
Preface

Over 2000 years ago in China, antibodies elicited by early forms of vaccination likely played a major role in the protection of the population from infectious agents. Vaccination has been further developed in Europe and described by Edward Jenner in the late-eighteenth century, then successfully implemented worldwide. The idea to use the active ingredient in the blood of vaccinated (or immunized) animals or humans for the treatment of diseases came a century later. It was made possible by a series of discoveries, such as the realization that the serum from animals immunized with toxins, for example, diphtheria toxin or viruses, is an effective therapeutic against the disease caused by the same agent in humans. In the 1880s, von Behring developed an antitoxin (anti-body) that did not kill the bacteria but neutralized the bacterial toxin. The first Nobel Prize in Medicine (1901) was given to him for the discovery of the serum therapy. A century later, 22 monoclonal antibodies (mAbs) are approved by the United States Food and Drug Administration (FDA) for clinical use, and hundreds are in clinical trials for the treatment of various diseases including cancers, immune disorders, and infections. The revenues from the top-five therapeutic antibodies reached \$11.7 billion in 2006, and major pharmaceutical companies raced to acquire antibody biotech companies with a recent example of MedImmune, Inc., which was acquired for \$15.6 billion by AstraZeneca in 2007.

This explosion of research and development in the field of therapeutic antibodies prompted the publication of the MiMB volume *Therapeutic Antibodies: Methods and Protocols*. The book's major goal is to present a set of protocols useful for researchers discovering and developing therapeutic antibodies. Current advances and future trends in the antibody therapeutics are analyzed in the lead-in review article. The road from identification or selection of appropriate targets to antibodies in clinical use is divided into five major stages: (1) recombinant antigens, (2) antibody libraries, (3) antibody discovery, (4) antibody engineering, and (5) antibody preclinical development. Also a low-cost antibody sequence database is described in the last chapter. Representative protocols for each stage are written by leading experts from academic laboratories and biotechnology companies. Protocols for antibodies as reagents are not included because of the existence of excellent books on methods for such antibody generation and characterization.

Part I includes several methods that have been successfully employed to produce, purify, and characterize soluble secreted versions of several viral envelope glycoproteins successfully used as antigens for selection of neutralizing human monoclonal antibodies. Part II details methods to create phage libraries of human synthetic single-chain antibodies, human antibody domains (V_H), and rabbit antibodies. It also details a method for construction of a large naïve human Fab library, which was successfully used for selection of potent neutralizing antibodies against viruses and cancer-related proteins. Part III contains protocols for selecting antibodies against intracellular targets, specific internalization fragments, antibodies with broad spectrum of binding

and neutralization, non-aggregating V_H binders from synthetic phage libraries, and IgGs from combinatorial libraries expressed in *Escherichia coli*. It also contains advanced methods for high-throughput screening of single-chain antibodies, identification of fully human antigen-specific antibody repertoire from plasma cells, and rapid screening platform for stabilization of single-chain antibodies. Part IV covers methods for antibody engineering including affinity maturation, construction of tetravalent bispecific antibodies, deimmunization of antibodies, and preparation and characterization of antibody conjugates for targeted cancer therapies. Part V describes several aspects of the antibody preclinical development including high-level production for laboratory studies, scaling up and production for preclinical animal studies, in vitro antibody potency and breadth of virus neutralization, and in vivo methods for establishing synergy between antibodies in cancer therapy in mice and passive immunization against HIV-1 in macaques.

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