Monoclonal Antibodies in Medicine

Discovery of antibodies

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Passive immunisation has been used in clinical practice since the end of last century, mainly for prophylaxis. Success of early treatments was marred by anaphylactic reactions and serum sickness because antibodies or antitoxins were not raised in humans. Recombination of gene segments during antibody synthesis means that specific antibodies for numerous antigens can be produced from a limited gene pool. Killer lymphocytes, phagocytes, and complement then bind to the constant region of the antibody facilitating elimination of the pathogen. Development of a method of obtaining large quantities of antibodies against a specific antigen (monoclonal antibodies) offers the possibility of initiating host defence mechanisms against any unwanted antigen, though some problems still remain in preventing the body from attacking the monoclonal antibody.

History of passive immunisation

The first use of passive immunisation is thought by some to have been on Christmas night 1891. The recipient is reported to have been a young boy in Berlin with diphtheria who was cured by injection of diphtheria antitoxin, although this cannot be proved today. Passive immunisation is based on work carried out by Emil Behring and Shibasabura Kitasato from Robert Koch’s Hygiene Institute in Berlin. On 4 December 1890 Behring and Kitasato published a landmark article in which they showed that serum from an animal actively immunised against diphtheria toxin could be used to neutralise even a fatal dose of the toxin in another animal. The same results were obtained with tetanus toxin, and the effects of these sera were shown to be specific in that tetanus antitoxin did not protect against a challenge with diphtheria toxin.

The potential for treatment in humans was immediately apparent. At that time diphtheria was a common and often fatal disease and Behring set to work trying to produce large amounts of antitoxin that would be effective against diphtheria in humans. The pharmaceutical company Farberwerke Hoechst scaled up production by immunising sheep, and the first large trials started in 1893 with good results. Immunisation of horses was then introduced and by 1894 the case mortality of diphtheria in Paris had already fallen from 52% to 25%. The first British patients were treated in the summer of 1894 with favourable results. Behring then suggested the combination of diphtheria toxin and antitoxin serum as a safe method of producing active immunity in humans. This was introduced in 1913 but superseded when inactivated diphtheria “toxoid” was found to induce immunity.

A major problem with passive serotherapy was its toxicity. Anaphylactic reactions often occurred after giving horse serum and serum sickness (fever, rash, and joint pains) was common. Behring believed that the antitoxin effect resided in the protein fraction of the serum and went on to show that ammonium sulphate precipitated a fraction (now known as gammaglobulin) that retained antitoxin activity. Precipitation reduced side effects but did not completely overcome the problem of serum sickness, which is now known to result from the deposition of circulating immune complexes. Cleavage of gammaglobulin by pepsin further reduced the incidence of serum sickness.

Large scale production of antiserum to diphtheria toxin was made possible through the work of the bacteriologist Paul Ehrlich, who moved to Koch’s institute in 1889. He took part in early work challenging rabbits with bacterial toxins and subsequently developed guinea pig assays to test the potency of different batches of antiserum. Ehrlich proposed the side chain theory of toxicity. He suggested that toxins mediated their effects on cells through preformed protein side chains and that immunity arose because of overproduction of these side chains. The side chains are reminiscent of what we now know as antibodies, although Ehrlich assumed (incorrectly) that their primary function was nothing to do with toxin neutralisation.

The introduction of human immunoglobulin preparations in 1944 as treatment for and protection against measles finally overcame the problem of serum sickness and today pooled human immunoglobulins are still used. Agammaglobulinaemic patients have benefited from the availability of relatively pure immunoglobulin preparations, which are given regularly to prevent infection. There are currently two types of preparation: normal immunoglobulin and specific immunoglobulin. Normal immunoglobulin is prepared from pools of at least 1000 random donations of human plasma and contains antibodies to hepatitis A, measles, mumps, and other viruses. It produces immediate protection that lasts four to six weeks. Specific immunoglobulins are prepared by pooling the blood of patients convalescing from specific diseases. Preparations are available against several pathogens, including hepatitis B virus, Clostridium tetani, and varicella-zoster virus. Other specific immunoglobulins are available or under development for example, antirhesus D antibodies raised by deliberate challenge of normal male volunteers with the rhesus D antigen are used routinely to prevent sensitisation of mothers negative for anti-D antibodies after delivery of an antibody positive baby. There is also some interest in passive immunisation for AIDS. Some antisera can still not be produced safely in humans and thus, for
Antibody structure

The active components of gammaglobulin that protect against the effects of diphtheria and tetanus toxins are now known to be immunoglobulins, also known as antibodies. IgG, the prototype antibody, is a glycoprotein with a molecular weight of 150,000 Dalton. The molecule consists of two identical heavy chain-light chain heterodimers linked by a disulphide bridge to form a Y shaped structure (fig 1). Each heavy chain comprises one variable (V) and three constant immunoglobulin (Ig) domains, whereas light chains consist of a single variable and a single constant Ig domain. Each domain comprises about 110 amino acids.

![Antigen binding sites diagram](http://www.bmj.com)

**FIG 1—Structure of antibodies. Antigen binding sites comprise two variable regions (V) and two constant regions (C), one each from the heavy and light chains. Fc region comprises two constant regions from the heavy chain**

From a functional perspective, the important features are the antigen binding site (Fab) and the Fc region (fragment crystallisable), which is responsible for initiating host defence mechanisms. The Fc region is the stem of the Y structure and the two antigen binding sites are at the tip of the outstretched arms. The hinge region of the antibody is flexible so the antigen binding sites are widely and variably separated, permitting the linking of two identical antibodies by the same antibody; many invading microorganisms such as bacteria and viruses have repeating subunits and this effect allows much more effective binding (the avidity effect). The multiple binding sites also allow aggregation and rapid clearance of antigens.

The antigen binding site also crystallises quite easily and this has allowed x-ray diffraction analysis of antibody structure. Several crystal structures are now known and they show important conserved features. The antigen binding site is formed by the apposition of two globular variable domains, one derived from the heavy chain (VH) and the other from the light chain (VL). The folds of each of the V domains form three short loops of amino acids—the hypervariable loops or complementarity determining regions (CDRs). At the antigen binding site all six loops (three each from the heavy and light chains) come together. The extraordinary specificity of antibodies to the vast numbers of possible antigens is possible because of the variability in both the length and the amino acid composition of these loops. Their configurations are, however, not entirely random and can be categorised into a limited number of recurring canonical forms. Alterations in residues of the underlying β sheet framework adjust the orientation of individual loops and also play some part in the structure of the binding site. Besides providing insights into the basis of antibody diversity, detailed analysis of the structural features of antigen combining sites provides a basis for new approaches to antibody construction.

Although an antibody may have a direct neutralising effect on a virus or toxin, Fc mediated defence systems are needed to eliminate the source of the antigen. Antibodies can sensitise a target cell for attack by killer (K) lymphocytes by coating (opsonising) the cell with IgG. K lymphocytes recognise coated target cells because they have receptors for the Fc region of IgG on their surface. This mode of killing is known as antibody dependent cellular cytotoxicity. Similarly, phagocytes (including neutrophils and macrophages) target an opsonised antigen through Fc receptors. The combination of antibody and antigen can activate the classical complement pathway and deposition of complement on the target cell can produce lysis or further opsonisation.

**ANTIBODY CLASSES**

There are five classes of immunoglobulin, classified according to the type of heavy chain they possess: IgG, IgA, IgM, IgD, and IgE. IgG comprises 70% of the immunoglobulin pool and is produced mainly in secondary immune challenges. There are four light subclasses (IgG 1-4) which vary in their capacity to initiate host defence systems. IgG1 and IgG3 mediate antibody dependent cellular toxicity and can bind complement whereas IgG2 and IgG4 are relatively deficient in these functions (IgG2 can bind complement, although less efficiently than IgG1 and IgG3). IgG is present in the intravascular and extravascular spaces and, importantly, can cross the placenta to protect the developing fetus and new born child. IgM, which forms 10% of the immunoglobulin pool, has a pentameric structure and is mainly confined to the intravascular space. It is a potent complement fixing antibody but cannot produce antibody dependent cellular cytotoxicity. IgA (secretory immunoglobulin) forms around 20% of the pool. It has a dimeric structure and is present in secretions such as saliva and milk. IgD forms less than 1% of plasma immunoglobulin and is mainly found on the surface of B cells. Its function is unknown. IgE binds to Fc receptors on the surface of basophils, eosinophils, and mast cells where cross linking by antigen triggers the release of pharmacological mediators that can cause immediate hypersensitivity reactions.

**Genetic basis of antibody diversity**

The finding that each antibody had its own amino acid sequence in the variable region suggested a separate gene for each antibody produced. If this were so, most of the human genome would be needed to code for the enormous number of antibody molecules that could exist. In 1965 Dreyer and Bennett hypothesised that each class of constant region is encoded for by one gene but that large numbers of genes exist for the variable domains (VH and VL). They correctly suggested that antibody genes would be formed by recombinations between these genes during B cell development. We now know that assembly of the genes for variable domains occurs by random joining of a number of gene segments (V, D, and J for heavy chains or V and J for light chains) and that additional diversity results from loss or addition of nucleotides at the junctions between these gene segments (fig 2). In the case of the heavy chain, there are about 75 VH segments, around 30 D segments and six JH segments...
on each chromosome.16 The V segment encodes most of the variable domain including the first and second complementarity determining regions (CDR1 and CDR2) whereas the highly variable CDR3 is encoded by D segments and by nucleotides at the V-D and D-J junctions. The VDJ segment is then apposed to the constant region at the messenger RNA stage, after editing of the large nuclear RNA transcripts produced. The number of unique immunoglobulin variable regions possible due to random recombination of V, D, and J gene segments is very large. Moreover, the joining positions of V1H, D1H, and JH segments are not always the same and the D1H segment may end up in any of its three possible amino acid reading frames. This, along with random loss or addition of nucleotides at the boundaries between the segments further increases diversity.17 The x and λ light chain loci also undergo recombination of Vx and Jx coding regions (there is no equivalent of the heavy chain D segment). Since any light chain is thought to be able to combine with any heavy chain an enormous diversity (estimated at over $10^{12}$ antibodies) can be produced from a relatively small number of DNA segments. Further diversity arises later through the introduction of scattered point mutations into the DNA of the assembled heavy and the light chains.17 This is known as somatic hypermutation and usually occurs during the immune response to foreign antigen (see below).

Humoral immune response

During a typical infection protective antibodies are produced against the invading organism. After an initial lag phase the level of antibody detectable in the bloodstream rises rapidly, reaches a plateau, and then falls to low levels once the pathogen has been eradicated. IgM is the first class of antibody to appear in this primary antibody response, followed by IgG, which has a longer lag period. Re-exposure to the same pathogen gives rise to a "memory" or secondary response that is stronger and has a much shorter lag phase; it is dominated by IgG and tends to be of higher affinity.

Cellular basis of the immune response

Antibodies are produced by B lymphocytes. These cells develop in fetal liver and subsequently in bone marrow. It is at these sites that immunoglobulin gene rearrangements occur and potentially harmful self reactive B cells are deleted from the repertoire. In its resting state each B cell displays an antibody of one specificity on its surface. At any one time there are about $10^8$ different B cell clones in the body, each displaying a unique antibody. When antigen enters the body it binds specifically to only a few of these resting B cells, stimulating them to divide and to mature into plasma cells that secrete large amounts of the immunoglobulin which was previously displayed on their surface. This model of clonal selection of B cells was originally proposed in the 1950s by McFarlane Burnett.16 During antigen driven B cell proliferation there is a switch in the main class of antibody produced from IgM to IgG, IgA, or IgE (class switching), and B cells that secrete higher affinity antibodies begin to emerge (affinity maturation). The B cell class switch is a complex process which is regulated by external factors including local secretion of cytokines by antigen specific helper T cells. Some of the proliferating cells do not become secretory but continue to display their surface antibody and continue to circulate long after the infection has cleared. These memory B cells (mainly derived from cells which have undergone class switching) are responsible for the more rapid onset and greater intensity of the secondary immune response.

Monoclonal antibodies

In 1975 George Köhler and César Milstein of the Medical Research Council Laboratory of Molecular Biology in Cambridge described an elegant system of obtaining pure antibodies of known specificities in large amounts (fig 3).18 An astute reporter from the BBC World Service immediately recognised the potential of the discovery of monoclonal antibodies but it was some time before they were widely used. In the original method, a mouse is immunised repeatedly with the desired antigen and the spleen, which contains proliferating B cells, is removed. B cells normally die in culture, but can be immortalised by fusion with a non-secretory myeloma cell. The resulting hybridoma can then secrete large amounts of the antibody encoded by

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**FIG 2**—Antibody diversity—the joining of V, D, J segments of the heavy chain. The genes are encoded by a relatively small number of germline gene segments but the random recombination gives rise to large numbers of expressed genes.

**FIG 3**—Production of monoclonal antibodies. Mice are immunised with antigen and their B lymphocytes are then fused with a non-secretory myeloma cell line. The myeloma cell line has a mutation in the hypoxanthine guanine phosphoribosyl transferase (HGPRT) gene and is therefore killed in hypoxanthine aminopterin thymidine (HAT) medium. During selection in HAT medium, un fused myeloma cells are killed and unfused mouse B cells die spontaneously. Spontaneous B cell myeloma cell hybrids (hybridomas) survive HAT selection since the B cell supplies a normal HGPRT gene. The antigen specific B cell also supplies immunoglobulin genes which encode its unique antibody. Screening of large numbers of hybridomas usually identifies a few clones which produce antibody of the desired specificity (against the immunising antigen).

**TABLE 1**—Summary of immunoglobulin classes and subclasses. Numbers of different Ig isotypes may vary between species and in some cases between individuals within a species. The amount of antibody of each class produced is largely determined by the antigenic stimulus. The standard model of plasma cell differentiation in response to T helper cell cytokines is shown.
its B cell fusion partner. Supernatants from the hybrids that survive the selection procedure outlined in figure 3 are tested for binding to the original antigen.

Alan Williams showed in 1977 that monoclonal antibodies could be raised against biologically interesting molecules and this triggered the development of a stream of useful monoclonal based diagnostic procedures. It soon became apparent, however, that rodent monoclonal antibodies were unsuitable or ineffective for treating humans because they initiate human defence systems poorly and are themselves the target of an immune response that can greatly shorten their circulating half life. One solution is to produce human monoclonal antibodies, but this is difficult for several reasons. Firstly, human B cells immortalised by fusion with a myeloma cell or by infection with Epstein Barr virus are unstable and can rapidly lose their capacity for antibody production. Even when they are successfully transformed with Epstein Barr virus antibody yields tend to be low and the virus usually transforms IgM secreting cells that produce lower affinity antibodies. Secondly, hyperimmunisation of human subjects is problematic, particularly against self antigens. In vitro immunisation—that is, antigenic stimulation of cultured human lymphocytes provides a partial solution to this problem, but antibody production is still unstable after immortalisation. The third problem is that the most convenient source of human lymphocytes is the peripheral blood, a poor source of B cells producing specific high affinity antibodies. Such cells are more abundant in the spleen, bone marrow, or lymph nodes.

The recent arrival of antibody engineering techniques (detailed in the second review of this series) provides several alternative solutions to the production of human monoclonal antibodies. We are therefore beginning to see a renaissance of immunotherapy as a treatment after its eclipse by chemotherapy. In his address to the Naturalists Congress in 1895 Behring predicted, “I have no fear that the thought which forms the basis of serum therapy will ever disappear out of medicine” and 100 years later this prediction seems to be holding well.

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ANY QUESTIONS

Is there a risk of upper respiratory tract infections being passed on when children share β2 agonist inhalers?

I am not aware of any published work in relation to this question. Theoretically, however, it is possible that viral infections could be transmitted in this way under certain circumstances. Most viruses die as a result of exposure within a few hours, but it is likely that viable virus particles survive on the inhaler and may be transmitted in droplet form or as a contaminant on the hands or on the inhaler. Such transmission would be possible for the common cold and for other respiratory viruses, such as rhinovirus or respiratory syncytial virus. If two people used the same inhaler within a relatively short time—even an hour or so—these infections could be transmitted. Transmission of bacterial infection is unlikely as a consequence of shared use, but there may be a theoretical possibility of transmitting tuberculosis. Under the circumstances it seems sensible to continue to enforce the recommendation that such inhalers should not be shared.—A RICHARD MAW, consultant otolaryngologist, Bristol