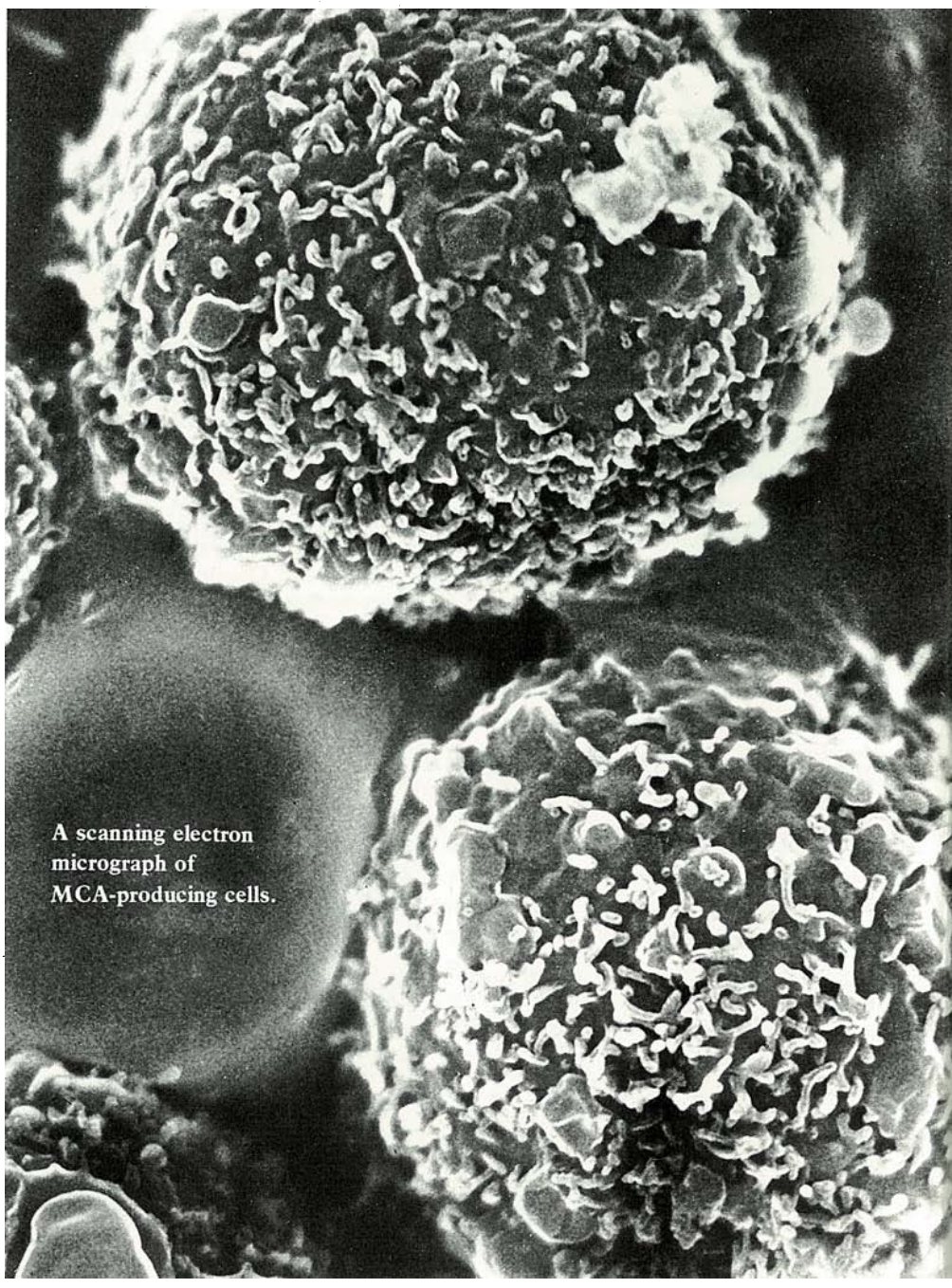
Exploiting monoclonal antibodies

Only a short 7 years ago Dr Cesar Milstein and Dr George Kohler, at Cambridge University in the United Kingdom, developed the techniques for producing monoclonal antibodies (MCAs) during the course of their studies into the genetic control of antibody synthesis. Their discovery of a way to make highly specific antibodies is already finding many applications in medical diagnosis and treatment, and the potential for further applications seems very great.



A scanning electron micrograph of MCA-producing cells.

When the human body is penetrated by a microbe or toxin, the white blood cells of the immune system — constantly circulating and scanning, on the look-out for molecules that are 'not-self' — quickly lock onto the foreigner. Reactions are many and varied, but the most common is that some of the white blood cells, as well as plasma cells in the spleen and bone marrow, synthesize and secrete antibodies that react with different parts of the foreign molecule. The antibodies bind to the foreigner and to each other to form large complexes that other specialist cells of the immune system, the macrophages, eventually clean up.

The same processes that operate in this natural bodily defence mechanism are put to work in disease diagnosis. Antibodies to particular disease agents can be manufactured in the laboratory. When added to a blood sample, they will bind only with the specific foreign molecules, more correctly termed antigens, that invoke their production in the body. Minute amounts of antibody can find and bind to equally minute amounts of antigen in a solution.

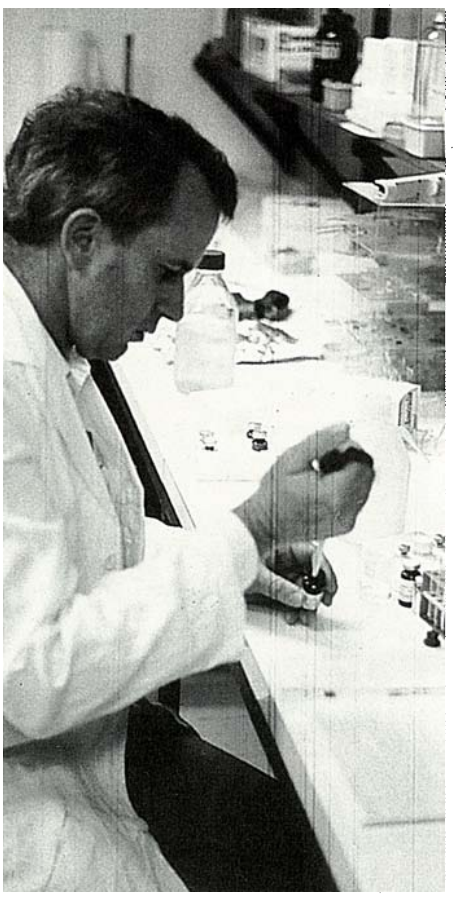
A perpetual source of a single, well-defined antibody had been produced.

If the antibody is labelled with a radioactive chemical, such as an isotope of iodine, chemical separation and measurement of the radioactivity in the various fractions can indicate how much antigen the original solution contained. This technique — radioimmunoassay (RIA) — has many important applications in medicine. It can be used to measure hormone levels as well as to detect disease agents, because human hormones also stimulate antibody production — but in other animals.

The antibodies needed for RIA are produced by laboratory animals, like goats, sheep, guinea pigs, rats, or rabbits, which are given injections of the antigen. As a result, a high concentration of the antibody active against the injected antigen builds up in the cell-free fraction of their blood, the serum. Serum diluted up to a million times is used in RIA.

However, that serum is really a cocktail of antibodies. It contains small, but measurable, quantities of antibodies that formed earlier in the animal's life — such as those for cold and flu viruses. More importantly, those produced in response to the antigen are also many and varied. This is because an antigen molecule commonly has a large number of sites, called antigenic determinants, that can react with antibody. The immune system is very obliging, producing antibodies for all the different determinants. It even doubles up the attack by fashioning different antibodies that fit, more or less well, a single determinant.

This is the crucial difference between conventionally produced and monoclonal antibodies; while the 'cocktail' from a laboratory animal may react with many determinants on the antigen, an MCA will bind to only one.



Measuring prolactin with a specific monoclonal antibody.

Antibodies derived from rats or rabbits are widely used in such medical tests as typing blood groups or tissues before transplantation, and detecting a range of human hormones, imbalances of which are implicated in a large number of serious disorders. However, as the rabbits or rats produce such a variety of antibodies, and the mixture varies from animal to animal and even from bleed to bleed in the individual animal, it is difficult to produce standards for any of these tests.

International standards are often available against which local preparations can be compared, but these are based upon large quantities of serum derived from one or a few animals; once that serum is exhausted, the standard has to change as well.

Obviously an inexhaustible supply of highly specific antibodies would simplify procedures. It could also open up new medical opportunities in specialist areas where antibody production and assay are so troublesome that their use has been severely restricted — usually to research.

Developing an MCA

For many years scientists have known that a continuous supply of antibody can be provided by growing in artificial culture single cells of a cancer, common to both man and animals, known as myeloma. This cancer involves the antibody-secreting plasma cells, so such a culture makes available a continuous harvest of a single antibody; the only trouble has been that, until recently, it was impossible to predict or control what antibody it would be!

The yield of antibody-producing clones is typically low.

During 1973 Dr Milstein and an Australian scientist, Dr Robert Cotton, while studying how antibody is constructed, fused cells from a mouse myeloma with those from a rat myeloma and found that the hybrid continued to secrete antibodies from both the rat and the mouse. This told the scientists a lot about where the antibody genes are located and how the products of those genes are built up into an antibody molecule. More importantly, it also suggested to Dr Milstein and Dr Kohler a way to produce continuous supplies of specific antibodies.

The key to developing MCAs was the discovery that a myeloma cell, when hybridized with another antibody-producing cell, could continue to produce the introduced cell's antibody as well as its own.

And when they fused myeloma cells with spleen cells from a mouse immunized against sheep red blood cells — an antigen chosen because it is easy to detect — they found that a good number of the hybrids were viable in artificial culture, and a few were secreting antibody directed against sheep red blood cells. A perpetual source of a single, well-defined antibody had been produced.

The simple technique the scientists devised has been modified only slightly

Prolactin prepares the breast for milk production and release and, ironically, is a major cause of infertility in women.

during recent years — the most important change being the use of a mutant myeloma cell line that doesn't secrete any of its own antibody. Using this procedure, biologists can produce highly specific single antibodies against any substance that is capable of inducing antibody formation.

Commercial development

Monoclonal antibodies can be used in routine diagnostic tests, or used to separate and purify chemicals that are present in very low concentrations (see the box on page 20). They also have a potential use in immunotherapy directed against a range of diseases, the most important being the various cancers. Specific MCAs, directed against the antigens present on the cancer cell membrane, could be used to enhance the patient's own immune response.

Alternatively, cell-killing drugs could be attached to the MCAs. These, as they would seek out the cancer cells, should result in more specific and effective chemotherapy than is normally possible, with a reduction in the adverse side-effects that follow cancer chemotherapy.

Because of the potentially large market for MCAs, the new technology has excited commercial interest. For example, the Queensland Institute of Technology and the Commonwealth Serum Laboratories are exploiting MCAs. A Sydney-based company called Australian Monoclonal Developments Pty Ltd is using MCAs to develop more-specific and accurate tests for brucellosis in cattle.

Another company, Bioclone Australia Pty Ltd, has entered into a joint venture with the CSIRO Molecular and Cellular Biology Unit and a research group attached to St Vincent's Hospital — the Garvan Institute of Medical Research — to develop and produce a range of MCAs.

The CSIRO group, led by Dr Anne Underwood, had both the facilities and the expertise to develop MCAs. The group had produced MCAs against strains of the influenza virus and used these to plot the evolution of the virus, and is investigating the production of antibodies against the regulatory proteins involved in gene expression. The Garvan Institute researchers, led by Dr Margaret Stuart, had specific expertise in the group of hormones whose detection was an important early commercial application for MCAs — the pituitary hormones.

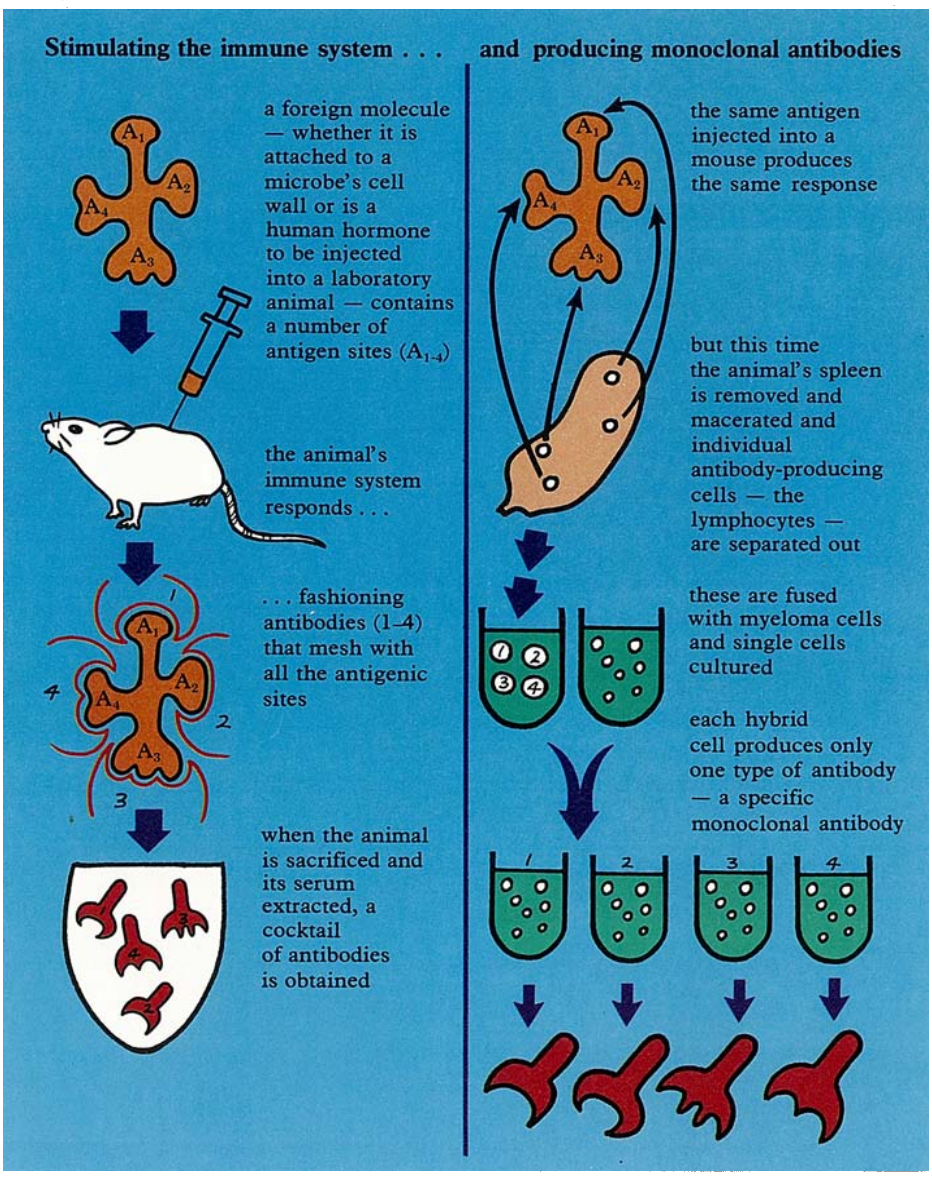
A hormone mix

The pituitary gland, situated at the base of the brain, secretes nine hormones and these small proteins have a powerful influence on human growth and development.

Growth hormone, for example, ensures that we all reach our full growth potential and any deficiency of the hormone results in cretinism; too much and we have gigantism. The thyroid-stimulating hormone (TSH) regulates the thyroid gland's activity, while follicle-stimulating hormone (FSH) and luteinizing hormone (LH) control the female's menstrual cycle. Prolactin prepares the breast for milk production and release, and high prolactin levels are responsible for the relative infertility that follows the successful completion of a pregnancy. Other hormones regulate the adrenal glands, kidney function, and the birth process.

As would be expected, imbalances in pituitary hormone levels are implicated in a wide range of disorders, and many tests — usually involving RIA — are made for diagnostic purposes.

For example, an elevated level of prolactin — associated with a small benign tumour of the pituitary gland — is one of the commonest causes of infertility in women. This problem responds readily



to drug therapy, and a check on prolactin levels is one of the first tests a gynaecologist would recommend for women experiencing difficulty in becoming pregnant. Yet the test is complicated and the results often uncertain because of the similarity between prolactin and some of the other hormones that the pituitary produces.

The pituitary hormones are generally composed of two amino acid chains that twine around each other to form the active structure. The three hormones FSH, LH, and TSH have one chain in common, and a similar chain appears in an important hormone secreted by the placenta — human chorionic gonadotrophin (HCG). Prolactin shares common sequences with growth hormone and another placental hormone, human placental lactogen. Because of these similarities a rabbit injected with LH, for example, will produce some antibodies that will also react with FSH, TSH, and HCG. This cross-reactivity obscures the results of any test for LH that exploits that rabbit's serum.

An MCA has obvious advantages over such a mix of antibodies, but before any MCA earns a role in routine testing for pituitary hormones it must itself pass a few tests.

Firstly, the hybrid cell that produces the specific antibody must be stable — a common phenomenon is that the hybrid starts to shed chromosomes, and antibody synthesis is often lost during this shedding. Secondly, the antibody involved must not cross-react with other similar hormones. Finally, for most purposes the antibody must have a strong affinity for the hormone involved. However, weakly binding antibodies are preferred if the MCA is to be used for purification of the hormone using 'affinity columns' (see the boxes).

Although the production of MCAs appears a straightforward exercise, the above represent fairly demanding criteria for most clonal lines; and many cannot match those demands. For example, a mouse's spleen contains about 150 million cells that are potential MCA-producers, but the yield of antibody-producing clones is typically low. Most laboratories can produce viable antibody-secreting clones — but once they apply the three tests mentioned in the previous paragraph they reject most of them.

The research group ran into the problem of low yield of specific hybrid clones when they were attempting to raise an MCA against prolactin. Of the 384 hybrids they produced, just over half continued to grow, and only eleven secreted antibodies active against prolactin. From those eleven, eight lost the ability to synthesize antibody, which meant they had only three clones available for tests on cross-reactivity and affinity. Two of these were found to be suitable for use in affinity chromatography, but only one was ideal for use in hormone assays.

An Australian MCA

The 'hybridoma' line secreting antibody against prolactin was an obvious first choice for commercial development by Bioclone. And once the decision was made to proceed it took only 12 months to produce bulk quantities of antibody suitable for use in diagnostic kits.

The speed of this development stemmed largely from the fact that scaling-up for production of bulk quantities of MCAs is a relatively simple affair. Because the antibody is so active, only small quantities are necessary — so there is no need for massive and sophisticated fermentation vessels.

Hybridomas can be grown in a liquid culture medium built around animal serum and essential nutrients, or grown *in vivo* — the hybridoma being injected into a mouse's peritoneum where it proliferates and secretes massive quantities of antibody that are tapped at regular intervals. Typically, one cancerous mouse can produce as much antibody as a 6-litre culture, and this is enough for about half a million assays. Scale-up is thus essentially an exercise in picking the right mouse or the right size in glassware.

Scaling-up for production of bulk quantities of MCAs is a relatively simple affair.

In November 1982, Bioclone announced the development of its prolactin assay kit and testing the kit is continuing in major teaching hospitals and testing laboratories throughout Australia. Preliminary results suggest that it is as good as the imported product, and Bioclone expects to begin regular production of the kits during 1983.

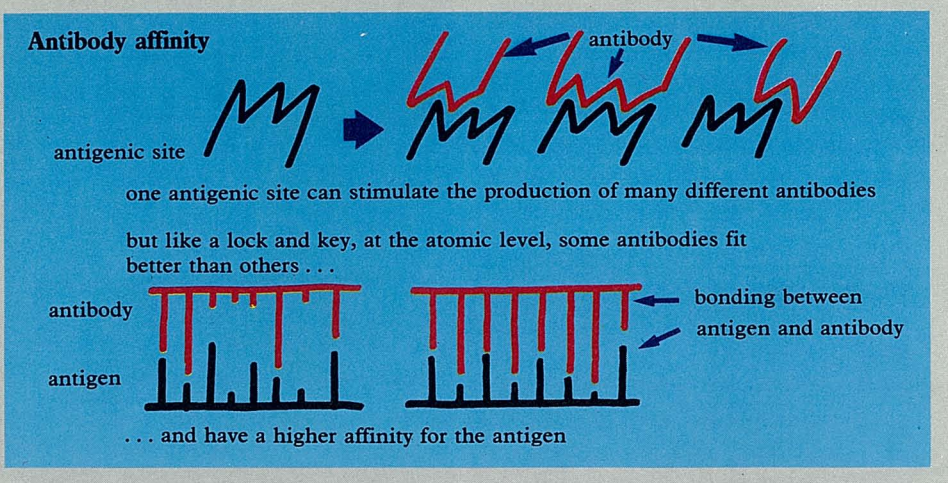
Also, Bioclone has sold trial quantities of bulk antibody to kit manufacturers in the United Kingdom, Sweden, Italy, the United States, and Canada. As a result, the company recently received the first

Tests that MCAs must pass

An MCA raised against prolactin has to pass tests for cross-reactivity and affinity; both involve labelling a pure preparation of human prolactin with radioactive iodine.

In the cross-reaction test a similar hormone — growth hormone from humans, sheep, cattle, or pigs, or prolactin from the animals — is incubated with the labelled human prolactin and the MCA under test. The amount of antibody-labelled antigen complex is then compared with the amount that is formed in the absence of a competing hormone. If there is little difference between the two, then little cross-reaction has occurred and the MCA should be useful in diagnostic tests.

In affinity tests, the labelled antigen and the antibody under test are incubated together and at set times the amounts combining are measured; a high level of antibody–antigen complex early in the incubation period indicates a high affinity. Conversely, if the reaction proceeds only slowly the MCA is not strongly attracted to the antigen and its uses may be restricted.

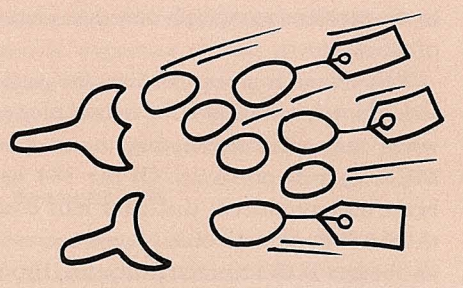


Two uses for MCAs

Protein hormones can be detected and their concentration estimated by a number of techniques that utilize antigen–antibody binding; however, the most commonly used technique is radioimmunoassay (RIA).

In RIA, the first step involves working out what dilution of antibody will bind about 50% of a hormone that has been labelled with radioactive iodine. This determines the standard for the test. In the next step the diluted antibody is mixed with the quantity of labelled hormone that produces 50% binding. Then this mixture is added to varying dilutions of the solution — usually a blood extract — in which hormone levels are to be measured.

The unlabelled hormone from the blood sample now competes with the labelled hormone for the limited number of antibody molecules. After a period of incubation, when the system has reached equilibrium, the antibody-bound and free hormones in the solution are separated. (A number of methods can be used for this job.) Measuring the radioactivity of the recovered antibody–hormone complex gives an indication of how much of the labelled hormone has been displaced by the unlabelled hormone and, working backwards, an estimate of the quantity of hormone in the test sample.



For the RIA test, a relatively pure source of hormone is necessary for comparison's sake. Pure extracts are also useful for research purposes. However, because of their low levels in natural sources — concentrations are typically of the order of one part per hundred billion in blood and urine — their extraction is usually difficult.

Through their use in affinity chromatography, MCAs can help overcome this difficulty. In this technique the antibody can be chemically bound to various substances and the mixture packed into a column. When dilute or impure solutions containing the hormone are passed through the column, the antibody binds to the hormone and selectively removes it from the solution. Acid or alkaline solutions can then be flushed through the column to release the hormone in a relatively pure form.

overseas order for bulk prolactin antibody, and expects more to follow.

In addition to its use in assays, the prolactin antibody can be employed in affinity chromatography to separate out the antigen from impure and very dilute solutions. Some affinity columns have already been sold to researchers and regular production and sales will also begin in 1983.

Bioclone, in conjunction with CSIRO and the Garvan Institute, expects to develop monoclonal antibodies, for kit use, against all the pituitary hormones within the next few years. Kits for detecting growth hormone and human chorionic gonadotrophin (HCG) are following closely behind the prolactin kit.

Antibody to growth hormone is used in the early detection of dwarfism and gigantism, as well as the diagnosis of acromegaly — a related disorder of older people.

The HCG antibody's most popular use will be in pregnancy testing — both for normal and ectopic pregnancies. And it can also be used in the diagnosis of choriocarcinoma — a particularly malignant cancer involving the placenta — and in following up the results of surgery and chemotherapy. In other cancers, a good proportion of tumours — for example, about 30% in lung cancer — secrete HCG, so the antibody assay kit also has a potential role in monitoring therapy in these cases.

Research roles

Looking further ahead, the group is attempting to develop serum-free tissue cultures for use in MCA production. *In vitro* culture gives a purer product than *in vivo* culture, and if researchers can induce the hybridomas to grow without the aid of animal serum they may be able to use monoclonal antibodies, raised against the antigens on the surface of cancer cells, in immunotherapy without the complications induced by animal sera. This would require MCAs of human origin rather than those from laboratory animals, and only in recent years have human myelomas, suitable for MCA production, been cultured.

Research still has an important role in the further development of MCA technology. The CSIRO and Garvan Institute teams have already provided one notable example of the sort of refinement that can be made.

According to Dr Underwood, low yields of hybridomas have retarded developments in the field. Her group's experience with prolactin provides a typical example of the low yields that can be expected.

However, when the scientists looked more closely at the process they were using they found that a number of steps affected the final yield. These included immunization schedules, the timing of spleen extraction, the conditions under which fusion occurred, and the way in which subsequent hybrids were cultured. By manipulating these various factors, the group has managed to increase the hybridoma yield up to thirtyfold — an increase that will help ensure that useful MCAs can be quickly selected and developed.

Wayne Ralph

More about the topic

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