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Two Distinct Serum Mannose-Binding Lectins Function as β Inhibitors of Influenza Virus: Identification of Bovine Serum β Inhibitor as Conglutinin

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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. We have previously shown these β inhibitors to be mannose-binding lectins that apparently act by binding to carbohydrate on the viral hemagglutinin, blocking access of the receptor-binding site to receptors on host cells (E. M. Anders, C. A. Hartley, and D. C. Jackson, Proc. Natl. Acad. Sci. USA 87:4485–4489, 1990). For the H3-subtype virus A/Memphis/1/71 × A/Bel/42 (H3N1), sensitivity to β inhibitors is determined by the oligosaccharide at residue 165 of the hemagglutinin, this glycosylation site being lost in a resistant mutant selected by growth in the presence of bovine serum. In the present study, we sequenced the hemagglutinin genes of additional bovine serum-resistant mutants derived from influenza viruses A/Philippines/2/82 (H3N2) and A/Brazil/11/78 (H1N1). The results confirm the importance of carbohydrate at residue 165 for inhibitor sensitivity of H3 viruses and implicate carbohydrate at residue 87 (94a in the H3 numbering system) as an important determinant in the sensitivity of H1-subtype viruses to the bovine inhibitor. Unlike the two H3 mutants, which had also gained resistance to hemagglutination inhibition by mouse serum, the H1 bovine serum-resistant mutant remained sensitive to the mouse β inhibitor, suggesting that inhibition by the two types of sera is mediated by distinct mannose-binding lectins. In support of this hypothesis, the β inhibitors in bovine and mouse sera were shown to differ in their pattern of inhibition by monosaccharides and in their sensitivity to 2-mercaptoethanol. In these and other properties, the bovine inhibitor closely resembled conglutinin, a Ca^{2+}-dependent *N*-acetylglucosamine- and mannose-binding lectin present in bovine serum but absent from the serum of other species. Furthermore, polyclonal and monoclonal anticonglutinin antibodies abrogated the hemagglutination-inhibiting activity of bovine serum. Direct binding of conglutinin to the parent viruses and reduced binding to their respective mutants were confirmed by radioimmunoassay.

Normal sera contain a variety of nonspecific inhibitors of influenza virus. These have been classified as α, β, and γ inhibitors on the basis of various properties including their sensitivity to inactivation by heat, neuraminidase, or periodate treatment, their possession of virus-neutralizing as well as hemagglutination-inhibiting (HI) activity, and their spectrum of activity against influenza A viruses of different subtypes (16, 27). Inhibitors of the α and γ classes are sialylated glycoproteins whose mode of action against influenza virus is to compete with sialylated cell surface receptors for binding to the viral hemagglutinin (HA). The γ inhibitors in horse and guinea pig sera have been identified as α_2-macroglobulin (35); those in pig and rabbit sera have not been identified but appear to be distinct from that in horse serum (37).

β inhibitors, on the other hand, are not sialylated and their mode of action has remained unclear for many years. β inhibitors possess both virus-neutralizing and HI activity, are Ca^{2+} dependent, and are present at a high titer in bovine serum and, to a lesser extent, mouse and other sera. They inhibit type A influenza viruses of the H1 and H3 subtypes but not the H2 subtype (12), although H1 viruses that have been passaged in mice are reported to be resistant (7, 8).

We have recently shown the β inhibitors of bovine and mouse sera to be mannose-binding lectins (1). For the H3-subtype virus $Mem71_H$-Bel_N, the carbohydrate side chain at residue 165 of the HA molecule is critical in determining sensitivity of the virus to β inhibitor; this glycosylation site is absent from the HA of a resistant mutant which was selected in the presence of bovine serum. In H3 HA, the high-mannose carbohydrate side chain at residue 165 lies across the interface between HA_1 subunits extending close to the receptor-binding site on the adjacent HA monomer (42, 48, 49). Binding of β inhibitor to this carbohydrate side chain is presumed to block access of cell surface receptors to the receptor-binding site.

In the first part of this report, we extend our study of β inhibitor-resistant mutants of influenza A viruses to include mutants of a second H3-subtype virus, A/Philippines/2/82 (H3N2), and A/Brazil/11/78 (H1N1). The results of sequencing the HA genes of these mutants confirm the critical importance of carbohydrate at HA residue 165 for β inhibitor sensitivity of late as well as early H3 viruses and implicate carbohydrate at residue 87 (94a in H3 numbering) as an important determinant in the sensitivity of H1-subtype viruses to the bovine inhibitor.

Two types of Ca^{2+}-dependent mannose-binding lectins that have been described in mammalian sera and are thus candidates for the β-inhibitor molecule are serum mannose (or mannan)-binding protein (21, 22, 25, 34, 44) and conglutinin (28). Both belong to the family of mammalian C-type lectins possessing collagenlike sequences, but they differ in their size and molecular architecture and in their fine specificity for different sugars (13, 46).

In further characterizing the β inhibitors of bovine and

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mouse sera, we found them to differ in a number of properties, including their reactivity with the bovine serum-resistant mutant of A/Brazil/11/78 (H1N1) influenza virus and their pattern of sensitivity to inhibition by monosaccharides. These differences indicate that the bovine and mouse β inhibitors are distinct molecular species. Further experiments are described which identify the major bovine β inhibitor as conglutinin.

MATERIALS AND METHODS

Viruses. The type A influenza viruses used were A/Philippines/2/82 (H3N2), A/Brazil/11/78 (H1N1), and the reassortant $Mem71_H$-Bel_N (H3N1), bearing the HA of A/Memphis/1/71 (H3N2) and the neuraminidase of A/Bel/42 (H1N1). Bovine serum-resistant mutants Phil82/BS, Brazil78/BS, and $Mem71_H$-Bel_N/BS (previously denoted $Mem71_H$-$Bel_N\beta^R$ [1]) were selected by growth of the parent viruses in eggs in the presence of bovine serum as described previously (1).

Nucleotide sequencing of HA gene. Nucleotide sequences of the HA_1 portion of the HA gene of parent and mutant viruses were analyzed by the dideoxy chain termination technique (38), using procedures described previously (1). The oligonucleotide primers used were complementary to residues 19 to 33, 304 to 318, and 516 to 530 of the HA gene of A/Brazil/78 virus (36) and, for A/Philippines/82 virus, residues 411 to 425 and 771 to 785 of the HA gene of Phil82 (32) and residues 13 to 27 from the sequence of A/Memphis/1/71 (33).

Sera and antisera. Bovine, mouse, and horse sera were collected from freshly clotted blood and stored at −20°C (bovine and mouse sera) or −70°C (horse serum, for use as a source of complement). Rabbit antiserum to bovine conglutinin was a gift from P. Lachmann, Medical Research Council Centre, Cambridge, England.

Hemagglutination titrations and HI tests. Hemagglutination titrations and HI tests were performed by standard procedures with 96-well microtiter plates and 1% (vol/vol) chicken erythrocytes. The diluent was either TBS (0.05 M Tris, 0.15 M NaCl, pH 7.2) containing 50 mM $CaCl_2$ or Veronal-buffered saline (pH 7.2) containing 1.8 mM $MgCl_2$ and 0.25 mM $CaCl_2$ (complement fixation test diluent, Oxoid, London, England). The effect of different monosaccharides on HI activity was assayed in WHO macrotrays with 5% erythrocytes since, in the presence of sugar, shields of agglutinated erythrocytes were found to be more stable in the larger wells.

Conglutinin assay. Sheep erythrocytes coated with antibody and complement were prepared as described by Coombs et al. (10), using heated (56°C, 30 min) bovine serum as the source of Forssman antibody and horse serum as the complement source. Conglutinin was assayed by the resuspension method (10), using complement fixation test diluent throughout. The conglutinin titer was the reciprocal of the highest dilution of serum causing distinct clumping of cells.

Isolation of mannan-binding fraction of bovine serum. Early studies had shown the bovine β inhibitor to precipitate with the euglobulin fraction of serum (24). Bovine serum was dialyzed against distilled water for 48 h at 4°C. The euglobulin precipitate was recovered by centrifugation (10,000 × *g*, 30 min), dissolved in 1/10th of the original serum volume of loading buffer (0.04 M imidazole-HCl [pH 7.8], 1.25 M NaCl, 0.05 M $CaCl_2$, 0.1% NaN_3), heated for 30 min at 56°C (see Discussion), and dialyzed against the same buffer overnight at 4°C. After centrifugation (10,000 × *g*, 15 min), 5 ml of supernatant was applied to a 30-ml column of Sepharose 4B-CL (Pharmacia) to which had been coupled mannan from *Saccharomyces cerevisiae* (Sigma). The bound fraction was eluted with buffer containing 0.04 M imidazole-HCl [pH 7.8], 1.25 M NaCl, 0.1% NaN_3, and 2 mM EDTA. The eluate was concentrated by vacuum dialysis, dialyzed against TBS containing 0.1% NaN_3, and stored at 4°C. Compared with the original serum, this mannan-binding fraction showed a 5,000-fold and 2,000-fold increase in the specific activity of conglutinin and HI activity, respectively.

Production of an MAb to conglutinin. BALB/c mice were immunized in the hind footpads with 24 μg of the mannan-binding fraction of bovine serum in Freund's complete adjuvant (Commonwealth Serum Laboratories, Parkville, Victoria, Australia). After 15 days, cells from the inguinal and popliteal lymph nodes were pooled and fused with Sp2/0-Ag14 myeloma cells (40), and hybrid cells were isolated by the hypoxanthine-aminopterin-thymidine selection procedure (23). Culture supernatants of developing hybridomas were assayed by enzyme-linked immunosorbent assay for binding to the immunogen. Cells from positive wells were cloned by limiting dilution and grown as ascites in BALB/c mice. One monoclonal antibody (MAb), 3H1, was shown to be specific for conglutinin by its ability to inhibit conglutination by bovine serum (see Results).

Binding of conglutinin to influenza virus. The binding of conglutinin to different strains of influenza virus was measured by radioimmunoassay. Wells were coated overnight with 25 μl of purified influenza virus (1,000 HA units/ml) in TBS, blocked for 1 h with 10 mg of bovine serum albumin (BSA) per ml, and washed with TBS containing 0.05% Tween 20 (TBS-T). Serial dilutions (25 μl) of the mannan-binding fraction of bovine serum in TBS-T containing 5 mg of BSA per ml and 50 mM $CaCl_2$ (BSA_5-TBS-T-Ca^{2+}) were incubated in the wells overnight, and then the wells were washed. Bound conglutinin was detected by the addition of anticonglutinin MAb 3H1 (1/100 dilution of ascitic fluid in BSA_5-TBS-T-Ca^{2+}) for 4 h and then, after washing, ^{125}I-labeled rabbit anti-mouse immunoglobulin (Dako Immunoglobulins, Glostrup, Denmark) in the same diluent (100,000 cpm/25 μl, 1 h). The amount of radioactivity bound to individual wells was determined in a gamma counter. In specificity controls, ^{125}I-labeled antibody failed to bind in wells receiving an irrelevant MAb in place of MAb 3H1 and in wells in which the mannan-binding fraction was diluted in BSA_5-TBS-T containing 2 mM EDTA. To confirm that each pair of mutant and parent viruses had bound to the wells with equal efficiency, a further set of virus-coated wells was titrated with serial dilutions of mouse antiserum against the parent virus and developed as above.

RESULTS

Isolation and sequencing of bovine serum-resistant mutants of A/Brazil/78 (H1N1) and A/Philippines/82 (H3N2) viruses. Bovine serum-resistant mutants of Brazil78 and Phil82 viruses were selected by growing the parent virus in eggs in the presence of bovine serum (1). The resultant viruses, denoted Brazil78/BS and Phil82/BS, respectively, were, like the mutant $Mem71_H$-Bel_N/BS (previously denoted $Mem71_H$-$Bel_N\beta^R$ [1]), markedly more resistant to neutralization and HI by bovine serum than their respective parent viruses (Table 1), although the resistance of Brazil78/BS was less complete than that of the other two mutant viruses. Phil82/BS and $Mem71_H$-Bel_N/BS were also resistant to HI by the β inhibitor in mouse serum, whereas Brazil78/BS remained sensitive to the mouse inhibitor.

TABLE 1. Reactivity with bovine and mouse serum β inhibitors and amino acid sequence differences in HA_1 of parent and mutant viruses

Virus	Virus titer (log_{10} EID_{50}/ml)[a]		HI titer of serum[b]		Amino acid substitution in mutant HA_1
	Without serum	With bovine serum	Bovine	Mouse	
$Mem71_H$-Bel_N	9.5	4.0	2,560	160	
$Mem71_H$-Bel_N/BS	8.5	7.3	20	<20	Thr-167 → Asn
Phil82	9.0	3.0	3,840	240	
Phil82/BS	9.5	8.5	<20	30	Thr-167 → Leu
Brazil78	9.5	3.0	2,560	160	
Brazil78/BS	10.0	6.5	80	160	Thr-89 → Ile

[a] Dilutions of virus were incubated with an equal volume of diluent or undiluted bovine serum for 1 h at room temperature before inoculation into 10-day-old embryonated hen eggs. EID_{50}, dose of virus infecting 50% of eggs.
[b] HI titer is the reciprocal of the highest dilution of serum inhibiting 4 HA units of virus.

TABLE 2. Inhibition of HI activity of bovine and mouse sera by monosaccharides[a]

Sugar	Minimal concn (mM) of sugar required for complete inhibition of HI activity of:	
	Bovine serum	Mouse serum
N-Acetyl-D-glucosamine	3	6
D-Mannose	50	3
L-Fucose	100	6
D-Glucose	100	12
D-Galactose	200	50
L-Rhamnose	>200	>200
N-Acetyl-D-galactosamine	>200	>200

[a] Serial dilutions of sugars were tested for their ability to inhibit the HI activity of 4 HI units of bovine or mouse serum assayed on $Mem71_H$-Bel_N virus in WHO plates with 5% chicken erythrocytes. One HI unit is the minimal activity inhibiting 4 HA units of virus.

The viral RNA coding for the HA_1 subunit of each pair of parent and mutant viruses was sequenced by the dideoxy chain termination procedure (Table 1). The nucleotide sequence of Phil82 HA was identical to the published sequence (6, 32). The HA gene of the mutant Phil82/BS differed from that of the parent by a single base substitution ($A^{576} \rightarrow G$) giving rise to the amino acid substitution Thr → Leu at residue 167. This is the same amino acid residue that is altered in the mutant $Mem71_H$-Bel_N/BS, the change in that virus being Thr → Asn (1). In both cases, the change results in loss of the glycosylation site Asn-165–Val-166–Thr-167 at the tip of the HA spike.

The nucleotide sequence obtained for the HA of the parent Brazil78 virus was identical with the sequence published previously (36), except for a single base change ($T^{486} \rightarrow G$). The mutant Brazil78/BS differed from the parent in a single nucleotide ($C^{349} \rightarrow T$), resulting in the amino acid substitution Thr → Ile at residue 89 of HA_1 and loss of the potential glycosylation site Asn-87–Gly-88–Thr-89. Asn-87 of H1 HA corresponds to residue 94a in the H3 numbering system and is located on the globular head of the HA molecule, in the vicinity of the receptor-binding site.

Distinct properties of bovine and mouse serum β inhibitors. The β inhibitors present in bovine and mouse serum are similar in many respects, including their Ca^{2+} dependence and sensitivity to inhibition by D-mannose (1) and their sensitivity to collagenase (unpublished observations). Furthermore, loss of carbohydrate from residue 165 of H3 HA rendered the mutant viruses $Mem71_H$-Bel_N/BS and Phil/BS resistant to HI by mouse, as well as bovine, serum (Table 1). However, the observation that the H1 mutant Brazil/BS remained sensitive to the mouse inhibitor indicated that β inhibition by bovine and mouse sera may be mediated by different types of mannose-binding molecules. To examine this question further, we compared the bovine and mouse inhibitors for their pattern of sensitivity to inhibition by different monosaccharides.

Both types of β inhibitor were sensitive to inhibition by *N*-acetylglucosamine, fucose, and glucose (Table 2), as well as by mannose as reported previously (1), whereas *N*-acetylgalactosamine and rhamnose were without effect. The two inhibitors showed a distinct difference in their fine specificity, however, with the bovine inhibitor being much more strongly inhibited by *N*-acetylglucosamine than by mannose and fucose, whereas the mouse inhibitor showed a similar sensitivity to all three sugars.

A further difference between the two inhibitors was observed in their sensitivity to 2-mercaptoethanol. When HI titrations against $Mem71_H$-Bel_N virus were done in the presence of 0.1 M 2-mercaptoethanol, the titer of bovine serum remained unchanged, whereas the titer of mouse serum was reduced from 320 to 10. Taken together, these data indicated that the β inhibitors in bovine and mouse sera are not identical and that there are two types of serum mannose-binding lectin that can act as β inhibitors.

Evidence for the identity of bovine β inhibitor and conglutinin. Resistance to 2-mercaptoethanol, sensitivity to collagenase, and the pattern of sugar specificity (*N*-acetylglucosamine >> mannose > fucose) exhibited by bovine β inhibitor are all characteristic properties of conglutinin, a Ca^{2+}-dependent lectin present in bovine serum but absent from the serum of other species (17, 22, 28, 29, 30). Conglutinin was identified originally by its ability to cause agglutination (conglutination) of erythrocytes coated with antibody and complement. As further support for a link between conglutinin and bovine β inhibitor, a direct correlation was found between the HI and conglutinin titers of 11 individual bovine serum samples obtained from animals ranging in age from 4 months to >3 years (Fig. 1). The lower levels of each activity were found in sera from the younger animals.

A rabbit antiserum against bovine conglutinin, provided by Peter Lachmann, was found to inhibit the HI activity, as well as the conglutinating activity, of bovine serum (Table 3). The serological cross-reactivity of conglutinin and β inhibitor was confirmed with an MAb prepared against the mannan-binding fraction of bovine serum (see Materials and Methods). MAb 3H1, shown to be specific for conglutinin by its ability to inhibit conglutination, also inhibited the HI activity of bovine serum (Table 3). A control MAb, 1E6, derived from the same fusion but with no inhibitory activity against conglutinin, had no effect on the HI activity of bovine serum.

Binding of conglutinin to sensitive and resistant strains of influenza virus. MAb 3H1 was used in a radioimmunoassay to examine the binding of conglutinin to $Mem71_H$-Bel_N, Phil82, and Brazil78 viruses and to their bovine serum-resistant mutants. All three parent viruses were shown to bind conglutinin in a Ca^{2+}-dependent manner (Fig. 2). Conglutinin bound only very poorly to the H3 mutant viruses

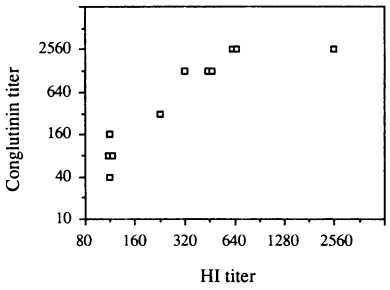

FIG. 1. Comparison of the titers of conglutinin and of HI activity against $Mem71_H$-Bel_N virus in 11 individual bovine serum samples. Conglutinin titers are the reciprocals of the highest dilution of serum giving distinct clumping of antibody- and complement-coated erythrocytes. HI titers are reciprocals of the highest dilution of serum inhibiting 4 HA units of virus. Spearman rank correlation coefficient (r_s) = 0.96; $P < 0.01$ (41).

$Mem71_H$-Bel_N/BS and Phil/BS, indicating that the carbohydrate side chain at residue 165 lost from the HA of these mutants represents the major site of binding of conglutinin to the parent virus. The H1 mutant virus Brazil/BS, on the other hand, retained significant binding of conglutinin, although again the level was substantially lower than binding to the parent virus.

DISCUSSION

The results of this study show bovine and mouse serum β inhibitors of influenza virus to be distinct mannose-binding lectins and identify the major bovine serum β inhibitor as conglutinin. Conglutinin is a member of the group of Ca^{2+}-dependent mammalian lectins that contain collagenlike sequences linked to a C-type lectin domain (11, 13, 46). It was discovered early this century as a component of normal bovine serum that caused the agglutination of erythrocytes bearing fixed complement (4, 5), a process which is now known to be mediated by binding of conglutinin to a particular high-mannose carbohydrate side chain on iC3b, a degradation product of the major complement component C3 (17, 18). In addition to high-mannose-type oligosaccharides,

TABLE 3. Inhibition of HI activity of bovine serum by anticonglutinin antibodies

Serum or MAb	Inhibitory titer against:	
	HI activity[a]	Conglutinin[b]
Rabbit anticonglutinin antiserum	4,800	1,600
Normal rabbit serum	<100	<100
MAb 3H1[c]	640	320
MAb 1E6[d]	<10	<10

[a] Titer is expressed as the reciprocal of the highest dilution of antibody which abrogated the HI activity of 4 HI units of bovine serum assayed on $Mem71_H$-Bel_N virus. One HI unit is the minimal activity inhibiting 4 HA units of virus in the standard microtiter plate assay.

[b] Titer is expressed as the reciprocal of the highest dilution of antibody which completely inhibited the activity of 8 conglutinating units of bovine serum. One conglutinating unit is the minimal activity causing distinct clumping of antibody- and complement-coated sheep erythrocytes in the standard conglutinin assay.

[c] Conglutinin-specific MAb raised by immunization of mice with the mannan-binding fraction of bovine serum.

[d] Control MAb derived from the same fusion as 3H1 but lacking inhibitory activity against conglutinin.

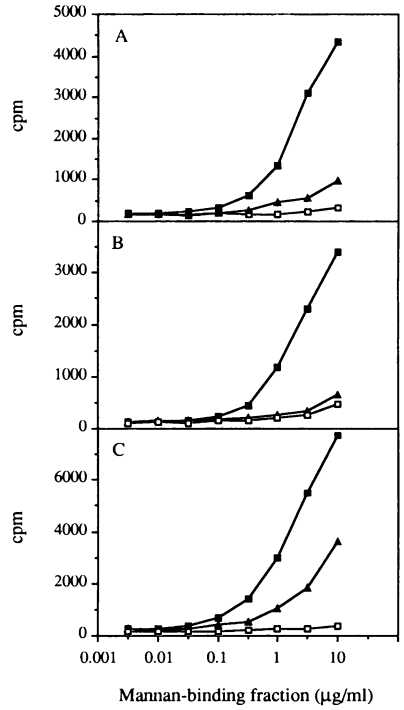

FIG. 2. Radioimmunoassay of binding of conglutinin to the parent virus (■) and bovine serum-resistant mutant (▲) of $Mem71_H$-Bel_N (A), Phil82 (B), and Brazil78 (C). The mannan-binding fraction of bovine serum was titrated on virus-coated wells, and bound conglutinin was detected with MAb 3H1 and ^{125}I-labeled rabbit anti-mouse immunoglobulin as described in Materials and Methods. Binding to the parent viruses in the presence of 2 mM EDTA is also shown (□).

conglutinin binds also to complex- and hybrid-type oligosaccharides bearing terminal *N*-acetylglucosamine or mannose residues (31). In ultrastructure, conglutinin is a large spider-like molecule, composed of four arms linked at a central hub, with a globular lectin domain at the end of each arm (43, 46). The diameter of the extended molecule is approximately 90 nm, similar to that of an influenza virus particle (100 to 120 nm). Conglutinin was originally thought to be restricted to sera of members of the family Bovidae (28), but has more recently been detected in the plasma (but not serum) of humans and mice (3, 45).

The stability of conglutinin to heating at 56°C for 30 min (28) led us initially to overlook this molecule as a possible candidate for the bovine β inhibitor, since β inhibitors are generally regarded as heat labile (24, 27). However, others (12, 39) reported bovine β inhibitor to be relatively heat stable, and we have found this property to be rather variable, with the loss of HI titer on heating samples from a single batch of bovine serum at 56°C for 30 min ranging from 20-fold (1) to less than 2-fold on different occasions. The factors contributing to this variability are not understood. In our initial (unpublished) attempts to enrich for the bovine β inhibitor by mannan-affinity chromatography of unheated serum (see Materials and Methods), the HI activity of the isolated mannan-binding fraction was found to be very labile on storage at 4°C, a problem also encountered by others in early attempts to isolate this inhibitor by different procedures (24, 26). This problem was overcome by the use of heated serum, as is standard practice for the isolation of

conglutinin (28). The stabilizing effect of heat may be due to inactivation of serum proteases, since isolated conglutinin is known to be very sensitive to the action of enzymes such as trypsin and papain (28). The possible presence of a second, heat-labile β inhibitor in bovine serum is not excluded by the present study. However, since the data on correlation of HI titers with conglutinin titers (Fig. 1) and the inhibition of HI activity by anticonglutinin antibodies (Table 3) were obtained with unheated, unfractionated bovine serum, it appears that if a second inhibitor is present, it contributes only a minor fraction of the total β inhibitor activity of bovine serum.

For H3-subtype viruses, the oligosaccharide attached at residue 165 of HA is critical in determining sensitivity of the virus to both the HI and neutralizing effects of bovine serum (Table 1). This glycosylation site, which is conserved throughout viruses of the H3 subtype, is lost from the HA of the mutant viruses $Mem71_H$-Bel_N/BS and Phil82/BS selected by growth in bovine serum. Direct binding studies showed that these mutant viruses bind substantially lower levels of conglutinin than their respective parent viruses. Thus, although the Mem71 and Phil82 HA molecules carry a total of 7 and 10 potential glycosylation sites, respectively (6, 32, 33, 47), and the neuraminidase glycoprotein carries a further 4 to 5 sites (9), the carbohydrate side chain at residue 165 of HA appears to represent the major site of binding of conglutinin to the parent virus of each strain. The oligosaccharide side chain at residue 165 is of the high-mannose type and lies across the interface between HA_1 subunits of the HA trimer, close to the receptor-binding site on the adjacent HA_1 monomer (42, 48, 49). Binding of conglutinin to this carbohydrate side chain may thus block access of cell surface receptors to the receptor-binding site and perhaps also cause aggregation of the virus. Additional consequences may include interference with virus internalization and/or uncoating by host cells, effects which could contribute to virus neutralization if the efficiency of blocking of virus attachment to host cells is less than 100%.

The crystal structure of H1-subtype HA has not yet been solved but is expected to be similar to that of H3-subtype HA, based on the position of conserved amino acids including cysteine residues and residues within the receptor-binding site (49, 50). The sites of carbohydrate attachment on HA of the H1 subtype differ from those on H3 HA. In A/Brazil/78 virus, there are eight potential N-linked glycosylation sites on HA_1 (36), of which four, at residues 94a, 131, 158, and 163 (in H3 numbering), are located on the globular head of the HA molecule and in the vicinity of the receptor-binding site. The mutant virus Brazil78/BS was found to have lost the glycosylation site at residue 94a, this loss being accompanied by a marked reduction in sensitivity to both neutralization and HI by bovine serum as well as in the ability to bind conglutinin. The fact that the reduction in each of these properties was partial rather than complete implies that conglutinin can bind to at least one other carbohydrate side chain on Brazil78 HA. Our attempts to isolate a mutant of Brazil78/BS that has gained full resistance to bovine serum have so far been unsuccessful.

The loss of carbohydrate from residue 94a of Brazil78 HA did not affect sensitivity of the virus to HI by mouse serum. This observation provided the first indication that bovine and mouse β inhibitors may be distinct lectins, a conclusion supported by demonstration of a difference in the monosaccharide inhibition profiles and sensitivity to 2-mercaptoethanol of the two inhibitors. The molecular identity of the mouse β inhibitor remains to be established, but in several of its properties it resembles the serum mannose-binding proteins (21, 22, 25, 34, 44) and mouse Ra-reactive factor, a Ca^{2+}-dependent, mannose-binding lectin in mouse serum that is bactericidal for the Ra-chemotype *Salmonella* strain in the presence of guinea pig complement (19).

The biological role of conglutinin and other serum mannose-binding lectins is not known, but in recent years interest has focussed on their possible role in first-line host defense against microbial infections (14). Conglutinin has been shown to possess antibacterial activity in vivo (15, 20) and to act as an opsonin in vitro, enhancing the uptake by phagocytes of bacteria bearing fixed complement on their surface (15). The results of the present study, and of a recent study by Andersen et al. (2), suggest that a protective role for conglutinin also extends to viral infections. Andersen et al. (2) demonstrated direct binding of bovine conglutinin to recombinant gp160 of human immunodeficiency virus type 1, with resultant blocking of binding of gp160 to cell membrane CD4. The finding of conglutinin in the plasma of humans and mice (3, 45) extends the relevance of this molecule to species other than cattle.

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