The Breakdown of Immunological Tolerance

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Much of the work involving the experimental breakdown of immunological tolerance can be understood only if the distinction between antigenicity and immunological specificity is clearly recognised. Antigenicity is the capacity of a substance to induce an immune response: specificity defines the particular chemical grouping against which the immune response is directed. In general, antigenicity is a property confined to proteins or polypeptides; specificity can involve almost any variety of organic or inorganic chemical grouping, or even a single element.

In many instances the specific groups on an antigen are a part of the antigenic protein itself: such an antigen may be designated simple. In contrast, the specific groups of many antigens are not part of the protein itself but are composed of some entirely different chemical grouping referred to as a hapten. Such antigens are termed complex. They may be either natural (e.g. the ABO blood group substances in which the determinant, or haptenic group, is polysaccharide) or artificial, in which the non-protein moiety has been linked in vitro or in vivo to the carrier protein. A widely used example is dinitrobenzene.

This distinction between the antigenicity of the carrier protein and the specificity of the hapten is fundamental to our understanding of many of the instances in which immunological tolerance appears to break down. The reason is that, in general, tolerance is a phenomenon related to antigenicity, not to specificity; that is, it relates to the carrier protein and slightly, if at all, to haptens. This is readily shown by the experimental induction of tolerance with a protein-hapten conjugate. Reimmunisation of the animal with the same conjugate induces no response, but specific antibodies to the same hapten are readily obtained if immunisation is performed with a conjugate of the same hapten but with a different protein.

It is now easy to see one method by which autoantibodies can be experimentally induced. If, for example, rabbits are immunised with a fine suspension or homogenate of rat liver, antibodies are produced that react not only with the immunising tissue but also with extracts of rabbit liver, whereas similar
immunisation of rabbit with rabbit liver is without effect. This is presumably because some of the haptenic determinants on the two hepatic antigens are identical or closely related, and consequently the antibody raised in the rabbit against rat reacts with both. No response is obtained on immunisation with rabbit liver because the animal is tolerant to the protein carrying these haptenic determinants (Asherson and Dumonde, 1962).

A similar but far more important example of this principle is shown by immunisation of experimental animals with a variety of micro-organisms that carry cross-reacting antigens of various kinds. The first is the antigen in several strains of β haemolytic streptococci that cross-reacts with an antigen in human and rabbit myocardium. This can be shown by several immunological techniques but most strikingly by immunofluorescence. Rabbits immunised with such a strain of streptococcus produce antibodies that give excellent fluorescent staining with both human and rabbit myocardium, including that of the immunised rabbit itself (Kaplan, 1962). The second example is the induction, again in rabbits, of antibodies reacting specifically with an antigen in the colon as a result of immunisation with certain strains of E. coli. (Holborow et al., 1963).

In both these examples it is thought that the successful induction of autoreactive antibodies depends upon the immunological cross-reactivity of the hapten associated with the organisms, on the one hand, and the tissue, on the other. The role of these bacterial-born haptens in the genesis of human autoimmune disease is still unknown but there can be little doubt that the anti-myocardial antibodies found in a high proportion of patients with acute rheumatic fever arise in response to the immunological stimulus of the preceding streptococcal infection (Kaplan and Svec, 1964).

Autoimmunisation is also theoretically possible if an endogenous hapten should by chance become linked to an exogenous protein such as a bacterium. This can certainly arise experimentally: for example, plant polysaccharides such as agar are not antigenic when given alone but excite an excellent immune response in rabbits when given as a vaccine adsorbed on to streptococci (Glynn and Holborow, 1952). Attempts to induce a similar immune response to connective tissue polysaccharides, such as hyaluronic acid, have proved consistently unsuccessful, which suggests that a form of immunological tolerance can also involve at least some types of hapten such as autochthonous polysaccharides. Here, however, the failure to obtain an immune response may perhaps be attributed to the concurrent presence of free hapten which contrasts with the tolerance to protein antigens that seems to outlast considerably the presence of free antigen in the body.

From what has already been said it could be considered that the genesis of
an effective antigen by the interaction of a foreign hapten with a host protein is unlikely, since the host is tolerant to its own protein. It is evident, however, from the vastness of the problem of the industrial dermatoses, and of drug reactions in general, that potent antigens can and do arise from such interactions, despite the tolerance to the carrier protein. It must, therefore, be presumed that the interaction between the protein and the hapten results in sufficient alteration of the surface configuration to render it no longer recognisable as self by the immunological apparatus. This brings us to the important question: 'How much alteration must an endogenous protein undergo before it ceases to be recognisable as self and, therefore, excites an immunological response?' This may well vary from protein to protein, and certainly varies from individual to individual. It would be most remarkable if such a complex biological reaction did not show the range usually found with biological variables. Therefore, individuals may be accepted as varying in their sensitivity to change in self antigens from the highly resistant, requiring a considerable change, to the highly sensitive, requiring very little. This range in sensitivity is well brought out by a study of the incidence and nature of the rheumatoid factors, so called because of the frequency with which they occur in patients with rheumatoid arthritis (Ball and Lawrence, 1961). These factors are now known to be antibodies directed against determinants present in gammaglobulins, and revealed by various degrees of uncoiling of the molecule, i.e. denaturation (Glynn et al., 1957). Since some degree of denaturation of gammaglobulin is a common occurrence, it is almost certain that we are all being exposed to this type of antigenic stimulus; yet only a small proportion of individuals develops these antibodies that, in a few, may reach extremely high titres.

It may well be objected that antibodies against foreign haptens arising as a result of alteration of endogenous proteins in consequence of the hapten-protein interaction can hardly be regarded as auto-antibodies. This would be strictly true if the specificity of the immune response were restricted to the exogenous haptenic determinants. There is, however, no reason why this should be so. Once the carrier protein has been sufficiently altered to allow it to pass the 'self recognition' screen, other determinants belonging to the protein itself may then become involved in the specificity of the response. Let us consider, for example, an endogenous antigen with potential specificities A, B, C, D, E. No immune response occurs because the individual is tolerant to the protein that carries these determinants. Now we envisage an interaction to occur between this carrier and some foreign hapten, such as chlorodinitrobenzene. As a result of this reaction, not only has a new determinant been added to the molecule, but the carrier portion has been sufficiently altered to render it
no longer recognisable as 'self'. The immunological response may, therefore, involve not only the newly added haptenic determinants but some of those present on the unaltered molecule, i.e. A, B, C, D, or E. Some of the resulting antibodies could therefore react with these determinants on the unaltered native antigen, i.e. the response can be classified as autoimmune. An excellent example has recently been provided by Weigle (1965). Rabbits were immunised with rabbit thyroglobulin coupled to two haptenic determinants, arsanil and sulfanil. The antibodies formed reacted not only with the altered but also with the unaltered thyroglobulin, and injections of the latter were even able to boost the titre of the autologous antibodies. This leads to the extremely important conclusion that some autoimmune diseases might be induced by an immune response to an altered body constituent, and could be maintained by the unaltered constituent even after the initial alteration had been corrected.

**The Liberation of Antigens Normally Without Access to the Antibody-Forming Tissue**

Specific tolerance to one's own antigens implies their access at some stage to the antibody-forming cells. With most tolerated antigens this probably occurs soon after their first appearance in the foetus. An endogenous antigen could fail to achieve this access either because it is normally incarcerated within its cells of origin, or because it only appears late in ontogeny, presumably after cells capable of synthesising the corresponding antibody have passed their phase of special vulnerability. Typical of the first are antigens present in cardiac muscle fibres. Damage to the fibres with release of antigen is therefore often followed by the appearance of humoral antibodies, as in the postcardiotomy syndrome or after myocardial infarction (Dressler, 1959). Antigens associated with the myelin of the central nervous system are also probably of this kind, and it is therefore not surprising that corresponding antibodies can frequently be found in a considerable variety of demyelinating diseases. Of the antigens appearing late in individual development the most important in relation to autoimmunisation are those associated with the sex organs, especially the testis. Autoimmune responses to spermatozoa were among the first to be recorded, and the frequency of sperm-immobilising antibodies in patients with azoospermia suggests an important causal relationship (Rümke and Hellinga, 1959).

Of still greater importance, because of the frequency with which the immune response is associated with undesirable clinical effects, is the absence of tolerance to antigens in the lens of the eye. The release of some of these antigens during surgery for cataract, and the subsequent immune reaction to them,
provides convincing evidence of their immunological 'foreignness' (Irvine, 1957).

Any discussion of the breakdown of tolerance must include the role of immunological adjuvants, substances usually incorporated with antigen to enhance the immunological response. The most important of these, Freund’s adjuvant, consists of a water-in-oil emulsion with the antigen in the aqueous phase. Two varieties are used, termed complete or incomplete, depending upon the presence or absence respectively of acid-fast bacilli. The complete form of adjuvant is particularly associated with the experimental production of autoimmune reactions. Thus, experimental thyroiditis, encephalitis, orchitis, and adrenalitis are all most readily induced by immunisation with the appropriate antigen incorporated in Freund’s complete adjuvant. Unfortunately, the mode of action of this adjuvant is still largely obscure, but its success in the induction of autoimmune responses raises the question of whether other infectious agents, or even metabolites, could act in a similar fashion. Pertussis vaccine, for example, has been used successfully in association with adrenal or testis extracts to induce adrenalitis or orchitis respectively. Of even greater interest, in view of their isolation from the joints of some patients with rheumatoid arthritis, are certain diphtheroids whose ability to substitute for mycobacteria in Freund’s adjuvant has been shown in the experimental production of chronic arthritis in rabbits (Glynn, 1968). The manner in which adjuvants help to overcome tolerance is obviously germane to the whole problem of autoimmune disease. Because of the variety of autochthonous substances rendered antigenic by a single adjuvant it is more probable that the effect is upon the immunological apparatus rather than on the antigenic material itself. The granuloma at the site of injection of antigen with adjuvant lends support to this view, as it is characterised by a dense aggregation of macrophages and lymphocytes, two cell types known to belong to the immunological cellular apparatus.

This brief account of the mechanisms thought to be operating in the breakdown of immunological tolerance, natural or experimentally induced, make no claim to be comprehensive. It probably includes, however, the majority of those operating in naturally acquired autoimmune reactions.

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Genetic factors in Autoimmune Disease

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Familial concentration has been observed in several clinical manifestations of autoimmune disease. This type of evidence presents difficulties for any formal genetic analysis. It is possible to probe a little further into the genetic background of some autoimmune conditions in experimental animals. Such analyses raise questions about the genetic control of antibody synthesis itself, and the genetic control of susceptibility to infectious agents, especially viruses, which may play a part in the pathogenesis of supposedly autoimmune processes.

Familial concentration of autoimmune manifestations in man

In Graves' disease there is a concordance rate of over 50 per cent in monozygotic, compared with 9 per cent in dizygotic, twins of the same sex (Harvald and Hauge, 1956). Cases have been recorded as discordant when one twin had goitre without overt thyrotoxicosis, but modern methods might well have shown latent Graves' disease or underlying thyroiditis. Several recent reports reviewed by Doniach and Roitt (1969) show that in monozygotic twins affected by autoimmune thyroid disorders there is a close similarity in the antibodies detected and their respective titres. Hall et al. (1964) found thyroid antibodies in 56 per cent of siblings of patients with autoimmune thyroiditis, and concluded that one or both parents could have transmitted the tendency to thyroid autoimmunity in 17 out of 19 families of young patients with thyroiditis. Similar results have been obtained in seven different family studies in Hashimoto's disease and thyrotoxicosis (Doniach and Roitt, 1969). Masi et al.