New Antibody Weapons against an Old Foe

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ABSTRACT **Antibodies have been used in a diagnostic capacity for many diseases and for identifying serotypes within single species of pathogens, notably between the multiple capsular polysaccharide serotypes of *Streptococcus pneumoniae*. For many years, the functions of antibodies in infection were thought to be limited to the opsonization of microorganisms followed by phagocytosis and to the fixing of complement. The thought that antibodies could have other functions has emerged only recently. The study by Yano and coworkers from the laboratory of Liise-anne Pirofski published in *mBio* [M. Yano, S. Gohil, J. R. Coleman, C. Manix, and L.-A. Pirofski, mBio 2(5):e00176-11, 2011] identifies one mechanism whereby nonopsonic antibodies enhance the transformation competence of two *S. pneumoniae* serotypes, which leads to an increase in genetic exchange and bacterial variability with a resulting population reduction through fratricide. These new and revealing antibody functions will add another chapter to the burgeoning story of the diversity and versatility of the immune response to bacteria.**

Since antibodies were first described late in the 19th century, antibodies have become the single most highly recognized mediators of immunity. During the past century, the general public has also become familiar with the practice of passive immunization for the treatment of a variety of infections that include tetanus and rabies. The downside of antibody therapy, a form of hypersensitivity known as serum sickness that develops after exposure to heterologous immunoglobulins (i.e., from other species), is also well-known.

Antibodies have been used in a diagnostic capacity for many diseases and for identifying serotypes within single species of pathogens (including distinguishing between the multiple capsular polysaccharide serotypes of *Streptococcus pneumoniae*), a job which they still discharge remarkably well. For many years, the functions of antibodies in infection were thought to be limited to opsonization of microorganisms followed by Fc receptor-mediated phagocytosis and to the fixing of complement. The thought that antibodies could have other functions has only recently emerged.

Today, it is rare to find an issue of a medical journal that does not include a report that uses chimeric monoclonal antibodies as therapy for any one of a broad spectrum of diseases (1). Consequently, therapeutic monoclonal antibodies are undergoing an unprecedented and fast expansion in the drug market. This antibody "renaissance" is due to the extraordinary success of molecular biology that permitted (i) the grafting of the Fab fragments from animal sources onto the Fc-bearing domains of class-specific human immunoglobulins, and (ii) the creation of transgenic mice that can produce human immunoglobulins for the creation of hybridomas to many diverse immunogens (2).

Other approaches using antibodies have been tested with various degrees of success and hold much hope of further development for specific infectious and noninfectious diseases. Antibodies have been linked to drugs and radioisotopes to target specific organisms and cells without having the effects of broad toxicity. The specificity of the antibody combined with the targeted toxicity of a drug or isotope clearly limits undesirable side effects to nontarget tissues. This concept of the "magic bullet" is not new, but it has received a new jump start with increasing knowledge of the production and properties of antibodies. In addition, it is now possible to create single-chain fragments of variable regions (scFV) and single domains or nanoantibodies that retain therapeutic activity and other desirable properties such as specificity. The innovative technologies that are applied to monoclonal antibody therapy are now indispensable for treatment of diseases that had no known cure and for improved modalities against certain types of infection where rapid intervention is required.

Not unexpectedly, in the last few years, new mechanisms of action for antibodies have been discovered. The versatile collection of antibody functions that clear microorganisms includes new findings such as complement independent-bacteriolytic immunoglobulins, which kill several species of *Borrelia* (3, 4), and direct antimicrobial effects on gene expression in fungi (5), among others.

As mentioned above, antibodies are used to identify the serotypes of *S. pneumoniae* that are critical for the formulation of the current pneumococcal vaccines. The most effective type of host response to *S. pneumoniae* is centered on antibody binding to the pneumococcal capsular polysaccharide followed by Fc receptor-mediated phagocytosis. Moreover, this traditional mechanism of opsonization-phagocytosis is also thought to be essential for the response to active immunization with both the 7- and 23-valent pneumococcal capsular polysaccharide vaccines (6).

In contrast to the traditional understanding that opsonization-phagocytosis is necessary for pneumococcal clearance, we now know that there are a number of nonopsonic antibodies to the capsular polysaccharides that have the capacity to protect both experimentally and clinically. A number of these nonopsonic antibodies have been identified and are both polyclonal and monoclonal, can be derived from humans and mice, and protect against pneumonia and sepsis in experimental models.

So, how do these nonopsonic antibodies work? The study by Yano and coworkers in the laboratory of Liise-anne Pirofski published in *mBio* (7) identifies one mechanism that was heretofore

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unappreciated: the nonopsonic antibodies enhance the transformation competence of two *S. pneumoniae* serotypes, which leads to an overall increase in genetic exchange and bacterial variability and sharply lowers the number of organisms. While the bactericidal end result has obvious therapeutic relevance, the road taken to elucidate this mechanism is also of much biological interest and one that crisscrosses microbiology and immunology at many points.

A protective nonopsonic monoclonal antibody (1E2, $1gG1^k$ specific for *S. pneumoniae* serotype 3) induced a higher transformation frequency in the appropriate strains when added to competence-stimulating peptide (CSP) than CSP alone or the other opsonic subclass-matched monoclonal antibodies that were used as controls. Moreover, a human monoclonal nonopsonic IgM had the same effect as 1E2, indicating that this mechanism is not specific to the immunoglobulin class. Similar effects obtained with antibodies to *S. pneumoniae* serotype 8 also showed that the induction of transformation efficiency could be obtained with more than one pathogenic strain of pneumococcus and with antibodies derived from both human and mouse hybridomas. Agglutination of the pneumococcus appeared to be a factor in the induction of higher transformation frequency. Interestingly, agglutination, at least in *S. pneumoniae*, appears to be a necessary precondition for achieving the competent phenotype.

Competence-stimulating peptides (CSP) are pheromones secreted by *S. pneumoniae* for interbacterial communication through the activation of the Com pathway that regulates genetic transformation and therefore induces "competence" in these bacteria, the physiological state that allows incorporation of exogenous DNA. In general, CSP released into the medium activates a two-component system (ComDE) that results in the expression of *comX*, leading to the upregulation of genes associated with competence (8).

Along these lines, the 1E2 monoclonal antibody induced late competence and altered gene expression. Incubation of CSP and antibody with the appropriate specific type 3 pneumococcus resulted in an induction of *comX* expression after 8 minutes of incubation, representing a new second wave of expression that followed the peak expression induced by CSP alone after 2 min. The entire process of competence development in *S. pneumoniae* occurs rapidly, within 15 minutes, a period of time that can easily encompass the 2- and 8-min observation of upregulation of *comX* in organisms exposed to CSP and the nonopsonic antibody in the Yano et al. study (7). Likewise, the *com*-induced regulon has genes that function at different times (9), and these genes are also induced by exposure to the nonopsonic antibodies.

Yano et al. (7) also reveal some interesting findings regarding the competent state of *S. pneumoniae* and the production of lytic factors that are capable of eliminating the cells that do not become competent following exposure to CSP (7). Eliminating noncompetent cells supports the idea that permissiveness to accept exogenous DNA is the preferred condition following an episode of stress. This killing phenomenon was characterized as "fratricide" (10) and results in the release of DNA and a number of virulence factors. Yano and colleagues show that induction of *comX* expression by exposure of *S. pneumoniae* to CSP and nonopsonic antibodies was followed by marked upregulation in the expression of genes associated with fratricide (7). The 1E2 antibody alone increased expression of *blp* bacteriocin genes, irrespective of CSP. These genes are expressed in stationary cultures and in fratricide of noncompetent cells. It could be argued that enhancing fratricide, particularly if directed to cells that cannot accept new genetic information, may also work in favor of preserving the competent cells by the acquisition of resistance factors to the effects of the nonopsonic antibodies. Likewise, 1E2 increased by 2-fold the mortality of pneumococcus over and above the mortality achieved by CSP alone.

If conditions are such that the majority of cells in an *S. pneumoniae* culture can accept DNA more readily than before, those cells could well acquire new resistance factors. In this scenario, the random acquisition of new genes could result in the expression of new antigens not recognized by the antibodies, new exogenous proteases that can cleave immunoglobulins, or a number of other possible factors that would enhance the establishment and continuation of an infection. There may be an advantage to *S. pneumoniae* to respond to the stress and danger of specific antibodies with the ability to acquire new DNA. This is a prime area for expansion for the Pirofski laboratory following their observations published in *mBio* (7).

The fact that an antibody can induce fratricide begs the question of who enjoys the advantages of this interaction, the host or the pathogen? Can *S. pneumoniae* benefit from eliminating noncompetent cells in the hope that the surviving competent organisms can have a chance to acquire a factor that would make them resistant to the antibodies? This is another question to be answered as a follow-up to these intriguing observations. Moreover, what does this mean for pneumococcus? Is this bacterium helped or harmed by the action of these antibodies? It would appear that the altered gene expression consistent with fratricide could result in fewer organisms, which in turn could act synergistically in the clearing capacity of the opsonizing antibodies, benefiting the host. On the other hand, an antibody effect that bestows increased fitness on the capacity for genetic exchange could result in acquired resistance in much the same manner as it occurs with antibiotics, benefiting the pathogen.

Indeed, the comparison with antibiotics in this antibody mechanism is unavoidable. The data presented by Yano and coworkers on the mechanism of action of the nonopsonic antibodies is reminiscent in general scope to the mechanism of action of bacteriostatic antibiotics (7). Bacteriostatic antibiotics work indirectly by weakening the bacteria so that the host response is more effective. In fact, the functional similarity between certain types of antibodies and antibiotics is already more than theory. The term "antibiobodies" has been used to characterize anti-idiotypic recombinant antibodies that have toxin-like activity against fungal pathogens (11–13).

Nonopsonic antibodies to *S. pneumoniae* also work indirectly to trigger fratricide of at least part of the population and thus allow the host to clear the infection faster. Further studies of these new and revealing antibody functions will likely add another chapter to the burgeoning story of the diversity and versatility of the immune response to bacteria.

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REFERENCES

1. **Reichert JM, Dewitz MC.** 2006. Anti-infective monoclonal antibodies: perils and promise of development. Nat. Rev. Drug Discov. **5**:191–195.
2. **Casadevall A, Dadachova E, Pirofski LA.** 2004. Passive antibody therapy for infectious diseases. Nat. Rev. Microbiol. **2**:695–703.
3. **Connolly SE, Benach JL.** 2005. The versatile roles of antibodies in Borrelia infections. Nat. Rev. Microbiol. **3**:411–420.

4. **LaRocca TJ, et al.** 2010. Cholesterol lipids of *Borrelia burgdorferi* form lipid rafts and are required for the bactericidal activity of a complement-independent antibody. Cell Host Microbe **8**:331–342.
5. **McClelland EE, Nicola AM, Prados-Rosales R, Casadevall A.** 2010. Ab binding alters gene expression in *Cryptococcus neoformans* and directly modulates fungal metabolism. J. Clin. Invest. **120**:1355–1361.
6. **Romero-Steiner S, et al.** 1997. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. Clin. Diagn. Lab. Immunol. **4**:415–422.
7. **Yano M, Gohil S, Coleman JR, Manix C, Pirofski L.** 2011. Antibodies to *Streptococcus pneumoniae* capsular polysaccharide enhance pneumococcal quorum sensing. mBio **2**(5):e00176-11.
8. **Claverys JP, Håvarstein LS.** 2007. Cannibalism and fratricide: mechanisms and raisons d'être. Nat. Rev. Microbiol. **5**:219–229.
9. **Claverys JP, Prudhomme M, Martin B.** 2006. Induction of competence regulons as a general response to stress in gram-positive bacteria. Annu. Rev. Microbiol. **60**:451–475.
10. **Håvarstein LS, Martin B, Johnsborg O, Granadel C, Claverys JP.** 2006. New insights into the pneumococcal fratricide: relationship to clumping and identification of a novel immunity factor. Mol. Microbiol. **59**: 1297–1307.
11. **Casadevall A, et al.** 1994. Molecular and idiotypic analysis of antibodies to *Cryptococcus neoformans* glucuronoxylomannan. Infect. Immun. **62**: 3864–3872.
12. **Magliani W, et al.** 2011. Killer peptide: a novel paradigm of antimicrobial, antiviral and immunomodulatory auto-delivering drugs. Future Med. Chem. **3**:1209–1231.
13. **Polonelli L, et al.** 1996. Human natural yeast killer toxin-like candidacidal antibodies. J. Immunol. **156**:1880–1885.