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REVIEW ARTICLE

Monoclonal antibodies. Basic features

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Abstract
Introduction: Monoclonal antibodies are a powerful tool in laboratory diagnosis and are increasingly used in the treatment of several diseases.
Development: Antibody development and characterization has a long history and goes back to immunology itself. The present article provides a historical introduction to humoral immunity until the discovery of monoclonal antibodies and reviews concepts relating to the structure and function of antibodies, as well as to the generation of diversity, activation and maturation of B lymphocytes.The main techniques for producing monoclonal antibodies are outlined and some of their applications in human disease are described.
Conclusions: Since their discovery , monoclonal antibodies have revolutionized the diagnosis and treatment of numerous diseases. The use of human and humanized monoclonal antibodies has markedly improved their tolerability. Current technology for manufacturing these antibodies allows new designs that may broaden their possible applications in medicine.

PALABRAS CLAVE
Inmunidad humoral;
Anticuerpos monoclonales

Anticuerpos monoclonales. Aspectos básicos

Resumen
Introducción: Los anticuerpos monoclonales son una poderosa herramienta para el diagnóstico de laboratorio y un instrumento cada vez más utilizado en el tratamiento de diversas enfermedades.
Desarrollo: El descubrimiento y caracterización de los anticuerpos tiene una larga historia, que es la de la propia inmunología. En este artículo se hace una introducción histórica sobre la inmunidad humoral hasta el hallazgo de los anticuerpos monoclonales y se revisan conceptos relativos a la estructura y funciones de los anticuerpos, así como a la

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generación de diversidad, activación y maduración de los linfocitos B. Se mencionan las principales técnicas de producción de anticuerpos monoclonales y se enumeran algunas de sus aplicaciones en patología humana.
Conclusiones: Los anticuerpos monoclonales han producido desde su descubrimiento una revolución de gran calado en el diagnóstico y el tratamiento de numerosas enfermedades. La utilización de anticuerpos monoclonales humanizados y humanos ha mejorado notablemente su tolerancia. La tecnología actual de fabricación de estos anticuerpos permite nuevos diseños que pueden ampliar sus posibles aplicaciones en medicina.

Introduction

Antibody discovery and characterization has a long history; in fact, its history is as long as that of immunology itself, dating back to the end of the 19th century. At that time, microbiologists were studying the organism's defence mechanisms against microbial agents, more specifically, against bacterial toxins. In the last decade of the 19th century, Von Behring and Kitasato laid the foundations of humoral immunity when they discovery that serum produced substances that antagonized toxins such as the toxins responsible for diphtheria or tetanus. Ehrlich, at the end of the century, consolidated the idea that the toxins generated serum antitoxins that behaved according to the laws of chemistry; blood cells were capable of producing side chains that reacted specifically against the toxins, much in the same way as a key to its lock. [1] Depending on the different properties the antitoxins had to react, they received different names: agglutinins, opsonins, etc.

During the third decade of the 20th century, Landsteiner, the discoverer of the ABO system, identified all those functions and focused them in a single molecule, the antibody, while at the same time, he began to replace the name "toxin" by the term "antigen". Years later, this same author, together with Pauling, developed the instructionist theory of antibody formation, according to which antigens would determine the conformation of antibodies, adapting it to its structure. At the end of the forties, the cell origin of antibodies in B cells and plasma cells is discovered and years later, light chains are described and A, D, and E immunoglobulins are discovered.

In contrast to the instructionist theory, during the fifties, Jerne proposed that antibodies pre-existed in the organism and that the function of antigens would be that of selecting the most appropriate antibody.[2] Shortly afterward, Burnett and Talmage put forward the theory of clonal selection [3] that perfects and expands Jerne's ideas, presupposing that each B cell produces a single type of antibody with a particular specificity, which is generated by random somatic mutations during the process of cell maturation; later, exposure to antigens causes these B cells to proliferate.

During the sixties, the concept of idiotype is described and, during the seventies, the theory of idiotype/anti-idiotype networks is coined, although the revolution in the world of antibodies does not take place until 1975 in Cambridge, when Milstein and Köhler discover monoclonal antibodies. In 1976, Tonegawa describes the somatic recombination of immunoglobulins. [4] Since then, research has consummated our knowledge about molecular genetics of antibodies and the mechanisms by which its diversity is generated.

Structure and basic characteristics of antibodies

Each molecule of an antibody is made up of 4 chains, 2 light and 2 heavy, each of which is identical to one another and joined by disulphide bridges forming a spatial structure that is similar to a Y. Antibodies have 2 basic functions, that of recognizing and binding to antigens, which they perform through the amino terminal ends of the chains, as well as an effector function, carried out by the carboxyl terminal end of the heavy chains (fig. 1).

The light chains have a variable portion, on which specificity depends, as well as a constant region that differs according to whether they are κ or λ light chains.

Likewise, the heavy chains also have a variable region and a constant region, which will determine the main immunoglobulin (Ig) classes or isotypes: γ, μ, α, δ, and ε, which will form IgG, IgM, IgA, IgD, and IgE, respectively. Furthermore, IgA has 2 subclasses, IgA1 and IgA2, and IgG is divided into 4 subclasses: IgG1, IgG2, IgG3, and IgG4. The properties of the Ig depend on each class and subclass. Once secreted, Ig are monomers, with exception of IgA, which forms dimers, and IgM, which forms pentamers.

The variable regions of the light and heavy chains are juxtaposed to form the binding site to the antigen; consequently, there are 2 antigen binding sites in each antibody molecule.

Within the structure of the Ig chains, repeated structures of 110 amino acids (AA) with a beta fold are called domains. Light chains have 1 domain in the variable region (VL) and 1 in the constant region (CL). In turn, heavy chains have 1 domain in the variable region (VH) and 3 or 4 in the constant region (CH), depending on the class of Ig. The CH1 and CH2 domains contain an area known as the constant chain hinge area that confers it flexibility and allows for adaptable spatial coupling.

In the variable regions of light and heavy chains, there are 3 hypervariable segments containing 10 juxtaposed AA that form the antigen binding site, called CDR 1, 2, and 3,

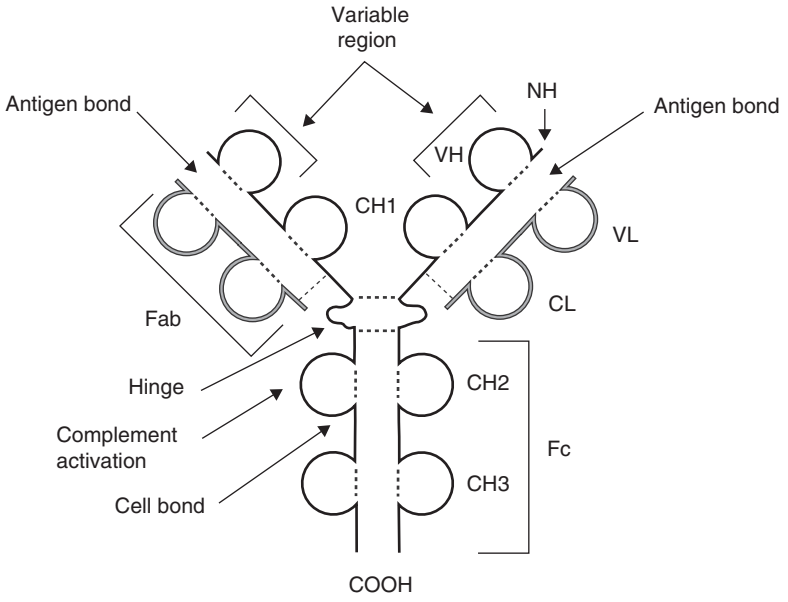

Figure 1 Outline of the structure of an immunoglobulin molecule. The heavy chains appear in black and the light ones in light grey. CH: domains of the constant region of the heavy chain; CL: constant domain of the light chain; COOH: carboxyl terminal end; Fab and Fc: fragments resulting from proteolysis; NH: aminoterminal end; VH: variable domain of the heavy chain; VL: variable domain of the light chain; - - -: disulphide bridges.

given that they are complementary to their sequence; the most variable of them all is CDR3. These structures form loops on the antibody surface by means of which they interact with antigens. The rest of the variable domain is known as FR.

Ig molecule proteolysis produces different fragments according to the substance used; the F fragment (Ab')2 is generated after treatment with pepsin which splits the molecule at the level of the hinge, leaving the upper part of the Y with 2 F fragments(Ab) bound to one another. Papain digests the molecule further up and leaves 3 fragments behind, 2 F(Ab) fragments and one constant fragment, Fc. The term F fragment(Ab') is used when it includes the hinge region of the heavy chain.

Antibodies are capable of generating any number of responses after binding to antigens. These effector responses depend on the carboxyl terminal end of each isotype which determines the type of binding to certain cell membrane receptors and complement fixing capacity.

Immunoglobulin synthesis. Generation of diversity. Activation and maturation of B lymphocytes

B cells that, during their mature stage, express IgM and IgD molecules on their membranes are in charge of Ig production. When activated, low rate Ig production begins, the isotype changes, and affinity maturation commences. In the plasma cell stage, there is a high rate of high affinity Ig secretion with scant presence of membrane Ig.

The mechanisms that control antibody diversity [5] are highly complex and have been the focus of researchers' activity for decades. In order to make a very long story short, we can say that there are 2 basic stages to this process: a somatic recombination stage, in which different gene segments present in the variable regions of the light and heavy chains combine and end up forming a functional gene in charge of the AA sequence of the variable portion of the Ig molecule, giving rise to tremendous diversity of molecules in what is known as the primary repertoire of antibodies; the second basic stage consists of somatic hypermutation during the response to antigens, which corresponds to point mutations of the variable sequence once it is formed and these end up allowing for greater binding affinity. Moreover, as the immune response matures, there is a change in the isotype by means of which the reorganized variable segment can combine with any of the constant segments of the Ig. The final effector characteristics of the Ig molecule secreted will depend on this second stage.

The genes of the light chains are grouped in 2 gene segments of the variable region, V (variable) and J (binding), and a different constant segment (C) depending on whether we are dealing with κ or λ chains. Heavy chains have 3 segments in the variable regions: V, D (diversity), and J, and a different C segment according to the isotype of each Ig.

In human beings, the light chain κ depends on a region in chromosome 2 that groups the V , J, and C segments. The same gene segments responsible for the λ chain are found on chromosome 22. Segments V , D, J, and C of the heavy chains are located in an area of chromosome 14. The number of V, D, J, and C segments of the chains and the likelihood of combination are listed in detail in table 1.

In order for B cells become activated, they need to be come into contact with antigens. It is important to point out that, unlike what happens with T cells, B cells recognize a host of protein and non-protein antigens. Macromolecules

Table 1 Possible combinations in the primary repertoire of antibodies

	κ chains	λ chains	Heavy chains
V segments	40	31	51
D segments	0	0	27
J segments	5	4	6
Possible combinations	200	124	8,262

Total number of combinations possible: (200+124) x 8,262=2,676,888.
Taken from Álvarez-Vallina et al., 2004.

stimulate the antibodies by means of antigenic determinants or epitopes that may be linear or conformational, that is to say, juxtaposed in a fold of the protein. If the protein is transformed, it can bring about new antigenic determinants. Binding with the antigen is reversible and the strength of the bond is called affinity. The overall strength of binding to the antigen is known as avidity, which depends on the number of binding points available. Specificity is the ability to recognize small antigenic differences in an antibody.

The IgM and IgD molecules anchored on B cell membranes act as antigen receptors. In the case of protein antigens, T cells are needed to help in activating the B cell, which implies triggering intracellular signals with the activation of genetic transcription and expression factors, changing isotype, and differentiation toward an antibody-producing cell. In the case of non-protein, non-thymus dependent antigens, T cell co-operation is not necessary. Activation stops by means of complex inhibitory signals when a sufficient amount of antibodies has been produced.

During activation and maturation, B cells migrate to follicles in lymph glands and the spleen, where they mature by means of somatic hypermutation with ever more affinity for the antigen. In the end, only those having the greatest affinity with the antigen presented by the dendritic cells survive and, finally, migrate toward secondary lymphatic organs, although a small portion of them remains in the form of memory B cells that recirculate between lymph glands and the spleen. Each plasma cell clone produces a single type of antibody.

Production of monoclonal antibodies

Monoclonal antibodies were discovered in the first half of the seventies by Milstein and Köhler in the molecular biology laboratory in Cambridge (United Kingdom). These authors were researching the molecular mechanisms of the generation of diversity of antibodies and they needed to produce an immortal B cell with known specificity with which to analyze Ig gene mutations in depth. To this end, they fused a murine myeloma cell line, sensitive to certain drugs, with spleen cells of an immunized animal. Through this procedure, they were able to select only the hybrid cells and the clones with known specificity. Their work was published in Nature in 1975 [6] and 9 years later, they were awarded the Nobel prize for this discovery. It was enormously significant since, for the first time, it was possible to have unlimited amounts of antibodies with precise specificities.

All the myeloma tumour cells in mice came from a cell line created by Michael Potter in the seventies known as MOPC21, deficient in key enzymes for the synthesis of oligonucleotides by means of the rescue pathway. The initial fusing agent was the Sendai virus, but it was soon substituted by polyethylene glycol. The B cells came from lymph glands or from the spleen of mice that had been repeatedly and effectively immunized with the desired antigen. These cells were cultured with the myeloma cells and the fusing agent in a special culture medium (HAT) that does not allow non-hybrid myeloma cells to survive; the unfused B lymphocytes also died and the fused cells were left. Specificity was analyzed in the supernatants of the culture dish wells, choosing only the desired ones and, finally, they were cloned by limiting dilution or other means.[7,8]

The hybridomas created can be preserved indefinitely in dimethyl sulphoxide and the monoclonal antibodies are purified from the supernatants. The culture yield is not high; hence, the production technique using ascitis in mice has been developed using intraperitoneal injection of hybridomas, a method that is not accepted in all countries, or in vitro procedures by means of fermenting mammalian cell cultures using bioreactors and continuous perfusion culture systems.

The first therapeutic use in humans took place in 1982 to treat a lymphoma. [9] Murine monoclonal antibody use was soon seen to entail a problem of tolerance with the production of human anti-murine antibodies that diminished their effectiveness. Various alternatives have been explored in an attempt to overcome these difficulties, the most important of which have been chimerization and humanization. Chimerization was developed in 1984. [10] Chimerization denotes the production of monoclonal antibodies in which only the variable region is murine, while the remaining heavy and light chains are human. In humanized antibodies, only the hypervariable regions of the light and heavy chains are murine. [11,12] Half of all the monoclonal antibodies used to treat humans are chimeric or humanized (fig. 2).

Another alternative is to use human monoclonal antibodies produced in transgenic animals carrying human Ig genes; the transgenes include fragments of the variable regions in germ lines, which facilitates the ability to recombine the human antibodies. Miniloci, artificial yeast or human chromosomes, and P1 vectors are the avenues of introduction of those segments. The animals can have their own endogenous Ig genes inactivated. [13,14] Human monoclonal antibodies offer more advantages because they are less

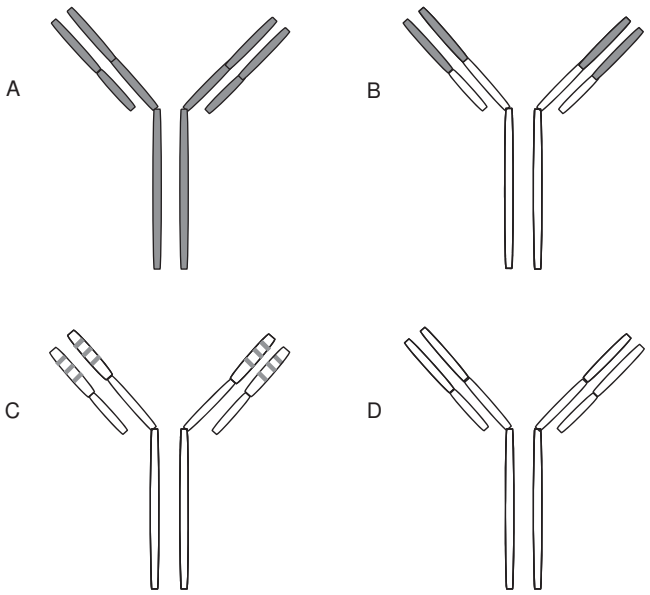

Figure 2 Chimerization and humanization of monoclonal antibodies. A) Murine monoclonal. B) Chimeric monoclonal in which the variable regions are of murine origin and the rest of the chains are of human origin. C) Humanized monoclonal: only includes the hypervariable segments of murine origin. D) Human monoclonal.

antigenic and better tolerated; furthermore, they have a longer circulation time in comparison with chimeric antibodies.

The technology of library-derived antibody fragments deployed on surface proteins of f lamentous phages, introduced in the last decade of the 20th century, is another possibility for producing large repertoires of genes of the variable regions of human Ig.[15]

It is important to point out that the recombinant technology currently available also enables the manufacture of several types of antibody-derived fragments, including $F(ab')_2$ lacking the Fc region, the Fab fragments, the bivalent or diabodies, or even trimers or tetramers, called triabodies and tetrabodies. These fragments make it possible to overcome some of the problems related to the complete molecule of the antibody, to improve avidity and enhance binding to certain targets.

Usefulness and application of monoclonal antibodies in human disease

Regardless of their use in diagnostics, which has represented a true revolution in the f eld of histopathology or enabled the development of laboratory techniques such as f ow cytometry, there are ample possibilities for application to the treatment of human disease. [16] Depending on the Fc region, the binding of the monoclonal antibody to the antigen it is designed to target can facilitate the production of antibody-dependent cell cytotoxicity (ADCC) or complement-system activation cytotoxicity . The very binding to the antigen itself can block cell membrane receptors, binding to factors present in the serum and keeping it from binding to receptors, or inducing intracellular signals. The f nal consequences of these interactions are many and have found application in very different areas.

One way to modify the effector capacity of monoclonal antibodies is through conjugation with cytotoxic molecules, through toxins, radiation drugs or cytokines; this last possibility has been a widely used strategy in oncology through the creation of fusion proteins incorporating IL-2, IL-12, or GM-CSF genes, among others. The conjugation of enzymes capable of turning a prodrug into a drug with monoclonal antibodies targeting tumour cells has made it possible for the drug in question to exert a highly selective action in tumour tissue.

Without a doubt, oncology is the most important area of therapeutic application. Antibodies targeting HER2 are widely used in breast cancer , and anti-epidermal growth factor (EGF) or anti-vascular endothelial growth factor (VEGF) antibodies are used in various types of tumours; anti-CD20 or anti-CD52 are also used to treat lymphomas/leukaemias.

Autoimmune diseases are the next important group of human disease in which these products have been most widely used, and, in great measure, in rheumatoid arthritis, inf ammatory bowel disease, multiple sclerosis, lupus erythematosus, as well as in rejection of transplants and graft versus host disease. The most widely used have been anti-cytokine antibodies, above all, TNF-α and anti-VLA4, but also anti-CD20 and anti-CD25, among others.

Monoclonal antibodies have also been used for other purposes; for instance, in the treatment of septicaemia, prevention of complications of viral diseases, or in the treatment of drug poisoning. This article does not seek to provide a detailed review of the present applications of monoclonal antibodies currently approved and of many more in varying stages of therapeutic development. Undoubtedly, the availability of these antibodies and of the technology to ref ne and perfect them forms a fundamental part of our therapeutic arsenal today and even more so in the future.

Conclusions

Beyond the impact on laboratory diagnostics, monoclonal antibodies represent an extremely powerful treatment tool. Their high degree of specif city enables us to approach very precise targets that can determine very diverse cell changes; moreover, depending on the Fc region in question, they can be designed to facilitate different types of effector responses. The use of humanized and human antibodies has gone a long way towards improving their clinical tolerance. The manipulation of antibodies through binding to other molecules or designing new antibody fragments opens the door to a wide range of possible applications in medicine.

Conf ict of interest

The author declares no conf ict of interest.

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