Immunological Recognition of Self

Such recognition suggests a relationship with processes through which functional integrity is maintained

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I like to think that when Medawar and his colleagues showed that immunological tolerance could be produced experimentally, the new immunology was born. This is a science which to me has far greater potentialities, both for practical use in medicine and for the better understanding of living process, than the classical immunochemistry which it is incorporating and superseding.

In this article I shall be concerned almost exclusively with theoretical aspects of immunity. Medawar has spoken of the experimental aspects of acquired immunological tolerance and other types of immunological nonreactivity, and he has touched on the impossibility, in natural chimeras, of demonstrating that the genetically alien cells are treated in any way differently from cells that are genetically proper to the body. For me, acquired immunological tolerance means simply that the content of self-components in the body has been enlarged by an experimental manipulation. Basically, I shall deal in this article with a single problem: How does the vertebrate organism recognize self from not-self (in the immunological sense), and how did this capacity evolve?

Nature of Antigen and Antibody

The production of antibody is not the only, nor I believe the most important, manifestation of immunity, but for reasons both historical and of experimental convenience antibody is likely to remain the touchstone of immunological theory. Any formulation of theory must cover the nature of antibody and lay down the conditions under which it will or will not be produced. In this article I am concerned for obvious reasons only with antigens derived from the cells of other vertebrates and tested for antigenicity in a defined species of mammal; in experimental work rabbits and pure-line mice are the most usual, but much work on the border line between therapy and experiment has also been carried out in man.

Bovine serum albumin is antigenic in a rabbit, rabbit serum albumin is not. Both have presumably the same function in their proper species, and the difference responsible for antigenicity can be regarded genetically as an example of neutral polymorphism. Superficially at least, the differences seem to have no relevance to survival. Serum albumin is a well-defined protein, but no laboratory has yet attempted to ascertain its full chemical structure. At present there are only two proteins whose primary polypeptide structure is known, insulin and ribonuclease, and only in the case of insulin have we information as to how structure varies according to the species from which the protein is derived. Insulin is a very poor antigen—otherwise we could not use bovine insulin successfully for the treatment of diabetes. Nevertheless it *can* function as an antigen in man, and it is known that when immunological resistance to beef insulin develops, replacement by pig insulin will usually allow effective therapy.

Since Sanger's work (*1*) it has become well known that species differences between insulins involve primarily a group of three amino acid residues, numbers 8, 9, and 10, on the A chain. Human insulin differs from other mammalian types by having a different C-terminal amino acid on the B chain (*2*).

The immunological difference between beef insulin and human insulin, which is presumably responsible for the antigenicity of the former in some human beings, is thus limited to a very small portion of the whole molecule. It may be either the actual difference at positions A8, 9, and 10 or some change in the secondary structure of the molecule dependent on this difference that gives rise to the effective antigenic determinant.

This consideration of insulin as the only available antigen whose chemical structure is known leads to a conclusion which could be supported by many other pieces of evidence—that is, that an antigenic determinant has very much the quality of a gene. Its existence can only be recognized by virtue of its *difference* from something else of the same general quality. A protein or other type of macromolecule is antigenic because it carries one or more chemical configurations (antigenic determinants) which differ from any configurations of the same general quality that are present in the animal being immunized.

There is evidence which I need not particularize that an antigenic determinant, like the active patch on an antibody molecule with which it combines, is small (perhaps 100 to 200 A^2) and that to be active it must be part of an appropriate carrier macromolecule and in an accessible situation in the molecule. There is no evidence as to how many potential antigenic determinants there are in an insulin molecule. One could guess that there were some hundreds of different patterns produced by knots of three to five amino acids accessible on the surface of the molecule, any one of which might serve as an antigenic determinant, but until we know more about the requirements for antigenicity we cannot be sure that it is not a much smaller number. In practice, of course, all these potential determinants have the same structure as the corresponding substance in the immunized animal and are therefore inert.

If my last statement is correct, and I believe most immunologists would accept it, then it allows us to pose the basic problem of immunology in a spe-

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cific form. How can an immunized animal recognize the difference between an injected material like insulin or serum albumin from another species and its own corresponding substance?

Immunological Information

Clearly this is a problem of information. It is conceivable that a substance could be recognized as foreign if it were built up of chemical configurations insusceptible to enzymic breakdown by the available mechanisms of the animal involved. This may have some relevance to microorganismal antigens but not to the substances of vertebrate origin that are our present concern. Their recognition, in the sense in which we are using the word, requires that there be available in the body a large volume of accessible "information" with some superficial analogies to a dictionary. In other words, there must be something against which a configuration can be compared, and on the basis of which a decision can be made as to whether it corresponds or not. We find somewhere a combination of letters *RAXE*, and we use an English dictionary to find that there is no such word in English. If the body is to differentiate between self- and not-self configurations, the only general form of solution that has so far been thought of requires the presence of a complete set of complementary steric patterns in some accessible form which correspond to either (i) all configurations *not* present in body components, or (ii) all configurations *present* in body components, or (iii) all configurations, but in two categories corresponding to (i) and (ii).

Of these alternatives the first is obviously the most attractive, providing a *positive* recognition of any configuration against which reaction will be necessary. It is the only one which I will elaborate here; neither of the others has been seriously considered by anyone. I should agree with Jerne that the information needed may be compared to a "purged xenotypic dictionary" (*3*).

To clarify this concept we might adopt the currently popular convention of discussing "coding" problems of polypeptide synthesis by identifying amino acid residues with letters of the alphabet. If the small specifically patterned areas of an antibody molecule are constructed of a small segment or knot of a polypeptide chain, we could legitimately simplify matters by regarding all specific antibody patterns as being four-letter words—*axqb*, for example—each corresponding to an antigenic determinant represented by the upper case form of the same letters, *AXQB*. We could generate the type of information we require in the alphabetical analog by first requiring a computer to produce, say, 10^7 random four-letter combinations. The combinations are scrutinized as they are produced by a team of English speakers who eliminate every combination which forms an English four-letter word. All other combinations are stored in the computer's memory to be called into activity whenever the corresponding upper-case group is fed into the machine.

Translated into biological terms this requires some process of randomization to provide the primary array of complementary steric patterns. The elimination of self-reactive patterns would, by hypothesis, result when prenatal contact with self-components occurred. The residue would be available to react with and "recognize" foreign configurations entering during the period of independent life.

Two suggestions have been made as to the carriers of the patterns. Jerne postulated the circulating globulins, Talmage (*4*) and I (*5*) both preferred mesenchymal (lymphoid) cells. I believe that circulating globulin can be categorically eliminated in view of the phenomena of graft-versus-host reactions and that any attempt to give an observable basis to the concept must be concerned with the immunologically competent cell.

This is a term which is used differently by different immunologists. I prefer to define an immunologically competent cell as one which is specifically stimulated to some reaction (either observable or in principle observable) by contact with an appropriate antigenic determinant. In order to illustrate this concept of the immunologically competent cell, I shall make a brief diversion from theory to experiment. For the last three years we have been interested in the graft-versus-host reaction that is shown when normal fowl leucocytes are inoculated onto the chorioallantoic membrane of chick embryos. Figure 1 shows four membranes, all from eggs laid by a single hen and fertilized by artificial insemination from the same cock. Both birds are from the same highly inbred strain of White Leghorns.

On each membrane we inoculated about 2×10^6 leucocytes from the cock and reincubated the embryos for another 4 days. As harvested, two of the membranes show no lesions; the others show between 100 and 200, with marked opaque foci about 1 millimeter across. These lesions mark areas of cellular proliferation in which both the embryonic (host) cells and the mature (donor) cells and their descendants play a part. The foci represent an immunological response initiated by individual immunologically competent cells; antibody production is not involved. We believe that the difference between positive and negative membranes is due to the presence of a single antigenic determinant in the embryos showing lesions and to its absence in the embryos that show none (*6*). There are several interesting features about these foci: (i) they are immunological in character; (ii) they are produced by *normal* lymphocytes from completely normal birds; (iii) each lesion is almost certainly induced by a single cell, but only about one lymphocyte in 10^4 can induce lesions; (iv) the lesion is initiated either immediately or within a few hours of depositing the cells on the membrane.

There could hardly be a more direct demonstration of the potentiality of the immunologically competent cell, and although with sufficient ingenuity the facts can be pressed into the mold of an instructive theory, they fulfill naturally and easily the requirements of a theory calling for cellular carriage of previously generated information that will allow recognition of a given antigenic determinant.

At the present time I believe there is very little doubt among immunologists that some form of selective theory of this general form is needed. The whole domain of homograft immunity and tolerance, graft-versus-host reactions, and histocompatibility genes demands a cellular basis of immunity and a "selective" rather than an "instructive" origin of immunological specificity.

By adopting the idea of randomization of pattern we imply that during embryonic life a very large range of patterns is synthesized in such a fashion that in later life any one of the patterns can be produced in large numbers on demand. If the patterns are carried eventually in lymphoid cells, we must presumably look for some process of differentiation or somatic mutation in the primitive cells ancestral to the lymphoid series. Most geneticists and

immunologists would probably prefer to look for randomization of pattern in a hypermutability of one or more genetic loci at some stage during embryonic life, with a relative stabilization subsequently. This is in line with the general dogma that the pattern of a protein is determined in the last analysis by the pattern of a segment of chromosomal deoxyribonucleic acid.

In this way we can picture clones of cells arising which carry the capacity to synthesize, under appropriate stimulus, one, two, or more specific patterns which, either as a cell receptor or as the active patch on an antibody molecule, could react each with a specific antigenic determinant.

There are two ways at least in which the functional elimination of patterns reactive with self-components could be implemented. If a cell or clone is limited to one or two patterns, then it is practical to postulate that any clone carrying either one or two self-reactive patterns is eliminated, leaving only clones carrying patterns corresponding to configurations not present in the body. This is the form taken by the clonal selection theory, and provided two is adopted as the usual number of patterns for a diploid somatic cell, it provides a reasonable interpretation of the facts.

As both Lederberg (*7*) and Monod (*8*) have pointed out, there is no special reason why only two patterns should be produced by a process of hypermutation. It is obvious, however, that any increase above two will make it progressively more difficult to sort out patterns corresponding to self- from those corresponding to not-self configurations by elimination of clones. If there are 10 or 20 random patterns per clone, the elimination of reactivity against self-configurations must be accomplished by some process of inhibition that still leaves potential activity with those patterns which are complementary to foreign antigenic determinants.

Many immunologists are impressed with the fact that, in general, tolerance induced in the perinatal period only persists when the antigen in question remains present in the body. They feel that this points to an inhibitory or blocking action rather than the elimination of the cells concerned.

It is not difficult, in fact, to picture an inhibitory process of the type needed, but to do so requires a little preliminary discussion. The difference between a primary and a secondary immune response is known to everyone who has ever been concerned with practical immunization procedures. Modern work suggests that there are several levels of physiological reactivity that can be manifested by a clone of immunologically competent cells. At least three, which we can call grades 0, 1, and 2, are probably necessary, grades 1 and 2 corresponding to the cells responsible for immune responses of primary and secondary type, respectively.

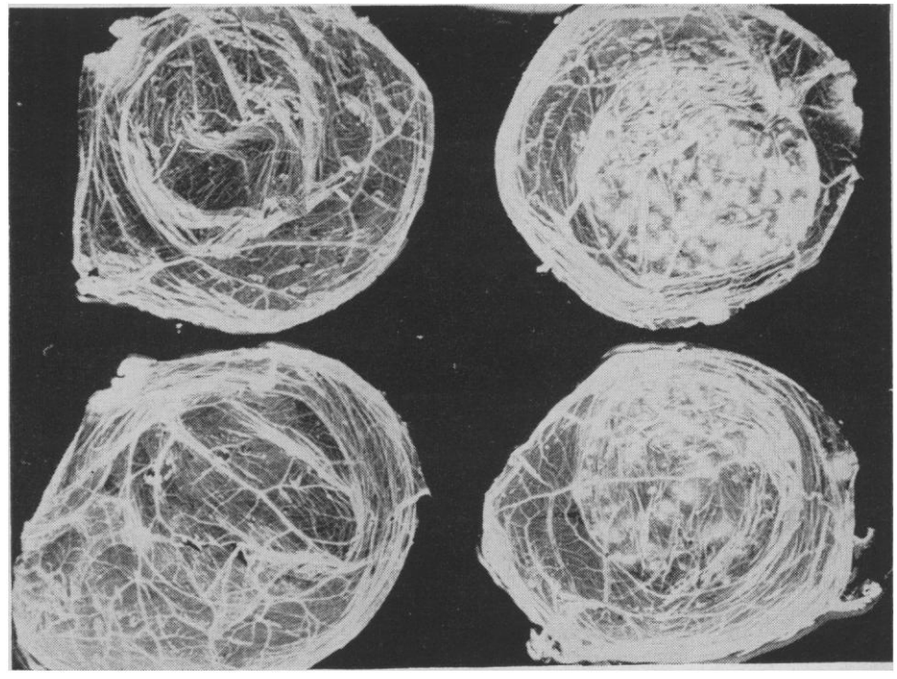

Fig. 1. Typical graft-versus-host foci on the chorioallantoic membrane. Leucocytes from a cock of an inbred strain were placed on four membranes, each from an egg laid by a hen of the same inbred line and fertilized by the donor cock. Note the segregation for the factor, which allows the appearance of foci.

In grade 0, characteristically but not exclusively present in embryonic life, the only reactivity that need be postulated is an inhibition of part or all of the cellular activity by contact with the antigenic determinant. One assumes that, after birth, initial contact of antigen with a grade 0 cell gives rise to grade 1, perhaps directly, perhaps by way of proliferation. In grade 1 we have cells capable of specific proliferation after contact with antigenic determinant and capable also of producing reactions of delayed-hypersensitivity type. For antibody production, grade 2 cells must be produced, presumably by antigenic stimulation of grade 1 cells. The essential lesion in agammaglobulinemia is a failure of the change from grade 1 to grade 2 to occur.

Any cellular theory of immunity demands the presence of cell receptors which, by making an antibody-like union with antigenic determinant, can provoke reaction of one sort or another. The difference between the grades of reactivity could well depend on the number and accessibility of these receptors. In grade 0 in the embryonic phase or its equivalent, the receptors are few and, perhaps because of their situation, are readily blocked for a prolonged period by molecules carrying the antigenic determinant. This appears to be the type of reaction that Smith (*9*) favors as an explanation of his experiments on acquired tolerance in rabbits. If all cells carrying the embryonic grade 0 receptors for antigen X have all these receptors blocked so that they can neither react with any further antigen nor mature to a higher grade, this would provide as adequate an explanation of tolerance as elimination of the clones concerned. A qualifying hypothesis would probably have to be added, to the effect that when a receptor was released by metabolic breakdown of the antigenic determinant, it would remain in the nonreactive phase long enough for it to be found and again inhibited by other molecules of the blocking antigenic determinant.

Given sufficient time (perhaps a few days) free from antigen, a receptor would presumably mature to grade 1 and behave like normal unstimulated receptors. Once grade 1 is reached,

specific contact with a receptor becomes a stimulus to proliferation and perhaps, in special environments, to plasma-cell development and antibody production.

Such a concept can be represented in diagrammatic form for a cell (or clone) assumed to have two patterns corresponding to self-components 1 and 2 and two corresponding to non-self patterns *a* and *b* (Fig. 2). The advantages of a hypothesis of this sort are that it (i) provides a simpler interpretation of the necessity for the continuing presence of antigen if tolerance is to be maintained indefinitely, and (ii) allows the existence of a complete range of immunological patterns with a much smaller number of clones than would be required if every clone carrying a self-pattern had to be annihilated. Unfortunately from the point of view of experimental test, a hypothesis in which the number of patterns available to a clone is, or may be, large soon becomes experimentally indistinguishable from an instructive-theory hypothesis.

I am concerned with immunological theory primarily only in so far as it deals with the problem of self-recognition. It is obvious, however, that any theoretical formulation must also be acceptable as an interpretation of the other significant aspects of immunity. A brief reference should therefore be made to the possibility, which cannot be altogether excluded, that genetic information can be transferred from one somatic cell to another, by some process analogous to processes known to operate in bacteria. If, after a primary elimination of self-reactivity along one or other of the lines described, antibody-producing capacities could be transferred from one clone to another, this would have some obvious advantages in relation to immunological memory.

To summarize this discussion of the basis of self-recognition and tolerance, I have given reasons for believing that the only possible type of approach is by a "selective" theory of immunity which must be developed on a cellular, and probably on a clonal, basis. Within these limitations there are several possible alternatives in regard to the number of potential patterns carried by a single cell or clone and the means by which patterns complementary to body components can be inhibited or eliminated.

This is not the place to elaborate other aspects of immunological theory, nor would I have any novelty to offer if I did so. It is only in relation to the first stage of the immune process—the recognition of foreignness with its implication of pre-adapted patterns—that there is serious controversy. Once the immunological competence of a cell has been unmasked (according to selectivity theory) or produced by antigen (according to instructive theory), the interpretation of phenomena arising subsequently, including antibody production and immunological memory, hardly differs from one theory to the other.

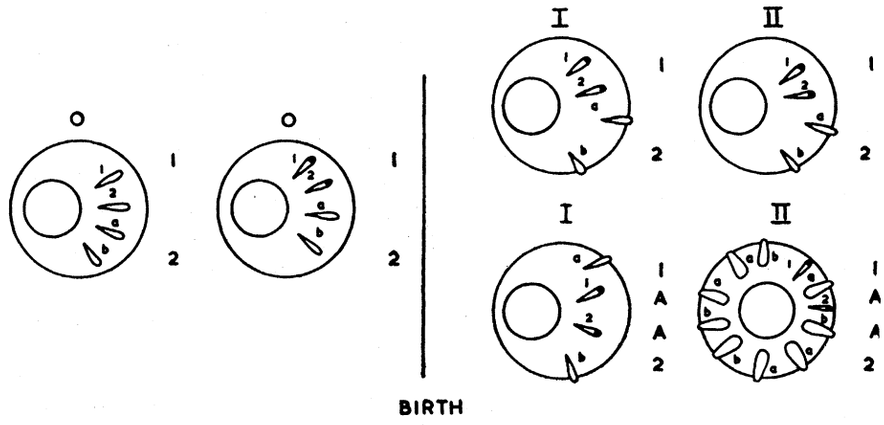

Fig. 2. Diagram indicating the possible validity of a clonal-selection hypothesis, with fairly numerous potentialities per cell. (1, 2) "Self"-type antigenic determinants and the corresponding reactive units in the cell. (*A*, *B*) Foreign antigenic determinants; (*a*, *b*) the corresponding cellular units. The changes on exposure to the antigenic determinants shown outside the cells are described in the text.

Evolution of the Immune Process

To anyone with a speculative turn of mind there are very interesting problems in the evolutionary origin of the processes we have been considering. It is not difficult to persuade oneself that the development of immunity against pathogenic microorganisms is of survival value, but for many years I have found this a rather unsatisfying and naive approach. The phenomena of tolerance and of the nonantigenicity of self-components seem to be more basic than those of postinfectious immunity. I cannot conceive that they evolved from an earlier process concerned only with protection against recurrent infection, whereas I *can* conceive that the converse took place.

The question then becomes, Why and how, in the evolutionary sense, did warmblooded vertebrates develop the capacity to recognize the presence of foreign configurations in the body and to initiate a process of eliminating any cells so recognized?

There are several possible lines of thought here, but the only one I find attractive concerns the significance of somatic mutation in metazoan organisms, particularly in complex, large, and long-lived vertebrates.

It is axiomatic that mutation supplies the raw material for evolution. In other words, the whole evolutionary process depends on the possibility of error in replication that is necessarily associated with mitotic division. This possibility of error must be at least equally present in the replication of somatic cells. One of the requirements, therefore, for the success of a large multicellular animal is that any potentially dangerous mutations in proliferating somatic cells should be eliminated before they can cause serious damage in the evolutionary sense. The most serious effect that could be due to a somatic mutation or series of mutations is, of course, malignancy, but there are other possibilities as well which might have undesirable effects in special situations.

According to present-day thinking, every mutation must result in the appearance of a protein of pattern different in some respect from a normal protein. This follows simply from the absence of any known way in which a change in nucleic acid structure can influence phenotype except via a protein, usually pictured as an enzyme. The existence of immunological changes or deletions in somatic mutant (malignant) cells has been described on many occasions, and there is evidence of serological and cellular immunological responses to some spontaneous tumors. There could well be survival advantage in being able to recognize the presence of cells carrying wrong molecular con-

figurations and to eliminate them from further proliferation. It would profit the organism to maintain a surveillance over the orthodoxy of its chemical structure and to stamp out heresy before it could spread. To be able to do this would require just such a mechanism as is called for by the facts of immune tolerance. On this view, the faculty of immunological recognition becomes an intrinsic part of the homeostatic controls that maintain the body as a going concern. And once in existence, it could clearly provide the basis for the development of anti-infectious immunity.

To provide an evolutionary interpretation of a physiological process, however, requires something more than the demonstration that it has survival value to the possessor. We must also offer some hint as to how it might have developed from pre-existent faculties. Here there is an obvious suggestion that immunological recognition is an inevitable derivative of the basic requirement for any integrally organized, multicellular organism—the existence of an elaborate system of information and control, of receptor, effector, and feedback mechanisms, that is needed to maintain morphological and functional relationship between cells. Some of this—perhaps a large proportion—must be mediated, as Paul Weiss has suggested, by complementary pattern relationships between macromolecular constituents. This may seem to be a very thin speculation which could not possibly stimulate a line of experimental inquiry. It may be foolish to attempt to interpret immunity in terms of processes like differentiation and morphogenesis that we know extremely little about, but the converse possibility, that light might be thrown on differentiation from work in the more experimentally amenable field of immunity, is too inviting to be neglected. There has been a recent suggestion, moreover, at the experimental level which seems to point in just this direction.

During the last few months I have been deeply interested in an obscure organ of the chick embryo. The bursa of Fabricius is a diverticulum from the hind-gut which develops a complex of folds covered with entodermal epithelium and containing loose mesodermal cells and frequent regions of hemopoietic tissue. About the 14th to 16th day of incubation, nodules of rapidly growing epithelium develop and expand into the mesodermal tissue. According to Ackerman and Knouff (*10*) (and all our own observations are in accord), these epithelial cells begin to lose their epithelial packing, and about the 18th or 19th day, the center of the nodule becomes indistinguishable from a germinal center of lymphoid tissue. Subsequently, a lymphoid structure somewhat analogous to the thymus develops and appears to play an important part in antibody production in the chicken. Like the thymus, it reaches a maximum when the chicken is about 4 months of age and thereafter atrophies. When we take into account the epithelial origin of the thymus, we have a shadow of justification for wondering whether the antibody-producing system, the immunologically competent cells, may not have been derived, phylogenetically and ontogenetically, from cells which had once had, as it were, morphological responsibilities. The immunological significance of the thymus and the bursa of Fabricius is one of our present main areas of investigation.

Conclusion

My part in the discovery of acquired immunological tolerance was a very minor one—it was the formulation of a hypothesis that called for experiment. The clinical and experimental facts that have been recognized since Medawar and his colleagues opened the way have emphasized again and again the importance of self-recognition in immunology. This, I believe, is something which in its turn calls for new hypotheses. In this article I have tried to present as briefly as is consistent with reasonable clarity my thoughts about the theoretical implications of immunological tolerance and self-recognition. The hypotheses that have been stated are modifications of earlier hypotheses, modifications enforced by the advance of experiment and observation. I have only at two points introduced new factual material, and I have done this to illustrate that the approach being used is not meaningless speculation but suggests experiments that may lead to its modification or rejection.

I have introduced ideas about the evolution of the process of self-recognition because, as a biologist, I believe we know less about the processes of differentiation and morphogenesis than about any other major field in biology. There is an insistent suggestion that immunological self-recognition is derived from the processes by which morphological and functional integrity is maintained in large and long-lived multicellular organisms. This may be a mere cobweb of phantasy, but in my more optimistic moments I can hope that it may also function like Ariadne's thread to guide us effectively through part of that biological labyrinth, the process of differentiation.

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