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Antibody-independent, complement-mediated enhancement of HIV-1 infection by mannosidase I and II inhibitors

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Summary

Human immunodeficiency virus type 1 (HIV-1) infectivity and cytopathic effect require proper maturation of the viral envelope glycoprotein carbohydrate moieties. We have found that fresh human serum enhances the infectivity of HIV-1 in MT-2 cell infection assays when virus is synthesized in the presence of the mannosidase I inhibitor, 1-deoxymannojirimycin, or the mannosidase II inhibitor, swainsonine, but has no enhancing effect on virus synthesized in the presence of the glucosidase I inhibitors, castanospermine and 1-deoxynojirimycin, or the glucosidase II inhibitor, bromoconduritol. Enhanced infections were characterized by cytopathic effect, antigen synthesis and reverse transcriptase release, all which occurred sooner than in control-infected cultures. This enhancement of infection was also observed in C1q-deficient serum but was not observed in serum that was heat-inactivated or depleted of complement components C3 or factor B, thus suggesting a requirement for the alternate pathway of complement.

HIV infection-enhancement; Glycosylation; Complement

Introduction

Human immunodeficiency virus type 1 (HIV-1) infectivity and cytopathicity require proper maturation of the carbohydrate moieties of the viral envelope glycoproteins, gp120 and gp41. A major functional role for HIV-1 surface carbohy-

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drate in the infectivity and pathogenesis of the virus was first suggested by studies utilizing carbohydrate-specific lectins (Lifson et al., 1986; Robinson et al., 1987) and enzymatic removal of gp120 carbohydrates (Matthews et al., 1987). Later it was found that HIV-1 infectivity and cytopathicity could be blocked if viral proteins were synthesized in the presence of inhibitors of certain glycoprotein carbohydrate processing enzymes (Gruters et al., 1987; Montefiori et al., 1988a; Tyms et al., 1987, 1988; Walker et al., 1987). These enzymes are responsible for final maturation of glycoprotein carbohydrate moieties via the N-glycosylation pathway (Elbein, 1987). The inhibitors shown to block HIV-1 infectivity and cytopathicity in vitro include castanospermine (Cs) and 1-deoxynojirimycin (dN) as inhibitors of glucosidase I, and 1-deoxymannojirimycin (dMM) as an inhibitor of mannosidase I but not the glucosidase II inhibitor bromoconduritol (BCU) or the mannosidase II inhibitor swainsonine (Sw).

Blocking HIV-1 infectivity and cytopathicity with inhibitors of N-linked glycosylation represents a unique mechanism of attack for potential therapeutic application. In order for these agents to be effective in vivo, however, they must work in the presence of human serum components. As an example, the alternate pathway of complement recently was shown to mediate antibody-dependent enhancement of HIV-1 infection (Robinson et al., 1988a, b; 1989) and is known to be activated by particulate glycoprotein lacking sialic acid (McSharry et al., 1981). Although envelope glycoproteins of infectious HIV-1 are at least partially sialylated (Abel et al., 1987; Geyer et al., 1988; Mizouchi et al., 1988), inhibitors of the N-glycosylation maturation pathway block sialylation as the terminal step in glycoprotein carbohydrate processing. Non-sialylated HIV-1 virions, in turn, may be capable of activating complement, thereby leading to complement-mediated enhancement of HIV-1 infection by cells bearing suitable receptors in an antibody-independent mechanism. We have investigated this possibility in vitro using an MT-2 cell infection assay. The results of these experiments demonstrated that altered N-glycosylation can result in antibody-independent, complement-mediated enhancement of HIV-1 infection and that this enhancement abolishes the anti-HIV-1 activity of at least one inhibitor of N-glycosylation. The implication of these findings to the clinical potential of such agents in the control of HIV-1 infection are significant.

Materials and Methods

Cells and virus

MT-2 cells (Harada et al., 1985) were used as targets for infection with HIV-1 synthesized by H9/HTLV-III$_B$ cells (Popovic et al., 1984). Growth medium was RPMI-1640 containing 12% heat-inactivated fetal bovine serum and 50 μg gentamicin/ml. Virus was prepared from conditioned culture fluids made cell-free by low speed centrifugation and 0.45 micron filtration. Virus was quantitated by 50% tissue culture infectious dose values obtained by end-point microtitration on MT-2 cells as described (Montefiori et al., 1988b).

Glycosylation inhibitors and sera

All glycosylation inhibitors were purchased from Boehringer-Mannheim (Indianapolis, IN) except Sw which was provided by Dr. Harry Broquist, Vanderbilt University, Nashville, TN. Fresh, pooled, unfractionated human serum and human C1q-deficient and factor B-deficient sera were obtained lyophylized from Sigma Chemical Co. (St. Louis, MO). Complement component C3-deficient serum was obtained lyophylized from Organon Teknika Corp., Cappel, Malvern, PA.

Infection assays

Infection assays were performed in either 96-well microdilution plates or 25 cm^2 culture flasks. The microdilution plate method was used for cytopathic effect (CPE) assays as described (Montefiori et al., 1988b). 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 (A_{540}) between test wells and virus control wells divided by the difference in absorbance between cell control and virus control wells. Infection assays were also performed in flask cultures in order to provide sufficient quantities of cells and culture supernatants to perform indirect immunofluorescence assays (IFA) and reverse transcriptase (RT) assays. For infection, 10 ml cell suspensions were challenged with 2 ml of virus for 2 h, then unadsorbed virus was removed by 2 washes in growth medium. The cultures were then incubated in 20 ml of fresh growth medium for the remainder of the incubation period. In both assays, cells were challenged in the presence and absence of either untreated, heat-inactivated or complement component-deficient human sera using virus synthesized in the presence and absence of various glycoprotein carbohydrate processing inhibitors as described in the figure legends. All challenges were at an MOI of 1–3 infectious virions per cell.

Indirect immunofluorescence assay (IFA)

IFA was performed on acetone/methanol-fixed cells as described (Montefiori and Mitchell, 1986) using a 1:50 dilution of serum from a healthy HIV-1 seropositive individual (positive for all major HIV-1 antigens by Western blot, DuPont Co., Wilmington, DE) and a 1:200 dilution of fluorescein-conjugated, IgG fraction, goat anti-human IgG (heavy and light chains specific, Organon Teknika Corp., Cappel).

Reverse transcriptase (RT) assay

RT assays were performed on polyethylene glycol-precipitated virus as described (Poiesz et al., 1980) using poly(A)·$(dT)_{15}$ as template primer and 25 μCi [*methyl*-^{3}H]dTTP (80.1 Ci/mmol, New England Nuclear) per reaction.

Results

Cultures of washed H9 cells chronically infected with the HIV-1 isolate HTLV-III_B were incubated for two days in the presence and absence of inhibitors and then glycosylation-modified versus wildtype virus was used to challenge MT-2 cells in 96-well microdilution plates. Reverse transcriptase measurements indicated that

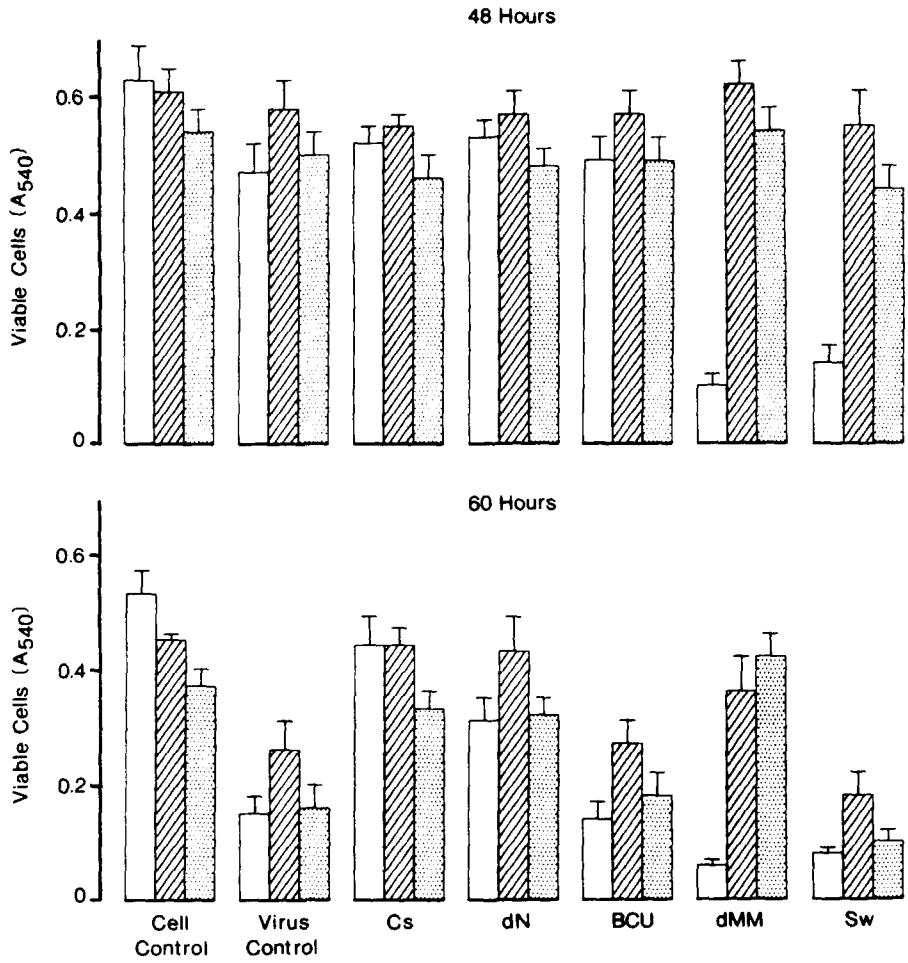

Fig. 1. Complement-mediated enhancement of HIV-1 infection as measured by cytopathic effect. Virus was synthesized by H9/HTLV-III_B cells in the presence and absence of indicated inhibitors and used to challenge MT-2 cells in 96-well microdilution plates as described in Materials and Methods. Infections were in the presence of either fresh human serum (open bars), heat-inactivated (56°C, 1 h) human serum (hatched bars) or complement component C3-deficient human serum (stippled bars). Virus preparations were preincubated for 1 h in a 1:40 dilution of respective human serum in growth medium prior to the addition of cells. CPE was quantitated and reported as ± 1 standard deviation after 48 and 60 h by vital dye uptake. Concentrations were 0.5 mM for Cs, dN, dMM and Sw, and 0.25 mM for BCU.

viral yields in all producer cultures were nearly equivalent (less than 20% variability). Cells were challenged in the presence of either fresh human serum, heat-inactivated human serum or C3-deficient human serum. Infection end-points were measured by vital dye (neutral red) uptake for CPE. The results are shown in Fig. 1. Cells challenged with virus synthesized in the presence of 0.5 mM dMM or Sw demonstrated enhanced infection in the presence of fresh human serum after 48 h where more than 75% of cells exhibited cytolysis compared with less than 25% in the control. This enhancement of infection was not observed in the presence of heat-inactivated serum or C3-deficient serum, thus indicating that complement was involved. In contrast, no enhancement of infection was observed for virus synthesized in the presence of Cs (0.5 mM), dN (0.5 mM) or BCU (0.25 mM). Twelve hours later, cells challenged with control virus exhibited significant CPE, whether in the presence or absence of complement (Fig. 1, 60 h). At that time, antiviral activity was observed for Cs and dN under all three serum conditions. Antiviral activity was also observed for dMM but only in the presence of heat-treated or C3-deficient human serum and not in the presence of fresh human complement serum. BCU and Sw, as previously reported (Montefiori et al., 1988a), did not attenuate HIV-1 infectivity.

Enhanced infections were further characterized by IFA for viral antigen synthe-

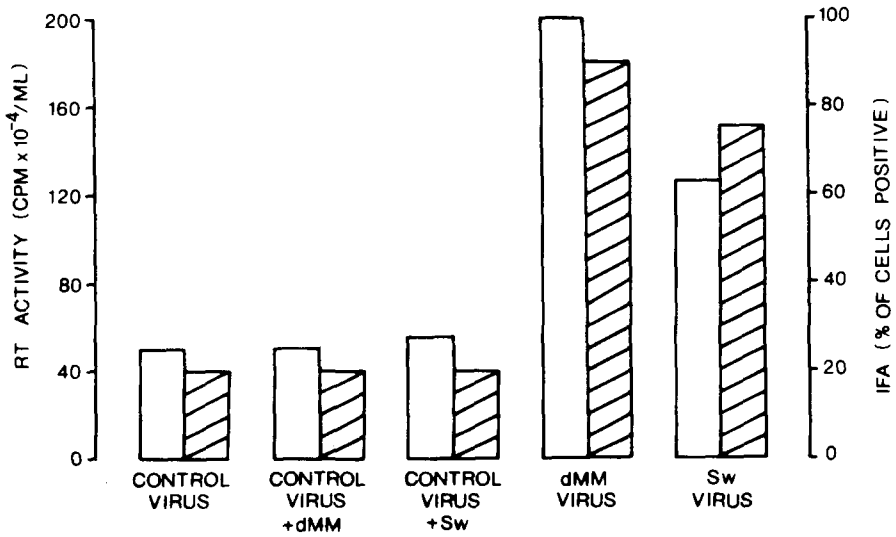

Fig. 2. Demonstration by antigen synthesis and reverse transcriptase release of enhanced HIV-1 infection. Virus was synthesized by H9/HTLV-III$_B$ cells in the presence and absence of dMM (0.5 mM) or Sw (0.5 mM) and used to challenge cultures of MT-2 cells in 25 cm^2 culture flasks as described in Materials and Methods. Challenges with control virus were in the presence and absence of 0.125 mM dMM and Sw to adjust for the concentration of these inhibitors present during challenge with test virus. Virus preparations were preincubated in a 1:40 dilution of fresh human serum in growth medium prior to the addition of cells. Cells and culture fluids were harvested for IFA and RT assays 60 h post viral challenge. IFA, open bars; RT, hatched bars.

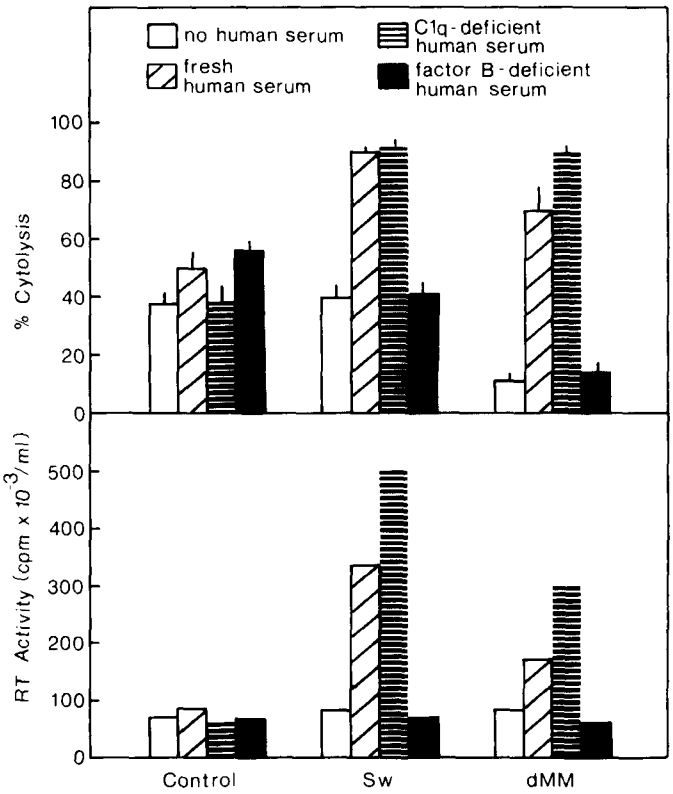

Fig. 3. Requirement for the alternative pathway of complement. HTLV-III_B synthesized in the presence and absence of Sw or dMM was used to challenge MT-2 cells in microdilution plates in the presence and absence of either fresh human serum, C1q-deficient human serum or factor B-deficient human serum. Virus preparations were preincubated in a 1:40 dilution of respective serum in growth medium prior to the addition of cells. After 3 days, infections were monitored by vital dye uptake and RT assays as described in Materials and Methods. For % cytolysis, values are given with 1 standard deviation, $N=6$. The contents of all 6 wells for each test were combined for the determination of RT activities. Concentrations were 0.5 mM for Sw and dMM.

sis and by the release of RT activity into culture fluids as a measure of virus production. Virus was synthesized in the presence and absence of dMM or Sw and then used to challenge cultures of MT-2 cells which were harvested 60 h later for IFA and RT. Reverse transcriptase measurements demonstrated that equivalent amounts of virus were used for each challenge (less than 10% variability). Results of these experiments are shown in Fig. 2. After 60 h, all control cultures were in an early stage of infection where 20% of cells were positive by IFA and levels of RT activity were relatively low (450–475 $\times$ 10^3 cpm/ml). Thus, having dMM or Sw present during challenge with control virus had no effect on the outcome of the infection. In contrast, infections by virus synthesized in the presence of dMM or Sw were more advanced, where IFA and RT levels were 2–4 times greater than controls.

Two additional complement component-deficient sera (i.e., C1q and factor B-deficient) were used to help identify the complement pathway responsible for the enhancement of HIV-1 infection observed with Sw and dMM. Infections in these sera were compared with infections in the presence and absence of fresh, unfractionated human serum as measured by vital dye uptake and release of RT activity into the culture fluids. The results are shown in Fig. 3. Sw and dMM once again caused an enhancement of infection in fresh human serum as compared to the absence of human serum. Here, cytopathic effects produced by virus synthesized in the presence of Sw or dMM were nearly twice as great and release of RT activity was 3–5 times greater in the presence of fresh, unfractionated human serum. Similar enhancement of infections were observed for Sw and dMM in C1q-deficient serum but not in factor B-deficient serum.

Discussion

We have demonstrated that the infectivity of HIV-1 synthesized in the presence of the mannosidase I inhibitor, Sw, or the mannosidase II inhibitor, dMM, is enhanced in the presence of fresh human serum and that this effect abolishes the anti-HIV-1 activity observed for dMM in the absence of fresh human serum (Figs. 1–3). HIV-1 infection, enhancement by Sw and dMM was also observed in complement component C1q-deficient serum (classic pathway deficient) but not in human serum that was heat-treated (a process that inactivates both complement pathways) or depleted of complement components C3 (required for both pathways) or factor B (required for the alternate pathway). These results suggest that the alternate pathway of complement is responsible for the enhancement of N-glycosylation-modified HIV-1 infection. This is very similar to the complement-mediated, antibody-dependent enhancement of HIV-1 infection that we described earlier (Robinson et al., 1988a,b; 1989) with the exception that HIV-1 antibody is apparently not required when glycosylation is affected by mannosidase I or II inhibitors.

The mechanism for glycoprotein carbohydrate-dependent, complement-mediated enhancement of HIV-1 infection could involve attachment of virus to cell surface receptor(s) and/or virus uncoating once inside the cell. Under normal conditions, the CD4 surface antigen on lymphocytes such as MT-2 cells acts as the receptor for HIV-1 (Dalgleish et al., 1984; Klatzmann et al., 1984; McDougal et al., 1986a). If normal glycosylation is altered and sialylation is prevented, activation of the alternate pathway of complement is favored (McSharry et al., 1981). Activated complement components could subsequently facilitate binding of HIV-1 to complement receptors or a complex of complement and CD4 receptors. Internalized virions opsonized by complement components may also be routed more efficiently to lysozomes for uncoating. Using monoclonal antibodies, the MT-2 cells used in this study have been demonstrated by IFA and flow symmetry to have CR2 but not CR1 or CR3 complement receptors (Robinson et al., 1989), thus suggesting that CR2 or a CR2–CD4 complex is the binding site for complement-mediated enhancement of HIV-1 in these target cells.

Glucosidase I inhibitors, such as Cs and dN, appear to retain their potential as anti-HIV agents since their antiviral activity was not abolished by fresh human serum (Fig. 1). This may be because the glycoprotein carbohydrate moieties that arise in the presence of glucosidase I inhibitors are unable to elicit complement activation. Another possibility is that HIV-1 gp120 synthesized in the presence of glucosidase I inhibitors loses its affinity for the viral transmembrane gp41 as suggested by earlier findings (Montefiori et al., 1988a). In this event, even if the modified gp120 were to activate complement, the HIV-1 would most likely remain attenuated since gp120 would be incapable of effectively bridging HIV-1 to cell receptors. Furthermore, if this were the case, the natural weak affinity of gp120 for gp41 (McDougal et al., 1986b; Schneider et al., 1986) would seem to favor an acceptable therapeutic index for glucosidase I inhibitors since cellular functions are likely to be less sensitive to a decrease in this enzymatic activity.

Glycoprotein processing inhibitors offer a new therapeutic approach to HIV infection that is unique and which may be valuable not only for single agent therapy but also for combination therapy as demonstrated by the synergistic anti-HIV-1 activity of Cs with Ampligen in vitro (Montefiori et al., 1989). It is clear, however, that normal human serum components must be considered when evaluating glycoprotein processing inhibitors for antiviral activity in vitro because of the possibility for infection-enhancement. It is conceivable that similar considerations may also apply to other therapeutic strategies, such as the use of soluble CD4 or gp160-derived sequences, that are based on glycoprotein-mediated processes.

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