The Institute of Biology's
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Immunobiology

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Preface

The immune system, as developed during the course of vertebrate evolution, is of crucial importance to the health of mankind. Its fascination, however, spreads far beyond the confines of medical science. In its capacity to distinguish between self and non-self it provides for the biologist a unique example of cellular recognition, reflecting a capacity which may be universal among living organisms and which has become adapted to serve many different functions. Vertebrate immunity also provides the cell biologist with insights into the regulation of cellular physiology and cellular interactions while the production of antibody serves as a model for protein synthesis in general. Elsewhere in biology, antibodies themselves have proved a useful if not essential tool in taxonomy, histochemistry and in radioimmunoassays for blood concentrations of hormones and other molecules.

The purpose of this book is to provide a brief survey of vertebrate immune mechanisms, setting them in their wider biological context. Because immunological research is moving very rapidly, new questions are raised with every old question that is answered and, rather than indulge in too much speculation, some arguments are left open-ended in the text. Inevitably, also, some aspects of immunology will be left out, particularly since the bias is biological rather than clinical. Nevertheless it is hoped that this introduction will be useful not only to biologists but also to medical students who wish to understand the fundamentals of the subject.

I should like to record my thanks to my colleague, Dr H. S. Micklem, for his help in the preparation of this book.

Edinburgh, 1980

C.J.I.

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1 Immunological Strategy

1.1 Phagocytic cells and immunity

All multicellular animals are equipped with defensive mechanisms which afford protection against invasion of the body by micro-organisms or other parasites. In the simplest Metazoa, these defences may depend almost entirely on phagocytic cells, whose major function is to engulf and digest foreign organisms or their harmful secretions. On the other hand, in higher animals, complex immune systems are found which involve a variety of cell types together with specialized organs such as the vertebrate spleen, bone marrow, thymus and liver. Despite the evolution of these additional mechanisms, however, phagocytes remain a fundamental means of defence throughout the Metazoa as well as providing an immunological link between the most lowly and the most advanced groups. While their role in immunity has become augmented during the course of evolution, mammalian phagocytes still share with those of the sponges and the coelenterates the basic properties of ingestion and digestion of foreign or unwanted cells, particles or molecules.

Phagocytic cells provide what has come to be known as *natural* or *non-specific* immunity. In the first demonstration of immunity in action at the cellular level, this particular system was discovered by the Russian biologist Metchnikoff in the 1880s, when he noticed that small splinters, implanted into molluscs and other invertebrates, were surrounded and engulfed by wandering amoeboid cells. Among the mammals, non-specific immunity is provided by two families of phagocytic cells which are related in that they both derive, in adults, from stem cells in the bone marrow. The first of these groups is comprised of motile, blood-borne *monocytes* (Fig. 1–1) and relatively sessile *macrophages* which are found in organs such as the lungs, spleen and liver. The second category also consists of blood-borne cells known as *granulocytes*, or sometimes as polymorphonuclear leukocytes (often shortened to polymorphs), the former name owing its origin to the prominent cytoplasmic inclusions which are characteristic of this class (Fig. 1–1). On the basis of their staining characteristics, granulocytes are further divided into neutrophils (the most frequent), eosinophils and basophils. The roles of these two families of phagocyte in immunity are often interlinked. For instance, if a localized infection becomes established in the skin, granulocytes quickly find their way to the spot and engulf large numbers

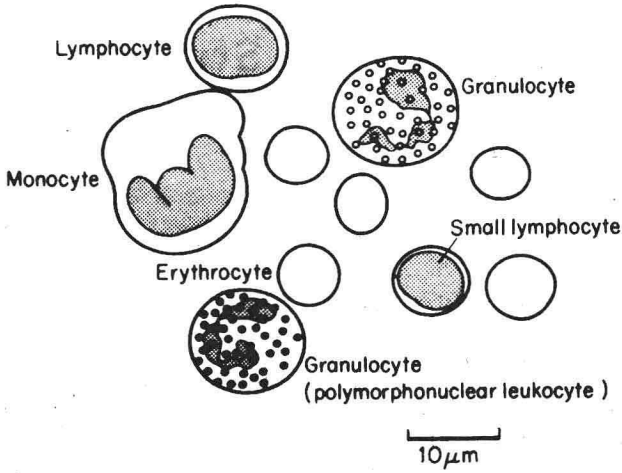

Fig. 1–1 Diagram to show the types of leukocyte (white blood cells) involved in immune mechanisms in mammals. Some red cells are given for comparison. Normally they are far more numerous, about 5×10^6 mm^{-3} compared with 5×10^3 mm^{-3} for leukocytes.

of bacteria, often succumbing in the process and forming pus. At a later stage, monocytes enter the lesion and engulf and digest both surviving bacteria and the remains of other cells, a sort of mopping-up operation which limits tissue damage and brings the infection to a halt.

The term 'non-specific immunity' in this context derives from the fact that phagocytic cells show no inherent specificity for foreign material. Any phagocyte is potentially able to recognize and respond to any foreign cell or particle. This characteristic stands in contrast to the high degree of specificity which is associated with those cells, the *lymphocytes*, which mediate the other major category of immunity, *acquired immunity*.

1.2 Lymphocytes

Lymphocytes are small to medium-sized cells, often with very small quantities of cytoplasm (Fig. 1–1), which are found in the blood stream and the lymphoid tissues of the body (see Chapter 4 for a detailed discussion of their characteristics and behaviour). Like phagocytes they are derived in mammals from stem cells in the bone marrow. The cellular

basis of acquired immunity has been exhaustively investigated among the vertebrates, since it is characteristic of this group of animals. However, immune mechanisms which show certain similarities to vertebrate acquired immunity have been discovered in some invertebrate phyla, and it remains of great interest to determine whether lymphocytes have an evolutionary history which extends back into the protochordates and beyond; and, indeed, whether any analogue of lymphocytes exists in protostomate invertebrates such as the annelids or arthropods.

The functional feature which best distinguishes lymphocytes from phagocytes is the fact that each lymphocyte normally responds to one foreign agent, or *antigen*, only. Hence the alternative term, *specific immunity*. The complete spectrum of specific immune responses in vertebrates is due to the provision of a large population of lymphocytes, only a small proportion of which will react to any one antigen. It is also a property of lymphocytes that having once encountered an antigen, they or their descendants are capable of a more rapid and more efficient *secondary response* if they encounter it a second time. The system of specific immunity thus exhibits immunological memory.

The characteristics of immunological memory have been recognized since the time of the Greeks, when it was appreciated that individuals who recovered from smallpox were unlikely to suffer again from the disease. Crude immunization procedures against smallpox were current in the seventeenth century, and in the late eighteenth century Jenner showed that immunization with the agent causing benign cowpox (the virus *Vaccinia*) protected against the more virulent, but antigenically related, smallpox (*Variola*). The property of immunological memory is the basis of all immunization procedures against bacterial and viral infections in man and domestic animals.

1.3 Antibodies and graft rejection: two sorts of immunity

Acquired immunity in vertebrates takes two distinct forms (Fig. 1–2), and it is now known that these are mediated by separate families of cell, commonly known as B- and T-lymphocytes (see Chapters 4 and 5). The first of these systems is *humoral immunity* in which B-lymphocytes, stimulated by contact with an antigen, produce daughter cells which secrete proteins with the capacity to bind specifically to that antigen. These proteins are called *antibodies*. Their production can be initiated by viruses, bacteria, or bacterial secretions such as tetanus or diphtheria toxins. The earliest clear demonstration of the presence of blood-borne (humoral) factors capable of protecting against disease was in 1891 when von Behring injected a young diphtheria patient with serum from

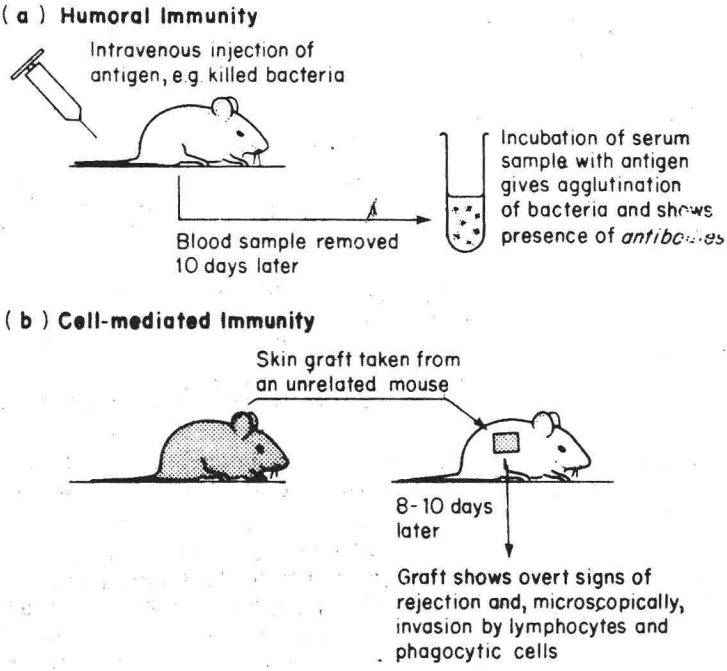

Fig. 1–2 Two sorts of acquired immunity. Humoral immunity involves production of antibodies by lymphocytes and is the typical response to systemic infections or inoculation with bacteria, viruses of foreign proteins. Cell-mediated immunity involves the direct destruction of foreign grafts, but the same mechanism may be activated following infection by intracellular parasites, or in some allergies.

an immunized sheep. The child recovered, and von Behring was awarded the first Nobel Prize for Medicine.

On the other hand, certain immune responses can occur which, although specific, do not involve antibodies. This is true, for instance, of the rejection of transplants of some foreign tissues such as skin; here, lymphocytes can kill cells of the graft by direct contact. Although antibodies *may* be produced at the same time, they are not necessary for graft rejection. As a consequence, this form of response is known as *cell-mediated immunity*. As well as graft rejection it also embraces certain hypersensitivity reactions (allergies), and the response to some intracellular parasites such as the tubercle bacillus.

Both humoral and cell-mediated types of immunity exhibit the specificity and generation of memory which are characteristic of

acquired immunity. Thus a secondary antibody response is usually characterized by more rapid synthesis of antibody, and, frequently, higher antibody concentrations in the blood, while a second skin graft is rejected more rapidly than a previous one from the same donor.

1.4 Relationship of natural and acquired immunity

Although natural and acquired forms of immunity rely primarily on different cell populations in the vertebrates, a close working relationship has become established between them. For instance, a major biological function of anti-bacterial antibodies is to improve the rate of phagocytosis of bacteria, particularly those such as species of *Pneumococcus* whose polysaccharide coat renders them resistant to ingestion by phagocytes. This function has been aided by the evolution of receptor sites within the macrophage plasma membrane. These can either bind antibodies directly or an ancillary molecule, one of a series of serum proteins which are activated following antigen–antibody binding. A summary of these interactions, and their role in phagocytosis is given in the next chapter, in Fig. 2–2. Other interactions between phagocytes and lymphocytes will be dealt with at a later stage.

The two cell populations, phagocyte and lymphocyte, also have a common feature in that they are able to distinguish what is antigenic or foreign: to tell 'self' from 'non-self'. A sensitive recognition system is absolutely fundamental to both types of immunity. Although, as will be seen, the molecular mechanisms for recognition differ between the two cell types, they probably both represent adaptations of a feature which is essential for successful multicellular life – a system of cell surface molecules which allows recognition and interaction between cells and hence the proper organization of the body into tissues and organs.

2 Evolution of Recognition Mechanisms

2.1 Cell interactions and recognition mechanisms

Recognition of foreign-ness is a cellular characteristic of most living organisms, and is certainly not confined to the immune systems of higher animals. The process of fertilization, for instance, well illustrates the need for cellular recognition mechanisms, and these may be found to operate before gamete fusion in both plants and animals. Among the angiosperms molecular mechanisms have been discovered which prevent both interspecific fertilization and, in some families, self-fertilization; in both instances pollen tube development is inhibited or restricted as a consequence of some recognition event (see HESLOP-HARRISON, 1978). Similar mechanisms operate during mating fusions in Protozoa such as *Paramecium*, and are equally important in metazoan species such as *Hydra* or *Nereis* where gametes are simply shed into the aquatic surroundings, and must be able to 'distinguish' those of the same species from the great variety of gametes which they may encounter. Even in higher animals where internal fertilization and behavioural isolating mechanisms serve to prevent interspecific gamete contact, the molecular mechanisms of gamete recognition still persist.

In a similar way, cellular recognition mechanisms are of great importance in all multicellular animals in determining the orderly development and arrangement of tissues and organs. This is particularly true during embryogenesis, where a great deal of cell movement and redistribution occurs, but it also applies during adult life, and lymphocytes themselves are a good example of cells whose re-circulation and tissue distribution are both carefully regulated (see section 4.5). The proper organization of the body, which can easily be taken for granted, would be most unlikely without the specific interactions which determine cell behaviour.

Interactions of this sort, at the level of cell–cell contact, have been best worked out in embryonic systems, and one of the more interesting results shows a parallel between the behaviour of dissociated tissues from vertebrate embryos (usually the chick) and the behaviour of dissociated sponge cells. Sponges are a sort of half-way house between colonial and true multicellular organization, and represent an evolutionary byway, but if their tissues are dissociated they show the ability to reform by reaggregation of the isolated cells. It has long been

established that the reaggregation is species specific, and that if cell suspensions from two separate species are mixed, then the cells sort themselves out according to their original colonies. Within each colony there is also some sorting out according to cell type. Likewise, with the chick, if two embryonic tissues (such as liver and neural retina) are dissociated and their cells then mixed, reaggregation takes place, but also sorting out, so that the reforming complexes are specific for one or another of the tissues involved (Fig. 2–1).

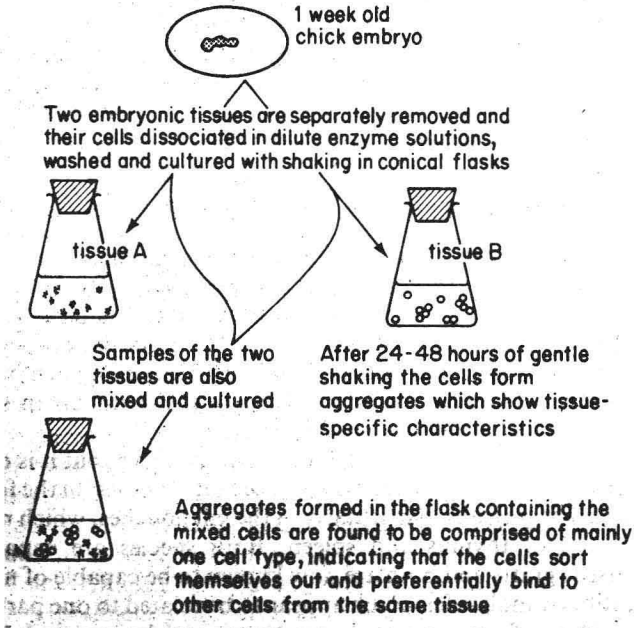

Fig. 2–1 Reaggregation and sorting out of mixed-tissue suspensions from chick embryos. Tissues used in these studies have included liver, retina, kidney, skin and limb-bud.

Reaggregation in both sponge and chick embryo systems is promoted by soluble factors which bind to tissue-specific receptor sites on the cell surfaces. There is not complete agreement at present as to the molecular nature of the factors involved, but it is clear that they have the common property of allowing 'like' cells to bind together while excluding 'unlike' cells. Although certain tissues are particularly amenable to this sort of

experiment, it is not unreasonable to suggest that the integrity of many different tissues is due to this type of mechanism.

Whatever the details of the mechanisms involved, it is apparent that, from the earliest multicellular animals, cells have possessed surface molecules with the capacity to bind to ligands (either molecules or other cells), and to respond to this interaction in a specific way. With the evolution of more complex systems these mechanisms have multiplied to form, for instance, the basis of many endocrine systems (those involving polypeptide hormones), neurochemical transmission at synapses or neuro-muscular junctions, host-parasite specificities and so on. It is against this sort of background that the evolution of immune systems must be seen, and it should be said that a number of theoretical attempts have been made to explain the origins of immune recognition, particularly with respect to lymphocytes, in terms of pre-existing cell surface molecules with a tissue-recognition function. From these, receptors for foreign antigens might have evolved.

2.2 Evolutionary origins of vertebrate immunity

Mechanisms of acquired immunity can be traced back through the vertebrate line to the most primitive group, the jawless fish of the class Agnatha, represented in modern times only by the hagfish and the lamprey. In these animals we apparently see the beginnings of a system which increases in complexity throughout vertebrate evolution (see, for instance, section 4.4) and which gives rise to the mammalian system described in outline in the last chapter.

Lymphocytes are found in both hagfish and lamprey, but it is only in the latter species that these cells are organized into 'tissues' in the form of gut associated aggregates, particularly in the gill pouches, which may be the precursors of thymus and spleen. Both species are capable of rejecting tissue grafts and both are now known to be capable of making specific antibody molecules which appear to be related to one particular class of antibody found in jawed vertebrates and known as IgM (see Chapter 3). However antibody production in the hagfish in particular is not the physiologically straightforward affair that it is in mammals since the animal must be maintained at the near-lethal temperature of 18°C and repeatedly immunized before antibody molecules appear. It is difficult to see the adaptive advantage of a defence system which only comes into play at quite unnatural temperatures, quite apart from the means by which it could have been selected in the evolutionary sense. As a consequence some workers have suggested that the mechanisms of graft rejection preceded that by which antibodies are manufactured and secreted.

Although graft rejection is a feature of some invertebrate groups, the

Agnatha represent a true turning point in the evolution of immune mechanisms. While, in their possession of lymphocytes and antibodies, they foreshadow the more complex systems of higher vertebrates, they also show a variety of non-specific mechanisms which are more characteristic of the non-chordate phyla and to which we should briefly turn our attention.

2.3 Mechanisms of invertebrate immunity

As was indicated in Chapter 1, the characteristic immune effector cell in invertebrate phyla is the phagocyte, often referred to in this context as a haemocyte or an amoebocyte. This cell shows two characteristic responses to foreign material, phagocytosis and encapsulation, and together these provide an efficient defence against micro-organisms or their secretions and against invasion by metazoan parasites.

Phagocytosis proceeds in a similar manner to that shown by vertebrate phagocytes, and depends first on adhesion, for example of a bacterium to the cell membrane. While phagocytic cells can bind micro-organisms directly, particularly by interactions between carbohydrate chains on the two cell membranes, soluble factors known as *opsonins* are found in both vertebrates and higher invertebrates, and characteristically these promote adhesion and subsequent ingestion.

Among the vertebrates, some classes of antibody molecule and one of the components of the complement system (see section 3.5) are especially effective as opsonins, partly because some phagocytes have evolved specific cell membrane receptors for these molecules (Fig. 2–2). Factors which serve the same function have been identified in the body fluids of molluscs and arthropods in particular, but chemically these molecules seem to bear little relationship to their vertebrate counterparts. They are usually non-inducible substances, found in the body fluids of normal (un-immunized) animals, protein in nature but of variable size. Although they are clearly not antibodies, they frequently show the experimental property of being able to agglutinate bacteria or vertebrate red blood cells, sometimes with a high degree of specificity. The adhesiveness by which these natural agglutinins bind to recognized sites on the surface of, say, bacteria is undoubtedly of functional importance; for example, the mollusc *Aplysia* (the sea hare) is able to clear from its body fluids by phagocytosis those species of bacteria for which it possesses natural agglutinins, while bacteria for which no agglutinins exist can survive in the circulation.

It is clear that in some situations opsonins such as these are important for effective phagocytosis. However, experiments in which phagocytes are cultured in artificial media have shown that, for many organisms, opsonins added to the culture enhance phagocytic activity, but are not

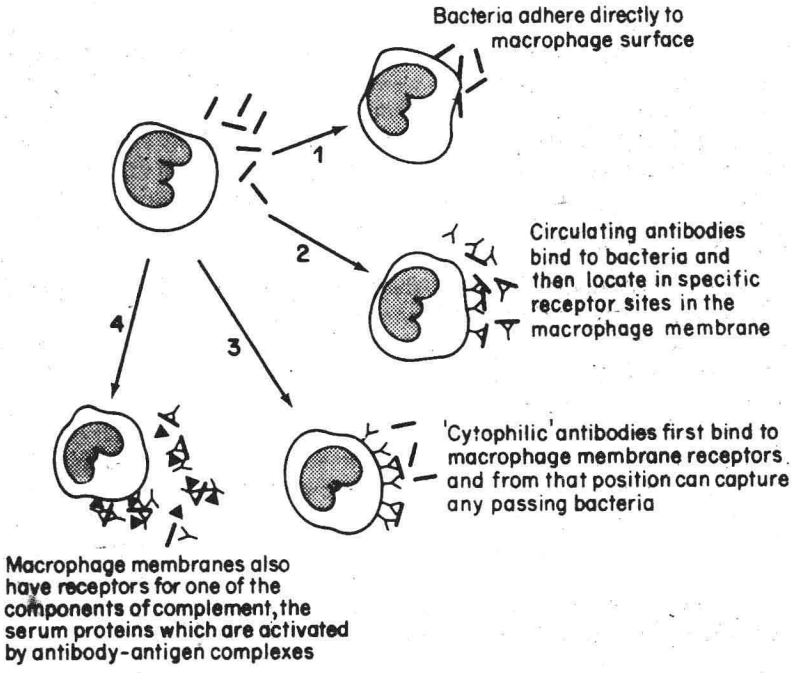

Fig. 2–2 Phagocytosis and opsonins in vertebrates. Macrophage membranes contain a number of receptor sites for molecules whose attachment to antigen thus improves phagocytosis. Not all antibodies are cytophilic or can fix complement, but a straightforward coat of antibody can often neutralize strong electrostatic charges on bacterial surfaces and make them more easily ingested.

essential for it. These observations agree with the evolutionary appearance of phagocytic cells in groups such as the sponges, before the development of vascular systems and of body fluids.

A further interesting feature of phagocytic activity in many invertebrate groups is the expulsion from the body of cells carrying ingested material. Thus in sponges, erythrocyte-laden amoebocytes have been observed to migrate to the excurrent canals, while in molluscs, micro-organisms may be removed by migration of phagocytes across epithelia to the exterior, as well as by normal intracellular digestion.

The second characteristic defence activity of invertebrate phagocytes is encapsulation, a response which may follow invasion by parasites, but which may also be experimentally induced by transplantation, for

example, of nervous tissue or even small lengths of nylon into the haemocoel of insects. Encapsulation starts as a clustering of haemocytes around the foreign organism or tissue, but the cells eventually die, the tissue mass becoming first fibrous and then calcified. Isolation of the enclosed material thus becomes complete.

It is evident then that the invertebrate phyla have a well developed and versatile defense system based on phagocytic cells, but which may receive support from blood-borne opsonins in the higher groups. As in vertebrates, phagocytes are also responsible for the ingestion and removal of old or unwanted 'self' tissues. This is seen, for instance, in the insects during metamorphosis, but it is also a feature of lower groups and is found even in freshwater sponges where the flagellated cells of the free-swimming larva are phagocytosed when it adopts a sessile way of life. With the exception of encapsulation the general properties of phagocytic cells extend throughout the animal kingdom and, by and large, the system in invertebrates is as capable of discriminating between self and unwanted material as are the phagocytes of vertebrates.

2.4 Acquired responses in invertebrates

In addition to the mechanisms described above, a number of inducible or acquired responses have been identified among the invertebrates and some of these show features which are typically associated with lymphocyte-mediated mechanisms in vertebrates, for example, some sort of memory. The evolutionary relationship between these invertebrate forms of immunity and specific immunity in vertebrates is a biological question of considerable interest. However it must be remembered that the animal family tree split at a very early stage into two major lines, the deuterostomes, of which the echinoderms and the chordates are the best known phyla, and the protostomes which contain the molluscs, annelids and arthropods among other groups. There is only the most distant relationship then between higher invertebrates, as represented by insects, crustacea and arachnids, and the various vertebrate groups. Any similarities in their immune systems must either be due to the retention in both lines of primitive but worthwhile features, as seems to be the case with phagocytic cells, or to the evolution on more than one occasion of mechanisms which serve the same function. Taking the animal kingdom as a whole, this has certainly been the case with flight, air-breathing, and with the development of the segmented body plan which we see in chordates on the one hand and annelids and arthropods on the other. It may equally be true of the graft-rejection mechanisms which these same groups display.

Although molecules similar to vertebrate antibodies have not been isolated from the body fluids of invertebrates, a number of humoral

factors have been discovered which have a role in immunity, including the natural agglutinins mentioned above. While the agglutinins are generally not inducible, others of these substances are. However close investigation shows a number of important differences between their induction and antibody synthesis.

One of the best studied molecules in this respect is a factor which was first identified after infection of the insect *Oncopiltus fasciatus* (the milk weed bug) with cultures of the bacterium *Pseudomonas aeruginosa*. It was found that a bacteriolytic agent was rapidly released into the haemolymph, its appearance after only four hours standing in marked contrast to the relatively slower rate of antibody production in vertebrates and suggesting that cell division (see section 4.6) was not involved. Although this factor, once isolated, could be used to transfer protection to non-infected insects, it showed a further distinction from antibody molecules in the fact that it was secreted as promptly after mechanical damage as after exposure to bacterial infection.

Factors with similar properties have been identified in other groups, and in the lobster, for example, a specific memory component has been demonstrated. This more sophisticated response may have evolved from a generalized reaction to physiological trauma in the broad sense. However the actual bases of memory and of the specificity by which bacterial lysis is achieved (but not, presumably, lysis of the host's own cells) remain to be explained.

The invertebrate response which shows the closest relationship to its vertebrate counterpart is that of graft rejection, and the ability to recognize and respond to foreign tissue grafts has proved to be very widespread among the invertebrate phyla, including more lowly groups such as the Coelenterata. It is therefore a phenomenon which is associated, in the evolutionary sense, with the whole history of multicellular life and with the sort of tissue interaction and recognition mechanisms which were discussed earlier. However it should be made clear that graft rejection in the more lowly groups is often a sluggish affair and also that the situation in coelenterates differs from that seen in vertebrates in one important respect; no memory component has yet been demonstrated. On the other hand, rejection responses in earthworms, which have been particularly well studied, result in the rapid destruction of primary grafts when tissue is exchanged between worms of different genera within the family Lumbricidae, and clear evidence of specific memory (Fig. 2–3). The response in worms is less rapid when grafts are exchanged between different individuals within a single species, and the incidence of successful rejection may not exceed 15%. This is in contrast to the situation in man, where intraspecific grafts (heart, kidney or skin transplants) are invariably rejected with great speed unless most careful steps are taken to 'tissue match' donor