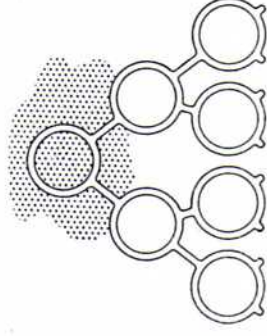


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THE CLONAL SELECTION THEORY OF ACQUIRED IMMUNITY



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IV

THE CLONAL SELECTION THEORY OF ANTIBODY PRODUCTION

In the last chapter I gave a short account of what I regarded as the important experimental and observational facts of the immune responses. In this, I want to discuss the possible ways in which a general theoretical account of the findings can be constructed. It would be difficult, presumptuous, and inappropriate to this series of lectures to attempt to deduce directly from the facts the most likely theory of antibody production. Instead, I shall consider only points of view that have already been expressed, beginning with Ehrlich's side-chain theory.

1. *The side-chain theory of Ehrlich*

This is, of course, based on an old-fashioned picture of cell metabolism, but its essence can be expressed in terms that would be intelligible today. Ehrlich assumed that all foreign antigens, and especially the bacterial toxins with which he was primarily concerned, damaged body cells by combining with pre-existent chemical patterns (side chains) normally concerned with some metabolic function. The union was specific and irreversible and the damage to the cell could only be overcome by casting off the blocked side chain and replacing it with a new one. Such regeneration was regarded as conforming to the general law of over-compensation that had been formulated by Weigert, and would therefore tend to produce an excess of side chains which were liberated into the body fluids.

The side-chain theory implies the existence of cellular patterns complementary to all possible antigenic determinants. With Landsteiner's studies of serological specificity

it became clear that an enormous variety of cell receptors would need to be provided, so many that Ehrlich's theory was discarded. Landsteiner in 1936 concluded that antibodies are produced by some modification of the process of protein synthesis by which the proteins (antibodies) are adapted as closely as possible to the immunizing antigen.

2. *Haurowitz-Pauling (direct template) theory*

With the development of a better understanding of protein chemistry, Landsteiner's work became the basis for the first of the current theories of antibody production, for which Alexander, Mudd and Haurowitz are usually given joint credit. Its most fully developed form is due to Pauling (1940) and it is probable that most immunologists still find it the most convenient framework for thought on practical matters. Following Talmage (1957), the theory can be called the 'direct template' theory. It holds that antibody molecules have their specificity determined by being synthesized against a template of the antigen molecules themselves. In Haurowitz's (1952) view the specificity is conferred not at the synthesis of the polypeptide chain but at a later stage when the folding of the chain is taking place. In this way a complementary configuration is developed at one or more, probably two, sites on the coiled-up globulin, which 'fits' accurately with the antigen or its particular determinant against which it was moulded.

It is doubtful whether anyone has attempted to cover all the main fields of immunology in relation to this view. Clearly accessory hypotheses not very closely dependent on the primary one are needed to deal with normal antibodies, immunological tolerance, homograft immunity, etc. Its chief virtue has been the stimulus it has provided for the experimental study of specificity of chemical structure, and of the persistence of foreign organic material in various tissues. The important implications of this theory in which it differs from others are:

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- (i) antibody can be produced only while antigen or its determinants remain as such in the tissues:
- (ii) antibody can be produced against any type of organic pattern provided it is presented on an appropriate carrier macromolecule, most commonly protein in character.

3. *Burnet-Fenner indirect template theory*

In 1949, Burnet and Fenner published a comprehensive discussion of the available information in terms of what has been referred to as an 'indirect template' theory. This was elaborated but not basically altered by Burnet in 1956.

The theory was an attempt to include in a general formulation those features for which the direct template theory has no point of contact. We held, for instance, that the two most important features of immune reactions were (a) that body components are immunologically inert and that an equivalent tolerance to foreign antigens can be demonstrated if they are introduced at an appropriate stage in embryonic life and (b) that antibody production can continue long after the effective antigen has disappeared from the body.

The indirect template theory still adopted the view that antibody production was an active response of cells to the entry of foreign organic material, and for this theory the existence of natural antibody was an accidental and irrelevant circumstance. The new features were essentially three in number:

- (i) To account for the non-antigenicity of body components these were assumed to carry 'self-markers'; at some point in the antibody-producing sequence a 'recognition unit' was postulated to act as a means of detecting material carrying self-markers and deflecting it from the possibility of immune response;
- (ii) To account for the persistence of antibody-producing capacity it was postulated that a 'genocopy' of the antigenic determinant was incorporated in the genome

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of the stem cell concerned, so allowing the indefinite production of descendant antibody-producing cells;

(iii) This incorporation of pattern determinants into the genetic structure of antibody-producing cells provided some basis for the changes in antibody character that may result from secondary antigenic stimuli or simple lapse of time.

This theory had its main success in predicting that immunological tolerance following prenatal injection of appropriate antigens should be experimentally demonstrable. On the other hand, the concept of self-markers and recognition units was seen to be a clumsy one that could only be a rough paraphrase of the actual mechanism.

4. *Jerne's natural selection theory*

In 1955 Jerne published a new and strikingly different conception of antibody production in which, for the first time since Ehrlich, natural antibodies were seriously considered in relation to 'true' antibodies. [Jerne discarded altogether the view that antibody production was a direct result of the entry of an antigen into body cells.] He held that the gamma globulin molecules of the plasma represent a population comprising carriers of all the reactive sites needed to unite with *any* potential antigenic determinant except those already existing in accessible components of the body. The function of the antigen which enters the body from without is to act as 'a selective carrier of spontaneously circulating antibody to a system of cells which can reproduce this antibody'. It is assumed that once antibody is taken into cells of the antibody-producing system, replicas of this natural antibody will be produced. With the liberation of this crop of new antibody a second injection of antigen will find many more antibody-producing cells and give a stronger 'secondary' stimulus to antibody production. Like the direct template theory, the natural selection theory leaves a considerable range of questions unanswered. It is not clear, for instance,

whether the multiplicity of antibody reactivities in gamma globulin molecules is a random character due to a simple indeterminacy of pattern in the molecules produced or whether there are clones of cells which produce pattern *a*, others pattern *b*, and so on for the thousands of patterns required. This second possibility would naturally also demand a corresponding range of coding for pattern in the nucleic acids of the cells forming the clones in question. Jerne appears to favour the first alternative and to assume that when a (partially denatured) globulin molecule which happens to have pattern *x* is brought to any one of the antibody-producing cells this cell is stimulated to produce globulin of *definitive* pattern corresponding precisely to the random pattern of the 'natural' antibody molecule. This appears to be out of line with any of the current ideas on protein synthesis and is one of the main points which led to the development of a 'clonal selection' theory.

5. *The clonal selection theory*

The great contribution of Jerne's theory was that it drew attention to the theoretical possibility that the recognition of self from not-self could be achieved in another fashion than by the recognition of 'self-markers'. As Talmage (1957) points out, Ehrlich's side-chain theory was in many ways the logical equivalent of Jerne's concept. The side-chain theory was quietly shelved as evidence accumulated of the vast variety of antibodies that could be produced, some against non-biological determinants such as arsenic acid. It seemed, and to most immunologists still seems, inconceivable that all types of antibody could be pre-existent in the normal complement of gamma globulin molecules. Nevertheless, if Jerne is correct that a comprehensive range of molecules corresponding to all organic patterns other than those of body components is present in the gamma globulin population, this would be an effective and much more elegant way of accounting for the differentiation of self from not-self.

The outstanding difficulty in accepting Jerne's theory is the claim that when a given type of natural antibody molecule is brought to a cell by antigen, the cell then proceeds to make more natural antibody molecules of the same type. The facts that, in general, union with specific antigen results in partial denaturation of antibody globulin, that there is no nucleic acid in antibody and that homologous antibody is very rapidly broken down when it is taken into a cell (Humphreys and Macfarlane, 1954), all speak against the concept. Talmage (1957) pointed out that it would be more satisfactory if the replicating elements essential to any such theory were cellular in character *ab initio* rather than extracellular protein which can replicate only when taken into an appropriate cell. He did not elaborate this view but clearly regards it as the best current basis for immunological theory. Our own view is that any tenable form of Jerne's theory must involve the *existence of multiple clones of globulin-producing cells*, each responsible for one genetically determined type of antibody globulin. This immediately poses the question of how the antibody-antigen complex can reach the cells, which are genetically determined to produce the corresponding type of antibody molecule. Clearly it would simplify matters a great deal if the antigen were in a position to react with natural antibody or a pattern equivalent thereto on the surface of the cell which produced it.

* [This is the crux of the clonal selection hypothesis. It assumes that in the animal there exist clones of mesenchymal cells, each carrying immunologically reactive sites corresponding in appropriate complementary fashion to one (or possibly a small number of) potential antigenic determinants. This provides a population of cells which, when an appropriate stage of development has been reached, are capable of producing the population of globulin molecules which collectively provide the normal antibodies. When an antigen is introduced it will make contact with a cell of the corresponding clone, presumably a lymphocyte, and by so doing

stimulate it to produce in one way or another more globulin molecules of the cell's characteristic type. The obvious way of achieving this is to postulate that stimulation initiates proliferation as soon as the cell in question is taken into an appropriate tissue niche, spleen, lymph node or subacute inflammatory accumulation.

The reasons for temporarily or permanently discarding the 'indirect template' hypothesis in favour of this clonal selection approach were cumulative and largely indirect.

(i) In discussing the biology of malignant disease the importance of clonal phenomena in the expendable cells of the body became increasingly evident (Burnet, 1957*b*).

(ii) Interest in the auto-immune complement fixation test developed in my laboratory by Gajdusek (1957) led to the tentative conclusion that the 'antibody' concerned was an adventitious mixture of globulin molecules produced by clones of cells which had undergone some type of somatic mutation.

(iii) Workers in the field of adaptive enzyme production in bacteria are now unanimous that the pattern of the adaptive enzyme is genetically determined and not a 'transcript' of pattern introduced by substrate or inducer molecule. This destroyed the significance of any analogy between adaptive enzyme production and the indirect template hypothesis of antibody production.

(iv) Changing views on the life-history of the lymphocyte have made it admissible to postulate that a lymphocyte appropriately stimulated could give rise to a clone of descendant cells. In particular, work from Florey's laboratory (Gowens, 1957) shows that lymphocytes can undergo more than one cycle between tissue and circulation. Simonsen (1957), working with avian material, showed that adult fowl blood contained circulating cells which could settle and proliferate in

