ANTIGENIC SIMILARITIES BETWEEN BRAIN COMPONENTS AND BACTERIA CAUSING MENINGITIS

Implications for Vaccine Development and Pathogenesis

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Summary Glycopeptides containing polysialic acid units were isolated from human and rat brain and tested for reactivity with antibodies against meningococcal capsules. The polysialosyl glycopeptides bound specifically to horse antiserum against meningococcus group B. The interaction was inhibited by capsular polysaccharides from meningoccus group B but not groups A or C. The capsular polysaccharide of Escherichia coli K1, which is immunochemically similar to the group B polysaccharide, also inhibited binding. These findings could explain the failure to develop efficient vaccines against group B meningococcus or E.coli type K1 and also suggest that immunological tolerance could be a factor in the pathogenesis of meningitis caused by these bacteria. The presence of the cross-reactive brain component calls for caution in efforts to develop capsular polysaccharide vaccines from these bacteria or the proposed use of passively administered antibodies as immunotherapy of neonatal meningitis.

Introduction

BACTERIAL meningitis continues to be a medical and public health problem, with a mortality of 5% or more, and a high rate of serious sequelae.1-3 Vaccines against the epidemic forms caused by meningococci of serogroups A and C have proved effective.4,6 However, the acceptance of these vaccines for general use has been slow, owing largely to the fact that most non-epidemic bacterial meningitis in industrialised countries is caused by organisms for which vaccines are not available. Of these, one of the most common is group B meningococcus; the development of an effective vaccine against this agent is an urgent research priority.6,8

The successful vaccines against meningitis caused by group A or C meningococci are directed towards their serogroup-specific capsular polysaccharides. Group B meningococci also have a polysaccharide capsule, but efforts towards vaccine development have been disappointing. The purified polysaccharide is a very poor immunogen in both human beings and animals, and attempts to improve its immunogenicity by conjugation to protein carriers have been unsuccessful.5,6 The properties of the meningococcus B (MenB) polysaccharide appear to be the principal cause of these difficulties, since another bacterium, Escherichia coli of the capsular type K1, has an immunochemically identical capsule with equally poor immunogenicity.12,13 This bacterium is also one of the major causes of meningitis and septicaemia in newborn infants.14

We now report that glycoproteins of human and rat brain contain oligosaccharide side-chains that cross-react immunologically with the capsular polysaccharides of group B meningococci and E.coli type K1. The presence of such cross-reactive tissue components suggests that a breakdown of the natural tolerance caused by an artificial vaccine or transfusion of antibodies15 might have adverse effects which should be carefully assessed.

Methods

Total glycopeptides were prepared by extensive pronase treatment of delipidated human and rat brain tissue as described previously,16 and fractionated according to molecular size by gel filtration on a column of "Bio-Gel P-100".17 The column eluates were analysed for neuraminic acid (absorbance at 580 nm). The purified polysialosyl and control glycopeptides were labelled by N-[3H] acetylation of the peptide portion.16 Horse antiserum against Neisseria meningitidis group B (anti-MenB) and control horse antiserum against N meningitidis group A (anti-MenA) were kindly supplied by Dr J. B. Robbins (FDA, Bethesda, USA).

Binding of glycopeptides to antiiserum was investigated by mixing 50 µl labelled glycopeptides (2.5 pmol) in phosphate-buffered saline with 50 µl aliquots of the antiserum serially diluted in fetal calf serum. After incubation for 2 h at 23 °C antigen-antibody complexes were precipitated18 by the addition of 200 µl cold 20% polyethylene glycol 6000 in 50 mmol/1 sodium phosphate buffer, pH 7·4, and the samples were incubated at 4 °C for 1 h. 1 ml of a 2/1 mixture (by volume) of the polyethylene glycol solution and phosphate-buffered saline was added and the samples were centrifuged at 10 000 g for 2 min. The pellets were dissolved in 150 µl water and the percentage of glycopeptides bound to antibodies determined by liquid scintillation counting. Inhibition studies were carried out by including the capsular polysaccharides—N meningitidis group B (MenB) and group C (MenC) (Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania); MenB (Connaught...
Laboratories, Swiftwater, Pennsylvania); and *E. coli* K1 (colominic acid) (Sigma Chemical Company, St Louis, Missouri)—at the final concentrations indicated in the incubation mixture containing 1/2 diluted anti-MenB serum. The values given are the means of duplicate samples; duplicates differed by only 0–6%.

**Results**

A small but significant proportion of the glycopeptides from fetal brain was of a high molecular weight (fig 1). A similar pattern was observed for another sample of human fetal brain (not shown; 18 weeks' gestation), whereas glycopeptides of the postnatal brain sample contained only small amounts of the early-eluting, high-molecular-weight glycopeptides (fig 1). A similar difference has been observed between glycopeptides of developing and adult rat brain; the early-eluting substances were polysialosyl glycopeptides containing N-acetylneuraminic acid residues bound by α2-8 linkages. Our analyses of the sugar composition of the early-eluting glycopeptides of fetal human brain (data not shown) showed that they were polysialosyl glycopeptides similar to those present in developing rat brain.

The polysialosyl glycopeptides reacted with horse anti-MenB but not anti-MenA serum (fig 2A). The MenB capsular polysaccharide is a polymer of α2-8-linked sialic acid, whereas the MenA polysaccharide is a structurally unrelated polymer of N-acetylmannosamine phosphate. The specificity of the binding was further investigated by means of inhibition with various polysaccharides (fig 2B). Of the three *N. meningitidis* polysaccharides studied only that of group B inhibited binding. As would be expected, the similar polysaccharide from *E. coli* K1 was also inhibitory.

The reactivity of human and rat brain glycopeptides with the anti-MenB serum is shown in the table. Significant binding was observed only for the fractions containing the polysialosyl glycopeptides; the normal glycopeptides of the same sources did not bind. A further indication of the specificity of the interaction was that polysialosyl glycopeptides desialised by mild acid treatment did not bind to the antisera (table). Furthermore, the proportion of the polysialosyl fraction of glycopeptides bound to antisera was higher in human fetal brain (21.7%) than in postnatal brain (3.8%), which accords with chemical data showing a fall in the content of α2-8-linked sialic acid units in these glycopeptides during development.

**Discussion**

Suggested causes of the poor immunogenicity of the capsular polysaccharides of *N. meningitidis* group B and *E. coli* K1 include differences in the terminal sugar residues, which are more extensively sialylated in the postnatal brain, and the presence of the polysialosyl glycopeptides, which are not present in the capsular polysaccharides of these meningococcal species.
KI include intrinsic deficiency of the vaccines used, degradation of the polysaccharide by tissue neuraminidase, intramolecular esterification, and tolerance due to some cross-reactive tissue component.9–12,22 Our findings strongly support the latter possibility; they show that brain glycoproteins contain polysialosyl chains which chemically and immunologically closely resemble the bacterial polysaccharides. The immunological cross-reaction is highly specific; the MenC capsular polysaccharide, which differs from the MenB polysaccharide only in that the linkage of the sialic acid units is α-2-9 instead of α-2-8,20 did not inhibit the binding of the brain glycopeptides to anti-MenB serum. The anti-MenB antibodies also seem to require a fairly long stretch of α-2-8-linked sialosyl units for binding (unpublished). Thus, although brain gangliosides contain α-2-8-linked disialosyl and trisialosyl units,24,25 an immunological cross-reaction with MenB polysaccharide has not been reported.

Although many examples of immunological cross-reactions between bacterial antigens and tissue components have been detected and consequences for the host-parasite interactions postulated,26,27 there are few data showing a biologically significant effect. The bacteria for which we have shown an immunological cross-reaction with tissue components are important human pathogens. The cross-reaction poses severe restrictions to the immune defence against these bacteria. Although anti-MenB antibodies can usually be demonstrated in adults, they are of low affinity and of the IgM class only.28,29 Furthermore, they are poorly bactericidal;30 serum bactericidal activity is considered an important defence mechanism in meningococcal infections.31 IgM antibodies do not cross the placenta, thus the newborn infant will remain without antibodies against the capsules of group B meningococcus or E coli K1. Possibly the lack of placental transfer is a physiological mechanism to prevent adverse effects from reactions of the antibodies with the polysialosyl chains that occur in high concentration during the fetal and neonatal phases of brain development.

The lack of protective antibodies may explain the high incidence of E coli capsular type K1 in infections in newborn infants: 30–40% of septicaemia and more than 80% of cases of meningitis are caused by E coli K1 in newborn infants, as compared with approximately 15% E coli K1 in the gut flora.32,33 The excess of K1 in meningitis relative to septicaemia suggests that other factors may favour the localisation of the bacteria in the brain.

In the absence of any demonstrated cross-reactivity between normal tissue components and the MenB polysaccharide, attempts have been made to increase its poor immunogenicity by chemical modifications and coupling to carrier molecules.9–11 Treatment of neonatal E coli K1 meningitis by the administration of the specific antcapsular antibodies has also been suggested.15 An important consequence of our findings is that caution should be exercised in these efforts. It is conceivable that breaking the natural tolerance to this polysaccharide structure could initiate an autoimmune process.34 In addition, unexpected adverse effects could be due to interference with the physiological function of the brain polysialosyl structures: a large proportion of the brain polysialosyl chains occurs in a glycoprotein involved in neuronal cell adhesion.22

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