

Friend or Foe?

The Biological Discrimination

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Living creatures touch each other, and touch themselves. This applies even at the cellular level when two cells reach such proximity that the molecules on the surface of the cell membrane can meet. Certain protein molecules in the cell membrane are specialized “feelers” possessing the ability, often very selectively, to form a close spatial connection with other molecules outside the cell, especially when the latter are presented on the surface of a neighbouring cell. Such specialized molecular tactile tools are often termed “receptors” which precisely describes their general function: to “receive” particular molecules which approach sufficiently near to the cell for close spatial contact to be established. On the other hand, the term receptors is non-committal as regards the more specific chemical structure of both the receptor molecule itself and the molecule it attaches to and for which it is said to have affinity. The phenomena which I shall discuss here belong for the most part to immunology, where we are now familiar with some of the cellular receptors, while others are as yet almost – or totally – unknown as regards their structure.

Meanwhile, let us first consider the theoretical possibilities of the membrane molecules establishing such a close spatial contact. Two main possibilities must be considered: namely identity and complementarity (this is very simply illustrated in Fig. 1). In everyday language, the identity principle can be compared to a stack of soup plates which can easily be placed because they are identical, while the complementarity principle is analogous to that

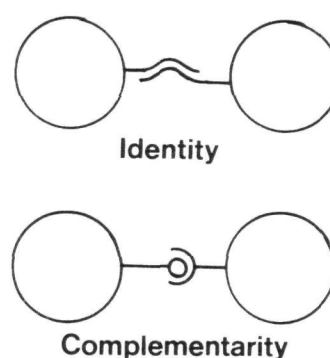


Fig. 1. Schematic illustration of the means by which two cells recognize each other with the aid of either identical or complementary molecules of the cell membranes.

of the lock and key, where the receptor molecule acts as the lock. (The lock and key simile is an illustration which has served biologists in many contexts in the hundred years since it was first used by Emil Fischer to describe the specificity of an enzyme to its substrate).

Whether Nature, in its inventive experimentation with the possible, has ever used the principle of the soup plate in the communication between cells and their surroundings, I do not know. It is a possibility that has been discussed, particularly in connection with the processes that hold identical cells together in colonies – low life forms such as marine fungi, or in the organs of more developed animals. On the other hand, the complementarity principle is known with certainty to function, and in the following I shall discuss, among other things, two quite different categories of protein molecules which utilize, with great efficiency, this principle in various receptor functions: namely antibodies and lectins.

Whatever their precise construction, these molecules – known as receptor molecules – are believed to establish their contact with other molecules by means of so-called “weak physical forces”. This is very different from covalent binding which links together amino acids in a protein molecule. The “weak physical forces” are operative only over very short distances. Hence key and lock must make a close fit.

The immune system

“The immune system defends the organism against invasion by ‘enemies’, among which can be included pathogenic bacteria and viruses. Such infections, as well as organized immunization against them, lead to the formation of antibodies”.

I consider that this very brief characterization of the immune system more or less describes what most people think they know about it, and thus it is fortunate that the description is not exactly erroneous – albeit most inadequate.

Firstly, antibodies are not the only (and not even the most important) means with which the immune system fights infection. Immune cells form the most important line of defence, and only some of these produce antibodies.

Secondly, both the formation of immune cells and of antibodies are reactions confined to vertebrates. Invertebrates have developed alternative defence mechanisms.

Thirdly, the cellular and biochemical processes which we lump together under the heading “immune response” or “immune reaction” are by no means directed against “enemies” only. Many people are hypersensitive (allergic) to their dearest pets, or to pollen from flowering trees and grasses. Others suffer from so-called autoimmune diseases which have this in common: that an immune response to the organism’s own tissue components is developed. Some females react to pregnancy by forming antibodies to their own foetus – in extreme cases causing its death. Practically everyone (with the sole exception of unizygotic twins) reacts immunologically to cells, tissues or organs transplanted for therapeutic purposes. All these are as valid examples of the immune

response as is the development of immunity to measles and smallpox.

Fourthly, the immune system has a peculiar relationship to enemies and friends. In a sense, enemies are only recognized when introduced by friends; I admit this sounds a little cryptic, but I will explain later.

The dramatis personae in the immune response

The leading role in every specific immune response is performed by the lymphocyte. These cells are found everywhere in the body, but they are especially numerous in the so-called lymphoid organs, which I will briefly describe.

Fig. 2 schematically illustrates the lymphoid organs of a chicken (immunologically, birds are more symmetrically structured than mammals). The positions of four organs are shown, namely bone marrow, thymus, bursa Fabricii and spleen. These organs are part of a fixed sequence in the development and life of the lymphocyte. Its life starts in the bone marrow, where a multitude of diverse cell types proliferate – among them the stem cells of the future lymphocytes. The stem cell, or perhaps a slightly more advanced form, is carried by the blood to the next station, which is either the thymus or the bursa. These two organs are functionally parallel, although different. They could be described as lymphocyte schools, each

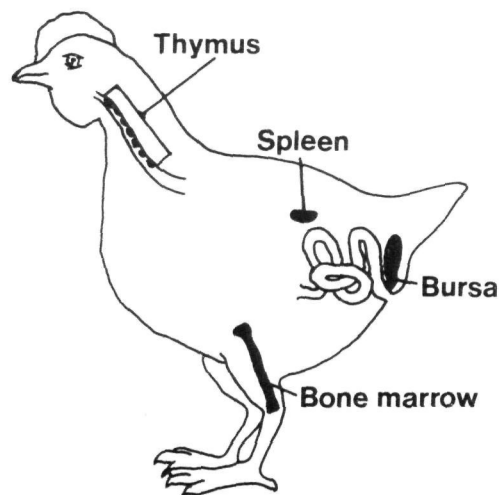


Fig. 2. Sites of lymphoid organs in a bird: bone marrow, thymus, bursa Fabricii, and spleen.

educating its separate kind of pupil. For the sake of symmetry and the human memory it was decided to call the product of the thymus the T lymphocytes and that from the bursa the B lymphocytes. Thus the most basic classification of lymphocytes is represented by the T and B forms which, although morphologically identical, are functionally very different.

The newly formed T and B lymphocytes exit via the blood and may reside temporarily in the spleen which, in all vertebrates, is the body's largest single depository of fully developed lymphocytes of both categories. In mammals (which lack bursa Fabricii) there exist large numbers of lymph nodes, which can be considered as being coordinated with the spleen. Also lymph nodes are meeting places for T and B lymphocytes, as well as other cells types of which I shall only name the supposedly most important, the macrophage, whose precursors are, incidentally, also formed in the bone marrow.

It is important to appreciate that all lymphoid organs are stages in the life-story of individual lymphocytes. Both the spleen and lymph nodes are involved in a perpetual process of exchange of their lymphocytes with those of the blood.

A considerable proportion of the blood lymphocytes, both T and B, in their passage through spleen and lymph nodes, leave the blood vessels through specialized areas in the venous system (high-endothelial venules) and travel via partly unknown routes through the organized structures of these organs. This journey, which averages several hours' duration, returns the lymphocyte to the blood circulation, directly in the case of the spleen, or indirectly via afferent lymph ducts in the case of lymph nodes, whence it embarks upon a new journey through the same, or some other lymphoid organ. The back-and-forth process, which may be repeated numerous times, is referred to as lymphocyte recirculation. What is its function?

Division of labour in lymphocytes

In all probability recirculation must be seen in connection with the fact that lymphocytes em-

ploy division of labour. They differ as to their supply of receptors, and this applies to both T and B lymphocytes. These two main categories can in fact be divided into thousands, or more likely millions of qualitatively different subgroups, each with its own receptor molecules. Lymphocytes of the same subgroups (also called clones) are, as far as is known identical in that they contain identical receptor molecules in the cell membrane, which differ from similar receptor molecules in all other clones in the same individual. Let us assume that a person has approximately 2×10^{12} lymphocytes more or less equally divided between T and B lymphocytes. Let us further assume that in each category these can be divided into 10^6 (one million) different clones. Thus each clone will on average contain 10^6 lymphocytes which taken together weigh approximately 1 mg and occupy the volume of a pin head. Let us imagine that a clone representing this tiny amount of tissue were to be permanently localized to a single lymph node, e.g. on an arm, and that this particular clone specialized in combatting a bacterium such as cholera bacillus which infects the intestine. This would not be very ingenious and nature has experimented too well and for too long make do with such an inappropriate model. An alternative model, although also unsatisfactory, would be to distribute all clone members more or less randomly between all the lymphoid organs and let them remain in place, just as liver cells keep to the liver and brain cells to the brain, existing alongside the same neighbouring cells throughout their lifetime. This would entail that no matter which pathway the bacteria chose, the most effective defenders would be too few in number to do anything in the first phase.

On the other hand, recirculation of lymphocytes is an extremely effective way of organizing a defence which can exploit the lymphocytes' clonally distributed reactivity. The most effective clones can thus be rapidly mobilised via the blood and lymph vessels to meet any danger, no matter where in the organism members of these clones may primarily be found.

B lymphocytes and antibodies

Antibodies, or immunoglobulins (Ig), have been recognized for almost 100 years as serum proteins with specific ability to react against foreign and often harmful substances, e.g. bacterial toxins. However, it is only in the last 20 years that we have reached a deeper understanding of these interesting and important molecules' structure, formation and function. Antibodies will be discussed here in connection with B lymphocytes for two good reasons: B lymphocytes make them and employ them as receptors.

Antibodies are synthesized by both B lymphocytes and by plasma cells, which represent the final stage of differentiation which, as described, began with a stem cell in the bone marrow and continued development in the bursa. In mammals, which have no bursa, it is not yet clear where this process of maturation to a functional and recirculating lymphocyte occurs. Until 20 years ago the plasma cell was in fact considered to have no relationship to the lymphocyte. Today we know better and consider the plasma cell as the highly specialized end cell, which with maximal efficiency implements the B lymphocytes' potential: to produce and distribute antibodies en masse. It is reckoned that a single plasma cell can synthesize and pump into the blood some 2,000 antibody molecules per second. These are all alike and also, in their specificity, identical to the immunoglobulins which the plasma cell's predecessor could manufacture in smaller quantities when it was still a B lymphocyte.

Apart from the physiological role of normal antibody producer, plasma cells have played an important role in research into the biochemistry of antibodies, as they can form tumours without losing the ability to produce antibodies. Each such plasma cell tumour produces only one type of immunoglobulin, as it has probably originated from a single B lymphocyte. Being transplantable in the inbred animal strain of origin, such tumours can be turned into small living "factories" each producing great quantities of a completely uniform product: An immunoglobulin of a specific structure.

Another close association between B lympho-

cytes and antibodies is the fact that B lymphocytes use their own synthesized immunoglobulins as receptors built into the cell membrane. Thus each B lymphocyte possesses its own receptor whose specificity is different from those of other B lymphocytes, unless these belong to the same clone. Due to its specific receptor molecules, a lymphocyte can now select a particular toxin, or other antigenic molecule from among the millions of other protein molecules in the neighbourhood.

The lymphocyte's reaction with the antigen, via its specific Ig-receptors, initiates a new stage in its life, leading both to renewed division and to further differentiation into a plasma cell. Renewed division creates more B lymphocytes of the same type (memory cells) which of course reinforce this particular part of the defence system.

It is thus understandable that a single plasma cell only manufactures one type of antibody, i.e. the same type its predecessor used as a receptor molecule in the membrane. Division of labour in antibody production thus dates back to the B lymphocyte stage. How B lymphocytes came to differ from each other in different clones involves another and more complicated process which we cannot go into, until we have looked at the biochemical structure of the Ig molecule.

Antigens and immunoglobulins

Without antigens there is no immune reaction of any kind. This is simply a matter of definition, as the word antigen is a collective term for all substances that react specifically with antibodies or immune cells. There is no common chemical denominator for antigens. Practically anything can be rendered antigenic and be incorporated in an immune reaction with a high degree of specificity, but the great majority of known antigens in nature are large molecules which are proteins, carbohydrates or lipids, or are compounds such as glycoproteins or glycolipids.

Any protein may be artificially modified by a variety of chemical methods. The modified protein will usually lead to formation of antibodies

which differ from those elicited by the natural protein. Thus it seems that antibodies can also be produced against antigens which Nature has never before encountered and which she could not have been "prepared" for.

How can such a wealth of possibilities be generated?

Fig. 3 shows schematically the structure of an immunoglobulin molecule. It is entirely symmetrical along a longitudinal axis which separates two identical halves, each consisting of an L (light) and an H (heavy) polypeptide chain. In each half, the L and H chains are covalently bound with an S-S bond, and the two halves are mutually linked by S-S bonds between the heavy chains.

In principle, the most important feature of the molecule's structure is that both the L and H chains possess a so-called variable and a so-called constant component. The constant part of, e.g. the H chain is so called because it has been shown to contain the same number of the same amino acids in the same sequence no matter which kind of specificity the Ig in question possesses, whether it functions as an antibody to tetanus toxin or to cat hair. The same applies to the constant part of the L chain, while the constant parts of the L and H chains are very different from each other. The variable parts lying at the N-terminal end at the left of the drawing behave quite differently. It is exactly variations in the amino acid sequence in this part of the molecule which determine that Ig in B cells and plasma cells from different clones differ in specificity to the almost endless variety of antigens. Using a very mechanistic analogy,

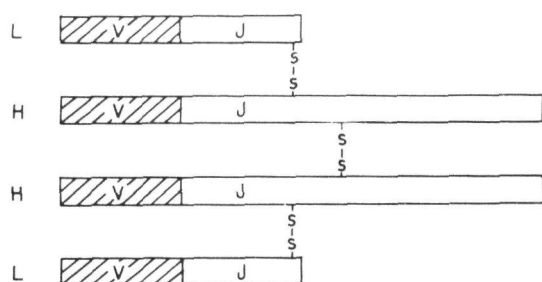


Fig. 3. Schematic illustration of a single molecule of an immunoglobulin. The molecule comprises two identical H-chains and two identical L-chains which each possess a variable (V) and a constant (C) part. These are mutually joined by S-S binding.

the repertoire of different Ig molecules in a single individual is very like an adjustable screw-driver, where you use the same handle but can change the more specific part of the tool to fit various types of screw. Elaborating a little on this very much simplified analogy: man's known stock of immunoglobulins has not one, but 10 different handles which in principle can all combine with each of the various blades, thereby increasing the number of different molecules by a factor 10, though not increasing the number of different specificities. Why do we need different handles? Not surprisingly, these determine physiological differences, such as the ability to pass from mother to foetus, or to fix to other types of cells, such as mast cells.

Now we can confront somewhat more closely the fundamental problem: how can a single individual produce so many different clones of Ig-producing cells that it can cover virtually the whole universe of antigens and can distinguish such slight differences between various macromolecules that no chemical or physical analytical method can compete?

Let us assume that the number of Ig-producing clones in man is about 10^6 (the real figure is perhaps 10 times that, but this is of slight importance for this argument). Among all genetic possibilities there are two extremes: a) that the entire genome comprises 10^6 different genes coding for Ig, b) that there is only a single gene which has undergone so many changes during the differentiation of the B-lymphocytes that shortly after birth it has resulted in 10^6 different Ig genes in this cell population. We know today that both these extremes are wrong.

The correct answer is not yet definitely known but the outlines are beginning to take shape. The essential point seems to be that there is not just one unbroken strand of DNA which codes for one Ig molecule, but that during differentiation of B lymphocytes, processes occur whereby different DNA strands are spliced together and transcribed in one piece to a primary messenger RNA, from which the final messenger molecule is generated by splicing at the RNA level.

We are now back to the analogy of the com-

plex toolkit. The variable part of both the L and H chains are coded for by at least two DNA strands designated V and J which are spliced together during differentiation. The point now is that the genome in fact contains several different copies of each, let us say 200 for V and five for J (which at the moment seems a reasonable estimate). This thus gives $200 \times 5 = 10^3$ possible VJ sequences after splicing. On a combinatorial basis, 10^3 different L chains and 10^3 different H chains (which, by the way, are coded from different chromosomes, transcribed to separate mRNA molecules and only joined together in the multichain structure after translation) can make $10^3 \times 10^3 = 10^6$ different Ig molecules. This impressive repertoire is achieved for a "DNA price" of a few hundred genes. Such delightful ingenuity can make the production of 10^6 different antibodies seem a simple matter, and it is entirely possible that even so humble a creature as the tadpole is able to produce them. If our actual need is closer to 10^7 it need not require much more DNA.

T lymphocytes and transplantation

While the B lymphocyte's claim to fame, as we have seen, is bound up with the formation of antibodies, that of T lymphocytes has roots in their ability to finish off transplanted cells from other individuals of the same species. This must sound absurd to those who are not acquainted with the subject but who attempt to reason logically. Why on earth do we need lymphocytes (and almost half our stock at that) if their main function is to reject transplants? Kidney transplantation, etc. are modern inventions which have played no part whatsoever in the development of the species. The only natural transplantation we can think of is impregnation in mammals, which results in a certain number of weeks or months, depending on the species, during which the mother in fact bears a semi-foreign embryo. We shall return later to the mysteries of reproduction, confining ourselves at the moment to noting with wonder how Nature has seen fit to arm us with a means to fight transplants when she has provided us no transplants at all except our unborn children.

In defence of Nature, it must provisionally be added that T lymphocytes have uses other than the rejection of transplants, and that their receptors are apparently so constituted that they can only recognize antigens in connection with one or other transplantation antigen; i.e., under normal conditions with the organism's own antigens. I imagine that this must sound bizarre but, as it seems to be true, we might as well go into transplantation antigens, as it is otherwise impossible to grasp the basis of modern immunology.

Transplantation antigens

The biological role of these antigens has only in recent years begun to dawn upon us, and no one knew of their existence until they were discovered during transplantation experiments. Hence their vernacular name. The term of histocompatibility antigens, shortened to H antigens, is useful as long as we remember that it does not refer to the above-mentioned H chains in antibody molecules.

H antigens are, like receptor molecules, built into the cell membrane and are thus exposed on the cell surface. A lymphocyte always has both types of molecule, but normally will not damage itself or the other lymphocytes of the organism. Some H antigens are confined to particular tissues, e.g. skin, others are common to several tissues, and yet others are found in almost all cells in the body.

In all cases we are dealing with macromolecules which are primarily the common inheritance of the species but which can also vary in detail from one individual to another. It is those details we are concerned with. If a protein in the cell membrane is composed of, say, 200 amino acids, and has had just one of these replaced by another as a result of a point mutation, this would seem to be a bagatelle. However, this alteration can sometimes be crucial to the protein's function, whatever that is, or it can lead to qualitative change in the molecules antigenic determinants. The lymphocytes may apprehend the change, and if they do, we in fact have an H antigen. H antigens are among the clearest examples available of com-

plete genetic control of traits which vary between individuals. Blood groups are well-known examples among the general category of H antigens.

There are many hereditary traits which can now be identified as H antigens. The most thoroughly explored experimental method of detection is skin transplantation between inbred strains of mice. Already, more than 30 different loci (i.e. positions in the genome which alternate genes can occupy) have been pin-pointed, each of which can provoke rejection of a skin transplant if the donor and recipient have different genes on one of these loci. Theoretical calculations indicate that the total number of loci for this type of incompatibility may be counted in the hundreds, but we will not go into this here. First of all it is important to realize that among all these loci there are a few key ones that are linked and which occupy a position of central importance in virtually the whole field of immunology. These are the loci known under the collective term of MHC (major histocompatibility complex).

MHC and transplant reactions

The so-called MHC genes are located on the same chromosome, and in such proximity that they are closely linked, hence usually inherited as a whole. Even very closely linked genes can eventually be separated by the process of crossing-over, which occurs during the first meiotic division, preceding the formation of gametes in the male and female gonads. If crossing-over occurs in a DNA section situated between two linked genes, these will enter two separate spermatozoa or two separate ova. The frequency of such cross-overs is used to measure the proximity of genes: the rarer the crossing-over, the greater the proximity.

It is not known exactly how many genes the MHC chromosomes of the various animal species contain, but they appear to increase the more the species has been subject to controlled experimentation. This is to say, the most widely employed experimental mammal – the mouse – probably provides the most precise estimate. It is improbable (at least as regards mammals)

that the various species differ to any great extent in the number of these genes they possess. In the MHC of the mouse about 20 loci have so far been recognized, each of which can be occupied by alternate genes (alleles). In man, not nearly as many loci have been identified to date: however, more alleles have been identified at the individual loci – probably a reflection of the much greater motivation to type the humans of this world than the wild mice.

We can now consider some of the consequences which ensue when the MHC barrier is breached during transplantation experiments. It is evident, on the basis of the foregoing, that differences in MHC between donor and recipient can cause rejection of a skin transplant. Although this also applies to many antigenic differences outside the MHC, it usually occurs more rapidly when MHC differences are involved.

The most striking expression of MHC incompatibility is seen when the recipient, for one reason or another, is incompetent to reject a transplant even though the latter contains T lymphocytes fully competent to react against the recipient's foreign MHC antigens. Such reactions can have violent, often lethal results – collectively termed GVH (graft versus host) reactions, since they are unleashed by the transplant turning against the host organism.

Factors which can reduce the host's reactive ability and lead to a serious GVH reaction include physiological immaturity in foetal and perinatal life. Suppose a newborn mouse receive a transplant which contains T lymphocytes (e.g. spleen or lymph node). These will enter the above-mentioned lymphocytic recirculation system and, in passage through the host animal, will be stimulated by its MHC antigens and transform into aggressive cells that will attack the host's cells and eventually kill the host. The process is not completed in only one or two days – it may take several weeks. The host animal will be retarded in growth compared with a normal young mouse and will usually develop severe diarrhoea and excematous lesions of the skin before it dies. Fig. 4 illustrates these conditions. Such extreme



Two mouse siblings from the same litter. Immediately after birth the small weak looking mouse had been injected with spleen cells from an adult mouse with a foreign MCH type, while the larger normal mouse had received cells of its own MHC type.

reactions are provoked only by the MHC antigens and not by the much more numerous but weaker H antigens outside MHC.

What are the grounds for believing that the GVH reaction is caused by the T lymphocytes? As previously mentioned, both the spleen and lymph nodes also contain B lymphocytes.

There are in fact several compelling reasons for believing that the T lymphocytes are the guilty party. One of the clearest is based on experiments with animals which selectively lack either T or B lymphocytes. There is a widely used experimental animal, commonly called the nude mouse, which through genetic defects has neither hair nor a thymus. The latter defect, as theory would suggest, entails that the T lymphocyte precursors in the bone marrow do not mature. If such an animal is used as a donor in a transplant of lymphoid tissue there will be no GVH reaction. If used as a recipient in skin transplantation the skin will not be rejected. Even skin from totally unrelated species will be accepted, e.g. it is possible to produce a nude mouse with chicken feathers!

In contrast, a chicken which lacks a bursa is an experimental animal devoid of B lymphocytes but possessing T lymphocytes. Such animals are quite competent to reject skin transplants from other chickens, just as their lymphocytes can provoke a GVH reaction upon transfer to MHC incompatible chicken embryos.

MHC restriction of T lymphocytes

It has been of enormous importance to the developing study of cellular immunology that we have learned how to simulate the transplant reactions of T cells outside the organism, i.e. in experiments employing cell cultures. The hostility they display towards the host's MHC antigens in GVH reactions can, for example, be mimicked in the so-called MLR (mixed lymphocyte reaction). In an MLR, T lymphocytes are mixed with lymphocytes from a genetically foreign donor. By the mixed culturing of the two individuals' lymphocytes, two separate processes can be observed within a few days.

1) Certain small, stimulated T lymphocytes

are converted into blast cells which actively synthesize DNA, RNA and protein. The intensity of this process is usually quantitated by the amount of radioactively labelled thymidine incorporated in the DNA of the reacting cells. A difference in MHC is normally a prerequisite for this reaction to occur and these reacting T lymphocytes are called, not altogether aptly, T helper cells (T_H).

2) Certain other T lymphocytes differentiate into another cell type called T killer cells (T_K), since they are in fact capable of killing cells from another individual. This can be shown by the addition of fresh target cells from the other individual, having first labelled them with radioactive chromium chloride which is absorbed in the cytoplasm of living cells and released into the surrounding fluid following their destruction by T_K cells. Such cells are also produced by the intact animal following transplantation of foreign tissue and can be detected in a mixed culture of labelled target cells from the donor together with cells harvested from the lymphoid organs of the sensitized recipient.

It has now been definitely established that the two processes described above are attributable to two separate classes of T lymphocytes, which differ both as regards own membrane markers (so-called Ly antigens in mice) and the repertoire of foreign MHC antigens recognized by their receptors. The respective MHC molecules which are recognized are encoded by two different families of genes within the MHC and are widely different in chemical structure, although they are all glycoproteins. T_H and T_K cells, respectively, recognize preeminently MHC molecules termed class II and class I.

As to the less obvious name, T helper cells, I must explain that cells of this type play a decisive role in the interaction between the different cell types involved in the immune system. For example, they are necessary for the stimulation of B cells to produce antibodies against most antigens (not only transplantation antigens). The mechanism is not clearly understood, but by some means or another, in order to initiate normal production of IgG antibodies, an antigen must be recognized not only by a B lymphocyte but also by a T_H cell.

Moreover, this cooperation between B and T lymphocytes seems essentially to require that these cells have some of the MHC genes in common, namely class II which in transplantation experiments is just the class recognized by these T helper cells.

On the other hand, it makes no difference if all other H genes are different. B and T lymphocytes are quite able to cooperate under experimental conditions, despite being genetically very different, with the proviso that they are similar as regards a very few MHC genes. This is the phenomenon known as MHC restriction. Under natural conditions no problem arises since the T and B lymphocytes are from the same individual.

Another, and no less intriguing MHC restriction has been shown for T_K cells.

Let us, for example, consider an experimental situation in which an inbred mouse belonging to strain A is infected with smallpox virus which in mice is relatively harmless. The immune system will naturally react and among the measurable changes is the production of T_K cells which have the following interesting properties: they will kill the virus-infected cells of some mice, but not those of others, and the selectivity can be pinpointed with precision. It is restricted to the MHC class I molecules, which are what T_K cells react to in transplantation experiments, as mentioned above.

How can this be shown? Let us assume that A and B represent two inbred mouse strains. Within each strain the mice are genetically identical, in principle just like identical twins. Let us further assume that the entire difference between A and B lies in the MHC while the remainder of the genome is identical. Several decades of patient application have produced such nearly identical mouse strains, known as congenic strains. In addition, genetic recombinations have occurred between congenic strains which permit analysis of the significance of the individual genes or regions within MHC. Thanks to this mouse genetic treasure in its entirety, it has in principle been relatively simple to dissect MHC restrictions and localize them to definite genes.

Let us now assume that our A and B mice

only differ as regards MHC genes belonging to class I which as said, can be recognized by T_K cells. We would then find that T lymphocytes from an infected A mouse can, in tissue culture, kill A cells infected with smallpox virus, but not similarly infected B cells. Neither will T lymphocytes from an A mouse kill infected cells from a third strain, C, unless by chance C has at least one class I antigen in common with A. Non-infected A, B and C cells are not killed, nor are cells infected by an unrelated virus. Apparently, infection with this particular virus has produced a form of T_K cell-bound specific reactivity which is not directed against the virus alone, but rather against virus presented on a specific background of MHC molecules.

What does this mean? Could not these properties just as well be described by saying that the T_K cells from mice belonging to strain A have acquired specific immunity against smallpox virus as such? The fact that cell death can only be seen if the immune A cells encounter the same virus on an A target cell could mean that the two cells must come in close contact before a reaction is provoked, and it could then be imagined that such close contact depends particularly on the MHC molecules, whose presence is apparently so vital. Perhaps it is simply that Nature has used the "soup plate" principle shown in Fig. 1 and made some molecules which effect cellular contact with the help of identity.

This theory is unsound, which is perhaps more easily seen if one considers what happens when a mouse which is both A and B is infected. Such mice are easily produced, being the F_1 generation of a cross between the A and B strains, and they will be identical and will have inherited an MHC chromosome from both A and B. If such an A/B mouse is infected with smallpox, naturally this will induce production of T_K cells which can attack the virus presented on both A and B target cells.

However, it has been shown – by methods which would take too long to describe here – that in reality there are two different populations of T_K cells from the A/B mouse which attack the virus on A and B cells. It is possible to render one of these populations inactive without

inactivating the other. This could not be done if contact between A/B T_K cells and A and B target cells were effected by the identity principle because all A/B cells are, and remain, equally A and B as regards the phenotype of MHC molecules. This in turn means that we must consider the MHC-restricted recognition as a process in which both the viral antigen and MHC antigen are recognized via complementary molecules in the lymphocyte membrane. Schematically, this position might be resolved in one of two ways:

1) T lymphocyte have two kinds of complementary receptor molecule, one used to recognize self-MHC product and the other to recognize the viral antigen. In the literature this hypothesis goes under the name "Dual receptor model".

2) T lymphocytes, like B lymphocytes, have one type of receptor molecule per cell. However, the repertoire of possible receptors on T lymphocytes is, in reality, like variations on a single theme, consisting of self-MHC. The variations are supplied by whatever foreign material is present, for example viral antigen, in close association with the MHC coded molecules in the membrane. This hypothesis is known as "Altered self model".

I really see no reason to suppose that the two models should be incompatible. As we have seen, the immunoglobulin molecule consists of gene products from different parts of the genome (the H and L chains are not coded for by the same chromosome). One could quite well visualize a structure for a T lymphocyte receptor molecule in which one part of the molecule recognized its own MHC while another part was responsible for the recognition of the foreign determinant. Receptor molecules of T lymphocytes have not yet been structurally identified. At present it is widely believed that their variable parts are encoded by the same pool of genes which determines the variable parts of antibody H chains, but this has not been proven.

Acquisition of recognition

It applies to both types of T lymphocytes, as explained above, that recognition of self MHC molecules is essential for their normal functioning. It seems, moreover, that this self-recognition is acquired through receptors, which are complementary to certain antigenic determinants of their MHC molecules in the cell membrane. On the other hand, it is not at all clear how it normally comes about that each individual is equipped with just those T lymphocyte receptors required for self-recognition.

In the last few years some extremely interesting, but at the same time difficult experiments have been undertaken to elucidate the acquisition of T cell recognition. Unfortunately, the results are often contradictory, but they do show that the thymus plays an important role. The aforementioned "nude mice" are especially suitable for such experiments as they have no thymus but have a normal production of immature T lymphocyte precursors in the bone marrow. By transplanting the thymus from a normal newborn mouse to a nude mouse it is possible to restore the T lymphocytes, as the precursors are now provided with the type of thymic tissue needed in order to mature. It has been shown in these experiments that both T_H and T_K cells can mature in the transplanted thymus, but their acquired self-recognition is not independent of the donor thymus MHC. Thus, what is provided by the thymus transplant is not a population of donor T cells, but the missing environment in which the nude mouse T cell precursors can be educated.

In my opinion the most critical experiments to date indicate that, during maturation in the thymus, self-recognition cannot be acquired for MHC products other than those already possessed by the T lymphocytes. On the other hand, the genetic potential can be limited by the thymus, as indicated in grafting of thymus from strains A or B newborns to nude mice of A/B genotype. Even though matured T lymphocytes are themselves A/B they have only acquired self-recognition for one set of MHC molecules, namely that which was present in the thymus, i.e. either A or B. This would seem to point to

the conclusion that it is not sufficient for a T lymphocyte to possess the appropriate genes; these must also be "approved" in the thymus and preferably by the appropriate "arbiter". In other words, the transplanted thymus is most efficient in inculcating self-recognition in those T lymphocytes which, genetically, could have been its own. The molecular mechanism in this maturation process is completely unknown.

Acquisition of cellular xenophobia and tolerance

In the world of the T lymphocyte, xenophobia (which in immunology is rather termed allo-aggression) is congenital and extends to all members of the same species which harbour genes in their MHC other than those of the individual in question. Thus xenophobia is present in the genes but, as with self-recognition, is subject to modification. Such modifications can operate in either direction. If earlier exposure to another individual's H antigen results in increased reactivity against them, one usually speaks of immunization or sensitization.

However, some 30 years ago it was observed that, under certain circumstances, contact with foreign H antigens could weaken or abolish reactivity upon renewed exposure to the same antigens. Phenomena of this general description have been analysed from many angles and published under various names, of which acquired immunological tolerance (P. B. Medawar) is probably the most popular. The classical demonstrations of tolerance involved a first exposure to transplantation antigens in embryos or newborn animals, before the immune system had reached maturity.

It is presumably a similar mechanism which leads to tolerance in the adult, nude mouse which we shall now come back to.

Instead of transplanting a thymus to a nude A/B mouse from a newborn A (or B) mouse, we now transplant from a newborn A/B to a nude A (or B) mouse. The immature T cells now come from A and are to be educated in an A/B thymus. As already indicated this does not provide self-recognition of B which is genetically alien to A with regard to MHC. The immature T lymphocytes only acquire self-recog-

inition of the MHC components in the thymus for which they themselves have the genes. Nevertheless, odd things happen as regards the foreign MHC components of the thymus transplant: they are tolerated. This has been illustrated by, for example, the above-mentioned MLR reaction which will be negative when T cells of the reconstituted nude A is mixed with B cells (the thymus donor strain) but positive when mixed with C, D, etc. (other foreign strains). This latter control is essential and shows that the nude mouse in general has had its T lymphocyte reactivity restored. The acquired tolerance is specific, i.e. limited to the B strain.

Among T lymphocytes there is thus a demonstrable difference between self-recognition and tolerance, even though both qualities are acquired within certain genetic frameworks and both can be acquired in the thymus. Even if one can learn to tolerate foreign cells (A tolerating B), this does not mean that the latter are being accepted as self (A is still restricted to A, and not to B, in cellular cooperation).

Nature's own transplant

If Our Lord had consulted an immunological archangel before creating mammals He would probably have gone no further.

Mammalian ancestors and relatives among vertebrates: fish, amphibians, reptiles and birds also have both lymphocytes and antibodies, but all have managed reproduction by laying eggs and thereby avoided the direct intimate contact between mother and foetus. Among mammals, however, the embryo is a transplant, half of whose genes – including the MHC genes – stem from the father. Furthermore, some transplantation antigens differentiate early and are present from the earliest stages of the embryo's development. During pregnancy the mother is perfectly capable of reacting against them and, to a certain extent, she does (rhesus antibodies are a well-known example of this). The mother does not develop immunological tolerance towards her child. Animals have also been ex-

amined to ascertain whether the interior of the uterus might in some way be a privileged site for a transplant. This is not so. Only the foetus is privileged. Why?

As yet there is no wholly satisfactory explanation but two important considerations may be mentioned: 1) Nature has prudently separated the blood of the mother from that of the foetus by setting up barriers in the placenta which keep the two blood systems from each other. It is true that these barriers are not wholly impermeable as blood cells in particular pass from foetus to mother, but by and large the lymphocytes of the mother are prevented from entering the embryo. If they were allowed free passage hardly any offspring would be born in conditions of MHC incompatibility – and MHC incompatibility almost always predominates in nature in general, not only in man. 2) As the two circulatory systems are kept more or less separate, the main point of contact between the mother and foetus is a special layer of cells in the placenta called the trophoblast layer. The trophoblasts are derived from the foetus and also contain its transplantation antigens. Nevertheless they are unusual in being both inept at immunizing and highly resistant to an immunity which is produced by immunizing with other cells containing the same transplantation antigens (e.g. father's cells).

All in all, a pregnancy is in fact a matter of balancing on the edge of immunological abysses, but nature is incredibly good at getting the equilibrium right.

Lectins

I would like briefly to describe here a group of proteins (or glycoproteins) which also seem able to act as receptor molecules in the cell membrane. They are very different from immunoglobulins and their importance reaches far beyond the limits of immunology. The word lectin is really a common term for every protein (short of antibody) in the animal and plant kingdoms which can bind specifically to carbohydrate. Sugars are ubiquitous in animal cell membranes where the proteins and lipids often contain side-chains composed of oligo-

saccharides. There are only nine different monosaccharides in animals to choose from as building blocks, but in principle these can be combined in an incredible array of differently structured oligosaccharides. In fact the theoretical possibilities of combination are far greater than for proteins, all of which are constructed of 20 different amino acids. While for example, a given series of 12 amino acids in a given order will yield a single well-defined molecule, a given 12 monosaccharides in a given order could actually be combined in several million different ways.

The classic lectins are of plant origin. They are most easily extracted from legumes such as peas, beans or lentils. Thus substances are easily extracted from these seeds which agglutinate mammalian erythrocytes and thus have a superficial resemblance to blood group antibodies. Certain lectins also stimulate T lymphocytes to cell division in tissue culture thereby offering functional resemblance to MHC antigens.

A classic example of such a lectin is Con A (extracted from *Concanavalia ensiformis*) which up to the moment is the only lectin whose biochemical structure is as well known as that of immunoglobulins. Con A is a protein built up of four identical sub-units each comprising 237 amino acids, one calcium atom and one manganese atom. Each sub-unit has a binding site with affinity to a few closely related monosaccharides, also when they are built into the oligosaccharide chain in the cell membrane. Some lectins of the pea family, especially legumes, have been partly analysed as regards amino acid sequence and show a certain relationship to Con A. The composition of other lectins in the animal and plant kingdoms is not nearly as well known.

It is obvious that the importance of lectins for legumes has nothing to do with human or other blood cells. Legumes, however, are among plants of great specific ecological importance; they assimilate nitrogen from the atmosphere and convert it into protein. Nitrogen-assimilating plants can only perform this feat in symbiosis with certain bacteria, among which the *Rhizobium* family is of great importance. In fact

a remarkable exclusivity has developed such that a given species of plant flourishes in conjunction with a given species of bacteria.

It is only very recently that we have acquired a sound basis for the assumption that symbiosis between plants and bacteria is effected by the plant lectins. These function as receptors for bacteria. For example, the fine root hairs of the white clover plant contain the lectin trifolin which binds specifically to the monosaccharide 2-deoxyglucose which is found on the surface of the preferred bacteria. The binding of this bacterium to the root hair can be prevented by free 2-deoxyglucose, just as would be expected from the hypothesis of lectin binding with specificity for this sugar.

As already mentioned, lectins are nearly ubiquitous and are probably involved in many important biological processes. They are found in many viruses, e.g. influenza virus where they undoubtedly mediate binding to the host cell. Lectin binding is thought to be involved in the reproductive processes of lower and higher forms of plant life.

A very widespread phenomenon among many families of flowering plants is self-sterility which is genetically controlled by a single locus, S, with many allelic genes. In order to allow fertilization, the pollen grain germinating on the stylus must form a tube which passes down to the ovum thus enabling the sex cells to unite. In plants practising self-sterility this is only possible if the pollen is from another plant because, paradoxically, the S genes must be incompatible to allow the pollen tube to develop. The biochemical mechanisms involved in this phenomenon are far from clear, but it is obvious that there must be a very specific form of cellular discrimination between own and foreign gene products of the S genes.

In the animal kingdom lectins are present not only in all invertebrate animals studied but also in vertebrates. Both mammals and birds, for example, have sugar binding proteins in the liver cells which enable them to recognize changes in the structure of the above-mentioned oligosaccharides which are positioned as side-chains on many proteins. It seems quite possible that this plays a role in the normal

homeostasis in getting rid of effete cells and cell components.

The role of the immune system in the development of the species

In the foregoing remarks on lectins I have deliberately ventured beyond the realm of immunology in the usual sense. In my opinion it is unnecessarily restricting if immunologists only grudgingly acknowledge that there are forms of cellular distinction between self and foreign other than those encompassed by immunoglobulins and lymphocyte receptors. However, if one wishes to use these blinkers I believe it can be argued convincingly that the immune system came into being with the first vertebrates. Even the most primitive contemporary fish such as the hagfish and lampreys (cyclostomes) form antibodies, while antibodies have never been found among the motley crowd of invertebrates.

A similar situation holds for lymphocytes and thymus which cannot be identified in anything below the level of cyclostomes. However, it is clear that contemporary invertebrates and their often strikingly similar looking ancestors – known to us from fossils formed hundreds of millions of years ago – must have developed alternative means of combatting microbial infections. We know they have done this, but it is surprising how little we actually know about the biochemical background of these systems, and the same is true of the entire plant kingdom. Some of our scant knowledge is at least consistent with the view that lectins could be operative factors in these primitive defence mechanisms.

Let us leave aside the biochemistry of receptor molecules and pose instead the simple phenomenological question: when did cellular aggression towards those who were related but different (allo-aggression) come into being?

No date can be given, but one thing is certain: allo-aggression is far, far older than the vertebrates. The phenomenon is seen among representatives of practically all the invertebrates, e.g. earth worms, corals, starfish and many more. I find it of particular interest to consider the

situation as regards those creatures which zoologists consider are our closest contemporary relatives, the tunicates. Probably the first fish evolved from the ancestors of these marine animals more than 400 million years ago. Evolution thereby took a decisive step which led to amphibians, reptiles, birds and mammals.

The larval stage in tunicates is reminiscent of the tadpole, they are free-swimming, provided with eyes and a chorda, while in the adult stage they are blind and sessile, attached to the sea bed, and form a collective of identical individuals produced by asexual reproduction of a single animal. Two larvae hence do not produce two adult individuals but two adult colonies. A colony can be cut in half and the two halves placed side by side, and the colony will grow together again as if nothing had happened.

On the other hand, if two halves from different colonies are placed side by side this can lead to violent reciprocal rejection, although this does not always happen. Two different colonies can be either compatible or incompatible, though they are usually the latter, especially if they come from different locations. Whether there is compatibility or incompatibility is apparently determined by a single genetic locus (or conceivably by several closely linked genes, an MHC of the tunicates in which to date only a single locus has been identified). This locus, called the F locus, has several alleles. What are these for? The principal explanation will probably be found in regard to the sex life, which seems to be governed by the same genes according to the following interesting rules: all the adult animals are hermaphrodites which produce both spermatozoa and eggs but are unable to fertilize themselves. Why not? Because fertilization, which takes place inside the animal, requires incompatibility regarding the F genes (formally just as in the above-mentioned flowering plants which have self-sterility genes). Only spermatozoa which have an F gene different from the two F genes carried by the adult colony are able to penetrate the egg. "Compatible" spermatozoa are stopped before they reach the egg, or reach it more slowly than those which are "incompatible".

Whatever the biochemical mechanism, there

is obviously some form of genetic control which favours incompatibility in fertilization. It is easy to imagine that this must have been of crucial importance for development. Without this discrimination in favour of foreign spermatozoa, evolution might have been hampered since spermatozoa produced by the colony itself are closest, and fertilization by its own sperm would considerably reduce the possibilities of nature testing different genetic combinations. Perhaps our remote ancestors, the tunicates, would never have progressed further without allo-aggression.

The stratagems of nature and man

Now and then, when they consider it opportune, the powers that be will promise to do something for the good of mankind, and one of the items on their list is the granting of money to fight cancer. All forms of cancer taken together are responsible for approximately one sixth of all deaths in a typical industrial country such as the United States. This means in absolute numbers about 300,000 per year, the greater part being persons over 60 years old. If we reckon that the population of the United States represents about a fifth of that of all industrial countries (including USSR and Japan), by multiplying the number by five we probably get a fair idea of the number of deaths caused by cancer in that part of the world where almost all medical and biological research takes place.

Most cancers are predominantly diseases of the older age group. For the United States, John Cairns has estimated that complete eradication of cancer in a future balanced society – in which there are an equal number of births and deaths per year – would affect the age distribution so that the 60-year-olds would make up 18% of the population compared with 16% if the cancer death rate remains unchanged.

Let us now suppose that the sum of biomedical research resources was constructively used to investigate the global incidence and importance of diseases; cancer research would still of course be important but would be very much over-shadowed by research into other problems.

The overwhelmingly dominant problems are malnutrition and parasitic diseases. I will end by discussing the last mentioned group of diseases, more exotically called tropical diseases because they to an eminent degree present unsolved scientific riddles concerning cellular recognition.

Tropical diseases, which form a large and varied group, are caused chiefly by animal parasites, some unicellular (protozoa) and some multicellular wormlike animals belonging to several invertebrate phyla. All these parasites have a complicated life cycle in which man is unfortunate enough to play a role, but usually also involving an auxiliary from elsewhere in the animal kingdom. Insects, especially certain mosquitoes, are probably the commonest vectors (malaria, filariasis). I believe that as a fairly general rule there are two great scientific challenges involved in each of these diseases: 1) the biological nature of host-parasite preference and 2) the immunological defence mechanism and the possibilities of manipulating it to our own advantage, for example with a suitable type of vaccine.

The preference certain parasites show for certain host constellations often seems rather bizarre. Expressed more conservatively: it is difficult to see the logic and to understand the mechanisms. Just take the example of schistosomiasis: why does the flatworm which causes the disease have to employ a particular type of watersnail as intermediate host while it cannot use others? From a national viewpoint this is a good example as this is a field in which a Danish laboratory is trying to solve a problem which, apart from being intriguing, is also relevant to hundreds of millions of people in the world who, in the course of their labours in rice paddies are exposed to infection by this chronic, crippling and often fatal disease. If varieties of snails could be found which could oust the common types without themselves providing suitable intermediate host material, this would probably prove a brilliant stratagem.

This same flatworm (*Schistosoma mansoni*) has its own stratagem, too. A highly vulnerable stage in its life cycle occurs when it has left the snail and just penetrated the skin of a human

being (or other suitable mammal). Indeed, after all I have said about our immune system, one would suppose this to be a dangerous place for a flatworm to venture into. Just consider all the T lymphocytes which are so ready to reject a kidney or skin transplant from another human being. What about a transplanted flatworm! Unfortunately the expected does not happen regularly enough, otherwise there would be no resultant disease. In practice most of the worms probably die at this stage, but not all of them; if they survive just a few days they will have penetrated the lungs and undergone peculiar changes which will have rendered them practically invulnerable to our immune system. It seems in fact that the worms are able to appropriate the host's antigens and incorporate them in their own "skin". For example, in human beings the worms can incorporate glycolipids carrying the known A and B blood groups, but apparently not the Rhesus groups which are not in any case linked with glycolipids. By incorporating the host molecules into its own "skin" it is apparently so well disguised that it can avoid or suppress aggression by the immune system. (Lectins might be suspected of assisting in the appropriation since it is the sugar-containing blood groups which are intercepted, but this is only a guess on my part).

Other parasites have also evolved their own strategies. For instance the unicellular *Trypanosoma brucei* which causes African sleeping sickness can avoid succumbing to the patient's immune response by continuously varying the molecules it synthesizes itself and incorporates in its surface membrane. It probably has a large number (perhaps several hundreds) of genes to choose from which code for different glycoproteins and it "knows" how to change as a developing immune response makes it prudent to "change coats". Something similar apparently applies to malarial parasites.

Up to now, parasites have used more effective stratagems than scientists. Nevertheless, there is every reason to believe that it is scientifically possible to pick up enough of the parasites' tricks to devise an effective defence.

Even though in the past decade considerable progress has been made (especially in Britain

and the USA) in research into the biology of certain tropical diseases, there is no doubt that this area has been neglected. The degree of neglect would be unthinkable if the diseases in question were "our own", that is to say common in those countries which pay for and carry out research. The World Health Organization is well aware of this but WHO's own research funds are severely limited. When at least 500 million people in the underdeveloped countries suffer from malaria, schistosomiasis and filariasis, to mention only the largest disease groups, it seems a great pity that the powers that be pay such scant attention to tropical diseases. I sympathize and agree with the sarcasm employed in a recent WHO publication (Tropical Diseases) which sets the annual global investment in research into tropical diseases at around 30 million dollars, and states that, in costs, this tallies up with a few miles of motorway.

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