The Road to Toxin-Targeted Therapeutic Antibodies

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ABSTRACT Once an infection by a toxin-producing bacterium is well established, therapies such as antibiotics that target bacterial growth may have little impact on the ultimate patient outcome. In such cases, toxin-neutralizing antibodies offer an opportunity to block key virulence factors. New work by A. K. Varshney, X. Wang, J. L. Aguilar, M. D. Scharff, and B. C. Fries [mBio 5(3):e01007-14, 2014, doi:10.1128/mBio.01007-14] highlights the role of the antibody isotype in determining the efficacy of toxin-neutralizing antibodies *in vivo*. Varshney et al. examined the role of antibody isotype for protection in murine models of staphylococcal enterotoxin B (SEB)-induced lethal shock and sepsis produced by SEB-producing *Staphylococcus aureus*. Murine antibodies of the IgG2a isotype were more protective than antibodies of the IgG1 and IgG2b isotypes that have identical variable regions and binding activity. These results add to the complexity inherent in the selection and optimization of antibodies for anti-infective passive immunization and emphasize the need to use relevant *in vivo* models to evaluate potential therapeutic monoclonal antibodies.

Antibodies play a central role in the defense against many microbial pathogens. The use of antibodies to treat disease began with passive administration of serum from immunized animals for the treatment of diseases such as diphtheria and pneumococcal pneumonia. Antibody therapy expanded dramatically with the development of monoclonal antibody (MAb) technologies and antibody engineering. MAbs now play a central role in the treatment of various forms of cancer, autoimmune disease, and infectious disease. The application of passive immunization for the treatment of disease is limited primarily by the need to identify suitable targets for antibody binding and gain a greater understanding of how antibodies function within the complex *in vivo* milieu.

Our understanding of antibody structure-function has its foundation in the studies of Porter and Edelman. Porter found that treatment of rabbit gamma globulin with the enzyme papain yielded three fragments (1). Two of the fragments retained the ability to recognize antigen (Fab) but lost precipitating activity. The third fragment was readily crystalized (Fc) and had most of the antigenic sites of the original molecule but had no antigen-recognizing activity. Edelman and coworkers determined the amino acid sequences of several myeloma proteins to identify variable and constant regions and identified domain structures within the constant regions of the heavy chains (2). These latter observations anticipated the differences between heavy-chain domains of antibodies of different isotypes that ultimately accounted for many of the biological functions of the heavy chain. G. Edelman and R. R. Porter shared the 1972 Nobel Prize for Physiology and Medicine.

The identification of unique antigenic determinants on different immunoglobulins and the structures of the respective antibodies led to the recognition of the five major classes of immunoglobulins. Further study led to the identification of isotypes or subclasses of IgG and other immunoglobulin classes. These isotypes reflect relatively minor antigenic differences in the Fc portion of the molecule due to distinct amino acid sequences in the constant region of the heavy chain. In the case of human IgG, the isotypes are IgG1, IgG2, IgG3, and IgG4. In the case of the mouse, the isotypes are IgG3, IgG1, IgG2b, and IgG2a. Isotype switching in mice occurs via a spontaneous recombination process that generates antibodies with the heavy-chain constant regions of each isotype that share the variable region of the original antibody. Antibodies of different IgG isotypes have different functional and biological activities that include the ability to activate the complement cascade, opsonization via Fc receptors (FcγR), NK cell antibody-dependent cell-mediated cytotoxicity (ADCC), serum half-life, and segmental flexibility. As a consequence, the isotype of an antibody can dramatically affect its biological activity.

A critical distinction between antibodies of different IgG isotypes is their differential abilities to interact with Fc receptors on effector cells that include macrophages, neutrophils, dendritic cells, and NK cells. Four major classes of Fc receptors have been identified for murine and human immune effector cells: FcγRI, FcγRII, FcγRIII, and FcγRIV (reviewed in reference 3). In mice, these receptors have been classified as activating (FcγRI, FcγRIII, and FcγRIV) or inhibitory (FcγRIIB) receptors. The four murine IgG isotypes differ in their binding to murine FcγRs. The high-affinity FcγRI only binds IgG2a. In contrast, the low-affinity FcγRIII binds IgG1, IgG2a, and IgG2b. FcγRIV binds IgG2a and IgG2b with intermediate affinity but does not bind IgG1 or IgG3.

A further wrinkle in the contribution of isotype to antibody activity is the contribution of the heavy chain constant region to the affinity of some antibodies. Antibody binding is classically viewed as a function of the Fab portion of the molecule, which contains the variable region that is directly associated with binding. However, several recent reports indicate that the antibody constant region can influence affinity and specificity in families of antibodies that have identical constant regions but different isotypes (4–7).

The neutralization of toxins by antibody has been generally viewed as the ability of antibody to block the binding of toxin to a cellular receptor, because toxin neutralization can often be

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achieved by Fab fragments. However, a recent report by Abboud et al. identified a central role for the Fc fragment and host FcγRs in the neutralization of *Bacillus anthracis* protective antigen by a murine MAb (8). *In vivo*, the activity was influenced by the IgG isotype (IgG2a > IgG2b > IgG1). Moreover, passive immunization protected wild-type mice but not FcγR-deficient mice from a normally lethal *B. anthracis* infection, thus identifying a key role for FcγRs in *in vivo* protection.

Studies by Varshney et al. (9) of the role of antibody isotype in protection against lethal shock induced by staphylococcal enterotoxin B (SEB) and *Staphylococcus aureus* sepsis in mice lend further evidence in support of the critical role played by the Fc region in the *in vivo* biological activity of toxin-specific MAbs. Previous studies by this group found that MAb 20B1, an IgG1 MAb, neutralized the mitogenic effects of SEB in *in vitro* T-cell proliferation assays and in two murine models for SEB-induced lethal shock (10). The key finding in their more recent report is an evaluation of the effect on protective efficacy of isotype switching of the original IgG1 MAb 20B1 to the IgG2a or IgG2b isotype, each of which has the same variable region sequence. A comparison of the protective effects *in vivo* found that the IgG2a MAb was markedly more protective than the variable-region-identical IgG1 or IgG2b antibody in both SEB-induced lethal shock and the *S. aureus* sepsis model in mice. This result is similar to the results of studies of an isotype switch family of MAbs that are reactive with *B. anthracis* protective antigen (8); the IgG2a protective antigen MAb was more effective *in vivo* than its variable-region-identical IgG1 or IgG2b siblings.

SEB MAbs of different isotypes showed similar affinities for SEB; hence, the role of isotype in protection is a consequence of the effect of the constant region on a biological activity other than antibody binding. This conclusion is supported by the finding that MAbs of the IgG1, IgG2a, and IgG2b isotypes showed similar abilities to neutralize SEB activity *in vitro*. In contrast, there was an isotype-dependent difference in protection *in vivo* which correlated with early levels of inhibition of proinflammatory cytokine production. Clearly, there is an isotype-dependent component to the blockade of *in vivo* effects that is not observed in *in vitro* neutralization assays. These results emphasize the need to use relevant *in vivo* models when predicting the protective effects of passive immunization. To be sure, there can be important *in vitro* correlates of protection, but these *in vitro* correlates need to be founded in *in vivo* studies.

Antibody affinity has long been viewed as a major determinant of antibody efficacy in toxin neutralization. The studies by Varshney and others have clearly identified antibody isotype as a second critical variable that determines *in vivo* activity. This is a potential fork in the road to toxin-targeted therapeutic antibodies. Indeed, there is likely a nexus of affinity- and isotype-dependent biological activity that must be optimized to achieve the maximum level of protective or therapeutic activity. Identification of the molecular and cellular basis of the role of isotype in protection will be critical to the production of next-generation antibody-dependent vaccines and therapeutics.

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