Clinical implications of the specialised B cell response to polysaccharide encapsulated pathogens

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Box 1: Summary of main points
- Encapsulated bacteria (meningococci, pneumococci, and *H influenzae* type b) are major causes of respiratory and meningeal infections in infancy worldwide.
- Factors that predispose to recurrent infection with encapsulated organisms reflect the importance of B cell receptor signalling and production of complement fixing, opsonising IgG antibodies for host defence against these pathogens.
- Investigation of patients with recurrent infection with encapsulated bacteria hinges on identification of defects in antibody production (specific B cell defects, hyposplenism) and opsonisation defects (complement deficiency).
- Capsular polysaccharide antigens evoke type 2 thymus independent (TI-2) antibody responses but fail to generate conventional B cell memory.
- Antibody unresponsiveness to TI-2 antigens can be overcome by inducing T cell help for responses to polysaccharide antigens (for example, with conjugate vaccines).

Microbiology of encapsulated bacteria
Polysaccharide capsules confer virulence, in part because they enable bacteria to evade adaptive and specific immune defence mechanisms. Capsules inhibit phagocytosis, obscure phosphoryl-choline residues in the cell wall from recognition by C reactive protein, and offer resistance to the lytic action of complement. Capsules also hide immunogenic proteins and lipids present in their outer cell wall, including lipopolysaccharide in Gram negative bacteria such as *H influenzae* type b and meningococcus. Thus, production of opsonising antibody towards capsular polysaccharides that fix complement is critical for early host defence against these organisms.

Predisposition to infection with encapsulated bacteria
(1) Specific antibody unresponsiveness to polysaccharides
The most significant cause of failure of B cells to respond to encapsulated organisms is young age. Humans are capable of generating antibodies to protein antigens from birth and therefore can respond to most pathogens. However, an ability to make antibodies to polysaccharide antigens does not start to develop until after 2 years of age, and does not reach adult levels until approximately 5 years of age. This period of polysaccharide unresponsiveness during infancy coincides with the peak incidence of invasive infections with encapsulated organisms. The reason for this unresponsiveness is still not known. Several hypotheses have been put forward, including immaturity of B cells, lack of diversity of the neonatal B cell repertoire, absence of B cells with a marginal zone phenotype, lack of a stromal component in the neonatal spleen, or deletion of polysaccharide specific B cells to prevent autoimmunity from recognition of cross reactive neuronal antigens.

While most individuals acquire the capacity to respond to encapsulated organisms during childhood, specific failure to produce antibodies to polysaccharides may persist. Inability to produce antibodies against polysaccharides may occur in the context of primary immunodeficiency disease, including X linked agammaglobulinemia, Wiskott-Aldrich syndrome and ataxia telangiectasia, although these conditions are rare (box 2). Recurrent encapsulated bacterial infection may herald the development of common variable immunodeficiency in adults. Finally, isolated failure to produce antipolysaccharide antibodies may persist after infancy as a discrete but poorly understood
immune defect. This form of immuno-
deficiency may account for a proportion of
adults with normal splenic function and intact
complement who present with recurrent infec-
tions with encapsulated bacteria.

2) HYPOSPLENISM
Absence of the spleen increases both the risk of
infection with encapsulated organisms and the
risk of mortality from invasive disease.6–7 Sepsis
in patients with hyposplenism is most com-
monly caused by pneumococcus, H influenzae
type b, meningococcus, and less commonly
babesia and Capnocytophaga canimorsus. The
incidence of post-splenectomy sepsis is influ-
enced by the indication for the splenectomy,
ranging from 1%–2% in trauma patients, up to
10%–20% for B cell activation by polysaccha-
ride antigens patients with Hodgkin’s disease
or thalassaemia.8–9

3) COMPLEMENT DEFECTS
Each pathway of complement activation as well
as assembly of the membrane attack complex
have been implicated in normal clearance of
encapsulated bacteria (fig 1). Defects of the
classical complement cascade predispose to
infections with encapsulated bacteria, espe-
pecially pneumococcus because of failure to gen-
erate opsonising C3 degradation fragments.10–13
Abnormalities that result in excessive C3 con-
sumption and functional C3 deficiency, such as
factor I deficiency or C3 nephritic factor (an
autoantibody that may accompany mesangi-
ocapillary glomerulonephritis), predispose to
the same spectrum of infections.14 15 Properdin
deficiency, which prevents activation of the
alternative pathway, predisposes to recurrent
infection and fulminant meningococcaemia.16
There is evidence to suggest that some
polymorphisms of mannose binding lectin
confer an increased risk of meningococcal dis-
 ease.17 Assembly of the membrane attack com-
p lex from the late complement components
(LCC, C5–9) is particularly important for host
resistance to invasive neisserial disease and
defects of this complex predispose to meningo-
coccaemia, meningococcal meningitis, and
disseminated gonococcal infection, rather than
invasive infections with encapsulated organ-
isms per se.18 19

Box 2: Causes of selective antibody unresponsiveness to polysaccharide antigens

Infancy
Primary immunodeficiency
- X linked agammaglobulinaemia (brk
mutation).
- XLA phenocopy (normal brk): BLNK
(SLP-65) deficiency; phosphoinositol-3
kinase deficiency*; protein kinase C-γ
deficiency*; phospholipase C-γ2
deficiency*.
- Common variable immunodeficiency.
- Wiskott-Aldrich syndrome†
- Ataxia telangiectasia†
Secondary immunodeficiency†
- Cytotoxic/myeloablative therapy.
- HIV infection.
- Chronic lymphocytic leukaemia.
- Multiple myeloma.
- After bone marrow transplantation.
* Only described in mice so far.
† Often associated with other immune
defects.

Figure 1 Complement defects and susceptibility to encapsulated bacteria. Summary of the three pathways of complement
activation, which generate C3 convertases (C4b2b and C3bBb), opsonising C3 degradation products (C3bi, C43dg), and
the membrane attack complex (MAC). Defects that predispose to infection with encapsulated organisms are indicated (P =
properdin; D = factor D).
Basic immunology: response to polysaccharides

(1) B CELL ACTIVATION BY POLYSACCHARIDES

Immunologists classify polysaccharides as type-2 thymus independent (TI-2) antigens, whereas lipopolysaccharide (endotoxin) is a TI-1 antigen, and proteins are thymus dependent (TD) antigens (fig 2). The defining characteristics of TI-2 antigens are their high molecular weight, repetitive epitopes, and resistance to degradation in vivo. They cause extensive cross linking of B cell receptors (BCRs), but they are poorly internalised by B cells.20 The potency and persistence of the signal through BCRs after ligation by TI-2 antigens probably obviates the requirement for T cell help (hence the term “thymus independent”), which in any case is unavailable because B cells do not process and present epitopes from polysaccharides to T cells. By contrast, conventional or TD protein antigens only induce antibody production after B cells have received T cell help, which is elicited when B cells process and present peptides to primed T cells.

T cell priming takes one to three days and is required before T cells can provide help to B cells for responses to TD antigens. As this is unnecessary in responses to TI-2 antigens, B cell activation and antibody production occurs earlier than in TD B cell responses.21 While this rapid antibody response may be crucial for host defence, TI-2 antigens fail to stimulate high affinity memory B cells so repeated exposure to TI-2 antigens does not evoke an anamnestic response. This is because germinal centres, which are the sites of memory B cell formation, are unusual in responses to TI-2 antigens, and when they do form they appear to involve before memory cells are generated.22 Nevertheless, TI-2 antigens stimulate a long lived antibody response, probably due to ongoing B cell activation by persistent polysaccharide antigen.21

Classification of polysaccharides as TI-2 antigens originates from the observation that they fail to elicit B cell responses in the CBA/N (X linked immunodeficiency, Xid) strain of inbred mice, which respond normally to both TD and TI-1 antigens.23 The cause of this murine immunodeficiency has been identified as a point mutation in the gene encoding Bruton’s tyrosine kinase (Btk).24 Btk participates in several signalling pathways downstream from the BCR, which are critical for survival and differentiation of activated B cells in response to polysaccharide antigens (fig 3). Investigations of Btk signalling pathways have proved informative about susceptibility to encapsulated organisms.

Point mutations of btk in humans cause X linked agammaglobulinaemia (XLA), which is characterised by B cell deficiency and low or absent serum immunoglobulin. Interestingly, rare circulating B cells have been isolated from patients with XLA and shown to be selectively unresponsive to TI-2 antigens.25 This is consistent with evidence that the bulk of morbidity in XLA is due to respiratory tract infections with encapsulated bacteria. Some viruses have been shown to stimulate TI-2 responses revealing that they are not only evoked by polysaccharides.26 For example, enteroviruses...
stimulate TI-2 responses by virtue of the regular spacing of the epitopes on the viral envelope. Failure to clear enteroviral infections is another characteristic of XLA patients. Early antibody response to all of these pathogens is probably important for prevention of haematogenous spread and infection of the central nervous system and other vital organs. Recent studies have identified patients presenting as XLA phenocopies but who harbour non-Btk gene defects. For example, patients with mutations in the gene for the linker protein, BLNK, present with clinical features typical of XLA. Identification of these defects promises further clarification of the biology of TI-2 responses. Mouse studies have shown that selective TI-2 unresponsiveness also occurs after deletion of phosphoinositol-3 kinase or protein kinase C-γ genes (box 2, fig 3). Interestingly, each of these defects also impinge on Btk signalling pathways. The clinical significance of these findings is that patients with selective susceptibility to encapsulated bacteria may be found with similar signalling defects.

(2) THE IMPORTANCE OF THE SPLEEN AND MARGINAL ZONE B CELLS
The increased risk of infection conferred by hyposplenism reflects the fact that antibody responses to polysaccharides occur more readily in the spleen than lymph nodes. Some studies have suggested that specialised splenic macrophages are crucial for removing antibody coated encapsulated organisms from the blood. However, TI-2 responsiveness is maintained even after depletion of these cells from mice. By contrast, studies have shown that when mice or rats are rendered selectively deficient of marginal zone B cells, there is a significant impairment of the response to polysaccharides. Thus, a more plausible explanation for the importance of the spleen is that marginal zone B cells comprise about 30% of human splenic B cells, but are rare in most lymph nodes other than mesenteric lymph nodes. A practical consequence of this is that encapsulated bacterial infection via the nose and throat or through the skin is unlikely to produce a strong antibody response in the draining nodes.

(3) THE IMPORTANCE OF OPSONISATION AND IMMUNOGLOBULIN CLASS SWITCHING
A competent immune response to encapsulated organisms depends not only on production of antcapsular antibodies but also on the interaction of these antibodies with serum complement and opsonic receptors for immunoglobulin and complement fragments on phagocytes. Although natural antibodies,
which comprise low affinity IgM and are produced in the absence of immunisation, have been suggested to play a part in responses to encapsulated bacteria, production of antcapsular IgG appears to be more important. This is because phagocytes bear receptors for the Fc portion of IgG molecules (termed Fcγ receptors), and these are critical for opsonisation. Studies using mice with selective deficiencies of secreted IgM (natural antibody) support this concept because they have normal TI-2 responses.

After immunisation with polysaccharide, B cells undergo immunoglobulin switch recombination predominantly to IgG2 and IgG1. Much of the total serum IgG2 in healthy individuals is derived from TI-2 responses. The explanation for predominance of IgG2 when switching occurs in the absence of T cell help during responses to polysaccharides remains unclear, since IgG2 antipolysaccharide antibodies are no more effective than other IgG subclasses in protecting against pneumococcal infections. Furthermore, IgG1 is at least as efficient as IgG2 at opsonisation; conjugate H influenzae type b vaccine (see below) induces predominantly IgG1, which is protective. Combined genetic deletion of IgG1 and IgG2 appears to be compatible with normal health so IgG3 may also provide protection against encapsulated organisms. In summary, although IgG2 is the predominant class of immunoglobulin produced in response to polysaccharides, this class of antibody does not appear to be especially effective for TI-2 immune responses.

IgM and IgG1–3 antcapsular antibodies can activate the classical complement pathway. The alternative pathway may be activated by direct contact with bacterial cell wall components, but not after contact with the capsule. Complement degradation products (C3bi and C3dg) enhance antibody opsonisation significantly (fig 1). This is illustrated by the finding that induction of polysaccharide specific antibodies, either by passive transfer of antcapsular IgG, or by polysaccharide immunisation, fails to normalise either protection from encapsulated organisms or phagocytosis when complement deficiency is present. The clinical implication is that both complement defects and Ig defects predispose to infection with encapsulated organisms.

**Applied immunology: diagnosis and prevention**

(A) IDENTIFYING PATIENTS WITH SUSCEPTIBILITY TO INFECTION WITH ENCAPSULATED ORGANISMS

Complement fixing IgG antibody formation by marginal zone B cells is crucial for normal host defence against polysaccharide encapsulated organisms. In patients with recurrent infections involving these pathogens, investigations should be directed towards these key mechanisms.

(1) Specific antibody defects and IgG subclass deficiency

Although there have been numerous reports of increased risk of infection with encapsulated bacteria in patients with low levels of IgG2, other studies have shown that IgG2 gene deletions are compatible with normal health. As discussed above, there is no solid biological explanation of why IgG2 deficiency should predispose to infection. The response generated by conjugate vaccines is predominantly IgG1, which represents compelling evidence against a specific requirement for IgG2. Thus, when IgG2 deficiency occurs in patients with recurrent encapsulated bacterial infections it is probably the result of a fundamental defect in responsiveness to polysaccharide antigens, rather than the cause. It follows that the preferred test for heightened susceptibility to infection with encapsulated bacteria is measurement of the capacity to generate polysaccharide specific antibodies after immunisation with a polysaccharide vaccine such as Pneumovax, rather than measurement of IgG subclass levels.

(2) Complement defects

Inherited complement defects are rare (combined prevalence of <0.1%) and predisposition to infection (unlike autoimmunity) is only manifest in homozygotes. However, among adults presenting with sporadic meningococcal infection, the prevalence of LCC deficiency is 10%–15%. It is important to identify this subset of patients because they suffer a high rate of recurrent infections. Screening for complement deficiency is by total haemolytic complement (CH50) assay. Absent haemolytic activity indicates deficiency of a component of the classical or terminal pathways. Assaying specific serum complement components can identify the precise deficiency. Properdin deficiency, which often leads to fulminant meningococcal disease in infancy, is X linked. Identification of this defect necessitates a screen for the integrity of the alternative pathway, the so-called AH50.

(3) Hyposplenism

The presence or possibility of hyposplenism is most often determined by obtaining a history of splenectomy, or of a condition that predisposes to functional hyposplenism (box 3). Congenital asplenia is exceedingly rare. The Hox-11 gene has been shown to control spleen development in mice, although analogous defects have not been identified in asplenic humans. Furthermore, most cases of human

<table>
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<th>Box 3: Causes of hyposplenism</th>
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<td>• Congenital asplenia.</td>
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<td>• Splenectomy.</td>
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<td>• Gastrointestinal disease: coeliac disease, inflammatory bowel disease.</td>
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<td>• Autoimmune disease: primary biliary cirrhosis, thyroid disease, rheumatoid arthritis, systemic lupus erythematosus.</td>
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<td>• Sickle cell disease.</td>
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<td>• Infiltration: amyloid, sarcoid.</td>
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<td>• Graft versus host disease.</td>
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Asplenia occur in the context of more complicated developmental defects involving abdominal contents, heart, lungs, and face. There is also evidence that a polysaccharide vaccine booster abrogates the benefit of conjugate vaccination. Together, these findings raise the possibility that polysaccharide vaccination may exhaust the immune response by inducing terminal differentiation to plasma cells of antigen specific cells B cells without replenishing the memory B cell population. Together, these data suggest that conjugate vaccines are preferable for prevention of infections with encapsulated organisms.

(2) Conjugate vaccines
While there are concerns about the efficacy of polysaccharide vaccines, experience with polysaccharide conjugate vaccines has been spectacularly successful. Conjugate vaccines comprise capsular polysaccharide (for example, *H influenzae* type b polyribosylribitol phosphate) conjugated with protein (for example, diphtheria toxoid, a non-toxic mutant of diphtheria toxin (CRM197), tetanus toxoid, or the outer membrane protein complex of *Neisseria meningitidis*). Consequently, they stimulate TD responses to polysaccharide and are immunogenic for infants (fig 4). The widespread use of conjugated *H influenzae* type b vaccines has virtually eliminated this disease from developed countries.

Group C meningococcal vaccine was released recently, but a vaccine for group B strains, which account for about 60% of meningitis in the Western countries, remains elusive. This may be due to a similarity between group B capsular antigen and self antigens in neonatal brain, in which case self reactive B cells would be purged from the neonatal repertoire, precluding responsiveness to vaccine (and pathogen). As with other encapsulated organisms, meningococcal infections are most common in infancy. However, adolescents are also at risk of fatal invasive meningococcal infection, even though they are immunocompetent. This second peak of incidence correlates with an increased rate of nasal carriage. Conjugate vaccination offers the possibility of reducing disease because long lived, high affinity antibody would be expected to prevent invasive disease, and may also reduce the incidence of nasal carriage. On the other hand, there is also a risk that an effective group C vaccine may alter meningococcal ecology and increase the prevalence of invasive disease due to group B strains. Production of a conjugate vaccine against pneumococcus has been problematic because there exist over 90 serotypes (that is, >90 different polysaccharide capsules). Nevertheless, a heptavalent conjugate vaccine is now available, which theoretically could protect against the majority of invasive disease episodes in young children living in Western countries, although as with meningococcal vaccination, there is a risk that oligovalent vaccines will result in an increased prevalence of non-vaccine serotypes.

(b) MANIPULATING THE IMMUNE RESPONSE TO POLYSACCHARIDES
(1) Polysaccharide vaccines
At present, polysaccharide vaccines are the main preventive measures for pneumococcus and group A meningococcus. The 23 valent pneumococcal polysaccharide vaccine (Pneumovax) is recommended for people who are at increased risk of invasive pneumococcal disease. This recommendation cannot apply to infants, who are unresponsive, and the efficacy of this vaccine in certain other high risk groups is questionable. Evidence that vaccination may actually increase the risk of infection in the elderly and HIV infected individuals is of particular concern. The presence of Howell-Jolly bodies, as well as basophilic stippling of the red cells on the blood film usually identifies hyposplenism. Asplenism may be demonstrated by technetium-99m sulphur colloid scanning because this normally identifies both liver and spleen, whereas only the liver is visualised in the asplenic abdomen.

(3) Surrogate T cell help
Conjugate vaccines demonstrate the value of manipulating the immune system to elicit TD
Box 4: Future directions

- Identification of B cell signalling defects other than Btk and BLNK that cause specific unresponsiveness to polysaccharide antigens. Candidate molecules emerging from mouse studies are PI3-kinase, PLC-γ2, and PKC-γ2.
- Clarification of the efficacy of polysaccharide vaccines, their indications, and their interactions with conjugate vaccines.
- Expansion of the role of conjugate vaccines including development of an immunogenic type B meningococcal polysaccharide vaccine.
- The H influenzae type b and meningococcus type b genomes, which have been sequenced, may reveal genes encoding highly conserved proteins for use in conjugate vaccines.

responses to polysaccharide antigens. Given the obstacles to making conjugates of all the clinically significant polysaccharides, an alternative strategy is to provide surrogate T cell help in the form of agonistic anti-CD40 antibody treatment at the time of polysaccharide immunisation. This strategy has been shown to enhance responses to polysaccharide antigens. 95 Studies in mice sound a note of caution, however, for anti-CD40 antibodies result in substantial splenomegaly associated with massive proliferation of lymphoid and myeloid derived cells, but fail to generate either germinal centres or memory responses when given with TI-2 antigen.96

Summary

It is difficult to exaggerate the impact of encapsulated bacteria on human health. Our understanding that polysaccharide antigens activate B cells in a T independent manner, and that infants are unable to generate TI-2 responses explains the pattern of invasive disease due to these organisms, and has led to successful intervention with conjugate vaccination. Recent developments in B cell biology hold the promise of explaining selective TI-2 unresponsiveness in adults.

Box 4 shows future directions.

MCC is supported by grants from the Sylvia and Charles Viertel Foundation and the University of Sydney. CGV is a recipient of an ARC Clinical Research Fellowship.