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# Bovine and mouse serum β inhibitors of influenza A viruses are mannose-binding lectins

(β inhibitor-resistant mutant/hemagglutinin/carbohydrate/receptor-binding site/antigenicity)

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**ABSTRACT** **Normal bovine and mouse sera contain a component, termed β inhibitor, that inhibits the infectivity and hemagglutinating activity of influenza A viruses of the H1 and H3 subtypes. To investigate the nature of the interaction of β inhibitors with influenza A viruses we isolated a mutant of the virus Mem71$_H$-Bel$_N$ (H3N1) that could grow in the presence of bovine serum. The mutant virus was resistant to hemagglutination inhibition by mouse serum as well as by bovine serum and had undergone changes in the receptor-binding and the antigenic properties of its hemagglutinin (HA) molecule. Sequence analysis of the HA genes of parent and mutant viruses revealed a single nucleotide change in the mutant, resulting in the substitution Thr → Asn at residue 167 of the HA$_1$ chain of HA. This change leads to loss of the potential glycosylation site Asn-165–Val-166–Thr-167 at the tip of the HA spike, which in viruses of the H3 subtype is known to bear a high-mannose (type II) carbohydrate side chain N-linked to Asn-165. The association of β inhibitor resistance with loss of this carbohydrate side chain suggested that β inhibitors may be lectins. In support of this hypothesis, treatment of the β inhibitor-sensitive parent virus Mem71$_H$-Bel$_N$ with periodate converted it to the resistant state. Furthermore, the inhibitory activity of both bovine and mouse sera for the parental virus was abrogated by D-mannose. We conclude that the β inhibitors in bovine and mouse sera are mannose-binding lectins that inhibit hemagglutination and neutralize virus infectivity by binding to carbohydrate at the tip of the HA spike, blocking access of cell-surface receptors to the receptor-binding site on HA.**

The interaction of influenza virus with sialylated cell-surface receptors is subject to inhibition by a variety of nonspecific inhibitors present in normal human and animal sera (for review, see refs. 1 and 2). β inhibitors of influenza virus were identified in the late 1940s as heat-labile components of normal sera that had virus-neutralizing as well as hemagglutination-inhibiting activity (3–5). These and other properties distinguished them from the other class of serum inhibitor known at the time, the so-called Francis or α inhibitors (6), which were heat stable and inhibited hemagglutination but not virus infectivity. A third class of inhibitor, γ inhibitor, was identified after the emergence in 1957 of the Asian (H2N2) influenza subtype (7, 8). γ inhibitors had many of the properties of α inhibitors but, like β inhibitors, also possessed virus-neutralizing activity.

Inhibitors of the α and γ classes are sialylated glycoproteins; their mode of action is to compete with cell receptors for binding to the hemagglutinin (HA) of influenza virus. γ inhibitors are active against human influenza A viruses of the H2 and H3 subtypes but not the H1 subtype (8, 9). γ inhibitors are present at high titer in horse and guinea pig sera and have been identified as $\alpha_2$-macroglobulins. Their activity is stable to heat, destroyed by periodate, but not affected by neuraminidase due to the presence of the unusual sialic acid, 4-*O*-acetylneuraminic acid, which resists hydrolysis by this enzyme (10–12).

The receptor-binding site on HA has been identified by x-ray crystallographic studies as a pocket of conserved amino acids at the tip of the HA molecule (13, 14). Mutants of H3 influenza viruses selected for resistance to horse serum inhibitor have provided insight into the relationship between an amino acid substitution within the receptor-binding site and the fine specificity of receptor binding. Resistance to horse serum inhibitor is conferred by the single amino acid substitution Leu-226 → Gln in HA$_1$, which changes the receptor-binding specificity from preferential recognition of sialic acid in the α2 → 6Gal linkage to one that binds sialic acid-linked α2 → 3Gal (15).

Much less is known about the chemical nature and mode of action of β inhibitors. Those active against influenza A viruses are particularly abundant in bovine serum and, to a lesser extent, mouse serum. They inhibit viruses of the H1 and H3 subtypes but not of the H2 subtype (16), although H1 viruses that have been passaged in mice are also resistant (5, 17, 18). β inhibitors are heat labile, $Ca^{2+}$-dependent, protein in nature, and resistant to both neuraminidase and periodate (1). The mode of action of β inhibitors would thus appear not to involve sialic acid and consequently to be quite different from that of α and γ inhibitors.

To gain insight into the nature of the interaction of β inhibitors with influenza virus, we isolated a mutant of the influenza A virus Mem71$_H$-Bel$_N$ (H3N1) that was resistant to neutralization by bovine serum. This mutant was shown to have also gained resistance to the β inhibitor in mouse serum and to have undergone changes in the receptor-binding and the antigenic properties of its HA molecule. Sequencing of the HA gene revealed a single nucleotide difference between mutant and parent viruses, leading to loss in the mutant of the glycosylation site at amino acid residue 165 at the tip of the HA spike. Further experiments are described that indicate that the β inhibitors in both bovine serum and mouse serum are lectins that block access to the receptor-binding site by binding to carbohydrate at the tip of the HA spike.

## MATERIALS AND METHODS

**Viruses.** The influenza type A virus Mem71$_H$-Bel$_N$ (H3N1) is a reassortant bearing the HA of A/Memphis/1/71 (H3N2) and the neuraminidase of A/Bel/42 (H1N1). A bovine serum-resistant mutant, Mem71$_H$-Bel$_N$β$^R$, was selected by growth of Mem71$_H$-Bel$_N$ virus in eggs in the presence of bovine serum. The resultant virus was passaged twice in eggs at high dilution in the absence of serum and then plaqued on Madin–Darby canine kidney cells, and a single plaque was used to

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Abbreviations: HA, hemagglutinin; HI, hemagglutination inhibition; RBC, erythrocytes; RDE, receptor-destroying enzyme; mAb, monoclonal antibody.

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Table 1. HI of Mem71$_H$-Bel$_N$ virus by bovine and horse sera

| Exp. | Treatment of serum | Diluent | HI titer of serum: Bovine | Horse |
|---|---|---|---|---|
| 1 | None | PBS | 3840 | 480 |
| | 56°C, 30 min | PBS | 160 | 1280 |
| | $KIO_4$ | PBS | 5120 | <20 |
| 2 | None | 0.15 M NaCl | 2560 | 480 |
| | | +0.025 M sodium citrate | <10 | 480 |
| | | +0.025 M sodium citrate + 0.05 M $CaCl_2$ | 1920 | 480 |
| | | +0.025 M sodium citrate + 0.05 M $MgCl_2$ | 10 | 480 |
| | | +0.05 M $CaCl_2$ | 1920 | 640 |

Exp., experiment.

prepare egg-grown stocks. A horse serum-resistant mutant, Mem71$_H$-Bel$_N$HS$^R$, had been isolated in this laboratory (19). Viruses were grown in the allantoic cavity of 10-day embryonated hens' eggs by standard procedures and stored at −70°C. Viruses used for nucleic acid sequencing were concentrated from allantoic fluid by precipitation with 8% (wt/vol) polyethylene glycol 6000 (BDH) and purified by rate zonal sedimentation on 25–70% (wt/vol) sucrose gradients.

**Normal Sera.** Fresh bovine serum was obtained from Leigh Corner, Commonwealth Scientific and Industrial Research Organization, Division of Animal Health, Parkville, Victoria, Australia. Horse serum was purchased from the Commonwealth Serum Laboratories, Parkville, Victoria, and mouse serum was obtained from the animal facility of this Department. All sera were stored at −20°C.

**Hemagglutination Titrations and Hemagglutination Inhibition (HI) Tests.** Hemagglutination titrations and HI tests were performed by standard procedures with 96-well Microtiter plates and 1% (vol/vol) chicken erythrocytes. To test the effect of simple sugars on the inhibitory activity of normal sera, assays were performed in standard W.H.O. (macro) plates with 5% chicken erythrocytes because, in the presence of sugars, shields of agglutinated erythrocytes were found to be more stable in the larger wells than in microwells. HI titers obtained with the macromethod were ≈4-fold higher than those obtained with the micromethod.

**Periodate Treatment of Serum and Virus.** One volume of serum was treated with 3 vol of 0.011 M $KIO_4$ for 15 min at 20°C. The $KIO_4$ was then inactivated by the addition of 6 vol of 0.22% (wt/vol) glycerol in phosphate-buffered saline, pH 7.2 (PBS). Periodate treatment of virus was carried out similarly, by using 1 vol of virus-containing allantoic fluid and incubating with $KIO_4$ for different periods before adding glycerol. For mock treatment, the periodate and glycerol were mixed before virus addition.

**Treatment of Erythrocytes with Receptor-Destroying Enzyme (RDE).** Chicken erythrocytes were treated with increasing concentrations of *Vibrio cholerae* RDE (Commonwealth Serum Laboratories, Parkville, Australia), as described by Yewdell *et al.* (20).

**Nucleotide Sequencing of the HA Gene.** RNA was extracted from purified virions by treatment with proteinase K and SDS, followed by phenol/chloroform extraction and ethanol precipitation. Nine synthetic DNA oligomers complementary to nucleotides 13–27, 201–215, 411–425, 597–611, 798–812, 945–959, 1078–1092, 1296–1310, and 1533–1547 of the HA gene of Mem71 virus (21) were used as sequencing primers in dideoxy chain-terminating reactions. The sequencing protocol was essentially the same as described by Naeve *et al.* (22), except that [$^{35}$S]dATP was used instead of [$^{32}$P]dATP.

## RESULTS

**Sensitivity of Mem71$_H$-Bel$_N$ Virus to β and γ Inhibitors in Serum.** Hemagglutination by Mem71$_H$-Bel$_N$ virus was sensitive to inhibition by normal bovine and horse sera. As shown in Table 1, the inhibitory activity present in bovine serum was heat labile, resistant to oxidation by periodate, abrogated in the presence of citrate, and restored by the addition of $Ca^{2+}$ ions but not by the addition of $Mg^{2+}$ ions. These properties are characteristic of β inhibitors and contrast with those of the well-characterized γ inhibitor in horse serum, equine $\alpha_2$-macroglobulin, which is periodate sensitive, independent of calcium, and enhanced, rather than destroyed, by heating (Table 1).

**Selection of a β Inhibitor-Resistant Mutant of Mem71$_H$-Bel$_N$ Virus.** Bovine serum also strongly inhibited the infectivity of Mem71$_H$-Bel$_N$ virus, such that its infectivity titer was reduced $10^6$-fold when grown in eggs in the presence of bovine serum. A mutant of Mem71$_H$-Bel$_N$ virus, resistant to neutralization by bovine serum, was selected and cloned as described in *Materials and Methods*. This mutant, referred to as Mem71$_H$-Bel$_N$β$^R$, was highly resistant to HI by bovine serum and by mouse serum, which also contains a β inhibitor, but remained sensitive to the γ inhibitor in horse serum (Table 2). Furthermore, a horse serum-resistant (HS$^R$) mutant of Mem71$_H$-Bel$_N$ virus, isolated in this laboratory (19), retained sensitivity to β inhibitor.

**Receptor-Binding Properties of the β Inhibitor-Resistant Mutant.** To examine whether the change to β inhibitor resistance of Mem71$_H$-Bel$_N$ virus was accompanied by a detectable change in the receptor-binding properties of the virus, mutant and parent viruses were compared in two different assays of hemagglutination using (*i*) erythrocytes (RBC) from different animal species, and (*ii*) chicken RBC treated with increasing concentrations of *V. cholerae* RDE. The latter treatment leads to the progressive desialylation of the RBC receptors for influenza virus, ultimately rendering the erythrocyte nonagglutinable.

Table 2. Resistance to β inhibitor is independent of resistance to γ inhibitor

| Virus | HI titer of normal serum: Bovine | Mouse | Horse* |
|---|---|---|---|
| Mem71$_H$-Bel$_N$ | 2560 | 160 | 1280 |
| Mem71$_H$-Bel$_N$β$^R$ | <20 | <20 | 1280 |
| Mem71$_H$-Bel$_N$HS$^R$ | 2560 | 160 | <20 |

*Horse serum was heat inactivated (56°C, 30 min) before use.

Table 3. Agglutination of different species of RBC by mutant and parent viruses

| Virus | RBC of different species, HA titer: Chick | Duck | Guinea pig | Sheep | Cow | Horse |
|---|---|---|---|---|---|---|
| Mem71$_H$-Bel$_N$ | 32 | 24 | 64 | <2 | <2 | <2 |
| Mem71$_H$-Bel$_N$β$^R$ | 32 | 24 | 64 | 12 | <2 | 12 |

Tests were performed in Microtiter trays with 1% suspensions of RBC.

Table 4. Hemagglutination of RDE-treated chicken RBC by mutant and parent viruses

| Virus | RDE dilution | | | | | |
|---|---|---|---|---|---|---|
| | 1/320 | 1/640 | 1/1280 | 1/2560 | 1/5120 | no RDE |
| $Mem71_H$-$Bel_N$ | − | − | − | − | + | ++ |
| $Mem71_H$-$Bel_N\beta^R$ | − | ++ | ++ | ++ | ++ | ++ |

Tests were performed in Microtiter trays by using 1% suspensions of RDE-treated or untreated chicken RBC and two agglutinating doses of virus. −, No agglutination; +, partial agglutination; ++, complete agglutination.

By both assays, a clear distinction was seen between mutant and parent viruses. Thus (*i*) $Mem71_H$-$Bel_N\beta^R$ differed from the parent virus in being able to agglutinate both sheep and horse erythrocytes (Table 3), and (*ii*) an 8-fold higher concentration of RDE was required to render chicken erythrocytes nonagglutinable by $Mem71_H$-$Bel_N\beta^R$ than by the parent virus (Table 4). These changes in receptor-binding characteristics may reflect an increased avidity and/or a change in fine specificity of the mutant HA for erythrocyte receptors.

**Antigenic Properties of the β Inhibitor-Resistant Mutant.** The antigenic properties of $Mem71_H$-$Bel_N\beta^R$ and its parent virus were compared in HI tests with a panel of anti-HA monoclonal antibodies (mAbs) whose specificity for antigenic sites A, B, D, or E on the HA molecule (23, 24) has been determined (ref. 25; L. E. Brown, J. M. Murray, D. O. White, and D.C.J., unpublished data). mAbs directed against antigenic sites A, B, or E reacted equally well with both viruses (data not shown). mAb 40, however, whose binding to HA is known to be affected by single amino acid substitutions within the receptor-binding site (residue 226) or in the interface region of HA (site D, residue 205 or 218), failed to inhibit hemagglutination by $Mem71_H$-$Bel_N\beta^R$ but reacted strongly with the parent virus (titers of <200 and 4800, respectively). The failure of mAb 40 to bind to $Mem71_H$-$Bel_N\beta^R$ was confirmed by ELISA (data not shown).

**Nucleotide Sequence Analysis of Parental and Mutant HA Genes.** To understand the molecular basis for the differences between the parent and mutant HA, the nucleotide sequence of viral RNA coding for the HA of both viruses was determined by the dideoxy chain-termination procedure. The sequence obtained for the parent virus $Mem71_H$-$Bel_N$ was identical with the published sequence for Mem71 HA (21) except for three silent base changes ($G^{431} \rightarrow T$, $C^{794} \rightarrow G$, and $G^{797} \rightarrow A$). The mutant $Mem71_H$-$Bel_N\beta^R$ differed from the parent virus by a single point mutation—namely, a change in nucleotide 577 from cytosine in the parent to adenine in the mutant. This change results in the amino acid substitution Thr → Asn in residue 167 of the $HA_1$ chain of HA and consequent loss of the potential glycosylation site Asn-165–Val-166–Thr-167 at the tip of the HA spike. In the closely related viruses X-31 (1968) and A/Memphis/102/72, this site is known to bear a high-mannose (type II) carbohydrate side chain N-linked to Asn-165 (26, 27).

Table 5. Effect of periodate treatment of $Mem71_H$-$Bel_N$ virus on sensitivity to β inhibitor

| Treatment of virus* | HI titer of normal serum | | |
|---|---|---|---|
| | Bovine | Mouse | Horse† |
| None | 1920 | 80 | 1280 |
| Mock | 2560 | 80 | 1280 |
| $KIO_4$ (5 min) | 640 | 60 | 2560 |
| $KIO_4$ (10 min) | 160 | 40 | >2560 |
| $KIO_4$ (15 min) | 60 | 20 | >2560 |
| $KIO_4$ (20 min) | 30 | <20 | >2560 |

*Virus was treated with $KIO_4$ for the indicated times or mock-treated; neither treatment had any significant effect on HA titer of the virus.

†Horse serum was heat inactivated (56°C, 30 min) before use.

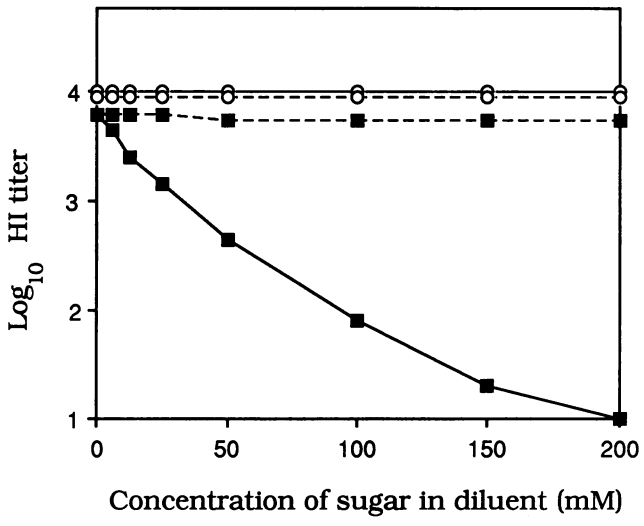


FIG. 1. Effect of D-mannose and L-rhamnose on inhibitory activity of bovine and horse sera for $Mem71_H$-$Bel_N$ virus. HI titers of bovine (■) and horse (○) sera were determined in a series of assays in which the diluent contained different concentrations of D-mannose (—) or L-rhamnose (- - -), as shown. The particularly high inhibitory titers obtained in the absence of sugars (cf. Tables 1, 2, and 5) were due to the use of the macro-, rather than the micromethod of HI titration (see *Materials and Methods*).

**Effect of Periodate Treatment of Virus on Sensitivity to β Inhibitor.** The association of β inhibitor resistance with loss of the carbohydrate side chain at residue 165 of $HA_1$ raised the possibility that the β inhibitors in bovine and mouse sera may be lectins that, by binding to carbohydrate near the tip of the HA spike, block access to the receptor-binding pocket. If so, oxidation of the carbohydrate side chains on HA might be expected to have the same effect of converting a β-sensitive virus to β-resistance. Table 5 shows that this was, indeed, the case. Treatment of the parental virus $Mem71_H$-$Bel_N$ with 0.011 M $KIO_4$ for periods up to 20 min had little or no effect on its HA titer but progressively reduced its sensitivity to inhibition by bovine serum and mouse serum. No such effect was seen with γ inhibitor: periodate-treated virus was, in fact, more susceptible to the horse serum inhibitor than was untreated virus.

**The Activity of β Inhibitor Is Inhibited by D-Mannose.** If β inhibitor is a lectin, its activity should be inhibitable by the appropriate sugar ligand. Fig. 1 shows that the inhibitory activity of bovine serum for $Mem71_H$-$Bel_N$ virus was abrogated in a dose-dependent manner by D-mannose but was unaffected by similar concentrations of L-rhamnose (6-deoxy-L-mannose). Mouse serum inhibitor was similarly blocked by D-mannose (data not shown), whereas neither sugar had any effect on the inhibitory activity of horse serum (Fig. 1).

## DISCUSSION

Although serum β inhibitors of influenza A viruses were first described over 40 yr ago, their mode of action has not been understood. Results of this study indicate that β inhibition by bovine and mouse sera is mediated by mannose-binding molecules. For the H3 subtype virus $Mem71_H$-$Bel_N$, the carbohydrate side chain at residue 165 of HA is critical in determining sensitivity of virus to the inhibitor; this glycosylation site is absent from the HA of the resistant mutant, $Mem71_H$-$Bel_N\beta^R$, which was selected in the presence of inhibitor.

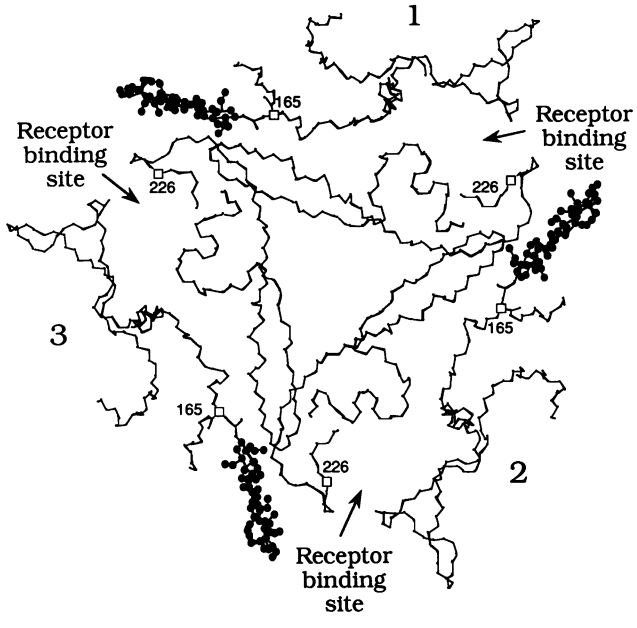


FIG. 2. The oligosaccharide attached at Asn-165 of X-31 (1968) HA is oriented towards the receptor-binding site on the adjacent HA monomer. Viewer is looking down the 3-fold axis of symmetry from the distal end of the main chain of influenza virus X-31 $HA_1$ from residues 122–169 and 187–228. Monomers 1, 2, and 3 are indicated, with the α-carbon atom of residue Asn-165 shown (□) and also the partial (*N*-acetylglucosamine-*N*-acetylglucosamine-mannose-mannose) structure of the attached oligosaccharide (●). The α-carbon atom of residue 226, which is in the receptor-binding site of HA, is also indicated (□). This figure was drawn by using the software package Chem3D Plus (Cambridge Scientific Computing) from coordinates deposited in the Brookhaven Protein Data Bank (October 1988) (code 1HMG).

Orientation of the high-mannose oligosaccharide side chain at residue 165 of H3 HA has been precisely determined for X-31 (1968) virus by x-ray crystallography (13, 14, 28, 29). Located near the tip of the HA spike, this side chain lies across the interface between $HA_1$ subunits, extending very close to the receptor-binding site on the adjacent monomer (Fig. 2). The β inhibitors thus would appear to block access to the receptor-binding site by binding to the carbohydrate at residue 165. Conservation of this carbohydrate attachment in all H3 subtype viruses sequenced so far, including human isolates from 1968 to 1986 (30–33), would account for the general susceptibility of H3 viruses to β inhibitor.

In viruses of the H1 and H2 subtypes, the sites of carbohydrate attachment on HA differ from those of H3 viruses (34–36). Presumably, the sensitivity of H1 viruses and the resistance of H2 viruses likewise reflect their respective patterns of glycosylation in the vicinity of the receptor-binding site of HA. If the β inhibitor can bind only to high-mannose (type II) and not to complex (type I) oligosaccharides, the resistance of H2 viruses may be explained by the absence of type II oligosaccharides from their $HA_1$ subunit, as reported for A/Jap/305/57 and A/RI/$5^-$ viruses (36, 37). Alternatively, the β inhibitor may bind to both types of oligosaccharide, but the carbohydrate attached near the tip of H2 HA may not be oriented towards the receptor-binding site. In A/Jap/305/57 HA, this glycosylation site is at residue 164 (36), which is equivalent to residue 169 in the H3 molecule (35).

For H1 viruses, the original studies on β inhibitors established the sensitivity of pre-1957 strains that had not been mouse-adapted (5, 17), and we have found post-1977 H1 strains to be sensitive also. The particular carbohydrate side chain involved has not yet been identified but may be revealed when we sequence the HA gene of a β-resistant mutant of A/Brazil/11/78 (H1N1) that we have recently isolated. The changes in HA that accompany adaptation of influenza virus to growth in mice are also of interest because mouse adaptation, at least of the pre-1957 H1N1 strains, invariably resulted in selection for β inhibitor resistance (5, 17, 18). Gitelman *et al.* (38, 39) adapted A/USSR/90/77 (H1N1) virus to growth in mice and found the adapted virus had acquired two successive amino acid substitutions in HA at residues 127 and 89 of $HA_1$, which correspond to residues 131 and 96, respectively, in the H3 molecule. It is noteworthy in the present context that both these changes are situated in the vicinity of the receptor-binding site, and both resulted in the loss of potential glycosylation sites.

In addition to its resistance to β inhibitors, the mutant virus $Mem71_H\text{-}Bel_N\beta^R$ differed from the parent virus in its receptor-binding properties and in the antigenicity of its HA molecule. While these altered phenotypes could result from the primary-amino acid substitution in $HA_1$ of Thr-167 → Asn, it seems more probable that, like β inhibitor resistance, these phenotypes are a consequence of loss of carbohydrate at the tip of the HA spike. Residue 167 itself is not situated near the receptor-binding site, whereas, as discussed above, the carbohydrate side chain at residue 165 in H3 viruses extends across the $HA_1$ subunit interface, close to the receptor-binding site on the adjacent monomer (Fig. 2). Loss of this carbohydrate may facilitate access of cell-surface receptors to the receptor-binding pocket, allowing a higher affinity of interaction with the same types of receptors as are bound by the parent virus. Alternatively, binding to sialic acid linkages not normally bound by the parent virus may be permitted in the resistant mutant. A similar association between loss of a carbohydrate attachment site from the tip of the HA spike and a demonstrated or inferred alteration in receptor-binding properties of influenza virus has been noted in a number of other systems (40–42).

The antigenic change in $Mem71_H\text{-}Bel_N\beta^R$ is characterized by loss of binding of mAb 40, an antibody that has an epitope known also to be affected by substitution in residue 226 in the receptor-binding site and residues 205 and 218 in the interface region of HA (site D) (L. E. Brown, J. M. Murray, D. O. White, and D.C.J., unpublished data). Amino acid residue 167 itself is unlikely to form part of the epitope of mAb 40 because this area of HA is masked by the carbohydrate attached at residue 165 (28, 29). If, instead, the antigenic change results from loss of the carbohydrate side chain from residue 165, this would suggest either that carbohydrate forms part of the epitope of mAb 40 or that loss of this carbohydrate alters the conformation of the $HA_1$ subunits at the interface in such a way as to destroy the integrity of the epitope for mAb 40.

Past attempts to purify and characterize β inhibitors from serum have led to conflicting conclusions as to their molecular nature (for review, see ref. 1). A number of early studies sought evidence for the involvement of the complement system, particularly properdin, but were not substantiated (4, 43, 44). Recently, however, Yamamoto *et al.* (45) concluded that a β-like inhibitor in guinea pig serum active against influenza B viruses is a component of the classical complement pathway. This inhibitor was not active against influenza A viruses, however, and its relationship to the β inhibitors in bovine and mouse sera is not known.

Our finding that β inhibitors of bovine and mouse sera are mannose-binding lectins opens the way to their purification by affinity chromatography. Of particular interest is the possible relationship of these inhibitors to other $Ca^{2+}$-dependent mannose-binding proteins that have been described in the serum and liver of humans and rodents. The recent reports by Ezekowitz and coworkers (46) that the human serum mannose-binding protein functions as an op-

sonin for certain Gram negative bacteria and inhibits *in vitro* infection by human immunodeficiency virus (47) suggest that these lectins may be part of a first-line host defence against microbial infection.

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