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ACKNOWLEDGMENT

The author would like to thank Dr. Robert L. Hunter for his assistance and guidance in the writing of this manuscript.

Monoclonal Antibodies by Somatic Cell Fusion

Christine M. Grimaldi and Deborah L. French

INTRODUCTION

Immunoglobulins or antibody molecules are synthesized by B-lymphocytes in response to a spectrum of biological macromolecules. Antibodies are bifunctional molecules that bind specifically to antigenic determinants on macromolecules and eliminate foreign substances through recruitment of effector mechanisms. The core structure of an antibody molecule comprises two identical, covalently-linked heavy chains that, in most instances, are covalently-linked to two identical light chains. Two distinct areas of the core structure, designated variable and constant regions, mediate the antigen binding and effector functions. The heavy and light chain variable region domains form the antigen binding site and the heavy chain constant region contains the recognition sites for a variety of effector systems. Due to the functional assembly and expression of single heavy and light chain alleles, each B cell expresses a homogenous population of antibody molecules of a single antigenic specificity. The ability to immortalize individual B-lymphocytes that produce monoclonal antibodies of a particular antigenic specificity is the basis of the somatic cell fusion or hybridoma technology (Köhler and Milstein, 1975). Monoclonal antibodies are standard tools and reagents in numerous scientific disciplines and their availability has expanded applications in diagnostics and therapeutics.

The generation of rodent monoclonal antibodies by the hybridoma technology or somatic cell fusion has been very successful. This technology has resulted in the generation of thousands of monoclonal antibodies creating groups of antibodies with precisely defined antigenic specificities. This article presents an overview of monoclonal antibody production by somatic cell fusion using the mouse as the prototype animal model. Discussions of myeloma fusion partners, antigen choice and preparation, and in vivo and in vitro immunization are included. Genetic and in vitro manipulations for isolating isotype switch variants for functional and in vivo use will also be discussed. Detailed discussions of hybridoma methodology and applications of monoclonal antibodies can be found in excellent reviews (Galfrè and Milstein, 1981; French et al., 1987) and books (Harlow and Lane, 1988; Ritter and Ladyman, 1995).

Christine M. Grimaldi, B.S., M.S, is with the Department of Pathology, State University of New York at Stony Brook. Deborah L. French, Ph.D., is an assistant professor with the Department of Medicine, Mount Sinai School of Medicine, New York, New York.

B-CELL DEVELOPMENT AND ANTIBODY SYNTHESIS

The desired goal of generating monoclonal antibodies is to obtain a panel of antibodies with the appropriate antigen

specificity, affinity, and heavy chain isotype. The generation of monoclonal antibodies is dependent on numerous criteria that include sources of B cells, immunization schemes, B-cell immortalization procedures, stability of hybrid clones or cell lines, and production of sufficient quantities of antibody for purification (Borrebaeck, 1989). The first step in obtaining antigen-specific monoclonal antibodies that are of high affinity is to expand the desired B-cell clones by immunization with appropriate antigen. To understand the process of B-cell proliferation and differentiation, a brief review of B-cell development and antibody synthesis highlights essential features important in the expansion of antigen-specific B-cell clones.

In adult mammals, B-lymphocyte differentiation occurs in the bone marrow in stages defined as antigen independent and antigen dependent (Kincade, 1987). During the antigen independent stage of development, sequential rearrangement and expression of immunoglobulin heavy and light chain genes occurs (Alt et al., 1987). The immunoglobulin heavy chain genes are rearranged and expressed first followed by light chain gene rearrangement and expression. The heavy chain of IgM (μ) appears first in the cytoplasm of developing B-lymphocytes and is followed by the appearance of κ or λ light chains. The heavy and light chains are expressed on the cell surface as a monomeric membrane complex that forms the antigen receptor on B-lymphocytes. This cell surface receptor is required for the antigen dependent stage of B-cell development. The activation of antigen-specific B-cell clones is initiated by cross-linking membrane immunoglobulin with antigen. This process results in the expansion and differentiation of antigen-specific clones of B cells, which forms the basis of the clonal selection theory of B-lymphocyte development (Burnet, 1959). The expansion of antigen-stimulated B-cell clones defines the repertoire or number of different antigenic specificities in an immune response. The final stage of B-cell development is the differentiation of mature B cells to terminally differentiated plasma cells that function to secrete large quantities of antigen-specific antibody molecules into the circulation.

Upon initial exposure to antigen, a primary immune response develops in which antibodies of the IgM isotype are generated. IgM is secreted as a large pentameric molecule that has high avidity due to ten antigen binding sites, but low affinity. Upon re-exposure to antigen, a secondary immune response develops in which high-affinity bivalent IgG molecules are generated. This response depends on the presence of long-lived memory B and T cells (MacLennan and Gray, 1986) that are generated during the antigen-dependent phase of an immune response. Memory B cells express surface immunoglobulin and both memory B and T cells reside in lymphoid organs. In a secondary immune response, re-exposure to the same antigen activates and expands memory cell clones, which results in the immediate appearance of circulating antibody without a lag phase that occurs in a primary immune response.

During repeated exposures to antigen, two essential processes in B-cell development occur: (1) affinity maturation of an immune response (Eisen and Siskind, 1964) and (2) heavy chain switching of antibody isotypes (Shimizu and Honjo, 1984). The affinity maturation of an immune response is achieved by the mechanism of somatic hypermutation (Tonegawa, 1983; French et al., 1989). This mechanism generates nucleotide substitutions within heavy and light chain variable region genes (Weigert et al., 1970) that can result in amino acid substitutions affecting the affinity of the antibody combining site (Berek and Ziegner, 1993). Immunoglobulin isotype switching results in the association of different heavy chain constant region domains with the same variable region domain (Rothman et al., 1989). Progeny B-lymphocytes switch from IgM, the first isotype expressed in all primary immune responses, to another heavy chain class or subclass resulting in populations of antibody molecules that have the same antigen binding specificities associated with different isotypes such as IgG, IgA, or IgE. The different isotypes or constant regions of the antibody molecule carry distinct recognition sites for receptors of a variety of effector systems.

The events associated with affinity maturation, clonal expansion, and isotype switching require help from subsets of T-lymphocytes (Coffman et al., 1988; Snapper and Mond, 1993). Through direct cell contact or secreted soluble cytokines, these cells provide signals that help B cells to proliferate and differentiate. By crosslinking the T-cell receptor (TCR) with antigen, T-lymphocytes are activated and can help in the expansion and maturation of B-lymphocytes. The TCR recognizes antigen that is expressed on the surface of antigen presenting cells (APC) such as macrophages, dendritic cells, or B-lymphoctyes. The APC enzymatically process antigen into small peptides that sit in a groove formed by major histocompatibility complex molecules that are expressed on the cell surface (Bjorkman et al., 1987). In choosing antigen and immunization schemes for generating specific monoclonal antibodies, the activation and expansion of B- and T-lymphocytes is an essential feature for generating high affinity antibodies that tend to be of IgG isotypes.

SOMATIC CELL FUSION OR HYBRIDOMA TECHNOLOGY

In 1975, Köhler and Milstein demonstrated that fusion of murine B cells to myeloma cells resulted in the generation of immortalized cell lines that secreted antibody of a predefined specificity. This seminal discovery is the foundation for monoclonal antibody production. The development of the hybridoma technology has resulted in panels of monoclonal antibodies specific for a wide array of complex antigens and peptides. Excellent reviews of this procedure have been written and can be referred to for in-depth discussions of theory and protocols including choice of animal, antigen and immunization, and screening assays (Galfrè and Milstein, 1981; Harlow and Lane, 1988; Coligan et al., 1991; Ritter and Ladyman, 1995). The mouse is the best choice and most common host for generating monoclonal antibodies by the

hybridoma technology. Rats have also been used successfully and are a good second choice. For practical reasons, rabbits are the best choice for routine production of polyclonal antibodies. Up to 500 ml of sera can be safely and easily obtained and rabbit antibodies are well characterized and easily purified (Harlow and Lane, 1988; Coligan et al., 1991). Each laboratory has its own variation of the basic hybridoma methodology and we use a modification (French et al., 1987) of an earlier protocol (Fazekas de St. Groth and Scheidegger, 1980). The goal of the procedure is to generate hybridoma cell lines from immunized B-lymphocytes that produce the desired monoclonal antibody and to immortalize these cells by fusing them to myeloma tumor cells. Excellent mouse and rat myeloma fusion partners are available (Galfrè and Milstein, 1981), thus making these animals the best choice for the procedure. We routinely use the NSO myeloma cell line which was generated from the inbred mouse strain BALB/c and does not express antibody heavy or light chains. Most myeloma tumor cells used as fusion partners are derived from BALB/c mice or LOU rats, thus these strains are frequently used for immunization and monoclonal antibody production.

Myeloma cell lines can be selected in a medium containing hypoxanthine, thymidine, and aminopterin (HAT), thus nonfused myeloma cells die and only those cells fused to normal cells survive. Myeloma fusion partners are deficient in an enzyme required for the salvage pathway of nucleotide synthesis. These cells will die in HAT-containing medium because aminopterin blocks normal nucleotide synthesis and the enzyme deficiency blocks utilization of hypoxanthine or thymidine in the salvage pathway. If myeloma and normal cells fuse, the resulting hybridoma will live indefinitely in culture because the normal cell supplies the missing enzyme for selection in HAT-containing medium and the myeloma cell immortalizes the cell line. Unfused normal lymphocytes will only survive in tissue culture for approximately 1 week before they die. The choice of a myeloma cell fusion partner should be genetically compatible with the immunized B-cell source because hybridomas generated from cells of the same species are more stable than hybridomas generated from different species.

The somatic cell fusion or hybridoma procedure in the mouse is schematically represented in Figure 1. Hybridoma cell lines are generated by fusing splenocytes from an immunized mouse to a mouse myeloma cell fusion partner using polyethylene glycol (PEG). The cells are resuspended in HAT-containing medium and plated in microtiter plates. Usually within 2 weeks of selection, hybrid clones are visible and culture supernatants can be screened for antigen-specific antibody. Wells containing the desired antibody can be identified by a number of different assay systems, of which the most commonly used are direct or indirect enzyme-linked immunosorbent assay (ELISA), whole cell ELISA, immunoprecipitation, or immunoblot. Detailed explanations of these assays can be found elsewhere (Harlow and Lane, 1988; Coligan et al., 1991). The ease and reproducibility of the screening assay is an essential feature of a successful fusion

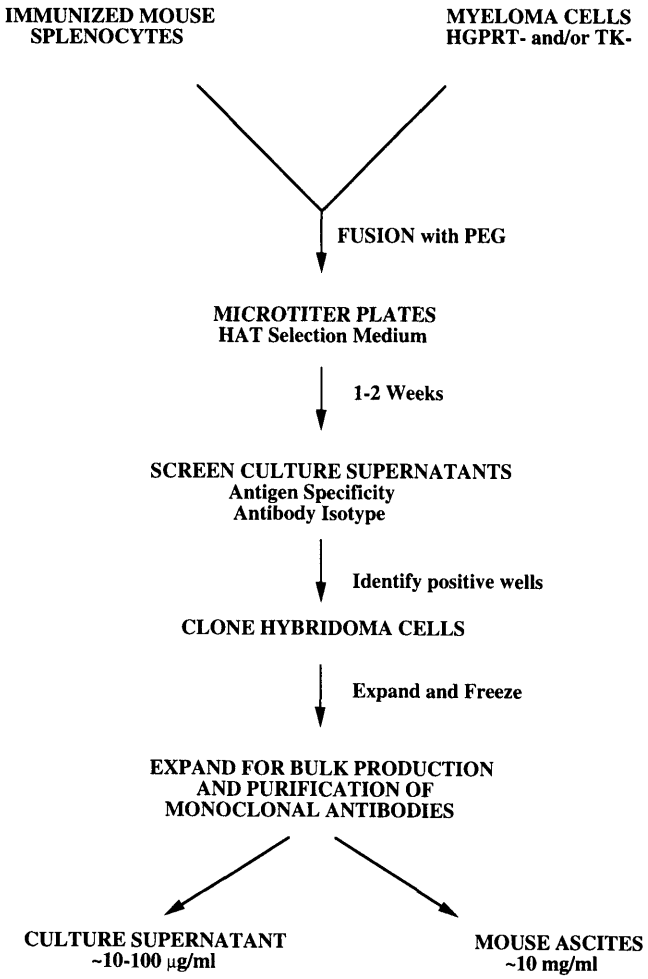

FIGURE 1 Hybridoma technology. Schematic of monoclonal antibody production in the mouse by somatic cell fusion. Abbreviations: HGPRT=hypoxanthineguanine phosphoribosyltransferase; TK=thymidine kinase; PEG=polyethylene glycol; HAT=hypoxanthine, aminopterin, thymidine.

and should be reliable and functional at this stage of the procedure. Since a single well may contain multiple hybridoma clones, the isolation of a single hybridoma cell from a positive well is performed by cloning in soft agar (French et al., 1987; Coligan et al., 1991) or limiting dilution (Coligan et al., 1991). To ensure that a single hybridoma cell is isolated that generates the monoclonal antibody of interest, each hybridoma cell line is usually cloned twice. Once hybridoma cells are successfully cloned, the cells are expanded for freezing and generation of stock solutions of monoclonal antibody. Bulk solutions of monoclonal antibodies are generated by tissue culture or by ascites production in mice (Harlow and Lane, 1988; Coligan et al., 1991). Antibody synthesis from hybridoma cells is variable and antibody concentrations in tissue culture supernatants are usually in the range of 10-20 μg/ml, but can be as high as 100 μg/ml from very high producing clones. Large amounts of monoclonal antibody can be generated as ascites fluid in a mouse that is MHC compatible with the myeloma fusion partner and spleen cell donor. The hybridoma cell line grows in the peritoneal

cavity of a mouse where ~10 mg/ml of monoclonal antibody is secreted into the abdominal ascitic fluid.

ANTIGEN CHOICE, PREPARATION, AND IMMUNIZATION

Soluble and Membrane Antigens

The generation of monoclonal antibodies by the hybridoma technology is dependent on immortalization of B-cell clones by fusion (Galfrè and Milstein, 1981). A key feature in the success of this procedure is the immunization and expansion of antigen-specific B-cell clones to high enough numbers such that the frequency of targeting events for successful immortalization will be increased. The expansion and isolation of B-cell clones expressing antibody molecules of a desired antigen-specificity and affinity is modulated by the immunogenicity of the antigen used for immunization. Immunogens are defined as molecules that can elicit an immune response. Features that contribute to the immunogenicity of a molecule include size, complexity, and non-self or foreignness to the host. In general, immunogens must have a molecular weight greater than 10,000 daltons and globular proteins are usually more immunogenic than carbohydrates, lipids, or nucleic acids. The immunogenicity of a molecule is not always known and in these situations immunizations can be performed and test bleeds analyzed for antibody production, specificity, and isotype expression. When possible, test bleeds should always be analyzed before any procedure to generate monoclonal antibodies because a high titer, specific response of the appropriate isotype indicates clonal expansion. In addition, utilization of the same screening assay that will be used for selection of monoclonal antibodies will ensure specificity, ease, and reproducibility of the assay system.

The immunogenicity of antigen molecules can be enhanced by modification procedures and purification schemes. If monoclonal antibodies are to be used for recognition of native protein and particularly if anti-functional antibodies (Ramos-Desimone et al., 1993) are desired, a good immunizing source is native antigen. The antigenic determinants on native antigen that are recognized by antibodies are usually conformational epitopes (Lerner, 1982; Davies et al., 1988). These epitopes are formed by amino acid residues that are distant from each other in the primary sequence and are brought together in the native structure. Monoclonal antibodies that recognize conformational determinants may not recognize denatured protein, thus all monoclonal antibodies must be tested for reactivity to native and denatured protein in standard ELISA, immunoblot, and immunoprecipitation analyses (Coligan et al., 1991).

If microgram to milligram quantities of specific antigen are present in crude, semicrude, or pure preparations for immunization, the immunogenicity of antigen can be enhanced by conjugation to immunogenic carrier molecules (such as bovine serum albumin), emulsification in adjuvant, or both. Soluble proteins are emulsified in adjuvants, such as Freunds adjuvant or RIBI Adjuvant System®, which create reservoirs of antigen that are released slowly over time and induce inflammatory responses resulting in the generation of nonspecific cytokines that activate immune cells. Conjugation procedures of soluble antigens to particulate molecules, such as agarose beads, stimulate phagocytosis of the complex resulting in processing and presentation of antigenic peptides to T-lymphocytes (Unanue, 1984; Townsend and Bodmer, 1989). In addition, the process activates antigen presenting cells such as macrophages to secrete cytokines that enhance clonal expansion.

Methodologies to semi-purify a protein of interest and concentrate proteins in low abundance include SDS-PAGE and immunoblot in which gel slices or pieces of nitrocellulose containing the protein of interest can be used for immunization. The acrylamide or nitrocellulose acts as an adjuvant inducing an inflammatory response. Immunogenicity can be enhanced by dipping or emulsifying the piece of nitrocellulose in adjuvant and injecting (Knudsen, 1985) or laying the piece of nitrocellulose directly on the spleen.

Particulate antigens such as whole cells and cell lysates are good immunogens because of quantity, complexity, and foreignness particularly if the antigen and immunizing host are of different species. Immunogenicity can be enhanced by emulsification of the whole cell or lysate in adjuvant. The success of obtaining monoclonal antibodies to specific cellular proteins depends on the abundance and immunogenicity of the intracellular or surface protein or receptor. In addition, the screening assay must differentiate between monoclonal antibodies specific for the desired protein and those specific for other proteins expressed in or on the surface of the cell. Mice immunized with purified human platelets emulsified in adjuvant, resulted in the successful isolation of monoclonal antibodies recognizing platelet surface receptors (Coller et al., 1983a) and complex-dependent anti-functional antibodies that inhibit platelet aggregation (Coller et al., 1983b; Coller, 1985). In this situation, the identified platelet proteins are in high abundance on the cell surface.

In other situations, cellular proteins are expressed in low abundance on the cell surface, on a subset of cells at particular stages of development, or in a disease state such as on tumor cells. In these cases, additional manipulations may be required to activate and expand the desired antigen-specific B-cell clones. A methodology that has proven successful in the isolation of monoclonal antibodies specific for cell surface proteins of low abundance and immunogenicity on metastatic tumor cells is subtractive immunization (Williams et al., 1992). This procedure resulted in the isolation of a panel of monoclonal antibodies that inhibited metastasis in an in vivo chick embryo model (Brooks et al., 1993). The basis of this procedure is the elimination of antigen-specific B-cell clones that recognize cell surface proteins on all subsets of cells by using cyclophosphamide. Animals are initially immunized with a population of cells that express cell-surface proteins that are in abundance and common to all cells. Injections of cyclophosphamide are given to eliminate these antigen-activated B-cell clones. Animals are then immu-

nized with a subset of cells expressing the antigen of interest and test bleeds are analyzed for production of specific antibody and isotype expression. Somatic cell fusions are performed during this window of reactivity to immortalize the expanded antigen-specific B-cell clones. An alternative to this approach is to immunologically tolerize an animal to one set of antigens before immunization with the antigen of interest (Iman et al., 1994). This procedure resulted in the generation of monoclonal antibodies that identified a previously unknown luminal epithelial antigen on normal breast epithelium.

Bacterial Expression Systems to Purify Antigens

The use of soluble or cellular proteins for immunization is advantageous but may not be possible or provide the best results due to insufficient quantity, low immunogenicity, or protein-protein interactions that may mask important antigenic determinants. Molecular biology provides a powerful approach to isolate antigens that are in low abundance, difficult to purify, weakly immunogenic, or unknown. If partial or complete sequences of known or unknown proteins are identified, bacterial expression systems can be used to isolate proteins that are to be used as immunogens. Bacterial expression systems have been developed in which a gene or partial gene sequence is cloned into a specially designed vector and expressed as a fusion protein that has been designed for easy purification. The glutathione S-transferase (GST) gene fusion system (Smith and Johnson, 1988) results in high level expression of a desired protein fused to GST. The expressed protein is purified by affinity chromatography and cleaved from the fusion product using a site-specific protease. Another clever design for easy purification of bacterially expressed proteins is metal-chelate affinity chromatography (Hochuli et al., 1988; Smith et al., 1988). This protocol uses histidine-tail fusion proteins in which nucleotides encoding these amino acid residues are added to the cloned insert using the polymerase chain reaction (PCR) or are already incorporated into specially designed vectors for this purpose. Proteins are purified on nickel-containing resins and incorporation of protease cleavage sites in the vector sequence enables the removal of the fusion tail after protein purification. Besides overexpressing the protein and yielding sufficient quantities for immunization, the fusion proteins themselves, such as GST, are immunogenic and can potentially boost an immune response for the specific protein. Screening for antigen specificity of the monoclonal antibodies must be performed with purified protein lacking the fusion partner. An alternative in vivo approach to obtain high level expression of an antigen of interest is genetic immunization (Barry et al., 1994). Expression vectors containing the gene or sequence of interest are injected as DNA-coated microprojectiles into animals using a propelling device (Vahlsing et al., 1994). This technique has been successful in the production of monoclonal antibodies specific for native and denatured forms of human growth hormone (Barry et al., 1994).

Peptide Antigens

Another approach to immunizing with proteins that are in low abundance or difficult to purify, such as transmembrane proteins, has been the use of peptides (Lerner, 1982). Peptides have been successfully used to elicit monoclonal antibodies that neutralize virus (Chin et al., 1994; Fraisier et al., 1994) and recognize native antigen (Lerner, 1982; Wilson et al., 1994). The goal of this approach is to develop systems in which antipeptide monoclonal antibodies are elicited that cross-react with native antigen. Due to their small size, peptides are not immunogenic. An immune response will be elicited after conjugation to carrier proteins such as bovine serum albumin or keyhole limpet hemocyanin and a variety of cross-linking reagents are commercially available for this purpose. The use of peptide conjugates or fusion proteins as immunogens results in the expansion of peptide-specific B-cell clones and clones specific for the conjugated or fused protein. The number of clones specific for the peptide will probably be small compared to the number of clones specific for immunogenic carrier or fusion proteins. In addition, any coupling procedure may modify the peptide and destroy or create epitopes. To generate peptide-specific monoclonal antibodies without the use of carrier proteins, the multiple antigenic peptide (MAP) system was developed (Tam, 1988). This methodology was developed for vaccine strategy and involves the direct solid-phase synthesis of peptides on a core of eight lysine residues. The bulk of molecular weight is contributed by the peptides and a small percent of the molecule is represented by the lysine core. Studies have demonstrated that the exposed ends of the peptides are immunogenic and no response is generated to the lysine core or conjugation sites (Tam, 1988). By using this system in conjunction with adjuvant, anti-peptide monoclonal antibodies were generated, some of which recognized the native structure of the β chain of the T-cell receptor (Posnett et al., 1988).

Since adjuvants are toxic and inflammatory to the animal, alternatives to adjuvant use are being developed. One approach for the immunization of unconjugated peptides has been the use of liposomes containing a non-toxic adjuvant, monophosphoryl lipid A (MPLA) (Friede et al., 1993). Surface bound peptides in liposomes containing a neutral charge generate a long-lasting polyclonal IgG response to native protein. Another interesting approach for peptide presentation and immunization has been the genetic engineering of peptide sequences into the variable region of an antibody molecule (Brumeanu et al., 1995). Viral epitopes from Influenza A and Human Immunodeficiency Virus (HIV) have been engineered into critical regions of the antibody combining site and the antibody molecule has been used as the immunizing vehicle. The half-life of the injected antibody has been increased by derivatization of the molecule using monometho-

xypolyethylene glycol that reacts with lysine residues on the antibody molecule. The derivatized molecules are immunogenic, elicit antibodies specific for the peptide, and do not require toxic adjuvants to stimulate the response. Another approach uses antigen presenting cells as the immunizing vehicle. Syngeneic dendritic cells pulsed in vitro with antigen induce a strong antibody response in mice upon challenge with soluble antigen (Snorasse et al., 1992). This method of immunization was as good as adjuvant in generating antigen-specific monoclonal antibodies of the IgG isotype.

In Vitro Immunization

As an alternative to in vivo immunization, in vitro immunization with antigen has been successfully used (Borrebaeck, 1989). This method was devised to enrich the percentage of antigen-specific B cells prior to immortalization and to escape potential in vivo regulatory mechanisms that may inhibit a particular antibody response (Hengartner et al., 1978). This procedure is beneficial when antigen is in limited supply and previous attempts at in vivo immunization have yielded negative results possibly due to a similarity to self antigens or to the weak immunogenicity of the antigen (Bunse and Heinz, 1994). Other advantages of this approach are that fewer animals are required, the immunization period is 4-5 days compared with weeks and months for in vivo immunization, it is possible to monitor and control the immune response without the immune regulatory mechanisms that occur in vivo, and monoclonal antibodies can be generated against toxic agents. Using culture systems containing non-immune mouse splenocytes that include B cells and APC, the addition of T cells or T-cell derived cytokines with antigen in vitro may result in the clonal expansion and maturation of antigen specific B cells that are representative of cells in a secondary memory B-cell response. The in vitro immunization of non-immune mouse splenocytes with inactivated viral proteins was performed in the presence of T-cell derived cytokines (Stäuber et al., 1993). Antigen-specific monoclonal antibodies were generated in which 14% were IgG, 25% were IgM, 18% typed both IgG and IgM, and 43% were not classified. Another study used a commercial kit for in vitro immunization and generated neutralizing monoclonal antibodies to an immunomodulatory protein in which 20% were IgM, 16% were IgG, and 6% were IgA (Guzman et al., 1995).

GENETIC AND IN VITRO MANIPULATION OF MONOCLONAL ANTIBODIES

The constant region of an antibody molecule is responsible for recruitment of a variety of effector functions. For in vivo applications, the half-life of an antibody molecule is determined by the constant region (Pollock et al., 1990). Situations arise in which monoclonal antibodies may have appropriate antigen specificities, but different isotypes or constant regions may be desired for purification purposes, to increase half-life, or to perform specific effector functions. Methodologies have been developed to isolate switch variants in culture or genetically engineer an antibody molecule to express a desired isotype. One study generated switch variants in culture in which IgA monoclonal antibodies were isolated from an IgG secreting hybridoma cell line (Steinmetz et al., 1994). These antibodies were generated for passive immunization into mice to study the local defense mechanisms in upper respiratory tract infections.

The isolation of switch variants from hybridoma cell lines utilizes sib selection (Cavalli-Sforza and Lederberg, 1956) coupled with a number of different screening assays including fluorescence activated cell sorting (FACS) and ELISA (Radbruch et al., 1980; Dangl and Herzenberg, 1982; Müller and Rajewsky, 1983; Spira et al., 1984). The incidence of heavy chain class switching in hybridoma cell lines is low, but a new selection scheme using the ELISA spot assay (Czerkinsky et al., 1983) has improved the methodology (Spira et al., 1993). The identification of switch variants within a large population of cells is more reliable and sensitive and rates of heavy chain class switching can be determined. The use of this method has identified subclones of cells within a population of hybridoma cells that undergo switching at high rates comparable to B-cell lines and normal B cells (Spira et al., 1994).

Another approach to heavy chain class switching is genetic engineering. This approach requires the cloning of desired variable region genes into specially designed vectors containing constant region genes (Morrison et al., 1984). These vectors are used routinely for the production of chimeric antibodies in which rodent variable region genes are expressed with human constant region genes. This approach guarantees the expression of an antibody with a particular isotype. A precaution before switching any monoclonal antibody from IgM to IgG is first to consider the affinity of the IgM molecule for antigen. An IgM molecule may have high avidity due to the pentameric structure of the secreted molecule, but the affinity of the individual antibody-combining site may be below the limits of antigen binding. A quick analysis to determine if switching IgM to IgG will result in antibodies that bind antigen is to test antigen binding reactivity of monomeric IgM from cell lysates. These approaches provide the means to switch isotypes of existing fully characterized monoclonal antibodies and eliminate the generation of new monoclonal antibodies in hopes of identifying the desired isotype.

CONCLUSION

The somatic cell fusion or hybridoma technology continues to be used today for the generation of antigen-specific, high affinity mouse and rat monoclonal antibodies of a variety of isotypes. Numerous immunization schemes and protocols have been devised to modulate the immune response and increase the chances of obtaining antibodies to the desired antigenic specificity. The use of genetic manipulations

coupled with established conventional immunization and immortalization protocols provides the means to obtain monoclonal antibodies of desired specificity, affinity, and isotypes.

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Recombinant Antibody Technology

Alexander E. Karu, Christopher W. Bell, and Tina E. Chin

INTRODUCTION

Monoclonal antibody methods give us the ability to derive individual antibodies of invariant specificity and selectivity, and to immortalize the antibody-producing cells, ensuring a virtually infinite supply (Köhler and Milstein, 1975). However, hybridoma technology requires substantial time, labor, expense, specialized cell culture facilities, the use of animals, and the expertise to prepare and screen large numbers of hybridomas to select the best ones. The number of substances that are immunogenic in mammals is limited, and the maximum diversity expected from the mammalian immune response is on the order of 6×10^6 different antibodies (Harlow and Lane, 1988, p. 16). More important, there is no practical way to alter the properties of antibodies produced by hybridomas.

Interest in isolating and expressing antibody genes developed after the first descriptions of hybridoma production by Köhler and Milstein (1975). Recombinant antibody technology is based on advances in the understanding of antibody structure and function, the biology of bacteriophage replication, and new techniques for DNA manipulation and mutagenesis. After the DNA sequences of many immunoglobulin heavy and light chain variable domains (V_H and V_L) were determined, consensus oligonucleotide primers were designed to recover the genes from new antibodies (Orlandi et al., 1989; Coloma et al., 1991). The polymerase chain reaction (PCR) was used to amplify the genes from a single-stranded DNA copy of the antibody messenger RNA (mRNA) (Mullis, 1990). This was accompanied by the development of new plasmid and bacteriophage cloning vectors for the selection and expression of antibodies. Additional necessary methods were quickly adapted from other applications.

In this article we present a brief review of antibody architecture; an introduction to the major steps in deriving, selecting, and expressing recombinant antibodies; an overview of the present status of antibody engineering and semi-synthetic combinatorial antibody libraries; and our perspectives on how these emerging technologies will affect the use of animals in research.

Alexander E. Karu, Ph.D. is a research biochemist and director of the Hybridoma Facility in the College of Natural Resources at the University of California, Berkeley. Christopher W. Bell, Ph.D. is a postdoctoral research fellow, and Tina E. Chin, B.S. is an assistant specialist in the Hybridoma Facility.

GENERAL PRINCIPLES

Antibody Structure

This article includes references to monoclonal antibodies (MAbs), Fabs, and Fv fragments. These are diagrammed in Figure 1. Although antibodies are very specific in their rec-