Current Practice

GROWING POINTS

Autoimmune Disease—I

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In some human diseases antibodies react with human tissue *in vitro*. These antibodies are called *autoantibodies*, and are found in rheumatic fever, Hashimoto's thyroiditis, systemic lupus erythematosus, and other diseases. The presence of autoantibodies is compatible with the view that tissues are damaged by an autoimmune process directed against components of the patient's own tissues. For this reason Hashimoto's thyroiditis and systemic lupus erythematosus are called *putative* autoimmune diseases.

For readers wishing to learn more about autoimmunity the recent books by Humphrey and White (1964) and Glynn and Holborow (1965) are recommended. The bibliography for the statements made in this article is given by Asherson (1965).

Tissue Damage due to Immune Reactions

The tissue damage caused by autoimmune reactions to the components of an animal's own tissue is a special example of tissue damage due to immune reactions. There are two main types of immunological tissue damage: that due to humoral factors (antibodies) and that due to cellular factors. The former is called *immediate hypersensitivity*; the lesions characteristically appear within minutes or hours of exposure to antigen, and histological examination shows exudation of plasma and infiltration with polymorphs in some cases. The latter is called *delayed hypersensitivity*; the lesions reach a maximum 24 to 48 hours after exposure to antigen, and histology shows infiltration with chronic inflammatory cells such as histiocytes and lymphocytes.

Immediate hypersensitivity reactions can be transferred passively from one person to another with serum (passive transfer). In the Prausnitz-Kustner reaction intradermal injection of serum from an allergic person causes local weal and flare when antigen is injected into the same area. In contrast the delayed hypersensitivity reaction—for example, the tuberculin reaction—cannot be transferred by serum alone, and cells such as peripheral white cells, or lymph-node, spleen or peritoneal exudate cells are required for its passive transfer from one animal to another.

Immediate Hypersensitivity

Patients injected with penicillin sometimes produce antibodies against the drug, so that when they meet penicillin again anaphylactic shock or urticaria develops. Other antibodies cause vascular damage with polymorph infiltration of arteriolar walls, where they react with their corresponding antigen. This is known as the *Arthus reaction*, and may be important in the pathogenesis of certain types of arteritis.

Antibodies may also be directed against components of the animal's own tissue. Patients with Hashimoto's thyroiditis have a cytotoxic antibody which, in the presence of complement, kills thyroid cells in tissue culture. Yet other antibodies are unable to cause tissue damage, either because they have the wrong biological properties or because they are directed against an inappropriate or inaccessible antigen. Many apparently healthy people have autoantibodies against thyroglobulin which are not thought to cause tissue damage. Babies of mothers with systemic lupus erythematosus have circulating antibodies against nuclei (*antinuclear factor*) which has crossed the placenta: these do not cause tissue damage and cannot penetrate the nuclei of intact cells. (See Fig. 1.)

FIG. 1.—Antinuclear factor demonstrated in the serum of a patient with systemic lupus erythematosus by immunofluorescence using calf thyroid (by courtesy of Dr. E. J. Holborow).

At the other extreme certain antibodies are protective. After they have recovered from experimental allergic (autoimmune) encephalomyelitis rats have an antibody which protects other rats from the encephalomyelitis which would otherwise follow injection of brain in Freund's adjuvant. The antibody acts by blocking the induction or the damaging effect of immune responses, and this protective effect is called *immune enhancement*.

Complement

Complement is the name given to a group of factors in normal serum which are necessary for the haemolytic, cytotoxic, and certain other damaging actions of antibody. Thus red cells are agglutinated *in vitro* by certain physicochemical types of antibody, but they are lysed only in the presence of

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complement. Indeed, red cells coated with antibody (sensitized red cells) are used to detect complement.

At suitable concentrations many antibodies will form precipitates with soluble antigens. When the concentration of antigen is high, however, these complexes are soluble, and soluble antigen-antibody complexes are thus formed. These complexes often fix—that is, combine with—the components of complement, and so cause glomerular and vascular damage. They may be responsible for renal damage in systemic lupus erythematosus.

In the presence of complement antigen-antibody complexes release a soluble factor which promotes the migration of polymorphs. This may be important in causing the polymorph infiltrate in the Arthus reaction and the damaging effect of soluble antigen-antibody complexes.

Delayed Hypersensitivity

The Mantoux reaction is the classical example of delayed hypersensitivity. The site of injection of tuberculin becomes infiltrated with lymphocytes and histiocytes, and the maximal reaction occurs after 24 to 48 hours. In animals this lesion can be transferred by cells but not by serum alone. This indicates that delayed hypersensitivity is not due to a conventional circulating antibody. It is sometimes explained by postulating a hypothetical antibody which is bound to the cells.

Despite the development of certain *in-vitro* tests in man delayed hypersensitivity has only been detected by skin tests.

In autoimmune disease it is customary to attribute tissue damage to delayed hypersensitivity, partly because of its importance in experimental autoimmune disease, and partly because there is a poor correlation between the presence of circulating antibody and tissue damage in diseases such as Hashimoto's thyroiditis. Though serum alone will not transfer delayed hypersensitivity in animals, cells and serum are synergic in transferring delayed hypersensitivity to several protein antigens. The implication is that in autoimmune disease tissue damage may be due to both cellular and humoral factors rather than to either singly.

Mode of Production of Autoimmune Responses

Autoimmune responses are a special example of immune responses. They are those responses in which the antibody will combine with the animal's own tissue components *in vitro*, or in which delayed hypersensitivity can be produced with the animal's own tissue components. Tissue damage is not a constant feature of the autoimmune response, either because the type of response is inappropriate or because the antigen is inaccessible. By definition it is not necessary that the production of an autoantibody should be stimulated by the animal's own tissue components. Indeed, tissue from other species (*heterologous* tissue) is usually better than tissue from the same species (*homologous* tissue) in causing the production of autoantibodies. Autoimmune responses should be distinguished from *isoimmune* responses¹—for example, the blood-group antibodies, which react with the red cells of some but not all humans and which do not react with the subject's own red cells.

Immune Tolerance

The first exposure to an antigen may alter the effect of the second exposure to it in several different ways. The classical

effect of the first injection of antigen is to increase the magnitude and speed of the response to the second injection: this is known as the *secondary response*. When soluble antigen is given in neonatal life, however, the response to the second injection is depressed. For instance, the injection of bovine serum albumin into newborn rabbits prevents the production of antibody which otherwise follows the injection of bovine serum-albumin in later life: this is known as *immune tolerance*. Some antigens are also able to suppress immune responses in adult animals in long-continued low dosage or very high single doses, while intermediate doses favour antibody production: this is known as *immune paralysis*, and it is thought to be the same type of phenomenon as immune tolerance.

The conditions for suppressing delayed hypersensitivity are less exacting than the conditions for suppressing the production of antibody. This selective diminution of delayed hypersensitivity is called *immune deviation*, and it may be important in limiting tissue damage even when autoantibodies are formed.

Normally animals do not produce antibodies against their own tissue components because of immune tolerance, though there are three situations in which autoantibodies are characteristically produced.

Antigens Similar to but not Identical with Self

When animals are injected with tissue similar to but not identical with their own tissue components autoantibodies are formed and sometimes tissue damage occurs. This is a general and important mechanism. Weigle showed that rabbits injected with rabbit thyroglobulin failed to produce autoantibodies or tissue damage. On the other hand, injection of chemically altered thyroglobulin (without adjuvant) led to the formation of autoantibodies against rabbit thyroglobulin and to the production of thyroiditis. This provides direct evidence that *altered* self components can cause tissue damage. Another possible example is the formation of autoantibody which occurs in rats whose livers are damaged by the injection of carbon tetrachloride.

Certain bacteria have antigens similar to those in mammalian tissue. The group A beta-haemolytic streptococcus has antigens similar to human heart, and the nephritogenic type 12-streptococcus has antigens similar to human kidney. *Escherichia coli* has antigen similar to human colon. These bacteria will cause autoantibody formation against heart and colon in animals, and it is thought that the antibodies against heart and colon found in rheumatic fever and ulcerative colitis may arise through this mechanism.

Immunization with Adjuvant-Antigen Mixtures

Certain materials (*adjuvants*) increase the intensity and change the nature of the immune response. The best-known example is Freund's complete adjuvant, which consists of a creamy emulsion of an aqueous solution of antigen, paraffin oil, and detergent, to which dead tubercle bacilli are added. Freund's *complete* adjuvant—i.e., with tubercle bacilli—has four distinctive actions, which do not occur when Freund's *incomplete* adjuvant—i.e., without tubercle bacilli—is used. These are:

- (1) Increased delayed hypersensitivity.
- (2) Increased production of cytotoxic antibody.
- (3) Increased production of antibody which adheres to macrophages (cytophilic antibody).
- (4) Increased production of autoimmune disease and autoantibodies when antigens such as brain, testis, peripheral nerve, thyroid, adrenal, kidney, labyrinth, uvea, and muscle are injected.

The role of Freund's complete adjuvant in producing experimental autoimmune disease has been used as evidence that

¹ The nomenclature used in transplantation work is different. Antigens identical with those of an animal are called *isologous*, while those differing are called *homologous*. The new terms *syngeneic* and *allogeneic*, respectively, avoid this confusion.

delayed hypersensitivity is important in the pathogenesis of autoimmune disease. It is not clear whether Freund's complete adjuvant favours autoantibody formation by altering the immune system, or whether it also causes the antigen to be altered so that it becomes similar but not identical with self.

Familial Factors

New Zealand black mice (NZ/B mice) spontaneously develop haemolytic anaemia and glomerulonephritis associated with antibodies against red cells and antinuclear antibodies. Germinal centres which appear in the thymus resemble those seen in systemic lupus erythematosus and myasthenia gravis. The disease provides a useful model of systemic lupus erythematosus. Recent evidence shows that these mice carry a leukaemia virus which may be important in their disease.

Classification of Human Autoimmune Disease

Three major groups of autoimmune disease may correspond to the three groups which can be produced in animals:

(1) Diseases in which micro-organisms cause an autoimmune process. Possible examples are rheumatic fever, acute glomerulonephritis, and primary atypical pneumonia.

(1a) Diseases (or autoantibody production) due to exposure to altered or sequestered tissue components. Examples are the production of antibody to heart, spermatozoa, and pancreas after damage to the corresponding organ.

(2) Hashimoto's thyroiditis and diseases such as pernicious anaemia and Addison's disease (non-tuberculous adrenalitis), which show clinical, serological, and familial overlap with it. The autoantibodies found characteristically react with *organ-specific* antigens in the thyroid, stomach, and adrenal. Autoantibodies reacting with widely distributed antigens (*non-organ-specific* autoantibodies) are rarely found.

(3) Systemic lupus erythematosus and other diseases, apparently affecting many systems, which sometimes merge clinically with systemic lupus erythematosus. These diseases usually show a high incidence of antinuclear factor and other autoantibodies against widely distributed antigen. Organ-specific antigens of the type seen in the Hashimoto group of diseases have a normal or only slightly raised incidence.

Detection of Autoantibodies

The finding of autoantibodies is important in the diagnosis of putative autoimmune diseases. There are three main classes of immunoglobulins: IgM (macroglobulin), IgG (7S gamma-globulin—the common type of antibody), and IgA (7S gamma-globulin, which appears in secretions). Different types of antibody have different biological properties and are detected by different means.

Gel Precipitation.—Many antibodies precipitate with their corresponding antigen when they are mixed in the right proportions. When serum containing antibody diffuses towards antigen in an agar plate precipitin lines may form where the antibody and antigen meet. This method will detect antibodies against antigen occurring in the liver and many other organs in Sjögren's disease. Large amounts of antibody against thyroglobulin can sometimes be found with this method in Hashimoto's thyroiditis.

Tanned-red-cell Haemagglutination.—The precipitation test can be made very sensitive if the antigen is attached to an inert carrier such as a red blood cell. An important example is the tanned-red-cell technique for measuring autoantibodies to thyroglobulin. When sheep red cells are treated with tannic acid their surface is altered so that thyroglobulin will adhere. The tanned red cells coated with thyroglobulin are agglutinated

by autoantibodies to thyroglobulin. These antibodies may show titres up to several million in Hashimoto's thyroiditis. This technique can be used to detect antibody against heart in the post-cardiotomy syndrome, and against muscle in myasthenia gravis.

Latex-fixation Test.—Antigen can also be attached to latex (rubber) particles, which are then agglutinated by antibody. Latex particles coated with human gammaglobulin are agglutinated by the rheumatoid factor (commonly found in the serum of patients with rheumatoid arthritis), which behaves like an antibody to altered human gammaglobulin.

In the *Rose-Waaler reaction*, sheep red cells are coated with rabbit gammaglobulin by mixing them with a subagglutinating amount of rabbit anti-sheep-red-cell serum. These cells are agglutinated by those rheumatoid factors which react with animal gammaglobulin. (See Fig. 2.)

Complement Fixation.—When certain antibodies combine with antigen the resultant complex will react with a group of factors in fresh mammalian serum which are collectively known as complement. Sheep red cells coated with antibody are lysed by complement and are used as a detector system to show

FIG. 2.—Mechanism of the Rose-Waaler reaction for rheumatoid factor.

The Rose-Waaler reaction is sometimes called the sheep cell agglutination test (S.C.A.T.) or the differential agglutination test (D.A.T.).

whether complement fixation has occurred. The complement-fixation test is used to detect autoantibody against thyroid cytoplasm, stomach, and adrenal gland in Hashimoto's thyroiditis, pernicious anaemia, and Addison's disease. These are examples of organ-specific autoantibodies.

Complement-fixing autoantibodies can also be demonstrated against a widely distributed antigen which occurs in liver, kidney, and many other organs. This is called the *autoimmune complement-fixation test* and it is an example of a non-organ-specific autoantibody. These autoantibodies are found in many patients with systemic lupus erythematosus, primary biliary cirrhosis, lupoid hepatitis, and Sjögren's disease, and in a few patients with rheumatoid arthritis and idiopathic pulmonary fibrosis.

Autoantibodies which react with the Wassermann antigen (alcoholic extract of beef heart) in some patients without

syphilis are responsible for the biologically false-positive reaction. Transient biologically false-positive reactions are sometimes due to infection, such as glandular fever or infectious

FIG. 3.—Immunofluorescence.

I. Preparation of Fluorescent Antihuman Gammaglobulin.

II. Immunofluorescent Test for Antibody to Nuclei.

NOTE:

- (1) Immunofluorescence may also be used to detect the site of binding of complement. The section is treated with human serum and complement (fresh human or guinea-pig serum) and the adherence of complement is detected by a fluorescent anticomplement serum. This is a microscopic complement-fixation test.
- (2) Bound gammaglobulin refers to gammaglobulin found in sections which have not been treated with human serum *in vitro*. Immunoglobulins can be shown in antibody-producing cells. In other sites the finding of bound immunoglobulin provides some evidence of an *in vivo* antibody-antigen reaction if the gammaglobulin is unaccompanied by other serum proteins.
- (3) Specific antisera can be prepared against different human immunoglobulins—such as IgG, IgA, and IgM—and these can be used to distinguish between different types of antibodies by immunofluorescence.

hepatitis, but about a sixth of all patients with chronic biologically false-positive reactions (positive reactions for over six months, with a negative treponema-immobilization test for syphilis) develop serious disease resembling rheumatoid arthritis or systemic lupus erythematosus.

Immunofluorescence.—Immunofluorescence (Coons technique) is based on the fact that antibodies are gammaglobulins and are good antigens (see Fig. 3). Rabbits immunized with human gammaglobulin produce antibodies which react specifically with human gammaglobulin. These rabbit antisera can be conjugated with fluorescent dye, such as fluorescein, and used to detect human gammaglobulin.

Many autoantigens survive freezing of tissue and being cut into histological sections in a specially refrigerated cabinet or cryostat (at $-20^{\circ}\text{C}.$). The sections are treated with serum being tested and then with the fluorescein-conjugated anti-human gammaglobulin. Microscopic examination in ultraviolet light shows fluorescence wherever the autoantibodies adhere.

Immunofluorescence is used to detect antibodies against nuclei—antinuclear factor. These are found in nearly all patients with systemic lupus erythematosus, and in many patients with lupoid hepatitis and Sjögren's disease, and in a variable number of patients with rheumatoid arthritis, polyarteritis nodosa, scleroderma, and dermatomyositis. Antibodies against the cytoplasmic constituents of thyroid, stomach, and adrenal can be demonstrated in diseases of the Hashimoto group. Antibodies occur against salivary-duct cells in Sjögren's disease, against heart and muscle in myasthenia gravis, and against mitochondria in primary biliary cirrhosis.

Immunofluorescence can be adapted to recognize the three types of immunoglobulin (IgM, IgG, and IgA) and complement-fixing antibodies. It can be used to detect gammaglobulin and complement which has become bound to human tissue *in vivo*. This uncommon occurrence provides some evidence of *in-vivo* antibody-antigen reactions. Bound gammaglobulin is found in the auricle of the heart in some cases of rheumatic fever, and in the kidney in systemic lupus erythematosus and many cases of nephritis.

Coombs Consumption Test.—Antibody against human gammaglobulin is known as *Coombs reagent*. The *Coombs consumption test* is an ingenious method of measuring antibody. Particulate antigen is mixed with the test serum and then washed repeatedly. It is assumed that antibody will adhere despite repeated washing, while non-specifically adhering gammaglobulin will be washed off. The ability of the treated particulate antigen to reduce the titre of an antihuman-gammaglobulin serum (Coombs reagent) is then measured. Some workers have found antibody against joint tissue in rheumatoid arthritis by this means.

(Part II of this article, with References, will appear in next week's issue.)