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Complement-dependent neutralization of influenza virus by a serum mannose-binding lectin

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The nature of the β inhibitor in guinea-pig serum and its mechanism of neutralization of influenza virus have been investigated. This inhibitor was shown to be a mannose-binding lectin serologically related to human serum mannose-binding protein. Ca^{2+}-dependent binding of the guinea-pig lectin to influenza virus or to mannan could be detected with polyclonal or monoclonal antibodies against human mannose-binding protein in an ELISA. Furthermore, the monoclonal antibody inhibited both the haemagglutination-inhibiting and virus-neutralizing activities of the guinea-pig lectin. The lectin was active against influenza viruses of both type A and type B. In haemagglutination inhibition it acts independently of complement, apparently by sterically hindering access to the receptor-binding site on the viral haemagglutinin through binding of the lectin to carbohydrate side-chains in the vicinity of this site. Neutralization by the lectin, however, was shown to require activation of the classical complement pathway. To our knowledge, the neutralization of influenza virus by a serum lectin plus complement represents a previously unrecognized mechanism of complement-dependent viral inactivation that may be important in first-line host defence against a variety of enveloped viruses.

Introduction

The infectivity of influenza virus is neutralized by normal sera from a number of different animal species (Krizanova & Rathova, 1969). Neutralization is independent of antibody and is mediated by at least two types of inhibitory molecule, both of which also inhibit haemagglutination by the virus. The so-called γ inhibitors are heat-stable, sialylated glycoproteins that act by competing with cell-surface receptors for binding to the viral haemagglutinin (HA). The γ inhibitor in horse and guinea-pig serum has been identified as α_2-macroglobulin (Pritchett & Paulson, 1989); those in pig and rabbit serum have not been identified but appear to be distinct from that in horse serum (Ryan-Poirier & Kawaoka, 1991).

β inhibitors, on the other hand, are Ca^{2+}-dependent, non-sialylated and generally heat-labile. They occur at high titre in bovine serum, but are also present in mouse, guinea-pig, ferret and rabbit serum (Krizanova & Rathova, 1969). We have recently shown the bovine and mouse β inhibitors to be related, but distinct, mannose-binding lectins (Anders *et al.*, 1990; Hartley *et al.*, 1992). Both belong to the group of mammalian C-type lectins bearing collagen-like sequences (Thiel & Reid, 1989), but they differ in a number of properties including their fine specificity for different monosaccharides, heat lability and sensitivity to 2-mercaptoethanol. The major bovine β inhibitor was identified as conglutinin (Hartley *et al.*, 1992), a lectin found only in bovine serum (Lachmann, 1967) although it has been found in the plasma (but not serum) of humans and mice (Baatrup *et al.*, 1987); the β inhibitor in mouse serum, on the other hand, shares many properties with the serum mannose-binding proteins (MBPs) that have been isolated from a number of different species including man (Kozutsumi *et al.*, 1980; Kawasaki *et al.*, 1983, 1985; Summerfield & Taylor, 1986; Oka *et al.*, 1988).

Haemagglutination inhibition (HI) by β inhibitors appears to result from steric hindrance of access to the receptor-binding site on the HA glycoprotein by binding of the lectin to carbohydrate side-chains on HA that lie in the vicinity of this site (Anders *et al.*, 1990; Hartley *et al.*, 1992). Whether this mechanism accounts also for the neutralizing activity of these inhibitors has yet to be established; virus penetration of and/or uncoating by host cells are additional steps that could be inhibited by

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the presence of the bound inhibitor. Furthermore, the mechanism of neutralization by bovine conglutinin and by the MBPs of mouse and other species may be different. Conglutinin is very large (Strang *et al.*, 1986; Thiel & Reid, 1989), the diameter of the extended molecule (90 nm) approaching that of the influenza virus particle itself. The mannose-binding proteins are smaller (Thiel & Reid, 1989), more comparable in size to an HA molecule, and furthermore they have the capacity to fix complement (Ikeda *et al.*, 1979; Schweinle *et al.*, 1989; Lu *et al.*, 1990; Ohta *et al.*, 1990; Matsushita & Fujita, 1992). The latter property is of particular interest in light of a study by Yamamoto *et al.* (1987) in which they concluded that a β-like inhibitor in guinea-pig serum that neutralized influenza B virus was a component of the classical complement pathway. The inhibitor in that study, however, was reported to be inactive against influenza A viruses.

In this study, we have characterized the β inhibitor of guinea-pig serum. We show it to be a mannose-binding lectin serologically related to human MBP and sharing a number of properties with the mouse β inhibitor. It is active against both type A and type B influenza viruses and, in HI, acts independently of complement. Neutralization of virus infectivity by this lectin, however, proceeds via activation of the classical complement pathway.

Methods

Viruses. The type A influenza viruses used were $Mem71_H$–Bel_N (H3N1), a genetic reassortant of A/Memphis/1/71 (H3N2) × A/Bel/42 (H1N1), $Mem71_H$–Bel_N/HS, a mutant of $Mem71_H$–Bel_N selected for resistance to the γ inhibitor in horse serum (Anders *et al.*, 1986), and $Mem71_H$–Bel_N/BS, a mutant of $Mem71_H$–Bel_N selected for resistance to the β inhibitor in bovine serum (Anders *et al.*, 1990). Influenza type B virus B/Hong Kong/8/73 was also used. Viruses were grown in eggs and purified from allantoic fluid as described previously (Anders *et al.*, 1990).

Sera and antisera. Guinea-pig serum (GPS) was collected from freshly clotted blood, absorbed with 0·1 volume packed sheep red blood cells for 30 min at 0 °C and stored at −70 °C. C4-deficient GPS (Calbiochem) was treated similarly. Rabbit antiserum and monoclonal antibody 6 (MAb#6) (IgG1) against human MBP were prepared by using human recombinant MBP as immunogen, as previously described (Super *et al.*, 1992). A control IgG1 MAb of irrelevant specificity, 6.6.H3, was provided by Georgia Kapaklis-Deliyannis of the Department of Microbiology, University of Melbourne, Australia. $F(ab')_2$ fragments of MAb#6 and MAb 6.6.H3 were prepared by digestion of IgG with 2·5% (w/w) pepsin (Worthington Biochemical) for 60 min as described by Parham (1983). Complete digestion was verified by analysis of the products by SDS–PAGE.

Virus neutralization assay. Neutralization of virus infectivity was measured by plaque reduction in Madin-Darby canine kidney (MDCK) cell monolayers cultured in six-well plates (Nunc). Dilutions of serum were prepared in RPMI-1640 medium supplemented with 100 U penicillin and 100 µg streptomycin per ml, and mixed with 150 p.f.u. of virus in a total volume of 300 µl. The mixtures were incubated at 37 °C for 30 min, then 100 µl of each mixture was inoculated to duplicate MDCK monolayers that had been washed with RPMI-1640. After adsorption of virus for 45 min at 34 °C, 3 ml of an overlay medium prewarmed to 45 °C was added. The overlay consisted of Leibovitz L-15 medium with glutamine (Gibco) supplemented with HEPES buffer (0·01 M pH 6·8), gentamicin (30 µg/ml), penicillin (100 U/ml), streptomycin (100 µg/ml), trypsin:TPCK treated (Worthington, 1 µg/ml) and agarose (0·9%). Plates were incubated at 34 °C in 5% CO_2 for 3 days and plaques were counted without staining.

To study the inhibition of neutralization by sugars or antibodies, a dilution of GPS that alone caused complete neutralization of virus was incubated for 15 min at 20 °C with increasing concentrations of the sugars or antibodies under test. Virus was then added and the mixtures were incubated at 37 °C and plated as in a standard neutralization assay.

Periodate treatment of serum. Serum was treated with KIO_4 as described previously (Anders *et al.*, 1990).

Absorption of serum with mannan–Sepharose. Mannan from *Saccharomyces cerevisiae* (Sigma) was coupled to CNBr-activated Sepharose 4B-CL (Pharmacia) to give 8 mg mannan per ml of packed beads. Serum was incubated with an equal volume of mannan–Sepharose beads in the presence of 0·5 mM-$CaCl_2$ for 4 h on ice with frequent agitation. The beads were removed by centrifugation, and the absorbed serum, now at a 0·5 × dilution of the original, was stored at −70 °C.

Complement titrations. Total haemolytic complement was determined by the modified Mayer technique described by Lachmann & Hobart (1978). Alternative pathway activity was measured by the ability of serum to lyse unsensitized rabbit erythrocytes (Nelson & Ruddy, 1979).

Treatment of serum with γ-inulin. To deplete GPS of alternative complement pathway activity, the serum was incubated with γ-inulin (0·5 mg/ml) at 37 °C for 30 min as described by Cooper & Carter (1986). Inulin was removed by centrifugation in a microfuge (5 min, 4 °C) and the supernatant was stored at −70 °C. The treated serum retained 50% of its total haemolytic complement activity but possessed no detectable alternative pathway activity (data not shown).

Detection of Ca^{2+}-dependent guinea-pig mannose-binding lectin by ELISA. The wells of a flexible polyvinyl microtitre tray were coated overnight with 50 µl mannan (50 µg/ml) or influenza virus (1000 haemagglutinating units/ml) in TBS (0·05 M-Tris–HCl, 0·15 M-NaCl, pH 7·2), blocked for 1 h with 10 mg BSA per ml, and washed with TBS containing 0·05% Tween-20 (TBS–T). Serial dilutions of GPS in TBS–T containing 5 mg 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. The binding of a lectin serologically related to human serum MBP was detected by the addition of rabbit antiserum against human MBP (1/600 dilution in BSA_5–TBS–T–Ca^{2+}) for 3 h, and then, after washing, horseradish peroxidase (HRP)-conjugated swine anti-rabbit immunoglobulin (Dako) in the same diluent for 1 h. Alternatively, the bound lectin was detected by the addition of anti-human MBP MAb#6, followed by HRP-conjugated rabbit anti-mouse immunoglobulin (Dako). In either case, after washing, 100 µl of the substrate 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulphonic acid) (0·2 mM in 50 mM-citrate buffer pH 4·0, containing 0·004% H_2O_2) was added and absorbance at 405 nm was read in a Titertek Multiskan (Flow Laboratories). In specificity controls, HRP-conjugated antibodies failed to bind in wells receiving normal rabbit serum in place of rabbit anti-MBP antibodies, or an irrelevant MAb in place of MAb#6, and in

Table 1. *Haemagglutination inhibition of Mem71$_H$–Bel$_N$ virus and its serum-resistant mutants by guinea-pig serum*

Serum treatment	Diluent	HI titre of guinea-pig serum against Mem71$_H$–Bel$_N$	Mem71$_H$–Bel$_N$/HS*	Mem71$_H$–Bel$_N$/BS†
None	CFT‡	1280	480	1280
56 °C, 30 min	CFT	10240	< 10	10240
KIO_4	CFT	240	320	< 10
None	CFT	960	< 10	960
	CFT +25 mM-sodium citrate	640	320	960
	CFT +25 mM-sodium citrate +50 mM-$CaCl_2$	960	10	960
	CFT +100 mM-D-mannose			

* A mutant of Mem71$_H$–Bel$_N$ virus selected for resistance to the γ inhibitor in horse serum.
† A mutant of Mem71$_H$–Bel$_N$ virus selected for resistance to the β inhibitor in bovine serum.
‡ Complement fixation test diluent (Oxoid; barbitone-buffered saline pH 7·2, 0·25 mM-$CaCl_2$, 1·8 mM-$MgCl_2$).

Table 2. *Comparison of some properties of guinea-pig β inhibitor with those of bovine and mouse serum β inhibitors*

Property of β inhibitor in HI tests	Guinea-pig	Mouse*	Bovine* (conglutinin)
Inhibition by different monosaccharides	Man > GlcNAc ≈ Fuc†	Man > GlcNAc ≈ Fuc	GlcNAc > > Man > Fuc
Sensitivity to heating 56 °C, 30 min	Labile‡	Labile§	Stable
Sensitivity to 0·1 M-2-mercaptoethanol	Sensitive‖	Sensitive	Resistant

* Experimental data for mouse and bovine β inhibitors can be found in Hartley *et al.* (1992).
† The minimum concentrations of sugars required to inhibit completely the HI activity of 3 HI units of GPS for Mem71$_H$–Bel$_N$/HS virus were as follows: D-mannose (Man), 6 mM; *N*-acetyl-D-glucosamine (GlcNAc), 12 mM; L-fucose (Fuc), 12 mM; D-glucose, 25 mM; D-galactose, 50 mM; L-rhamnose, > 200 mM. One HI unit of GPS is the minimum amount inhibiting 4 HA units of virus. Full details of the method can be found in Hartley *et al.* (1992).
‡ See Table 1.
§ HI titre reduced from 320 to < 10 after heating.
‖ HI titre for Mem71$_H$–Bel$_N$/HS virus reduced from 320 to 10 in the presence of 0·1 M-2-mercaptoethanol.

wells in which the GPS was diluted in BSA_5–TBS–T containing 2 mM-EDTA to chelate Ca^{2+} ions.

Results

Properties of guinea-pig serum β inhibitor as revealed by HI tests

GPS contains a β- and a γ-type inhibitor of influenza virus, both of which are active against strain Mem71$_H$–Bel$_N$ of the H3 subtype. In order to examine the properties of the guinea-pig β inhibitor we have used a mutant strain of virus, Mem71$_H$–Bel$_N$/HS, which is resistant to the effects of the γ inhibitor. As Table 1 shows, the inhibitory activity of GPS against Mem71$_H$–Bel$_N$/HS is heat-labile, resistant to oxidation by KIO_4, Ca^{2+}-dependent, and inhibited by D-mannose, characteristics that identify it as a β-type inhibitor, i.e. a mannose-binding lectin (Anders *et al.*, 1990). Conversely, the characteristics of the γ inhibitor (activated by heat, periodate-sensitive, Ca^{2+}-independent and mannose-insensitive) are revealed by assaying on Mem71$_H$–Bel$_N$/BS virus, a mutant that is resistant to HI by the β inhibitor in GPS. Together, these inhibitors account for the total HI activity of the serum.

We have shown previously that β inhibitors of influenza virus comprise at least two distinct types of mannose-binding molecules. The major β inhibitor in bovine serum, identified as conglutinin, differs in a number of properties from the inhibitor in mouse serum, including its fine specificity for different monosaccharides, heat lability, and sensitivity to 2-mercaptoethanol (Hartley *et al.*, 1992). When tested for these same properties, the guinea-pig β inhibitor was found to resemble the mouse inhibitor rather than the bovine inhibitor in all respects (Table 2). The sugar specificity of the mouse and guinea-pig inhibitors closely resembled

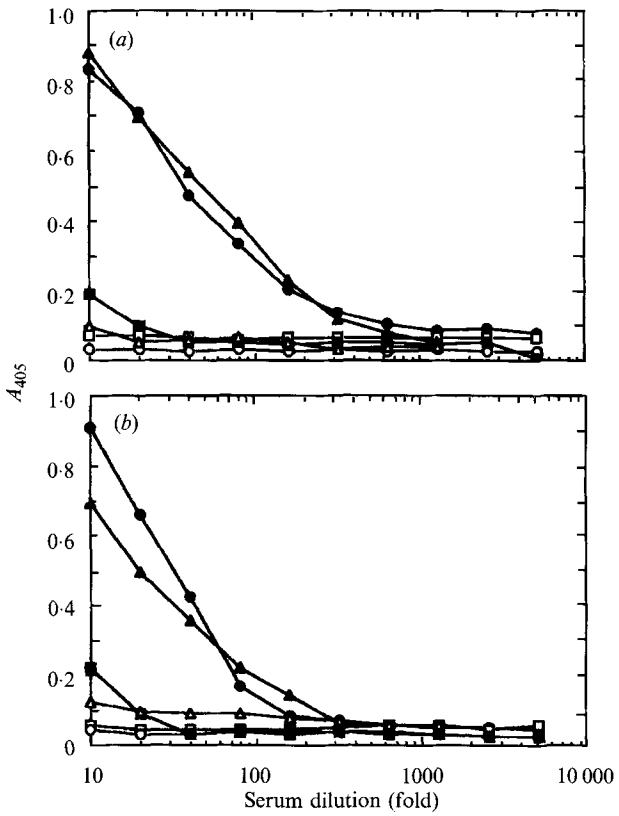

Fig. 1. Serological cross-reactivity of guinea-pig mannan-binding lectin with human MBP. GPS was titrated in the presence of Ca^{2+} on wells coated with mannan (*a*) or $Mem71_H$–Bel_N virus (*b*) and the plates were developed in an ELISA by using either polyclonal rabbit anti-human MBP serum (●), normal rabbit serum (○), MAb#6 (▲) or control MAb 6.6.H3 (△), followed by peroxidase-conjugated swine anti-rabbit immunoglobulin or rabbit anti-mouse immunoglobulin as appropriate. The binding of GPS in the presence of EDTA (□) and of GPS that had been absorbed with mannan–Sepharose (■), both developed with rabbit anti-MBP serum, is also shown.

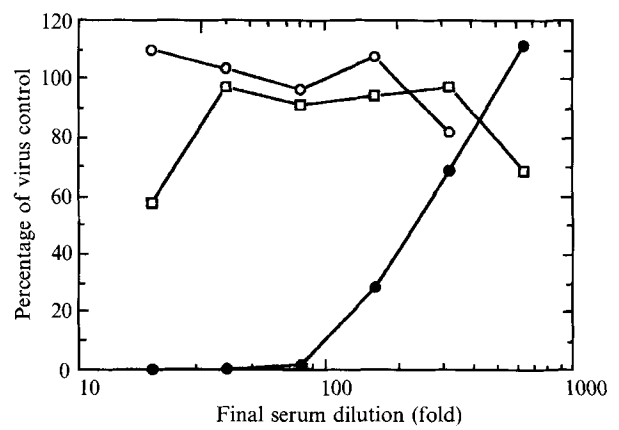

Fig. 2. Neutralization of $Mem71_H$–Bel_N/HS virus by GPS. Graph shows plaquing of $Mem71_H$–Bel_N/HS virus on MDCK cells (as a percentage of control plaque numbers) after treatment of virus (37 °C, 30 min) with serial dilutions of GPS (●), GPS preheated at 56 °C, 30 min (○) and GPS that had been depleted of mannan-binding activity by passage over mannan–Sepharose (□).

that of human MBP (Summerfield & Taylor, 1986; Kawasaki *et al.*, 1989).

Guinea-pig β inhibitor cross-reacts serologically with human serum MBP

Titration of GPS, in the presence of Ca^{2+}, on wells coated with mannan or with $Mem71_H$–Bel_N virus led to binding of a component that could be detected in ELISA by polyclonal (rabbit) or monoclonal (MAb#6) antibodies raised against human serum MBP (Fig. 1). Passage of GPS over mannan-coupled Sepharose 4B simultaneously removed the mannan-binding and virus-binding activities of the serum (Fig. 1) as well as its HI activity towards $Mem71_H$–Bel_N/HS virus (HI titre reduced from 240 to < 10). Furthermore, the HI activity of GPS for $Mem71_H$–Bel_N/HS was shown to be inhibited by MAb#6; the HI activity of a 1/80 dilution of GPS (containing 3 HI units) was completely abolished by preincubation with MAb#6 IgG at a concentration of 20 μg/ml, whereas up to 400 μg/ml of a control MAb of the same isotype (MAb 6.6.H3, IgG1) had no effect. These findings indicate that the mannan-binding lectin detected in GPS by ELISA with antibodies to human MBP is in fact the *β* inhibitor.

Neutralization of influenza virus by guinea-pig serum β inhibitor requires complement activity

GPS neutralized the infectivity of $Mem71_H$–Bel_N/HS virus (Fig. 2). Involvement of the mannose-binding lectin (*β* inhibitor) in the neutralization process was indicated by the following observations. The neutralizing activity was heat-labile (56 °C, 30 min) and was removed by passing the serum over mannan–Sepharose (Fig. 2), a procedure which depleted the serum of its mannan-binding activity (Fig. 1) but reduced the total haemolytic complement activity of the serum by less than twofold (data not shown). Neutralization was also inhibited by preincubation of the serum with simple sugars before the addition of virus (Fig. 3*a*), mannose and *N*-acetyl-glucosamine being the most active inhibitors, with fucose also exerting a strong inhibitory effect. Neutralization was not reversed, however, if sugars were added after the virus had been incubated with serum at 37 °C (data not shown). Most importantly, neutralization by GPS was inhibited in the presence of MAb#6 and of its $F(ab')_2$ fragment (Fig. 3*b*). The haemolytic complement activity of GPS was shown to be unaffected by incubation with any of the sugars or with MAb#6 IgG (data not shown).

Neutralization of virus is not mediated by the mannose-binding lectin alone, however. GPS that had been treated with KIO_4 failed to neutralize $Mem71_H$–Bel_N/HS virus (Fig. 4*a*), despite possessing full HI

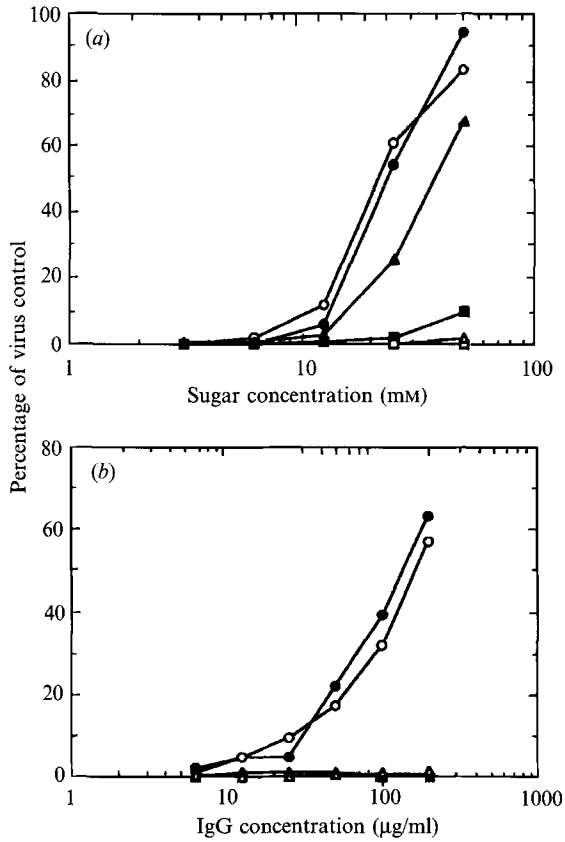

Fig. 3. Inhibition of neutralization by simple sugars and by MAb#6 against human MBP. A dilution of GPS (1/40 or 1/60) that alone gave complete neutralization was preincubated for 15 min at 20 °C with (*a*) sugars or (*b*) antibodies at the indicated concentrations before mixing with $Mem71_H$–Bel_N/HS virus in a standard neutralization assay. Plaque numbers are expressed as a percentage of the number of plaques obtained in a non-neutralized virus control. (*a*) D-Mannose (●), *N*-acetyl-D-glucosamine (○), L-fucose (▲), D-glucose (■), D-galactose (△) and L-rhamnose (□). (*b*) MAb#6 IgG (●), MAb#6 $F(ab')_2$ (○), control MAb 6.6.H3 IgG (▲) and MAb 6.6.H3 $F(ab')_2$ (△). The antibody concentrations shown are for IgG; $F(ab')_2$ preparations were used at the equivalent molar concentrations.

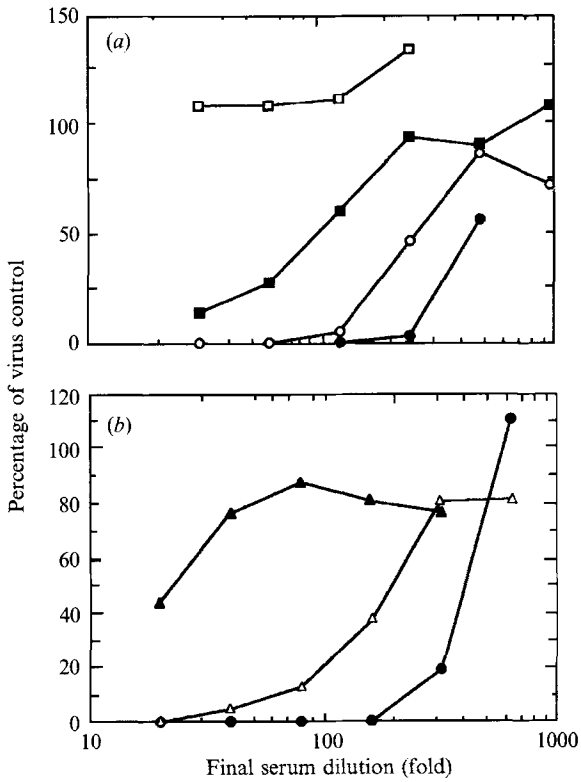

Fig. 4. Complement-dependence of neutralization by the guinea-pig lectin. (*a*) $Mem71_H$–Bel_N/HS virus was incubated (37 °C, 30 min) with serial dilutions of KIO_4-treated GPS as a source of lectin, and then for a further 30 min with either a 1/20 (final) dilution of mannan–Sepharose-absorbed GPS as a source of complement (■), or medium alone (□). Neutralization by untreated GPS (●) and mock-periodate-treated GPS (○) are included as positive controls. Results are expressed as the percentage of virus control in medium alone (●, ○, □) or in mannan–Sepharose-absorbed GPS alone (■). (*b*) Neutralization of $Mem71_H$–Bel_N/HS virus by GPS (●), C4-deficient GPS (▲) and γ-inulin-treated GPS (△).

activity and mannan-binding activity in ELISA (data not shown). In this preparation, haemolytic complement activity was undetectable. Neutralizing activity was restored when virus that had been incubated with KIO_4-treated serum was treated with mannan–Sepharose-absorbed GPS as a source of complement (Fig. 4*a*).

The MBPs of several species have been reported to fix complement by the classical pathway (Ikeda *et al.*, 1987; Kawasaki *et al.*, 1989; Lu *et al.*, 1990), and in the case of human MBP, also by the alternative pathway (Schweinle *et al.*, 1989). To determine which pathway of complement activation was required for neutralization of virus by the guinea-pig mannose-binding lectin, GPS deficient in one or other pathway was tested (Fig. 4*b*). GPS deficient in the classical pathway component C4 possessed normal levels of the mannose-binding lectin according to HI tests (titre 320) and ELISA (not shown) but showed a markedly reduced ability to neutralize $Mem71_H$–Bel_N/HS virus compared with normal GPS. GPS that had been treated with γ-inulin to inactivate the alternative complement pathway (see Methods) retained neutralizing activity (Fig. 4*b*). Taken together, the data indicate that neutralization of $Mem71_H$–Bel_N/HS virus by GPS requires both the mannan-binding lectin (β inhibitor) and at least the early components of the classical complement pathway.

Activity of guinea-pig β *inhibitor against influenza virus type B*

Type B influenza viruses are also susceptible to a β-like inhibitor in GPS. Yamamoto and coworkers (1987) characterized this activity as Ca^{2+}-dependent, heat-labile and requiring complement components C1 and C4 for its activity, and concluded that the inhibitor was a com-

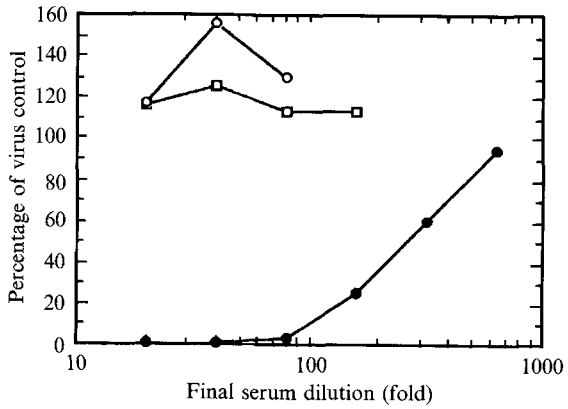

Fig. 5. Neutralization of type B influenza virus by GPS also involves the mannose-binding lectin. Neutralization of influenza virus B/Hong Kong/8/73 by GPS (●), GPS preheated at 56 °C, 30 min (○) and GPS that had been absorbed with mannan–Sepharose (□).

ponent of the classical complement pathway. As shown in Fig. 5, the heat-labile neutralizing activity of GPS for influenza virus B/Hong Kong/8/73 was removed by absorption of serum with mannan–Sepharose, indicating involvement of the serum mannose-binding lectin in neutralization of type B, as well as type A, influenza viruses.

Discussion

In this study we have demonstrated the presence in GPS of a Ca^{2+}-dependent mannose-binding lectin that acts as a potent inhibitor (β inhibitor) of influenza virus haemagglutination and infectivity. In HI, this lectin acts independently of complement, but virus-neutralizing activity was shown to depend upon activation of the classical complement pathway. From a number of its properties, including its specificity for different monosaccharides, its serological cross-reactivity with human MBP and its complement-fixing ability, this lectin, like its counterpart in mouse serum, appears to belong to the MBP family of mannose-binding molecules, and is distinct from conglutinin, the major β inhibitor in bovine serum (Hartley *et al.*, 1992).

Yamamoto and coworkers (1987) studied a β-like inhibitor in GPS that was active against influenza type B, but not type A, viruses and concluded that it was a component of the classical complement pathway. At that time, neither the ability of serum MBPs to activate complement (Ikeda *et al.*, 1987), nor the interaction of these lectins with influenza virus (Anders *et al.*, 1990) was known. We show here that neutralization of influenza B/Hong Kong/8/73 by GPS, like that of A/Mem71$_{H}$–Bel$_{N}$/HS, is dependent on the presence of the mannose-binding lectin. The lack of activity of the guinea-pig inhibitor against influenza A viruses reported by Yamamoto *et al.* (1987) may reflect the choice of virus strains tested. These included the early H1N1 viruses A/PR/8/34 and A/WSN/33, both of which have been mouse-adapted, a procedure which is known to select for β inhibitor-resistant variants of influenza virus (Chu, 1951; Briody *et al.*, 1955). In contrast, we have found a range of non-adapted influenza A viruses of the H1 and H3 subtypes to be sensitive to the guinea-pig β inhibitor (lectin), including A/Brazil/11/78 (H1N1), A/Chile/1/83 (H1N1), A/Port Chalmers/73 (H3N2) and A/Philippines/2/82 (H3N2) (unpublished observations).

Whereas HI can be explained by steric hindrance of access of erythrocyte receptors to the receptor-binding site on the HA molecule, this mechanism is not sufficient to effect neutralization of the virus by the guinea-pig lectin under the conditions of the plaque reduction assay used here. Binding of virus to the short sialylated receptors on erythrocytes may be more easily blocked by the lectin than binding to the longer sialylated structures that are believed to form the receptors for influenza virus on infectible cells (Dimmock, 1987). Furthermore, the amount of lectin required to be bound per virion to inhibit the formation of an agglutinated lattice of virus and erythrocytes may be less than that required to completely block binding of the virion to the surface of an infectible cell. Activation of complement by the HA-bound lectin could lead to virus neutralization in a number of ways. Deposition of complement proteins on to the HA molecule may result in more effective blocking of virus attachment, and perhaps also inhibition of later steps in the replication cycle, including viral entry and uncoating. Alternatively, neutralization may result from aggregation of virion–lectin complexes by complement, thereby reducing the number of infectious units; *in vivo*, such aggregation could increase the probability of phagocytosis and destruction by macrophages. As a further alternative, activation of the entire complement sequence may result in lysis of the lipid envelope of the virus; for neutralization by the guinea-pig lectin we have yet to determine whether the full lytic pathway is required. Whichever mechanism is operating, neutralization of influenza virus by lectin plus complement is irreversible and cannot be inhibited by the addition of mannose or other sugars after incubation of the virus with serum at 37 °C (unpublished observations). Precedents for each of the above mechanisms exist in the neutralization of different viruses by antibody plus complement (Linscott & Levinson, 1969; Oroszlan & Gilden, 1970; Oldstone *et al.*, 1974; Radwan & Crawford, 1974; Beebe & Cooper, 1981). In addition, certain retroviruses may be lysed by human serum without the participation of antibody, through activation of the classical complement pathway by direct binding of the virus to C1q (Cooper *et al.*, 1976). To our knowledge, the

neutralization of influenza virus by a serum lectin plus complement represents a previously unrecognized mechanism of complement-dependent viral inactivation.

The complement dependence of neutralization by MBP-like lectins may not be absolute at all lectin to virus ratios. The recent observation by Hartshorn *et al.* (1993) that the infectivity of influenza virus could be inhibited by purified human recombinant MBP alone may reflect the assay conditions used. In the present study, we measured complete neutralization of a low concentration of virus (500 p.f.u./ml) by dilutions of serum; under these conditions the lectin alone did not neutralize and complement activation was essential. In the experiments of Hartshorn *et al.*, purified recombinant human MBP was mixed with high concentrations of virus (10^8 to 10^9 p.f.u./ml) and the residual infectivity of the mixture was determined. Under these conditions, the formation of viral aggregates and blocking of virus attachment/entry to cells was apparently sufficient to give the 10- to 100-fold drop in infectivity observed, without the participation of complement.

Human recombinant MBP has been shown to bind also to the envelope glycoprotein of human immunodeficiency virus (HIV) type 1 (Ezekowitz *et al.*, 1989; Larkin *et al.*, 1989) and to inhibit infectivity of the virus for H9 lymphoblasts (Ezekowitz *et al.*, 1989). In light of the present findings, it will be important to determine whether neutralization of HIV by lower concentrations of MBP requires the participation of complement. Of interest is the report by Spear *et al.* (1991) that neutralization of HIV by non-immune guinea-pig, mouse and rabbit sera occurred via antibody-independent activation of the classical complement pathway. The mechanism of activation was not determined, but could possibly involve MBP since the sera of all three species are known to be good sources of the lectin.

The virus-neutralizing activity of serum mannose-binding lectins suggests an important role for these lectins in first-line defence against viral infection, in addition to their proposed role against bacterial and fungal infections (Ezekowitz, 1991). The antiviral activity of mannose-binding lectins may extend beyond influenza virus and HIV to the diverse range of other enveloped viruses that bear glycosylated surface antigens. Susceptible viruses need not be limited to those bearing high-mannose oligosaccharides, since MBP binds also to complex and hybrid oligosaccharides bearing terminal *N*-acetylglucosamine residues, and to oligosaccharides with fucose linked to subterminal *N*-acetylglucosamine residues as in the sequence Fucα1-4/3(Galβ1-3/4)-GlcNAc (Childs *et al.*, 1989). For influenza virus, the presence of this neutralizing activity in serum may account for the fact that virus is very rarely isolated from the blood of infected humans, or of animals infected with non-adapted virus strains (Sweet & Smith, 1980). Whether serum lectins can gain access to the lung is not known, but the related C-type lectins SP-A (Haagsman *et al.*, 1987; Thiel & Reid, 1989) and SP-D (Rust *et al.*, 1991; Shimizu *et al.*, 1992) present in pulmonary surfactant may serve a similar role at that site.

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