2016

Vaccines to combat meningococcal disease - definitive vaccines for elusive pathogens

BOSTON UNIVERSITY

GRADUATE SCHOOL OF ARTS AND SCIENCES

Thesis

VACCINES TO COMBAT MENINGOCOCCAL DISEASE – DEFINITIVE VACCINES FOR ELUSIVE PATHOGENS

by

GEORGE F. SANTOS

B.S., University of Connecticut, 1982

Submitted in partial fulfillment of the

requirements for the degree of

Master of Arts

2017

Approved by

First Reader _____

Ulla Hansen, Ph.D.
Professor of Biology

Second Reader _____

Thomas Gilmore, Ph.D.
Professor of Biology

VACCINES TO COMBAT MENINGOCOCCAL DISEASE –

DEFINITIVE VACCINES FOR ELUSIVE PATHOGENS

GEORGE F. SANTOS

ABSTRACT

Neisseria meningitidis (Nm) disease occurs worldwide. Disease incidence rates can vary from 1 to 1000 cases per 100,000 with the highest incidence found in the sub-Saharan Africa meningitis belt. Nm has evolved a number of mechanisms to evade host immunity. This includes the production of genetic variants through re-combinatorial events, which is thought to have contributed to the evolution of hyper-invasive lineages that are largely responsible for meningococcal disease. Antigenic diversity of Nm surface proteins has been the main limitation in the design of broadly protective vaccines, particularly against capsular serogroup B strains. To overcome this problem, several Nm genomes have been sequenced in an effort to find highly sequence-conserved surface antigens recognized by the human immune system in order to develop a vaccine, which would be broadly protective against disease. Nm genomes contain over 2 million base pairs that contain between 2000 and 2500 open reading frames. Add to this the difficulty of identifying highly conserved recombinant antigens with strong intrinsic immunostimulatory properties, makes vaccine design and development a daunting task. Recent advances in our understanding of the interactions between innate and acquired immunity, and the discovery of pattern recognition receptors, including

Toll-like receptors (TLRs), have ushered in a new set of adjuvant compounds, TLR agonists, which invoke strong humoral and cellular responses with nominal toxicity and adverse reactions. These insights have opened up new areas of vaccine research to combat invasive Nm disease.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

Epidemiology of *Neisseria meningitidis* Disease

Disease caused by *Neisseria meningitidis* (Nm) is a global health problem. Nm is pathogenic only in humans. Nm infection can result in mortality within 24 to 48 hours from the onset of disease symptoms. The mortality rates associated with meningococcal infection are 10–20%, which can vary by causative strain. Of those individuals that survive infection, approximately 20% experience significant sequelae including limb loss, hearing loss, chronic pain, and loss of neurological function (Pace & Pollard, 2012; Stein-Zamir et al., 2014). Disease incidence occurs mostly within three age groups (Figure 1): the highest rate in infants under the age of 1 years, with approximately two-thirds of Nm disease occurring in the first 6

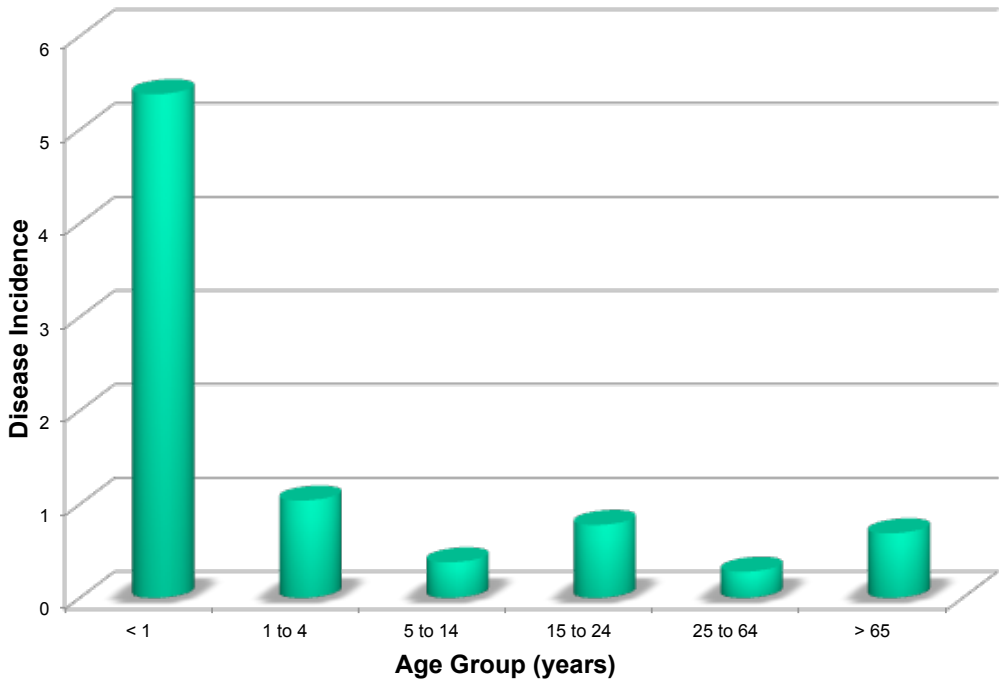

Figure 1: Estimated Annual Disease Rate in the United States, 1998-2007 (adapted from Cohn, 2010)

months due to absences or loss of maternal antibodies; a 2nd peak in adolescents and young adults between 15 and 24 years old; and, a 3rd peak seen in the elderly at 65 years and older shown (Thompson et al., 2006; Cohn et al., 2010; Chang et al., 2012).

Classification of Nm is historically based upon the immunochemistry of its capsular polysaccharide (serogroup). There are 13 serogroups that have been identified to date, with most disease occurrences resulting from serogroups A, B, C, W-135, X, and Y. Meningococci are further classified on the basis of their class 1 outer-membrane proteins (PorA, serosubtype), class 2 or 3 outer-membrane proteins (PorB,serotype), and lipo-oligosaccharides (immunotype). Molecular subtyping with the use of pulsed-field gel electrophoresis, or DNA-sequence analysis has been helpful in identifying closely related strains responsible for disease outbreaks (Frasch et al., 1985; Maiden et al., 1998). Globally, Nm disease cases are caused primarily by serogroups A, B, and C. The geographical variations observed likely result from differences in regional population immunity, and environmental factors. The incidence of disease can vary from between 0.5 and 1000 cases per 100,000 depending on geographical region of occurrence (Stephens et al., 2007; Caugant & Maiden 2009). The majority of cases in Europe and North America involve serogroups B, C and Y, while Serogroups A and C predominating throughout Asia and Africa (Connolly & Noah, 1999; Cohn et al., 2010; Jafri et al., 2013). Fluctuations in disease dynamics have been observed recently in the Sub-Saharan "meningitis

belt", where serogroup A was historically the cause of seasonal epidemics, however serogroup W-135 predominated in 2010 and 2011 (Halperin et al., 2012). In order to better understand the mechanisms underlying outbreaks, a genetics based characterization scheme referred to as Multi-locus Sequence Typing (MLST) was developed in which the sequences of seven housekeeping genes are analyzed to group disease-related isolates into sequence types and then larger clonal complexes. This strain typing system has allowed researchers and clinicians to better determine if local outbreaks and regional epidemics are due to a single strain or multiple unrelated strains circulating within the affected population (Brehony et al., 2007). This typing system has advanced the identification of disease-causing hypervirulent strains.

Neisseria meningitidis Disease Mechanisms

Colonization and Carriage

Nm related invasive disease is a multi-step process, which involves colonization and carriage in the nasopharynx, invasion of the respiratory tract epithelia and migration of the underlying endothelia, entry into the microvasculature, and then systemic dissemination via the bloodstream. Nm colonization and subsequent carriage in the human nasopharynx is typically asymptomatic and can be detected in up to 40% of the population at any particular time. Carriage is transient, can last

from days to months depending on the bacterial strain, and varies by season and age group (Caugant et al., 2007; Caugant & Maiden 2009). The first steps to nasopharyngeal colonization involves the initial adhesion of meningococci to the exposed epithelium which is mediated by Type IV pili, a multimeric scaffold/pore protein complex that spans the inner and outer membranes of Nm strains (Carbonnelle et al., 2006; Brown et al., 2010). The type IV pilus has also been reported in other infection related processes including adhesion to endothelial cells, bacterial aggregation, and migration, and natural transformation of exogenous DNA (Bernard et al., 2014; Imhaus & Dumenil, 2014). Adhesion is further mediated by additional meningococcal surface receptors such as: the opacity proteins, Opa, and Opc; the trimeric autotransporter NhhA; the Adhesion and penetration protein, App; and NadA, which collectively bind to extracellular receptors and matrix components, including carcinoembryonic antigen cell adhesion molecules (CEACAMs), heparan sulfate, and laminin (Virji, 2000; Hadi et al., 2001; Comanducci et al. 2002; Serruto et al., 2003; Capecchi, et al., 2005; Scarselli et al., 2006; Coureuil et al., 2010). The assortment of adhesion receptors with different specificities suggests a high level of binding cooperatively when targeting the same cell type, as well as at other stages of infection when binding endothelium, and other cells types for entry into various host tissues. Many of these membrane structures are possible target for the development of a vaccine to protect against meningococcal disease (Figure 2), however Nm strain diversity and an inclination to circumvent immune surveillance has made use of most of these antigens impractical (Sadarangani & Pollard, 2010).

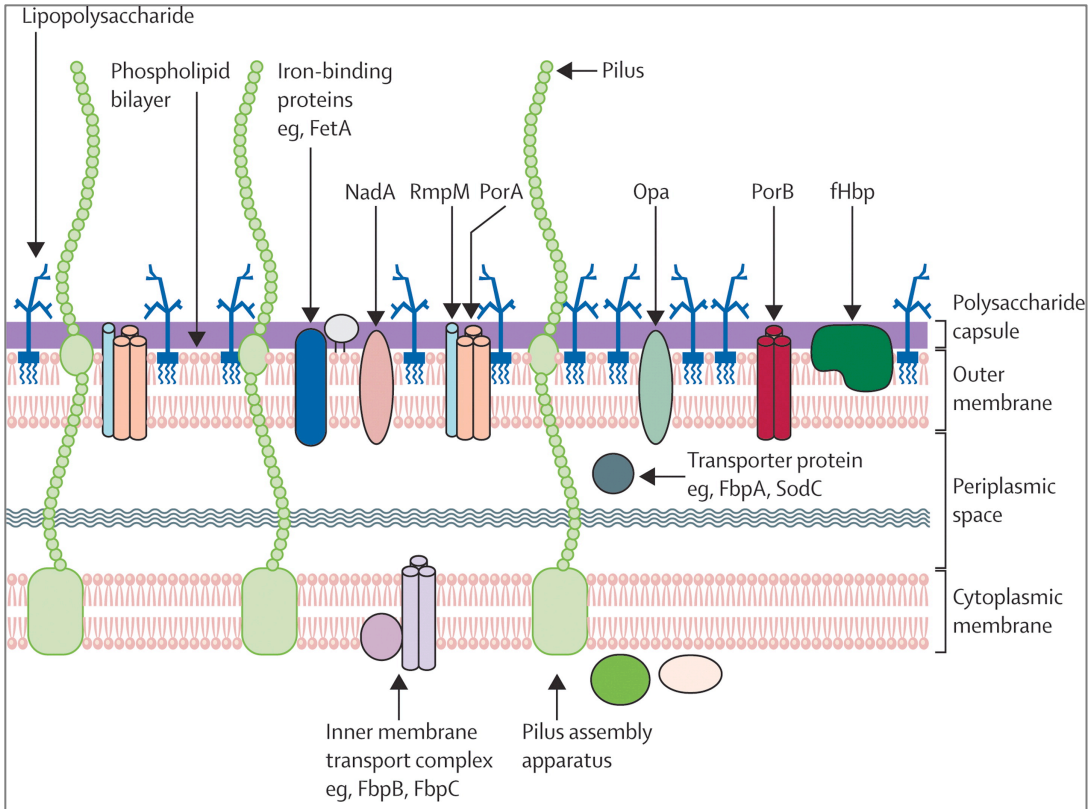

Figure 2: Nm surface structures. Membrane components that are relevant to invasion and immunological resistance as possible vaccine components. (taken from Sadarangani & Pollard, 2010)

Immune Evasion by *Neisseria meningitidis*

Several strategies are used by Nm to generate genetic variants in order to evade immunity by altering antigenic structure, or acquire and maintain antibiotic resistance when selective pressures are exerted in this manner (Spratt et al., 1992; Swartley et al., 1997). One such mechanism is horizontal gene transfer, which allows Nm to obtain large segments of DNA from other commensal and invasive Neisseria strains or unrelated species (Feil et al., 1999; Linz et al., 2000). Capsule switching is an example of this whereby the original infectious strain can achieve

immunologic escape from natural immunity, or acquired immunity via immunization, while maintaining in essence the same genetic lineage. In the United States, a large percentage of disease-causing Nm strains appear to have arisen via capsule switching including an outbreak of serogroup B disease in Oregon in the 1990s where serogroup C isolates were found to be otherwise genetically indistinguishable from the serogroup B outbreak strain (Diermayer et al., 1999; Harrison et al., 2010). Capsule switching was also likely the cause of a serogroup W-135 outbreak in the year 2000 at the Hajj in Mecca, Saudi Arabia (Mayer et al., 2002; Mustapha et al., 2016).

The importance of the capsule and lipo-oligosaccharide structures on the surface of meningococci for immune evasion and resistance to complement-mediated lysis was demonstrated by a large scale gene disruption study which suggested that these two components were major contributors to serum resistance during invasion and widespread dissemination in the blood stream (Geoffroy et al., 2003). Additionally, it was shown that Nm have three independent RNA thermo-sensors that are activated by an increase in temperature, such as during invasion from the naso-pharynx into the blood stream, or during the fever response in humans. The thermo-sensors are in the 5' untranslated regions (UTR) of genes necessary for capsule biosynthesis, sialylation of lipopolysaccharide, and the expression of the complement inhibitor factor H binding protein (fHBP). All three products are essential for resistance to host bactericidal responses (Loh et al., 2013). These

thermo-sensors form stem loop structures at the lower permissive temperatures, which obscures the ribosome-binding site (RBS) contained within the loop, and interferes with translation. When these loops are unwound at higher temperatures, the RBS becomes more accessible allowing increased expression of relevant proteins.

Recombinant gene conversion is employed by Nm to achieve antigenic variants allowing Nm to escape host immune detection without the acquisition of foreign DNA. Over 100 phase-variable genes have been identified (Moxon et al., 1994; Snyder et al., 2001) with the majority associated with meningococcal surface antigens including: the capsule (Hammerschmidt et al., 1996b); lipopolysaccharide (Jennings et al., 1995); the PilE component in Type IV pili which has in close proximity 8 truncated pseudogenes available for recombination with the pilE gene (Howell-Adams & Seifert, 2000; Saunders et al., 2000; Andrews & Gojobori, 2004; and, other outer membrane proteins, such as the porins and the opacity proteins (Sarkari et al., 1994; van der Ende et al., 2000). Moreover, antigenic variation has been shown to arise from a variety of insertion sequences, transposons, and Correia elements (Hammerschmidt et al., 1996a; Packiam et al., 2006; Elias & Vogel, 2007). Nm has been shown to also bind complement negative regulators to its surface as an immune evasion strategy, which allows Nm to further mask itself and increase its resistance to lysis by the complement (Madico, et al., 2006). This propensity toward the production of genetic variants through re-combinatorial events is thought to be

a primary mechanism underlying Nm evasion of host protective immunity, as well as the evolution of hyper-invasive lineages that are largely responsible for meningococcal disease.

Host Defenses against *Neisseria meningitidis*

Innate immune responses are the first line of defense against Nm, especially in the immature immune systems in human infants and toddlers, who are particularly susceptible to infection following the loss of maternal antibodies. Central to this innate protective response is the complement system, consisting of over 30 fluid phase factors, and membrane-bound regulators of complement, which plays a significant role in defense against meningococcal infection. The importance of the complement system in combating Nm disease is highlighted by the increased susceptibility of patients with complement deficiencies to Nm, in particular loss of terminal complement components, reduction in levels of circulating C3, and alterations in complement regulators (Figueroa et al., 1993; Garty et al., 1993; Schneider et al., 2007; Hellerud et al., 2010).

Three complement activation pathways have been identified to date in humans: the alternative pathway, which is activated by the binding of the complement component C3b covalently to the hydroxyl or amino groups of microbial surface structures that then recruits additional complement components, factor B, factor D

and properdin (P) to further activation; the lectin pathway, which is triggered when mannose-binding lectin (MBL) or ficolins bind to carbohydrates on microbial surfaces and recruit two MBL-associated serine protease (MASP1 and. MASP2); and, the classical pathway (C1q, C1r, C1s, C4, and C2 components), which is activated primarily by antibody–antigen interactions (Nesargikar et al., 2012). When triggered, all three converge to further produce C3b via two different C3-convertases, which catalyze the formation of C5-convertases, and sets off the subsequent cascade of downstream activation events (Figure 3). The end product of this cascade is the formation of the membrane attack complex (MAC) involving C5b, C6, C7, C8 and C9, which inserts pores into the surface of pathogens resulting in membrane disruption and pathogen lysis (Morgan et al., 1999; Nesargikar et al., 2012). Additional complement cleavage products, such as C3a, C4a, and C5a, are known to chemotactically recruit immune cells to sites of infections, as well as bind and opsonize microbial targets for clearance by phagocytic cells (Gasque, 2004).

In the case of Nm, the insertion of MACs into surface membranes and subsequent cell lysis has been established as the primary method of protection against disease. This was determined in a landmark study of Army recruits in the United States in the 1960s where the presence of serum bactericidal antibodies was shown to be the most important protective host factor against Nm infection (Goldschneider et al., 1969). These bactericidal antibodies are thought to be naturally acquired through carriage of commensal species, such as *Neisseria lactamica*, that express on their

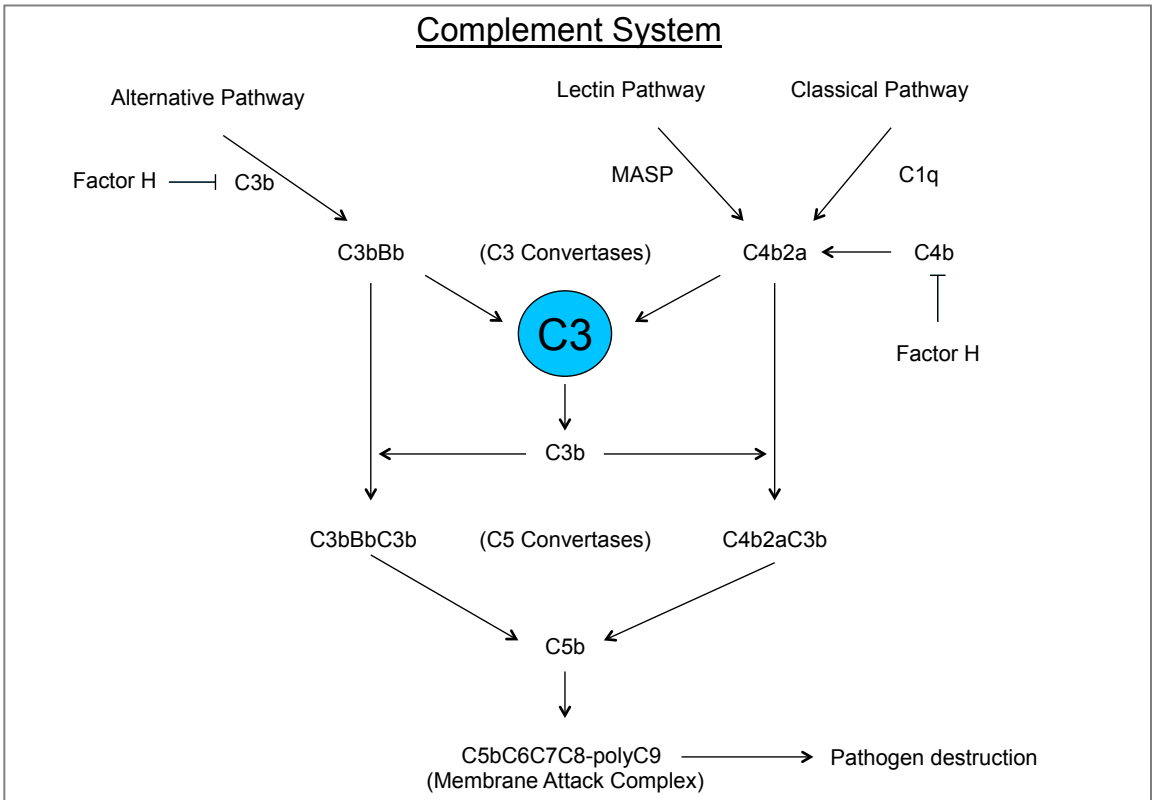

Figure 3: Complement activation. Key: C1q, antibody-antigen binding component; MASP, MBL-associated serine protease, MASP-1 and MASP-2

surface cross-reacting epitopes, as well as from colonization of non-invasive meningococci. This acquisition then provides increased protection against meningococcal infection through early childhood and adolescence (Gold et al., 1978; Pollard & Frasch, 2001; Sanchez S, et al., 2002; Troncoso et al., 2002). Given the high rate of Nm disease in infants however, the need to induce via vaccination a strong adaptive immune response in this population is clear and apparent.

Correlates of Protection

In the seminal study in the 1960s, the importance of bactericidal antibody for protection against Nm disease was shown using baseline serum samples from military recruits who were at high risk of acquiring serogroup C disease during training camp. A baseline serum bactericidal titer of ≥1:4 was a strong predictor of protection whereas individuals with baseline titers <1:4 were at highest risk to contract disease. A titer of 1:4 indicates that a dilution of serum down to 25% by volume, for use in the standard bactericidal assay, will result in a ≥50% reduction in bacterial colonies compared to the number of colonies measured at time zero. Furthering this link was data on age-specific incidence of meningococcal disease in the United States, which demonstrated that the proportion of individuals lacking serum bactericidal activity to serogroups A, B, and C was inversely related to the incidence of disease (Goldschneider et al., 1969; Frasch CE, 2009). These data established the bactericidal assay, with normal human serum as the complement source, as the immunologic correlate of protection against meningococcal disease. For serogroup B, a correlation between protection after vaccination and the level of bactericidal antibody was reported following the use of an Outer Membrane Vesicle (OMV) vaccine during a meningococcal epidemic in Norway (Holst et al., 2003).

Finding normal human serum lacking naturally acquired antibodies to Nm from adults is not trivial, as sera from many healthy adults often contain antibodies to group-specific polysaccharide, lipopolysaccharide, or outer membrane proteins that

can activate complement and interfere with assay results. In order to accurately model these early studies, serum complement from an untreated agammaglobulinemia patient, which is now rare in the population due to medical management, or from healthy adults who lacks intrinsic bactericidal activity against specific strains of interest, would be required. Because of the perceived challenges in obtaining human complement, infant rabbit sera that lack intrinsic bactericidal activity was selected for use in the standardization of the bactericidal assay (World Health Organization, 1976; Wong et al., 1977; Maslanka et al., 1997).[65–67] It was later reported that the use of rabbit complement resulted in much higher bactericidal titers for serogroup B and C meningococcal strains than the use of human complement (Zollinger & Mandrell, 1983; Mandrell, et al., 1995). Since the clinical relevance of higher bactericidal titers as measured in the standardized assay with rabbit complement was unknown, a direct comparison of the two complement sources using serogroup C post vaccination human sera after 1 dose determined that a threshold titer of approximately 1:128 when using rabbit complement was equivalent to 1:4 when using human complement (Santos et al., 2001).[70] This observation was confirmed and extended to serogroups A, W-135, and Y in a follow-up study (Gill et al., 2011).

The apparent difference in titer when using the two complement sources has been attributed at least in part to the species specificity of binding of human factor H, a complement regulatory molecule that down-regulates complement activation. Nm

binds human factor H to its surface as an immune evasion strategy, which allows Nm to mask itself and increase its resistance to bactericidal activity. Rabbit factor H does not bind Nm allowing unrestricted complement deposition onto its surface and bacteriolysis at significantly lower anti-Nm antibody concentrations (Schneider et al., 2007; Granoff et al., 2009). For serogroup B strains, species specificity regarding the source of exogenous complement for clinical assay use was not an issue because the lack of compatibility of baby rabbit complement in the assay system eliminated it as a viable option. Despite the perceived limitations, sufficient volumes of human complement were obtained for a four-site interlaboratory standardization of the bactericidal assay for Nm serogroup B (Borrow et al., 2005), as well as ongoing clinical trials.

As a correlate of protection, the utility of the bactericidal assay in assessing vaccine responses to new and novel antigen-adjuvant combinations is evident. There is an ongoing need however, to better understand the mechanisms underlying meningococcal disease, and develop additional laboratory correlates for testing the effectiveness of meningococcal vaccines in both pre-clinical studies and future human clinical trials. Among the model systems that have come forward, the ex vivo human blood bacteremia model, the infant rat model, the humanized mouse infection assay, and the meningococcal antigen typing system (MATS) are some of the in vitro systems showing early promise for the evaluation of protective immunity against Nm (Granoff et al., 1998; Toropainen et al., 1999; Gorringe et al.,

2005; Plested et al., 2009, Donnelly et al., 2010). The need for streamlined assays for use during clinical trials is especially apparent given that volumes of test samples are limiting, particularly in infants, and breadth of bacterial strains to be tested are large. Many normal human donors have intrinsic bactericidal activity that is strain specific, hence a specific complement donor or donor set must be found for each isolate. Assay miniaturization is an important issue for both trial specimen and complement reagent conservation with the aim of maintaining the established protective correlation (Mountzouros & Howell, 2000; Rodríguez et al., 2003; Mak et al., 2011).

Developments in *Neisseria meningitidis* Capsular Antigen Vaccines

Despite the prompt use of antibiotic to combat meningococcal infection upon clinical presentation, vaccination is clearly the best strategy to prevent the high fatality rate and significant sequelae associated with meningococcal disease. This is primarily due to the rapid onset of disease with death occurring often within 24 to 48 hours. Early vaccines developed against Nm were based upon purified capsular polysaccharide antigens targeting serogroups A and C (Gotschlich et al., 1969). This was subsequently followed by a four-component polysaccharide vaccine, which covered serogroups A, C, W-135, and Y vaccine, licensed in the United States in the 1980s. Although these vaccines were safe and immunogenic and greater than 85% efficacious for the A and C components, polysaccharide vaccines in general were

largely ineffective in the population most susceptible to Nm disease, namely children under 2 years of age (Cadoz, 1998). Because polysaccharides are T-cell independent antigens that induce only short-lived humoral immunity with no memory response, a new generation of protein-conjugated polysaccharide vaccines emerged that largely remedied these limitations (Bilukha & Rosenstein, 2005; Pollard et al., 2009). Protein-conjugated polysaccharides elicit both B-cell and T-cell responses along with immunologic memory in all age groups including children under 2 years of age (Harrison, 2006). Polysaccharide–protein conjugate vaccines were first introduced into the United Kingdom in 1999 to protect against Nm serogroup C strains in the ST11 clonal complex (ET37 complex C2a) that had spread there following appearances in Canada, Spain, and the Czech Republic (Miller et al., 2001). These vaccines were shown to elicit bactericidal antibodies in all age groups including infants under 2 years old, and resulted in a significant decline in the incidence of serogroup C disease in the UK (Borrow et al., 2013). Serogroup C polysaccharide–protein conjugate vaccines were subsequently included into the schedule for routine infant immunizations. The success of this campaign later resulted in the development of quadrivalent polysaccharide–protein conjugate vaccines that protected against serogroups A, C, W-135, and Y by also inducing serum bactericidal antibodies, interrupting carriage transmission, and providing herd immunity against strains in the other 3 serogroups (Baltimore, 2006). Because of the high cost of the quadrivalent conjugate vaccine in the developed countries, to combat serogroup A disease in the meningitis belt in sub-Saharan Africa a low cost

conjugated serogroup A vaccine was developed in India. The serogroup A vaccine was launched in Burkina Faso and Chad starting in 2010, and resulted in a significant reduction of the incidence of disease along with a concurrent decline in serogroup A carriage (Daugla et al., 2013; Kupferschmidt, 2014; Gamougam et al., 2015; Meyer et al., 2015).

The Challenges of Vaccines Development against Serogroup B

Capsular polysaccharide conjugation to protein carriers has greatly improved the overall effectiveness of meningococcal vaccines against disease caused by serogroups A, C, W-135, and Y. The development of capsule based vaccines to protect against Nm serogroup B disease did not move forward however, due to the structural identity between the serogroup B capsule, an α2-8–linked polysialic acid, and the human neural-cell adhesion molecules NCAM, particularly the embryonic form (Finne et al., 1983; Finne et al., 1987; Nedelec et al., 1990).

A different strategy was undertaken to confront this problem, which led to the development of outer membrane vesicles (OMVs) as an antigen vehicle for vaccines against serogroup B disease. OMVs were used to control outbreaks of group B disease for many years however, the breadth of protection they offered was limited (Bjune et al., 1991; Sierra et al., 1991; de Moraes et al., 1992; Boslego et al., 1995; O'Hallahan et al., 2009).

OMVs contain a mixture of immunogenic outer-membrane antigens including the porins, PorA and porB, and the iron-regulated membrane protein, FetA, in a lipid based structure derived from deoxycholate detergent preparations of Nm cultures (van der Ley et al., 1991; Feavers et al., 1996; Thompson et al., 2003). The primary protein component that drives the initial immune response to OMVs, PorA, contains two immunodominant hyper-variable loops VR1 and VR2, which effectively limit protective immunity mainly to the epidemic/outbreak, strain PorA-type. By one estimate, a vaccine would have to contain 20 different PorA types in order to cover 80% of strains that cause endemic disease in the United States alone (Sacchi et al., 2000). Although OMV vaccines have been effective in outbreak type situations caused by a single PorA-type strain, the number of PorA variants globally makes this approach impractical in the long-term against a pathogen with an inherent propensity towards antigenic diversity (Harrison et al., 2006).[106]

Reverse Vaccinology and Serogroup B

Traditional biochemical methods of purifying and identifying membrane bound antigens for the purposes of developing a broadly protective vaccine against Nm serogroup B had been largely unsuccessful. An alternative strategy coined "reverse vaccinology" was launched in 2000 which involved the sequencing of the entire genome of the disease causing serogroup B strain, MC-58, in order to identify highly conserved outer membranes bound antigens for use in the next generation serogroup B vaccine. From the sequencing of a 2,272,351-base pair genome, 2158

predicted coding regions were identified (Tettelin et al., 2000). Over 300 candidate antigen sequences were expressed in E. coli and used to immunize mice, which resulted in the identification of proteins that were surface exposed, sequence conserved across a diverse collection of disease causing strains, and induced a bactericidal antibody response (Pizza et al., 2000). From this work five antigens were formulated: two protein–protein fusions, fHbp(GNA2091)-GNA1870 and NHBA(GNA2132)-GNA1030; and; NadA for use in human clinical trials.

The three target antigens fHbp, NHBA, and NadA, were selected due to their ability induced serum bactericidal antibodies against a diverse strain set. The two carrier proteins, GNA 2091 and GNA 1030, improved the immunogenicity of the coupled target antigens when fused with them. When tested in mice, this vaccine induced bactericidal antibodies against 78% of a globally diverse panel of 85 meningococcal strains. Interestingly, the addition of CpG oligonucleotides to an aluminum hydroxide based formulation, known to stimulate immune responses via toll-like receptor (TLR) activation, increased strain coverage to 90% (Giuliani et al., 2006).

The antigen fHbp, is an important virulence factor that allows Nm to bind soluble human factor H, down-regulate the complement amplification loop, and inhibit the insertion of the membrane attack complex on its surface. This makes fHbp a significant vaccine target given that antibodies raised against it can potentially block the binding of human factor H rendering strains more susceptible to the action of complement. The 2nd vaccine antigen is NadA which is an important target given its

role in Nm adhesion and invasion. Though highly sequence conserved, it is not universally expressed on all strains. Expression of NadA is known to be phase variable, and completely absent from the disease causing sequence type 41/44 serogroup B lineage. The 3rd vaccine antigen, NHBA, is expressed by most meningococcal strains, and shown to bind heparin, which is thought to aid the adhesion/invasion process. The vaccine includes the most common variant of NHBA, which induce antibodies that results in cross-reactivity with many of the other variants (Wang et al., 2011).

This set of antigens were taken into human trials, alone and also in combination with a PorA serosubtype P1.4 OMV, successfully used to combat an epidemic outbreak in New Zealand (Toneatto et al., 2011; Gossger et al., 2012). Concurrent to this work, a second research and development effort using more traditional antigen identification methods, independently identified fHbp as a potent antigen. In this case, a bivalent vaccine was developed containing elements of two Nm fHbp subfamilies shown to be effective in animal studies against 87% of strains tested (Jiang et al., 2010). Currently, both of these vaccines are independently licensed for use around the world including the United States, Europe, Canada, and Australia, selectively for infant through adults in varying countries.

Tweaking What's Innate - Toll-like Receptor Agonists as Adjuvants

Vertebrates have evolved an immune defense with two interacting branches, innate and acquired immunity, that function cooperatively to eliminate invasive pathogens. Innate immunity is an evolutionarily conserved system consisting of complement, chemokines, cytokines, and a variety of immune cells including neutrophils, phagocytes, natural killer cells, and dendritic cells that all act as a first line of defense against microbial organisms. Acquired immunity is characterized by an exquisite diversity of epitope recognition and specificity. This is made possible by somatic gene rearrangement, and subsequent clonal expansion of lymphocytes that express receptors to the vast array of epitopes in the environment. Innate immunity was long regarded as an ancient and relatively nonspecific system of immunity whose central function was the direct destruction of infectious microorganisms. The underlying complexity of the innate immune system was uncovered recently, in studies that revealed an intricate system of specific receptors coupled to signal transduction mechanisms. These innate pathways are responsible for initiating early danger signals that function to launch the cascade of initial host defenses against foreign substances, in particular invasive pathogens. A key component to this system are pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs), that detect pathogen-associated molecular patterns (PAMPs), and are found on a variety of immune cells including macrophages, dendritic cells, B and T cells, as

well as non-immune cells such as fibroblasts and epithelial cells (Janeway Jr & Medzhitov, 2002).

To date, 10 human and 13 mouse TLRs have been identified. Each TLR detects distinct PAMPs derived from viruses, bacteria, mycobacteria, fungi, and parasites (Table 1), which include: peptidoglycans (TLR1 and TLR2), viral double-stranded RNA (TLR3), lipopolysaccharides (TLR4), flagellin (TLR5), bacterial lipoproteins (TLR2 and TLR6), viral single-stranded RNA (TLR7 and TLR8), and bacterial and viral CpG (cytosine-phosphate-guanine dinucleotide) oligodeoxynucleotides (TLR9) (Akira et al., 2006). Meningocccal porB was also identified as having MyD88-dependent TLR2 stimulatory effects (Massari et al., 2002). Many TLRs are localized to the cell surface and associated with the extracellular membrane, such as TLRs 1, 2, 4, 5, and 6. However several that are localized internally, TLRs 3, 7, 8, and 9, require internalization of associated ligand before signal transduction events will occur (Akira et al., 2006).

Table 1: Recognition of Microorganisms by TLRs

Bacteria	**Species**	**Associated TLR**
LPS	Gram-negative bacteria	TLR4
Peptidoglycans	Gram-positive bacteria	TLR1/TLR2
Porins	*Neisseria*	TLR2
Flagellin	Flagellated bacteria	TLR5
CpG-DNA	Bacteria and mycobacteria	TLR9
Viruses		
DNA	Viruses	TLR9
dsRNA	Viruses	TLR3
ssRNA	RNA viruses	TLR7 and TLR8
Envelope proteins	RSV, MMTV	TLR4
Hemagglutinin protein	Measles virus	TLR2
Fungus		
Zymosan	*Saccharomyces cerevisiae*	TLR6/TLR2
Mannan	*Candida albicans*	TLR4
Parasites		
Glycoinositolphospholipids	*Trypanosoma*	TLR4
Hemozoin	*Plasmodium*	TLR9
Profilin-like molecule	*Toxoplasma gondii*	TLR11

TLRs are classified as type 1 integral membrane glycoproteins, which consist of a leucine-repeat rich extracellular N-terminal domain, and an intracellular C-terminal Toll/interleukin 1 receptor (TIR) domain. Upon dimerization, TLR monomers take on a M-shaped structure (Choe et al., 2005; Jin et al., 2007; Kang et al., 2009; Park et al., 2009), which then allows the intracellular TIR domains to recruit adaptor molecules that activate signaling pathways that up-regulate transcription factors, such as nuclear factor-kB and interferon regulatory factors. Known adaptor

molecules include myeloid differentiation primary response protein 88 (MyD88), TIR domain-containing adaptor protein (TIRAP), TIRAP inducing interferon β (TRIF), and TRIF-related adaptor molecule (TRAM) (Akira & Takeda, 2004). Current evidence provides support for the requirement of activation of innate immunity for the induction of acquired immunity. Transfection of a constitutively active mutant of a human Toll into human cell lines was shown to induce NF-kB expression and the genes controlled by NF-kB. TLR activation stimulates the production of the inflammatory cytokines IL-1, IL-6 and IL-8, along with the co-stimulatory molecule B7.1, required for the activation of naive T cells (Medzhitov et al., 1997; Akira et al., 2001; Medzhitov, 2001).

Recent advances in the identification of pattern-recognition receptors have opened up a new line of study on the therapeutic possibilities of PRR activation in the treatment of infectious, allergic, and immune diseases, as well as cancer. In the field of vaccine research, a growing number of compounds with immunopotentiation properties have been brought into use in an effort to exploit the variety of TLRs discovered in recent years. This has allowed for the development of safe and potent vaccine adjuvants and delivery systems for both prophylactic and therapeutic uses. This advance is particularly important as the use of recombinant protein subunits in vaccines, which may lack inherent immunostimulatory properties, continues to move forward. Investigations continue into novel TLR stimulating compounds alone or in combination with aluminum salts (alum), the vaccine adjuvant first

approved for human use (Lindblad, 2004). Alum itself principally stimulates T helper type 2 (Th2) biased immunity and is thought to dampen the production of protective immunity when using recombinant antigens against pathogenic microorganisms. The success of these compounds is based largely upon their abilities to skew immune responses more toward a T helper type 1 (Th1) biased response, and promote the interaction of the innate and adaptive immune pathways without raising serious adverse events (Lahiri et al., 2008).

Some of the compounds developed to date include: Monophosphoryl lipid A (MPL), a detoxified lipid A derivative of lipopolysaccharide from Salmonella enterica, and the AS04 adjuvant system consisting of MPL adsorbed on either aluminium hydroxide or aluminium phosphate, which minimizes the proinflammatory MyD88-dependent signalling pathway while stimulating Th1 and cell-mediated responses (Garcon, et al., 2007; Mata-Haro, et al., 2007; Casella & Mitchell, 2008); RC-529 (Ribi.529; Corixa, Seattle, WA, USA), a fully synthetic MPL mimetic and TLR4 agonist that has been used as a potent adjuvant in both preclinical and clinical studies (Mason et al., 2004; Dupont et al., 2006; Zhu et al., 2006); E6020, a synthetic molecule with a hexa-acylated acyclic backbone with the ability to promote Th1-biased antibody production (Hawkins et al., 2002; Przetak, et al., 2003; unmethylated CpG oligodeoxynucleotides, which elicit potent immunostimulatory responses through TLR9, primarily expressed on B cells and dendritic cells, and induce antigen-specific humoral and Th1-mediated cellular responses (Krieg et al.,

1995; Vabulas et al., 2000; Hornung, et al., 2002); polyriboinosinic polyribocytidylic acid (poly[I:C]) which is a synthetic analogue of viral double-stranded RNA molecules and targets TLR3 (Asahi-Ozaki et al., 2006); the imidazoquinolines, imiquimod (R-837) and resiquimod (R-848), which are synthetic small molecules recognized by TLR7, and TLR7 and TLR8, respectively, both shown to be potent Th1 adjuvants in mice and non-human primates (Vasilakos et al., 2000; Zuber et al., 2004; Wille-Reece et al., 2005); and, the protein Flagellin, a potent activator of the NF-kB signaling pathway through TLR5 which induces robust antibody responses even without supplemental adjuvants against a variety pathogens including influenza, Yersinia pestis, Plasmodium vivax, and L monocytogenes antigens (Tallant et al., 2004; Honko et al., 2006; Huleatt et al., 2007; Bargieri et al., 2008; Huleatt et al., 2008; Mizel et al., 2009; Skountzou et al., 2010).

Alternative approaches to this problem involved the use of an OMV vaccine prepared from recombinant strains that over-express recombinant factor H binding protein (fHbp) as the target antigen, and attenuated endotoxin, designed to target TLR4, was shown to stimulate broad serum bactericidal antibody responses (Koeberling et al., 2011); and the direct coupling of a TLR7 agonist to a serogroup C polysaccharide-conjugate vaccine (Donadei et al., 2016). Efforts to engage multiple TLR receptors and/or other pattern-recognition receptors in order to gain synergistic immune responses with little or no reactogenicity are of continuing interest (Lahiri et al., 2008; Chen et al., 2010; Hajishengallis & Lambris, 2016).

Similar approaches, such as combining immune evasion surface proteins as target antigens with compounds that possess strong immune agonist properties, may be instrumental in the eradication of other difficult to treat infections including gonorrhea, tuberculosis, HCV, and potentially HIV.

Bibliography

Akira S, Takeda K, Kaisho T. 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. Nature Immunology 2:675–680.

Akira S, Takeda K. 2004. Toll-like receptor signaling. Nature Reviews. Immunology 4:499–511.

Akira S, Uematsu S, Takeuchi O. 2006. Pathogen recognition and innate immunity. Cell 124:783–801.

Andrews TD, Gojobori T. 2004. Strong positive selection and recombination drive the antigenic variation of the PilE protein of the human pathogen Neisseria meningitidis. Genetics 166:25–32.

Asahi-Ozaki Y, Itamura S, Ichinohe T, Strong P, Tamura S, Takahashi H, Sawa H, Moriyama M, Tashiro M, Sata T, Kurata T, Hasegawa H. 2006. Intranasal administration of adjuvant-combined recombinant influenza virus HA vaccine protects mice from the lethal H5N1 virus infection. Microbes and Infection 8:2706–2714.

Baltimore RS. 2006. Recent trends in meningococcal epidemiology and current vaccine recommendations. Current Opinion in Pediatrics 18:58–63.

Bargieri DY, Rosa DS, Braga CJ, et al. 2008. New malaria vaccine candidates based on the Plasmodium vivax Merozoite Surface Protein-1 and the TLR-5 agonist Salmonella Typhimurium FliC flagellin. Vaccine 26: 6132–6142.

Bernard SC, Simpson N, Join-Lambert O, Federici C, Laran-Chich MP, Maïssa N4, Bouzinba-Ségard H, Morand PC, Chretien F, Taouji S, Chevet E, Janel S, Lafont F, Coureuil M, Segura A, Niedergang F, Marullo S, Couraud PO, Nassif X, Bourdoulous S. 2014. Pathogenic Neisseria meningitidis utilizes CD147 for vascular colonization. Nature Medicine 20:725–731.

Bilukha OO, Rosenstein N. 2005. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recommendations and Reports 54:1–21.

Bjune G, Hoiby EA, Gronnesby JK, Arnesen O, Fredriksen JH, Halstensen A, Holten E, Lindbak AK, Nøkleby H, Rosenqvist E. 1991. Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. Lancet 338:1093–1096.

Borrow R, Aaberge IS, Santos GF, Eudey TL, Oster P, Glennie A, Findlow J, Høiby EA, Rosenqvist E, Balmer P, Martin D. 2005. 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. Clinical and Diagnostic Laboratory Immunology 12:970–976.

Borrow R, Abad R, Trotter C, van der Klis FR, Vazquez JA. 2013. Effectiveness of meningococcal serogroup C vaccine programmes. Vaccine 31:4477–4486

Boslego J, Garcia J, Cruz C, Zollinger W, Brandt B, Ruiz S, Martinez M, Arthur J, Underwood P, Silva W, Moran E, Hankins, W, Gilly J, Mays J, the Chilean National Committee for Meningococcal Disease. 1995. Efficacy, safety, and immunogenicity of a meningococcal group B (15:P1.3) outer membrane protein vaccine in Iquique, Chile. Vaccine 13:821–829.

Brehony, K.A. Jolley, M.C. Maiden. 2007. Multilocus sequence typing for global surveillance of meningococcal disease. FEMS Microbiology Reviews 31:15–26.

Brown DR, Helaine S, Carbonnelle E, Pelicic V. 2010. Systematic functional analysis reveals that a set of seven genes is involved in fine-tuning of the multiple functions mediated by Type IV pili in Neisseria meningitidis. Infection and Immunity 78:3053–3063.

Cadoz M. 1998. Potential and limitations of polysaccharide vaccines in infancy. Vaccine 16:1391–1395.

Capecchi B, Adu-Bobie J, Di Marcello F, Ciucchi L, Masignani V, Taddei A, Rappuoli R, Pizza M, Aricò B. 2005. Neisseria meningitidis NadA is a new invasin which promotes bacterial adhesion to and penetration into human epithelial cells. Molecular Microbiology 55:687–698.

Carbonnelle E, Helaine S, Nassif X, Pelicic V. 2006. A systematic genetic analysis in Neisseria meningitidis defines the Pil proteins required for assembly: functionality, stabilization and export of Type IV pili. Molecular Microbiology 61:1510–1522.

Casella CR, Mitchell TC. 2008. Putting endotoxin to work for us: monophosphoryl lipid A as a safe and effective vaccine adjuvant. Cellular and Molecular Life Sciences 65:3231–3240.

Caugant DA, Tzanakaki G, Kriz P. 2007. Lessons from meningococcal carriage studies. FEMS Microbiology Reviews 31:52–63.

Caugant DA, Maiden MC. 2009. Meningococcal carriage and disease — population biology and evolution. Vaccine 27:B64–B70.

Chang Q, Tzeng YL, Stephens DS. 2012. Meningococcal disease: Changes in epidemiology and prevention. Clinical Epidemiology 4:237–245.

Chen WH, Basu S, Bhattacharjee AK, Cross AS. 2010. Enhanced antibody responses to a detoxified lipopolysaccharide-group B meningococcal outer membrane protein vaccine are due to synergistic engagement of Toll-like receptors. Innate Immunity 16:322–332.

Choe J, Kelker MS, Wilson IA. 2005. Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. Science 309:581–585.

Cohn AC, MacNeil JR, Harrison LH, Hatcher C, Theodore J, Schmidt M, Pondo T, Arnold KE, Baumbach J, Bennett N, Craig AS, Farley M, Gershman K, Petit S, Lynfield R, Reingold A, Schaffner W, Shutt KA, Zell ER, Mayer LW, Clark T, Stephens D, Messonnier NE. 2010. Changes in Neisseria meningitidis disease epidemiology in the United States, 1998–2007: implications for prevention of meningococcal disease. Clinical Infectious Diseases 50:184–191.

Comanducci M, Bambini S, Brunelli B, Adu-Bobie J, Aricò B, Capecchi B, Giuliani MM, Masignani V, Santini L, Savino S, Granoff DM, Caugant DA, Pizza M, Rappuoli R, Mora M. 2002. NadA: a novel vaccine candidate of Neisseria meningitidis. Journal of Experimental Medicine 195:1445–1454.

Connolly M, Noah N. 1999. Is group C meningococcal disease increasing in Europe? A report of surveillance of meningococcal infection in Europe 1993–6. Epidemiology and Infection 122:41–49.

Coureuil M, Lécuyer H, Scott MG, Boularan C, Enslen H, Soyer M, Mikaty G, Bourdoulous S, Nassif X, Marullo S. 2010. Meningococcus Hijacks a beta2-adrenoceptor/beta-Arrestin pathway to cross brain microvasculature endothelium. Cell 143:1149–1160.

Daugla DM, Gami JP, Gamougam K, Naibei N, Mbainadji L, Narbé M, Toralta J, Kodbesse B, Ngadoua C, Coldiron ME, Fermon F, Page AL, Djingarey MH, Hugonnet S, Harrison OB, Rebbetts LS, Tekletsion Y, Watkins ER, Hill D, Caugant DA, Chandramohan D, Hassan-King M, Manigart O, Nascimento M, Woukeu A, Trotter C, Stuart JM, Maiden MC, Greenwood BM. 2013. Effect of a serogroup A meningococcal conjugate vaccine (PsA-TT) on serogroup A meningococcal meningitis and carriage in Chad: a community study. Lancet 383:40–47.

de Moraes JC, Perkins BA, Camargo MC, Hidalgo NT, Barbosa HA, Sacchi CT, Landgraf IM, Gattas VL, Vasconcelos Hde G. 1992. Protective efficacy of a serogroup B meningococcal vaccine in Sao Paulo, Brazil. Lancet 340:1074–1078.

Diermayer M, Hedberg K, Hoesly F, Fischer M, Perkins B, Reeves M, Fleming D. 1999. Epidemic serogroup B meningococcal disease in Oregon: the evolving epidemiology of the ET-5 strain. JAMA: The Journal of the American Medical Association 281:1493–1497.

Donadei A, Balocchi C, Mancini F, Proietti D, Gallorini S, O'Hagan DT, D'Oro U, Berti F, Baudner BC, Adamo R. The adjuvant effect of TLR7 agonist conjugated to a meningococcal serogroup C glycoconjugate vaccine. European Journal of Pharmaceutics and Biopharmaceutics 107:110–119.

Donnelly J, Medini D, Boccadifuoco G, Biolchi A, Ward J, Frasch C, Moxon ER, Stella M, Comanducci M, Bambini S, Muzzi A, Andrews W, Chen J, Santos G, Santini L, Boucher P, Serruto D, Pizza M, Rappuoli R, Giuliani MM. 2010. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. Proceedings of the National Academy of Sciences of the United States of America. 107:19490–19495.

Dupont J, Altclas J, Lepetic A, Lombardo M, Vázquez V, Salgueira C, Seigelchifer M, Arndtz N, Antunez E, von Eschen K, Janowicz Z. 2006. A controlled clinical trial comparing the safety and immunogenicity of a new adjuvanted hepatitis B vaccine with a standard hepatitis B vaccine. Vaccine 24:7167–7174.

Elias J, Vogel U. 2007. IS1301 fingerprint analysis of Neisseria meningitidis strains belonging to the ET-15 clone. Journal of Clinical Microbiology 45:159–167. Erratum in: 45:1078, 2007.

Feavers IM, Fox AJ, Gray S, Jones DM, Maiden MC. 1996. Antigenic diversity of meningococcal outer membrane protein PorA has implications for epidemiological analysis and vaccine design. Clinical and Diagnostic Laboratory Immunology 3:444–450.

Feil EJ, Maiden MC, Achtman M., Spratt BG. 1999. The relative contributions of recombination and mutation to the divergence of clones of Neisseria meningitidis. Molecular Biology and Evolution 16:1496–1502.

Figueroa J, Andreoni J, Densen P. 1993. Complement deficiency states and meningococcal disease. Immunologic Research 12:295–311.

Finne J, Leinonen M, Makela PH. 1983. Antigenic similarities between brain components and bacteria causing meningitis: implications for vaccine development and pathogenesis. Lancet 2:355–357.

Finne J, Bitter-Suermann D, Goridis C, Finne U. 1987. An IgG monoclonal antibody to group B meningococci cross-reacts with developmentally regulated polysialic acid units of glycoproteins in neural and extraneural tissues. Journal of Immunology 138:4402–4407.

Frasch CE, Zollinger WD, Poolman JT. 1985. Serotype antigens of Neisseria meningitidis and a proposed scheme for designation of serotypes. Reviews of Infectious Diseases 7:504–510.

Frasch CE, Borrow R, Donnelly J. 2009. Bactericidal antibody is the immunologic surrogate of protection against meningococcal disease. Vaccine 27:B112–116.

Gamougam K, Daugla DM, Toralta J, Ngadoua C, Fermon F, Page AL, Djingarey MH, Caugant DA, Manigart O, Trotter CL, Stuart JM, Greenwood BM. 2015. Continuing effectiveness of serogroup A meningococcal conjugate vaccine, Chad, 2013. Emerging Infectious Diseases 21:115–118.

Garcon N, Chomez P, Van Mechelen M. 2007. GlaxoSmithKline Adjuvant Systems in vaccines: concepts, achievements and perspectives. Expert Review of Vaccines 6:723–739.

Garty BZ, Nitzan M, Danon YL. 1993. Systemic meningococcal infections in patients with acquired complement deficiency. Pediatric Allergy and Immunology 4:6–9.

Gasque P. 2004. Complement: a unique innate immune sensor for danger signals. Molecular Immunology 41:1089–1098.

Geoffroy MC, Floquet S, Métais A, Nassif X, Pelicic V. 2003. Large-scale analysis of the meningococcus genome by gene disruption: resistance to complement-mediated lysis. Genome Research 13:391–398.

Gill CJ, Ram S, Welsch JA, Detora L, Anemona A. 2011. 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 30:29–34.

Giuliani MM, Adu-Bobie J, Comanducci M, Aricò B, Savino S, Santini L, Brunelli B, Bambini S, Biolchi A, Capecchi B, Cartocci E, Ciucchi L, Di Marcello F, Ferlicca F, Galli B, Luzzi E, Masignani V, Serruto D, Veggi D, Contorni M, Morandi M, Bartalesi A, Cinotti V, Mannucci D, Titta F, Ovidi E, Welsch JA, Granoff D, Rappuoli R, Pizza M. 2006. A universal vaccine for serogroup B meningococcus. Proceedings of the National Academy of Sciences of the United States of America 103:10834–10839.

Gold R, Goldschneider I, Lepow ML, Draper TF, Randolph M. 1978. Carriage of Neisseria meningitidis and Neisseria lactamica in infants and children. Journal of Infectious Diseases 137:112–121.

Goldschneider I, Gotschlich EC, Artenstein MS 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. Journal of Experimental Medicine 129:1307–1326.

Gorringe AR, Reddin KM, Funnell SG, Johansson L, Rytkönen A, Jonsson AB. 2005. Experimental disease models for the assessment of meningococcal vaccines. Vaccine 23:2214–2217.

Gossger N, Snape MD, Yu LM, Finn A, Bona G, Esposito S, Principi N, Diez-Domingo J, Sokal E, Becker B, Kieninger D, Prymula R, Dull P, Ypma E, Toneatto D, Kimura A, Pollard AJ; European MenB Vaccine Study Group. 2012. 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: The Journal of the American Medical Association 307:573–582.

Gotschlich EC, Goldschneider I, Artenstein MS. 1969. Human immunity to the meningococcus. IV. Immunogenicity of group A and group C meningococcal polysaccharides in human volunteers. Journal of Experimental Medicine 129:1367–1384.

Granoff DM, Maslanka SE, Carlone GM, Plikaytis BD, Santos GF, Mokatrin A, Raff HV. 1998. A modified enzyme-linked immunosorbent assay for measurement of antibody responses to meningococcal C polysaccharide that correlate with bactericidal responses. Clinical and Diagnostic Laboratory Immunology 5:479–485.

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

Hadi HA, Wooldridge KG, Robinson K, Ala'Aldeen DA. 2001. Identification and characterization of App: an immunogenic autotransporter protein of Neisseria meningitidis. Molecular Microbiology 41:611–623.

Hajishengallis G, Lambris JD. 2016. More than complementing Tolls: complement-Toll-like receptor synergy and crosstalk in innate immunity and inflammation. Immunological Reviews 274:233–244.

Halperin SA, Bettinger JA, Greenwood B, Harrison LH, Jelfs J, Ladhani SN, McIntyre P, Ramsay ME, Sáfadi MA. 2012. The changing and dynamic epidemiology of meningococcal disease. Vaccine 30:B26–B36.

Hammerschmidt S, Hilse R, van Putten JP, Gerardy-Schahn R, Unkmeir A, Frosch M. 1996a. Modulation of cell surface sialic acid expression in Neisseria meningitidis via a transposable genetic element. EMBO Journal 15:192–198.

Hammerschmidt S, Muller A, Sillmann H, Muhlenhoff M, Borrow R, Fox A, van Putten J, Zollinger WD, Gerardy-Schahn R, Frosch M. 1996b. Capsule phase variation in Neisseria meningitidis serogroup B by slipped-strand mispairing in the polysialyltransferase gene (siaD): correlation with bacterial invasion and the outbreak of meningococcal disease. Molecular Microbiology 20:1211–1220.

Harrison LH. 2006. Prospects for vaccine prevention of meningococcal infection. Clinical Microbiology Reviews 19:142–164.

Harrison LH, Jolley KA, Shutt KA, Marsh JW, O'Leary M, Sanza LT, Maiden MC, Maryland Emerging Infections Program. 2006. Antigenic shift and increased incidence of meningococcal disease. Journal of Infectious Diseases 193:1266–1274.

Harrison LH, Shutt KA, Schmink SE, Marsh JW, Harcourt BH, Wang X, Whitney AM, Stephens DS, Cohn AA, Messonnier NE, Mayer LW. 2010. Population structure and capsular switching of invasive Neisseria meningitidis isolates in the pre-meningococcal conjugate vaccine era--United States, 2000–2005. Journal of Infectious Diseases 201:1208–1224.

Hawkins LD, Ishizaka,ST, McGuinness P, Zhang H, Gavin W, DeCosta B, Meng Z, Yang H, Mullarkey M, Young DW, Yang H, Rossignol DP, Nault A, Rose J, Przetak M, Chow JC, Gusovsky F. 2002. A novel class of endotoxin receptor agonists with simplified structure, toll-like receptor 4-dependent immunostimulatory action, and adjuvant activity. Journal of Pharmacology and Experimental Therapeutics 300:655–661.

Hellerud BC, Aase A, Herstad TK, Naess LM, Kristiansen LH, Trøseid AM, Harboe M, Lappegård KT, Brandtzaeg P, Høiby EA, Mollnes TE. 2010. Critical roles of complement and antibodies in host defense mechanisms against Neisseria meningitidis as revealed by human complement genetic deficiencies. Infection and Immunity 78:802–809.

Holst J, Feiring B, Fuglesang JE, Høiby EA, Nøkleby H, Aaberge IS, Rosenqvist E. 2003. Serum bactericidal activity correlates with the vaccine efficacy of outer membrane vesicle vaccines against Neisseria meningitidis serogroup B disease. Vaccine 21:734–737.

Honko AN, Sriranganathan N, Lees CJ, Mizel SB. 2006. Flagellin is an effective adjuvant for immunization against lethal respiratory challenge with Yersinia pestis. Infection and Immunity 74:1113–1120.

Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdörfer B, Giese T, Endres S, Hartmann G. 2002. Quantitative expression of toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. Journal of Immunology 168:4531–4537.

Howell-Adams B, Seifert HS. 2000. Molecular models accounting for the gene conversion reactions mediating gonococcal pilin antigenic variation. Molecular Microbiology 37:1146–1158.

Huleatt JW, Jacobs AR, Tang J Desai P, Kopp EB, Huang Y, Song L, Nakaar V, Powell TJ. 2007. Vaccination with recombinant fusion proteins incorporating Toll-like receptor ligands induces rapid cellular and humoral immunity. Vaccine 25:763–775.

Huleatt JW, Nakaar V, Desai P, Huang Y, Hewitt D, Jacobs A, Tang J, McDonald W, Song L, Evans RK, Umlauf S, Tussey L, Powell TJ. 2008. Potent immunogenicity and

efficacy of a universal influenza vaccine candidate comprising a recombinant fusion protein linking influenza M2e to the TLR5 ligand flagellin. Vaccine 26:201–214.

Imhaus AF, Dumenil G. 2014. The number of Neisseria meningitidis Type IV pili determines host cell interaction. EMBO Journal 33:1767–1783.

Jafri RZ, Ali A, Messonnier NE, Tevi-Benissan C, Durrheim D, Eskola J, Fermon F, Klugman KP, Ramsay M, Sow S, Zhujun S, Bhutta ZA, Abramson J. 2013. Global epidemiology of invasive meningococcal disease. Population Health Metrics 11:17.

Janeway CA Jr, Medzhitov R. 2002. Innate immune recognition. Annual Review of Immunology 20:197–216.

Jennings MP, Hood DW, Peak IR, Virji M, Moxon ER. 1995. Molecular analysis of a locus for the biosynthesis and phase-variable expression of the lacto-N-neotetraose terminal lipopolysaccharide structure in Neisseria meningitidis. Molecular Microbiology 8:729–740.

Jiang HQ, Hoiseth SK, Harris SL, McNeil LK, Zhu D, Tan C, Scott AA, Alexander K, Mason K, Miller L, DaSilva I, Mack M, Zhao XJ, Pride MW, Andrew L, Murphy E, Hagen M, French R, Arora A, Jones TR, Jansen KU, Zlotnick GW, Anderson AS. 2010. Broad vaccine coverage predicted for a bivalent recombinant factor H binding protein based vaccine to prevent serogroup B meningococcal disease. Vaccine 28:6086–6093.

Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, Lee H, Lee JO. 2007. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell 130:1071–1082.

Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, Han SH, Lee H, Paik SG, Lee JO. 2009. Recognition of lipopeptide patterns by Toll-like receptor 2-Toll-like receptor 6 heterodimer. Immunity 31:873–884.

Koeberling O, Seubert A, Santos G, Colaprico A, Ugozzoli M, Donnelly J, Granoff DM. 2011. Immunogenicity of a meningococcal native outer membrane vesicle vaccine with attenuated endotoxin and over-expressed factor H binding protein in infant rhesus monkeys. Vaccine 29:4728–4734.

Krieg AM, Yi AK, Matson S Waldschmidt TJ, Bishop GA, Teasdale R, Koretzky GA, Klinman DM. 1995. CpG motifs in bacterial DNA trigger direct B-cell activation. Nature 374:546–549.

Kupferschmidt K. 2014. A new vaccine vanquishes meningitis A in Africa. Science 345:1265.

Lahiri A, Das P, Chakravortty D. 2008. Engagement of TLR signaling as adjuvant: towards smarter vaccine and beyond. Vaccine 26: 6777–6783.

Lindblad E. 2004. Aluminium compounds for use in vaccines. Immunology and Cell Biology 82:497–805.

Linz B, Schenker M, Zhu P, Achtman M. 2000. Frequent interspecific genetic exchange between commensal Neisseriae and Neisseria meningitidis. Molecular Microbiology 36:1049–1058.

Loh E, Kugelberg E, Tracy A, Zhang Q, Gollan B, Ewles H, Chalmers R, Pelicic V, Tang CM. 2013. Temperature triggers immune evasion by Neisseria meningitidis. Nature 502:237–240.

Madico, G, Welsch JA, Lewis LA, McNaughton A, Perlman DH, Costello CE, Ngampasutadol J, Vogel U, Granoff DM, Ram S. 2006. The meningococcal vaccine candidate GNA1870 binds the complement regulatory protein factor H and enhances serum resistance. Journal of Immunology 177:501–510.

Maiden MCJ, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, Zhang Q, Zhou J, Zurth K, Caugant DA, Feavers IM, Achtman M, Spratt BG. 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proceedings of the National Academy of Sciences of the United States of America 95:3140–3145.

Mak PA, Santos GF, Masterman KA, Janes J, Wacknov B, Vienken K, Giuliani M, Herman AE, Cooke M, Mbow ML, Donnelly J. 2011. Development of an automated, high-throughput bactericidal assay that measures cellular respiration as a survival readout for Neisseria meningitidis. Clinical and Vaccine Immunology 18:1252–60.

Mandrell, RE, Azmi FH, Granoff DM. 1995. Complement-mediated bactericidal activity of human antibodies to poly alpha 2,8 N-acetyl-neuraminic acid, the capsular polysaccharide of Neisseria meningitidis serogroup B. Journal of Infectious Diseases 172:1279–1289.

Maslanka SE, Gheesling LL, Libutti DE, Donaldson KB, Harakeh HS, Dykes JK, Arhin FF, Devi SJ, Frasch CE, Huang JC, Kriz-Kuzemenska P, Lemmon RD, Lorange M, Peeters CC, Quataert S, Tai JY, Carlone GM. 1997. Standardization and a multilaboratory comparison of Neisseria meningitidis serogroup A and C serum bactericidal assays. Clinical and Diagnostic Laboratory Immunology 4:156–167.

Mason KW, Zhu D, Scheuer CA, McMichael JC, Zlotnick GW, Green BA. 2004. Reduction of nasal colonization of nontypeable Haemophilus influenzae following intranasal immunization with rLP4/rLP6/UspA2 proteins combined with aqueous formulation of RC529. Vaccine 22:3449–3456.

Massari P, Henneke P, Ho Y, Latz E, Golenbock DT, Wetzler LM. 2002. Cutting edge: Immune stimulation by neisserial porins is toll-like receptor 2 and MyD88 dependent. Journal of Immunology 168:1533–1537.

Mata-Haro V, Cekic C, Martin M, Chilton PM, Casella CR, Mitchell TC. 2007. The vaccine adjuvant monophosphoryl lipid A as a TRIF-biased agonist of TLR4. Science 316:1628–1632.

Mayer LW, Reeves MW, Al-Hamdan N, Sacchi CT, Taha MK, Ajello GW, Schmink SE, Noble CA, Tondella ML, Whitney AM, Al-Mazrou Y, Al-Jefri M, Mishkhis A, Sabban S, Caugant DA, Lingappa J, Rosenstein NE, Popovic T. 2002. Outbreak of W135 meningococcal disease in 2000: not emergence of a new W135 strain but clonal expansion within the electophoretic type-37 complex. Journal of Infectious Diseases 185:1596–1605.

Medzhitov R, Preston-Hurlburt P, Janeway Jr CA. 1997. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature 388:394–397.

Medzhitov R. 2001. Toll-like receptors and innate immunity. Nature Reviews. Immunology 1:135–145.

Meyer SA, Kambou JL, Cohn A, Goodson JL, Flannery B, Medah I, Messonnier N, Novak R, Diomande F, Djingarey MH, Clark TA, Yameogo I, Fall A, Wannemuehler K. 2015. Serogroup A meningococcal conjugate (PsA-TT) vaccine coverage and measles vaccine coverage in Burkina Faso–implications for introduction of PsA-TT into the Expanded Programme on Immunization. Vaccine 33:1492–1498.

Miller E, Salisbury D, Ramsay M. 2001. Planning, registration, and implementation of an immunisation campaign against meninococcal serogroup C disease in the UK: A success story. Vaccine 20:S58–S67.

Mizel SB, Graff AH, Sriranganathan N Ervin S, Lees CJ, Lively MO, Hantgan RR, Thomas MJ, Wood J, Bell B. 2009. Flagellin-F1-V fusion protein is an effective plague vaccine in mice and two species of nonhuman primates. Clinical and Vaccine Immunology 16:21–28.

Morgan BP. 1999. Regulation of the complement membrane attack pathway. Critical Reviews in Immunology 19:173–198.

Mountzouros KT, Howell AP. 2000. Detection of complement-mediated antibody-dependent bactericidal activity in a fluorescence-based serum bactericidal assay for group B Neisseria meningitidis. Journal of Clinical Microbiology 38:2878–2884.

Moxon ER, Rainey PB, Nowak MA, Lenski RE. 1994. Adaptive evolution of highly mutable loci in pathogenic bacteria. Current Biology 4:24–33.

Mustapha MM, Marsh JW, Krauland MG, Fernandez JO, de Lemos AP, Dunning Hotopp JC, Wang X, Mayer LW, Lawrence JG, Hiller NL, Harrison LH. 2016. Genomic Investigation Reveals Highly Conserved, Mosaic, Recombination Events Associated with Capsular Switching among Invasive Neisseria meningitidis Serogroup W Sequence Type (ST)-11 Strains. Genome Biology and Evolution 8:2065–2075.

Nedelec J, Boucraut J, Garnier JM, Bernard D, Rougon G. 1990. Evidence for autoimmune antibodies directed against embryonic neural cell adhesion molecules (N-CAM) in patients with group B meningitis. Journal of Neuroimmunology 29:49–56.

Nesargikar P.N, Spiller B, Chavez R. 2012. The complement system: history, pathways, cascade and inhibitors. European Journal of Microbiology & Immunology 2:103–111.

O'Hallahan J, McNicholas A, Galloway Y, O'Leary E, Roseveare C. 2009. Delivering a safe and effective strain-specific vaccine to control an epidemic of group B meningococcal disease. New Zealand Medical Journal 122:48–59.

Pace D, Pollard AJ. 2012. Meningococcal disease: Clinical presentation and sequelae. Vaccine 30:B3e9.

Packiam M, Shell DM, Liu SV, Liu YB, McGee DJ, Srivastava R. 2006. Differential expression and transcriptional analysis of the alpha-2,3-sialyltransferase gene in pathogenic Neisseria spp. Infection and Immunity 74:2637–2650.

Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO. 2009. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. Nature 458:1191–1195.

Pizza M. Scarlato V. Masignani V. Giuliani MM, Aricò B, Comanducci M, Jennings GT, Baldi L, Bartolini E, Capecchi B, Galeotti CL, Luzzi E, Manetti R, Marchetti E, Mora M, Nuti S, Ratti G, Santini L, Savino S, Scarselli M, Storni E, Zuo P, Broeker M, Hundt E, Knapp B, Blair E, Mason T, Tettelin H, Hood DW, Jeffries AC, Saunders NJ, Granoff DM, Venter JC, Moxon ER, Grandi G, Rappuoli R. 2000. Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. Science 287:1816–1820.

Plested JS, Welsch JA, Granoff DM. 2009. Ex vivo model of meningococcal bacteremia using human blood for measuring vaccine-induced serum passive protective activity. Clinical and Vaccine Immunology 16:785–791.

Pollard A.J., Frasch C. 2001. Development of natural immunity to Neisseria meningitidis. Vaccine 19:1327–1346.

Pollard AJ, Perrett KP, Beverley PC. 2009. Maintaining protection against invasive bacteria with protein-polysaccharide conjugate vaccines. Nature Reviews. Immunology 9:213–220.

Przetak, M, Chow, J, Cheng, H, Rose, J, Hawkins, LD, and Ishizaka, ST. 2003. Novel synthetic LPS receptor agonists boost systemic and mucosal antibody responses in mice. Vaccine 21:961–970.

Rodríguez T. Lastre M, Cedré B, Fajardo EM, del Campo J, Delgado I, Sierra G, Pérez O. 2003. Validation of colorimetric assay to detect complement-mediated antibody-dependent bactericidal activity against serogroups B and C Neisseria meningitidis. Biologicals 31:209–212.

Sacchi CT, Whitney AM, Popovic T, Beall DS, Reeves MW, Plikaytis BD, Rosenstein NE, Perkins BA, Tondella ML, Mayer LW. 2000. Diversity and prevalence of PorA types in Neisseria meningitidis serogroup B in the United States, 1992–1998. Journal of Infectious Diseases 182:1169–1176.

Sadarangani M, Pollard AJ. 2010. Serogroup B meningococcal vaccines: an unfinished story. Lancet. Infectious Diseases 10:112–24.

Sanchez S, Troncoso G, Criado MT, Ferreiros C. 2002. In vitro induction of memory-driven responses against Neisseria meningitidis by priming with Neisseria lactamica. Vaccine 20:2957–2963.

Santos GF, Deck RR, Donnelly J, Blackwelder W, Granoff DM, 2001. Importance of complement source in measuring meningococcal bactericidal titers. Clinical and Diagnostic Laboratory Immunology 8:616–23.

Sarkari J, Pandit N, Moxon ER, Achtman M. 1994. Variable expression of the Opc outer membrane protein in Neisseria meningitidis is caused by size variation of a promoter containing poly-cytidine. Molecular Microbiology 2:207–217.

Saunders NJ, Jeffries AC, Peden JF, Hood DW, Tettelin H, Rappuoli R, Moxon ER. 2000. Repeat-associated phase variable genes in the complete genome sequence of Neisseria meningitidis strain MC58. Molecular Microbiology 37:207–215.

Scarselli M, Serruto D, Montanari P, Capecchi B, Adu-Bobie J, Veggi D, Rappuoli R, Pizza M, Aricò B. 2006. Neisseria meningitidis NhhA is a multifunctional trimeric autotransporter adhesion. Molecular Microbiology 61:631–644.

Schneider MC, Exley RM, Ram S, Sim RB, Tang CM. 2007. Interactions between Neisseria meningitidis and the complement system. Trends in Microbiology 15:233–240.

Serruto D, Adu-Bobie J, Scarselli M, Veggi D, Pizza M, Rappuoli R, Aricò B. 2003. Neisseria meningitidis App: a new adhesin with autocatalytic serine protease activity. Molecular Microbiology 48:323–334.

Sierra GV, Campa HC, Varcacel NM, Varcacel NM, Garcia IL, Izquierdo PL, Sotolongo PF, Casanueva GV, Rico CO, Rodriguez CR, Terry MH. 1991. Vaccine against group B Neisseria meningitidis: protection trial and mass vaccination results in Cuba. NIPH Annals 14:195–210.

Skountzou I, del Pilar Martin M, Wang B Ye L, Koutsonanos D, Weldon W, Jacob J, Compans RW. 2010. Salmonella flagellins are potent adjuvants for intranasally administered whole inactivated influenza vaccine. Vaccine 28:4103–4112.

Snyder LA, Butcher SA, Saunders N.J. 2001. Comparative whole-genome analyses reveal over 100 putative phase-variable genes in the pathogenic Neisseria spp. Microbiology 147:2321–2332.

Spratt BG, Bowler LD, Zhang QY, Zhou J, Smith JM. 1992. Role of interspecies transfer of chromosomal genes in the evolution of penicillin resistance in pathogenic and commensal Neisseria species. Journal of Molecular Evolution 34:115–125.

Stein-Zamir C, Shoob H, Sokolov I, Kunbar A, Abramson N, Zimmerman D. 2014. The clinical features and long-term sequelae of invasive meningococcal disease in children. Pediatric Infectious Disease Journal 33:777e9.

Stephens DS, Greenwood B, Brandtzaeg P. 2007. Epidemic meningitis: meningococcaemia, and Neisseria meningitidis. Lancet 369:2196–2210.

Swartley JS, Marfin AA, Edupuganti S, Liu LJ, Cieslak P, Perkins B, Wenger JD, Stephens DS. 1997. Capsule switching of Neisseria meningitidis. Proceedings of the National Academy of Sciences of the United States of America 94:271–276.

Tallant T, Deb A, Kar N, Lupica J, de Veer MJ, and DiDonato JA. 2004. Flagellin acting via TLR5 is the major activator of key signaling pathways leading to NF-κB and proinflammatory gene program activation in intestinal epithelial cells. BMC Microbiology 4:33.

Tettelin H, Saunders NJ, Heidelberg J, Jeffries AC, Nelson KE, Eisen JA, Ketchum KA, Hood DW, Peden JF, Dodson RJ, Nelson WC, Gwinn ML, DeBoy R, Peterson JD, Hickey EK, Haft DH, Salzberg SL, White O, Fleischmann RD, Dougherty BA, Mason T, Ciecko A, Parksey DS, Blair E, Cittone H, Clark EB, Cotton MD, Utterback TR, Khouri H, Qin H, Vamathevan J, Gill J, Scarlato V, Masignani V, Pizza M, Grandi G, Sun L, Smith HO, Fraser CM, Moxon ER, Rappuoli R, Venter JC. 2000. Complete genome sequence of Neisseria meningitidis serogroup B strain MC58. Science 287:1809–1815.

Thompson EA, Feavers IM, Maiden MC. 2003. Antigenic diversity of meningococcal enterobactin receptor FetA, a vaccine component. Microbiology 149:1849–1858.

Thompson MJ, Ninis N, Perera R, Mayon-White R, Phillips C, Bailey L, Harnden A, Mant D, Levin M. 2006. Clinical recognition of meningococcal disease in children and adolescents. Lancet 367:397e403.

Toneatto D, Oster P, deBoer AC, Emerson A, Santos GF, Ypma E, DeTora L, Pizza M, Kimura A, Dull P. 2011. Early clinical experience with a candidate meningococcal B recombinant vaccine (rMenB) in healthy adults. Human Vaccines 7:781–791.

Toropainen M, Käyhty H, Saarinen L, Rosenqvist E, Høiby EA, Wedege E, Michaelsen T, Mäkelä PH. 1999. The infant rat model adapted to evaluate human sera for protective immunity to group B meningococci. Vaccine 17:2677–2689.

Troncoso G, Sanchez S, Criado MT, Ferreiros CM. 2002. Analysis of Neisseria lactamica antigens putatively implicated in acquisition of natural immunity to Neisseria meningitidis. FEMS Immunology and Medical Microbiology 34:9–15.

Vabulas RM, Pircher H, Lipford GB, Hacker H, Wagner H. 2000. CpG-DNA activates in vivo T cell epitope presenting dendritic cells to trigger protective antiviral cytotoxic T cell responses. Journal of Immunology 164:2372–2378.

van der Ley P, Heckels JE, Virji M, Hoogerhout P, Poolman JT. 1991. Topology of outer membrane proteins in pathogenic Neisseria species. Infection and Immunity 59:2963–2971.

van der Ende A., Hopman C.T., Dankert J. 2000. Multiple mechanisms of phase variation of PorA in Neisseria meningitidis. Infection and Immunity 68:6685–6690.

Vasilakos JP, Smith RM, Gibson SJ, Lindh JM, Pederson LK, Reiter MJ, Smith MH, Tomai MA. 2000. Adjuvant activities of immune response modifier R-848: comparison with CpG ODN. Cellular Immunology 204:64–74.

Virji M. 2000. The structural basis of CEACAM-receptor targeting by neisserial opa proteins: response. Trends in Microbiology 8:260–261.

Wang X, Cohn A, Comanducci M, Andrew L, Zhao X, MacNeil JR, Schmink S, Muzzi A, Bambini S, Rappuoli R, Pizza M, Murphy E, Hoiseth SK, Jansen KU, Anderson AS, Harrison LH, Clark TA, Messonnier NE, Mayer LW. 2011. Prevalence and genetic diversity of candidate vaccine antigens among invasive Neisseria meningitidis isolates in the United States. Vaccine 29:4739–4744.

Wille-Reece U, Flynn BJ, Lore K Koup RA, Kedl RM, Mattapallil JJ, Weiss WR, Roederer M, Seder RA. 2005. HIV Gag protein conjugated to a Toll-like receptor 7/8 agonist improves the magnitude and quality of Th1 and CD8+ T cell responses in

nonhuman primates. Proceedings of the National Academy of Sciences of the United States of America 102:15190–15194.

Wong KH, Barrera O, Sutton A, May J, Hochstein DH, Robbins JD, Robbins JB, Parkman PD, Seligmann EB Jr. 1977. Standardization and control of meningococcal vaccines, group A and group C polysaccharides. Journal of Biological Standardization 5:197–215.

World Health Organization. 1976. Requirements for meningococcal polysaccharide vaccine (requirements for biological substances no. 23). WHO Technical Report Series 594:72–73.

Zhu D, Barniak V, Zhang Y, Green B, Zlotnick G. 2006. Intranasal immunization of mice with recombinant lipidated P2086 protein reduces nasal colonization of group B Neisseria meningitidis. Vaccine 24:5420–5425.

Zollinger WD, Mandrell RE. 1983. Importance of complement source in bactericidal activity of human antibody and murine monoclonal antibody to meningococcal group B polysaccharide. Infection and Immunity 40:257–264.

Zuber AK, Brave A, Engstrom G Zuber B, Ljungberg K, Fredriksson M, Benthin R, Isaguliants MG, Sandström E, Hinkula J, Wahren B. 2004. Topical delivery of imiquimod to a mouse model as a novel adjuvant for human immunodeficiency virus (HIV) DNA. Vaccine 22:1791–1798.

CURRICULUM VITAE

GEORGE F. SANTOS

Contact information: gfsantos@bu.edu

Education:

B.S., Biological Sciences. University of Connecticut, 1982.

M.A., Molecular Biology, Cell Biology, and Biochemistry. Boston University, 2017.

Professional Positions:

1982 - 1983 *Research Assistant I, Yale University* - Investigated effects of chemotherapeutic agents on RNA expression and steroid receptor metabolism.

1983 - 1987 *Research Associate I/II, University of California San Francisco (UCSF)* - Investigated the transcriptional regulation of proto-oncogenes by anti-steroidal agents.

1988 - 1992 *Research Associate II, UCSF* - Investigated fibronectin structural domain requirements in the regulation of metalloproteinases and cytoskeletal organization.

1993 *Research Associate, Children's Hospital Oakland Research Institute (CHORI)* - Investigated the regulation Cystic Fibrosis Transmembrane Conductance Regulator (CFTR).

1994 *Research Associate II, CHORI* - Investigated the role of the cytoskeleton in the regulation of platelet activation.

1995 – 2000 *Research Specialist, Chiron Vaccines* - Preclinical and clinical studies in support of vaccine development to protect against meningococcal group C disease.

2000 – 2013 *Research Scientist / Head of Laboratory, Novartis Vaccines* - Preclinical and clinical studies in support of vaccine development to protect against meningococcal group B disease.

PATENTS

1. Publication number: 20110312510. High-Throughput Complement-Mediated Antibody-Dependent and Opsonic Bactericidal Assays. Filed: June 16, 2010, Publication date: December 22, 2011. Inventors: Puiying Annie Mak, George Santos, Jeffrey Eugene Janes, John J. Donnelly, III.

2. Publication number: 20100035234. Vaccine Assays. Filed: May 19, 2009. Publication date: February 11, 2010. Inventors: John Donnelly, Ping Wu, George Santos, Marzia Monica Giuliani, William Andrews, Jie Chen.

PUBLICATIONS

1. Benz, C., **Santos, G.**, Cadman, E.C. Tamoxifen and 5-fluorouracil in breast cancer: Modulation of cellular RNA. *Cancer Research*, 43:5304-5308, 1983.

2. Benz, C., **Santos, G.F**. Effects of cis- and trans-tamoxifen on RNA incorporation of human breast cancer cells. *Molecular Pharmacology,* 32:13-16, 1987.

3. **Santos, G.F.**, Scott, G.K., Lee, W.M., Liu, E., Benz, C. Estrogen-induced post-transcriptional modulation of c-myc proto-oncogene expression in human breast cancer cells. *Journal of Biological Chemistry,* 263:9565-9568, 1988.

4. Liu, E., **Santos, G.**, Lee, W.M., Osborne, C.K., Benz C.C. Effects of c-myc overexpression on the growth characteristics of MCF-7 human breast cancer cells. *Oncogene,* 4: 979-984, 1989.

5. Benz, C.C., Scott, G.K., **Santos, G.F.**, Smith H.S. Expression of c-myc, c-Ha-ras1, and c-erbB-2 proto-oncogenes in normal and malignant human breast epithelial cells. *Journal of the National Cancer Institute,* 81: 1704-1709, 1989.

6. **Santos, G.F.**, Reenstra, W.W. Activation of the cystic fibrosis transmembrane regulator by cyclic AMP is not correlated with inhibition of endocytosis. *Biochimica et Biophysica Acta*, 1195: 96-102, 1994.

7. Illek, B., Fischer, H., **Santos, G.F.**, Widdicombe, J.H., Machen, T.E., Reenstra W.W. Cyclic AMP-independent activation of CFTR chloride channels by

the tyrosine kinase inhibitor genistein. *American Journal of Physiology*, 268: C886-893, 1995.

8. Earnest, J.P., **Santos, G.F.**, Zuerbig, S., Fox, J.E.B. Dystrophin-related protein in the platelet membrane skeleton. Integrin-induced change in detergent-insolubility and cleavage by calpain in aggregating platelets. *Journal of Biological Chemistry*, 270:27259-27265, 1995.

9. Granoff, D.M., Maslanka, S.E., Carlone, G.M., Plikaytis, B.D., **Santos, G.F.,** Mokatrin, A, Raff, H.V. A Modified Enzyme-Linked Immunosorbent Assay for Measurement of Antibody Responses to Meningococcal C Polysaccharide That Correlate with Bactericidal Responses. *Clinical and Diagnostic Laboratory Immunology*, 5:479-485, 1998.

10. Ugozzoli, M., **Santos, G.,** Donnelly, J., O'Hagan, D. Potency of a Genetically Detoxified Mucosal Adjuvant Derived from the Heat-Labile Enterotoxin of *Escherichia coli* (LTK63) Is Not Adversely Affected by the Presence of Preexisting Immunity to the Adjuvant. *Journal of Infectious Diseases*, 183:351-354, 2001.

11. **Santos, G.F.,** Deck, R.R., Donnelly, J., Blackwelder, W., Granoff, D.M. Importance of Complement Source When Measuring Meningococcal Bactericidal Titers. *Clinical and Diagnostic Laboratory Immunology*, 8:616-623, 2001.

12. Halperin, S.A., McDonald, J., Samson, L., Danzig, L., **Santos, G.**, Izu, A., Smith, B., MacDonald, N. Simultaneous administration of meningococcal C conjugate vaccine and diphtheria-tetanus-acellular pertussis-inactivated poliovirus-Haemophilus influenzae type b conjugate vaccine in children: a randomized double-blind study. *Clin Invest Med.* 25:243-251, 2002.

13. Borrow, R., Aaberge, I.S., **Santos, G.F.**, Eudey, T.L., Oster, P., Glennie, A., Findlow, J., Hoiby, E.A., Rosenqvist, E., Balmer, P., Martin, D. . 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. *Clinical and Diagnostic Laboratory Immunology* 12:970-6, 2005.

14. Vu, D.M., de Boer, A.W., Danzig, L., **Santos, G.**, Canty, B., Flores, B.M., Granoff, D.M. Priming for immunologic memory in adults by meningococcal group C conjugate vaccination. *Clinical and Vaccine Immunology* 13:605-10, 2006.

15. **Santos, G.F.**, Giuliani, M., Santini, L., Adu-Bobie, J., Pizza, M., Rappuoli, R., Wacknov, W., Donnelly, J. Investigation on the effect of immune selection on resistance to bactericidal antibodies to group B meningococci in vitro. *Clinical and Vaccine Immunology* 16(11):1693-5, 2009.

16. Donnelly J., Medini D., Boccadifuoco G., Biolchi A., Ward J., Frasch C., Moxon E.R., Stella M., Comanducci M., Bambini S., Muzzi A., Andrews W., Chen J., **Santos G.,** Santini L., Boucher P., Serruto D., Pizza M., Rappuoli R., Giuliani M.M. Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. *Proc Natl Acad Sci USA*. 107(45):19490-5, 2010.

17. Koeberling O., Seubert A., **Santos G.**, Colaprico A., Ugozzoli M., Donnelly J., Granoff D.M. Immunogenicity of a meningococcal native outer membrane vesicle vaccine with attenuated endotoxin and over-expressed factor H binding protein in infant rhesus monkeys. *Vaccine.* 29(29-30):4728-34, 2011.

18. Toneatto, D., Oster, P., deBoer, A. C. W., Emerson, A., **Santos, G. F.**, Ypma, E. DeTora, L., Pizza, M., Kimura, A., Dull, P. Early clinical experience with a candidate meningococcal B recombinant vaccine (rMenB) in healthy adults. *Human Vaccines*. 7(7). 781-791, 2011.

19. Mak, P.A., **Santos, G.**, Kelly-Anne Masterman, Jeff Janes, Bill Wacknov, Kay Vienken, Marzia Giuliani, Ann E. Herman, Michael Cooke, Lamine Mbow, John Donnelly Development of an automated, high throughput bactericidal assay measuring cellular respiration as a survival readout for N. meningitidis. *Clinical and Vaccine Immunology* 18(8):1252-60, 2011.

20. Brito, L.A., Chan, M., Baudner, B., Gallorini, S., **Santos, G.**, O'Hagan, D., Singh, M. An alternative renewable source of squalene for use in emulsion adjuvants. *Vaccine*. 29(37):6262-8, 2011.

ABSTRACTS AND CONFERENCE PRESENTATIONS

1. Benz, C., Wiznitzer, I., and **G. Santos**. Tamoxifen-fluoropyrimidine synergy in cultured breast carcinomas: Modulation of RNA and specific estrogen binding. J. Steroid Biochem. 19:1105, 1983.

2. Benz, C., Wiznitzer, I., Hollander, C., and **G. Santos**. Preclinical studies on the effects of scheduling 5-fluorouracil with endocrine therapy in breast cancer. Amer. Soc. Clin. Oncology 3:C-152, 1984.

3. Benz, C., and **G. Santos**. RNA associated with specific estrogen binding in 5-fluorouracil with endocrine therapy in breast cancer cells. 7th Annual San Antonio Breast Cancer Symposium. 4:340, 1984.

4. Benz, C., Miller, B., and **G. Santos**. Novel approaches utilizing sequenced methotrexate and 5-fluorouracil in the treatment of endocrine-sensitive malignancies. 4th Mediterranean Congress of Chemotherapy, 1984.

5. **Santos, G.**, and C. Benz. Tamoxifen effects on nuclear and cytoplasmic RNA incorporation in human breast cancer cells. Amer. Fed. Clin. Res. 34: 1985.

6. Miller, B., **Santos, G.**, and C. Benz. Isolation of low molecular weight RNA associated with estrogen receptor in breast cancer cells. Amer. Assoc. Cancer Research 26:190, 1985.

7. **Santos, G.**, Lee, W., and C. Benz. Estrogen and antiestrogen effects on c-myc expression in human breast cancer cells. Breast Cancer Res. Treat. 8:86, 1986.

8. Liu, E., **Santos, G.**, Lee, W., and C. Benz. Phenotypic changes in MCF-7 cells after transfection with the human c-myc proto-oncogene. Amer. Assoc. Cancer Research, 27:1043, 1986.

9. **Santos, G.**, Lee, W., and C. Benz. Expression of c-myc is modulated by estradiol and tamoxifen in human breast cancer cells. Amer. Assoc. Cancer Research 27:260, 1986.

10. Benz, C., **Santos, G.**, Liu, E., and H. Smith. Short-term culturing of primary breast epithelium augments expression of c-myc and Ha-ras. Breast Cancer Res. Treat. 8:86, 1986.

11. Benz, C., **Santos, G.**, Liu, E., and H. Smith. Increased expression of growth-related oncogenes in short-term cultures of normal and malignant human breast epithelium. Amer. Fed. Clin. Res. 35: 521A, 1987.

12. **Santos, G.**, Lee, W., and C. Benz. Post-transcriptional regulation of biphasic c-myc induction following estrogen stimulation of MCF-7 breast cancer cells. Amer. Assoc. Cancer Research 28:88, 1987.

13. **Santos, G.**, Tremble, P., Schwartzbauer, J., Werb, Z., and C. Damsky. Structural features of fibronectin required for regulation of matrix

metalloproteinase expression and focal contact formation. Mol. Biol. Cell, 3s: 231a, 1993.

14. Reenstra, W.W., and **G.F. Santos**. cAMP Mediated Endocytosis in T84 Cells. FASEB J., 7:A427, 1993.

15. Reenstra, W.W. and **Santos, G.** cAMP Mediated Endocytosis and Chloride Channel Activity in T84 Cells. FASEB J., 7:A1230, 1994.

16. Fox, J.E.B., **Santos, G**., Zuerbig, S., and Saido, T.C. XVth Congress of the Int'l Soc. on Thromb. Haemost. 1995.

17. Raff, H., **Santos, G.**, Moos-Holling, R., Owens, M. Forrest, B., and D. Granoff. Correlation between ELISA and Bactericidal Activity in Infants and Toddlers Immunized with a MenC-CRM Conjugate Vaccine. 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. G85, p 158, 1996.

18. **Santos, G.**, Wacknov, B., Leong, W-P., Borrow, R., Danzig, L., Langenberg, A., Deck, R., and J. Donnelly. Comparison of Bactericidal Assays Used to Measure Immunological Responses Against Neisseria meningitidis Serogroup C. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. 53, p. 238 2000.

19. **Santos, G.F.**, Welsch, J.A., Granoff, D.M., Moyner, K., Aaberge, I.S., Wacknov, W., Danzig, L., Deck, R., Donnelly, J., Giuliani, M., Contorni, M., Morandi, M., Pizza, M., Rappuoli, R. Serum bactericidal responses in rhesus macaques immunized with novel vaccines containing recombinant proteins derived from the genome of *N. meningitidis*. 13th International Pathogenic Neisseria conference, p. 298, 2002.

20. **Santos, G.F.**, Wacknov, W., Danzig, L., Deck, R., Donnelly, J. Identification of human complement donors for use in the measurement of bactericidal activity against *Neisseria meningitidis* serogroups A, B, C. 13th International Pathogenic Neisseria conference, p. 299, 2002.

21. Martin, D., Glennie, A., McCallum, L., Rosenqvist, E., Hoiby, A., Aaberge, I., **Santos, G.**, Oster, P., Findlow, J., Borrow, R. Validating the serum bactericidal assay for group B meningococci for use in clinical trials. 7th Meeting of the European Monitoring Group on Meningococci, p 55, 2003.

22. Martin, D., Glennie, A., McCallum, L., Ruinje, N., Oster, P., Aaberge, I.S., Hoiby, A., Rosenqvist, E., Naess, L.M., **Santos, G.**, Findlow, J., Balmer, P.,

Borrow, R. Standardization and validation of the serum bactericidal assay for the measurement of immune responses to Serogroup B *Neisseria meningitidis*. 14th International Pathogenic Neisseria conference, p. 174, 2004.

23. Borrow, R., Aaberge, I.S., **Santos, G.**, Oster, P., Glennie, A., Findlow, J., Hoiby, E.A., Rosenqvist, E., Balmer, P., McCallum, L., Martin, D. Interlaboratory comparison of serum bactericidal titres against 44/76-SL before and after vaccination with the Norwegian MenBvac OMV vaccine. 14th International Pathogenic Neisseria conference, p. 157, 2004.

24. De Boer, A.W., Flores, B., Canty, B., Harmatz, P., Danzig, L.E., Izu, A.E., **Santos, G.F.**, Granoff, D.M. Kinetics of serum antibody responses to meningococcal C conjugate vaccine in adults previously immunized with meningococcal polysaccharide vaccine. 14th International Pathogenic Neisseria conference, p. 151, 2004.

25. Giuliani , M. M., Brunelli B., Biolchi A., Ferlicca, F., Santini, L., Luzzi, E., Wacknov, W., **Santos**, **G.,** Donnelly, J., Rappuoli, R., Pizza, M. Preclinical evaluation of a recombinant protein MenB vaccine with various adjuvants in mice and nonhuman primates. 9th Meeting of the European Monitoring Group on Meningococci, p. 92, 2007.

26. Donnelly, J., **Santos, G.**, Schaefer, M., Pizza, M., Rappuoli, R., Otten, G. *In vitro* studies of the biologic activity of components of a recombinant vaccine (MRB197) against *N. Meningitidis* group B. Meningococci *in vitro*. 16th International Pathogenic Neisseria conference, p. 279, 2008.

27. **Santos, G.F.**, Giuliani, M., Pizza, M., Rappuoli, R., Wacknov, W., Donnelly, J. Investigations on the effect of immune selection on resistance to bactericidal antibodies to Group B Meningococci *in vitro*. Meningococci *in vitro*. 16th International Pathogenic Neisseria conference, p. 157, 2008.

28. **Santos, G.**, Mak, A., Masterman, K., Wacknov, B., Janes, J., Herman, A., Cooke, M., Mbow, L., Valiante, N., Vienken, K., Giuliani, M., and Donnelly, J. Development of a high throughput meningococcal bactericidal assay. 10th Meeting of the European Monitoring Group on Meningococci, p. 109, 2009

29. **Santos, G.F.**, Jimenez, M., Goodman, R., Giuliani, M., Pizza, M., Rappuoli, R., Donnelly, J. Use of immunoglobulin depletion to develop a universal complement source for use in the meningococcal bactericidal assay. 17th International Pathogenic Neisseria conference, p. 170, 2010.

30. **Santos, G.**, Giuliani, M., Pizza, M., Rappuoli, R., Donnelly, J. Determination of serum bactericidal titers against group B meningococcus by flow cytometry using fluorescent viability dyes. 11th Meeting of the European Monitoring Group on Meningococci, p. 159, 2011

31. Granoff, D., Shaughnessy, J., Beernink, P., Vasudhev, S., **Santos, G.,** and Ram, S. Heterogeneity of binding of Rhesus macaque factor H to meningococcal factor H binding protein. 18th International Pathogenic Neisseria conference, p. 439, 2012.