Trans. Natl. Acad. Sci. & Tech., Philippines (2001) 23: 31-37

ENGINEERING ANTIBODIES FOR HUMAN THERAPY

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ABSTRACT

Antibodies have many potential uses in industry and medicine. Through protein engineering, the structure of an antibody can be altered and the molecule made more efficacious for human therapy. For example, the immunogenicity of a nonhuman antibody can be reduced by 'humanization' and improvements can be made on its pharmacodynamics. In addition, new properties and reactivities can be engineered into the molecule, such as multispecificity, multivalency, greater stability, etc.

INTRODUCTION

Our immune system responds to invasion by a foreign substance (antigen) by producing antibodies which bind specifically to the antigen, causing neutralization and eventual elimination. The immune system is quite versatile and antibodies can be produced against virtually any macromolecule, or, more precisely, against any accessible part of any macromolecule. The specificity of the immune response and the wide diversity of the specificities that can be generated make antibodies useful in the laboratory, e.g. in the detection and quantification of all sorts of substances, as well as isolation and purification of specific substances from complex mixtures with other molecules. The exquisite specificity of antibodies has many applications in medicine also. Indeed, antibodies have long been used as anti-toxins, as a diagnostic tool for a variety of diseases, as a means to prevent transplant rejection, etc. More recent applications include the treatment of cancer, asthma, autoimmune disease, and more.

The production of the large amounts of pure antibody needed for these applications has been made possible by the advent of hybridoma technology, by

recent advances in molecular biology, cell culture and genetic engineering techniques, and by the use of transgenic animals and plants for protein expression.

Antibody molecules are often engineered to provide them with more desirable properties. For example, an antibody derived from nonhuman source is usually first 'humanized', i.e. mutated to make it close to a human molecules as possible, prior to valence, its reactivity, even its stability, could be altered by judicious structural manipulations.

Considerable primary and three-dimensional structural information has become available for antibodies. This information has guided the successful engineering of antibody molecules.

Brief description is given here of what is currently known about antibody structure and how this information is being used to engineer antibodies for human therapy. A number of reviews have been written on the three-dimensional structure of antibody-antigen interactions (see Braden and Poljak, 1995; Davies and Cohen, 1996; Edmundson *et al.*, 1996; Padlan, 1994a, b, 1996; Wilson *et al.*, 1994). In addition, the systematic compilation and analysis of antibody sequences have been done by the late Elvin A. Kabat and co-workers (Kabat *et al.*, 1991), whose work still serves as the ultimate source of insights into the structure of the molecule. Antibody engineering protocols and examples of antibody therapeutics are described in several tomes [see the volumes edited by Harris and Adair (1997) and Kontermann and Dubel (2001)] to which the reader is referred.

The Structure of Antibodies:

The human immune system produces five different classes of antibodies, each with its own special properties and reactivities :IgA (of which there are two types:IgA1 and IgA2), IgD, IgE, IgG (of which there are four types: IgG1, IgG2, IgG3 and IgG4) and IgM. Antibodies are glycoproteins and are built from two types of polypeptide chains: a light (L) chain of approximately 210 amino acid residues and a heavy (H) chain about 450 to about 575 residues. The basic antibody molecule consists of two identical L chains and two identical H chains. Both L and H chains show tandem regions of sequence homology, or domains, with two domains in the L chain and four (in IgA, IgD, and IgG) or five (in IgE and IgM) in the H chain. The two NH2-terminal domains of both L and H chains are variable, i.e. they are different in different antibodies, while the other domains are constant, i.e. they are the same in chains of the same type.

The antibody combining site, i.e. the antigen-binding site, is built from the variable domains of the L and H chains (the VL and VH domains). This is the structural basis for the diversity for the antigen-binding specificities. The binding of antibody to antigen results in a variety of reactions (the so-called effector functions of antibodies), among which is the recruitment of certain cells and other molecules of the response. The effector functions involve the constant domains of the antibody.

The amino acid sequence of many thousand of both L and H chains have been determined (Kabat *et al.*, 1991). A comparison of antibody sequences had earlier shown the sequence variability is largely confined to the three regions – the so-called complementarity-determining regions, or CDRs (Wu and Kabat, 1970) - in both L and H chains. There is also variation in the number of amino acid residues in the CDRs of different antibodies.

Three-dimensional structural data on antibodies have been made available largely by x-ray crystallography. A survey made by this author in the summer of 2000 revealed 303 entries in the Protein Data Bank (Abola *et al.*, 1987), representing 165 different antibodies. Many of the crystal structures are of complexes with specific ligand. There is therefore a wealth of three-dimensional data that can be used to compare structures and to assess the structural significance of every part of the antibody molecule.

The x-ray analyses show that the antibody structure is modular, with each domain folding into a compact globular structure. All antibody domains display a bilayer structure consisting of two anti-parallel beta-sheets bridged by a disulfide bond. The domain structure is strong and the loops that connect the individual strands in the beta-sheets often vary in size and conformations with little or no effect on the structure of the bilayer. The VL and VH domains associate closely to form a compact module (the Fv) which contains the antibody combining site. The constant domain of the L chain (the CL) and the first constant domain of the H chain (the CH1) also form a compact module (the CL:CH1 module) which probably helps in the stabilization of the Fv structure. The other constant domains of the H chains associate as homologous pairs and together form the so-called Fc fragment. The Fv and CL:CH1 modules constitute the Fab, or antigen-binding fragment. The two Fabs and the Fc are loosely joined by a 'hinge' region.

Studies of antibody-antigen complexes reveal that the interaction between antibody and antigen mainly involves the CDRs, with some contribution from the non-CDR, or framework, residues of the variable domain. A close complementarity between the interacting surfaces of antibody and antigen is observed. This clearly, is the structural basis for the exquisite specificity of antibody binding to antigen. Since the antibody combining site is primarily built from the CDRs, variation in the structure of the CDRs, brought about by different sequences and different number of amino acids in these regions, can potentially result in a very large number of different antigen-binding site structures.

A closer examination of the interaction between antibody and antigen reveals that not all of the residues in the CDRs are actually involved in the binding. Only about one-quarter to one-third of the CR residues are in actual contact with the ligand. To distinguish them from the rest of the CDR residues, we call the antigen-contacting residues the specificity-determining residues, or SDRs (Padlan *et al.*, 1995).

The structural data also reveal that the antibodies from different animals have very similar structures. Indeed, homologous domains are superposable

regardless of isotype (class or subclass) or species origin, emphasizing the importance of preserving structure to preserve function. The major differences in structure are in the CDRs, which are found to be exposed loops congregating at the tip of the Fv. Yet, even large structural changes in the CDRs are clearly tolerated by the strong scaffolding provided by the framework regions.

The strength of the framework scaffold and the conservation of domain structures permit the structural manipulations that are performed during antibody engineering.

Antibody Engineering:

Judicious alterations in the structure of an antibody can provide the molecule with new properties and reactivities (Winter,1989). Among these are a different effector function, greater avidity for antigen, greater stability, greater transport across barriers, reduced immunogenicity, improved pharmacodynamics, etc.

The different antibody isotypes exhibit different effector functions [see, Janeway *et al.*, 1999]. For example, the IgG1, IgG3 and IgM isotypes are particularly effective in activating the complement system, i.e. triggering a cascade of reactions involving a system of proteins some components of which help in phagocytosis or in the lysis of invading cells. Also, IgG1 and IgG3 are the isotypes which recruit the participation of natural killer cells in immune response. On the other hand, the dimeric form of IgA is the molecule that is readily transported across epithelium, while IgE is the isotype that recruits eosinophils to attack larger parasites. Thus, an antibody of a particular specificity could be provided with a desired effector function simply by splicing the variable domains to the appropriate constant regions.

An antibody with multiple specificities has many potential uses. Also, a multivalent antibody, like IgM, is often desirable because the effective affinity (avidity) of an antibody for its specific ligand is greater when there are more binding sites. Multispecificity and multivalency are easily engineered into an antibody [see Pluekthun and Park, 1997; Ridgway *et al.*,1996; Santos *et al.*, 1999; Segal *et al.*, 1999].

The basic antibody molecule has two combining sites that are identical and which have the same binding specificity. But, one can create a bispecific molecule by coupling a half molecule (one L and one H chain) of one specificity with another half molecule of a different specificity. One possible use of a bispecific antibody is to bring two entities into close proximity. For example, one specificity could be for a tumor cell marker while the other could be for a molecule on the surface of a cytotoxic T cell; bringing the two cells together can lead to the death of the tumor cell (Gilland *et al.*, 1998). Such an antibody would be useful against cancer.

There are various ways of achieving multivalency. One way is to construct a molecule with several antigen-binding regions (Fvs, or Fabs) in tandem using

appropriate linkers. Another way is to attach antigen-binding regions to oligomerization domains, e.g. leucine zippers (Plueckthun and Park, 1997), or the streptavidin core which normally forms a homotetramer (Ernst *et al.*, 1999).

One or more domains of the antibody could be excised to reduced the size of the molecule or to remove unwanted reactivities (e.g. Slavin-Chiorini *et al.*,1997). This can be done because of the modular structure of antibodies. Such truncation could result in a molecule that has better pharmacodynamics, or one that is capable of greater penetration into target tissue.

A very important use of antibody engineering is the reduction of the immunogenicity of therapeutic antibodies. Antibodies directed against human antigens are readily obtained from rodent sources. However, rodent antibodies are of limited use in human therapy because the patient's immune system will recognize them as foreign and will try to neutralize and eliminate them. To be effective in human therapy, nonhuman antibodies first have to be 'humanized'.

A significant reduction in the immunogenicity of a nonhuman antibody can be achieved by transplanting the nonhuman variable domains onto human constant regions (Boulianne *et al.*, 1984; Morrison *et al.*,1984). Further reduction can be achieved by transplanting only CDRs onto a human framework (Jones *et al.*, 1986; Reichmann *et al.*, 1988; Winter and Harris, 1993). The greatest reduction is achieved by transplanting only those residues which are involved in the interaction with the antigen, the SDRs (Padlan *et al.*, 1995). Alternatively, the exposed residues of the nonhuman antibody could be replaced with the analogous human residues so that the surface of the antibody would appear 'human-like' to the immune system (Padlan, 1991). Many 'humanized' antibodies are in clinical trials and several have been approved for human use.

An example of a successful 'humanization' is the work on CC49, a murine antibody directed against the tumor-associated antigen, TAG 72, which is a high-molecular weight mucin found on many different kinds of cancer cells. CC49 has been humanized by grafting CDRs, as well as by transplanting only the SDRs onto human framework regions (Kashmiri *et al.*, 1995, 2001; Tamura *et al.*, 2000).

The existence of antibodies specific for tumor markers permits the delivery of radioactivity or of cytotoxic drugs to cancer cells. The cytotoxic drugs may be conjugated directly to an antibody (FV or Fab) to generate an immunotoxin (Pastan, 1997), or encapsulated in an antibody-targeted liposome (an immunoliposome) [see Lopes de Menezes *et al.*, 2000; Maruyama, 2000; Mastrobattista *et al.*, 1999; Parker *et al.*, 1997]

Antibody Engineering in a Philippine Setting:

We have the knowledge and expertise to perform antibody engineering in the Philippines. Indeed, a group of Filipino scientists is currently exploring the possibility of using immunoliposomes to deliver anti-tumor drugs to cancer cells. This group, called AMOR (Antibody and Molecular Oncology Researchers), is

composed of scientists from several disciplines and institutions working together towards a common goal of contributing to the development of a treatment for breast and other cancers. Funding for the efforts of AMOR is being provided by the Department of Science and Technology through the Philippine Council for Health Research and Development.

AMOR was conceived in February, 1998 when the author and Dr. Gisela P. Concepcion of the Marine Scince Institute of the University of the Philippines, Diliman, decided to collaborate in an effort to develop an antibody-based treatment for cancer using Dr. Concepcion's potential anti-cancer natural products from Philippine marine samples (Concepcion *et al.*, 1995). Other Filipino scientists were recruited to participate in the AMOR project and many have joined. Those who have contributed in one way or another to the effort include: Rowena R. Antemano, Francisco S. Chung, Jr., Fabian M. Dayrit, Romulo S. de Villa, Mary Ann A. Endoma, Jose Mariano L. Escaner, Noreen R. Gonzales, Amelia P. Guevara, Glenson A. Hidalgo, Sonia D. Jacinto, Daniel A. Lagunzad, Jose Enrico H. Lazaro, Ramon R. Miranda, Virginia D. Monje, Rodmar C. Pulido, Bernadette S. Ramirez, Amy V.D. Roberto, Nina Rosario L. Rojas, Portia G. Sabido, Dennis L. Sacdalan, Rhea V. Samonte, Ameurfina D. Santos, James A. Villanueva, and Ma. Luisa A. Virata.

Following the concept of AMOR, two other groups of Filipino researchers, again from diverse disciplines and institutions, are being put together: one group to develop diagnostics kits for use in the early detection, monitoring and prognostics of cancer and the other to explore the possibility of using immune-based techniques for the protection of agricultural crops.

We hope that useful products will result from the efforts of AMOR and the other groups. Further, we hope that these Philippine-based and Philippine-manned efforts will serve as training ground for more Filipino scientists.

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