Abstract

As polysialic acid (PSA), the capsule of Group B meningococcus (GBM) and Escherichia coli K1, is a component of mammalian glycopeptides, there is concern that vaccines against PSA could induce immunopathology. Purified PSA is not immunogenic; however, as a component of bacteria or bound to proteins, it induces protective antibodies. In this review, we did not unearth data indicating an association of IgG anti-PSA with immunopathology in experimental animals or humans. We found no increased incidence of autoimmunity from GBM infections in our review of the natural history/sequellae of Neisseria meningitidis infections. Accordingly, we propose that clinical trials of PSA conjugate vaccines, be considered.
1. Introduction

Despite effective antibiotics and supportive therapy, group B meningococci (GBM) continue to cause epidemics and outbreaks with high rates of morbidity, mortality, and permanent sequelae throughout the world [1–11]. In 1983, Finne et al. suggested that the antigenic similarities between brain components and the GBM capsular polysaccharide (PSA) could induce immunopathology should this antigen be considered for a vaccine [12]. This observation stimulated an extensive literature and a reluctance to consider PSA as a vaccine component.

Meningococci are classified into serogroups according to their capsular polysaccharides (CP) [13]. Of the 13 reported meningococcal disease. These CPs are essential virulence factors because they inhibit the protective actions of complement and are protective antigens because a critical level of serum IgG antibodies specific to these CPs induces complement mediated lysis of serogroups A,C,W135 and Y but not for GBM [13]. Compared to A, C, W135, and Y, GBM causes a disproportionately large number of infections in infants and young children [18].

PSA is also a surface component of many fetal and adult mammalian tissues and of the neural cell adhesion molecule (N-CAM) [28–30], prompting the concern that vaccine-induced, natural, disease-acquired or maternally derived anti-PSA may exert autoimmune pathol-
ogy [12]. Although PSA antibodies bind to many fetal and adult tissues in vitro, there is no evidence for in-vivo binding or associated pathology. This conclusion is illustrated by the report of Saulkomen et al. in which IgG PSA antibodies were shown to bind, when directly applied, to cryostat sections of newborn rat brains but not when the same antibodies were injected into the mother 2 days before parturition [31].

Efforts have been directed towards developing vaccines using non-capsular antigens including outer membrane proteins, lipopolysaccharide, iron-binding proteins, and other antigens newly identified by examination of the organism’s DNA [32–38]. Many of these proposed vaccines are heterogeneous, complex, subject to antigenic variation, and may not be representative of all GBM. Furthermore, none will be useful for E. coli K1 or P. haemolytica A2. Based upon the per-
formance of the Haemophilus influenzae type b, Salmonella typhi (Vi), pneumococcal and group C meningococci (GCM) vaccines, a PSA conjugate that induced protective levels of serum IgG anti PSA would be simple, easy to standardize and close to 100% effective at all ages [39].

As the development of a safe and effective vaccine against GBM is an important priority and a CP based vaccine would likely be highly effective and protect against other PSA-containing pathogens including E. coli K1, we reviewed the literature to evaluate the potential for autoimmune sequelae of PSA antibodies [40–45]. Thus far, experimental and retrospective clinical studies support the feasibility of a safe, immunogenic and protective CP vaccine against PSA.

2. Widespread occurrence of PSA-containing bacteria

Bacteria possessing the PSA CP are common in the upper respiratory and intestinal tracts of humans at all ages. GBM are inhabitants of and pathogens for humans only and are common in the nasopharynx of children and adults. Among 414 marines in a military base free of meningitis, meningococci were isolated from 64.5%:58% of the isolates could be grouped, and of those, GBM were the most prevalent [46]. Similar results have been found in children and young adults throughout the world [47–49]. Strains of E. coli K1 are also inhabitants of and pathogens for other vertebrates [50–54]. In one study of stool cultures from healthy pregnant females, E. coli K1 was the most common aerobic bacterium in 38% of the participants [55]. It is likely that most individuals carry GBM in their pharynx or an E. coli K1 in their intestine repeatedly during their lifetime.

2.1. Immunologic properties of PSA

Purified PSA is not immunogenic when injected into adult humans [56]. This property has been explained by its resemblance to host structures (self antigen) [12,13]. Nevertheless, PSA antibodies can be induced through other methods. Multiple intravenous injections of inactivated GBM or E. coli K1 into animals induced high-titered anti-sera to PSA [20,21,50–52,57–59]. PSA forms non-covalent complexes, presumably by electrostatic bonds, with outer membrane proteins of GBM [59–63]. When adsorbed onto aluminium hydroxide (alum) these complexes elicited PSA antibodies. Zollinger et al. observed that PSA-serotype protein complex vaccines that elicited PSA antibodies were administered to more than 500 adults and more than 2000 children in South Africa without ill effects [63]. In another study, GBM serotype six outer membrane protein–PSA complexes adsorbed onto alum elicited PSA antibodies in adult males [64]. Aside from local reactions and fever that lasted less than 48 h in about 10% of the vaccinees, none had any systemic reaction attributed to autoimmunity. Passive-immunization with these complex-induced PSA antibodies conferred protection to 6–8-week-old mice challenged with 32 LD<sub>10</sub> of GBM. Of interest was that the mice had “natural” pre-existing PSA antibodies that also conferred a low level
of protection. Covalently bound to a protein, PSA conjugates induced IgG antibodies with bactericidal and protective activities in mice and in non-human primates [65–67]. Jennings et al. modified PSA by changing the N-acetyl moiety on C4 of the sialic acid residues to N-propionyl [68]. In mice, the N-propionyl derivative induced antibodies that did not react with PSA but had protective activity against GBM. This modified PSA was shown to be safe in adult humans [69]. As with other meningococcal serogroups, convalescence from meningitis GBM results in increased levels of both IgM and IgG PSA antibodies and can thus serve as a proxy for active immunization with PSA-based vaccines [70–80]. Granoff et al. reported significant increases in IgM and IgG anti-PSA levels following systemic GBM infection [70–80]. As with other meningococcal serogroups, convalescence from meningitis GBM results in increased levels of both IgM and IgG PSA antibodies and can thus serve as a proxy for active immunization with PSA-based vaccines [70–80].

3. PSA antibodies in the healthy population

PSA antibodies are found in most healthy individuals [33,56,60,64,65,69–73,77,95]. The presence of these antibodies is likely the result of prolonged repeated exposure to organisms expressing PSA, such as pharyngeal carriage of GBM [1,7,9,14,45–49,79] and intestinal carriage of E. coli K1 [55]. In one report, IgG PSA antibodies were detected in most paired maternal and cord sera [65]. It is evident that healthy individuals and fetuses are routinely exposed to PSA antibodies without suffering any identifiable immunopathology [49].

4. Monoclonal PSA antibodies

Considering the “non-immunogenicity” of purified PSA, Frosch et al. induced an IgG monoclonal antibody (mAb) by intraperitoneal injection of 3-week-old “autoimmune” NZB mice with 10⁷ live GBM in complete Freund’s adjuvant [81]. Booster injections of 5 × 10⁸ live GBM were administered intraperitoneally twice a week for 4 weeks. At the end of the immunization scheme, the mice were splenectomized, their spleen cells fused with X63-Ag8.653 myeloma cells and an IgG anti-PSA was isolated. The same procedure failed to elicit a PSA mAb in BALB/cAnN mice. Later, as the technology for preparing hybridoma cultures improved, PSA mAbs of all three major isotypes were elicited in other mouse strains.

Kabat et al. discovered a mAb paraprotein anti-PSA, designated IgMNOV, in an 84-year-old man hospitalized with a renal infection [82]. This individual had no history of a meningococcal infection. His serum level of IgMNOV ranged from 13 to 26 mg/mL, or about 3 logarithms higher than normal levels. IgMNOV conferred 100% protection to mice challenged with E. coli K1 or GBM, induced in vitro complement-dependent lysis of GBM (rabbit complement source) and bound to the fixed tissues of newborn rats. Azmi et al. reported anti-PSA IgM paraproteins in 7 of 359 human paraproteins (159 IgM and 200 IgG) [83]. Aside from their gammapathies, none of the seven patients had disease symptoms suggestive of neuropathology or of autoimmunity. Passive administration of these IgM monoclonal paraproteins protected mice against bacteremia and induced complement-mediated bacteriolysis of E. coli K1 strains (rabbit complement source). Our interpretation of these data is that the presence of mAbs in humans, of considerably higher levels than “normal” anti-PSA, confers protection against GBM and E. coli K1 but is not associated with autoimmunity.

5. PSA and host structure

N-CAM is a widely abundant membrane glycoprotein that regulates cell–cell adhesion, and modulation of this adhesion is related to the length of the PSA chain [2–30,84–92]. N-CAMs with longer PSA chains, abundant in embryonic neural tissue, are more reactive with PSA antibodies [90–92]. Although most adult N-CAMs have shorter PSA chains, expression of high PSA content and chain length N-CAM has been identified in certain adult brain tissues and neural tumors [93]. Embryonic and adult N-CAMs have been detected in adult neural, kidney, heart and other muscle tissues. In vitro studies have shown that glycoproteins containing PSA bind anti-PSA antibodies. Post GBM infection sera, containing PSA antibodies, reacted with embryonic N-CAM and were able to lyse cells presenting embryonic N-CAM in a complement-dependant cytotoxic assay (rabbit complement source) [94]. But abundant evidence suggests the safety of PSA antibodies in vivo: first, primates with high levels of conjugate vaccine-induced IgG anti-PSA did not show immunopathology [67]. Second, full-term newborn rats exposed to in-utero to maternal IgG antibodies did not demonstrate binding of these antibodies to their tissues [31]. Third, intracranial injections of IgG anti-PSA into newborn and pregnant rats did not demonstrate binding or lesions in their brain tissue [31]. Fourth, PSA antibodies are present in a high proportion of healthy adults without detectable pathology [33,26,58,60,64,69–72,74,75,77–80].

What could be the explanation for the ability of PSA antibodies to confer immunity to systemic infection with GBM and E. coli K1 yet fail to induce tissue inflammation or pathology? From examination of the literature, we propose three unique properties of PSA that may act in concert: one factor may be poor activation of the later components of homologous complement [95]. Rather than by antibody-initiated complement-mediated bacteriolysis, anti-PSA inactivates GBM and E. coli K1 by stimulating opsonophagocytic killing [15,16]. High concentrations of PSA can have an inhibitory effect on the complement pathway and have been reported to promote the cleavage of C3b to iC3b, hemolytically inactive but recognized by the CR3 receptor on phagocytes [96,97]. PSA could inhibit the binding of complement components beyond C5 and thus allow anti-PSA activated C3 and C5 to facilitate opsonophagocytic killing. Failure to activate the later homologous complement components could explain...
the binding of anti-PSA to the PSA of glycoproteins without tissue injury. The second factor could be the lower binding energy of anti-PSA to PSA at 37°C compared to other polysaccharide antibodies [73]. This lesser binding could be sufficient to induce opsonophagocytosis of GBM and E. coli K1 but too low to induce tissue injury. The third could be the comparatively low rigidity or lack of structure of PSA compared to other polysaccharides. Jennings et al. showed that at least 10 sialic acid residues were required to exhibit helical structure and to inhibit the binding of PSA to its antibody [98]. In contrast, only five sialic acid residues of GCM CP, consistent with the size of an antibody combining site, demonstrated helical structure and exerted the same inhibition similar to that shown for other saccharides and peptides [99]. The helical structure detectable in a pentasaccharide of GCM CP was detected only in a decamer of PSA [100]. These properties explain why PSA of tissues must be fixed on a rigid structure, such as glass for microscopy or to plastic for ELISA, to demonstrate antibody binding [101].

6. Sequelae of meningococcal infection with different serogroups

Since systemic infection stimulates the development of anti-PSA, a population recovered from GBM meningitis can be used as proxy to study the risk associated with these antibodies. If PSA antibodies react pathologically with host PSA, as has been hypothesized, there would be an increased incidence of sequelae, such as autoimmune disorders following GBM meningitis than with other meningococcal serogroups. As meningococcal meningitis results in inflammation and central nervous system damage leading to both short and long term sequelae, a control group must differentiate between sequelae due to meningitis and GBM-specific sequelae. GCM CP differs from PSA only by linkage (PSA is 2→8 linked and GCM CP is 2→9 linked) [13,18]. Although they are closely related, PSA and GCM CP do not cross-react immunologically. GCM, therefore, may serve as a control for sequelae unique to the PSA of GBM [73].

We reviewed of articles identified by Medline using key-words Neisseria meningitidis, meningococcal, meningococcus, infection, bacterial meningitis, sequelae, serogroup, serotype, and autoimmune as well as cross referencing from other bibliographies. Studies were included if they compared cases of GBM infection to those of at least one other meningococcal serogroup as well as to a control group.

Five studies met these criteria and are included in this review:

1. All cases of GBM and GCM disease in four health centers in Ireland from 1995 to 2000 were reviewed retrospectively in a cohort study designed to determine the influence of serogroup on rates of sequelae [40]. Cases were endemic to the region over the 5-year period and the study preceded the routine introduction of the group C conjugate vaccine. A total of 407 patients were reviewed (303 of GBM and 104 of GCM). The patients’ ages ranged from 1 month to 18.4 years with a median age of 1.5 years for GBM and 2.5 years for GCM. Within both groups the highest proportion of patients was <4-year-old. There were no statistically significant differences (P > 0.05) between the two serogroups for rates of mortality and sequelae with infection as well as for other demographic characteristics (Table 1).

2. A similar retrospective study was conducted in Quebec, Canada for 1990–1994 [41]. All confirmed cases of meningococcal disease in Quebec: a total of 471 (167 GBM and 304 GCM), were examined. The median age was 2 years for GBM and 14 years for GCM (P < 0.05). The highest incidence was in <1-year-old for GBM and between 10 and 19 years for GCM. Case fatality rates were 7% for GBM and 14% for GCM (P < 0.05). Deaths and major complications were observed in 12% of GBM and 30% of GCM cases. Any complications, including minor ones, were observed in 37% of GBM and 59% of GCM cases (P < 0.05). With regard to all categories of sequelae and disease outcome, GBM cases showed rates equal to or lower than GCM.

3. A sample of meningococcal cases in the Netherlands from 1959 to 1963 was studied retrospectively for serogroup associations [42]. 1221 patients were reviewed (784 GBM, 209 GCM, 176 group A, and 52 other). The case fatality rate for the entire study population was 5.1% with serogroup A 2.3%, GBM 5.1% and GCM 4.8% (NS) among the three groups. The rate of any sequelae for the entire study population was 7.9% with serogroup A 9.9%, GBM 7.3% and GCM 6.5% (NS). GBM was associated with a significantly lower rate of hearing loss compared that of the other serogroups (1.9% versus 5.5%, P = 0.001), and lower rates of focal neurological signs compared to groups A and GCM (13% versus 18%, P = 0.03).

4. Rates of hearing loss following meningococcal disease were compared in a case control study in Germany [43]. From 1966 to 1992, 30 patients with disease caused by an uncommon serogroup (X, Y, Z, W135, 29E) were age and sex-matched with 30 control patients with GBM [43]. Of the 60 patients, 15% had complete or unilateral deafness: 26.6% among those with uncommon serogroups and only 3.3% among GBM patients (P < 0.01).

5. The four studies listed above were limited to short-term sequelae following infection. A case-control study in Norway considered long-term sequelae of meningococcal disease from 1967 to 1979 (3–15 years following infection with approximately 50% of cases infected with GBM [44,45]). Seventy-one cases and 64 age-matched healthy controls were studied. All subjects were 18–25-year-old at the time of infection. No statistically significant differences were found between the groups regarding sequelae of possible autoimmune etiology including...
Table 1

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<tr>
<th>Study</th>
<th>Serogroup</th>
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<th>Death</th>
<th>Any sequelae</th>
<th>Neurological sequelae</th>
<th>Hearing loss</th>
<th>Renal failure</th>
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<td>B</td>
<td>303</td>
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<td>C</td>
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<td>Erickson and De Wals [41]</td>
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<td>167</td>
<td>12 (7.2)</td>
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<td>C</td>
<td>304</td>
<td>42 (13.8)</td>
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<td>Spanjaard et al. [42]</td>
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<td>40 (5.1)</td>
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<td>A and C</td>
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<td>Mayatepek et al. [43]</td>
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<td>Uncommon groups</td>
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<td>8 (26.6)</td>
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* Only marked differences are statistically significant at \( P < 0.05 \).

arthritis, muscle pain, chronic skin disease, asthma, and other allergic reactions.

7. Conclusion

Few studies have compared severity of infection and outcome among different meningococcal serogroups, and those often consisted of small sample sizes or were biased with regard to factors, such as age, time to treatment, or treatment protocol and diagnosis. Further, there is no mention of “autoimmune” diseases, such as Guillain–Barre, multiple sclerosis, etc. in published studies of sequelae of meningococcal meningitis patients. Bearing the limitations in mind, these studies have not supported the hypothesis that PSA antibodies initiate autoimmune pathology. Our review shows equal or lower rates in nearly every category of sequelae and of mortality associated with GBM meningitis compared to those of other meningococcal serogroups [40–50]. Speculation about the similarity between PSA and tissue saccharides, but not clinical data, warned that development of a PSA-based vaccine could induce autoimmunity [12]. Realizing the difficulty, if not the impossibility, of evaluating a null hypothesis, there is no epidemiological or clinical evidence to associate pathology with PSA antibodies.

A wealth of data shows that serum IgG anti-PSA, induced by active immunization or administered passively as monoclonal or polyclonal antibodies, confers protection against challenge of experimental animals with GBM or \( E. coli K1 \) [102]. These PSA antibodies induce complement-dependent opsonophagocytic activity.

Most of the evidence presented here speaks to short-term sequelae and further investigation is needed to examine the possibility of long-range pathologies following GBM meningitis. We are planning a retrospective cohort study of meningococcal patients to examine evidence for autoimmunity associated with the short and long-term presence of PSA antibodies.

The comparative lack of tertiary structure of PSA with molecular sizes of less than 18 monomers that fail to functionally occupy the antigen binding site of antibody at 37°C and activate the later complement components is our best explanation of the unique biological effects of this immunologic system. This relative immunologic inertness of small size PSA may explain why this structure is so widely distributed as a surface saccharide throughout nature.

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