

Heberden Oration, 1972

Mechanisms of tolerance and autoimmunity

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The problem of autoimmunity is of clinical importance as well as theoretical interest. Although many autoantibodies, such as are commonly found in old people, are not associated with disease, some are unquestionably harmful. Among these are autoantibodies against erythrocytes, which produce haemolytic anaemia, and antibodies against renal glomerular basement membrane in Goodpasture's syndrome, which can be eluted from the kidneys of affected individuals and produce lethal nephritis when inoculated into monkeys. In systemic lupus erythematosus complexes of DNA and autoantibody are deposited in the kidneys and give rise to serious immunopathological glomerulonephritis. Many other cases of glomerulonephritis in man are associated with immune complex deposition; only rarely (as in malaria) are the antigens identifiable, and they may often be autoantigens. Rheumatoid factor is of course an autoantibody, and complexes of rheumatoid factor and immunoglobulin, as well as of other antibodies and antigens, are demonstrable in rheumatoid synovia and may play an important part in the genesis of lesions. Indeed, C. Cardella, P. Davies, and I have found that immune complexes added to cultures of macrophages bring about a marked release of lysosomal hydrolases from the cells into the surrounding medium. The cells are not killed, and continue to synthesize and release hydrolases over long periods. In view of the mononuclear infiltrate in the synovium in rheumatoid arthritis, we believe that continued lysosomal hydrolase release from macrophages, stimulated by immune complexes, makes an important contribution to the inflammation and tissue damage which are characteristic of this condition.

There are thus good reasons for the practising clinician, including the rheumatologist, to be interested in autoimmune reactions, quite apart from the theoretical challenge which the problem poses. Ehrlich (1906), with his usual perceptiveness, drew attention to the remarkable fact that, although vertebrates can readily be immunized with cells or body fluids from other animals, they do not as a rule make antibodies against their own tissue constituents. He

termed the phenomenon 'horror autotoxus' but was unable to advance any satisfactory explanation for it. While developing the clonal selection theory of immunity, Burnet (1959) postulated that autoantigens ('self' antigens) are either secluded from the immune system or that immunocompetent cells exposed to autoantigens early in the course of ontogenetic development are eliminated or inactivated. Autoimmunity was thought to follow the proliferation of 'forbidden clones' of lymphocytes with specificity for autoantigens.

Burnet's postulates attracted widespread interest, but have run into serious difficulties. With the development of sensitive methods for quantitation, notably radioimmunoassay, antigens thought to be secluded have been demonstrated in circulating blood. Thus, thyroglobulin is found in serum from normal human newborns and adults in concentrations of about 10–100 ng. per ml. (Torrighiani, Doniach, and Roitt, 1969). Thyroglobulin will be taken as a model autoantigen in this paper because of the ease with which autoantibodies against this protein can be elicited, for example, by immunization with autologous thyroglobulin in the presence of Freund's complete adjuvant or by immunization with heterologous thyroglobulins. Formation of autoantibodies against thyroglobulin in experimental animals is often accompanied by thyroiditis. Such autoimmune reactions are not confined to thyroglobulin: for example, immunization with isologous testicular or brain extracts leads to autoimmune orchitis or encephalomyelitis. It is difficult to understand how such procedures could rapidly induce the proliferation of 'forbidden clones' of lymphocytes able to react with the appropriate autoantigens.

Tolerance in T and B lymphocytes

Several findings of the past few years have allowed reconsideration of the problem. The first and most important is the distinction between thymus-dependent (T) and other (B) lymphocytes, the latter

cells and their progeny responsible for antibody synthesis and release while the former participate in cell-mediated immunity and exert helper effects in the formation of antibodies against most antigens (see Roitt, Greaves, Torrigiani, Brostoff, and Playfair, 1969; Miller and Mitchell, 1969). The second relevant finding is that, in the absence of adjuvants, administration of heterologous serum proteins at intermediate dosage results in antibody formation whereas administration of high doses or repeated low doses of the same antigens results in tolerance (Dresser and Mitchison, 1968). The tolerant animals are unable to synthesize antibody even when antigens are subsequently administered in a highly immunogenic form (usually in adjuvant), although their immune responses to other antigens are normal. Hence there is no general impairment of immune responses, but an induced antigen-specific unresponsiveness. It was then found by Taylor (1969) and by Chiller, Habicht, and Weigle (1971) that, in mice made tolerant by repeated low doses of bovine serum albumin or human gamma globulin, T lymphocyte responses to the antigens are markedly depressed whereas B lymphocyte responses are normal or nearly normal. This was demonstrated by the capacity of bone-marrow lymphocytes (or splenic B lymphocytes) from low-dose tolerant mice to reconstitute an immune response against the tolerogen when transferred to irradiated syngeneic mice together with normal thymus cells, in contrast to the poor immune response when thymus cells from tolerant animals were

used together with bone marrow cells from normal donors in irradiated recipients (Fig. 1). After a high dose of antigen bone marrow cells also become unresponsive; moreover, unresponsiveness is rapidly induced and is persistent in T lymphocytes and is more slowly induced and transient in B lymphocytes.

These findings led to the postulate by Allison (1971b) and independently by Weigle (1971) that, with circulating soluble autoantigens, two types of tolerance are present.

When antigens circulate in low concentrations, such as thyroglobulin, there will be the equivalent of low-dose tolerance; specific T cells become unresponsive but specific B cells are present in normal numbers and are able to respond to autoantigens suitably presented to them. The B cells can be stimulated by immunization with cross-reacting antigens, in which case autoantibodies are made only against those autoantigenic determinants shared with the cross-reacting antigens. Alternatively, autoantibodies would be formed by immunization with autoantigen in the presence of suitable adjuvants which provide non-antigen-specific T cell stimulation (see Allison, 1973). Other situations allowing stimulation of autoantigen-reactive B cells are discussed below.

In contrast, by analogy with high-dose tolerance, it can be postulated that, in the case of soluble autoantigens circulating in high dose, such as serum albumin, both B and T cells become unresponsive. In that case no manipulation would give rise to auto-antibody formation.

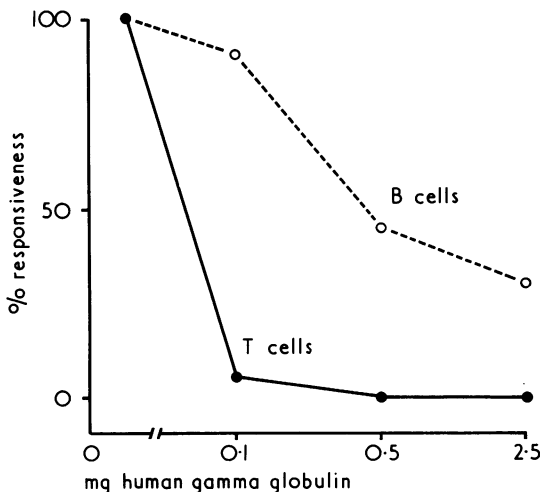


FIG. 1 *Effect of different doses of human γ -globulin on the responsiveness of T and B lymphocytes as measured by their capacity to co-operate in antibody formation when transferred to irradiated syngeneic recipients. The responses are expressed as a percentage of that observed in untreated donors. Note that the dose of antigen is on a logarithmic scale (data of Chiller and others, 1971)*

T cell helper effects in immune responses

The requirement for T cell helper effects in immune responses is now well defined. Helper effects are dose-dependent, being most marked when the antigen dose is small, and they occur with a wide range of antigens. A minority of naturally occurring and synthetic antigens with repeating antigenic determinants in appropriate configurations, do not require T cell helper effects, and are known as thymus-independent antigens. Examples are pneumococcus capsular polysaccharide, lipopolysaccharide (endotoxin) of Gram-negative bacteria, levan, and polyvinylpyrrolidone. However, with most naturally occurring antigens, T cell helper effects are marked; this is known, for example, in formation of antibody against serum albumin, immunoglobulin, thyroglobulin, and erythrocytes.

The third finding that has relevance to autoimmunity concerns the role of T and B lymphocytes in antibody formation against haptens attached to immunogenic carriers. Owing largely to studies in the laboratories of Mitchison (1971) and of Katz and Benacerraf (1972), it has been shown that anti-hapten antibody is synthesized and released by B cells, but

for this to occur an immune response by helper T lymphocytes against the carrier is normally required. This is shown in Fig. 2, line 1. A development of this approach has been the demonstration by Iverson (1970) and others that animals can also be sensitized so that T cells react against a hapten and that in such animals administration of a normally non-immunogenic protein (e.g. a myeloma immunoglobulin in an

inbred mouse) coupled to the hapten results in formation of antibody (anti-idiotypic). This is illustrated in Fig. 2, line 5.

Autoantigen-binding lymphocytes in normal subjects

If the hypothesis of self-tolerance presented above is correct, it should be possible to identify B cells but

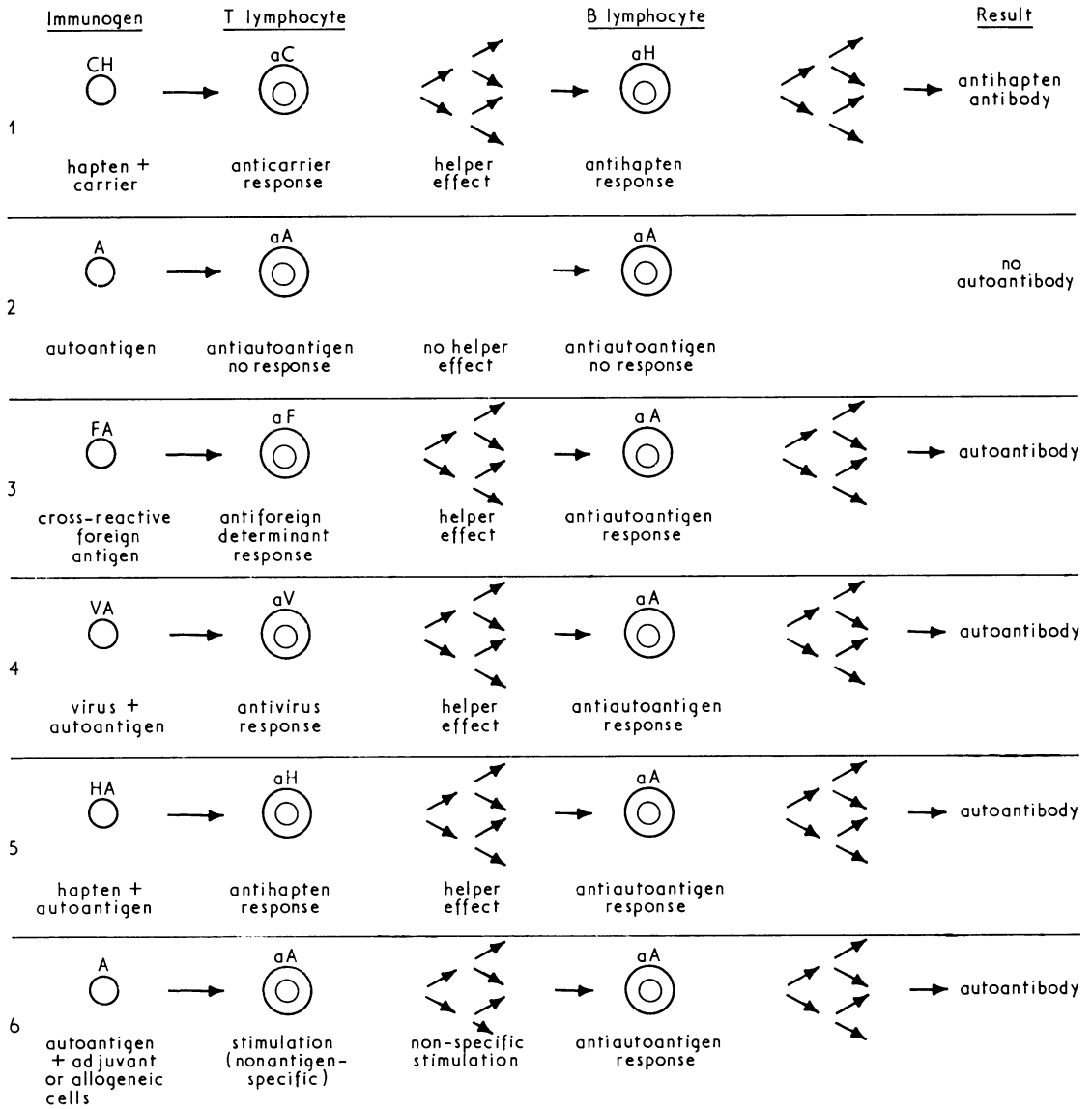


FIG. 2 Antigenic determinants: H—hapten; C—carrier; A—autoantigenic determinant, shared with a foreign antigen; F—foreign antigenic determinant not shared with autologous antigen; V—virus antigen
 Lymphocyte receptors for antigen: aH—anti-hapten, etc. Slanted arrows show proliferation and differentiation of cells, accompanied, in the case of B lymphocytes, by antibody production and in the case of T lymphocytes, by a helper effect. B lymphocytes exposed to antigen (arrow) respond only when there is a helper effect.

not T cells capable of reacting with autoantigens circulating in low concentration, such as thyroglobulin, whereas neither B nor T cells able to react with autoantigens circulating in high concentration, *e.g.* serum albumin, should be demonstrable. Experiments were therefore undertaken to determine, by sensitive autoradiographic techniques, the binding of homologous thyroglobulin and serum albumin by human lymphocytes (Bankhurst, Torrigiani, and Allison, 1973). Peripheal blood lymphocytes from normal human subjects were allowed to bind high specific-activity ^{125}I human thyroglobulin and human serum albumin. The B lymphocytes were identified by their large amount of surface immunoglobulin, as shown by binding of radioactive anti-immunoglobulin. Selective removal of B cells from the population of lymphocytes was achieved by passing them through columns of beads coated with antibody against human immunoglobulin. The B lymphocytes are retained on such columns, presumably because their large amount of surface immunoglobulin reacts with the antibody on the beads. Studies of the cells that have passed through the columns show marked depletion or absence of B lymphocytes.

Nine out of eleven normal subjects had peripheral blood lymphocytes which bound ^{125}I -thyroglobulin (Table I). In contrast, no lymphocytes which bound human serum albumin were found in three normal human subjects. Lymphocytes binding thyroglobulin were absent after passage through the column retaining B cells in three experiments, and were markedly depleted in a fourth experiment. Thus the antigen-binding cells are identified as B lymphocytes.

Table I *Lymphocytes binding homologous ^{125}I antigens in normal subjects (Bankhurst and others, 1973)*

Type of labelled antigen†	No. of labelled cells*	
	Individual subjects	Average‡
Human thyro-globulin	175, 455, 150, 0, 100 80, 380, 0, 500, 298, 240	216
Human serum albumin	0, 0, 0	0

* Number of labelled lymphocytes per 10^6 lymphocytes.

† 5×10^6 lymphocytes were reacted for 30 min. with 270–500 ng. labelled antigen in a 0.5 ml. volume. Autoradiographs were exposed for 8 to 24 days.

‡ Average calculated from the nine positive subjects

Antigen-binding lymphocytes have been characterized in the mouse, using radioiodinated antigens, by Ada and by Sulitzeanu and their colleagues (see Ada and Cooper, 1971). Antigen-binding lymphocytes are primarily B lymphocytes: their number is not reduced by treating spleen cells with anti-theta serum and complement, and there are normal numbers of antigen-binding lymphocytes in the congenitally athymic mouse. The antigen-binding cells are also the antigen-

sensitive lymphocytes: radiation-induced death of lymphocytes which bind a highly radioactive antigen specifically abrogates the immune response to that antigen while leaving other immune responses intact.

A general criticism of the work carried out on antigen-binding cells is that the cells have been incubated with less than saturating amounts of labelled antigen. However, the concentrations of thyroglobulin used in our experiments were of the same order as found in serum and as are required for radiation-induced death of reactive lymphocytes. The conditions used for studying binding presumably identify the cells on which interest should be focused, namely antigen-binding immunocompetent cells which are precursors of cells producing reasonably high-affinity antibody. Dr I. Goldsmith has also found cells binding homologous thyroglobulin in normal human subjects; the number of binding cells is increased in patients with autoallergic thyroiditis. Moreover, Ada and Cooper (1971) have found lymphocytes binding homologous thyroglobulin in normal rats, and Clagett (1972) has found them in normal mice. Since inbred animals were used, these results confirm that the reactions must be with auto-ologous rather than homologous antigenic determinants.

Several recent findings support the view that the antigen-binding B lymphocytes just described are involved in the production of autoantibody against thyroglobulin, and that T cell helper effects are required to elicit such an immune response. Suicide of CBA mouse spleen cells with highly radioactive isologous thyroglobulin before transfer to irradiated recipients leaves intact antibody formation against heterologous thyroglobulins but abolishes the formation of the thyroglobulin autoantibodies which are seen when no suicide has occurred. If the suicide is carried out with highly radioactive heterologous thyroglobulin, no antibodies against thyroglobulin—heterologous or homologous—can be elicited by immunization with the heterologous protein. In mice which have been thymectomized, irradiated, and reconstituted with syngeneic bone marrow, antibodies against heterologous thyroglobulin and autoantibodies are seen only when recipients have also received a graft of thymus cells. Thus our postulate that tolerance to an autoantigen circulating in low dose, thyroglobulin, is selective for T lymphocytes is borne out by appropriate experiments.

Playfair and Clarke (1973) has also found that repeated inoculations of mice with rat erythrocytes leads to the production of autoantibodies against erythrocytes. Helper T cells reacting against common antigenic determinants may well be involved, since thymectomy abolished autoantibody formation. The strain which responded best was C57B1, in ageing members of which a relatively high incidence of

erythrocyte autoantibodies has been reported by Linder, Pasternak, and Edgington (1972). These authors have shown that complexes of some erythrocyte antigens and antibodies accumulate in the kidneys, from which it appears that small amounts of the antigens may normally circulate in soluble form, thereby inducing selective T cell unresponsiveness.

The reason for the absence of lymphocytes binding human serum albumin in normal human subjects is open to speculation. The simplest explanation is that serum albumin is found in very high concentration in extracellular fluids and saturates all the receptor sites for antigen, despite washing of cells. An alternative explanation is that there are very few or no lymphocytes able to bind a soluble autoantigen present in high concentration, and that self-tolerance under these conditions involves a different mechanism, namely, the elimination or inhibition of multiplication of the antigen-sensitive B lymphocyte clones. Observations in experimental animals support the latter interpretation. Unanue (1971) has been unable to find in the lymph nodes of the mouse lymphocytes binding autologous albumin, although cells binding autologous growth hormone are observed; the latter would circulate in low dose. Naor and Sulitzeanu (1969) have reported that, in mice made tolerant to heterologous albumin, the number of antigen-binding lymphocytes falls. Similar findings in mice made tolerant to human gamma globulin have been reported by Louis, Chiller, and Weigle (1973). In contrast, mice tolerant to haemocyanin, flagellin, and *E. coli* lipopolysaccharide have shown normal or increased numbers of antigen-binding cells (Ada, 1970). Thus the nature of the antigen and dosage schedule seem to determine whether specific B cells are eliminated or made unresponsive, on the one hand, or remain demonstrable in the recipient.

Allogenic cell stimulation, adjuvants, and autoantibody formation

Katz (1972) has shown that injections of allogeneic immunocompetent cells under conditions that produce mild graft-versus-host reactions abolish the need for co-operation of carrier T cells in a hapten-carrier system. Evidence has accumulated that in such animals host T cells are stimulated in a non-antigen-specific fashion. By analogy, inoculations of allogeneic cells should stimulate the formation of autoantibodies. Boyse, Bressler, Iritani, and Lardis (1970) have found that mice injected with allogeneic cells produce autoantibodies against thymocytes. Fialkow, Gilchrist, and Allison (1973) have recently found that repeated injections of F1 mice with parental cells rapidly induces the formation of antinuclear antibodies; allotype markers were used to establish that these were produced by host and not donor B cells. These results support the view that allogeneic cell

stimulation can result in autoantibody formation, and the role of this phenomenon in chronic graft-versus-host disease deserves further study.

The role of adjuvants in eliciting autoantibody formation in experimental animals is well known. Many adjuvants increase proliferation of T cells, and T cells are required for the increase by several adjuvants of antibody formation by B cells (see Allison, 1973). Freund's complete adjuvant may in addition exert a carrier effect if mycobacterial antigens are able to form complexes with host antigens. The human counterpart is the finding of antinuclear and other autoantibodies in leprosy—in which patients carry a heavy mycobacterial load, which may have adjuvant activity—and also in malaria, syphilis, and other infections (references in Allison, Denham, and Barnes, 1971).

Idiotypic determinants of immunoglobulins were discovered by Oudin and Michel (1963) as a result of immunizing animals with bacteria coated with homologous antibodies. The bacteria may well have been active as carriers (their antigens stimulating T cells, which presented the idiotypic determinants of antibacterial antibodies correctly to responsive B cells), and perhaps also as adjuvants: non-antigen-specific stimulators of immunocompetent cells. In the mouse, bacterial lipopolysaccharide (endotoxin) is a powerful direct stimulator of B lymphocytes, and it may well be that one of the consequences of bacterial infection in man is an adjuvant effect.

Virus infections

It follows from what has been said that, if it were possible to bypass the requirement for T cells responsive against autoantigens, autoantibody formation might be elicited. One way by which this might be achieved is by virus infection. Virus-specific antigens are often found on the membranes of infected host cells, and host antigens in the envelopes of lipid-containing viruses. Thus virus antigens and autoantigens could form common immunogenic units which function in a manner analogous to the hapten-carrier system, as shown in Fig. 2, line 4.

An analogous principle has been used by Lindemann and Klein (1967) to increase immunity against tumour-specific antigens by immunizing mice with influenza virus grown in the tumour cells. Harboe and Haukenes (1966) have found that chickens immunized with influenza virus containing an antigen from the chorioallantoic cells in which it was cultured produce autoantibody against the same antigen present in liver and bile. Toniatti, Oldstone, and Dixon (1970) have reported that infection of NZB, NZW, and NZB/NZW hybrid mice with polyoma virus or lymphocytic choriomeningitis virus accelerates the onset of autoimmune manifestations and increases

their incidence. As infectious mononucleosis infections wane, a variety of autoantibodies are often found, and human infections with several other viruses, including influenza, measles, varicella, Coxsackie, and herpes simplex viruses, are sometimes followed by autoallergic manifestations, including antibody-mediated thrombocytopenia and positive Coombs tests. The development of cold autoagglutinins, often directed against the I blood group, after *Mycoplasma pneumonia* infections, may have a similar explanation.

Effects of haptens and drugs

As mentioned above, another way in which the requirement for specifically reactive T cells can be bypassed is to sensitize an animal against a hapten (such as oxazolone or dinitrophenol) and couple the hapten to host constituents before re-injection into the same or a syngeneic animal. Such procedures have been used to produce autoantibody against thyroglobulin (Weigle, 1971) and antibody against specific (idiotypic) determinants of a monoclonal immunoglobulin in a syngeneic system (see above). This may be the way by which, in human patients, exposure to certain drugs is followed by autoantibody production. Two possible examples are the formation of autoantibodies against red cells (often directed against Rh blood groups) in patients treated with α -methyl dopa and the presence of antinuclear factors in patients treated with hydrallazine, isoniazid, procaineamide, and other drugs (Cannat and Seligmann, 1968). The postulate is that patients will have T cell reactivity against the drugs or their metabolites and that the drugs or metabolites should be associated as haptens with erythrocyte membranes or nucleoproteins, respectively. Both these postulates are testable, especially since the antinuclear factor can be reproduced in experimental animals.

Control of immune responses by T cells

If tolerance to autoantigens is due to selective T cell unresponsiveness, it is inevitably precarious, since it can be abrogated by several mechanisms already listed. It is therefore likely that an additional 'fail-safe' mechanism for preventing autoimmunity should exist. A second hypothesis put forward by Allison

and others (1971) is that T cells can exert specific feedback control on the synthesis of antibodies by B cells and that relaxation of this control—especially in ageing humans and experimental animals—may be an important factor in the development of autoimmunity. A role of T cells in immunological surveillance against malignant cells is supported by observations of an increased incidence of tumours (especially those that are virus-induced) in experimental animals with depressed cell-mediated immunity (Allison, 1971a) and the raised probability of developing lymphoreticular neoplasms in humans with immunodeficiency syndromes or immunosuppressed after kidney transplantation (Penn and Starzl, 1972). Immunological reactions are known to be subject to feedback control, the most fully studied case being the specific inhibition of antibody formation by administration of antibody (Uhr and Möller, 1968). We suggest that a similar inhibition can be exerted by T cells, and that this provides a surveillance mechanism against aberrant immune reactions (Fig. 3).

Evidence summarized by Allison and others (1971) comes from experiments of Denman and his colleagues with NZB mice, which develop a Coombs-positive haemolytic anaemia from about the age of 4 months. If spleen cells are transferred from old to young NZB mice, about one-half of the recipients show positive Coombs tests which usually disappear in a few weeks (Fig. 4, opposite).

If the recipients are given antilymphocytic globulin (ALG), more recipients develop positive Coombs tests and these remain positive usually until the death of the animals. The simplest explanation of these results is that T cells in the young animals exert an inhibitory influence on the B cells from the spleens of old animals that are producing antibody against homologous erythrocytes, as shown by the arrow in Fig. 3, and that T cell control is abolished by ALG administration. In keeping with this interpretation, Playfair has found that the transfer of thymus cells from 2-week-old NZB mice monthly to NZBs from the age of 1 month significantly delays the onset of positive direct Coombs tests. Further evidence for the positive nature of the control comes from experiments in which spleen cells from NZB mice are transferred into irradiated BALB/c mice; the recipients develop

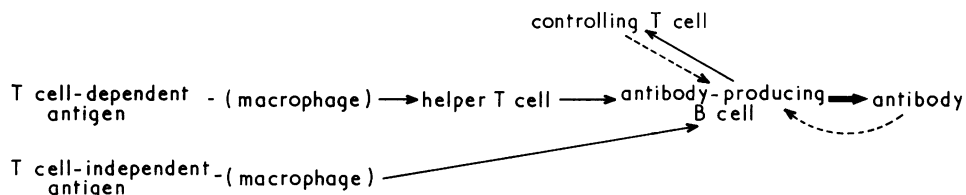


FIG. 3 *Concept of T cell control of antibody formation or release by B cells. Stimulatory interactions shown by thin arrows, products by thick arrows, and inhibitory interactions by dotted arrows*

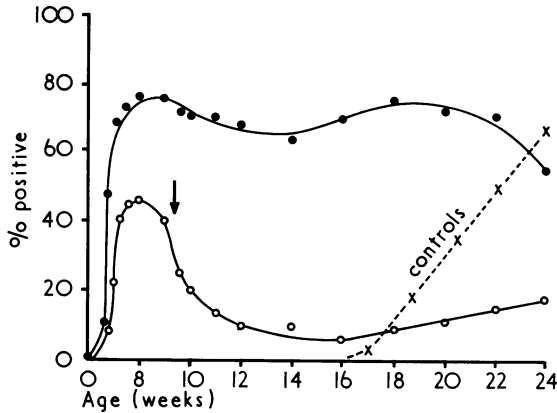


FIG. 4 Adoptive transfer of positive antiglobulin (Coombs) reactions to young NZB mice (From Allison and others (1971), by permission of the editor of the *Lancet*).

- Received ALG 2 mg. four times intraperitoneally followed by $200-250 \times 10^6$ spleen cells intraperitoneally from old Coombs-positive NZB donors.
- Received normal rabbit IgG (NGR) followed by similar injections of spleen cells.
- ×—× Received NRG only.

positive Coombs tests only if treated with ALG (Denman, Russell, and Denman, 1970). Adoptive transfer of renal disease within the NZB/NZW strain is likewise achieved only in ALG-treated recipients (Denman, Russell, and Denman, 1969). Thus suppression of autoantibody formation in adoptive transfer experiments dominates over expression, but the suppression can be relieved by ALG. Additional evidence for a role of suppressor T cells in this situation comes from experiments of Playfair in which spleen cells from NZB mice were transferred to BALB/c \times NZB F1 hybrids. No positive Coombs tests were observed in the recipients unless the transferred cell population was treated with anti-theta serum and complement, in which case recipients rapidly developed autoantibodies against erythrocytes.

In general, evidence has accumulated that NZB mice and some of their hybrid offspring are especially prone to develop autoimmunity for two reasons. Their T cells are unusually resistant to tolerance induction, as judged by reactions against foreign serum proteins and erythrocytes. Moreover, their T cell function declines rapidly with age, as judged by capacity to mount graft-versus-host reactions and in other ways. Hence there is a combination of both factors favouring autoimmunity.

Teague and Friou (1969) have found that ageing mice of strain A frequently develop antibody against deoxyribonucleoprotein (antinuclear factor—ANF). Transfers of thymus cells from young to old syngeneic animals that had developed ANF resulted in decreased levels or disappearance of ANF. These

workers have also suggested that cells in the thymus and spleen of young mice may participate in homeostatic control of autoantibody formation, and that this control may be less effective in ageing animals. The age-dependence of autoantibody formation in humans is well documented.

Two other groups of investigators independently postulated in 1971 the existence of suppressor T cells to explain experimental findings in their laboratories. Since the concept is still relatively novel and of general interest, evidence in support of it will be reviewed briefly. The Herzenbergs and their colleagues have been concerned with the phenomenon of allotype suppression in mice. Suppression of immunoglobulin allotypes is a remarkable example in higher organisms of the regulation of gene expression by antibodies directed against the products of these genes. In rabbits and mice, prenatal and/or early postnatal exposure to anti-allotype antibody suppresses the formation of the allotype in animals genetically able to produce it. In many mouse strains the suppression is transient, but in F1 offspring of the SJL strain, and in rabbits, long-term suppression lasting the life of the animal often occurs. Sensitive tests show that suppression is not due to antibody against the suppressed allotype, or to the elimination of B cells able to synthesize the allotype (see Jacobson and Herzenberg, 1972). It is due to the presence of T cells which suppress the expression of the allotype. For example, if spleen cells from allotype-suppressed animals are transferred to irradiated recipients, there is a brief burst of synthesis of the suppressed allotype followed by re-establishment of the suppression. If the cells are treated with anti-theta serum and complement before transfer, the suppressed allotype emerges permanently in the recipient. Admixture of cells from suppressed animals with those of normal donors before transfer prevents the expression of the allotype in irradiated recipients; as in the NZB example, suppression dominates over expression. Suppression has also been demonstrated *in vitro*. If heterozygous mice are immunized against sheep erythrocytes, approximately equal numbers of cells releasing antibody of each allotype are demonstrated by the Jerne plaque technique. If T cells from suppressed animals are added to the system, the proportion of cells producing the suppressed allotype is significantly reduced.

For several years it has been known that inoculations of large numbers of foreign erythrocytes in rats and mice induces tolerance to this antigen, and McCullagh (1970, 1972) has provided evidence that this is due to the presence in tolerant rats of cells suppressing the reaction. If mixtures of lymphoid cells from normal and tolerant animals are transferred to irradiated recipients, tolerance dominates over responsiveness. Gershon and Kondo (1971) have reported similar results in mice and obtained evidence

that the suppressor cells are T lymphocytes. Cooper and Ada (1973) have found that tolerance to haemocyanin in mice is due to the presence of suppressor T cells, and Basten (1973) has obtained similar results with fowl gamma globulin in mice. Thus several well-studied examples of T cell suppression of antibody formation have come to light, and the phenomenon may be quite general. Other findings suggest a role for suppressor T cells in graft-versus-host reactions (Gershon, Cohen, Hencin, and Liebhaver, 1972) and contact hypersensitivity against a chemical sensitizer in mice (Asherson, Zembala, and Barnes, 1971).

A role for T cell suppression of autoimmunity in chickens genetically predisposed to thyroiditis (obese chickens) is suggested by the work of Wick, Kite, and Witebsky (1970), who found that thymectomy accelerated and aggravated thyroiditis whereas early bursectomy abolished it. Presumably the helper effects were already established at the time of thymectomy.

Two mechanisms by which T cells may exert their suppressor effects can be postulated:

(1) T cells are able to recognize the specific (idiotypic) determinants of immunoglobulin receptors that distinguish one B cell from another; they can then react against the corresponding B cells and suppress immunoglobulin synthesis. Three lines of evidence—none of them yet entirely conclusive—suggest that T cells can recognize idiotypic determinants of syngeneic mice:

(a) Experiments on the use of monoclonal immunoglobulins as carriers in the production of antibody against haptens suggest that T cells can react against idiotypes.

(b) Hannestad, Kao, and Eisen (1972) found that BALB/c mice given injections of monoclonal immunoglobulins in complete adjuvant become immunized against plasmacytomas producing that immunoglobulin. In the immunized mice, there was strong selection for tumour variants with defective production of immunoglobulin heavy chains. It is likely that T cells in the immunized mice are able to react against idiotypic determinants to which heavy chains make an important contribution.

(c) The third argument follows from the postulate of Ramseier and Lindenmann (1969) that F1 animals are able to react against parental strain immunocompetent cells because of the presence of a receptor on the latter for antigens of the other parental strain. If such an immunological reaction against receptors occurs, it has wide implications, so that the possibility should be tested rigorously by acceptable methods.

(2) A second possible mechanism by which T cell products may suppress B cell activity arises from the work of Feldmann and Nossall (1972). They have advanced evidence in support of the view that T cells exposed to antigen release a factor (perhaps an immunoglobulin) which attaches to the surface of a

macrophage, where it can stimulate a response by B cells in the vicinity. In the absence of macrophages, or in the presence of excess T cell product, the product reacts directly with B cells, and inhibits their response. On this hypothesis, suppressor T cells would give rise to an excessive release of product, with inhibitory consequences. It should be possible to distinguish between these alternatives (for example, on Feldmann's hypothesis, transfer of many T cells from suppressed animals to irradiated recipients should result in suppression, whereas smaller numbers should exert a helper effect); but the appropriate experiments have not yet been performed.

Congenital virus infections

The work of Traube in the 1930s showed that, in mouse colonies, vertical transmission from mothers to offspring of lymphocytic choriomeningitis (LCM) virus establishes a symptomless lifelong infection in which no antiviral antibody is demonstrable in the circulating blood. Infection of adult mice with LCM leads to an immunopathological disease and antibody formation. The lack of antibody after congenital LCM virus infection was one of the observations which led Burnet and Fenner (1949) to postulate the existence of immunological tolerance. More recently Oldstone and Dixon (1969) have demonstrated the accumulation in the kidneys of mice congenitally infected with LCM of immune complexes containing viral antigen and antibody. Hence the congenitally infected mice are able to make some antibody against viral antigens. Volkert, and Hannover-Larsen (1965) found that, if spleen cells from LCM-immune mice (infected as adults) are transferred to syngeneic congenitally infected carrier animals, very high levels of antibody are formed, but no immunopathological disease results.

The simplest interpretation of these findings appears to be that LCM viral antigens produce a tolerance resembling that occurring with autoantigens in low dose, namely that T cells specific for viral antigens become unresponsive while specific B cells remain able to respond to antigen. In the absence of specific helper cells, only a small amount of antibody is produced, and this combines with antigen liberated into the circulation to form immune complexes which accumulate in the kidney, leading eventually to immunopathological glomerulonephritis. However, when virus-specific T cells are supplied by adoptive immunization, a helper effect greatly increases antibody formation in the recipient animals. A helper role for T cells in antibody formation against LCM viral antigens is demonstrated by the recent experiments of Cole, Nathanson, and Prendergast (1972). Adoptive immunization of congenitally infected LCM virus carrier mice with spleen cells from syngeneic immune donors resulted in high antibody

levels, as already described, but treatment of the spleen cells with anti-theta serum before transfer virtually abolished this effect (Table II). Thus, even in the presence of B cells from immune donors, little antibody is formed unless sensitized T cells are also present.

Table II *Antibody against lymphocytic choriomeningitis virus antigens in BALB/c mice neonatally infected and adoptively immunized (Cole and others, 1972)*

Treatment	Complement-fixing antibody		Viraemia
	Mean	Range	
None	0	0	4.0
10 ⁸ immune spleen cells	3,000	200–12,000	0.5
10 ⁸ immune spleen cells treated with anti-theta and C'	0	0–25	3.3

Analogous results have been obtained in other congenital virus infections: for example, accumulation in carrier mice of complexes of antibody and antigens of murine leukaemogenic viruses (Hirsch, Allison,

and Harvey, 1969; Oldstone, Tishon, Tonietti, and Dixon, 1972).

Conclusion

The concept that most autoantigens circulate in low dose and induce unresponsiveness in specific populations of T but not B cells explains many observations that were difficult to reconcile with the clonal deletion of antibody-forming cells postulated by Burnet. Enough evidence has accumulated to make it likely that the concept of selective unresponsiveness of T cells is correct, at least for those autoantigens so far analysed in detail. This includes antigens of vertically transmitted viruses as well as autoantigens. The concept is also useful in clinical immunology to explain autoantibody formation after virus and other microbial infections, by certain drug treatments and in other situations. However, other problems still remain without explanation, *e.g.* the mechanism by which prolonged exposure to low doses of antigens induces T cell unresponsiveness, and why tolerogenic exposure to some antigens leads to depletion of antigen-binding B cells while exposure to other antigens does not. Enough information has also accumulated to support the validity of the suppressor T cell hypothesis and the role of these cells in the control of autoimmune reactions.

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