CLINICAL INVESTIGATION

# Defining efficacy in meningococcal vaccine trials

**Clin. Invest.** (2012) 2(6), 589–601

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The incidence of meningococcal carriage and disease is, or will soon be, declining in many countries as the result of effective vaccination campaigns. The low incidence of disease requires the use of surrogate markers of protection in clinical trials of meningococcal vaccines. Assays of serum bactericidal activity (SBA) remain the primary efficacy outcome. Evidence that SBA assays are predictive of immune protection in an individual derives from prospectively studied epidemics; evidence that SBA assays are predictive of protection at the population level come from vaccine efficacy studies. Other immune mechanisms also play important roles, particularly in the large and possibly increasing proportion of meningococcal cases occurring in individuals with complement deficiencies. The declining incidence of asymptomatic carriage will result in less natural boosting of meningococcal immunity with a resulting shorter duration of protection. Our changing epidemiologic circumstances should prompt an ongoing re-assessment of our approach to evaluating vaccine efficacy.

**Keywords:** bactericidal activity • biomarker • complement • complement deficiency • efficacy • meningococcus • Neisseria • surrogate marker • vaccine

## The impact of disease epidemiology on the conduct of meningococcal vaccine trials

The development of vaccines for meningococcal disease should reflect the current and potential future epidemiologic situations in which *Neisseria meningitidis* is likely to cause disease. Unfortunately, our understanding of meningococcal epidemiology is incomplete. While molecular epidemiologic techniques have done much to elucidate mechanisms behind the emergence of meningococcal strains, reasons for the decline or disappearance of pathogenic strains are less clear [1] and our ability to predict future pathogenic strains is limited [2]. While the incidence of meningococcal disease in the USA is at an all-time low, successful vaccination campaigns in other parts of the world seem poised to bring us into a new era of declining disease burden globally.

The meningococcus appears to have made its first appearance in Geneva, Switzerland, in 1805 [3]. The predilection for those under the age of 30 years in this epidemic suggests that older individuals were protected, perhaps by previous exposure to a similar organism. However, the subsequent pattern of 'epidemic cerebrospinal meningitis', particularly its spread among soldiers in garrison rather than on campaign [4] and its spread to neighboring civilian populations in times of peace [5,6] as well as war [7], distinguish it from typhus, typhoid and other camp fevers, and suggest that this was indeed a new pattern of disease if not a new organism altogether.

The global spread of meningococcal disease and the successful use of capsular and outer membrane vesicle (OMV)-based vaccines to combat it are familiar success stories that are still being written about [8,9]. Due to the rapid clinical

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course and high-case fatality rate of meningococcal disease, prevention of disease will continue to be the preferred approach, and vaccination strategies will continue to be pursued even in low-incidence areas, as the experience in the USA suggests.

The incidence of meningococcal disease has been declining in the USA for several decades [10]. This decline began prior to the introduction of routine meningococcal vaccination, making it difficult to measure the impact of this intervention [11]. Presumably, this has been accompanied by a decline in the rate of asymptomatic colonization, in part due to the use of conjugate vaccines, though other behavioral factors have also been postulated [10].

The characteristics of the population at risk for meningococcal disease may also be changing. One recent study in Tunisia [12] supports earlier studies in Europe [13] and the USA [14], which showed the prevalence of complement deficiency among adults with sporadic meningococcal disease at 15–25%. The current prevalence in the USA may actually be higher because of the overall decline in disease incidence. Over two decades ago, Figueroa and Densen proposed that as the population incidence of meningococcal disease declines, the proportion of cases occurring among those with inherited or acquired deficiencies of the complement system should increase. Based on several studies done under varying epidemiologic circumstances, they constructed a curve predicting that when the incidence of meningococcal disease drops below one case per million per year, the proportion of those cases occurring in individuals with complement deficiencies could exceed 50% (Figure 1) [15]. The estimated incidence of meningococcal disease in the USA in 2009 was 3.2 per million [16]. If one goal of further meningococcal vaccine development is to decrease the incidence of disease further, as opposed to merely preventing future epidemics, it may be prudent to consider the nature of the population at greatest risk and the vaccine-inducible mechanisms available to protect them.

Disease epidemiology has a strong influence on the conduct of meningococcal vaccine trials. Early vaccine trials were carried out in attempts to abort epidemics. In the era before chemoprophylaxis, vaccines of unproven efficacy were used out of desperation and efficacy was inferred by comparing disease incidences before and after the vaccination campaign [17], or by comparing the incidences in vaccinated and unvaccinated populations in an uncontrolled fashion [18]. By the time effective meningococcal vaccines were developed in the USA, the incidence of group A disease had declined dramatically [19], necessitating the use of *in vitro* markers of vaccine efficacy against this serogroup [20]. Surrogate markers of vaccine efficacy have continued to provide a pathway for vaccine development [201,202], with demonstrations of clinical efficacy deferred until after licensure and large-scale vaccination [21,22], if at all.

Clinical trials of meningococcal vaccines currently proceed along several fronts. Trials of experimental vaccines are conducted along the path to licensure for group B [23,24] and other serogroups that may be emerging [25]. The safety and efficacy of new vaccine preparations must be defined in anticipation of large-scale vaccination campaigns [26]. Trials of licensed vaccines are also required to determine how best to incorporate them into the vaccine schedule in order to protect infants [27] and the immunocompromised [28,29], and to confirm their efficacy and safety when administered in combination with other vaccines [30,31]. How these end points are measured is the subject of this review.

## Defining efficacy in meningococcal vaccine trials

The gold standard for determining efficacy for any vaccine is a reduction in the number of cases in vaccinated versus unvaccinated individuals in a controlled field trial [32]. It was feasible to collect such clinical efficacy data for a group C meningococcal vaccine in US Army recruits in the late 1960s, when the incidence of disease reached 0.5 per 100 per 4-week training period [33]. Inferences about the efficacy of a group A vaccine were initially based on *in vitro* tests [20], but efficacy was clinically demonstrated soon thereafter when controlled field trials were carried out in Sudan and Egypt, where the incidence of group A disease was significantly higher [34,35]. Licensure of the original group C and group A polysaccharide vaccines was therefore based directly on clinical efficacy [36]. The circumstances under which the efficacy of other meningococcal vaccines could be demonstrated have been more limited, and therefore licensure has been based on *in vitro* data, under the assumption that the same surrogate markers of protection that were used for groups A and C polysaccharide vaccines should apply [37–39].

## Serum bactericidal activity assays

The serum bactericidal activity (SBA) assay, in one form or another, has been the accepted surrogate marker for meningococcal vaccine efficacy since the mid-1970s [40,201]. A lack of SBA against circulating strains of meningococcus was associated with an increased risk of infection by Goldschneider *et al.* [33] based on two key types of data: ecological and predictive.

### ■ Ecological studies associate SBA with immune

**protection**

The first key observation of Goldschneider *et al.* was based on seroprevalence data. They determined the age-specific prevalence of SBA to groups A, B and C meningococci in children (newborn to 12 years of age) and from Army recruits (19–26 years of age). When graphed along with the age-specific incidence of meningococcal disease in the general population at the time, the two curves seemed to vary inversely, implying that the presence of SBA was associated with protection against meningococcal disease. This is the basis for the 'ecological argument' for SBA being a principle mechanism of immune protection. Similar studies performed in the UK showed an inverse relationship between the prevalence of SBA and the incidence of group C disease, although this was less pronounced [41]. For group B disease, an inverse relationship was even less evident [42] and for groups Y and W-135, an inverse relationship was not seen [43]. On its own, therefore, this argument is not compelling.

Additional ecological evidence for a protective role for SBA has also been sought based on clinical and serologic responses to vaccination. Following vaccination campaigns against groups B and C in various countries, age-dependent vaccine efficacy has been shown to match the age-specific efficacy predicted by measurement of SBA in clinical trials and these data have been used to establish the minimum SBA titer considered protective [44–46]. For the conjugate group C vaccine in the UK, SBA titers in infants were shown to decline significantly within a year of vaccination [47] and vaccine effectiveness in children initially also appeared to decrease after a year [22]. As the overall incidence of disease has declined in the UK, so too has the incidence of vaccine failures [48]. Herd immunity now appears to be obscuring the ecological relationship between seroprevalence and the incidence of disease.

## ■ SBA predicts immune protection

The second key observation made by Goldschneider *et al.* was that baseline SBA predicted immune protection from meningococcal disease during basic military training (Table 1) [33]. Among three training companies, five out of 13 individuals who lacked bactericidal activity and became colonized with the epidemic group C strain developed disease compared with 0 out 11 who became colonized but had bactericidal activity at baseline. Based on these numbers, it can therefore be said that the SBA lacks sensitivity in identifying individuals who are putatively immune (only 11/19, or 58% of putatively immune individuals were SBA positive), but is highly specific

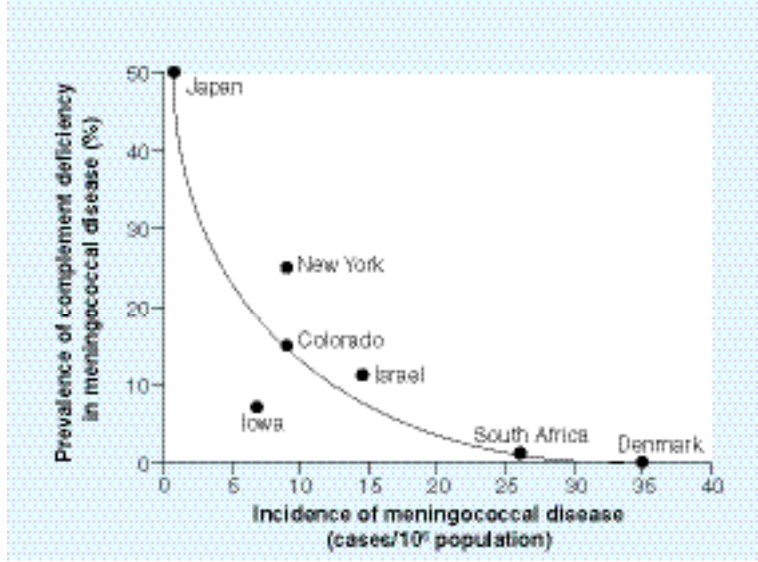


**Figure 1. Relationship between prevalence of complement deficiency and incidence of meningococcal disease.**
Reprinted with permission from [15].

(5/5, or 100%, of individuals who were infected were SBA negative). Considering the entire population of recruits to be at risk of disease without assessing colonization, three out of 54 cases had detectable SBA at baseline, giving a slightly lower specificity of 94% [33]. Although these numbers are small, this is the basis for the 'predictive argument' for SBA being a primary mechanism of immunity, by which is meant that SBA status can be used to predict clinical outcome for an exposed individual. The particular circumstances of very high incidence over a short duration in a defined community have not occurred since this study, and attempts to replicate these findings in other epidemiologic circumstances have yielded data that are much less robust due to significantly lower colonization rates and the small number of epidemic cases that occur in an era of effective chemoprophylaxis [49].

A predictive argument in favor of SBA as a primary mechanism of immune protection can also be made by the observation that individuals who lack SBA because of inherited or acquired defects of the terminal complement cascade are at a greatly increased

**Table 1. Clinical outcome of recruits colonized with the epidemic strain according to baseline serum bactericidal activity titer.**

| SBA titer | Putatively immune (colonized, not infected) | Susceptible (infected) |
|---|---|---|
| ≥1:4 | 11 | 0 |
| <1:4 | 8 | 5 |

SBA: Serum bactericidal activity.
Data taken from [33].

risk of meningococcal disease [13]. While this experiment of nature dramatizes the importance of SBA, the fact that individuals with terminal cascade defects do benefit from vaccination [50,51] also suggests that other vaccine-induced immune mechanisms can protect from meningococcal disease.

### Performance of the SBA assay

The SBA assay involves combining serial dilutions of test sera (potentially containing bactericidal antibodies) with meningococci in liquid media. To this, complement is added from a source that lacks intrinsic bactericidal activity against the strain being investigated. After a defined incubation time, the mixture is plated out, further incubated (typically overnight) and the resulting colonies counted. The lowest dilution of sera that decreases colony counts by at least 50% compared with a control culture incubated without serum defines the bactericidal titer [202]. Various methods are published that vary with respect to the number of colony-forming units of bacteria per well, buffers used, assay incubation times, sources of complement, complement concentrations and starting dilutions of serum [52]. A high-throughput partially automated version of the assay has been described [53].

There are several factors to consider when choosing what titer or increase in titer should be considered indicative of a vaccine response. When serial twofold dilutions of test sera are used in the assay, results can vary by a factor of two when repeated under the same conditions. Therefore, a fourfold increase in titer is considered the minimum criterion for establishing a response to vaccination [201] and some investigators increase this further to ensure this degree of protection falls within a margin of error [24]. The ecological and predictive arguments of Goldschneider *et al.* considered bactericidal activity to be present if their SBA assay gave a titer of 1:4 or greater [33]. A number of individuals possess protective SBA titers prior to vaccination; whether or not such individuals should be considered vaccine failures if they do not increase their titers further, may be an academic question, but one that can have implications for research investment and vaccination policy. When using the absolute postvaccination titer rather than the increase in titer as a marker of immune protection, a titer of 1:8 is often preferred, since it allows for some margin of error [54].

While Goldschneider *et al.* showed that a titer of 1:4 predicted immunity to meningococcal infection over the subsequent 8-week training period of the recruits in his study, a longer duration of protection is expected from routine vaccination. Individuals who mount a higher initial response to vaccination have been shown to retain protective titers longer [55]. While age at vaccination is an important factor in determining the durability of the immune response [44,56], asymptomatic colonization with meningococci also plays a role in boosting immunity [57]. As the incidence of asymptomatic colonization declines, the duration of SBA persistence and therefore vaccine efficacy may be less than what has been observed in the past. Higher postvaccination titers may become necessary to maintain long-term protection.

### Sources of complement for the SBA assay

Early studies measuring SBA froze blood soon after phlebotomy to preserve the complement activity in the sample being assayed (intrinsic human complement) [58]. However, because complement proteins are heat-labile, variations in collection techniques and sample handling can affect the resulting bactericidal titers [59]. The method used by Goldschneider *et al.* added sera from human donors that were prescreened to rule out intrinsic bactericidal activity and processed in a standardized fashion (human SBA [hSBA]) [33]. Prescreening the human sera used as a complement source is necessary because most adults have developed antibodies to various meningococcal antigens, if not by vaccination, then by prior colonization with *N. meningitidis* [60], *Neisseria lactamica* [61], or other bacteria expressing crossreacting antigens [62]. Such antibodies may, by themselves, or in combination with antibodies in the test sera, fix complement and result in bacterial lysis, increasing SBA titers and yielding false-positive results. Screening donor sera for bactericidal activity must be done for each strain of meningococcus to be tested and should be repeated with subsequent donations since the donor may become colonized and develop antimeningococcal antibodies at any time. Some strains may not be killed by sera from multiple donors when tested separately, but may be killed when they are pooled together. For some strains, a compatible human source of complement cannot be identified [63].

Using animal sources of complement, specifically baby rabbits, has become a standard practice since this is more amenable to standardization [40,201]. Serum from adult rabbits has also been used [64], though with less success. Even though rabbits are not natural hosts for meningococci, adult rabbit serum may have intrinsic bactericidal activity, perhaps due to crossreactivity with other bacterial species. In general, SBA titers generated using rabbit as a source of complement (rabit SBA [rSBA]) correlate with the hSBA, but tend to give significantly higher titers [65]. After the use of baby rabbit sera

had become standard practice for polysaccharide vaccines for groups A and C [201], it was discovered that antibodies induced to the group B polysaccharide could give a positive SBA reaction when using rabbit, but not human, complement and could significantly overestimate vaccine efficacy [66]. This is due in part to the fact that meningococci possess a factor H binding protein (fHbp) that downregulates complement activity on the outer membrane. Meningococcal fHbp is specific for human factor H and is not able to down-regulate rabbit complement activity, making the meningococci more susceptible to killing in the rSBA assay [67,68]. Studies of group B vaccines currently rely solely on the hSBA assay, but even for the other serogroups, a significant number of individuals, including unexposed and presumably susceptible infants, are consistently found who are negative by the hSBA but have detectable rSBA activity [65,69,70].

As the rSBA assay consistently gives higher titer results than the hSBA [46 ,202], one approach to improving its specificity has been to use a higher titer as a cutoff to indicate a vaccine response [67,65]. This approach still leads to categorizing a significant number of vaccinees as vaccine responders who do not have detectable hSBA [71], but the resulting efficacy rates predicted by this model have agreed with the ecological data on vaccine efficacy in the UK [48].

Due to the weight of predictive versus ecological evidence, the hSBA can be regarded as the 'gold standard' for defining immune protection in an individual vaccine recipient [71]. If regarded as merely a marker for hSBA against group C, the sensitivity and specificity of the rSBA are not particularly impressive [71]. In a recent analysis, results generated in parallel from matched patient sera for serogroups A, W-135 and Y showed the correlations between hSBA and rSBA to be even weaker than for group C [70]. However, in the ecological setting of a large vaccination campaign, the application of population-based data is more appropriate. Furthermore, the commercial availability of baby rabbit sera as a complement source makes standardization of the assay on a large scale feasible. In this context, the rSBA assay has become associated with considerable success and may be considered now to have a track record independent of the hSBA assay.

Candidate group B vaccines continue to be evaluated using hSBA [24] and conjugated polysaccharide vaccines for group C are tested with rSBA [203]. Clinical trials of a conjugate group A vaccine, in anticipation of large-scale administration in sub-Saharan Africa, are also measuring rSBA [26,72]. Recently, licensed tetravalent conjugated polysaccharide vaccines have been evaluated using either the hSBA exclusively [39] or the hSBA in children and the rSBA in adults [38,73].

## Opsonophagocytic activity assays

The number of recruits in the study by Goldschneider *et al.* with putative immunity to the epidemic strain who lacked SBA and the clinical benefit complement-deficient individuals derive from meningococcal vaccination, suggest that additional mechanisms of immune protection exist. A more sensitive test or combination of tests to determine vaccine efficacy must be theoretically possible.

The opsonophagocytic activity (OPA) assay is analogous to the SBA assay but quantifies the ability of activated neutrophils to engulf and destroy bacteria that have been opsonized [74]. In this assay, meningococci are incubated with sera along with a prescreened complement source and neutrophils purified from the test patient or another donor. Following incubation, the degree of opsonaphagocytosis can be determined microscopically or with the use of flow cytometry [75]. The use of a complement source that has been depleted of $C_6$ and is therefore incapable of generating a bactericidal response, has been used to measure OPA that is independent of SBA [74].

OPA assays measure both antibody- and complement-mediated phagocytosis. They require a standardized source of complement (lacking intrinsic opsonophagocytic or bactericidal antibody activity), as well as a standardized source of human neutrophils. Neither condition is easily met. While this assay allows assessment of this pathway of protection, it is neither simpler nor any more readily standardized than the SBA assay and has not been validated with either ecological or predictive data.

## Whole-blood activity assays

The whole-blood activity (WBA) assay is analogous to the SBA assay but uses Whole-blood instead of serum. The assay is performed by inoculating strains of meningoocci into Whole-blood and incubating them together. Early versions of this assay used blood 'as it comes from the vessel' [76]. More recent investigators have used fresh blood anticoagulated with lepirudin, which, unlike other anticoagulants, does not cause intrinsic activation of complement. [77]. The decrease in the concentration of colony-forming units from the inoculated blood after incubation is compared with that from before and the proportion killed is estimated [78]. This assay determines total antimeningococcal activity due to bactericidal activity, opsonophagocytosis and cytokine production, and serial samples of the culture can be further

analyzed to estimate the relative contributions of these different mechanisms [79]. One comparative study showed significantly more adults possessed WBA against a strain of group B meningococcus than SBA [80]. In studies of children convalescing from meningococcal disease [81] and in vaccine clinical trials [82], the WBA assay shows a greater proportion of individuals increase their ability to kill meningococci than the SBA assay.

A version of the WBA assay, the passive protection assay, uses Whole-blood from a donor to assess immune protection in previously collected sera. This assay also shows a greater increase in the ability to inhibit meningococcal growth than the SBA or OPA alone [77]. Neither the WBA nor the passive protection assay is simpler than the SBA nor are they readily standardized.

### Immunoassays for antibody measurement

Goldschneider *et al.* chose the SBA assay because it was a sensitive means of detecting specific antibodies, not because bactericidal activity was considered the only means by which antibodies confer protection [33]. A number of field trials conducted soon thereafter, including some by the same investigators, used other assays to measure the antibody response to vaccination [83]. These included the radioactive precipitin method of Gotschlich [83], the radioactive antigen binding test of Farr [83] and latex agglutination [84]. More recent clinical trials have used ELISA [26] and bead-based assays are also described [85]. Depending on the vaccine construct, antibody levels may be measured to whole bacteria [33], capsular polysaccharide [26], outer membrane proteins [86], OMV [87] or individual subcapsular antigens [88].

Levels of anticapsular antibody have at times correlated well with the hSBA and a number of early studies used them as the primary end point of efficacy [89,90]. However, they have not always correlated with protection in field trials [91]. When levels of antibody to polysaccharide and hSBA do not correlate, this is presumably due to the presence of low avidity or subclass-restricted antibodies that are detectable by ELISA, but are not efficient at fixing complement [92]. Purified polysaccharide vaccines, as T-independent antigens, particularly when used in infants, may not produce enough of an increase in antibody avidity to provide acceptable correlation with the hSBA and may explain why such trials have shown poor correlation between antibody levels and clinical outcome [91].

For conjugated vaccines, measurements of anticapsular IgG after a single dose do not correlate well with SBA but after the full series and a booster, the correlation improves, reflecting an increase in antibody avidity [93]. The addition of a 'chaotropic' agent, such as ammonium thiocyanate, which inhibits antigen–antibody interactions in a dose-dependent fashion, has been used to inhibit binding of low-avidity antibodies to antigen. This results in a better correlation between ELISA and SBA assays [94]. Low-avidity antibodies may also affect the correlation between antibody to OMV and hSBA. Figure 2 shows the correlation between IgG to OMV measured by ELISA and hSBA in volunteers from a clinical trial of a group B native OMV vaccine [87]. Prevaccination antibodies, possibly induced by colonization with crossreacting organisms and of low avidity for OMV from the vaccine strain, correlate poorly with the hSBA (Figure 2A). After vaccination, the correlation improves visibly (Figure 2B). This degree of correlation with SBA is comparable to what has been reported for antibodies to polysaccharide induced by polysaccharide vaccines [95].

Apart from its limited role as a marker for functional antibody activity, antibody measurement allows levels of antibody to specific antigens to be quantified, which allows specific vaccine characteristics to be compared without the need to replicate SBA assays.

### Selection of meningococcal strains for efficacy testing

Strain selection for immunogenicity assays of vaccines based on capsular polysaccharides is relatively straightforward. Group C capsular vaccines that protect from one group C organism should protect against others expressing the same capsular polysaccharide. However, differences in immunogenicity do exist and have been shown to depend in part on the acetylation status of the polysaccharide, with *O*-acetylated strains being more immunogenic [96,97]. Other features of capsule structure that may vary with culture conditions are also important [52], as is the strain lipooligosaccharide type, which can affect the degree of natural immunity induced by crossreacting bacteria [98,99]. Generally, the use of reference strains from which the vaccine polysaccharide is derived is preferred, as this allows standardization of the assay and comparisons between vaccines and between laboratories [71].

Evaluating vaccine efficacy directly against all pathogenic group B strains is not possible [204]. Subcapsular antigens, and therefore susceptibility to bactericidal activity, vary naturally in both sequence and expression levels between strains. In addition, expression levels can vary significantly as a result of slight differences in how the organisms are cultured or in how the reaction is mixed [100,101]. Geographically

distinct strains that appear phenotypically similar can give very different results with the same sera in SBA assays [102]. Instead of pursuing a large and constantly changing panel of pathogenic isolates, immunogenicity testing for subcapsular vaccines is directed toward characterizing the performance of individual vaccine antigens. Correlations can then be drawn between antigen reactivity (a function of both expression levels and degree of homology with the vaccine antigen) and susceptibility to postvaccination SBA. Disease isolates can then be characterized with respect to their expression of these vaccine antigens and susceptibility can be predicted accordingly [204]. One such method is the Meningococcal Antigen Typing System, which was developed for the Novartis group B vaccine [103]. Transferring this method to national reference laboratories in advance of large-scale vaccination will allow predicted strain coverage to be monitored in real-time [205]. For this approach, threshold levels of antigen expression that predict bactericidal killing have to be recalculated for every new vaccine construct. This method is yet to be validated clinically.

## Measuring vaccine response in complement-deficient individuals

Many of the complement deficiencies associated with susceptibility to Neisseria infections do not have other apparent immune deficits and may not be diagnosed until they, or a family member, develop meningococcal infection. Meningococcal infections associated with complement deficiencies are more likely to occur in adolescence or young adulthood [104], so these cases could theoretically be prevented by routine childhood meningococcal vaccination if such vaccines induced an appropriate protective response.

Deficiencies of factors proximal to $C_3$ (mannose binding lectin, $C_2$, $C_4$, factor D, factor B) compromise specific recognition pathways. Recurrent disease is unusual with these deficiencies, implying effective immunity can develop and one recognition pathway can compensate for a defect in another [104]. Deficiencies of properdin, which is required for the alternative pathway lytic cascade, as well as amplification of the classic pathway bactericidal response, is occasionally associated with recurrent disease [104]. Deficiencies of components of the late pathway, from $C_3$ (including factor H and factor I) through the formation of the membrane attack complex ($C_5$–$C_9$), are disorders of complement-mediated killing. These individuals with terminal complement component deficiencies (TCCD), even with intact meningococcal recognition, are at risk of recurrent disease and vaccine failure, demonstrating the importance of bactericidal activity in defense from Neisseria.

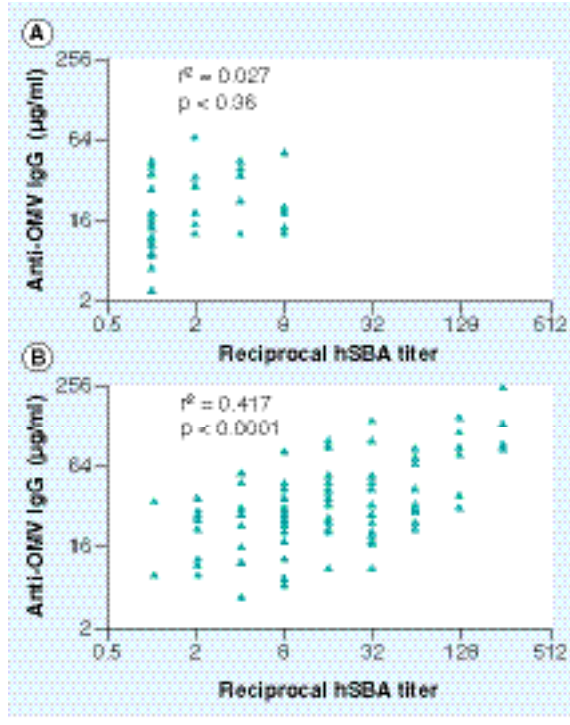


**Figure 2. Serum IgG as determined by ELISA to outer membrane vesicles from the vaccine strain versus reciprocal human serum bactericidal activity titers to the same strain. Both axes are shown on a logarithmic scale. (A)** Prevaccination, where antibodies presumably have low avidity, there is no correlation (n = 34). **(B)** Postvaccination with a native OMV vaccine, the correlation improves significantly. Serum was drawn 2 weeks after each of three vaccinations (n = 101) of the same 34 volunteers (one patient withdrew prior to the last sample being drawn).
hSBA: Human serum bactericidal activity; OMV: Outer membrane vesicle.
Details of this vaccine and clinical trial are provided in [87].

The fact that complement-deficient individuals derive clinical benefit from meningococcal vaccination is one reason to suspect that immune mechanisms other than bactericidal activity can be protective. One open trial compared clinical outcomes in 31 vaccinated TCCD patients with 14 unvaccinated TCCD patients. Three episodes of meningococcal disease with serogroups included in the vaccine developed in each group. Survival analysis showed a significant benefit from vaccination [51], although the incidence of disease in vaccinated TCCD patients is still significantly higher than that of vaccinated individuals without TCCD [105]. Measuring OPA and antibody levels in this population shows significant

vaccine responses, but also greater variability and more rapid loss of OPA and anticapsular antibody levels than nondeficient controls [50]. It has been suggested that measuring anticapsular antibody in postvaccination sera is an effective way to screen for immune protection in this population, with some suggesting 1–2 µg/ml [106] and others 5 µg/ml as the lower limit of protection [51]. The assumption is that anticapsular antibodies are more reliably opsonophagocytic than they are bactericidal, and that a sufficiently high level should correlate with protection via the opsonophagocytic pathway. However, when compared side-by-side, OPA assays and antibody levels do not correlate well [50,105]. Neither measurement has robust ecological or predictive data as a marker for immune protection in TCCD.

Partial complement component deficiencies [107] or complete deficiencies of some complement components [108] may retain some residual hemolytic function. For such individuals, a WBA with intrinsic complement may be a more relevant *in vitro* measure of efficacy. Indeed, one early investigator using a WBA discovered that his own blood consistently lacked the ability to inhibit growth of meningococci by this technique, and he later succumbed to meningococcal disease [76], providing evidence that this assay can detect a clinically significant lack of protection. Neither WBA nor OPA assays are widely available, however, and ELISA as the sole measure of vaccine response in complement-deficient individuals remains unproven.

This group of individuals would benefit from a vaccine that induces protection from all serogroups of meningococci to which they are susceptible. Ideally, such a vaccine would have a predictable, if not indefinite, duration of protection. In the short term, pursuing such unique immunogenicity criteria may seem like an 'orphan vaccine' project, but one outer membrane protein known to specifically elicit opsonophagocytic killing has already been identified as a vaccine component [54,109]. A vaccine that adequately protects individuals with TCCD could plausibly be incorporated into vaccines administered routinely.

Current guidelines from the US Centers for Disease Control and Prevention recommend that individuals with complement deficiencies receive a two-dose primary series of meningococcal conjugate vaccine followed by boosting every 5 years [110]. Whether or not this will be adequate for this population remains to be seen [111]. Since many complement deficiencies are also associated with infection by serogroups not represented in currently licensed vaccines [112], additional strategies, such as antibiotic prophylaxis or self-treatment of prodromal symptoms, seem prudent.

## Future perspective

*N. meningitidis* has been a burden on mankind for over 200 years [3] and vaccines have been pursued for over a century [113]. The use of effective vaccines, in combination with other factors, has decreased the incidence of meningococcal disease in many areas. As the incidence of meningococcal disease declines, the resources allocated to reduce the incidence further will likely decline as well. Meningococcal vaccines already represent a considerable proportion of the total cost of routine immunization in the USA [114], and adding a vaccine for group B or introducing a multidose regimen into the infant vaccine schedule would increase that proportion even further. As the epidemiology of meningococcal infection changes, so do the relevant questions and our resources for answering them. Determining vaccine efficacy in clinical trials is an ongoing challenge.

The relatively low incidence of meningococcal disease in most countries requires surrogate markers to be used in clinical trials as measures of vaccine efficacy. The ideal surrogate would identify all individuals who are immune from meningococcal disease, be amenable to standardization, and be technically and financially feasible to use on a large scale. Both ecological and predictive evidence supports use of SBA assays. These appear to underestimate protection and require complement sources to be prescreened. The hSBA is considered the gold standard for defining immune protection in an individual; considerable ecological evidence has associated the rSBA with successful large-scale vaccination campaigns for group C in the UK and now group A in sub-Saharan Africa. Measurement of antibodies via ELISA performs well as a marker for SBA in some limited circumstances and allows the immunogenicity of specific vaccine antigens to be compared without replicating the SBA assay. WBA using intrinsic complement may be more sensitive than SBA assays by measuring all mechanisms of immune protection, and may be useful in defining immunity in complement-deficient individuals. More widespread use of this assay in ecological studies and clinical trials would allow its performance characteristics to be better understood and perhaps set the stage for automation and standardization on a larger scale. Such assays would not only further the development of an orphan vaccine, capable of providing long-lasting protection against the broad range of serogroups that infect individuals with complement deficiencies, but may also improve on the sensitivity with which we can define any individual as immune from meningococcal disease.

With widespread vaccination of the meningitis belt against group A [9] and vaccines for group B approaching licensure [24], future prospects for control of meningococcal disease look bright. Though talk of global eradication of meningococcal disease may be premature, progress is being made on the fronts we can control, and trends are encouraging on those we cannot. As the end-game approaches, there is potential for the rules to change regarding the populations at greatest risk of disease and the immune mechanisms that are available to protect them, as well as the duration of protection that can be expected in the

## Executive summary

**The impact of disease epidemiology on the conduct of clinical vaccine trials**

- The current incidence of meningococcal disease in the USA is at an historic low. The incidence in many other areas is also decreasing as a result of vaccination.
- The low incidence of meningococcal disease requires us to rely on surrogate markers of vaccine efficacy.
- As meningococcal disease incidence declines, the proportion of cases occurring among individuals with complement deficiencies may increase. Such individuals are incapable of developing serum bactericidal activity (SBA).

**Defining efficacy in meningococcal vaccine trials**

- Polysaccharide vaccines for groups A and C were demonstrated to be effective using clinical end points in field trials in areas where the incidence of disease made this possible.
- Tetravalent vaccines covering groups Y and W-135, and conjugated polysaccharide vaccines, have been licensed based on surrogate markers of efficacy.

**SBA assays**

- The evidence for SBA being the primary means of immune protection derives from ecological and predictive data.
- As originally reported, the sensitivity of the SBA as a marker of meningococcal immunity was only 58%.
- Other immune mechanisms likely play important roles.

**Performance of the SBA assay**

- The titer of SBA considered protective may depend on whether immediate or long-term protection is required.
- The duration of vaccine-induced protection may decrease with a decline in asymptomatic colonization.

**Sources of complement for the SBA assay**

- The SBA can be performed using prescreened human sera as a source of complement (hSBA), or sera from baby rabbits (rSBA).
- Based on predictive data, the hSBA may be considered the gold standard for defining immune protection in an individual.
- The rSBA has been associated with successful vaccination campaigns against groups C and A.

**Opsonophagocytic activity assays**

- An assay measuring opsonophagocytosis by neutrophils is described.

**Whole-blood activity assays**

- An assay using whole-blood accounts for bactericidal, opsonophagocytic and cytokine activity against meningococci.

**Immunoassays for antibody measurement**

- Antibody levels do not correlate with clinical outcomes in polysaccharide vaccination of infants.
- In cases where high-avidity antibodies are induced, antibody levels may correlate with SBA.

**Selection of meningococcal strains for efficacy testing**

- Strain differences in capsular structure may lead to differences in immunogenicity.
- For subcapsular vaccines, strains used in efficacy testing are chosen to demonstrate specific features of immune protection.
- A meningococcal antigen typing system has been developed to predict strain coverage by a group B vaccine based on multiple subcapsular antigens without requiring postvaccination serum.

**Measuring vaccine response in complement-deficient individuals**

- Antibody levels and opsonophagocytic assays have both been used as measures of vaccine response in complement-deficient individuals.
- Whole-blood assays using intrinsic complement may be the most relevant measures of efficacy in this population.
- Current US CDC guidelines for meningococcal vaccination in complement-deficient individuals are for a two-dose primary series followed by boosting every 5 years.

**Future perspective**

- The declining incidence of disease may result in fewer resources being allocated to meningococcal vaccine research, a greater proportion of cases occurring in complement-deficient individuals and a shorter duration of protective titers as a result of decreased natural boosting.

absence of natural boosting from asymptomatic colonization. With diminishing resources allocated to the prevention of meningococcal disease in areas of low incidence, significant challenges remain.

## Acknowledgements

*The authors would like to acknowledge the assistance of E Moran, formerly of the Walter Reed Army Institute of Research, who performed the assays presented in* Figure 2*, and W Zollinger, formerly of the Walter Reed Army Institute of Research, who critically reviewed an early draft of this manuscript.*

## Disclaimer

*The findings and opinions expressed herein belong to the authors and do not necessarily reflect the official views of the Walter Reed Army Institute of Research, the Walter Reed National Military Medical Center, the US Army, Navy, Air Force or Department of Defense.*

## Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

*No writing assistance was utilized in the production of this manuscript.*

## References

Papers of special note have been highlighted as:

- ■ of interest
- ■■ of considerable interest

1 Harrison LH, Trotter CL, Ramsay ME. Global epidemiology of meningococcal disease. *Vaccine* 27(Suppl. 2), B51–B63 (2009).

2 Kriz P, Wieffer H, Holl K, Rosenlund M, Budhia S, Vyse A. Changing epidemiology of meningococcal disease in Europe from the mid-20th to the early 21st century. *Expert Rev. Vaccines* 10(10), 1477–1486 (2011).

3 Vieusseux G. Mémoire sur la maladie qui a régné à Genève au printemps de 1805. *J. de Méd. Chir. Pharm.* 11, 163–182 (1805).

■ **This is widely cited as the first report of an epidemic of meningococcal disease.**

4 Broussais C. *Histoire des méningites cérébro-spinales qui ont régné épidémiquement dans différentes garnisons de France, depuis 1837 jusqu'en 1842; d'aprè s les documents recueillis par le conseil de santé des armées* . von Schjerning O (Ed.). Moquet et Hauquelin, Paris, France, 1–10 (1845).

5 Transactions of the Medical Society of the King and Queen's College of Physicians. *Dublin QJM Sci.* 44(87), 172–227. (1867).

6 Brown JW, Condit PK. Meningococcal infections: Fort Ord and California. *Calif. Med.* 102, 171–180 (1965).

7 Saleun G, Ceccaldi J. Etude des méningocoques en Afrique Equatoriale Française et vaccination antiméningococcique. *Bull. Soc. Pathol. Exot. Filiales.* 29, 996–1006 (1936).

8 Gasparini R, Panatto D. Meningococcal glycoconjugate vaccines. *Hum. Vaccin.* 7(2), 170–182 (2011).

9 Frasch C, Preziosi MP, Laforce FM. Development of a group A meningococcal conjugate vaccine, MenAfriVac™. *Hum. Vaccin. Immunother.* 8(6) (2012) (In Press).

10 Harrison LH. Epidemiological profile of meningococcal disease in the United States. *Clin. Infect. Dis.* 50(Suppl. 2), S37–S44 (2010).

11 Cohn AC , MacNeil JR , Harrison LH *et al.* Changes in *Neisseria meningitides* disease epidemiology in the United States, 1998–2007: implications for prevention of meningococcal disease. *Clin. Infect. Dis.* 50, 184–191 (2010).

■ **Shows the incidence of meningococcal disease in the USA decreased to historic lows prior to implementation of routine vaccination.**

12 Kallel-Sellami M, Abdelmalek R, Zerzeri Y *et al.* Deficits hereditaires en proteines du complement au cours des meningites purulentes: etude de 61 patients adultes tunisiens et revue de la litterature. *Arch. Inst. Pasteur. Tunis.* 83(1–4), 25–34 (2006).

13 D'Amelio R, Agostoni A, Biselli R *et al.* Complement deficiency and antibody profile in survivors of meningococcal meningitis due to common serogroups in Italy. *Scand. J. Immunol.* 35(5), 589–595 (1992).

14 Ross SC, Densen P. Complement deficiency states and infection: epidemiology, pathogenesis and consequences of neisserial and other infections in an immune deficiency. *Medicine* 63(5), 243–273 (1984).

15 Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies. *Clin. Microbiol. Rev.* 4(3), 359–395 (1991).

■ **This review article on complement deficiencies suggests that the proportion of sporadic meningococcal disease occurring in complement-deficient indviduals should increase as the overall incidence decreases.**

16 Centers for Disease Control and Prevention (CDC). Summary of notifiable diseases: United States, 2009. *MMWR Morb. Mortal. Wkly. Rep.* 58(53), 1–100 (2011).

17 Pergher G, Portois F. Note sur l'épidémiologie et la prophylaxie de la méningite cérébro-spinale au Ruanda-Urundi. *Ann. Soc. Belg. Med. Trop.* 16, 343–366 (1936).

18 Bruynoghe R, Walravens P. La vaccination antimeningococcique. *Comptes Rendus des Seances de la Societe de Biologie et ses Filiales.* 95, 872–874 (1926).

19 National Communicable Disease Center. Current trends: meningococcal infections – United States. *Morb. Mortal. Wkly Rep.* 18(16), 135–140 (1969).

20 Gotschlich EC, Goldschneider I, Artenstein MS. Human immunity to the meningococcus. IV. Immunogenicity of group A and group C meningococcal polysaccharides in human volunteers. *J. Exp. Med.* 129(6), 1367–1384 (1969).

21 de Moraes JC, Perkins BA, Camargo MC *et al.* Protective efficacy of a serogroup B meningococcal vaccine in Sao Paulo, Brazil. *Lancet* 340(8827), 1074–1078 (1992).

22 Trotter CL, Andrews NJ, Kaczmarski EB, Miller E, Ramsay ME. Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction. *Lancet* 364(9431), 365–367 (2004).

23 Santolaya ME, O'Ryan ML, Valenzuela MT *et al.* Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a Phase 2b/3 randomised, observer-blind, placebo-controlled study. *Lancet* 379(9816), 617–624 (2012).

24 Gossger N, Snape MD, Yu LM *et al.* Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. *JAMA* 307(6), 573–582 (2012).

25 Delrieu I, Yaro S, Tamekloé TA *et al.* Emergence of epidemic *Neisseria meningitidis* serogroup X meningitis in Togo and Burkina Faso. *PLoS One* 6(5), e19513 (2011).

26 Sow SO, Okoko BJ, Diallo A *et al.*

Immunogenicity and safety of a meningococcal A conjugate vaccine in Africans. *N. Engl. J. Med.* 364(24), 2293–2304 (2011).

27 Bryant KA, Marshall GS, Marchant CD *et al.* Immunogenicity and safety of *H. influenzae* type b-*N. meningitidis* C/Y conjugate vaccine in infants. *Pediatrics* 127(6), e1375–e13785 (2011).

28 Spoulou V, Tzanakaki G, Lekka S, Chouliaras G, Ladis V, Theodoridou M. Natural and vaccine-induced immunity to *Neisseria meningitidis* serogroup C in asplenic patients with β-thalassemia. *Vaccine* 29(27), 4435–4438 (2011).

29 Siberry GK, Williams PL, Lujan-Zilbermann J *et al.* Phase I/II, open-label trial of safety and immunogenicity of meningococcal (groups A, C, Y and W-135) polysaccharide diphtheria toxoid conjugate vaccine in human immunodeficiency virus-infected adolescents. *Pediatr. Infect. Dis. J.* 29(5), 391–396 (2010).

30 Kitchin NR, Southern J, Morris R *et al.* Evaluation of a diphtheria-tetanus-acellular pertussis-inactivated poliovirus-*Haemophilus influenzae* type B vaccine given concurrently with meningococcal group C conjugate vaccine at 2, 3 and 4 months of age. *Arch. Dis. Child.* 92(1), 11–16 (2007).

31 Reyes MR, Dimaano E, Macalalad N *et al.* The investigational meningococcal serogroups A, C, W-135, Y tetanus toxoid conjugate vaccine (ACWY-TT) and the seasonal influenza virus vaccine are immunogenic and well-tolerated when co-administered in adults. *Hum. Vaccin. Immunother.* 8(7) (2012) (In Press).

32 Baylor NW, Midthun K. Regulation and testing of vaccines. In: *Vaccines* (5th Edition). Plotkin SA, Orenstein WA, Offit PA (Eds). Saunders Elsevier, London, UK, 1611–1627 (2008).

33 Goldschneider, I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J. Exp. Med.* 129, 1307–1326 (1969).

■■ **Classic paper presenting the two lines of evidence (ecological and predictive) supporting serum bactericidal activity as a marker for immune protection.**

34 Erwa HH, Haseeb MA, Idris AA, Lapeyssonnie L, Sanborn WR, Sippel JE. A serogroup A meningococcal polysaccharide vaccine: studies in the Sudan to combat cerebrospinal meningitis caused by *Neisseria meningitidis* group A. *Bull. World Health Organ.* 49(3), 301–305 (1973).

35 Wahdan MH, Rizk F, el-Akkad AM *et al.* A controlled field trial of a serogroup A meningococcal polysaccharide vaccine. *Bull. World Health Organ.* 48(6), 667–673 (1973).

36 Centers for Disease Control. Recommendation of the Public Health Service Advisory Committee on Immunization Practices: meningococcal polysaccharide vaccines. *Morb. Mortal. Wkly Rep.* 24(45), 381–382 (1975).

37 Miller E, Salisbury D, Ramsay M. Planning, registration, and implementation of an immunisation campaign against meningococcal serogroup C disease in the UK: a success story. *Vaccine* 20(Suppl. 1), S58–S67 (2001).

38 Menomune®, package insert. Sanofi Pasteur, PA, USA.

39 MENVEO®, package insert. Novartis Vaccines and Diagnostics, Sovicille, Italy.

40 Wong KH, Barrera O, Sutton A *et al.* Standardization and control of meningococcal vaccines, group A and group C polysaccharides. *J. Biol. Stand.* 5(3), 197–215 (1977).

41 Trotter C, Borrow R, Andrews N, Miller E. Seroprevalence of meningococcal serogroup C bactericidal antibody in England and Wales in the pre-vaccination era. *Vaccine* 21(11–12), 1094–1098 (2003).

42 Trotter C, Findlow J, Balmer P *et al.* Seroprevalence of bactericidal and anti-outer membrane vesicle antibodies to *Neisseria meningitidis* group B in England. *Clin. Vaccine Immunol.* 14(7), 863–868 (2007).

43 Trotter CL, Findlow H, Borrow R. Seroprevalence of serum bactericidal antibodies against group W135 and Y meningococci in England in 2009. *Clin. Vaccine Immunol.* 19(2), 219–222 (2012).

44 Boslego J, Garcia J, Cruz C *et al.* Efficacy, safety, and immunogenicity of a meningococcal group B (15:P1.3) outer membrane protein vaccine in Iquique, Chile. Chilean National Committee for Meningococcal Disease. *Vaccine* 13, 821–829 (1995).

45 Holst J, Feiring B, Fuglesang JE *et al.* Serum bactericidal activity correlates with the vaccine efficacy of outer membrane vesicle vaccines against *Neisseria meningitidis* serogroup B disease. *Vaccine* 21, 734–737 (2003).

46 Andrews N, Borrow R, Miller E. Validation of serological correlate of protection for meningococcal C conjugate vaccine by using efficacy estimates from postlicensure surveillance in England. *Clin. Diagn. Lab. Immunol.* 10, 780–786 (2003).

47 Borrow R, Balmer P, Miller E. Meningococcal surrogates of protection – serum bactericidal antibody activity. *Vaccine* 23(17–18), 2222–2227 (2005).

48 Campbell H, Andrews N, Borrow R, Trotter C, Miller E. Updated postlicensure surveillance of the meningococcal C conjugate vaccine in England and Wales: effectiveness, validation of serological correlates of protection, and modeling predictions of the duration of herd immunity. *Clin. Vaccine Immunol.* 17(5), 840–847 (2010).

49 Jones GR, Williams JN, Christodoulides M, Jolley K, Heckels JE. Lack of immunity in university students before an outbreak of serogroup C meningococcal infection. *J. Infect. Dis.* 181, 1172–1175 (2000).

50 Schlesinger M, Kayhty H, Levy R, Bibi C, Meydan N, Levy J. Phagocytic killing and antibody response during the first year after tetravalent meningococcal vaccine in complement-deficient and in normal individuals. *J. Clin. Immunol.* 20(1), 46–53 (2000).

51 Platonov AE, Vershinina IV, Kuijper EJ, Borrow R, Käyhty H. Long term effects of vaccination of patients deficient in a late complement component with a tetravalent meningococcal polysaccharide vaccine. *Vaccine* 21(27–30), 4437–4447 (2003).

52 Maslanka SE, Gheesling LL, Libutti DE *et al.* Standardization and a multilaboratory comparison of *Neisseria meningitidis* serogroup A and C serum bactericidal assays. *Clin. Diagn. Lab. Immunol.* 4(2), 156–167 (1997).

53 Mak PA, Santos GF, Masterman KA *et al.* Development of an automated, high-throughput bactericidal assay that measures cellular respiration as a survival readout for *Neisseria meningitidis*. *Clin. Vaccine Immunol.* 18(8), 1252–1260 (2011).

54 Granoff DM. Relative importance of complement-mediated bactericidal and opsonic activity for protection against meningococcal disease. *Vaccine* 27(Suppl. 2), B117–B125. (2009).

55 Jackson C, Lennon D, Wong S *et al.* Antibody persistence following MeNZB vaccination of adults and children and response to a fourth dose in toddlers. *Arch. Dis. Child.* 96(8), 744–751 (2011).

56 Perrett KP, Winter AP, Kibwana E *et al.* Antibody persistence after serogroup C meningococcal conjugate immunization of United Kingdom primary-school children in 1999–2000 and response to a booster: a Phase 4 clinical trial. *Clin. Infect. Dis.* 50(12), 1601–1610 (2010).

57 Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. II. Development of natural immunity. *J. Exp. Med.* 129(6), 1327–1348 (1969).

58 Frasch CE, Borrow R, Donnelly J. Bactericidal antibody is the immunologic surrogate of protection against meningococcal disease. *Vaccine* 27(Suppl. 2), B112–B116 (2009).

59 Borrow R and Carlone GM. Serogroup B and C bactericidal assays. In: *Meningococcal vaccines: Methods and Protocols.* Pollard A, Maiden M (Eds). Humana Press, Totowa, NJ, USA, 289–304 (2001).

60 Jäkel A, Plested JS, Hoe JC *et al.* Naturally-occurring human serum antibodies to inner core lipopolysaccharide epitopes of *Neisseria meningitidis* protect against invasive

meningococcal disease caused by isolates displaying homologous inner core structures. *Vaccine* 26(51), 6655–6663 (2008).

61 Snyder LA, Saunders NJ. The majority of genes in the pathogenic Neisseria species are present in non-pathogenic *Neisseria lactamica*, including those designated as 'virulence genes'. *BMC Genomics* 7, 128–139 (2006).

62 Sánchez S, Troncoso G, Ferreirós CM, Criado MT. Evaluation of cross-reactive antigens as determinants of cross-bactericidal activity in pathogenic and commensal Neisseria. *Vaccine* 19(25–26), 3390–3398 (2001).

63 Vu DM, Wong TT, Granoff DM. Cooperative serum bactericidal activity between human antibodies to meningococcal factor H binding protein and Neisserial heparin binding antigen. *Vaccine* 29(10), 1968–1973 (2011).

64 Weibel RE, Villarejos VM, Vella PP, Woodhour AF, Mclean AA, Hilleman MR. Clinical and laboratory investigations of monovalent and combined meningococcal polysaccharide vaccine, groups A and C. *Proc. Soc. Exp. Biol. Med.* 153(3), 436–440 (1976).

65 Santos GF, Deck RR, Donnelly J, Blackwelder W, Granoff DM. Importance of complement source in measuring meningococcal bactericidal titers. *Clin. Diagn. Lab. Immunol.* 8(3), 616–623 (2001).

66 Zollinger WD, Mandrell RE. Importance of complement source in bactericidal activity of human antibody and murine monoclonal antibody to meningococcal group B polysaccharide. *Infect. Immun.* 40(1), 257–264 (1983).

67 Welsh JA, Ram S. Factor H and Neisserial pathogenesis. *Vaccine* 26(Suppl. 8), I40–I45 (2008).

68 Granoff DM, Welsch JA, Ram S. Binding of complement factor H (fH) to *Neisseria meningitidis* is specific for human fH and inhibits complement activation by rat and rabbit sera. *Infect. Immun.* 77(2), 764–769 (2009).

69 Vesikari T, Karvonen A, Bianco V, Van der Wielen M, Miller J. Tetravalent meningococcal serogroups A, C, W-135 and Y conjugate vaccine is well tolerated and immunogenic when co-administered with measles-mumps-rubella-varicella vaccine during the second year of life: an open, randomized controlled trial. *Vaccine* 29(25), 4274–4284 (2011).

70 Gill CJ, Ram S, Welsch JA, Detora L, Anemona A. Correlation between serum bactericidal activity against *Neisseria meningitidis* serogroups A, C, W-135 and Y measured using human versus rabbit serum as the complement source. *Vaccine* 30(1), 29–34 (2011).

71 Borrow R, Andrews N, Goldblatt D, Miller E. Serological basis for use of meningococcal serogroup C conjugate vaccines in the United Kingdom: reevaluation of correlates of protection. *Infect. Immun.* 69(3), 1568–1573 (2001).

72 Kshirsagar N, Mur N, Thatte U *et al.* Safety, immunogenicity, and antibody persistence of a new meningococcal group A conjugate vaccine in healthy Indian adults. *Vaccine* 25(Suppl. 1), A101–A107 (2007).

73 284 Menactra®, package insert. Sanofi Pasteur, PA, USA.

74 Plested JS, Granoff DM. Vaccine-induced opsonophagocytic immunity to *Neisseria meningitidis* group B. *Clin. Vaccine Immunol.* 15, 799–804 (2008).

75 Michaelsen TE, Aase A. Antibody-Induced Opsonophagocytosis of Serogroup B Meningococci Measured by Flow Cytometry. In: *Meningococcal vaccines: Methods and Protocols*. Pollard AJ, Maiden MCJ (Eds). Humana Press, Totowa, NJ, USA, 331–337 (2001).

76 Heist GD, Solis-Cohen S, Solis-Cohen M. A study of the virulence of meningococci for man and of human susceptibility to meningococcic infection. *J. Immunol.* 7(1), 1–33 (1922).

■ **Early description of a whole-blood assay to identify protection from meningococcal disease, which concludes by describing the fatal meningococcal illness of the first author, whose blood consistently lacked antimeningococcal activity.**

77 Plested JS, Welsch JA, Granoff DM. *Ex vivo* model of meningococcal bacteremia using human blood for measuring vaccine-induced serum passive protective activity. *Clin. Vaccine Immunol.* 16(6), 785–791 (2009).

78 Ison CA, Heyderman RS, Klein NJ, Peakman M, Levin M. Whole-blood model of meningococcal bacteraemia – a method for exploring host-bacterial interactions. *Microb. Pathog.* 18, 97–107 (1995).

79 Ison CA. Whole-blood Model. In: *Meningococcal vaccines: Methods and Protocols*. Pollard AJ, Maiden MCJ (Eds). Humana Press, Totowa, NJ, USA, 317–329 (2001).

80 Welsch JA, Granoff D. Immunity to *Neisseria meningitidis* group B in adults despite lack of serum bactericidal antibody. *Clin. Vaccine Immunol.* 14(12), 1596–1602 (2007).

81 Ison C A, Anwar N, Cole MJ *et al.* Assessment of immune response to meningococcal disease: comparison of a whole-blood assay and the serum bactericidal assay. *Microb. Pathog.* 27, 207–214 (1999).

82 Morley SL, Cole MJ, Ison CA *et al.* Immunogenicity of a serogroup B meningococcal vaccine against multiple *Neisseria meningitidis* strains in infants. *Pediatr. Infect. Dis. J.* 20(11), 1054–1061 (2001).

83 Gotschlich EC, Rey M, Triau R, Sparks KJ. Quantitative determination of the human immune response to immunization with meningococcal vaccines. *J. Clin. Invest.* 51(1), 89–96 (1972).

84 Wyle FA, Artenstein MS, Brandt BL *et al.* Immunologic response of man to group B meningococcal polysaccharide vaccines. *J. Infect. Dis.* 126(5), 514–521 (1972).

85 Lal G, Balmer P, Joseph H, Dawson M, Borrow R. Development and evaluation of a multiplex flow cytometric assay for quantitation of serum antibodies to *Neisseria meningitidis* serogroups A, C, Y and W-135. *Clin. Diag. Lab. Immunol.* 11, 272–279 (2004).

86 Milagres LG, Ramos SR, Sacchi CT *et al.* Immune response of Brazilian children to a *Neisseria meningitidis* serogroup B outer membrane protein vaccine: comparison with efficacy. *Infect. Immun.* 62, 4419–4424 (1994).

87 Keiser PB, Biggs-Cicatelli S, Moran EE *et al.* A Phase 1 study of a meningococcal native outer membrane vesicle vaccine made from a group B strain with deleted lpxL1 and synX, over-expressed factor H binding protein, two PorAs and stabilized OpcA expression. *Vaccine* 29(7), 1413–1420 (2011).

88 Keiser PB, Gibbs BT, Coster TS *et al.* A Phase 1 study of a group B meningococcal native outer membrane vesicle vaccine made from a strain with deleted lpxL2 and synX and stable expression of opcA. *Vaccine* 28(43), 6970–6976 (2010).

89 Gold R, Lepow ML, Goldschneider I, Draper TL, Gotschlich EC. Clinical evaluation of group A and group C meningococcal polysaccharide vaccines in infants. *J. Clin. Invest.* 56(6), 1536–1547 (1975).

90 Gold R, Lepow ML, Goldschneider I, Draper TF, Gotshlich EC. Kinetics of antibody production to group A and group C meningococcal polysaccharide vaccines administered during the first six years of life: prospects for routine immunization of infants and children. *J. Infect. Dis.* 140(5), 690–697 (1979).

91 Sanborn WR, Bencic Z, Cvjetanovic B, Gotschlich EC, Pollock TM, Sippel JE. Trial of a serogroup A meningococcus polysaccharide vaccine in Nigeria. *Prog. Immunobiol. Stand.* 5, 497–505 (1972).

■ **Field trial of a group A vaccine in Nigeria**

**that failed to show protection in children despite an increase in antibody titer as measured by an antigen-binding assay.**

92 Jodar L, Cartwright K, Feavers IM. Standardisation and validation of serological assays for the evaluation of immune responses to *Neisseria meningitidis* serogroup A and C vaccines. *Biologicals* 28(3), 193–197 (2000).

93 Borrow R, Richmond P, Kaczmarski EB *et al.* Meningococcal serogroup C-specific IgG antibody responses and serum bactericidal titres in children following vaccination with a meningococcal A/C polysaccharide vaccine. *FEMS Immunol. Med. Microbiol.* 28(1), 79–85 (2000).

94 Granoff DM, Maslanka SE, Carlone GM *et al.* A modified enzyme-linked immunosorbent assay for measurement of antibody responses to meningococcal C polysaccharide that correlate with bactericidal responses. *Clin. Diagn. Lab. Immunol.* 5(4), 479–485 (1998).

■ **Study demonstrating how the correlation between antibody levels and human serum bactericidal activity can be improved be decreasing the contribution of low-avidity antibodies to the ELISA.**

95 Zangwill KM, Stout RW, Carlone GM *et al.* Duration of antibody response after meningococcal polysaccharide vaccination in US Air Force personnel. *J. Infect. Dis.* 169(4), 847–852 (1994).

96 Berry DS, Lynn F, Lee C-H, Frasch CE, Bash MC. Effect of O acetylation of *Neisseria meningitidis* serogroup A capsular polysaccharide on development of functional immune responses. *Infect. Immun.* 70, 3707–3713 (2002).

97 Giardina PC, Longworth E, Evans-Johnson RE *et al.* Analysis of human serum immunoglobulin G against *O*-acetyl-positive and *O*-acetyl-negative serogroup W135 meningococcal capsular polysaccharide. *Clin. Diagn. Lab. Immunol.* 12, 586–592 (2005).

98 Poolman JT, De Vleeschauwer I, Durant N *et al.* Measurement of functional anti-meningococcal serogroup A activity using strain 3125 as the target strain for serum bactericidal assay. *Clin. Vaccine Immunol.* 18(7), 1108–1117 (2011).

99 Moran, EE, Brandt BL, Zollinger WD. Expression of the L8 lipopolysaccharide determinant increases the sensitivity of *Neisseria meningitides* to serum bactericidal activity. *Infect. Immun.* 62, 5290–5295 (1994).

100 Borrow R, Aaberge IS, Santos GF *et al.* Interlaboratory standardization of the measurement of serum bactericidal activity by using human complement against meningococcal serogroup B, strain 44/76-SL, before and after vaccination with the Norwegian MenBvac outer membrane vesicle vaccine. *Clin. Diagn. Lab. Immunol.* 12(8), 970–976 (2005).

101 Borrow R, Carlone GM, Rosenstein N *et al.* *Neisseria meningitidis* group B correlates of protection and assay standardization – international meeting report Emory University, Atlanta, Georgia, United States, 16–17 March 2005. *Vaccine* 24(24), 5093–5107 (2006).

102 Findlow J, Holland A, Andrews N *et al.* Comparison of phenotypically indistinguishable but geographically distinct *Neisseria meningitidis* group B isolates in a serum bactericidal antibody assay. *Clin. Vaccine Immunol.* 14(11), 1451–1457 (2007).

103 Donnelly J, Medini D, Boccadifuoco G *et al.* Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. *Proc. Natl Acad. Sci. USA* 107(45), 19490–19495 (2010).

104 Densen, P. Complement deficiencies and infection. In: *The Human Complement System in Health and Disease*. Volanakis JE, Frank MM (Eds). Informa Healthcare, NY, USA, 409–421 (1998).

105 Fijen CA, Kuijper EJ, Drogari-Apiranthitou M, Van Leeuwen Y, Daha MR, Dankert J. Protection against meningococcal serogroup ACYW disease in complement-deficient individuals vaccinated with the tetravalent meningococcal capsular polysaccharide vaccine. *Clin. Exp. Immunol.* 114(3), 362–369 (1998).

106 Andreoni J, Käyhty H, Densen P. Vaccination and the role of capsular polysaccharide antibody in prevention of recurrent meningococcal disease in late complement component-deficient individuals. *J. Infect. Dis.* 168(1), 227–231 (1993).

107 Rameix-Welti MA, Régnier CH, Bienaimé F *et al.* Hereditary complement C7 deficiency in nine families: subtotal C7 deficiency revisited. *Eur. J. Immunol.* 37(5), 1377–1385 (2007).

108 Zoppi M, Weiss M, Nydegger UE, Hess T, Späth PJ. Recurrent meningitis in a patient with congenital deficiency of the C9 component of complement: first case of C9 deficiency in Europe. *Arch. Intern. Med.* 150(11), 2395–2399 (1990).

109 Welsch JA, Moe GR, Rossi R, Adu-Bobie J, Rappuoli R, Granoff DM. Antibody to genome-derived neisserial antigen 2132, a *Neisseria meningitidis* candidate vaccine, confers protection against bacteremia in the absence of complement-mediated bactericidal activity. *J. Infect. Dis.* 188(11), 1730–1740 (2003).

110 Centers for Disease Control and Prevention (CDC). Updated recommendations for use of meningococcal conjugate vaccines – Advisory Committee on Immunization Practices (ACIP). *MMWR Morb. Mortal. Wkly Rep.* 60(3), 72–76 (2010).

111 Committee on Infectious Diseases. Meningococcal conjugate vaccines policy update: booster dose recommendations. *Pediatrics* 128, 1213–1218 (2011).

112 Fijen CA, Kuijper EJ, Hannema AJ, Sjöholm AG, van Putten JP. Complement deficiencies in patients over ten years old with meningococcal disease due to uncommon serogroups. *Lancet* 2(8663), 585–588 (1989).

113 Davis DJ. Studies in meningococcus infections. *J. Infect. Dis.* 4, 558–581 (1907).

114 Kim JJ. Perspective:the role of cost–effectiveness in US vaccination policy. *N. Engl. J. Med.* 365(19), 1760–1761 (2011).

## ■ Websites

201 World Health Organization Expert Committee on Biological Standardization, technical report series No. 594. Geneva, Switzerland (1976). www.whqlibdoc.who.int/trs/WHO_TRS_594.pdf

202 World Health Organization. Standardization and validation of serological assays for the evaluation of immune responses to *Neisseria meningitidis* serogroup A/C vaccines (1999). www.who.int/vaccines-documents/DocsPDF99/www9927.pdf

203 EMEA Committee for Medicinal Products for Human Use. Opinion following an Article 29(4) referral for Menitoix, Annex II (2008). www.emea.europa.eu/docs/en_GB/document_library/Referrals_document/Menitorix_29/WC500008279.pdf

204 US FDA Vaccines and Related Biologic Products Advisory Committee. Approaches to licensure of meningococcal vaccines for prevention of serogroup B invasive meningococcal disease: briefing document (2011). www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics

205 Novartis Vaccines and Diagnostic, Inc. Briefing materials for the meeting of the Vaccines and Related Biological Products Advisory Committee, 7th April, 2011. www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/UCM249478.pdf