Monoclonal Antibodies

Heddy Zola, *Child Health Research Institute, Adelaide, Australia*

Introductory article

Article Contents

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Antibodies bind other molecules strongly and specifically and are therefore useful as reagents in research, diagnosis and therapy. Antibodies taken from the blood of immunised animals are a mixture of different antibodies produced by different cells (they are described as polyclonal). Monoclonal antibodies are antibodies with a unique specificity, generally made by cloning cells containing a particular antibody gene set to produce a population of identical cells, derived from a single cell, which all produce the same antibody. Monoclonal antibodies are therefore much more specific than polyclonal antibodies. Monoclonal antibodies can be made in cell culture and are therefore also more reproducible from batch to batch than polyclonal antibodies. Monoclonal antibodies have become the preferred reagents in many research and diagnostic applications and are increasingly used in therapy of cancer and immunological disorders, generating a multi-billion dollar industry.

Introduction

Vertebrates have evolved an immune system that protects them from invading microorganisms. A major component of the immune response to such organisms is the production of antibody molecules. These molecules possess a binding site for structures on the surface of the invading organism. In order to be effective, the immune system must be capable of making antibodies that can bind the enormous diversity of molecular structures expressed by viruses, bacteria and other parasitic organisms and must be capable of coping with mutations in these organisms. This challenge is met by the immune system in two ways. Firstly, B lymphocytes are capable of generating a diverse set of antibody structures (estimated at 10^{11}) by permutation and combination of a limited number of gene elements. Secondly, and uniquely in the body, the genetic elements that code for the antigen-binding structure of antibody are subjected to a high rate of mutation, coupled with a process that allows selection of cells that make antibodies that bind the antigen strongly. This process results in antibodies that bind their antigens with high specificity and high affinity. **See also**: Affinity of Antigen–Antibody Interactions; Antibodies; Antigen–Antibody Complexes; B Lymphocytes; Immunoglobulin Superfamily

The potential of antibody as a tool in medicine has long been recognised. Antibodies made in animals have been used to assay for the presence of hormones (e.g. in pregnancy tests) and even to neutralise toxic substances (as in the use of antibodies against snake venom proteins, made in horses, to treat human victims of snake bite). Antibodies made in animals have a number of limitations. The product of a single B cell is multiple copies of antibody with a unique binding site. However, when we immunise an animal and subsequently bleed it, we obtain, in the serum, a mixture of antibodies produced by the animal in response to the many antigenic molecules it encounters. Antisera made in animals are thus variable and of limited overall specificity. **See also**: Antibody Function; Antiserum; Immunization of experimental animals

In 1976, Georges Kohler and Cesar Milstein, while working in Cambridge, England, developed a procedure to isolate and propagate the individual B cells making antibody against the antigen of interest. They fused cells obtained from the spleen of an immunised mouse with myeloma cells. Myeloma is a tumour of antibody-producing cells, and myeloma cell lines are available in which the cells multiply rapidly and produce large amounts of antibody – although not usually of a specificity that is of use to us. Some of Kohler and Milstein's fusion products ('hybridomas') retained these properties of indefinite propagation and high antibody secretion rates but made antibody coded for by the antibody genes of the mouse spleen cells. The mixture of hybridomas still would make a variety of antibodies, but this mixture could be separated out by cloning – that is, individual cells isolated and allowed to proliferate into separate populations, or clones. Then, it was a matter of screening the many clones to see

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which ones made antibody against the antigen of interest. **See also**: Milstein, Cesar

This is the basis of the production of monoclonal antibodies. With relatively minor changes, this procedure has spawned an industry and revolutionised many aspects of medical diagnosis and research. Monoclonal antibodies are also being used increasingly as therapeutic agents. **See also**: Monoclonal Antibodies: Diagnostic Uses; Monoclonal Antibodies: Therapeutic Uses

Generation of Hybridomas

The process of generating hybridomas centres on the fusion reaction between immune spleen lymphocytes and myeloma cells, but the generation of these two components is critical and complex, involving several stages. The generation of hybridomas is illustrated schematically in **Figure 1**.

Strategies

Ideally, the target antigen, the molecular structure against which the antibody is to be made, will be available in pure form and in adequate quantity (a few milligrams). However, this ideal is often not achievable. Antibody against a complex multimolecular structure such as a virus or a blood cell may be desired, or the molecule may be known, but it may not be possible to purify it in sufficient quantity. Monoclonal antibodies provide excellent reagents to use in purifying molecules from complex mixtures; thus, it may be that the antibody is wanted in order to be able to purify the antigen.

Monoclonal antibodies against individual components of a complex mixture can be made by devising a selection strategy that will identify hybridomas making antibody against the antigen in question. The most effective strategy depends on the situation. Differential screening of hybridoma clones, against the mixture containing the antigen of interest and against another mixture, lacking the antigen but otherwise as similar as possible, is widely used. For example, in making antibodies that will specifically identify a particular peptide growth factor produced in culture, a culture lacking the stimulus that elicited the production of the growth factor may be a useful control. In most cases, these strategies will not identify antibodies against the antigen with certainty but will allow the selection of a group of hybridomas that are likely to include clones making antibody against the antigen of interest. Supplementary studies, for example, Western blotting to characterise the molecular weight of the molecule detected, precipitation of the antigen followed by partial sequence analysis by mass spectrometry of peptide fragments and functional inhibition studies to neutralise biological activity, will then be needed to identify the hybridomas secreting the antibodies in question. **See also**: Mass Spectrometry: Peptide Sequencing; Western Blotting: Immunoblotting

The principal consequence of these considerations is that a screening strategy must be in place before the immunisation of mice.

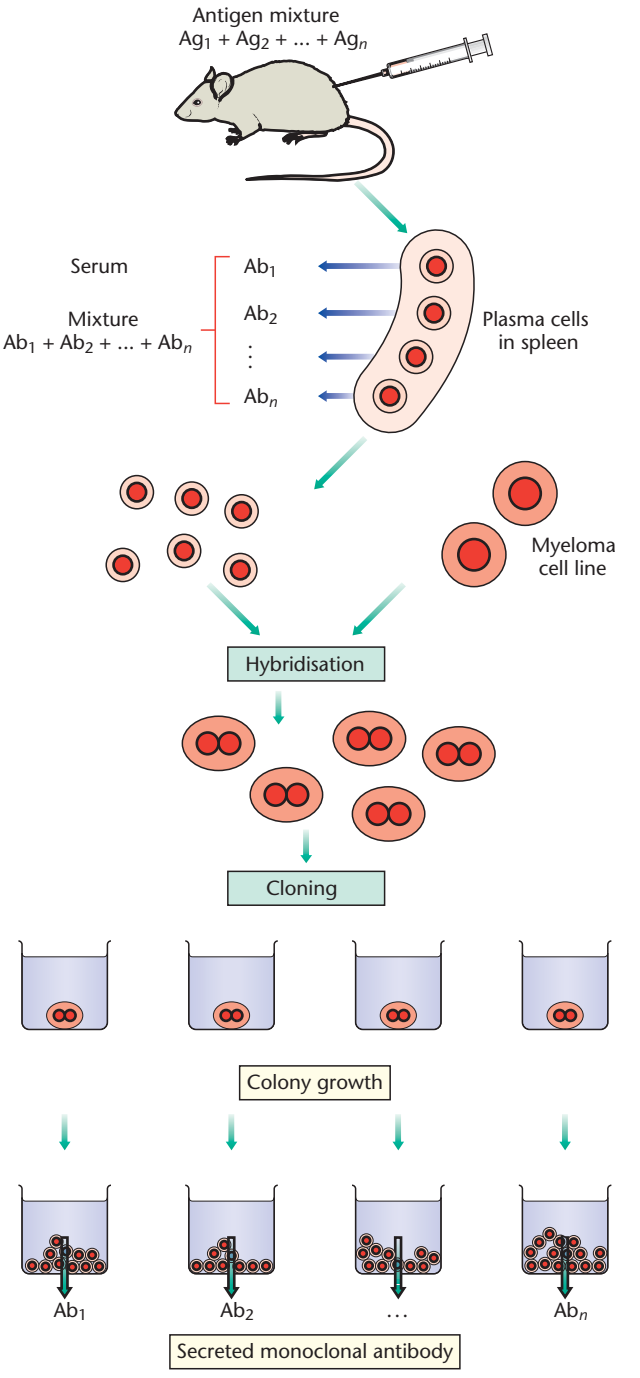


Figure 1 Schematic representation of the process of immortalising an antibody-producing clone by hybridisation, cloning and selection of clones producing the desired antibodies. *Notes*: Ab, antibody and Ag, antigen.

Immunisation

Virtually all hybridomas are made using immune cells from mice. Immunisation protocols vary widely depending on the nature of the antigen, but generally involve a priming dose injected subcutaneously with adjuvant to provide

a strong immune stimulus, followed about 4 weeks later by a booster dose, often given intravenously without adjuvant. It is sensible to use an immunisation protocol which has been described in the literature for a similar antigen. **See also**: Immunization of experimental animals

Myeloma cell line

A small number of mouse myeloma lines are available. The earlier lines are able to make their own antibodies, so that the hybridoma can make the light and heavy chains of both the myeloma and the spleen cell fusion partner. The light and heavy chains are made independently and assemble in the cell; hence, such a hybridoma can make a variety of antibody molecules, only one of which will have the desired binding sites. To avoid this heterogeneity, myelomas have been selected that have lost the ability to make their own light and heavy chains. **See also**: Antibodies

As myeloma cells grow continuously, a method is required to allow selective growth of hybridomas and suppress growth of the parent myeloma. The selection method used most widely depends on the use of myeloma cell lines that have lost the ability to make nucleotides by the salvage pathway. The main biosynthetic pathway can be blocked with the drug aminopterin, so that the myeloma cells cannot make deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and die. Hybridomas, on the other hand, have the enzymes for the salvage pathway (derived from the spleen lymphocyte) and can grow, provided they are supplied with the substrates for the pathway, hypoxanthine and thymidine. The selective system is named after the three substances that are added to the culture medium, hypoxanthine, aminopterin and thymidine (HAT). **See also**: Nucleotide Synthesis via Salvage Pathway

Myeloma cells are available for the production of hybridomas with rat, human and chicken lymphocytes, but most work has been carried out using the mouse, and extensive efforts to make human hybridomas for therapeutic purposes have yielded limited success. Heterohybridomas, using mouse myeloma and lymphocytes from a second species, have also had limited success. When human antibodies are required, interest has turned to the use of libraries of human antibody genes, genetic engineering methods and the use of mice genetically engineered to replace their mouse antibody repertoire by a human antibody repertoire. **See also**: Bacteriophage Display of Combinatorial Antibody Libraries

Fusion process

The reaction at the heart of the field of monoclonal antibodies, the fusion reaction, is surprisingly simple. Myeloma cells, taken from culture, are mixed with spleen cells isolated from an immunised mouse, at a ratio usually of 1 myeloma cell to 10 spleen lymphocytes. The cells are centrifuged together to form a pellet and resuspended in a small volume of a viscous solution of polyethylene glycol. After 1 min, the suspension is gradually diluted, care being taken not to break up small aggregates of cells, some of which will form the hybridomas. The cell suspension is washed and resuspended in culture medium. After a few hours in a tissue culture incubator (which provides a physiological pH and temperature), the cells are dispensed into small tissue culture wells at a concentration, which, from experience, is likely to yield single hybridoma colonies in the wells. The cells are then allowed to grow over the next 7–14 days, with occasional changes in medium. **See also**: Cell Culture: Basic Procedures

There are many minor variations of this procedure, and many critical points. Polyethylene glycol is a rather inert chemical and probably stimulates fusion passively, by allowing cells to stick together and excluding water from the junction. The cells should be in good condition before the fusion – the myeloma cells should be growing exponentially and the lymphocytes should not be subjected to traumatic processes during preparation. This requires a gentle procedure for disaggregating the spleen and for removal of red cells. The fusion procedure itself is critical; inadequate or overvigorous resuspension of cells will reduce yields. Postfusion dilution, washing and plating out again need to be carried out with an understanding of the objective – at this stage, the cells are not yet fused stably; there are doublets with adhering and perhaps partly fused membranes, and these should not be disrupted. **See also**: Lymphocytes

Growth and selection

Growth of hybridomas occurs gradually over the first 2 weeks after fusion. The yield may be improved by adding interleukin 6 (IL-6), which acts as a hybridoma growth factor. Other additives, found empirically to improve hybridoma yields, such as feeder cells or a variety of commercial supplements, are thought to act through IL-6. Cytokines have overlapping functions; thus, it is likely that other growth factors help hybridoma growth. **See also**: Interleukins

After a few days in culture, small colonies of hybridoma cells are seen using an inverted microscope, and, if all goes well, these colonies expand to the point where they are visible by eye and begin to affect the pH of the medium, turning the indicator dye yellow. About 1 week after the initial fusion, the unfused myeloma cells should be dead, and it is then helpful to gradually supplement the cells with medium lacking aminopterin. As hypoxanthine and thymidine are used up while aminopterin accumulates, the HAT medium should be replaced with HT (hypoxanthine and thymidine) medium.

Once the culture wells are showing visible colonies and the medium is turning yellow, it is time to test the supernatant for antibody. At this stage, there will be many colonies to test and maintain, perhaps several hundred, depending on the scale of the experiment. A priority is therefore to reduce the number of cultures by eliminating negative cultures. It is possible to test simply for immunoglobulin production, but this usually eliminates

very few. A more useful test is to screen for binding to the antigen by a simple assay capable of being run daily on large numbers of samples.

Selected cultures must be cloned because there is no guarantee that they arose from a single cell. Cloning is usually repeated several times over the first few weeks, because the fusion products are still genetically unstable and may produce loss mutants, which grow but do not produce antibody. Clones are grown into larger culture vessels to produce quantities of cells and antibody for more extensive evaluation.

At this stage, cells should be cryopreserved, so that if anything goes wrong there is a seed culture to go back to. Many things can go wrong, including contamination with bacteria or fungi; thus, cells should be cryopreserved as soon as there is evidence that the colony may be producing a useful antibody. However, cryopreservation requires a few million cells and hence cannot be performed until the culture has been sufficiently expanded. **See also**: Cryopreservation of Cells

Cryopreservation and long-term maintenance

The amount of work required to establish a hybridoma is considerable; the final product is unique and may not be reproduced exactly in a subsequent fusion. It is therefore essential to establish a secure 'bank' for hybridomas. This is achieved by storing ampoules of hybridomas in liquid nitrogen. The procedures for freezing down cells and for thawing them out to re-establish them in culture are straightforward; the critical issues are essentially administrative. It is important to freeze down at least 5–10 ampoules, to validate the 'deposit' by reconstituting one ampoule, to maintain adequate records and to lay down more ampoules when stocks become low. It is wise to store a set of ampoules in a separate laboratory.

Hybridomas may be maintained in culture essentially indefinitely, by 'splitting' the culture every 2–3 days, maintaining the cell concentration at between $2–3 \times 10^5$ and 10^6 cells mL^{-1}. However, it is more usual to grow them only when fresh antibody is required and re-establish them from cryopreserved stocks when needed. If they are maintained for long periods, it is necessary to check antibody production regularly, because loss of production may occur.

Antibody production

The amount of antibody secreted in small cultures such as microwells is adequate for screening. However, once a hybridoma has been found to make a useful antibody, larger amounts will be needed. Cells may be grown in conventional culture flasks, and supernatant may be harvested two to three times per week; such supernatants generally contain antibody at $1–5\,\mu g\,mL^{-1}$. This concentration is adequate for many assays; however, if the antibody needs to be purified, for example, to conjugate it to an enzyme or fluorochrome, larger amounts and higher concentrations are needed. These have historically been prepared by growing the hybridoma as an ascitic tumour in mice, yielding ascitic fluid with antibody at concentrations of $1–5\,mg\,mL^{-1}$. This procedure is increasingly unacceptable for ethical reasons, as alternatives become available. Furthermore, the ascitic fluid contains, in addition to the antibody made by the hybridoma, antibodies made by the mouse against environmental antigens. The antibody is no longer truly monoclonal and monospecific, and significant difficulties in interpretation may result. Fermenters and minifermenters are available for the production of monoclonal antibodies in culture at any scale from a few milligrams to the gram amounts required for clinical trials. Although there are still significant difficulties and uncertainties, and production costs are high, these methods have largely replaced ascites production in mice.

A number of methods are available for antibody purification in good yield and purity. For many purposes, however, conventional culture supernatant will work well, without the need for purification. **See also**: Immunoglobulin Purification

Applications

Monoclonal antibodies have a range of applications, which take advantage of the specific binding of their target antigen (**Figure 2** and **Figure 3**).

Analytical applications

Antibody may be used to detect an antigen, for example, in forensic applications, in microbiological testing of foodstuffs and in diagnostic testing of blood samples for toxins or infectious organisms. The antibody-based test for the presence of antigen may be rendered quantitative, providing an assay for antigen. Antibody-based assays are widely used in medicine to determine levels of growth factors, hormones, blood cells or malignant cells; the applications are essentially unlimited. Still in analytical mode, monoclonal antibodies may be used to locate antigen. Antibodies are widely used in conjunction with colour-forming labels and microscopy to localise antigens in tissue sections. This is known as immunohistochemistry. **See also**: Acquired Immune Deficiency Syndrome (AIDS); Enzyme-linked Immunosorbent Assay; Flow Cytometers; Immunohistochemical Detection of Tissue and Cellular Antigens; Monoclonal Antibodies: Diagnostic Uses

Preparative applications

Antibodies may also be used preparatively to purify molecules or cells from crude mixtures. Immunoaffinity-based preparative techniques are very powerful compared to more traditional biochemical purification methods, although in general a successful purification procedure will combine both affinity-based and conventional methods.

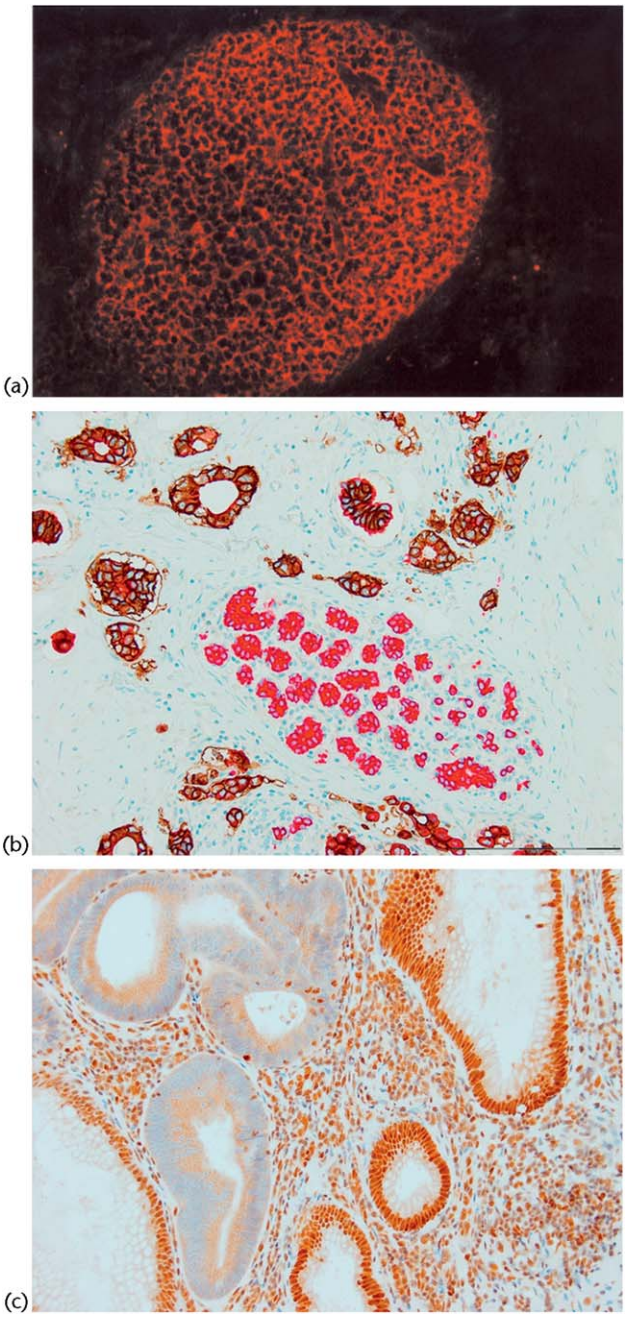
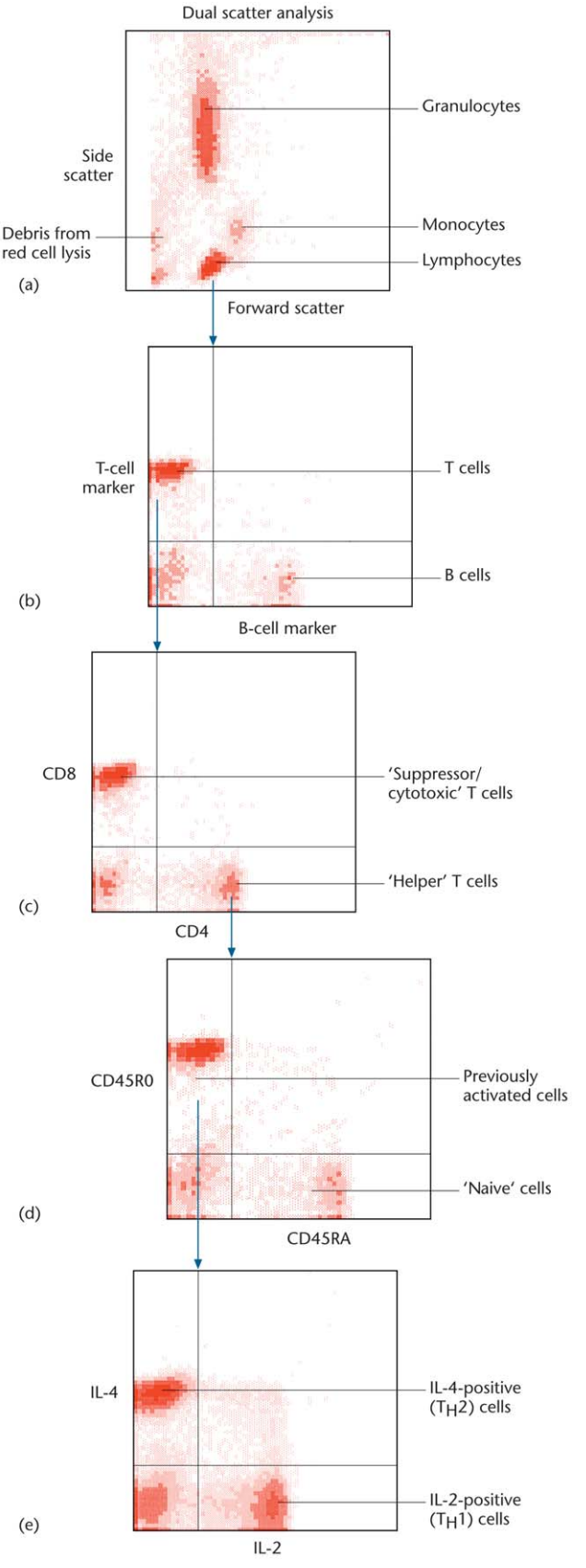


Figure 2 Examples of the use of monoclonal antibodies to identify particular molecules and cell types in tissues. (a) Staining of human lymph node tissue with a monoclonal antibody against a protein called CD19 and fluorescence microscopy. The protein is expressed on the surface of B lymphocytes, which are responsible for antibody production. The egg-shaped structure is a follicle of B lymphocytes, while the surrounding area (unstained) contains principally T lymphocytes, with a sprinkling of B lymphocytes. The follicle contains a germinal centre. Germinal centres develop in response to infection or other antigenic challenge. In the germinal centre, B lymphocytes divide rapidly, and the genes coding for the antibody made by the cell are mutated and selected to give strong binding to antigen. (b) Staining of breast cancer tissue section with two antibodies against different molecules, conjugated to different enzymes and detected using different colour-producing (chromogenic) enzyme substrates. Red staining is for the epithelial tissue marker AE1/3 and brown staining for the breast cancer marker Erb-b2. Courtesy of Dr Andrew Ruszkiewicz, SA Pathology, South Australia. (c) Staining of colonic cancer tissue section with antibody against MSH-2, a protein involved in DNA mismatch repair, showing abnormal expression. The neoplastic glands show loss of nuclear staining while non-neoplastic tissue including lamina propria lymphocytes show MSH-2 protein expression. Courtesy of Dr Andrew Ruszkiewicz, SA Pathology, South Australia.



Antibody-based purification methods are useful from the laboratory scale, where the aim is to purify nanogram to milligram quantities of biological substances from complex mixtures such as serum, to production of therapeutic substances, such as the blood-clotting factor VIII from large volumes of blood. The use of antibody to identify particular cell types in complex mixtures has been extended to preparative methods to purify these cells. One approach is to link antibody to magnetic particles. A magnet is then used to physically separate cells that bear the antigen from cells that do not. A more powerful technique uses the fluorescence-activated cell sorter, in which a cell may be identified on the basis of antibody tagged with fluorescent dye and physically separated from the other cells (**Figure 3**). Because the absolute differentiation of a particular cell type from all other cells may require several different markers, cell sorters can sort on the basis of up to 13, or more commonly 5, parameters simultaneously. **See also**: Immunofluorescence

Therapeutic applications

Potential therapeutic applications of antibodies include the neutralisation of toxins, the removal of infectious agents from the circulation and the destruction of body cells mediating disease, including autoimmune cells and cancer cells. **See also**: Autoimmune Disease: Treatment; Death Receptors at the Molecular Level: Therapeutic Implications; Immune System: Manipulation *In Vivo*; Immunosuppressive Drugs; Monoclonal Antibodies: Therapeutic Uses; Tumour Antigens Recognized by Antibodies; Tumours: Targeting of Monoclonal Antibodies for Imaging and Potential for Therapy

Polyclonal antisera have been used for many years in the treatment of snakebite and infections where the major threat to life is a toxin, such as tetanus. These conditions are relatively rare (bacterial infections such as tetanus generally being prevented by immunisation), so that an individual is unlikely to need repeated treatment. The immune system recognises antibody made in another species as a foreign protein and will make an antibody response against the protein. This means at best that second or subsequent treatment with antibody from the same species as the first treatment will be of limited effectiveness, because the protein is cleared rapidly; at worst, the resulting immune reaction can take the form of a life-threatening anaphylactic shock. **See also**: Antiserum; Venoms

Polyclonal antisera against human lymphocytes were developed in the 1970s to treat patients who were rejecting organ grafts. The principle was that the host immune response was responsible for the organ graft rejection; antibodies against key components of the immune system should suppress the rejection. These antisera have largely been superseded by a monoclonal antibody called OKT3, directed against a molecule expressed on human T cells and involved in T-cell function. OKT3 has been highly successful in reversing rejection episodes. The injected antibody is a mouse protein and is immunogenic, but the response is muted because the patients are immunosuppressed, both by the antibody itself and by other immunosuppressive therapy used to reduce the graft response. Nevertheless, OKT3, successful though it has been, has been perceived as limited in effectiveness partly by its immunogenicity. **See also**: Graft Rejection: Mechanisms; Immunosuppression: Use in Transplantation

For many years, the number of successful clinical trials with monoclonal antibodies was disappointing. This situation contrasted with the highly promising results in animal models, where antibodies can destroy established tumours, induce a state of permanent tolerance to transplanted organs, reverse autoimmune disease and rescue animals from acute toxicity caused by, for example, bacterial endotoxin. In comparison to the animal studies, therapeutic development of monoclonal antibodies for humans was slow to realise its full potential. However, in the past few years, the number of successful trials has increased sharply, and a number of monoclonal antibodies are now approved for therapeutic use, enjoying multi-billion dollar annual sales and providing successful therapies for previously intractable cancers or autoimmune diseases. Well-known examples include antibodies against CD20 in B-cell malignancies and arthritis, antibodies against the cell-surface receptor human epidermal growth factor receptor 2 (HER2) in breast cancer and antibodies against the inflammatory cytokine tumour necrosis factor (TNF) in autoimmune disease. **See also**: Death Receptors at the Molecular Level: Therapeutic Implications; Monoclonal Antibodies: Therapeutic Uses; Tumour Antigens Recognized by Antibodies

Figure 3 The analytical power of a panel of monoclonal antibodies against molecules on the surface of blood cells. (a) Light scatter distribution of blood cells analysed in a flow cytometer, allowing the selection of the lymphocyte population for further analysis. If monoclonal antibodies against a T-lymphocyte marker and a B-lymphocyte marker, each attached to a different fluorescent dye, are added to the blood sample, the pattern seen in (b) can be obtained, allowing the selection of T cell for further analysis. (c) Resolution of T lymphocytes into two populations: lymphocytes marked with a monoclonal antibody against CD4, identifying a population containing 'helper' cells, which provide positive signals in an immune response, and the CD8 population, which include suppressive activity. (d) If the CD4 lymphocytes are selected for further analysis, they may be further separated into cells (CD45RO-positive) that have previously been activated ('memory cells') and those that have not previously been activated ('naïve cells'). (e) These can be subdivided in turn according to the cytokines they make – cells that make IL-4 tend to favour antibody responses, while cells that make IL-2 tend to stimulate cell-mediated immunity. (Note that panels (a)–(d) show actual data, while panel (e) shows simulated data.) Technical limitations in flow cytometry instrumentation and the number of different fluorescent dyes available place limits on the extent of the analysis. Widely available instruments can analyse on the basis of three colours simultaneously (allowing, e.g., the identification or separation of CD4 cells for further analysis), while more sophisticated research methods allow up to 11 simultaneous antibody markers, more than adequate to conduct the entire series shown in the figure. In practice, some steps can be left out; there is no need, for example, to include a B-cell marker or CD8 or CD45RA in identifying the IL-4-secreting helper T cells.

Major factors that have limited the clinical applications of monoclonal antibodies have been

- immunogenicity;
- difficulty and cost of production on an adequate scale;
- unwanted biological activity – due, for example, to direct effects on cells of the immune system;
- limited binding affinity, which necessitates the injection of large amounts of antibody in order to achieve a therapeutic effect;
- lack of direct functional action, requiring conjugation of drugs or other biologically active materials and
- limited penetration into the target tissue – especially dense, poorly vascularised tumour tissue.

It is not clear in every case what technological developments have brought about the generation of successful therapeutic antibodies after decades of limited success, but several technological developments have contributed. Most antibodies for therapy are now genetically engineered to make them more human and less murine in structure, reducing immunogenicity. There are several patented genetically engineered mice that make human antibody. Biotechnology companies have invested in the molecular biological skills to manipulate antibodies so as to reduce immunogenicity, improve affinity and specificity, improve yield in fermentation, and improve or select ability to engage the immunological mechanisms of the host required to remove target cells. Tissue penetration remains an issue, but can be improved using genetic engineering to reduce the size of the antibody molecule. Importantly, all the engineering is done on the DNA coding the antibody, not on the antibody protein itself, and therefore, it needs to be done only once. **See also**: Monoclonal Antibodies: Therapeutic Uses

Antibody Engineering

Antibody is a protein that can be modified in a variety of ways: removing specific functional portions, reducing the overall size of the molecule, changing critical amino acids to increase affinity and linking to other functional molecules such as drugs, radioisotopes or toxins to add 'teeth'. It is generally easier in the long term to modify the DNA rather than the protein – this needs to be done once only, and the modified gene is then expressed to produce the modified protein.

Antibody engineering of hybridoma-derived antibody genes and technologies for identifying antibody-like binding molecules from large libraries are changing the field, providing much greater control over the final product. Important techniques include the following:

- preparation from existing hybridoma genes of genes coding for small proteins, which include the antigen-binding site but omit most of the rest of the molecule, including sequences responsible for some of the biological effects of antibodies;
- modification to increase antigen-binding affinity (affinity maturation);
- preparation of fusion proteins consisting of the antigen-binding site linked directly to, for example, a toxin, an enzyme, a sequence suitable for radioisotope labelling, another antibody sequence, to achieve increased or novel biological activities;
- modification to make the sequence more human-like and less immunogenic;
- generation of libraries of genes derived from human antibody genes, to avoid the immunogenicity associated with foreign protein;
- genetic modification of mice so that they produce human antibodies and then immunising and making hybridomas in the classical way and
- preparation of antibody fragments in bacterial culture, to increase yield.

Products of these techniques are undergoing clinical trial and some have been approved for patient use. Several have become established therapeutic modalities. **See also**: Affinity of Antigen–Antibody Interactions; Protein Domain Fusion

Relative Merits of Polyclonal Antisera, Monoclonal Antibodies and Genetically Engineered Antibodies

The major characteristic of antisera, prepared by immunising and subsequently bleeding animals, is the heterogeneity of the antibody preparation. This may be a strength or a weakness, depending on the application. An antiserum will contain a mixture of antibodies against the antigen of interest, together with antibodies against other antigens encountered by the animal. The latter may cause confusing reactions and may need to be removed by absorption. The multiple antibodies against the antigen of interest, reacting with a multiplicity of epitopes, showing a spectrum of binding affinity, and engaging multiple components of the host immune system in therapeutic applications, can be advantageous, giving a stronger reaction overall than is seen with a single monoclonal antibody.

There are important uses of antibodies where polyclonal antisera work very well. This is particularly true in radioimmunoassays, which have been established for many years; there is no pressing need to change. On the other hand, in immunohistochemistry, change was initially slow in coming, because polyclonal antisera apparently worked very well, until awareness of the increased specificity achievable by monoclonal antibodies became widespread. A polyclonal antibody may react with the antigen of interest in one tissue, but when applied to another tissue, it is not possible to know whether it is reacting with the same antigen. **See also**: Immunoassay

Monoclonal antibodies must always be preferred when specificity is important. However, monoclonal antibodies

can be cross-reactive. A rabbit antiserum may react with the antigen of interest and additionally with a completely dissimilar molecule against which the rabbit made an antibody without being asked to. This type of non-specificity is unlikely with a monoclonal antibody (unless it was made as ascitic fluid – see earlier discussion). Cross-reactivity of antibodies results from their reaction with epitopes with a similar shape, which may be found on unrelated molecules. This type of cross-reactivity is found with monoclonal antibodies and must be guarded against.

Apart from specificity, monoclonal antibodies possess a major advantage of reproducibility. Two rabbit antisera against a particular antigen will exhibit differences in affinity and in the mixture of antibodies against different epitopes of the antigen; they will also carry a different set of unrelated antibodies. Even two batches taken from the same rabbit may differ in activity and in cross-reactivity. The specificity of a monoclonal antibody, once established, should not vary. Differences in titre (amount of antibody) are relatively easy to adjust for. There remain subtle variations between batches of monoclonal antibody, depending on denaturation and aggregation during storage and processing, but these are minor compared to the variations between batches of rabbit antiserum.

In laboratory applications, the choice between polyclonal and monoclonal antibodies may best be summed up as follows: if there is a choice, use the monoclonal antibody. However, when there is no antibody available, it will sometimes be quicker to make a polyclonal antiserum; this should be replaced in due course with monoclonal antibody.

For therapeutic applications, the situation is sometimes radically different. A polyclonal antiserum against the venom of the Australian king brown snake will neutralise all of the toxic activities of this witches' brew – neurotoxins, anticoagulants, procoagulants and others we may not be aware of. To replace this, well-tried preparation with a cocktail of monoclonal antibodies would be a major undertaking. Polyclonal antisera have an assured future in some therapeutic applications. In others, monoclonal antibodies have proved disappointing, but, as discussed earlier, antibody engineering has greatly extending the therapeutic scope of antibodies. **See also**: Immunofluorescence; Immunohistochemical Detection of Tissue and Cellular Antigens

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