The Site of Bluetongue Virus Attachment to Glycophorins from a Number of Animal Erythrocytes

By BRYAN T. EATON* AND GARY S. CRAMERI

Australian Animal Health Laboratory, CSIRO, P.O. Bag 24, Geelong, Victoria 3220 Australia

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SUMMARY

Bluetongue virus (BTV) was shown to agglutinate human, ovine and porcine erythrocytes. Removal of neuraminic acid (NA) from erythrocytes by Vibrio cholerae neuraminidase prevented their agglutination. Haemagglutination was also inhibited by N-acetyl neuraminic acid (NANA), N-glycolyl neuraminic acid (NGNA) and N-acetyl neuramin-lactose. The ability of BTV to agglutinate trypsin-treated human erythrocytes, which lack the amino-terminal domain and the single N-linked oligosaccharide of glycophorin A, suggests that the virus bound to human erythrocytes via NANA-containing, O-linked oligosaccharides. Glycoproteins with NA-containing oligosaccharides of known structure such as mucin, fetuin, alpha 1-acid glycoprotein, ovomucoid and ovine, human and equine glycophorin were examined for their ability to inhibit BTV-mediated agglutination of human, ovine and porcine erythrocytes. All glycoproteins containing NANA- or NGNA2-6GalNAc were capable of inhibiting the agglutination of human and porcine erythrocytes. Treatment of human erythrocytes with Newcastle disease virus neuraminidase and of porcine erythrocytes with Clostridium perfringens neuraminidase to cleave preferentially the NANA- and NGNA2-3Gal linkages respectively, were shown to have little effect on the ability of the erythrocytes to be agglutinated by BTV. The results suggested that BTV binds to NANA- and NGNA2-6GalNAc residues in the O-linked oligosaccharides of human and porcine glycophorins respectively and indicated the presence of different binding sites on the virus for erythrocytes from other species.

INTRODUCTION

Many viruses have the ability to agglutinate a variety of mammalian erythrocytes (Lonberg-Holm & Philipson, 1974; Howe & Lee, 1972; Burness, 1981). The fact that haemagglutination is prevented by treatment of erythrocytes with bacterial neuraminidase implicates neuraminic acid (NA)-containing molecules as key sites for virus attachment. Most NA residues on erythrocytes are present in glycophorin, a relatively small, but heavily glycosylated protein (Tomita & Marchesi, 1975; Furthmayr & Marchesi, 1983; Krotkiewski, 1988). Glycophorins are integral membrane proteins whose extracellular amino-terminal domain contains a large number of O-linked NA-containing oligosaccharide side chains which show considerable heterogeneity in their structure and are linked to serine or threonine through N-acetylgalactosamine (GalNAc). Glycophorins from human and porcine erythrocytes also have one and two asparagine N-linked, NA-containing, complex oligosaccharide side chains, respectively (Yoshima et al., 1980; Honma et al., 1980). In contrast equine glycophorin does not contain N-linked oligosaccharides (Murayama et al., 1981).

Glycophorin has been shown to be the erythrocyte receptor for influenza virus (Marchesi & Andrews, 1971), encephalomyocarditis (EMC) virus (Enegren & Burness, 1977) and reovirus (Paul & Lee, 1987). Reovirus appears to bind to the terminal NA residues in the N-linked oligosaccharide of human glycophorin (Paul & Lee, 1987) whereas EMC virus attachment depends on the NA residues in O-linked human glycophorin oligosaccharides (Allaway &
Burness, 1986). Using human erythrocytes modified enzymically to contain cell surface oligosaccharides, Rogers & Paulson (1983) showed that human and animal influenza viruses may bind to cells containing NA2-6Gal or NA2-3Gal or to both cell types. None of the viruses examined agglutinated cells containing NA2-6GalNAc. A similar approach was used by Cahan & Paulson (1980) to show that polyoma virus agglutination of neuraminidase-treated erythrocytes was restored by the addition of NA in an a2-3Gal linkage but was not restored by NA linked a2-6 to either Gal or GalNAc. Similarly, agglutination of asialoerythrocytes by Newcastle disease virus (NDV) was achieved following the addition of NA in an a2-3Gal sequence (Paulson et al., 1979).

Bluetongue virus (BTV) is a member of the orbivirus genus in the Reoviridae family. The virus contains 10 segments of dsRNA surrounded by a double-shelled capsid (Verwoerd et al., 1970, 1972). The outer fibrillar layer of the virus contains two proteins, VP2 and VP5. BTV, like other members of the Reoviridae, agglutinates erythrocytes from certain species (Van der Walt, 1980; Hubschle, 1980; Tokuhisa et al., 1981) presumably through interaction with VP2 (Cowley & Gorman, 1987).

In sheep and cattle infected with BTV, the virus is found in association with erythrocytes (Collisson & Barber, 1983). This association may play a role in the dissemination of virus throughout the body of the vertebrate host and/or help maintain the virus within the gut of the Culicoides vector following a blood meal. In addition, blood from viraemic animals acts as the source of virus for isolation procedures using either embryonated chicken eggs or tissue culture. In order to understand better the nature of the BTV–erythrocyte interaction, we have attempted to determine the binding site for the virus on erythrocytes from a number of animal species. The results presented here suggest that BTV binds to human erythrocytes via NA2-6GalNAc-containing O-linked oligosaccharides of glycoporin.

METHODS

Materials. Vibrio cholerae type II and Clostridium perfringens type V neuraminidases, lithium 3,5-diiodosalicylate, 2,3-dehydroxy-3-dihydro-N-acetylmuramic acid (DDN), fetuin (type II), bovine submaxillary mucin (type 1-5), ovalbumin, human alpha-1-acid glycoprotein, trypsin inhibitor (type III-0; ovomucoid), N-acetyl neuraminic acid (NANA) (type IV), N-glycolyl neuraminic acid (NGNA), N-acetyl neuramin-lactose, galactose, galactosamine and N-acetyl galactosamine-1-phosphate were purchased from Sigma. Human type O and horse blood were kindly provided by the Department of Pathology, Geelong Hospital and the Veterinary Research Laboratories, Werribee, Victoria, respectively. Sheep and pig blood were obtained from animals held in the Australian Animal Health Laboratory (AAHL). Blood was collected, washed in Alsever's solution and stored as described by Van der Walt (1980). NDV, purified from infected allantoic fluid by lithium di-iodosalicylate-phenol extraction method described by Marchesi & Andrews (1971). This association may play a role in the dissemination of virus throughout the body of the vertebrate host and/or help maintain the virus within the gut of the Culicoides vector following a blood meal. In addition, blood from viraemic animals acts as the source of virus for isolation procedures using either embryonated chicken eggs or tissue culture. In order to understand better the nature of the BTV–erythrocyte interaction, we have attempted to determine the binding site for the virus on erythrocytes from a number of animal species. The results presented here suggest that BTV binds to human erythrocytes via NA2-6GalNAc-containing O-linked oligosaccharides of glycoporin.

METHODS

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Glycophorins. Glycophorins from human, equine, ovine and porcine erythrocytes were prepared using the lithium di-iodosalicylate–phenol extraction method described by Marchesi & Andrews (1971). Analysis by SDS-PAGE and Coomassie Brilliant Blue staining indicated that human and sheep glycophorins migrated with apparent Mr values of 80,000 and 55,000 respectively. These values are approximately double those estimated for glycophorins from human erythrocytes modified enzymically to contain cell surface oligosaccharides. Rogers & Paulson (1983) showed that human and animal influenza viruses may bind to cells containing NA2-6Gal or NA2-3Gal or to both cell types. None of the viruses examined agglutinated cells containing NA2-6GalNAc. A similar approach was used by Cahan & Paulson (1980) to show that polyoma virus agglutination of neuraminidase-treated erythrocytes was restored by the addition of NA in an a2-3Gal linkage but was not restored by NA linked a2-6 to either Gal or GalNAc. Similarly, agglutination of asialoerythrocytes by Newcastle disease virus (NDV) was achieved following the addition of NA in an a2-3Gal sequence (Paulson et al., 1979).

Virus and haemagglutinin preparation. An Australian isolate of BTV type 1 (BTV-1) (CS156) was grown in SVP cells in Medium 199 containing 5% foetal calf serum as described (Eaton et al., 1987). To prepare virus for haemagglutination, SVP cells in roller bottles were infected at an approximate multiplicity of 3 p.f.u./cell and incubated at 37 °C for 40 h. Cells were scraped from the substrate, pooled with those floating in the culture medium, pelleted, washed in phosphate-buffered saline (PBS) and lysed in 200 mm-Tris–HCl pH 8.0, containing 1% Triton X-100. The nuclear-cytoskeleton fraction was pelleted, resuspended in buffer and sheared in a Dounce homogenizer. Nuclei were removed by centrifugation. Cytoplasmic (i.e. Triton X-100-soluble material) and cytoskeletal fractions were pooled, layered over 40% sucrose in 200 mm-Tris–HCl pH 8.0, and centrifuged onto a 66% sucrose cushion at 39000 r.p.m. in a SW41 rotor at 4 °C for 1.5 h. The virus pellet was resuspended in 200 mm-Tris–HCl pH 8.0, made up to 3% in deoxycholate (Mertens et al., 1987) and sedimented through a sucrose column. Estimation of the haemagglutination activity and infectivity titre (as determined by plaque assay) indicated that such partially purified intracellular virus preparations had the same haemagglutination : infectivity ratio as preparations of virus particles which had been released into the culture medium of infected cells, precipitated with ammonium sulphate and purified by centrifugation through 40% sucrose columns as described above. However the comparison indicated that the intracellular concentration or virus haemagglutinin exceeded that released from
infected cells by a factor of three to four. Attempts to purify further the haemagglutinin using sodium lauryl sarcosine (SLS) and DTT as described by Mertens et al. (1987) resulted in a large decrease in both haemagglutination and infectivity titres. Examination of the protein content of crude, partially purified and SLS/DTT-purified virus preparations showed that there was no detectable loss of VP2 from the particles during purification (Eaton et al., 1988), indicating that the loss of haemagglutination activity was not due to a breakdown of the outer capsid layer during purification. Negative staining and electron microscopic examination of purified virus revealed the underlying capsomers of the inner capsid more clearly (Eaton et al., 1988) than in virus which had not been treated with SLS and DTT, confirming that such treatment lead to an alteration in the structure of the outer capsid layer of BTV-1 (Australia). Deoxycholate-treated, partially purified virus preparations were used as the source of viral haemagglutinin. Evidence for the virus-specificity of haemagglutinin was obtained from the observed haemagglutination inhibition (HI) by a bovine polyclonal anti-BTV-1 antiserum.

**Haemagglutination assay.** In microtitre plates, doubling dilutions of partially purified virus in TSAG (0.02 M-Tris–HCl pH 9.0, 0.14 M-NaCl, 0.001 M-CaCl2, 0.2% bovine serum albumin and 0.0025% gelatin) were mixed with an equal volume (25 μl) of TSAG and 50 μl of a 0.25% suspension of erythrocytes, as described by Van der Walt (1980). After 2 to 3 h at 22 °C, the reciprocal of the highest dilution producing complete haemagglutination was taken as 1 unit. In HI experiments, doubling dilutions of sugars, antiserum, glycophorins or glycoproteins in TSAG were mixed with 4 haemagglutination units of virus in an equal volume (25 μl) of TSAG. Following 20 min at 22 °C, 50 μl of a 0.25% suspension of erythrocytes was added to each well. Tests were read after 2 h.

**Neuraminidase and trypsin treatment of erythrocytes.** Human, ovine and porcine erythrocytes (5%) in either Earle's saline, PBS-containing 2 mM-calcium chloride or TSAG were incubated in the presence and absence of 1 milliunit/ml *V. cholerae or C. perfringens* neuraminidase. After incubation at 37 °C for 30 min, 1, 2 and 3 h, cells were washed twice and used for haemagglutination assays in the usual manner. NA released from erythrocytes by neuraminidase treatment was quantified as described by Roboz et al. (1981) and Warren (1959). Human erythrocytes in PBS (5%) were incubated at 37 °C with trypsin (1 mg/ml) for 30 min, 1 and 2 h. Trypsin-mediated cleavage of glycophorin A was confirmed by showing that *V. cholerae* neuraminidase released from trypsin-treated cells approximately 40% of the NA released from untreated cells under identical conditions.

**Neuraminidase treatment of human glycophorin.** Human glycophorin in PBS was treated with *V. cholerae* neuraminidase as described by Paul & Lee (1987).

### RESULTS AND DISCUSSION

#### BTV binds to glycophorin

To determine whether glycophorin is the receptor for BTV on a number of erythrocytes, an attempt was made to inhibit BTV-mediated haemagglutination with glycophorins isolated from human, porcine, ovine and equine erythrocytes. The data in Table 1 show that agglutination of human, porcine and ovine erythrocytes by BTV was inhibited, not only by the homologous glycophorin, but also with the three heterologous glycophorins.

**NA is required for erythrocyte agglutination by BTV**

A role for NA in the binding of BTV to erythrocytes was indicated by the results of three experiments. First, pretreatment of human glycophorin with *V. cholerae* neuraminidase destroyed its inhibitory effect on BTV haemagglutination (Table 1). Second, NANA, NGNA and N-acetyl neuramin-lactose were able to inhibit the BTV-mediated agglutination of human, ovine and porcine erythrocytes (Table 2). Third, pretreatment of erythrocytes with *V. cholerae* neuraminidase eliminated their ability to be agglutinated by BTV (Table 3). This effect was abolished in the presence of DDN, a neuraminidase inhibitor (Schauer, 1981) (data not shown).

**Location of the BTV binding site on human erythrocytes**

NA may be present in both N- and O-linked oligosaccharides in glycophorins. The structure of the N-linked oligosaccharides in porcine glycophorin is not known, nor is it clear whether ovine glycophorin contains N-linked oligosaccharides. However in the case of human glycophorin A, which constitutes approximately 80% of human glycophorin molecules (Bretscher, 1971), a single N-linked, complex oligosaccharide at amino acid position 26 contains terminal NaNAlz2-Galβ1-3GlcNAc residues (Yoshima et al., 1980). Glycophorin B, which comprises approximately 15% of the human glycophorins does not contain an N-linked oligosaccharide. Several lines of evidence suggested that NANA in the N-linked oligosaccharide was not involved in BTV agglutination of human erythrocytes. First, alpha 1-acid
Table 1. Effect of exogenous glycoproteins on BTV haemagglutination

<table>
<thead>
<tr>
<th>Glycoprotein</th>
<th>Maximum concentration used (µg/ml)</th>
<th>Erythrocyte agglutination*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovine glycophorin</td>
<td>125</td>
<td>(1)</td>
</tr>
<tr>
<td>Equine glycophorin</td>
<td>125</td>
<td>(1)</td>
</tr>
<tr>
<td>Porcine glycophorin</td>
<td>125</td>
<td>(2)</td>
</tr>
<tr>
<td>Human glycophorin</td>
<td>125</td>
<td>(1)</td>
</tr>
<tr>
<td>Neuraminidase-treated human glycophorin</td>
<td>125</td>
<td>+</td>
</tr>
<tr>
<td>Fetuin</td>
<td>250</td>
<td>(62)</td>
</tr>
<tr>
<td>Mucin</td>
<td>250</td>
<td>(8)</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>250</td>
<td>+</td>
</tr>
<tr>
<td>Ovomucoid</td>
<td>250</td>
<td>+</td>
</tr>
<tr>
<td>Alpha 1-acid glycoprotein</td>
<td>250</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A minus sign (−) denotes inhibition of agglutination and + denotes a failure of the glycoprotein to inhibit.
† The value in parentheses is the minimum inhibitory concentration in µg/ml.
‡ No added glycoprotein.

Table 2. Effect of exogenous sugars on BTV haemagglutination

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Maximum concentration used (mM)</th>
<th>Erythrocyte agglutination*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NANA</td>
<td>50</td>
<td>(12.5)</td>
</tr>
<tr>
<td>NGNA</td>
<td>50</td>
<td>(12.5)</td>
</tr>
<tr>
<td>N-Acetyl neuramin-lactose</td>
<td>50</td>
<td>(12.5)</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>50</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl galactosamine-1-phosphate</td>
<td>25</td>
<td>+</td>
</tr>
<tr>
<td>GalNAc</td>
<td>100</td>
<td>+</td>
</tr>
</tbody>
</table>

* A minus sign (−) denotes inhibition of agglutination and + denotes a failure of the sugar to inhibit.
† The minimum inhibitory concentration (mM).

glycoprotein which contains a trisaccharide identical to that in the human glycophorin N-linked oligosaccharide does not inhibit the BTV-mediated agglutination of human erythrocytes (Table 1). Second, treatment of human erythrocytes with trypsin which removes the amino-terminal portion of glycophorin A containing the N-linked oligosaccharide (Burness & Pardoe, 1981) did not reduce the ability of human cells to be agglutinated by BTV. Third, equine glycophorin which appears to have no N-linked oligosaccharides and does not contain Nα2-6Gal residues (Fukuda et al., 1980) is capable of inhibiting BTV-mediated agglutination of human erythrocytes (Table 1). Fourth, N-acetyl neuramin-lactose which is a mixture of NANAβ1-4Glc (85%) and α2-6Galβ1-4Glc (15%) is no more efficient than either NANA or NGNA in inhibiting agglutination of human erythrocytes by BTV (Table 2). These data suggest that BTV does not bind to the NANA in the N-linked oligosaccharide of human glycophorin and imply that binding occurs to human O-linked oligosaccharides. The ability of BTV to agglutinate equine erythrocytes (data not shown) suggests that the virus-binding site on these erythrocytes also resides in O-linked oligosaccharides.

The four glycophorins used in this study inhibit the agglutination of human, ovine and porcine erythrocytes by BTV (Table 1). They all contain a number of O-linked, NA-containing oligosaccharides with a majority of those from human, equine and ovine glycophorins consisting of the tetrasaccharide Nα2-3Galβ1-3 (Nαx2-6)GalNAc. O-linked oligosaccharides lacking either one of the NA residues have been identified in equine and human glycophorin (Krotkiewski, 1988). Porcine glycophorin differs from the other three in having an O-linked oligosaccharide structure, Galβ1-3 (Nαx2-6)GalNAc (Honma et al., 1980). The ability of
Table 3. BTV-mediated agglutination of neuraminidase-treated erythrocytes

<table>
<thead>
<tr>
<th>Erythrocyte</th>
<th>Source of neuraminidase</th>
<th>Time of neuraminidase digestion (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Sheep</td>
<td>V. cholerae</td>
<td>64*</td>
</tr>
<tr>
<td></td>
<td>C. perfringens</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Pig</td>
<td>V. cholerae</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>C. perfringens</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Human</td>
<td>V. cholerae</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>C. perfringens</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>NDV</td>
<td>32</td>
</tr>
</tbody>
</table>

* Haemagglutination titre.
† ND, Not determined.

porcine glycoporin to block agglutination of human erythrocytes by BTV suggests that the \( \text{NAc}_2-6\text{GalNAc} \), which is common to all four glycoporins, may be the site of BTV binding. The results in Table 1 also show that agglutination of human (and porcine) erythrocytes by BTV was inhibited by glycoproteins containing \( \text{NAc}_2-6\text{GalNAc} \) (mucin, fetuin), but not by those containing \( \text{NAc}_2-3\text{Gal} \) or \( \alpha_2-6\text{Gal} \) (alpha 1-acid glycoprotein, ovomucoid).

Further information on the nature of the BTV-binding site on human erythrocytes was obtained by using a linkage-specific neuraminidase. NDV neuraminidase exhibits strict specificity for \( \text{NANA}_2-3\text{Gal} \) residues (Paulson et al., 1982). Human erythrocytes treated with NDV were not agglutinated by that virus which binds to \( \text{NANA}_2-3\text{Gal} \) residues (Paulson et al., 1979) (data not shown), but retained their ability to be agglutinated by BTV (Table 3). This suggests that \( \text{NANA}_2-3\text{Gal} \) residues are not involved in BTV-mediated agglutination of human erythrocytes. The ability of porcine and equine glycoporins (which contain NGNA residues) to block agglutination of human erythrocytes (which contain only NANA residues) indicates that the acetyl residues are not critical for agglutination. This is confirmed by the data in Table 2 which show that NGNA and NANA are equally effective at inhibiting agglutination of human erythrocytes.

In summary, the evidence presented here suggests strongly that BTV binds to \( \text{NANA}_2-6\text{GalNAc} \) residues on the \( O \)-linked oligosaccharides of human glycoporin. The ability of BTV to agglutinate trypsin-treated human erythrocytes indicates that the site of the \( O \)-linked oligosaccharide(s) responsible may be associated either with glycoporin B or attached to glycoporin A at amino acid positions 44, 47 or 50 (Tomita & Marchesi, 1975).

The use of linkage-specific neuraminidase to provide evidence for the BTV binding site on porcine and ovine erythrocytes

The data in Table 3 show that treatment of human cells with \( C. perfringens \) neuraminidase resulted, within 30 min, in a complete loss of agglutinating ability. In contrast, ovine erythrocytes displayed partial resistance and porcine erythrocytes appeared to be completely resistant even after 3 h incubation with this enzyme. Increasing the enzyme concentration 10-fold did not alter this result (data not shown).

An explanation for the different effects of \( V. cholerae \) and \( C. perfringens \) neuraminidases on ovine, porcine and human erythrocytes may lie in the preferred specificities of the two enzymes and the nature of the NA in the glycoporins. Human glycoporins contain only NANA (Yoshima et al., 1980) whereas glycoporins from most other animals, including pigs, contain NGNA (Fukuda et al., 1980; Kawashima et al., 1982). Ovine glycoporin contains equal amounts of NANA and NGNA (Klimas et al., 1982). The preference of neuraminidases for NANA- rather than NGNA-containing substrates (Corfield et al., 1981) could account for the
observation that human erythrocytes are more rapidly inactivated by *V. cholerae* neuraminidase than are ovine and porcine cells (Table 3).

Using NGNA\(\alpha^{2-3}\) lactose (NGNA\(\alpha^{2-3}\)Gal\(\beta^{1-4}\)GI) and NGNA\(\alpha^{2-6}\)GalNAc as substrates, Corfield *et al.* (1981) demonstrated that *V. cholerae* neuraminidase was capable of cleaving NGNA from both substrates with approximately equal efficiency, whereas the *C. perfringens* enzyme exhibited a slow rate of cleavage of NGNA\(\alpha^{2-6}\)GalNAc linkages. This information and the resistance of BTV-mediated agglutination of ovine and porcine erythrocytes to *C. perfringens* neuraminidase suggests that BTV may bind to glycophorin in these cells via NGNA\(\alpha^{2-6}\)GalNAc residues or some other *C. perfringens* neuraminidase-resistant NA. The decreased haemagglutination titre with treated ovine erythrocytes may be due to the cleavage of NANA- but not NGNA\(\alpha^{2-6}\)GalNAc linkages in ovine glycophorin.

All the glycoporphins tested, including equine glycophorin which contains neither N-linked oligosaccharides nor NGNA\(\alpha^{2-6}\)Gal residues but does contain NGNA\(\alpha^{2-3}\)Gal and NGNA\(\alpha^{2-6}\)GalNAc residues (Fukuda *et al.*, 1980), block agglutination of human, porcine and ovine erythrocytes. This suggests that the NA responsible for BTV binding may lie in \(\alpha^{2-3}\)Gal and/or \(\alpha^{2-6}\)GalNAc linkages. The O-linked oligosaccharides in porcine glycophorin contain only NGNA\(\alpha^{2-6}\)GalNAc and the ability of this glycophorin to block BTV haemagglutination strongly suggests that the NA involved in ovine and porcine erythrocytes is also linked to 2-6GalNAc.

The ability of fetuin and mucin, both of which contain NANA\(\alpha^{2-6}\)GalNAc, to block human and porcine and not ovine erythrocyte agglutination, suggests that the BTV-binding site differs in human and ovine erythrocytes and that BTV may contain at least two erythrocyte-binding domains which recognize NAX\(\alpha^{2-6}\)GalNAc residues in glycophorin. One site may interact with human and porcine cells and is blocked by mucin and fetuin whereas the other may bind to ovine erythrocytes and is insensitive to these glycoproteins. The ability of glycoporphins from four erythrocyte species to block human (or porcine) and ovine haemagglutination suggests that in solution all these glycoporphins may present NAX\(\alpha^{2-6}\)GalNAc residues in conformations capable of binding to both sites on the virus. The presence of a further erythrocyte-binding site on BTV seems assured because BTV-1 also agglutinates bovine erythrocytes which lack NAX\(\alpha^{2-6}\)GalNAc in their O-linked oligosaccharides (Krotkiewski, 1988).

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REFERENCES


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