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*ImmunoHorizons* 2019, 3 (11) 519-530
doi: https://doi.org/10.4049/immunohorizons.1900031
http://www.immunohorizons.org/content/3/11/519

This information is current as of June 9, 2021.

**Supplementary Material**
http://www.immunohorizons.org/content/suppl/2019/11/05/3.11.519.DCSupplemental

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Effect of a C1s Inhibitor on the Efficacy of Anti-Capsular Antibodies against *Neisseria meningitidis* and *Streptococcus pneumoniae*

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**ABSTRACT**

Terminal complement pathway inhibition at the level of C5 alleviates symptoms of several diseases associated with complement overactivation. However, C5 blockade is associated with an increased risk of invasive meningococcal disease despite immunization. Targeting specific complement pathways proximal to C5 provides the theoretical advantage of leaving the other pathways (including the terminal pathway) intact for immune surveillance. We aimed to address the risk of *Neisseria meningitidis* and *Streptococcus pneumoniae* infections when inhibiting the classical pathway (CP) using a specific C1s inhibitor (TNT005). Addition of TNT005 to 20% normal human serum that contained anti-meningococcal capsular Ab decreased C4 deposition 8-fold and abrogated killing of *N. meningitidis*, despite leaving C3 deposition intact. TNT005 impaired killing of *N. meningitidis* in 78% nonimmune human plasma and 78% whole blood but permitted killing in both when specific anti-capsular Ab was added. Simultaneously inhibiting both the CP and alternative pathway (AP) blocked killing of Ab-coated *N. meningitidis* in whole blood. Blocking the AP alone abrogated C3 deposition, whereas TNT005 only partially inhibited (~40% decrease) C3 deposition on *S. pneumoniae* coated with anti-capsular Ab. Blocking either the CP or AP alone did not impair killing of pneumococci in whole blood containing specific Ab (~10% survival at 3 h); however, blocking both pathways resulted in ~35% bacterial survival. These data suggest that killing of *N. meningitidis* or *S. pneumoniae* in whole blood containing specific anti-capsular Abs is unimpeded by TNT005. Meningococcal and pneumococcal capsular conjugate vaccines may mitigate risk of these infections in patients receiving C1s inhibitors. *ImmunoHorizons*, 2019, 3: 519–530.

**INTRODUCTION**

In addition to its key role in innate immune defenses against invading pathogens (1), the complement system contributes to several other important physiological functions, including modulation of adaptive immune responses, Clearance of immune complexes and apoptotic cells, tissue regeneration and organogenesis, neural synaptic pruning, and surveillance of malignant cells (2–6). A delicate balance between activation and inhibition enables the complement cascade to perform functions such as marking pathogens while limiting damage to "bystander" host cells. Imbalances in this equilibrium that tilt the balance toward excessive activation

Received for publication April 17, 2019. Accepted for publication October 15, 2019.

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This work was supported by a sponsored research agreement between the University of Massachusetts Medical School (to L.A.L.) and True North Therapeutics. L.A.L. and S.R. were also supported by National Institutes of Health/National Institute of Allergy and Infectious Diseases Grants AI118161, AI141181, AI114790, and AI132296. L.A.L., S.R., and S.P. designed and performed experiments, interpreted results, and wrote the manuscript. L.A.L. performed experiments, R.B.D. and G.C.P. generated reagents. All authors discussed the results and commented on the manuscript.

Abbreviations used in this article: anti–FBb, mAb against the Bb fragment of human FB; anti–Sp, anti–type 4 pneumococcal typing sera; AP, alternative pathway; C₃, normal human complement; CP, classical pathway; FB, Factor B; NHS, normal human serum.

The online version of this article contains supplemental material.

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https://doi.org/10.4049/immunohorizons.1900031

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result in conditions such as atypical hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria and contribute to the pathology of age-related macular degeneration (7–11).

Aberrant complement activation is also observed in autoimmune diseases in which Abs against host cells trigger the classical pathway (CP) to drive pathology. In cold agglutinin disease, IgM autoantibodies (cold agglutinins) directed against erythrocytes activate the CP and lead to complement deposition and subsequent removal of complement-opsonized cells by the mononuclear phagocyte system, a process known as extravascular hemolysis (12–16). Previous work using an inhibitor that targets C1s, a CP-specific serine protease, has shown that CP inhibition prevents C3b/iC3b deposition on the surface of human erythrocytes, rescuing them from phagocytosis by macrophage-like cells (17). These in vitro results have translated into clinical efficacy, in which administration of the anti-C1s mAb sutimlimab to primary cold agglutinin disease patients resulted in rapid cessation of hemolysis and a subsequent rise in hemoglobin of nearly 4 g/dl (13, 18, 19).

However, given its central role in fighting infections, blockade of complement carries with it an increased risk of infections (1, 20, 21). Study of individuals with congenital deficiencies of complement components have provided an insight into the role complement, and in some cases, specific complement pathways, in host defense. Deficiencies in components of the alternative pathway (AP) and terminal pathways are almost exclusively associated with an increased risk of invasive meningococcal infection (1). Defects of CP components are associated with sinopulmonary infections, bacteremia and meningitis caused by pathogens including Streptococcus pneumoniae and Haemophilus influenzae (1, 20, 21). CP defects may also increase the risk of Neisseria meningitidis infections; of the 32 cases of C2 deficiency recorded by Densen and Figueroa (1), six individuals suffered invasive meningococcal infection.

Individuals being treated with complement inhibitors are likely to be at an increased risk of infection. Therefore, these patients are immunized against N. meningitidis, S. pneumoniae, and H. influenzae in an attempt to reduce the risk of infection (22). However, blockade of C5 with eculizumab is associated with an ~1000–2000-fold increased risk of invasive meningococcal infections, despite immunization with meningococcal vaccines (23–25).

In this study, we examine the effects of blocking CP activity using the anti-C1s inhibitor TNT005 on complement activation and killing of S. pneumoniae and N. meningitidis in an effort to define the potential risk of these infections in individuals on C1s inhibitor therapy.

MATERIALS AND METHODS

Bacterial strains
S. pneumoniae TIGR4 (26) and group C N. meningitidis strain 4243 (C:2a:PL5,2:ST-11) (27) have been described previously.

Human complement
Normal human serum (NHS) was obtained from healthy adult volunteers with no history of meningococcal infection who provided informed consent. Participation was approved by the University of Massachusetts institutional review board for the protection of human subjects. Serum was obtained from whole blood that was clotted at 25°C for 30 min followed by centrifugation at 1500 × g for 20 min at 4°C. To study the effects of specific anti-capssule Ab against S. pneumoniae or N. meningitidis without confounding natural anti-meningococcal or anti-pneumococcal Abs present in NHS, we depleted IgG and IgM from freshly collected human serum, as described previously (28). Briefly, EDTA (final concentration 10 mM) and NaCl (final concentration 1 M) were added to freshly prepared human serum, and treated sera were passed first over protein A/G plus agarose (Pierce), followed by passage through anti-human IgM agarose (Sigma-Aldrich); both columns were equilibrated in PBS containing 10 mM EDTA and 1 M NaCl. NaCl was added to minimize loss of C1q during passage of serum through the anti-human IgM column. The flow-through was collected, spun concentrated, and dialyzed against PBS/0.1 mM EDTA to its original volume using a 10-kDa cutoff Amicon Ultra-15 centrifugal filter device (MilliporeSigma, Bedford, MA), sterilized by passage through a 0.22-μm filter (MilliporeSigma), aliquoted, and stored at −70°C. Hemolytic activity was confirmed using a total complement hemolytic plate assay (The Binding Site, Birmingham, U.K.). This source of complement (NHS depleted of IgG and IgM) is referred to in this article as normal human complement (C').

C1q-depleted serum and purified C1q
C1q-depleted serum and purified C1q were from Complement Technology. In some experiments, C1q-depleted serum was reconstituted with C1q to a final concentration of 70 μg/ml.

TNT005 and isotype control F(ab′)2
TNT005 is an IgG2a mouse mAb that was discovered in a hybridoma library generated from mice immunized with human C1s. TNT005 F(ab′)2 and an isotype control F(ab′)2 were generated using the Pierce F(ab′)2 Preparation Kit (catalog number 44988; Thermo Fisher Scientific) per the suggested manufacturer's protocol. TNT005 F(ab′)2 was used in all experiments and is referred to as simply TNT005 throughout the article. The isotype control F(ab′)2 will be referred to as “F(ab′)2.”

Abs
Anti-type 4 pneumococcal typing sera (anti-Sp) was obtained from Cedarlane [product number 16747(SS)], and anti-meningococcal group C capsule mAb KS-C-1 (mouse IgG3; hereafter called anti-Nm) was kindly provided by Dr. Dan M. Granoff (Children's Hospital Oakland Research Institute, Oakland, CA) (29). Polyclonal sheep anti-human C3c FITC was from Bio-Rad (product code, 2222-6604F; formerly AbD Serotec). Polyclonal goat anti-human C4 FITC was from MP Biochemicals (catalog no. O. S5168). Note that the polyclonal anti-C3c Ab recognizes both C3b and its cleavage product, iC3b. Similarly, anti-C4 recognizes bacteria-bound C4b and its cleavage product C4d (cleavage of C4b releases C4c into solution; C4d remains bound to bacteria). The terms “C3 deposition” and “C4 deposition” used in this study refer to all fragments of C3 and C4 associated with bacteria.

https://doi.org/10.4049/immunohorizons.1900031
Factor B (FB) function in C’, plasma, and blood was blocked with a mAb against the Bb fragment of human FB (anti-FBb; catalog no. A2227, final concentration of 72 μg/ml; Quidel). Anti-FBb added to human serum to a final concentration of 125 μg/ml completely blocks C3 deposition on zymosan (30).

**Flow cytometry**
The amount of C3 and C4 deposited on bacteria was measured by flow cytometry, using previously published methods (31, 32). Data were acquired on either a FACSCalibur or LSRII flow cytometer, and data were analyzed using FlowJo software.

**Serum bactericidal assays**
Bactericidal assays were performed as described previously (33, 34). Approximately 2000 CFUs of *N. meningitidis* were incubated with 20% human complement (C’, IgG, and IgM depleted NHS) in the presence of anti–group C capsule Ab, with or without added TNT005 F(ab’)2 or control (concentration indicated for each experiment). The final volume of the bactericidal reaction mixture was 100 μl. Aliquots of 20-μl reaction mixtures were plated onto chocolate agar in duplicate at the beginning of the assay (t0) and again after incubation at 37°C for 30 min (t30). Survival was calculated as the number of viable colonies at t30 relative to t0.

**Whole blood killing assays**
Whole blood killing assays were performed as described by Konar and Granoff (35). In brief, blood obtained from adults not previously immunized with any meningococcal vaccine was anticoagulated with lepirudin (50 μg/ml final concentration). Plasma was obtained from anticoagulated whole blood following centrifugation. Bacteria (*N. meningitidis* or *S. pneumoniae*) were added to whole blood or plasma (78%) alone or with complement inhibitors as described: TNT005 F(ab’)2 (50 μg/ml), isotype control F(ab’)2 (50 μg/ml), and/or anti-FBb (catalog no. A2227, final concentration of 72 μg/ml; Quidel). Aliquots of the reaction mixtures were plated onto chocolate agar in duplicate at the beginning of the assay (t0) and again after incubation at 37°C for 60 min (t60) and 3 h (t180). Survival was calculated as the number of viable colonies at t60 and t180 relative to t0.

**Statistics**
Comparisons across multiple groups were made by ANOVA and pairwise comparisons were made by Tukey multiple comparisons test.

**RESULTS**

**The CP is essential for killing of *N. meningitidis* by anti-capsule Ab**
Complement-dependent bactericidal activity is a well-established correlate of protection against invasive meningococcal infection (36). *N. meningitidis* (strain 4243) showed >100% survival (i.e., growth) when incubated with normal human complement (NHS depleted of IgG and IgM) alone (Fig. 1). However, when first treated with anti–group C capsule Ab, exposure to 20% normal human complement resulted in 80% killing. The addition of TNT005 F(ab’)2 (referred to as TNT005 throughout this article) resulted in dose-dependent survival; only 43% survival was seen in the presence of 0.5 μg/ml of the drug, while >100% survival was seen at 5 or 10 μg/ml (Fig. 1). An isotype control F(ab’)2 did not alter survival of group C strain 4243 at any concentration tested.

**Anti-capsule Ab–mediated deposition of C4, but not C3, on *N. meningitidis* is decreased by TNT005**
Complement activation by vaccine Ab is critical for protection against invasive meningococcal disease (36). Whereas human
complement (NHS depleted of IgG and IgM) deposited minimal C4 on *N. meningitidis* above conjugate control levels, addition of anti–group C capsule Ab enhanced C4 deposition ~50-fold (Fig. 2). TNT005 decreased C4 deposition in a dose-dependent manner; the highest dose of TNT005 tested (10 μg/ml) decreased C4 deposition ~8-fold, with minimal residual C4 deposition seen on bacteria (~6-fold more than seen with complement alone). The isotype control F(ab')2 did not affect C4 deposition.

Opsonization of *N. meningitidis* with C3 fragments may also contribute to protection against invasive meningococcal infection (37). Therefore, we examined the effect of TNT005 on anti-capsule Ab-mediated C3 deposition. Complement alone did not deposit C3 above baseline conjugate control levels, however, the addition of specific Ab increased C3 deposition ~60-fold. Interestingly, the addition of TNT005 at 10 μg/ml did not decrease C3 deposition (Fig. 3A), despite the marked (8-fold) decrease in C4 deposition (Fig. 2).

To assess the contribution of the AP on C3 deposition on anti-capsule Ab coated *N. meningitidis*, we used an mAb directed against the Bb domain in FB, the proteolytic component of the AP C3 convertase, which prevents cleavage of FB by Factor D. As observed with TNT005 alone, anti-FBb alone did not inhibit C3 deposition on Ab-coated *N. meningitidis* (Fig. 3B). Interestingly, when both TNT005 and anti-FBb were added to the reaction, C3 deposition was reduced ~8-fold (Fig. 3B). These data suggest that kinetically overwhelming complement activation mediated by specific Ab when the CP is intact can drive maximal C3 deposition independently of the AP. However, when CP activation is reduced (as with TNT005 treatment), AP recruitment is critical for maximal C3 deposition. These data underscore the importance of Ab for C3 deposition, evidenced by the lack of detectable C3 on bacteria incubated with complement (NHS depleted of IgG and IgM) alone.

**TNT005 does not affect killing of *N. meningitidis* in whole blood containing anti-capsule Ab**

Although serum bactericidal activity is a correlate of protection against meningococcal disease, individuals who lack Ab titers that are considered protective may not contract invasive disease, raising the possibility that mechanisms other than direct membrane attack complex insertion and bacteriolysis (such as opsonophagocytosis) contribute to protection. Because TNT005 at concentrations ≥5 μg/ml abrogated killing of anti-capsule Ab–sensitized *N. meningitidis* by 20% complement (Fig. 1), we asked whether TNT005 interfered with killing of meningococci in whole blood containing anti-capsule Ab. In the absence of anti-capsule Ab, TNT005 enhanced survival of bacteria in whole blood (Fig. 4A). Findings in plasma (Fig. 4B) paralleled whole blood, suggesting that killing was mediated by membrane attack complex. As shown in Fig. 4C, 100% killing occurred when bacteria were incubated with whole blood plus anti–group C capsule Ab. When anti-capsule Ab was present, blocking the CP or the AP alone with the addition of TNT005 or anti-FBb, respectively, also resulted in 100% bacterial killing. However, in combination, TNT005 and anti-FBb permitted >90% bacterial survival (Fig. 4C). These data are in accordance with results presented in Fig. 3, where inhibiting both pathways simultaneously blocked C3 deposition. Of note, results in 78% plasma recapitulated those seen in whole blood (Fig. 4D), suggesting that membrane attack complex, but not phagocytes, killed meningococci in blood. We measured C4 and C3

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**FIGURE 2. TNT005 inhibits C4 deposition on *N. meningitidis* in a dose-dependent manner.**

(A) Group C *N. meningitidis* strain 4243 was incubated with complement (abbreviated as C'–; NHS depleted of IgG and IgM) alone, C' plus anti–group C capsular Ab (α-Nm), or C' plus anti–Nm plus increasing concentrations of TNT005 (from 0.5 to 10 μg/ml) or a control F(ab')2 molecule (10 μg/ml). The final concentration of C' was 20% in all assays. C4 deposition was measured by flow cytometry as fluorescence on a log10 scale (x-axis). The y-axis represents the number of events (counts). Numbers alongside each histogram represents the median fluorescence of the entire bacterial population. The conjugate control (gray shaded histogram) represents bacteria with anti-C4 FITC. (B) Quantitation of C4 deposition on *N. meningitidis* from two separate experiments. C4 deposition is represented as a percentage C4 deposited in the presence of C' plus anti-Nm. Differences across groups was significant (p < 0.0001 by one-way ANOVA). Pairwise comparisons across groups was made by Tukey posttest. ****p < 0.0001.
deposition on *N. meningitidis* in 78% plasma, and as described in Figs. 2 and 3, noted decreased C4 deposition but intact C3 deposition in the presence of TNT005 (Supplemental Fig. 1).

**C4 deposition on S. pneumoniae requires an active C1 complex**

The requirement for intact C1 complex to induce C4 deposition on *S. pneumoniae* was shown using C1q-depleted serum, which contains natural Ab but lacks the ability to activate the CP (left panel, Fig. 5). There was minimal C4 deposition in C1q-depleted serum (fluorescence of 13, ~1.4-fold above conjugate control levels). By contrast, C1q-depleted serum reconstituted with C1q showed ~19-fold more C4 deposition compared with C1q-depleted serum alone (histogram depicted by solid black line in graph on right; fluorescence of 246). The addition of specific anti-capsular Ab to C1q-depleted serum resulted in a small (~2-fold) increase in C4 deposition compared with C4 deposited by C1q-depleted serum alone, and was not appreciably affected in the presence of TNT005 or F(ab')2 isotype control (Fig. 5, right graph).

The addition of anti-capsular Ab to reconstituted C1q-depleted serum augmented C4 deposition only 1.3-fold compared with reconstituted C1q-depleted serum alone. The addition of TNT005 resulted in a dose-dependent decrease in C4 deposition; the highest concentration of TNT005 used (10 µg/ml) resulted in a ~3.3-fold reduction in C4 deposition but did not bring C4 deposition levels to that seen in C1q-depleted serum. As expected, the F(ab')2 control did not affect C4 deposition at 10 µg/ml, the highest concentration tested. We also measured C4 fragment deposition on *S. pneumoniae* using IgG- and IgM-depleted human serum (human complement) to assess the effects of anti-capsular Ab in the absence of natural Ab present in NHS (Supplemental Fig. 2). As expected, complement alone deposited minimal amounts of C4. The addition of specific anti-capsular Ab enhanced C4 deposition, which was inhibited by TNT005 in a dose-dependent manner. These data show that activation of the CP is required for C4 deposition on *S. pneumoniae*. Further, the absence of significant C4 deposition in C1q-depleted serum and in human complement (NHS depleted of IgG and IgM) suggests that the MBL and ficolin pathways do not contribute significantly to C4 deposition on *S. pneumoniae* TIGR4.

**Ab-dependent C3 deposition on S. pneumoniae is not affected by TNT005**

C3 fragments are critical to opsonize *S. pneumoniae* for uptake and killing by phagocytes (38–40). C1q-depleted serum (CP inactive, but contains natural Ab) when used alone deposited only small amounts of C3 on TIGR4 (fluorescence of 21, ~4-fold above conjugate control levels) (Fig. 6A, left graph). Reconstituting C1q-depleted serum with purified C1q increased C3 deposition (fluorescence of 175 (33-fold higher than conjugate control levels); histogram shown by black solid line in graph on right in Fig. 6A), which suggests that C3 deposited by natural Ab is CP dependent. The addition of TIGR4-specific anti-capsular Ab to C1q-depleted serum enhanced C3 fragment deposition ~10-fold compared with C3 deposition in C1q-depleted serum alone (fluorescence of 217 (solid green line), compared with 21 in the absence of added Ab), suggesting that specific anti-capsular Ab, as elicited by immunization with capsule-based pneumococcal vaccines, can enhance C3 deposition independently of the CP. Consistent with the dependence on the AP for anti-capsular Ab–mediated C3 deposition, blocking C1s function with TNT005 (solid blue lines in graph on left in Fig. 6A). As expected, the control F(ab')2 fragment did not affect C3 deposition.

The addition of anti-capsule Ab to reconstituted C1q-depleted serum further increased C3 deposition by 78% (fluorescence of 308 [solid green line] compared with fluorescence of 175 [solid black line]; Fig. 6A, right graph). Consistent with the ability of anti-capsular Ab to activate the AP and deposit C3 described above,
TNT005 did not inhibit C3 deposition. Again, the control F(ab')2 did not affect C3 deposition.

To assess the contribution of the AP for mediating anti-capsular Ab–dependent C3 deposition, we used anti-FBb. IgG and IgM depleted human serum was used as a complement source in these experiments to assess the function of anti-capsule Ab independent of natural Ab against *S. pneumoniae*. As shown in Fig. 6B, the addition of anti-FBb eliminated C3 deposition in the presence of the anti-capsule Ab. In contrast, C3 deposition was reduced by only ~47% in the presence of TNT005 at 10 μg/ml compared with C3 deposited in the presence of the F(ab')2 control. Collectively, these data highlight the importance of the AP in mediating anti-capsular Ab–mediated C3 deposition on *S. pneumoniae*.

**TNT005 does not interfere with Ab-dependent killing of *S. pneumoniae* in whole blood**

We next asked whether TNT005 (50 μg/ml) affected killing of *S. pneumoniae* in whole blood (contains natural Ab) alone or whole blood supplemented with specific anti-capsule Ab. As shown in Fig. 7, TIGR4 was killed 40% at 1 h and 89% over 3 h in whole blood alone; the addition of TNT005 or control F(ab')2 did not significantly alter killing. The addition of anti-capsule Ab to whole blood
increased killing (compared with whole blood alone) to 73 and 97% killing at 1 and 3 h, respectively. Again, the addition of TNT005 or control F(ab’2) did not significantly alter survival of S. pneumoniae. Blocking the AP alone with anti-FBb also did not alter killing of S. pneumoniae in whole blood or the presence of anti-capsule Ab. However, simultaneous blockade of both the CP and AP with TNT005 and anti-FBb significantly increased survival of bacteria, both in the presence and in the absence of specific anti-capsule Ab, reiterating the importance of the AP in host defense against S. pneumoniae. We measured C4 and C3 deposition on S. pneumoniae incubated with 78% plasma. Despite a significant decrease in C4 deposition to about baseline conjugate control levels in the presence of TNT005, C3 deposition to about half maximal levels was achieved (Supplemental Fig. 3), again demonstrating Ab-dependent AP activation.

**DISCUSSION**

An increasing appreciation of the role of aberrant complement activation in the pathogenesis of several diseases, coupled with a deeper understanding of structure–function relationships of several aspects of complement activation, has spurred the development of therapeutic inhibitors of complement (41). Eculizumab, which blocks activation of C5, was the first approved therapeutic complement inhibitor and revolutionized the management of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome (42, 43). However, complement forms an important arm of the innate immune defenses against infections, and long-term complement blockade may increase the risk of infections (1, 21). Indeed, C5 blockade with eculizumab has been associated with a 1000–2000-fold increase in invasive disease with N. meningitidis, highlighting the need for terminal complement activation in host defenses against meningococcal disease (24). Of 16 reported cases of meningococcal disease in eculizumab recipients, 11 were caused by nongroupable isolates that are not covered by capsular conjugate meningococcal vaccines (33). Nongroupable isolates very rarely cause invasive infections in complement-sufficient hosts (44–47), highlighting the unique risks posed by blocking the terminal complement pathway. The heightened risk of invasive infection that accompanies eculizumab treatment may also extend to N. gonorrhoeae (48, 49) Pathway-specific inhibition would permit complement activation through the other intact pathways to generate opsonic complement fragments such as C3b and iC3b and also allow the formation of the membrane attack complex, thereby theoretically reducing the risk of invasive meningococcal infections. In the case of TNT005, inhibiting C1s could permit complement activation through the alternative and lectin pathways, which promotes deposition of C3 in amounts sufficient for opsonization and activation of the terminal cascade. The complement pathways are not functionally independent—as an example, C3 deposited through the classical or lectin pathways can recruit the AP, which then further amplifies C3 deposition through its positive feedback loop. An example of “interplay” between complement pathways is zymosan; although traditionally viewed as an AP activator, the CP enhances C3 deposition on zymosan (50, 51).

The extent of complement inhibition defines the risk of infection; complete absence of a complement component greatly enhances the predisposition to infection, exemplified by individuals with congenital complement deficiencies (1). In contrast, subtotal complement deficiencies are associated with a lower risk of infection (52–54). Titrating complement inhibition to a level that controls disease symptoms yet provides enough complement activity to combat infections may substantially reduce the risk of infection, as opposed to completely blocking complement function. In this study, we show that TNT005 at concentrations that inhibit complement-mediated killing of Ab-coated N. meningitidis still permitted C4 deposition on S. pneumoniae and N. meningitidis in the presence of specific anti-capsular Ab. Although the amount of C4 deposited on anti-capsular Ab–coated N. meningitidis and S. pneumoniae in the presence of TNT005 was decreased 8-fold and 3.5-fold, respectively, near-maximal C3 deposition on bacteria occurred provided the AP was intact. Anti-pneumococcal Abs can activate complement even in the absence of an intact CP; Jönsson et al. (55) showed that anti-capsular Abs elicited by immunization supported opsonization of pneumococci in C2 deficient individuals as long as the AP was functional, likely through Ab-dependent C2 bypass activation of complement (56–58). Edwards and colleagues (59) showed that capsule-specific Ab against Type III group B streptococci could induce opsonophagocytic killing of bacteria in C2 depleted serum as well as in Mg-EGTA–treated serum (EGTA chelates Ca2+, thereby preventing C1s activity), but only when Ab was present at high titers. Bactericidal activity of anti-capsular Ab that was solely dependent on the AP was demonstrated on Haemophilus influenzae.

**FIGURE 5. C4 deposition on S. pneumoniae TIGR4 is dependent on an intact C1 complex and is inhibited by TNT005.** S. pneumoniae TIGR4 was incubated with C1q-depleted serum (C1q dep. serum; left graph) or C1q-depleted serum reconstituted with purified C1q (C1q dep. serum + C1q; right graph), either alone or with anti-Sp (α–Sp), control F(ab)2 (10 μg/ml) or isotype control F(ab)2 (10 μg/ml) as indicated to the right of the graph. Bacteria plus anti-C4 FITC served as the conjugate control. The concentration of C1q dep. serum in every instance was 20%. x-axis, fluorescence on a log10 scale; y-axis, counts. Numbers alongside each histogram indicates the median fluorescence of the entire bacterial population. One representative experiment of two reproducible repeats is shown.

https://doi.org/10.4049/immunohorizons.1900031
All these examples of Ab-dependent AP mediated killing of bacteria are restricted to anti-capsular Abs and require high titers of Ab. Thus, effective Ab-dependent AP activation in the absence of a fully functional CP requires high densities of specific surface bound Ab, underscoring the importance of immunizing individuals to ensure high titers of circulating immune Ab.

Activation of the AP by Ab was described almost 50 years ago by Sandberg and colleagues (62, 63), who showed that immune complexes of guinea pig IgG and dinitrophenylated bovine
γ-globulin induced complement deposition by a mechanism that bypasses C1, C2, and C4 (64). C3 activated through the AP is deposited on IgG; the majority of C3b in immune complexes is deposited on the H chain portion (C141 domain) of the F(ab′)2 region of IgG (65–68). Jelezarova et al. (69) proposed a model in which (C3b)2–IgG complexes can bind to FB and properdin, which can cleave C3 to amplify the AP. Abdominal aortic aneurysms that develop following elastase perfusion of mice requires the IgG and a functional AP, but not C4, and constitutes another example of Ab-mediated AP activation (70, 71). Similarly, we speculate that anti-capsular Ab may form a platform for assembly of a C3 convertase that functions independently of C4 activation. Another and not mutually exclusive mechanism of AP activation by anti-capsule Ab is interference with the anti-complementary properties of capsular polysaccharides.

With the exception cited above where meningococci are killed by the AP in the presence of high concentrations of anti-capsular Ab, killing of Neisseriae by human serum, requires a functional CP (72–74). Our data showing the survival of N. meningitidis in a bactericidal reaction mixture containing relatively low concentrations (20%) complement, anti-capsular Ab, and TNT005 at concentrations ≥5 μg/ml reiterate the importance of the CP in killing meningococci through membrane attack complex. Killing through anti-capsular Ab and the AP was unimpaired when plasma at a concentration of 78% was used. Complement activation on meningococci requires Ab; the lack of detectable C4 or C3 deposition on bacteria incubated with IgG- and IgM-depleted serum (complement alone) suggests that complement is not activated on group C meningococci in the absence of Ab. Upon the addition of anti-capsular Ab, however, C4 and C3 deposition on bacteria was greatly enhanced. Interestingly, the addition of TNT005 to complement plus anti-capsular Ab decreased C4 deposition markedly, but C3 recruitment through the AP remained intact. The reason why bacteria were not killed in 20% complement containing anti-capsular Ab and 10 μg/ml TNT005 (Fig. 1) despite high levels of C3 deposition under similar conditions is not clear. One possibility is failure to form C5 convertases because C3 convertases (C3bBb) may decay either spontaneously or through the action of FH prior to forming C5 convertases (C3bBbC3b). Meningococci bind FH via three distinct outer membrane proteins (33, 34, 75, 76) and, in addition, can regulate the AP through lipooligosaccharide sialic acid and groups B and C capsular polysaccharides (29, 31, 77), which all serve to limit AP activation. Another explanation may relate to the 6–9-fold lower catalytic rate of AP C5 convertase compared with CP C5 convertase (78), which may not result in membrane attack complex assembly at rates or amounts sufficient to kill bacteria. The higher concentrations of complement present in the whole blood and plasma assays were likely sufficient to permit formation of lytic membrane attack complexes even in the presence of TNT005, provided the AP was intact.

The CP alone is sufficient for complement-dependent killing of meningococci when Abs are directed against abundant surface Ags such as PorA, whereas killing by Abs directed against more sparsely distributed surface Ags such as Factor H–binding protein (FHbp) requires recruitment of the AP (79). Consistent with the abundance of capsular polysaccharide, anti–group C mAb was bactericidal even when the AP was blocked with anti-FHb. However, when activity of the CP is decreased, for example in the presence of TNT005, recruitment of the AP is necessary for bactericidal activity.

In summary, we present data to show that partial inhibition of the CP with TNT005 in sera that contains specific anti-capsular Ab still permits C3 activation through the AP that can facilitate bacterial killing in whole blood, which may in part mitigate the risk of infection. We emphasize the importance of administering appropriate capsular conjugate vaccines (pneumococcal, meningococcal, Vibrio cholerae) which are known to induce protective immunity.
and Haemophilus influenzae type b) to patients on C1s inhibitors to ensure development of high titers of bactericidal Ab. There are several complement inhibitors currently in clinical development (80), and patients and providers need to be vigilant for the development of invasive bacterial infections as more long-term safety data are gathered to define the incidence of infection in this clinical setting.

DISCLOSURES

L.A.L. and R.B.D. had research support from True North Therapeutics. S.P. and G.C.P. are former employees of True North Therapeutics, Bioverativ, and Bioverativ, a Sanofi company. S.R. serves as a paid consultant for Achillion Pharmaceuticals, Apellis Pharmaceuticals, and Ionis Pharmaceuticals. L.A.L. and S.R. are inventors on patent applications or on issued patents in the area of gonococcal immunotherapeutics or meningococcal vaccines. Rights to these inventions have been assigned to the University of Massachusetts.

REFERENCES

EFFECT OF C1s INHIBITION ON BACTERIAL KILLING


