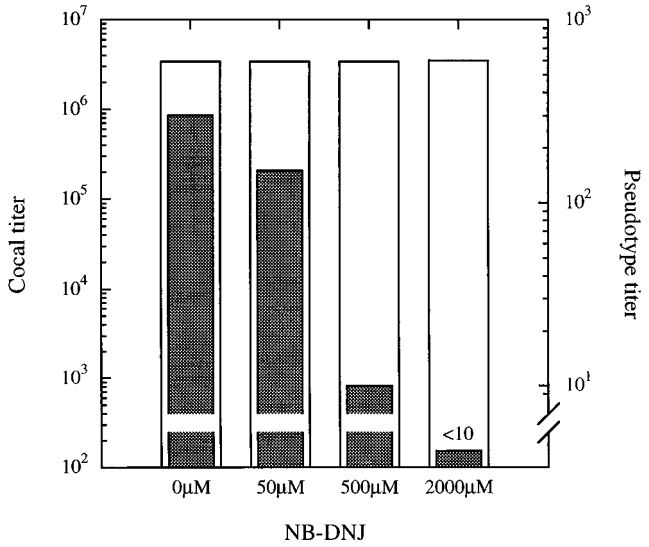

FIG. 1. Effect of NB-DNJ on the entry of Cocal(HIV) pseudotypes into HeLa CD4-positive cells. Cocal(HIV) pseudotypes were generated in the presence of various concentrations of NB-DNJ and titrated on HeLa T4 cells in the presence of various neutralizing antisera. The titer in the absence of neutralization is shown in the open bars, and the titer of the Cocal(HIV) pseudotypes is shown in the shaded bars as the difference between the titers observed in the presence of anti-Cocal antiserum and in the presence of anti-Cocal plus anti-gp120 antisera.

Effect of NB-DNJ on the time course of entry of pretreated virus into H9 cells. The entry of HIV grown in the presence of NB-DNJ was further analyzed by a PCR-based entry assay (3–5) analyzing the time course of the appearance of reverse transcripts (U3/U5) in cells postentry. Briefly, H9 cells were infected at time zero with DNase I-treated virus at a multiplicity of infection of 0.01. Samples were taken at time zero and at various time points thereafter, and DNA was prepared for PCR. PCR was performed using primers (U3/U5) detecting reverse transcripts containing a complete long terminal repeat, i.e., after first-strand exchange. As a control for the presence of DNA in the samples, primers detecting endogenous β-globin sequences were used.

Cells were infected with equal amounts of virus particles grown in the presence of various concentrations of NB-DNJ and standardized according to RT. No U3/U5 signals were detected at time zero immediately after the virus was mixed with the cells (Fig. 2). Virus generated in the absence of NB-DNJ (lines 1) showed a signal after 2 h, with maximum intensity being reached after approximately 8 h. Virus generated in the presence of 50 μM NB-DNJ (lines 2) showed a slight reduction in entry in the early stages of the infection, but after 4 h no significant difference in band intensity was observed relative to the control. Virus generated in 0.5 mM NB-DNJ (lines 3) did, however, show a significant impairment of entry, with no detectable reverse transcripts observed until 6 h postinfection. This was a delay of 4 h relative to the control, and the signal intensity did not reach that of the control, even 28 h postentry. A similar but greater effect was seen for virus generated in the presence of 2 mM NB-DNJ (lines 4). As a control for any direct effect of carryover NB-DNJ in the different virus preparations, cells were infected with the same amount of virus particles generated in the absence of NB-DNJ as described above, but in the presence of 2 mM NB-DNJ. As seen in lines 5, no significant direct effect of NB-DNJ was observed. Similar results were obtained in other independent experiments (data

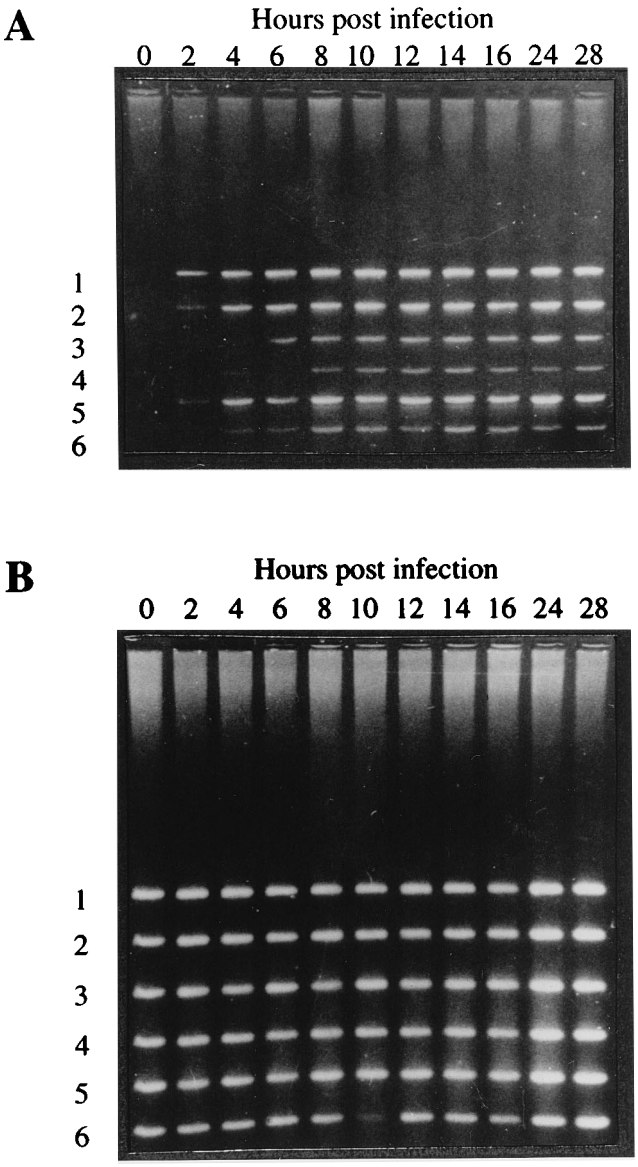

FIG. 2. Effect of NB-DNJ on the time course of entry of virus into H9 cells. H9 cells were infected at time zero with equal numbers of virus particles (standardized according to RT) grown in the presence of either 0 mM (lines 1), 0.05 mM (lines 2), 0.5 mM (lines 3), or 2 mM (lines 4) NB-DNJ. DNA was prepared from the cells at the time points indicated, and PCR was performed using either primers detecting reverse transcripts containing a full long terminal repeat (A) or endogenous β-globin primers (B). After amplification, the PCR products were loaded sequentially onto the same gel for ease of comparison. As controls, the cells were infected with virus generated in the absence of NB-DNJ in the presence of 2 mM NB-DNJ (lines 5) or at a multiplicity of infection of virus generated in the absence of NB-DNJ equivalent to that used for virus grown in 0.5 mM NB-DNJ (lines 6).

not shown). Finally, cells were infected with an amount of virus particles generated in the absence of NB-DNJ equivalent to the number of infectious virions present in the inoculum used in lines 3 for virus generated in 0.5 mM NB-DNJ (i.e., same number of infectious particles, as judged by the 50% tissue culture infective dose, but approximately 1/12 of the number of virus particles). A time course of entry similar to that obtained for virus grown in the presence of 0.5 mM NB-DNJ was seen. The lack of U3/U5 signals in panel A was not due to an absence of DNA in the samples (Fig. 2B).

Under the conditions used for this assay no U3/U5 PCR products are seen in $CD4^-$ cells or susceptible cells treated with zidovudine (10 μM) or the anti-CD4 monoclonal antibody Q425 or with virus treated with polyclonal anti-HIV antiserum (3–5). No effect of NB-DNJ (5 mM) on the entry of untreated virus in H9 cells pretreated for 36 h prior to infection was observed (data not shown).

Effect of NB-DNJ treatment on the kinetics of the interaction between rshCD4 and rgp120. To analyze whether the impairment of entry was due to a reduction in the affinity of the interaction between gp120 and CD4, we analyzed the kinetics of the interaction between recombinant soluble human CD4 (rshCD4) and recombinant gp120 (rgp120; BH10) produced in the presence or absence of 2 mM NB-DNJ by surface plasmon resonance (BIAcore; Pharmacia). rshCD4 (7) and rgp120 (17) were produced in CHO cells, as described elsewhere, and 3,650 BIAcore response units (arbitrary units) of rgp120 generated in the absence of NB-DNJ and 3,483 response units of rgp120 generated in 2 mM NB-DNJ were immobilized as described elsewhere (2). Briefly, flow cells (sensor chip CM5, research grade; Pharmacia) were activated with a 1:1 mixture of 0.1 M *N*-ethyl-*N*′-(3-diethylaminopropyl)carbodiimide and 0.1 M *N*-hydroxysuccimide in water. rgp120 was then immobilized at 20 μg/ml in 10 mM sodium acetate, pH 4.5, and the remaining binding sites were blocked with 1 M ethanolamine, pH 8.5 (Pharmacia). Finally, the flow cells were washed using 100 mM phosphoric acid (BDH). The running buffer used was HEPES-buffered saline (10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], 150 mM NaCl, 0.05% surfactant P20 [Pharmacia], pH 7.4), and all experiments were carried out at 25°C. Serial dilutions of rshCD4 in HEPES-buffered saline (100 μg/ml → threefold) were then passed over the immobilized rgp120, and sensograms were recorded. Phosphoric acid (100 mM) was used for regeneration between the different concentrations of rshCD4. The data were analyzed with the BIAevaluation program (Pharmacia).

Two representative sensograms are shown (Fig. 3A). A simple monophasic binding model could not be fitted to the data, implying that the binding has complex kinetics. Instead, a two-receptor model was fitted to the dissociation phase (Fig. 3B) and the association phase (Fig. 3C), giving a good fit with residuals below 1%. Trivial explanations for the complex kinetics are unlikely since (i) aggregates were eliminated from the preparation of rshCD4 by size exclusion chromatography prior to the binding analysis and (ii) complex kinetics were also observed when the binding was analyzed in the opposite orientation with rshCD4 immobilized and rgp120 in the fluid phase (data not shown).

The parameters obtained from the fitted models are summarized in Table 1. The parameters show that generation of gp120 in the presence of NB-DNJ had no effect on the binding of monomeric rshCD4 to monomeric rgp120.

Effect of NB-DNJ treatment on the binding of virions to H9 cells. Since NB-DNJ treatment might affect the interaction between oligomeric virion-associated gp120 and CD4 on the target cells without affecting the kinetics of the monomeric interaction, we analyzed the binding of ^{35}S-labelled virions generated in the presence of NB-DNJ to CD4-positive H9 cells.

^{35}S-labelled virus grown in the presence of either 0, 50, 500, or 2,000 μM NB-DNJ was produced as described above with the following modification. On day 5 the cells were harvested by centrifugation and resuspended at 10^7 cells per ml in 2 ml of methionine- and cysteine-free RPMI 1640 (ICN Flow, Costa Mesa, Calif.) containing 1% dialyzed fetal calf serum (Sigma, Poole, Dorset, United Kingdom), 2 mM L-glutamine (Gibco,

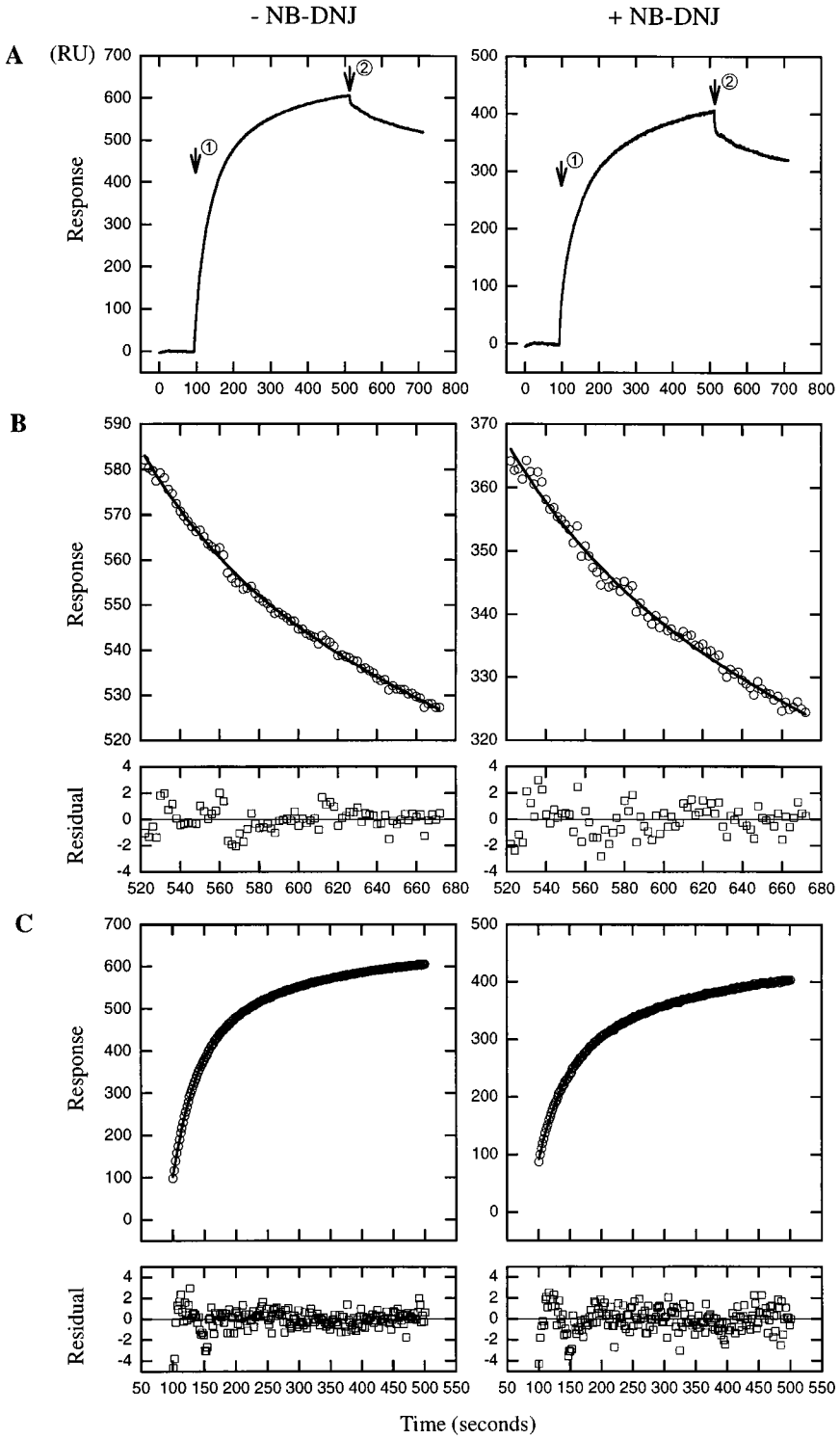

FIG. 3. Effect of NB-DNJ treatment on the kinetics of the interaction between rshCD4 and rgp120. rgp120 was produced in either the presence or absence of 2 mM NB-DNJ. rshCD4 was generated in the absence of NB-DNJ. The kinetics were analyzed by surface plasmon resonance (BIAcore). Serial dilutions of rshCD4 were passed over two different flow cells containing rgp120 generated in the absence or presence of NB-DNJ immobilized to the matrix, and sensograms were recorded (A) (14 μg of rshCD4 per ml). Binding is measured in arbitrary response units (RU). Arrow 1 indicates the start of the injection of rshCD4, and arrow 2 indicates the end of the injection. A two-receptor model ($A + B1 + B2 = AB1 + AB2$) was fitted to the dissociation phases (B) and to the association phases (C), and one in every four datum points was plotted. The actual datum points are shown as symbols, and the fitted curves are shown as lines. The residuals from the fits are plotted below each of the association and dissociation phase plots.

TABLE 1. Effect of NB-DNJ treatment on the kinetics of the interaction between rshCD4 and rgp120[a]

Recombinant gp120	k_{on1} (M^{-1} s^{-1})	k_{on2} (M^{-1} s^{-1})	k_{off1} (s^{-1})	k_{off2} (s^{-1})	K_1 (M)	K_2 (M)
− NB-DNJ	$(4.9 \pm 0.9) \times 10^4$	$(1.2 \pm 0.4) \times 10^4$	$(21 \pm 3.7) \times 10^{-3}$	$(3.8 \pm 0.6) \times 10^{-4}$	$(4.3 \pm 0.8) \times 10^{-7}$	$(3.5 \pm 1.6) \times 10^{-8}$
+ NB-DNJ	$(4.0 \pm 1.0) \times 10^4$	$(1.0 \pm 0.3) \times 10^4$	$(16 \pm 2.0) \times 10^{-3}$	$(3.3 \pm 0.8) \times 10^{-4}$	$(4.4 \pm 2.2) \times 10^{-7}$	$(3.5 \pm 1.5) \times 10^{-8}$

[a] Values are means ± standard deviations.

Paisley, United Kingdom), and antibiotics (50 U of penicillin per ml and 50 μg of streptomycin per ml) (Sigma), plus the appropriate concentration of NB-DNJ. The cells were then starved for 1 h 40 min at 37°C before the addition of [^{35}S]-Translabel (0.5 mCi; ICN Flow). After 3 h, 10 ml of complete RPMI 1640 was added, and 19 h later the supernatants were harvested by centrifugation and filtered (0.22-μm pore size). Virus supernatant (5 ml) was layered onto a 2-ml cushion of 30% glycerol in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.4) and spun at 14,000 × *g* for 1 h at 4°C. The pellet was resuspended in 200 μl of PBS containing 2 mg of bovine serum albumin (BSA) per ml and 0.1% NaN_3 on ice for 2 h and stored at −70°C until use. H9 cells were washed twice in PBS-BSA-N_3 and seeded in V-bottom 96-well plates with 10^5 cells per well. The cells were harvested by centrifugation and resuspended on ice in 50 μl of a threefold dilution series of ^{35}S-labelled virus (0, 50, 500, or 2,000 μM NB-DNJ) standardized according to RT. The cells were incubated with the virus for 45 min on ice and washed once in 100 μl of 10% BSA in PBS and twice in PBS-BSA-N_3. Finally the cells were resuspended in 4 M NaOH, and bound radioactivity was counted. As a control for gp120-dependent binding, wells incubated with virus dilutions preneutralized with anti-gp120 antibodies (ADP391 acitic fluid, 1/100; kindly provided by J. Cordell and C. Dean via the MRC AIDS Reagent Project) for 30 min on ice were assayed in parallel. As a control for nonspecific adsorption to the wells, wells containing no cells were assayed in parallel. The assay was performed in duplicate.

No difference in the binding to H9 cells by equal numbers of virions produced in the different concentrations of NB-DNJ was seen (Fig. 4). The purification scheme used for the virions resulted in a purification factor of approximately 85, as judged by the ratio of RT to total radioactivity in the different preparations. This purification lead to a ratio of soluble gp120 to virus-associated gp120 of less than 1% (data not shown). Identical results were obtained with other virus preparations (purification factor of 1 to 160). The gp120 dependency of the binding was demonstrated by preincubation of the virions with anti-gp120 antibodies, giving rise to an inhibition of between 55% (purification factor 1) and 95% (purification factor 141) of the binding. Background counts seen in wells containing virus only were approximately 10% of counts seen in wells containing both virus and cells.

Discussion. We have confirmed that, in contrast to many other viruses (1, 6, 29, 30, 34, 36), NB-DNJ treatment has only a minor effect on the output of virions from HIV-infected cells. In contrast, specific viral infectivity is greatly reduced in the presence of NB-DNJ. The impairment in infectivity of virus particles released in the presence of NB-DNJ was analyzed by a Cocal(HIV) pseudotype entry assay. This assay is ideal for studying entry of HIV, in that plaque-formation by the Cocal-(HIV) pseudotypes is totally dependent on entry via the HIV envelope. This allows the distinction between an effect on the viral envelope and an effect on the assembly of internal structures of the virus. The result that entry of Cocal(HIV) pseudotypes grown in the presence of NB-DNJ was greatly impaired conclusively demonstrates that the impairment of viral infectivity is due to an effect on the HIV envelope.

To study further the impairment of entry we used an independent, PCR-based entry assay analyzing the time course of entry by monitoring the appearance of reverse transcripts containing a full long terminal repeat postentry. When the entry of equal numbers of virus particles grown in the presence of different concentrations of NB-DNJ was studied, a clear inhibition in infectivity was observed at concentrations of NB-DNJ equal to or greater than 0.5 mM. The facts that some entry was observed in the later stages of the infection even at these concentrations and that this did not reach the same level as that of controls over a period of up to 28 h indicate that a relatively small proportion of the virus particles (about 4% at 2 mM) still contain an envelope sufficiently normal to enter the host cell. This is in accordance with findings from long-term cultures of HIV, in which, although greatly reduced compared with that in untreated cultures, some replication was seen even in the presence of high concentrations of NB-DNJ (2 mM) (data not shown). The observation that propagation of the host cells for up to 36 h in the presence of NB-DNJ prior to the infection had no effect on viral entry further supports the conclusion that the inhibition of viral replication by NB-DNJ in cell culture is due to an effect on the viral envelope and not on the host cell.

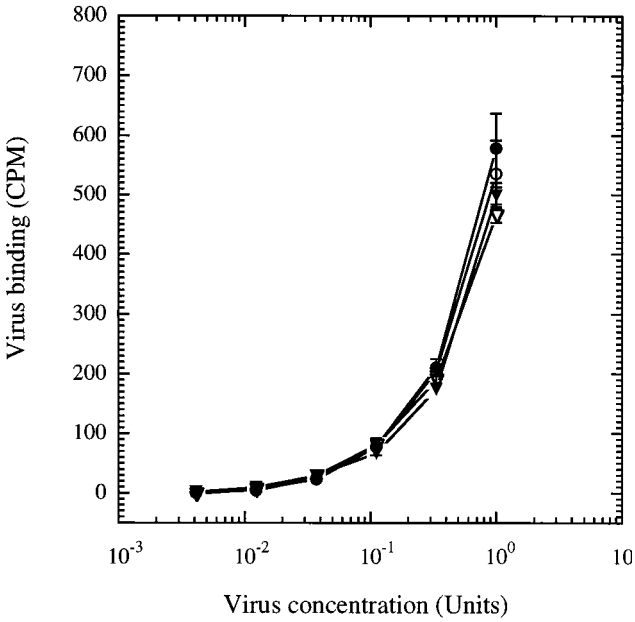

FIG. 4. Effect of NB-DNJ on the binding of virions to H9 cells. ^{35}S-labelled virus grown in the presence of 0 mM (○), 0.05 mM (●), 0.5 mM (▽), or 2 mM (▼) NB-DNJ was purified by ultracentrifugation, standardized according to RT, and incubated in threefold dilutions with H9 cells for 45 min on ice (highest concentration = 1 U). After washing, the cells were lysed and bound virus was counted. The background counts seen in the absence of cells were subtracted from the data.

The impairment of viral entry by NB-DNJ was not occurring at the level of gp120-CD4 binding. This was demonstrated by using surface plasmon resonance to study the kinetics of the monomeric interaction between rshCD4 and rgp120 generated in the presence or absence of NB-DNJ and by virion binding assays to study the interaction between multimeric gp120 on the virion surface and CD4-positive cells. This finding adds to the controversy that already exists about whether virus particles grown in the presence of α-glucosidase inhibitors display a decrease in their binding to $CD4^+$ cells. Two reports have showed a decrease in the binding of virions generated in the presence of α-glucosidase inhibitors by flow cytometry (8, 39), and two reports have showed no decrease in binding of virions generated in the presence of α-glucosidase inhibitors by either flow cytometric analysis or binding of radiolabelled virions to $CD4^+$ cells (32, 40). The studies using flow cytometry all used an HIV-positive serum for detection of bound viral antigens. A change in the glycan composition of gp120 from charged complex type oligosaccharides to neutral oligosaccharides has, however, been found to lead to a decrease in the binding of polyclonal antisera from both rabbits and humans to rgp120 in an enzyme-linked immunosorbent assay (25). The use of HIV-positive sera for detection of bound virions generated in the presence of NB-DNJ may therefore give rise to misleading results when comparisons are made to the binding of virions generated in the absence of NB-DNJ. Therefore, assays studying the direct binding of equal numbers of radiolabelled virions to cells provide a more accurate measure of the effect on NB-DNJ treatment on virus binding.

The detailed analysis of the kinetics of the monomeric interaction between rgp120 and rshCD4, using surface plasmon resonance, led to the finding that the interaction between gp120 and CD4 does not follow simple first-order kinetics. This is in accordance with previously suggested models for HIV entry (24). A detailed analysis of the complex kinetics observed was, however, beyond the scope of this paper, and for comparative purposes a two-receptor model was fitted to the data. The result does, however, imply that altering the *N*-glycans on gp120 with NB-DNJ has no effect on the complex kinetics of the interaction between gp120 and CD4.

The molecular basis for the inhibition of post-CD4 binding events by NB-DNJ remains to be elucidated. An increased incorporation of uncleaved, and nonfusenogenic (21), gp160 into the virions is an unlikely explanation, since uncleaved gp160 is likely to be retained inside the cell (41). Furthermore, no reduction in the amounts of gp120 found in the supernatant of HIV-infected cells grown in the presence of α-glucosidase inhibitors has been found (14, 32, 33, 40). A small effect of NB-DNJ treatment on the conformation of the V3 loop has been suggested. The result was, however, obtained using only a single monoclonal antibody (16), and its significance remains to be established.

In summary, the most likely mechanism of action of NB-DNJ as an inhibitor of HIV replication is an inhibition of viral entry at the level of post-CD4 binding. Further studies are needed to characterize the effect of a change in *N*-glycan composition of gp120 on these events, including possible effects on the conformation of gp120 and effects on CD4-induced conformational changes and shedding of gp120. A change in the glycan composition of gp41 may also lead to a reduction in its fusenogenic capacity.

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