

How antibody attacks cells

Last week at the National Institute for Medical Research, Mill Hill, joint work with the Imperial Cancer Research Fund was demonstrated, in which electron microscope studies have revealed how an antibody breaks the cell membrane so that the cell dissolves

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AT the turn of the century, when the nature of immunity against bacteria was first studied, the French biologist, Bordet, injected sheep red blood cells into the veins of a rabbit. The fresh serum obtained from the clotted blood of the rabbit would dissolve sheep blood. This activity (called "haemolysis") would disappear on standing the serum or exposing it to mild heat; however, it could be restored by the addition of fresh serum from an un.injected, normal rabbit. It became apparent, then, that there were two factors in the serum of the injected rabbit that caused sheep blood to dissolve. One factor was stable to heat, and depended on the prior injection of the rabbit with sheep blood; this we call "antibody". The other was sensitive to heat, could be obtained from the serum of animals of many different species and was eventually named "complement" by Bordet. Later, guinea pig serum was used as the source of complement in the laboratory. The phenomenon of dissolving sheep blood by antibody and complement was termed "immune haemolysis".

In recent years, the nature of complement has been studied intensively by Mayer, Rapp, Borsos and their co-workers at the National Institutes of Health in Bethesda. The activation of complement by antibody stuck to red cells ("antigen-antibody complex") has turned out to be a very complicated affair, at least six different components

having been identified so far. Each component triggers off the activity of the next in line. However, haemolysis, or the dissolving of the red blood cells, does not occur until the last component has been activated.

Red blood cells are small, sealed bags carrying haemoglobin; their exteriors are completely encased by the cell membrane. It was thought that complement acted by damaging part of the cell membrane, allowing potassium to leak out and water to leak in, until the cell swelled and burst. Mayer's group postulated that the damage caused by one molecule of triggered-off complement (its components having been activated sequentially) ought to be sufficient to dissolve one red blood cell. The "one-hit theory" was difficult to prove because the rate of activation of each component was different, leading to the false impression that a multiplicity of separate events was required to damage the cell. By studying the rates of reaction of the components separately, Mayer's group established that one complement "hit" is sufficient to damage a red cell so as to make it dissolve (or lyse).

Our interest in immune haemolysis arose in a roundabout way. In 1962, my colleagues, Dougherty and Harris and I (at the Imperial Cancer Research Fund, Mill Hill) published electron micrographs showing holes in the surface of membranes of red cells that had been exposed to a solution of

saponin (a poison known to dissolve red cells). Dr John Humphrey, of the National Institute for Medical Research at Mill Hill, suggested that we look at the surface of the membrane of red cells haemolysed in a variety of ways, one of which was by antibody and complement. The photographs obtained seemed to fulfil the predictions of Mayer and his coworkers. We saw holes 80 to 100 angstroms in diameter on the surface of the cell membranes (100 million angstroms=1 centimetre). Each hole was surrounded by an elevated ring (Figure 1). These features were seen for the first time, probably because a technique of electron microscopy was applied which was hitherto used for looking at the surface of viruses. This technique, called "negative staining", involves making a print of the surface to be examined by layering it with a very dense salt such as potassium phosphotungstate.

When Dr Tibor Borsos, in Mayer's laboratory, was told about these holes he wondered whether the lesions seen on the cell membranes were really the damage caused by complement. In other words, were they holes or merely pits? Also, could we confirm the "one hit" theory by electron microscopy? Working at Mill Hill, Borsos first prepared a suspension of sheep red cells treated with large amounts of antibody and complement, from which the last component had been removed. This preparation did not dissolve the cells. On examining the cell membranes in the electron microscope, we did not see holes. However, when Borsos added the last component of complement to his mixture, the red cells lysed instantly, and their membranes showed thousands of what we could now call holes with certainty, since they were directly related to cell membrane damage.

In order to confirm the "one-hit" theory of complement action, Borsos prepared a suspension of sheep red cells treated with a large excess of antibody and a measured dose of complement: in one experiment, thirty times the amount necessary to lyse the cells was used. When counts were made of the holes in the cell membranes in the electron micrographs approximately thirty were found per cell, thus confirming the suggestion that each "hit" was represented by one hole.

After Dr Borsos returned happily to Bethesda, Dr Humphrey designed some experiments to study the role of anti-

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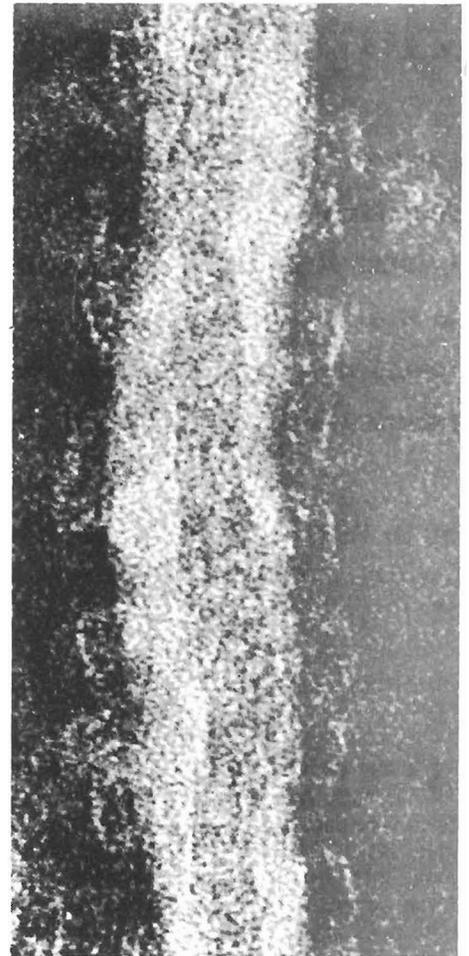
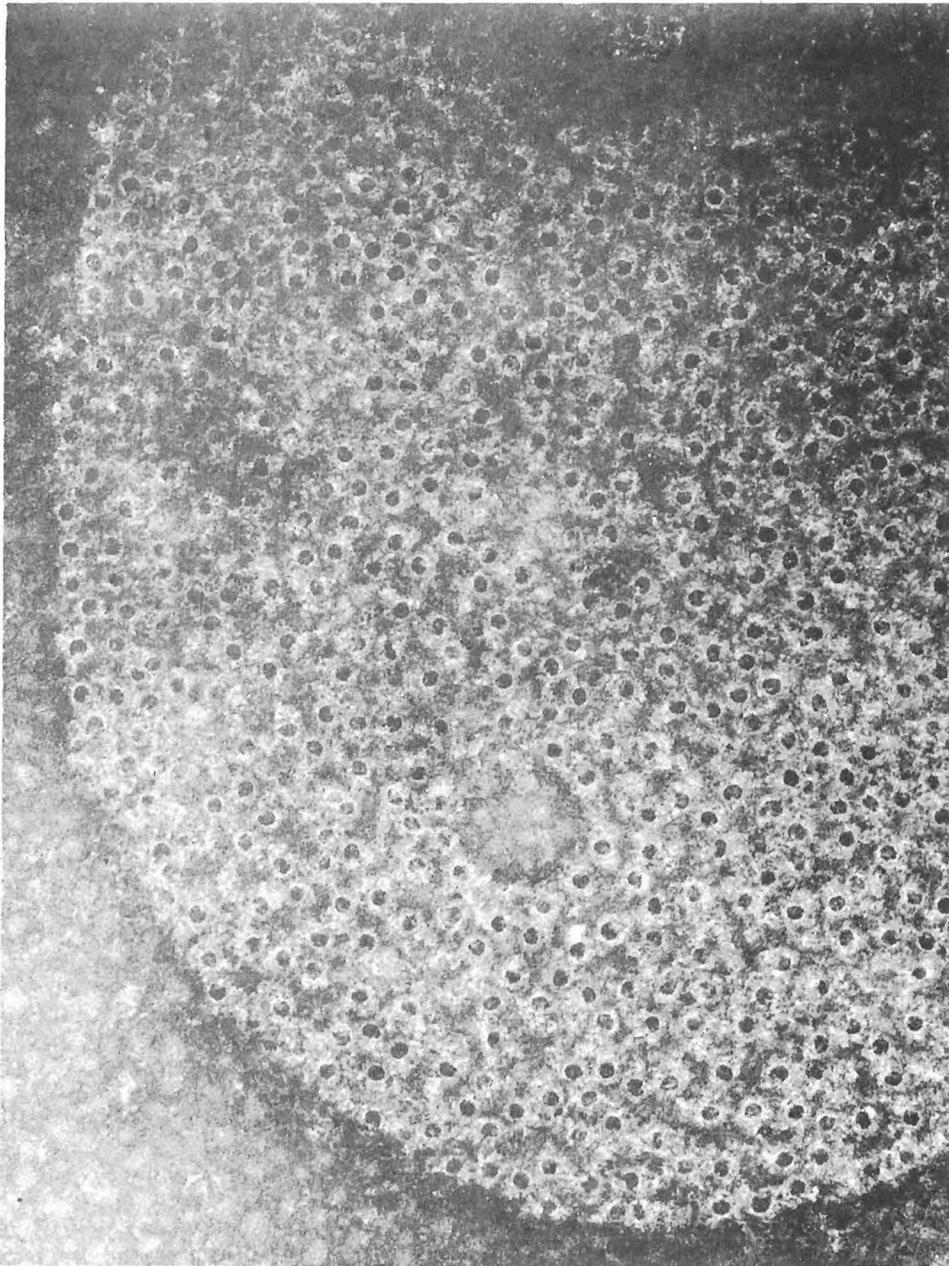


FIGURE 1. (Left) Red cell membrane after haemolysis by antibody and complement. Large numbers of holes, 80 to 100 angstroms in diameter, are seen as dark spots on the surface of the membrane (x 200 000)

FIGURE 2. (Above) 195 antibody attached to the surface of a piece of red cell membrane. The individual molecules appear as oblong structures lying on the membrane surface (x 640 000)

body in immune haemolysis. First of all, he purified the antibody in the serum of rabbits immunised against sheep red cells, by absorbing it with cells, collecting it from the cells with alkali, and further purifying it in the ultracentrifuge. Two separate types were obtained, labelled conveniently according to their rates of sedimentation as 195 (macroglobulin) and 75 (gamma-globulin). The 195 antibody, being the larger, was easily seen in electron micrographs when attached to bits of sheep cell membranes (Figure 2).

Dr Humphrey then labelled the antibodies with radioactive iodine, and, by using a measured amount of antibody and an excessive amount of complement, we were able to determine how many antibody molecules were neces-

sary to make one hole. The results for the two types of antibody were quite different. Not more than two, and probably only one, molecules of the 195 antibody was necessary for each hole, while 3000 to 6000 molecules of the 75 antibodies had to be applied to each cell to make a hole. Moreover, as more 75 antibody molecules were crowded on the cells, relatively fewer were necessary to make each hole. This result suggested that the formation of a hole depends on the chance juxtaposition of two or more 75 antibody molecules on the surface of the cell membrane. It is evident, then, that the two types of antibody are vastly different in their efficiency in dissolving red cells. This may be a clue as to their different functions.

This work may develop in several new ways. First of all, we are brought back to the observations of Bordet in 1902, and his German colleagues twenty years before. The action of complement was first noted by its killing effect on bacteria that had been treated with antibody. Are there similar holes in the membranes of bacteria killed in this way? What happens to the cells in organs or blood of individuals affected with diseases of "auto-immune" origin in which the body produces antibodies against its own tissues?—are their membranes damaged in the same way as in the test-tube experiments I have just described? In fact, can we tell where the antibody and complement have acted by looking at the damage they have caused?