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# Factor H and Neisserial pathogenesis

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## Abstract

Both *Neisseria gonorrhoeae* and *N. meningitidis* bind to factor H which enhances their ability to evade complement dependent killing. While porin is the ligand for human fH on gonococci, meningococci use a lipoprotein called factor H binding protein (fHbp) to bind to factor H and enhance their ability to evade complement dependent killing. This protein is currently being intensively investigated as a meningococcal vaccine candidate antigen. Consistent with the observation that meningococci cause natural infection only in humans, the organism resists human complement, and are more readily killed by complement from lower animals. This human species-specific complement evasion has important implications for evaluation of vaccine-elicited antibodies using non-human complement sources and development of animal models of disease.

## Complement inhibition by factor H

Complement forms an important arm of innate immune defenses against invading pathogens. Complement activation leads to deposition of C3 fragments (C3b and iC3b), which can enhance opsonophagocytosis of microbes. Subsequent activation of the terminal complement pathway results in direct complement-dependent killing of gram-negative bacteria. Microbes have developed several sophisticated mechanisms to limit complement activation on their surface to enable them to survive in their hosts.

One mechanism is binding of factor H, which is the main fluid-phase inhibitor of the alternative complement pathway. Factor H acts as a cofactor for the factor I-mediated cleavage of C3b to the hemolytically inactive iC3b molecule [1–3]. Furthermore, factor H can cause irreversible dissociation of C3b, Bb (alternative pathway C3 convertase) which is termed decay-acceleration [4,5]. Factor H is composed entirely of 20 short consensus repeat (SCR) domains and each domain is composed of about 60 amino acids [6]. The first four *N*-terminal SCRs are sufficient for these cofactor and decay accelerating activities [7]. Binding of factor H to host cell surfaces [8–10] and microbes [11,12] occurs through one or more of the 15 C-terminal SCRs. Thus, binding factor H through domains distinct from its functional domains (SCRs 1–4) allows microbes the use of factor H to limit complement activation on their surface.

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## Complement deficiencies and meningococcal infection

The critical role of the terminal complement pathway in innate immune defenses against Neisserial disease is well illustrated by epidemiological observations that persons deficient in terminal complement proteins (C5, C6, C7, C8 or C9) suffer a very high incidence of invasive meningococcal disease compared to normal individuals [13–15]. For example, a study reported that the risk of meningococcal disease among C7- and C9-deficient Japanese was approximately 10,000- and 1,400-fold greater, respectively, than that in the complement-sufficient Japanese population [16]. Interestingly, invasive meningococcal disease is the only infection that these individuals are more susceptible to. Alternative pathway deficiencies also are associated with a high incidence of meningococcal disease, as seen in families with properdin (X-linked recessive) [14,17–20] and factor D (autosomal recessive) [21] deficiencies.

Mortality in meningococcemia directly correlates with circulating endotoxin levels and the extent of complement activation [22]. Previous studies have shown that insertion of C5b-9 into the membrane of *E. coli* results in the release of lipid A from the bacterial membrane [23,24]. Consistent with this observation, an increase in circulating endotoxin levels were observed when a C6-deficient individual with meningococcemia was given fresh frozen plasma to correct coagulopathy, which also resulted in a temporary restoration of C6 levels [25]. Thus, at least one explanation for the lower mortality of persons with terminal complement levels may be lower lipid A release from meningococci with resulting lower circulating endotoxin levels. Persons with complement deficiency are more predisposed to invasive infections caused by less virulent serogroups such as W-135, Y, 29E and X, or by nongroupable isolates, which may also account for their lower mortality [14,15,26–28].

In contrast, properdin deficiency is associated with higher mortality [14]. Properdin stabilizes the alternative pathway C3/C5 convertases [29] and plays an important role in amplification of C3 deposition on the bacterial surface. This observation underscores the importance of the alternative pathway in defenses against meningococcal infections. Therefore, evasion of this pathway is important for the ability of the meningococcus to establish a niche in humans, its only known natural host.

## Factor H and meningococcal disease

Approximately 15 families with factor H deficiency have been reported [30]. Complete deficiency of factor H results in uncontrolled alternative pathway activation and consumption of complement components C3 and factor B and reduced or absent AP50 and CH50 levels. Uninhibited complement activation results in membranoproliferative glomerulonephritis (also called measangiocapillary glomerulonephritis), a condition characterized by thickening of the glomerular capillary wall together with hypercellularity in mesangial areas. Because of the lack of functional C3, such persons are also predisposed to invasive meningococcal disease.

Conversely, higher levels of factor H may be disadvantageous for the host because an excess of factor H relative to complement activatory molecules may impede optimal complement activation. A fine balance between the activatory and inhibitory arms of the complement system ensures that complement is activated appropriately on invading pathogens. While too much activation will result in host tissue damage, excessive regulation could facilitate microbial survival *in vivo*. Accordingly, a polymorphism in the NF-kB responsive element in the promoter region of factor H (C–496T) that results in higher factor H levels increases susceptibility to disease by a factor of ~2 [31]. The association of the C/C homozygous phenotype with serogroup C disease was even stronger (odds ration 2.9). Adding pure factor H to whole blood increased survival of serogroups A and C, but not serogroup B, meningococci [31].

## Lipooligosaccharide sialylation: the contrasts between gonococci and meningococci

Previous work has shown that *Neisseria gonorrhoeae* bind to factor H to down-regulate complement. Certain gonococcal isolates bind to factor H and become resistant to serum only when their lipooligosaccharide (LOS) is sialylated [32]. This is termed 'unstable serum resistance' because reversion to the serum sensitive phenotype occurs when the bacteria are passaged on media without added cytidinemonophospho-*N*-acetylneuraminic acid (CMP-NANA), the donor molecular for sialic acid. Two LOS structures can serve as acceptors for sialic acid (i) the lacto-N-neotetraose (LNT) LOS (Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→4Hep1) and the $P^K$-like LOS (Galα1→4Gal β1→4Glcβ1→4Hep1). Sialylation of only LNT LOS, but not the $P^K$-like LOS results in enhanced factor H binding [33]. Certain strains of *N. gonorrhoeae* that express the porin (Por) 1A molecule (now designated PorB.1A) can bind to factor H and are serum resistant even when their LOS molecule is not sialylated [34]. This is termed stable serum resistance and stably serum resistant strains often cause extragenital manifestations such as tenosynovitis and septic arthritis (disseminated gonococcal infection) [35,36].

While sialylation of gonococcal LNT LOS results in enhanced factor H binding, sialylation of LNT LOS in meningococci does not result in increased factor H binding. The reason for this difference among the two species is the requirement for the concomitant expression of gonococcal Por; allelic replacement of meningococcal PorB with gonococcal PorB results in increased factor H binding when meningococcal LOS is sialylated [37].

## Identification of the ligand for factor H on meningococci

Akin to gonococci, meningococci also bind to factor H and it was initially speculated that meningococcal PorB3 was the ligand for factor H based on its homology to gonococcal PorB. 1A [38]. While purified PorB3 was shown to bind to factor H in an ELISA assay [39], two lines of evidence suggested that PorB3 was not the acceptor for factor H on intact bacteria. First, deletion of PorB3 did not result in decreased factor H binding to bacteria [40,41], and second, allelic replacement of *porB2* of a factor H nonbinding strain with *porB3* from a factor H binding strain did not restore factor H binding [40].

Immunoblotting of meningococcal cell lysates and membrane preparations identified a ~29 kD target for factor H [40,41] and MALDI-TOF analysis identified the target as genome-derived Neisserial antigen (GNA) 1870 (also called LP2086) [40]. GNA1870 was confirmed to be a target for factor H on whole bacteria by (i) deleting *gna1870* and abrogating factor H binding and (ii) by blocking factor H binding with specific anti-GNA1870 mAbs [40].

Two classification schemes currently exist for GNA1870/LP2086 based on DNA sequence analysis and immunochemical reactivity. The system proposed by Masignani et al divided GNA1870 into variant 1, 2 and 3 proteins [42], while Fletcher et al classified LP2086 into A and B subfamilies [43]. Both groups are currently in Phase 1 and 2 clinical trials testing vaccines with formulations containing fHbp as a principal antigen. Regardless of the nomenclature used, factor H binds to all variants or families of GNA1870 or LP2086, respectively [40]. However, the expression level of GNA1870/LP2086 varies across meningococcal strains [42,43] and the amount of factor H binding to bacteria parallels fHbp expression levels; strains that express very low levels of the lipoprotein show no detectable factor H binding by flow cytometry [40]. We have found that select monoclonal antibodies specific to meningococcal fHbp inhibit this binding of factor H to fHbp.

### *N. gonorrhoeae* also possess *gna1870*

The gonococcal homologue of fHbp, NGO033, is not predicted to contain a signal sequence to direct membrane localization or lipid modification. In naturally occurring isolates, the presence of a "G residue" after position 36 (relative to the MC58 sequence) shifts the reading frame and results in loss of the signal peptidase II motif. Homology with the meningococcal fHbp is restored by an 8 bp insertion (CGGAGGCG) after position 73. When examined on western blot the gonococcal fHbp is approximately 6 kD larger than the meningococcal protein, consistent with a lack of signal peptide cleavage. Not surprisingly, deleting the fHbp homolog in gonococci did not result in decreased factor H binding or an alteration in serum resistance (our unpublished observations), consistent with the lack of a signal sequence which would predict that this protein is located in the cytoplasm. The function of the gonococcal fHbp homolog remains unclear.

## Functional significance of factor H binding to meningococci

GNA1870/LP2086 was renamed factor H binding protein (fHbp) after a series of studies elaborated the importance of this protein to *Neisseria meningitidis'* survival in human serum. In a bactericidal assay, Schneider et al showed increased killing of meningococci in human serum depleted of factor H compared to the same serum reconstituted with physiological amounts of factor H [41]. Similarly, insertional inactivation of *gna1870* deleting protein expression, prevented binding of factor H directly to the bacterial surface, and resulted in increased sensitivity of bacteria to complement-dependent killing in a serum bactericidal assay [40]. The dependence of a strain on fHbp for its survival in blood appears to vary with the level of fHbp expressed. When fHbp is deleted in high fHbp expresser strains, the strain is unable to survive in nonimmune human blood whereas the corresponding wildtype strain thrives [44]. In contrast, when fHbp is deleted from strains naturally expressing low amounts of the protein, the bacteria are able to grow in nonimmune human blood similarly to the corresponding wildtype strains (Fig. 1). Thus, although the functional significance of fHbp for the survival of the bacteria in the human host is clear, these data imply that there are additional alternate survival mechanisms employed by the low expressing strains. It is important to note that while binding of factor H to PorB.1A-bearing or sialylated PorB.1B gonococci results in high levels of serum resistance [32,34], factor H binding to meningococci that lack a capsule does not result in the same degree of serum resistance. Thus capsular polysaccharide expression remains necessary for high-level meningococcal serum resistance and for virulence [45–49].

## fHbp as a vaccine candidate

Prior to the discovery that the protein bound human factor H, fHbp was simultaneously identified by two different groups [42,43] as a highly immunogenic, universally expressed vaccine candidate against group B disease. As a vaccine fHbp is appealing because the expression of the protein by the bacteria enhances serum resistance. Thus, variants that do not express fHbp as a result of selection pressure by vaccine elicited antibodies will likely be relatively serum sensitive and at a survival disadvantage.

Expression levels of fHbp determine the bactericidal efficacy of anti-fHbp mAbs. Certain murine mAbs, and particularly those that belong to the IgG3 subclass such as JAR 3, were bactericidal against strains that express high levels of fHbp (such as strain H44/76) but not against low fHbp expressing strains (such as NZ98/254). A likely explanation for this observation is that epitope density on high fHbp expressing strains is adequate to permit C1q engagement by adjacent Fc domains with subsequent C4 activation and C4b deposition. In contrast, the distance between Fc domains on low expressers is too great to allow C1q binding. Consistent with this hypothesis, JAR 3 bound only to a high fHbp-expressing strain, but not to a low fHbp expresser, could mediate C4b deposition. However combination of anti-fHbp

mAbs (JAR 3 plus JAR 4) could overcome the obstacle of low epitope density and mediate C4b binding to the low fHbp expressing strain (Fig. 2) [50,51]. Additionally, mutants over-expressing fHbp are more susceptible to bactericidal activity of fHbp specific monoclonal antibodies [44]. Some of these mAbs inhibit binding of factor H to the surface of the bacteria, which could contribute to enhanced bactericidal activity of the mAbs due to increased serum sensitivity.

## Species specificity of factor H binding to Neisseriae and its implications

We have shown previously that serum resistance of *N. gonorrhoeae* is species-specific. *N. gonorrhoeae* bind to human complement inhibitory proteins such as C4bp-binding protein (C4BP; classical pathway inhibitor) and factor H in a species-specific manner. PorB.1B-bearing strains that can bind to human C4BP can also bind to chimpanzee C4BP and resist chimpanzee serum [52]. However, all serum resistant strains of *N. gonorrhoeae* strains tested so far are susceptible to killing by complement from other primates such as baboons and rhesus, and lower animals such as rabbits, guinea pigs and rats. Adding purified human C4BP or factor H to nonhuman sera restores the ability of *N. gonorrhoeae* to resist heterologous complement [52]. These results may in part explain the restriction of natural gonococcal infection to humans.

Akin to *N. gonorrhoeae*, *N. meningitidis* is a human specific pathogen. To investigate the underlying reason, we first compared binding of factor H present in human serum to *N. meningitidis* with factor H from other primates (chimpanzee, baboon and rhesus macaque). Compared to human factor H, we found weak binding to chimpanzee factor H and barely detectable binding to rhesus macaque and baboon factor H [53]. Furthermore, several meningococcal strains that express high levels of fHbp and resist killing by human complement are readily killed by infant rat complement. Adding purified human factor H to infant rat serum restores the ability of meningococci to resist complement-dependent killing.

Species-specificity of factor H binding has two important implications. First, baby rabbit complement which is often used as a complement source to evaluate the efficacy of killing by antibodies elicited by vaccination of humans yields higher killing titers than human complement [54,55]. These higher titers may result from lack of down-regulatory effects of rabbit factor H, which does not bind to the meningococcal surface. Consistent with this hypothesis, we have shown that adding purified human factor H to baby rabbit complement resulted in a 8- to 60-fold drop in serum bactericidal titers of polysaccharide vaccine recipients to levels that more closely resemble that seen with human complement. Second, any *in vivo* animal models of meningococcal bacteremia [56–59] are inherently flawed because of the inability of non-human factor H to bind to bacteria and inhibit complement activation on the bacterial surface. Absence of bound factor H will increase serum sensitivity of the bacteria, making the bacteria easier to kill than would be the case in a human system. We have observed that administering purified human factor H concomitantly with bacteria in the infant rat model increased the level of bacteremia. This observation provides the rationale to create transgenic animals that express human factor H in order to have a more biologically relevant *in vivo* model.

## CONCLUSIONS

Factor H plays a key role for both *N. gonorrhoeae* and *N. meningitidis* to evade complement dependent killing. We have described the different ligands for factor H on the two species. Gonococci lack a capsule and rely on binding of complement inhibitors to survive in the human host. On the other hand, meningococci rely on capsular polysaccharide expression for high-level serum resistance and virulence. The crucial role of fHbp is illustrated by the fact that all invasive isolates express this protein, albeit in varying amounts. High expressers of fHbp are dependent on binding factor H for survival in human blood. Although binding of factor H to

strains that are naturally low expressers of fHbp also may increase the ability of the organism to evade human innate host defenses, the low-expressing strains likely also possess additional mechanisms of complement evasion. Studies of fHbp expression in carriage isolates will shed light on the importance of this molecule in causing invasive disease. The species-specific binding of factor H to both *N. gonorrhoeae* and *N. meningitidis* provides another reason for the human-specific nature of Neisserial infection. Creation of transgenic animals that express human complement inhibitors will improve existing animal models of gonorrhea and meningococcemia. Finally, detailed structural analysis of factor H-fHbp interactions may aid development vaccines and therapeutic molecules.

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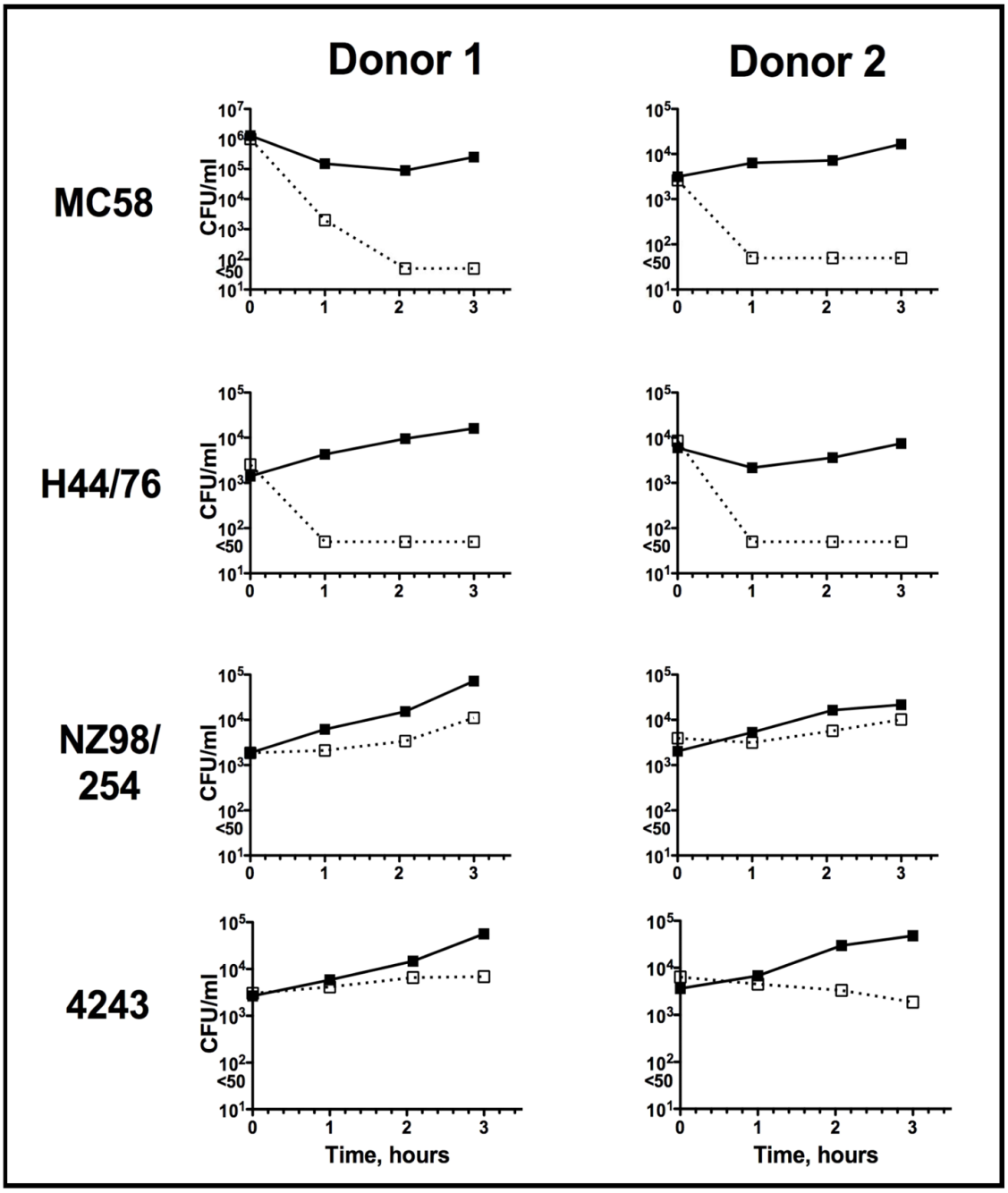


**Figure 1.**

Survival of wild-type meningococci and their fHbp deletion mutants in human blood. Meningococcal strains MC58 and H44/76 (high fHbp expressers) and NZ98/254 and 4243 (low fHbp expressers) and their fHbp deletion mutants were examined for their ability to survive in whole blood derived from two individual donors. Survival of the wild-type strains and their respective fHbp mutants are shown by the solid and broken lines, respectively. Loss of fHbp on only the high expressers, but not the low expressers, compromised bacterial survival. The data are from Ref. [44].

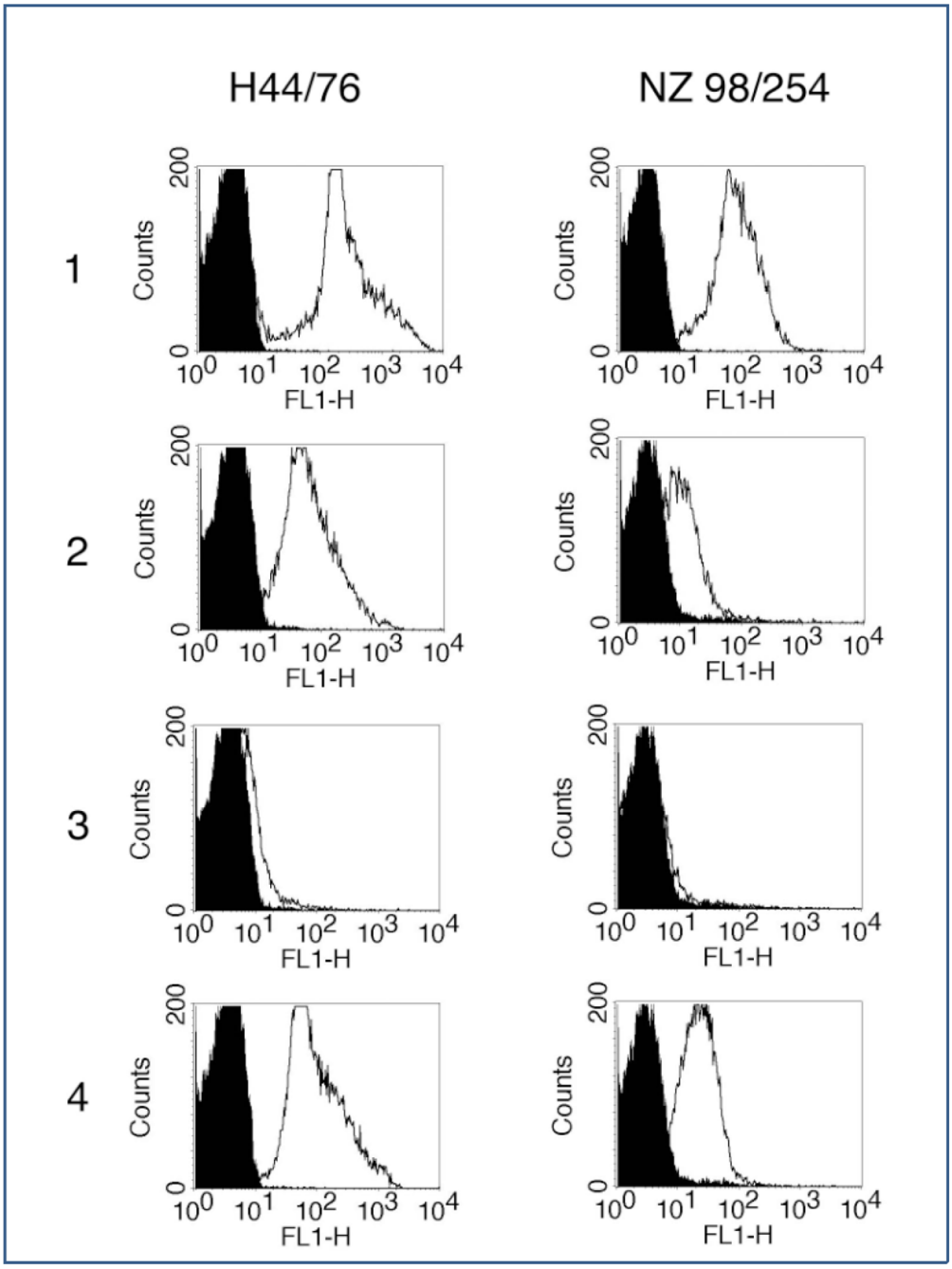


**Figure 2.**

Binding of human C4b to the surface of live encapsulated *Neisseria meningitidis* cells, as determined by indirect fluorescence flow cytometry. The left column shows results for the group B strain H44/76 (high fHbp expresser), and the right column shows results for the group B strain NZ98/254 (low fHbp expresser). Black areas in histograms indicate bacteria with 5% nonimmune human serum, and white areas indicate bacteria with monoclonal antibodies and 5% nonimmune human serum. Row 1: left, anti-PorA, P1.7, 2 μg/mL; right, anti-PorA, P1.4, 10 μg/mL. Row 2: JAR 3, 10 μg/mL. Row 3: JAR 4, 50 μg/mL. Row 4: JAR 3 plus JAR 4 (1 μg/mL for each). The data are adapted from Ref. [44].