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Review

# Serum bactericidal antibody assays – The role of complement in infection and immunity

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ABSTRACT

Complement is an essential component of the immune system and human pathogenic organisms have developed various mechanisms for evading complement mediated serum killing. The "gold standard" for measuring the ability of vaccine-induced antibody to kill *Neisseria meningitidis* is the serum bactericidal antibody (SBA) assay which measures complement mediated killing *via* antibody. This assay requires active complement, either intrinsic from the serum being tested or the addition of exogenous complement, either from a human or from another species such as rabbit. For serogroup C, an SBA titre of ≥4 was established as the correlate of protection when using human complement and ≥8 as the threshold when using rabbit complement, based on comparative assay results. Licensure of meningococcal vaccines, including polysaccharide protein conjugate vaccines and serogroup B vaccines has been based on the immune responses measured with the SBA assay, thus on a surrogate of vaccine efficacy. This review examines the use of complement and the SBA assay to assess immunity to meningococcal infection, and provides examples of vaccine trials in different age groups where various assays have been used.



## 1. Subversion of host defenses

The normal immune system is in a constant state of alertness. Circulating antibodies and memory B cells signify a state of responsiveness, and herald the elimination *in vivo* of invading organisms by targeting them, and alerting circulating immune cells to home in on the pathogen. The defence against capsulated bacteria relies mainly on naïve B-cells in the marginal zone of the spleen. These cells produce polyreactive antibodies that play a crucial role in this defence. The antibodies often perform this function with the aid of complement, especially if the invading organisms are capsulated bacteria. In patients with late complement component deficiencies, both capsulated and non-capsulated organisms, especially *N. meningitidis* are reported to cause disease such as sepsis and meningitis at a higher frequency than in the general population [1].

*Haemophilus influenzae* has developed various mechanisms, mostly involving outer surface structures such as lipooligosaccharide glycans and outer surface proteins, for *evading* the host's immune system [2]. Similarly, *Staphylococcus aureus* has the staphylococcal superantigen-like protein 7 (SSL7) that binds both immunoglobulin A and complement C5, in order to *inhibit* complement mediated haemolytic and bactericidal activity [3]. A surface-exposed neuraminidase is used by the oral spirochaete *Treponema denticola* both as a nutrient and as a decorating molecule, in order to *disguise* it from complement mediated serum killing [4]. *Borrelia* spp. uses CspA for binding of host-derived proteins and/or direct interaction with the formation of the terminal complement complex, as a mechanism for evasion [5] and [6]. These and other examples, including mechanisms used by *N. meningitidis*, are shown in Table 1 [2–16].

The meningococcus has several important adaptations that allow it to survive in the human host: the presence of the capsule and the ability to bind factor H (fH) which is an inhibitor of complement activation. Normally, factor H is one of the alternative pathway complement regulatory proteins that prevents excessive complement activation from injuring the host. Persons with genetic fH deficiencies have uncontrolled complement activation due to an

*Abbreviations:* anti-B PS IgM Ab, anti-serogroup B capsular polysaccharide IgM antibodies; BCA, bactericidal activity test; CFU, colony forming unit; ELISA, enzyme-linked immunosorbent assay; fH, factor H; fHbp, factor H-binding protein; GMT, geometric mean titre; IMD, invasive meningococcal disease; MCC, meningococcal serogroup C conjugate; OPA, opsonophagocytic assay; SBA, serum bactericidal antibody.

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**Table 1**
Mechanisms by which selected organisms evade complement mediated killing.

| Organism | Molecule(s) | Mechanism of evasion | Reference |
|---|---|---|---|
| *Neisseria meningitidis* | Polysaccharides and outer membrane proteins | Enhanced Opc expression associated with vitronectin binding and reduced membrane attack complex deposition; lipopolysaccharide immunotype switch; pile allelic exchange associated with enhanced autoaggregation | [7] |
| | Neisserial surface protein A (NspA) | Binds to factor H and enhances meningococcal resistance to complement | [8] |
| | Factor H binding protein (fHbp) | The organism becomes surrounded by factor H and is no longer identified as foreign | [9,10] |
| | Novel adhesin: meningococcal surface fibril (Msf) | Binding of Msf preferentially to activated vibronectin (in addition to Opc binding to vibronectin) | [11] |
| *Haemophilus influenzae* | Lipooligosaccharide glycans and outer surface proteins | Decrease cell surface binding of the complement activator IgM | [2] |
| | Vitronectin | Inhibition of complement | [12] |
| *Staphylococcus aureus* | Staphylococcal superantigen-like protein 7 | Binds immunoglobulin A and complement C5 | [3] |
| *Acinetobacter baumannii* | Secreted serine protease PKF | Resistance to complement killing and suppression of biofilm formation | [13] |
| *Pseudomonas aeruginosa* | 57-kDa dihydrolipoamide dehydrogenase (Lpd) | Surface exposed protein binds Factor H, Factor H-like protein-1, complement Factor H-related protein, and plasminogen | [14] |
| *Treponema denticola* | Surface-exposed neuraminidase | Nutrient and as a decorating molecule | [4] |
| *Borrelia* species | Orthologs of CspA | Binding of host-derived proteins and/or direct interaction with the formation of the terminal complement complex | [5,6] |
| Extraintestinal *Escherichia coli* (ExPEC) | Exopolysaccharide colanic acid | Protection from envelope stress whilst cell wall damage is repaired | [15] |
| *Yersinia pseudotuberculosis* | Outer membrane protein Ail | Recruitment of regulator of classical and lectin pathway (C4b-binding protein), and regulator of alternate pathway (factor H) | [16] |

inability to regulate the alternative pathway. Mutations in the gene are associated with diseases such as atypical hemolytic uraemic syndrome (aHUS) [17].

The meningococcus takes advantage of its ability to bind fH in order to shield itself from the action of the innate immune response. The meningococcus harbours at least three distinct proteins on its surface to exploit fH: factor H-binding protein (fHbp), Neisserial surface protein A (NspA) [8,10,18] and PorB [19,20]. With these proteins, the organism can bind human fH specifically – the organism becomes surrounded by fH which keeps complement C3b in check, thereby down-regulating complement activation by the human immune system. Consequently, the meningococcus is less susceptible to complement mediated antibody dependent lysis.

The meningococcal capsule is also known to play a major role in the evasion of complement-mediated lysis and in addition to outer membrane proteins such as fHBP binding to fH, the capsule itself down-regulates complement activation and enhances resistance to bactericidal activity [21,22]. Expression levels of the surface proteins which bind fH therefore affect the susceptibility of the organism: more expression of the protein on the bacterial surface equates with increased resistance to killing and *vice versa*: a decrease in expression of the proteins equates with increased susceptibility of the organism [23,24].

## 2. The serum bactericidal antibody (SBA) assay and correlates of protection

The origins of the SBA to measure meningococcal killing was in the 1960s when, during an outbreak of invasive meningococcal serogroup C disease at an army base, Goldschneider and colleagues measured functional antibody concentrations in newly-enlisted military recruits [25]. Goldschneider and colleagues demonstrated that recruits with a defined amount of circulating functional meningococcal antibodies were at lowest risk of disease, whilst recruits with antibody levels below this concentration were at highest risk. The group employed the SBA (sometimes referred to in the literature as the bactericidal activity test or BCA) assay to measure the concentration of these functional antibodies was the SBA. For the purposes of the present article, we take "SBA" to mean "serum bactericidal antibody". The SBA assay measures the ability of the antibodies to lyse bacteria in the presence of complement. In their analysis of the recruits, serum from both healthy recruits and those who succumbed to disease was used. The minimum titre that correlated with protection was the dilution 1:4. Thus the protective titre measured using human complement was defined as $\geq$1:4 although it should be noted that in this analysis it was an individual correlate based on natural immunity, not vaccine induced antibodies. A subsequent study of vaccinated individuals, the Norwegian protection trial with 172,000 adolescent subjects, came to a similar conclusion: that an hSBA titre of 4 is a good estimate of both short- and long-term protection [26].

To substantiate the conclusion that a titre of $\geq$4 was the correlate of protection against invasive meningococcal disease, Goldschneider also performed a population-based study, measuring SBA in individuals from infancy through to adulthood. An inverse relationship exists between the presence of SBA titres at 1:4 and the incidence of invasive meningococcal disease [25]. Of note, SBA was measured for serogroups A, B and –C, with all three inversely correlating with the incidence of disease, although disease at the time in the USA was primarily due to serogroups B and C only. The serum bactericidal activity as measured by SBA assay has become the "gold-standard" correlate of both infection and successful immunisation [27].

The SBA assay measures the ability of circulating antibodies to lyse meningococci in the presence of complement, otherwise known as complement mediated killing *via* the classical pathway of the immune response. The bactericidal titre is defined as the dilution of the test serum that results in at least a 50% decrease in colony forming units (CFUs) per mL of bacteria [28]. As such, it is a functional assay in that it measures the killing of live bacteria. The conditions of the assay have a considerable impact on the results, so defined and validated conditions need to be used, developed by a panel of experts convened by the WHO [29]. As meningococcal vaccines were under development, the guidelines for the assay included the use of baby rabbit complement, due to the availability of this reagent and the difficulty sourcing human complement [29].

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