Foundation Symposium

Ontogeny of Acquired Immunity

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Introduction

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One incentive for studying the ontogeny of the immune response is that it may help to illuminate the errors of development which lead to immune deficiency states in man. However, the interaction has not been entirely in one direction, for studies of human disease had already provided hints that the immune system was built up from components derived separately from the thymus and from some bursal equivalent, an idea strongly influenced by the anatomical separation of the two components in birds and finally vindicated by the discovery of marrow- and thymus-derived lymphocytes in rodents. This conference will provide an opportunity to examine whether the simple schemes derived from the study of immune responses in rodents apply to mammals generally and whether they provide a rational basis for the understanding and treatment of deficiency states in man. Another important topic for consideration will be the immunological relationship between the mother and the foetus. We must re-examine the privilege enjoyed by the foetus in the light of suggestions that blocking factors may be important in masking the immunity which develops in the mother against paternal antigens. This consideration will no doubt, in turn, lead us to discuss the possibility that blocking factors may also play a part in the mechanism of classic immunological tolerance.

We are all very grateful to the Ciba Foundation, and particularly to Dr Ruth Porter, for having conceived and organized this conference. Those fortunate enough to be enjoying the hospitality of the Foundation will be able to bring each other up to date on the impressive record of experimental work which has accumulated on the normal development of the immune response; they will also no doubt be equally impressed at the end of the meeting by the complexities which face clinicians when observing the consequences which follow the failure of normal development.

Contents

What precedes clonal selection?

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This paper is not concerned with the ontogeny of the anatomical and morphological arrangements of the cells that belong to or interact with the immune system, nor with the structure and pathway of the signals to which these cells respond. I shall restrict the term 'immune system' to the totality of antibody molecules and of lymphocytes that produce such molecules. I shall assume that all immunoglobulins are antibody molecules, including those that somehow function as receptors on the membranes of lymphocytes. I shall consider that lymphocytes of all sizes, thymocytes, antigen-sensitive cells, T cells, B cells, memory cells, plasma cells, etc., all belong to a dynamic population of clones of lymphocytes that interact amongst themselves and can respond to signals mediated by antigens and antibodies. By 'dynamic' I mean that the population is in continuous flux: new signals arise from stem cells, some cells are triggered, others are killed, some cells proliferate, some express their potentialities, others are suppressed, and so on. By the ontogeny of the immune system I shall understand all developments of this system from early embryogenesis until the death of the individual.

THE CLONAL SELECTION THEORY

The clonal selection theory (Burnet 1959) states that all antibody molecules synthesized by one lymphocyte are identical, particularly with respect to the specificity of their combining sites. More precisely, that one lymphocyte expresses only two v-genes, one for the variable region of the light chain and one for the variable region of the heavy chain. Furthermore, the theory postulates that a lymphocyte becomes committed to this restricted synthetic expression prior to the arrival of a fitting antigen. Thirdly, it states that the selection of

precommitted cells by antigen can lead to cell proliferation, and thereby to clonal amplification of selected synthetic potentialities. The clonal selection theory has successfully withstood all experimental attempts to disprove it. In fact, many experiments designed to test the theory have added support to its postulates. It has been shown by Dutton & Mishell (1967), Ada & Byrt (1969) and Wigzell & Andersson (1969) that a given antigen can remove a small fraction of antigen-sensitive lymphocytes from a lymphocyte population *in vitro*, leaving the remaining cells unresponsive to that antigen but capable of responding to other antigens. Several observations indicate that the cells of a clone breed true; that is, that the cells of one clone all produce the same species of antibody molecule. Thus, the continued production of homogeneous antibodies to streptococcal or pneumococcal polysaccharides (Krause 1970; Haber 1972) implies the expansion of large clones of cells secreting the same antibody product. Also, by serial transfer of one clone of antigen-sensitive and antibody-producing cells into successive irradiated recipient mice, Askonas, Williamson & Wright (1970) have shown a continued production through many cell generations of identical antibody molecules. This does not mean that mutant cells, or variant cells, synthesizing the product of a modified pair of v-genes, do not arise in a clone. Studies by Oudin (1969) of idiotypic specificities of antibodies at different times during the course of immunization imply that variant antibody molecules of the same idiotype arise and that some variant cells have selective advantages.

THE ANTIBODY REPERTOIRE

I shall assume the basic postulates of the clonal selection theory to be correct. When an antigen confronts the immune system, it impinges upon a repertoire of available antigen-sensitive lymphocytes. Each of these cells displays receptors of one antibody specificity only. The population of cells represents the repertoire of synthetic capabilities of the immune system at a given point in time. The repertoire will be subject to continuous qualitative and quantitative flux. New items will be added by the entry of differentiating stem cells and by mutation, others will be amplified by immunogenic and other mechanisms leading to cell proliferation. On the other hand, items may disappear from the available repertoire by cell death and by tolerogenic and other suppressive mechanisms. What is needed is an expansion of the clonal selection theory with a set of basic concepts concerning the ways in which the repertoire arises and the elements that govern its maintenance and variation.

Selection among the items of a repertoire requires the prior establishment of

a repertoire. A fundamental choice is needed between two types of theory, (1) a 'germ-line' theory claiming that (in spite of rare somatic mutations and variations) the overwhelming part of the available repertoire results from the expression of v-genes already present in the zygote from which the individual has arisen, and (2) a 'somatic' theory claiming that (in spite of the expression of a small number of v-genes already present in the zygote) the overwhelming part of the available repertoire results from the selection of cells expressing mutated or modified v-genes that have originated spontaneously in the descendants of stem cells before immunogenic stimulation, or among the cells of a clone responding to a stimulus, or both. It is clear that the available repertoire to some extent arises while the immune system functions, and that therefore the function of the system cannot be studied separately from the ontogeny of its repertoire. A discussion of these matters would be facilitated if we had some knowledge about the size of the repertoire; that is, about the order of magnitude of the number of different antibody molecules that the lymphocytes of one animal can produce.

There are various observations from which an impression of the size of the repertoire may be gained. The following indications suffice for the present discussion. Antibody assays show that the concentration of antibody molecules of a given reactivity in the serum of immunized animals can be several thousand or even several million times higher than their concentration in the serum in normal animals. If we assume that the gamma globulin of normal serum is a mixture of all molecular members of the repertoire, this finding suggests that the repertoire may exceed one million. A similar conclusion can be drawn from experiments by Kunkel (1970) showing that a given human myeloma idiotype occurs with a frequency of less than one in a million among normal serum globulin molecules. Considering the ease with which any rabbit can be induced to produce anti-idiotypic antibodies to the antibody molecules evoked by bacterial antigens in other rabbits (Oudin & Michel 1969; Kelus & Gell 1968), we could ask whether normal serum may contain antibody molecules reacting with the idiotypic determinants present on other antibody molecules in the same serum. The concentrations a and i molecules per ml of these reactants would be in equilibrium with c complexes per ml. If we permit 1 % of the antibody molecules carrying a given idiotypic determinant to form a complex with a fitting antibody molecule, the relation $ai = Kc$ would permit $a = 0.01\ K$. Considering only antibody molecules of an affinity to this idiotypic determinant corresponding to an equilibrium constant $K = 10^{12}$ molecules per ml (or 1.6×10^{-9} mole), the permissible concentration of this species of antibody molecules would be 10^{10} molecules per ml of normal serum. As normal human serum contains about 5×10^{16} immunoglobulin molecules per ml, the number of different antibody

populations would have to be larger than 5×10^6. It should be noted that more realistic models assuming the presence of a variety of antibody molecules of different affinity towards any given idiotypic determinant, all lead to estimates of a repertoire higher than 5×10^6 in man. Such models imply a degenerate network of idiotypic determinants and fitting antibody combining sites: a variety of different antibody molecules would fit any given idiotypic determinant whereas many different idiotypic determinants would fit any given antibody combining site.

It might be thought that the immune system develops tolerance to all idiotypic determinants of its own immunoglobulin molecules so that antibodies to idiotypic determinants present in the same serum do not occur. It should be clear, however, that this would imply an enormous purge of the potential repertoire (Jerne 1960), and would lead to much higher estimates of its size. The concept of a repertoire must be more clearly formulated before attempts can be made to arrive at more meaningful estimates of its size. We must distinguish between the *potential repertoire* of specificities that could arise given the genetic constitution of the zygote from which the animal develops, and the *available repertoire* embodied in the cells that can respond to antigens at a given moment in the life-time of the animal. The potential repertoire of animals of one inbred strain may be smaller than that of the entire animal species, because of v-gene polymorphism. The available repertoire at one point in time may be considerably smaller than the total repertoire available to an animal at one time or another during its entire life-time. It seems reasonable to assume that the size of the available repertoire increases during ontogeny and that it will tend towards a maximum in the normally functioning immune system of the adult individual. Furthermore, the question of the relation between T cell repertoire and B cell repertoire needs to be examined.

THE AVAILABLE REPERTOIRE

Rabbits immunized with a strain of *Salmonella* (Oudin & Michel 1963) or of *Bacillus proteus* (Kelus & Gell 1968) all make specific antibodies, but the sets of idiotypic determinants of the antibody molecules produced by any one rabbit differ from those of the antibody molecules produced by any other rabbit. In other words, each rabbit makes use of a different repertoire when responding to the same antigen. Though not inbred, many of these rabbits were of the same allotype. The idiotypes of the antibodies to a given antigen produced by first-generation offspring rabbits were no more similar to those occurring on the antibodies produced by a parent than to those occurring on the antibodies pro-

duced by unrelated individuals (Kelus & Gell 1968; J. Oudin & G. Bordenave, personal communication 1971). As an idiotypic determinant represents the antigenic properties of a given pair of variable regions of the polypeptide chains of an antibody molecule, it follows that the v-genes expressed by the responding cells of one rabbit are different from those expressed by the cells of another rabbit responding to the same antigen. These results not only demonstrate the enormous plasticity of the immune system in its ability to use different v-genes for producing different antibody molecules of similar specificity, but they also show that an individual makes use of only a small part of the potential repertoire which its inherited v-genes could have given rise to. A. R. Williamson & W. Kreth (personal communication 1971) have found that individual CBA mice, responding to a hapten (2,4-dinitrophenol, DNP, or 4-hydroxy-3-iodo-5-nitrophenyl acetic acid, NIP) attached to bovine gamma globulin, each produce more than a hundred different antibodies to the hapten and that the two sets of such antibodies to the same hapten produced by two mice are almost entirely different, so that there will be hardly more than one or two molecular species of antibody that occur in both sets. This experimental demonstration reinforces the conclusion that individual animals make use of widely differing repertoires when responding to an antigen, and that this is true even for the genetically virtually identical animals of the same inbred strain of mice, reared under the same conditions.

REPERTOIRE SUPPRESSION

How are we to interpret the findings (1) that the antibody repertoire available to an individual animal is very large (e.g. $> 10^6$), and (2) that each individual responding animal makes use of only a small part of the potential repertoire permitted by its germ-line genes? Two or three possibilities present themselves. The population of lymphocytes may, as it arises, express the entire potential repertoire. In that case, either the immune system does not make use of more than a small part of its available repertoire when responding to an antigen, or the repertoire is reduced drastically by suppressive mechanisms, leaving different available repertoires in different individuals. On the other hand, the entire potential repertoire may never be expressed in one individual, but only a sample of it. Or, thirdly, the repertoire actually used by a responding animal may be that which is left over after the expression by its lymphocytes of part of the potential repertoire, after a reduction of this expressed repertoire by suppression, and after a further reduction to the set of cells that antigen actually succeeds in stimulating. Various types of suppression are known. A rabbit

of allotypic genotype, say, a_1/a_3 produces antibody molecules of allotype a_1 as well as antibody molecules of allotype a_3. By immunizing the mother with globulin of paternal allotype, or by injecting anti-paternal-allotype antibody neonatally, the expression of the paternal allotype can be suppressed (Dray 1962; Mage 1967). This suppression lasts for many years and shows that practically half the lymphocytes that arise (those attempting to express this allotype) are suppressed. From a large variety of experiments in rabbits and in mice by Jacobson, Herzenberg, Riblet and Herzenberg (1972) it may be concluded (1) that the suppressed cells committed to the expression of an allotype are probably not eliminated, since the production of immunoglobulin of this allotype is resumed on transfer of cells from a suppressed animal to an irradiated recipient animal, and (2) that continued allotype suppression is probably effected by the presence of anti-allotypic T cells.

I wish to stress this suppressive effect involving the antigenic properties of antibody molecules, because these may play an important role in the development, maintenance, and shift of the repertoire available to an individual. We might generalize, tentatively, that both certain concentrations of antibodies, as well as the emergence of certain T cells, exhibiting antibody combining sites directed against antigenic determinants of antibody molecules (allotypes, idiotypes), can suppress the 'expression' of such molecules by B cells. If T cells can suppress such B cells, the target of this type of suppression would seem to be the antigenic determinants of the receptor molecules of these B cells, since these are the only targets that distinguish different B cells. Furthermore, it would seem that these targets are recognized by the combining sites of the T cell receptors. It is conceivable that the expression of many idiotypic determinants is normally suppressed in this same way, and that the available repertoire is correspondingly reduced. Conversely, we may conclude that antibodies (or B cell receptor molecules), by their allotypic determinants, suppress T cells of certain specificities that would emerge under conditions of allotype suppression. This could be taken as an example of induction of tolerance by antigens, including idiotypic antigenic determinants. Thus, Iverson & Dresser (1970) have shown that a mouse myeloma protein can be made immunogenic by attachment of hapten to the molecule and can provoke the formation of anti-idiotypic antibody in normal mice of the inbred strain in which the myeloma had arisen, whereas the injection of unaltered myeloma protein into such mice leads to tolerance to its idiotypic determinants.

The above examples (which could be multiplied) show that lymphocytes committed to the expression of a given antibody molecule A can be suppressed (1) by other antibodies, either humoral or functioning as receptors on other lymphocytes, possessing combining sites directed against the antigenic deter-

minants of A, and (2) by other antibodies possessing antigenic determinants fitting the combining sites of A. It therefore seems likely that antibodies arising from antigenic stimulation of a set of lymphocytes suppress other lymphocytes, and that the entire system represents a complex interacting 'network' of expression and suppression of potentialities. The available repertoire would represent the balance resulting from this continuing process.

In these considerations, I have left out all the many forms of induced tolerance, as well as other known examples of suppression of potentialities, such as the fact that immune responsiveness to a given antigen can be suppressed by passive IgG antibody directed against the same antigen, and the finding by Askonas & Williamson (1972) that established clones of cells producing a given antibody can prevent the same antigen from stimulating other cell clones. Another example is self-tolerance which implies (most obviously in F1 animals that are heterozygous for histocompatibility antigens) that part of the potential repertoire of the parental genes is suppressed. All in all, it is clear that the immune system exerts self-control by suppressive mechanisms, and that these suppressive actions restrict the available repertoire.

SOURCE OF THE REPERTOIRE

If, conceptually, we were to place the potential repertoire in the germ-line — that is, if we assume that all structural v-genes for the antibodies that an individual may potentially express are already present in the DNA of the zygote — then we would be tempted to conclude that only a small fraction of these are actually expressed in the available repertoire of an individual. Otherwise, genetically identical or related animals would be expected to produce, at least in part, identical antibody molecules to the same antigen.

This repertoire restriction appears to make a germ-line hypothesis untenable. We must admit that the number of v-genes required to encode an available antibody repertoire is already uncomfortably large, if it has to be located in the germ-line genome. The situation becomes worse if we consider that a germ-line theory would require the presence, in the genome of the zygote, of the entire potential repertoire, which is far larger. A collection of genes can be kept intact in evolution only if each gene is used and if its absence impairs survival to some degree. It is hard to believe that the presence of every gene in the large set that is required to encode the potential repertoire is essential. We cannot be quite certain of this, however, for even if a given light chain v-gene is not expressed in combination with any of several heavy chain v-genes, it may find expression in combination with other heavy chain v-genes. In spite of this consideration, the