Hitting the Target — with monoclonal antibodies

Contents: Reading and questions concerning the production and uses of monoclonal antibodies.

Time: 1 to 2 periods.

Intended use: GCSE Biology and Integrated Science. Links with work on disease and functions of the blood. More suitable for use with students likely to achieve grades A to C at GCSE.

Aims:

- To complement and revise prior work on the body's defences against disease
- To develop awareness of an important aspect of biotechnology, the production and uses of monoclonal antibodies
- To illustrate the process of scientific discovery and the technological potential of such discoveries
- To provide opportunities to practise skills in reading, comprehension and problem-solving.

Requirements: Students' worksheets No. 609

Background notes on monoclonal antibodies

The information given in the students' materials is highly simplified. In particular, production of antibodies is much more complex than how it is presented in the students' worksheets. It involves two types of lymphocytes, T cells and B cells. The T cells rapidly increase in number and then help the appropriate B cells increase in number. The B cells then produce the correct antibody. The process is not completely understood and is still an area of controversy.

Producing monoclonal antibodies in the laboratory is also more complex. The tumour cells used, for example, are deficient in an enzyme and could not survive without fusion with a lymphocyte. Because of the method of making the monoclonal antibodies, a number of different types of lymphocyte-tumour combinations are produced, as well as lymphocyte-lymphocyte and tumour-tumour combinations. The combination of interest has to be separated from the others.

Notes on some of the questions

Q.1 Having once been exposed to a particular pathogen, lymphocytes will respond more quickly to a subsequent exposure, and will rapidly produce the necessary antibodies and thus prevent the disease developing.

Q.2 Chicken-pox and measles are both viral diseases, but each requires a specific antibody.

Q.3 In the case of colds there are so many different forms of the virus responsible that it is impossible to gain immunity to all of them.

Q.5 See 'Background notes on monoclonal antibodies' above.

Notes on the problems

The problems are graded in approximate order of difficulty, easiest first. The 'solutions' given below are those that have been actually adopted, though students may produce other plausible solutions.

Problem 1 Make monoclonal antibodies specific to the protein (which is actually the hormone human chorionic gonadotrophin, HCG). Add antibodies to the urine and arrange for a suitable indicator to be present which will change colour in the presence of the HCG-antibody complex. (In fact, the kit contains a 'stick' impregnated in suitable antibodies. The stick is held in the urine, then dipped in an indicator solution.)

Problem 2 The bone marrow is removed and treated with monoclonal antibodies targeted on the cancer cells. These monoclonal antibodies have ricin attached to them.

Problem 3 Make monoclonal antibodies which are specific for Factor 8 and use them to pick out Factor 8 from blood. The antibodies are immobilized in a column through which the blood passes. Factor 8 attaches to these antibodies, and can later be removed from the column.

Problem 4 This problem is presented in a more open-ended way, and students may not arrive at the exact answer given here. The method used is to attach a weakly radioactive isotope to an antibody which is targeted on the cancer cells. Finding where the radioactivity is most concentrated then tells the doctor where to direct treatment.

Acknowledgements Figures 1 and 8 supplied by Imperial Cancer Research Fund.

HITTING THE TARGET with monoclonal antibodies

What are antibodies?

Antibodies are made by the body in response to attack by bacteria or viruses. This helps protect against infection. The antibodies do this by recognizing very accurately the proteins on the surface of the bacteria or microbe. Antibodies are produced by cells. By growing these cells in the laboratory, it is possible to produce large amounts of one type of antibody. These are known as **monoclonal antibodies.** To understand how this is done, let's first examine the body's natural mechanism for making antibodies.

Figure 1 Using an electron microscope to examine the action of antibodies

Where are antibodies made?

Antibodies are made by special cells called **lymphocytes.** Lymphocytes are made in the lymph nodes and spleen. You may have felt lumps in your armpits or on the side of your neck when you are ill. These are the lymph nodes hard at work producing antibodies to fight your illness. The lymph nodes can produce tens of thousands of different lymphocytes. Each one is able to produce a different antibody if necessary.

How do antibodies work?

Every microbe has uniquely shaped proteins on its surface. These proteins are called **antigens.** Antibodies have shapes which can lock onto a particular antigen. In this way antibodies recognize antigens, and attach themselves to them (Figure 2 on the next page).

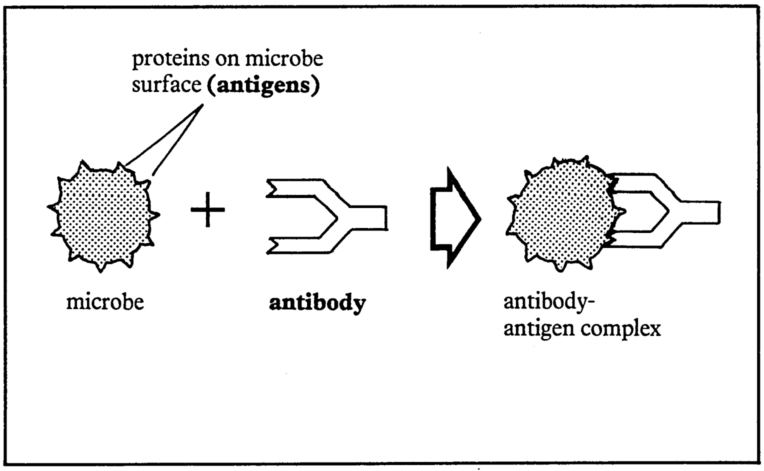

Figure 2 The antibody-antigen interaction

Whatever the shape of the antigen, an antibody exists which will fit it and make it harmless. The antibody-antigen complex can then be ingested and destroyed by the body's 'scavenger' cells — the **phagocytes** (Figure 3).

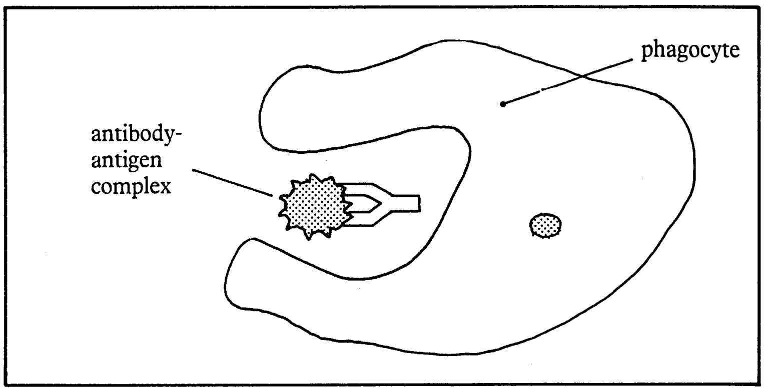

Figure 3 A phagocyte ingesting an antibody-antigen complex

Manufacturing antibodies

The body is usually invaded by thousands of microbes of the same type. So the body must be able rapidly to increase the production of the necessary lymphocytes.

As soon as new antigens are spotted in the body, action begins. The body rapidly increases the numbers of the lymphocytes that have the right shaped antibodies to lock onto the invading antigens. Many of these 'right shaped antibodies' can then be quickly produced. Only by this rapid response can the infection be successfuly fought (Figure 4 on the next page).

Questions

1 *For many diseases, once you have had the disease you are* **immune** *and cannot catch it again. Explain why.*

2 *Explain why antibodies produced to fight chicken-pox do not work against measles.*

3 *Suggest a reason why we do not get immune to colds.*

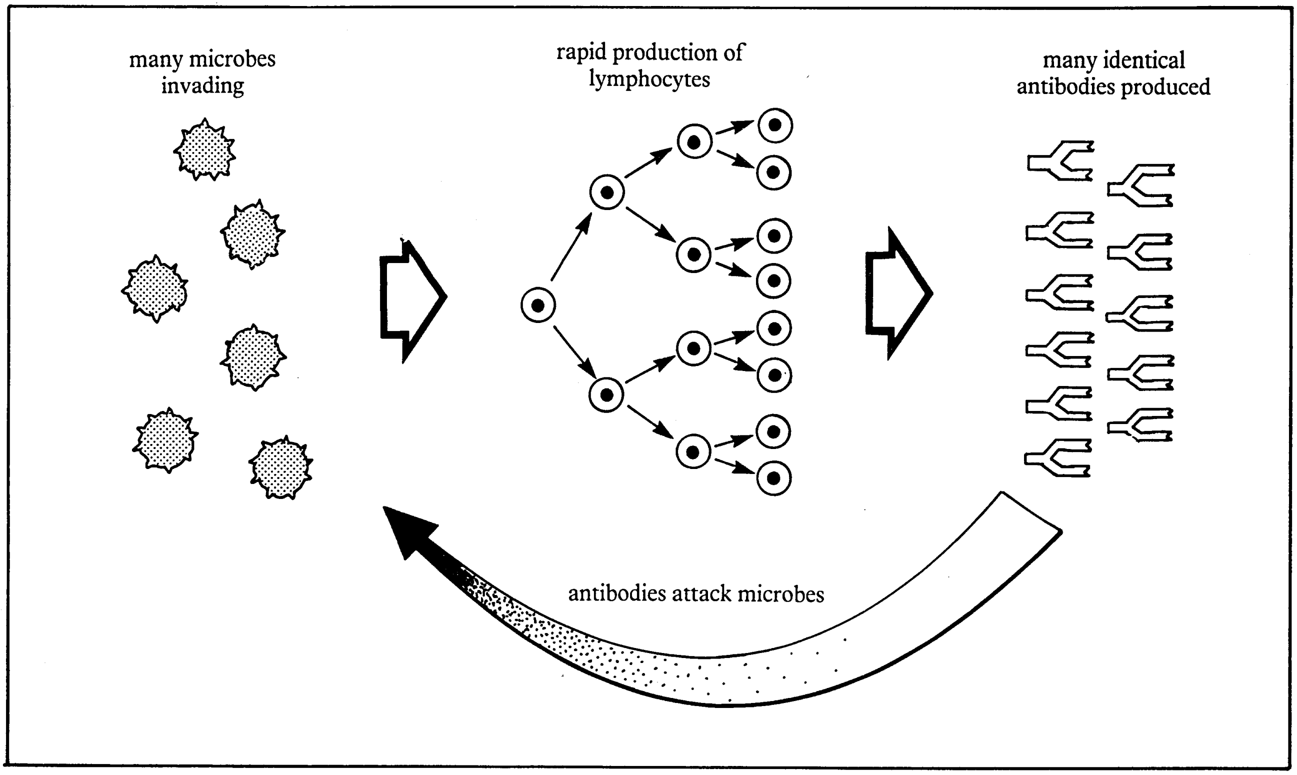

Figure 4 Manufacturing antibodies to fight invaders

Monoclonal antibodies

When large numbers of identical cells are produced they are called **clones.** When large numbers of *one type* of cell are produced, these cells are called **monoclonal.**

Scientists were interested in studying the antibody produced by a single type of lymphocyte. To do this, they had to persuade a lymphocyte to reproduce outside the body. It would then produce plenty of one type of antibody for them to study.

How are monoclonal antibodies produced in the laboratory?

Two Cambridge scientists, Cesar Milstein and George Kohler, were the first to produce monoclonal antibodies in the laboratory. In 1984 they received Nobel prizes for their work.

The big problem they had to overcome is that lymphocytes rapidly die outside the body. Milstein and Kohler had to persuade lymphocytes to go on living and reproducing in a test tube. To do this they used tumour cells. Tumours inside the body can be a serious problem and some tumours cause cancer. But tumour cells are able to go on living and reproducing outside the body. The scientists used them to make 'hybrid' cells. These 'hybrids' could produce antibodies like lymphocytes, but live outside the body like tumour cells.

Figure 5 illustrates the method.

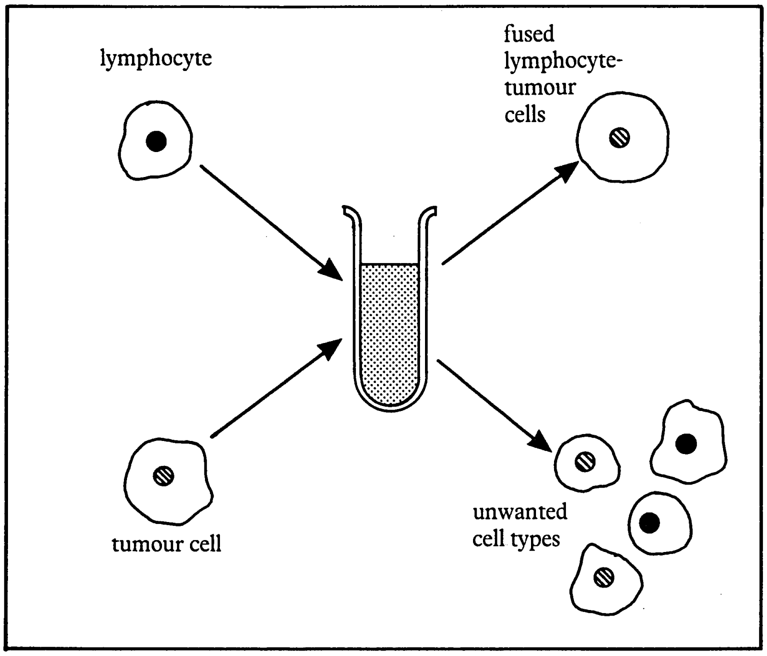

Figure 5 Making lymphocyte-tumour cells

They took lymphocytes from the body and mixed them with tumour cells in a test tube. They added a chemical to lower the surface tension, and gently shook the mixture. The lower surface tension allowed the cells to fuse (join together). Some of the lymphocytes fused with tumour cells. This 'hybrid' of lymphocyte and tumour cell was able to reproduce and live outside the body. All the scientists had to do then was separate the cells they wanted from the ones they did not. The 'hybrid' lymphocyte-tumour cells could then be used to make monoclonal antibodies.

Questions

4 Explain in your own words why hybrid tumour-lymphocyte cells were used instead of ordinary lymphocyte cells.

5 After shaking tumour cells and lymphocyte cells together, the scientists had to separate the cells they wanted from the cells they did not want. Which cells did they want? What unwanted cells would have been present?

Why the method was important

Milstein and Kohler originally made their monoclonal antibodies for scientific experiments. But it was soon clear that they would have important uses in medicine.

Antibodies work by recognizing a protein on the surface of a particular cell, and locking onto it. This means a particular cell can be made the 'target' of an antibody 'attack'. The antibody will attack that cell and no other.

Targeting drugs

For example, a drug could be attached to a monoclonal antibody. The antibody would be 'targeted' against proteins on a diseased cell. In this way the drug would be delivered to the diseased cell by the antibody (Figure 6). The drug would be concentrated in the area where it would do most good, instead of being spread throughout the body. This means that lower doses of the drug could be used.

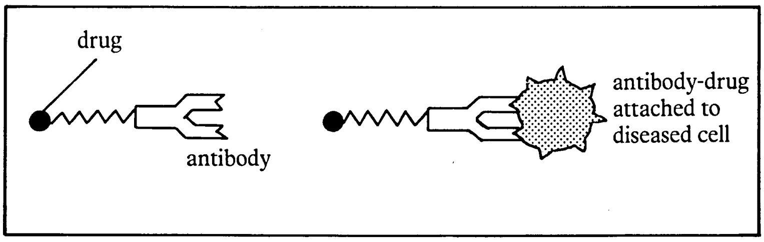

Figure 6 Targeting a drug using a monoclonal antibody

Purifying proteins

Monoclonal antibodies are not just used to destroy cells. They can be used to pick out useful substances from a mixture. Suppose you want to separate one protein from a mixture. An antibody is made which will pick out the required protein (Figure 7).

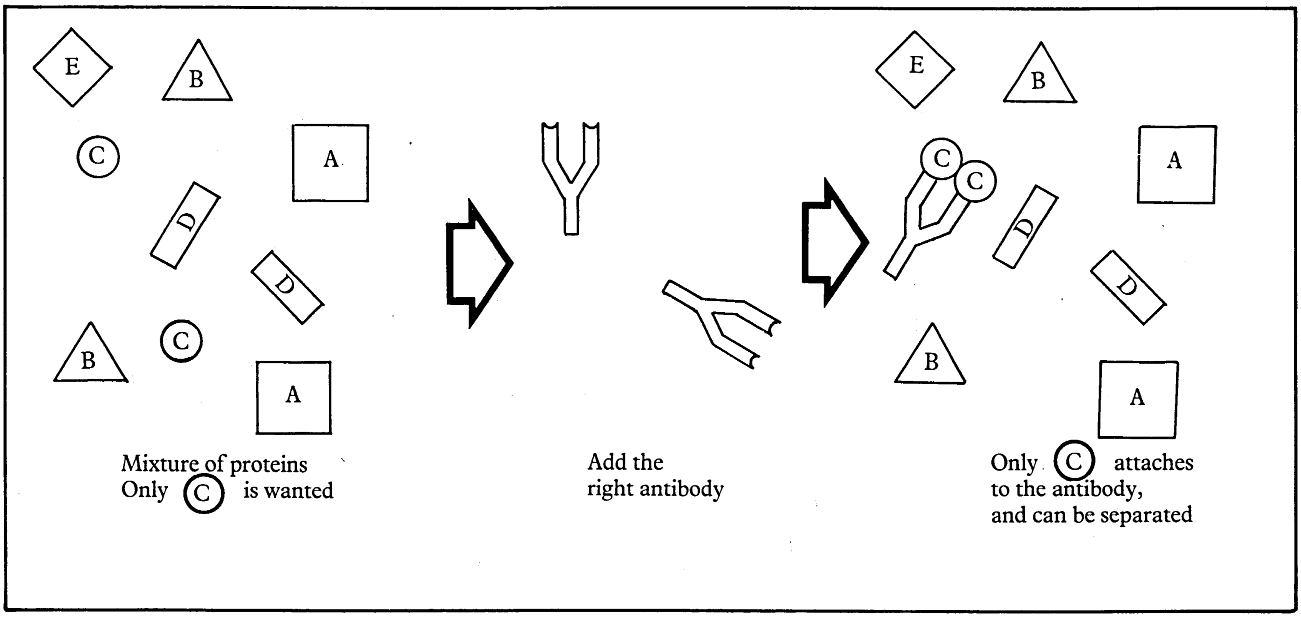

Figure 7 Using monoclonal antibodies to separate proteins

Detecting diseases

Kits can be produced which detect disease quickly and accurately. They contain monoclonal antibodies which react with antigens present on the microbe causing the disease. A solution containing the antibodies is added to a sample of the patient's blood or urine. If the microbes are present, the antibodies react with them. This in turn causes another chemical to change colour. The colour change indicates the patient has the disease.

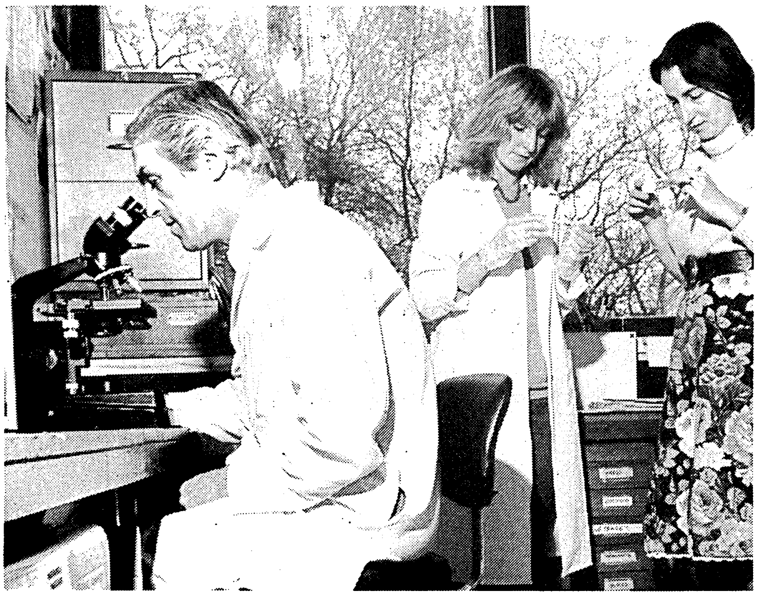

Figure 8 These research workers are examining lymphocyte-tumour hybrid cells

Solving problems using monoclonal antibodies

These are all real-life problems that have been solved using monoclonal antibodies. In each case, suggest a solution.

Problem 1 Pregnancy testing During pregnancy, certain proteins are released into the urine. How could monoclonal antibodies be used in a pregnancy testing kit?

Problem 2 Treating leukaemia Leukaemia sufferers have cancer cells in their bone marrow. These cells can be killed using a deadly poison called ricin. Unfortunately, ricin also kills normal bone marrow cells. How could monoclonal antibodies help?

Problem 3 Treating haemophiliacs Haemophiliacs have blood which does not clot. They risk bleeding to death when they cut themselves. This is because they lack a protein in their blood called Factor 8. Factor 8 is vital to the blood-clotting process, and haemophiliacs can be treated by giving them Factor 8. This can be done by giving them a transfusion of normal blood, which contains Factor 8. However, this means going to hospital, and there is always a chance that the blood given may contain harmful viruses, such as the virus that causes hepatitis. A better plan is to give haemophiliacs an injection of concentrated Factor 8. How could monoclonal antibodies be used to get pure Factor 8 from blood?

Problem 4 Finding cancers Before doctors can treat a cancerous growth, they need to know exactly where it is in the patient's body. Sometimes this is difficult to find out, even using X-rays. How could monoclonal antibodies help?