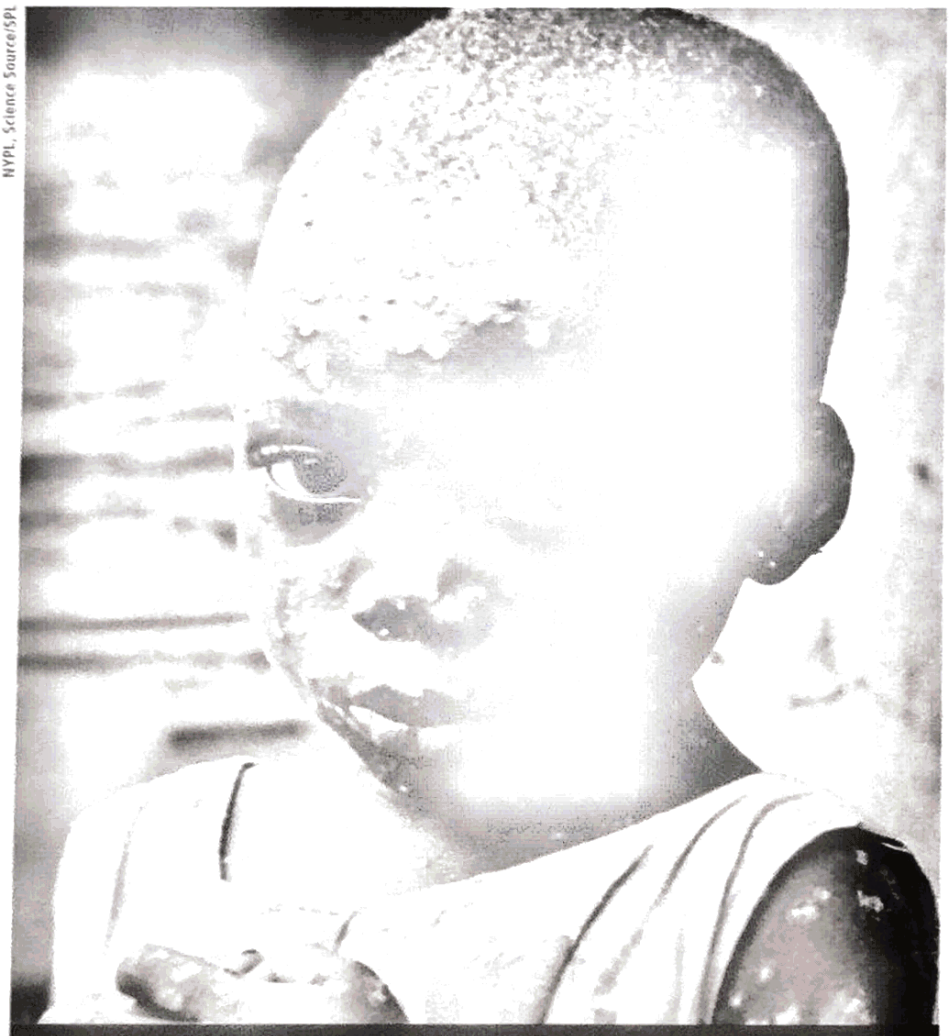
Linda Rolfe

Putting antibodies to work

Key words
Pathogens
Antibodies
Antigens
Monoclonal antibodies

Antibodies carry on a daily battle inside our bodies. They fight against diseases caused by pathogens. Recognition of pathogens and the body's response to them involves various types of white blood cells. This immune response is now well understood and research has led to many useful applications of antibody technology. Exciting new therapies for cancer have been developed that rely on the use of antibodies to target cancer cells specifically.



NYPL, Science Source/SPL

A Congolese child with smallpox in 1965. Smallpox was eradicated as an infectious disease in 1979, following a global vaccination programme. The vaccine induces antibodies, which protect against smallpox virus.

The study of immunity can be traced back to 1796. An English doctor, Edward Jenner, discovered that cowpox — *Vaccinia* virus — could be introduced into humans to give protection against the deadly smallpox virus. This is where the term vaccination came from. It describes the inoculation of healthy individuals with weakened strains of **pathogens** to provide protection against disease. Almost 100 years later, a Frenchman, Louis Pasteur, developed a rabies vaccine. Fortunately, it proved successful when tried for the first time on a boy who had been bitten by a rabid dog.

These initial successes led to a quest to understand how people are protected from infection. In 1890, biologists discovered that the plasma of vaccinated people contained substances that bound to the pathogen and thereby gave protection. They called these substances **antibodies**.

The formation of antibodies

Today we know that antibodies are proteins made by white blood cells called B lymphocytes. The B lymphocytes are formed in the bone marrow. The fact that they originate from bone marrow led to them being called B lymphocytes — to distinguish them from T lymphocytes, which develop in the thymus gland situated just above the heart. T lymphocytes elicit an immune response but do not produce antibodies.

Once fully formed, both types of lymphocytes enter the blood and travel to lymphoid tissue such as the tonsils, spleen and lymph nodes. Although

their numbers are particularly high in these parts of the body, lymphocytes continually circulate through the blood, lymph and lymphoid tissue (see Figure 1). When lymphocytes encounter a pathogen, they bind to it and then move to and become trapped in the lymphoid tissue. The accumulation of lymphocytes can cause the lymph node to enlarge, producing, for example, the painful swollen glands we associate with tonsillitis.

So how do lymphocytes recognise pathogens such as bacteria and viruses? It is down to **antigens** — specific molecules that all pathogens have on their outer surface. These antigens are recognised by the lymphocytes as being 'foreign' to the body.

B lymphocytes produce the humoral response (see Figure 2A). B cells bind with the antigen, and then get trapped in the lymphoid tissue. The B lymphocytes are stimulated to divide and produce many identical cells. These cells are capable of producing antibodies that are specific to the antigen that activated the B lymphocyte in the first place.

Some pathogens, such as viruses and some bacteria, replicate inside living cells. At first sight, this might seem a good way of avoiding detection by lymphocytes. However, antigens from the infective pathogen can be presented on the surface of the infected cell. These are recognised as foreign by the T lymphocytes, which produce an immune response (see Figure 2B). The T lymphocytes bind to the infected cell via specialised cell surface proteins called T-cell receptors. Once they bind to the infected cells they proliferate and eventually destroy the infected cell, by cell **lysis**.

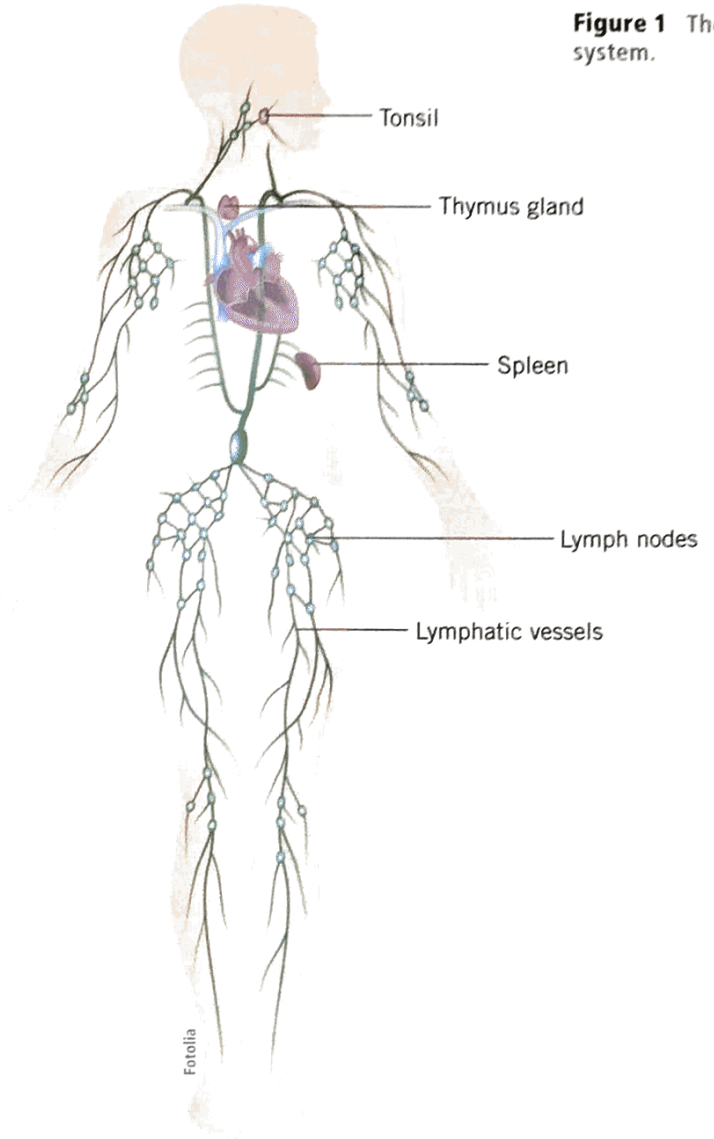


Figure 1 The lymphatic system.

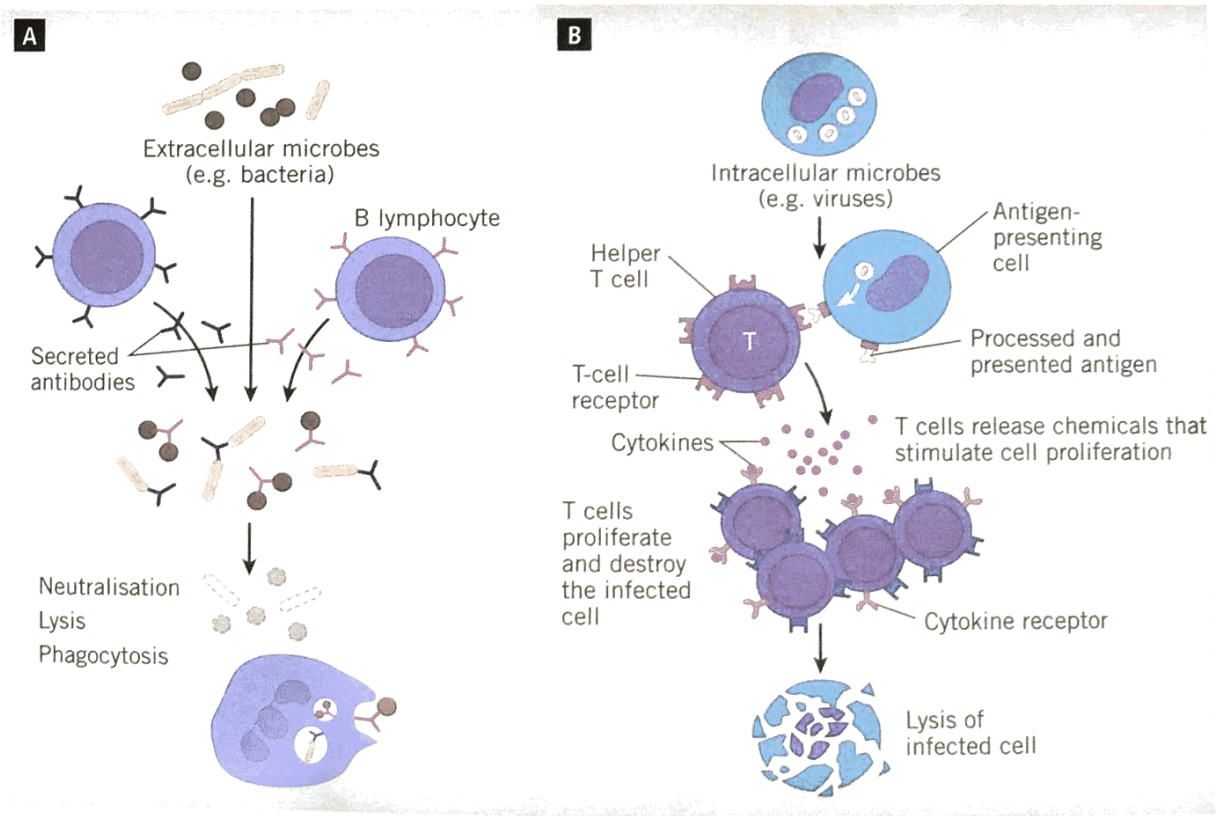


Figure 2 (A) Humoral immunity and (B) the T cell-mediated immune response.

Antibody structure

Antibodies are roughly Y-shaped molecules consisting of two types of polypeptide chains: heavy chains and light chains. There are two chains of each type and all four chains are held together by covalent linkages called disulphide bonds (see Figure 3). In any single antibody molecule, the two heavy chains and the two light chains are identical.

The arms of the Y consist of both heavy and light polypeptide chains. You might think that because antibodies are proteins (and hence genetically determined), the arms of the Y would always have the same amino acid sequence. However, this is not the case. Rearrangement of the DNA sequence coding for the variable regions of the heavy and light chains happens during development. This rearrangement results in an almost unlimited diversity in the amino acid sequence of the variable region of the antibody molecules, which then leads to a vast repertoire of antibodies that can bind to different antigens. The rest of the antibody sequence does not change between antibodies with different abilities to bind to antigens, so these are called constant regions.

How antibodies work

Antibodies identify antigens and their toxic products as being foreign to the body. They can do this in several ways. In a process called neutralisation, antibodies bind to pathogens, or their toxic products, and prevent them from entering cells. This method is effective against pathogens such as viruses that are only able to replicate inside living cells. An alternative method involves many antibodies attaching to antigens on the surface of the pathogens, leaving their constant regions exposed (see Figure 3). Phagocytic white cells recognise the constant region and ingest the pathogen. This method of covering the pathogen in antibodies is called opsonisation (see Biological Sciences Review, Vol. 18, No. 1, pp. 2–6 and pp. 2–6, this issue). Opsonisation is a useful defence against bacteria that would normally multiply outside cells.

Whatever the method, the end result is the same. The pathogen and its toxic products, having been clearly identified as foreign to the body, are engulfed, digested and eliminated by phagocytic white cells (see Figure 4).

Using antibodies in medicine

Antibodies have become major players in many developments in medicine. It is possible to produce

Terms explained

Antibodies Special proteins called immunoglobulins that are produced by B lymphocytes and which can bind to specific antigens.

Antigens Molecules (usually proteins) that stimulate lymphocytes to produce specific antibodies.

Chemotherapy Specific drug treatment used to kill cancer cells.

Endocytosis A mechanism whereby substances are transported into the cell inside vesicles formed from the cell surface membrane.

Lysis The destruction of a cell by breaking down its cell surface membrane.

Monoclonal antibody A single antibody that recognises a distinct part of an antigen. Monoclonal antibodies are distinct from polyclonal antibodies, which are several different antibodies that recognise different parts of the same antigen. Monoclonal antibodies can be made in large quantities in the laboratory.

Pathogen A microorganism that causes disease.

PET scanner Positron emission tomography scanner, an instrument that creates an image of areas of radioactivity in the body.

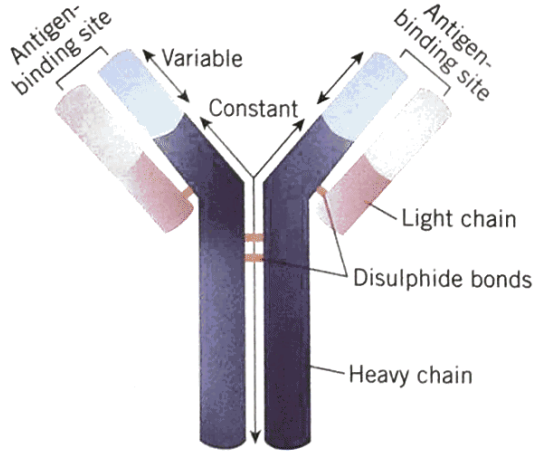


Figure 3 Structure of an antibody.

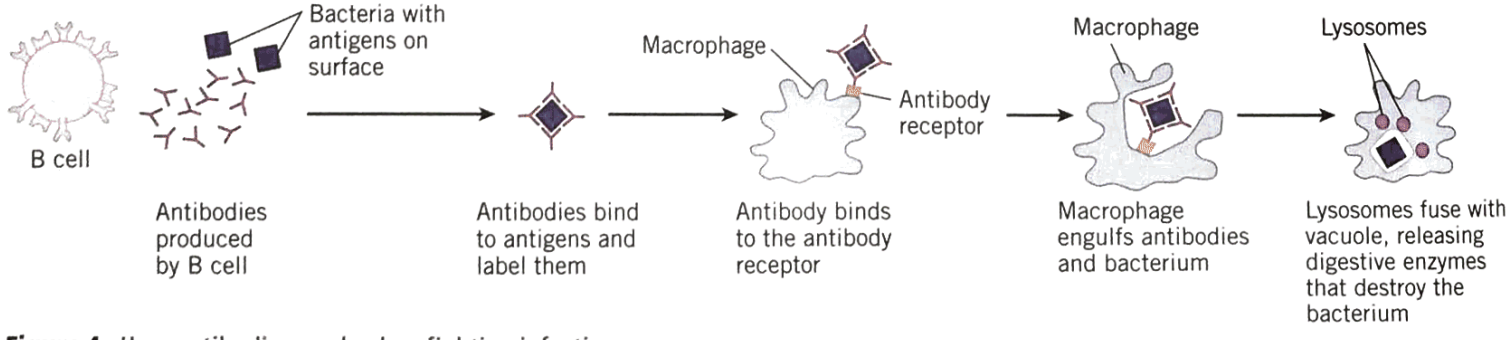


Figure 4 How antibodies work when fighting infection.

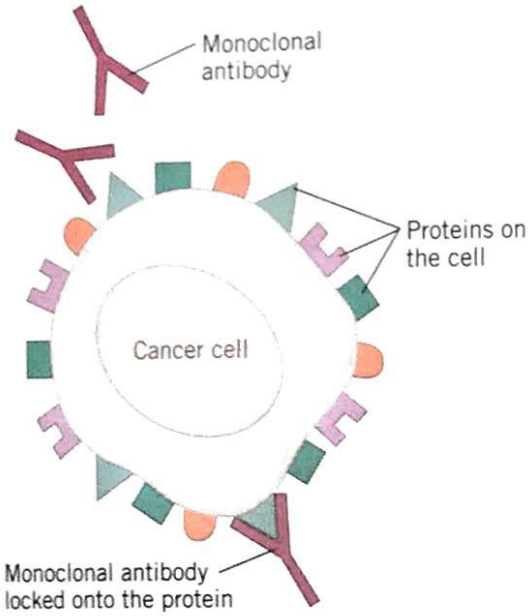


Figure 5 Monoclonal antibody attaches to a specific antigen that is only found on tumour cells.

Monoclonal antibodies are produced commercially in huge quantities.

antibodies that recognise and bind with a specific antigen — these are called **monoclonal antibodies**. Each monoclonal antibody recognises a specific antigen and this specificity can be put to use in many different ways.

Monoclonal antibodies can be combined with γ-emitting radioisotopes so that they can be used to image tumours. An antibody can be made that recognises a specific tumour (see Figure 5). An antibody with a radioisotope attached is injected into the blood, so it is transported all around the body. The antibody recognises an antigen that is only found on tumour cells and therefore binds only to tumour cells. The radiolabel accumulates at the site of the tumour and a **PET scanner** can detect this label, revealing the location of the tumour. This technology is extremely useful in producing images that allow accurate diagnosis and monitoring of the spread of tumours.

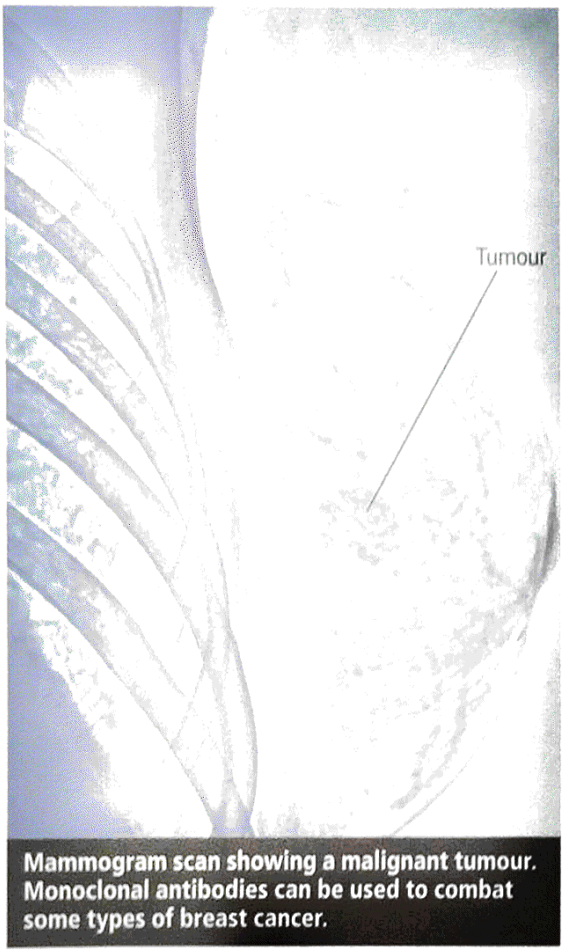
Chemotherapeutic drugs can be bound to monoclonal antibodies, so that the drug is delivered directly to the cell surface antigens on the tumour and is then taken up by **endocytosis**. Once inside the cell, the drug exerts its effects, either killing

BSIP, Raguet/SPL

A patient having a brain PET scan and doctors examining the results.

the cell or preventing it from dividing. Monoclonal antibodies have also been used to carry therapeutic radioisotopes to tumours, so concentrating their destructive effects exactly where they are needed. In both these approaches, the antibody acts like a magic bullet — seeking out only tumour cells, which leads to an effective cancer therapy. The antibodies themselves are the therapeutic agent, or drug. Huge sums of money and patients' lives are at stake, so the race is on between drug companies to produce and patent monoclonal antibodies for use against specific tumours.

One example of a monoclonal antibody that is already in use is Herceptin, which is used to treat women with a particular type of breast cancer called HER2 positive. It works by specifically targeting cells that produce too much HER2 protein. This protein is a natural receptor molecule found on the surface of cells. It plays a role in the normal cell cycle by signalling the cell to divide. Overproduction of HER2 produces more receptor sites, so resulting in more cell division and tumour growth (see Biological Sciences Review, Vol. 24, No. 1, pp. 38–41). Herceptin may work in a number of ways. It may attach to the HER2 proteins, causing them to be absorbed into the cell, thus preventing the signal for cell division, or it may bind to T cells, targeting them to kill the tumour cells. Herceptin can be used in conjunction with conventional **chemotherapy**, which damages the DNA in the nucleus of tumour cells. This combined therapy has increased survival rates in women with advanced breast cancer.



Visuals Unlimited Corbis

Mammogram scan showing a malignant tumour. Monoclonal antibodies can be used to combat some types of breast cancer.

These monoclonal antibodies do not affect healthy cells. This means that patients treated with Herceptin alone do not suffer from the unpleasant side effects often associated with chemotherapy, such as hair loss and fatigue. Unfortunately, Herceptin itself is not without side effects, such as nausea, pain and headache. It can also cause heart problems. Nevertheless, Herceptin is extremely useful in the fight against breast cancer.

Recent developments in monoclonal antibody technology may also bring relief to people suffering from the crippling disease rheumatoid arthritis. People with this disease have an excess of a protein called TNF-α, which accumulates in their joints and contributes to inflammation and destruction of the joint. A monoclonal antibody has been designed to block the activity of TNF-α, so reducing inflammation, pain and joint deformities. This drug has helped people suffering from rheumatoid arthritis to maintain an active and productive life.

In the future it is likely that drug companies will be able to produce fragments of antibodies that are easier to produce than the whole antibody. Such fragments are coded for by just part of a gene. The appropriate part of the gene, coding for the antibody fragment, can be incorporated into the DNA of *Escherichia coli* or yeast. The microorganism then produces substantial quantities of the antibody fragment. By using genetic engineering in this way, drug companies can produce large amounts of fragments relatively cheaply. Such fragments also have the advantage of being small, so they can penetrate deep into body tissues more effectively. One drug company already is producing antibody fragments against TNF-α in the hope that these fragments may be more effective than whole antibodies in the treatment of rheumatoid arthritis.

As our understanding increases — in particular the role of antigens in disease — it is likely that monoclonal antibody technology will play a greater part in the treatment of disease.

Further reading

McDermott, J. and Grencis, R. (2005) 'Fight for your life', Biological Sciences Review, Vol. 18, No. 1, pp. 2–6.

Gilmore, A. (2011) 'Can molecular biology cure cancer?', Biological Sciences Review, Vol. 24, No. 1, pp. 38–41.

Linda Rolfe teaches biology at Petroc in north Devon. Dr Rolfe has a particular interest in proteins and the application of monoclonal antibody technology.

Key points

- Humans have a defence system that protects them against harmful invading microorganisms (pathogens).
- Monoclonal antibodies can be used to produce images of tumours and to treat specific cancers.
- Genetic engineering can be used to produce large amounts of specific fragments of monoclonal antibodies that may be used as drugs.
- Monoclonal antibody technology may eventually lead to treatments for other diseases.