DIABETES

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PROCEEDINGS OF THE EIGHTH CONGRESS OF THE INTERNATIONAL DIABETES FEDERATION

Brussels, July 15–20, 1973

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1974

EXCERPTA MEDICA, AMSTERDAM

AMERICAN ELSEVIER PUBLISHING COMPANY, INC., NEW YORK

INTERNATIONAL CONGRESS SERIES No. 312

ISBN EXCERPTA MEDICA 90 219 0235 4
ISBN AMERICAN ELSEVIER 0 444 15074 9
LIBRARY OF CONGRESS CATALOG CARD NUMBER 74–77386

Publisher:

EXCERPTA MEDICA
335 Jan van Galenstraat
Amsterdam
P.O. Box 1126

Sole Distributors for the USA and Canada:

AMERICAN ELSEVIER PUBLISHING COMPANY, INC.
52 Vanderbilt Avenue
New York, N.Y. 10017

Printed in The Netherlands by Casparie Alkmaar B.V.

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IMMUNITY, AUTOIMMUNITY, AND DIABETES*

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It is easily thought, said and even written that diabetes mellitus, like other conditions of ill-defined etiology, may be (some say is) an autoimmune disease. Such a definite statement might correspond to some sort of fashionable thinking, rather than to a critical evaluation of proved facts. As professor Heremans (1973) recently said: 'Immunology is like a fungus the mycelium of which pervades the whole dignified building of medicine, suddenly producing crops of mushroom in areas where its presence was least expected, for instance endocrinology'. As far as diabetes is concerned, one may still wonder about the quality of this mushroom. To ascertain this, it seemed necessary to bring together not only diabetologists and pathologists interested in the subject but also, and foremost, basic immunologists. Thanks to the generous support of the Francqui Foundation, such a meeting was organized a few months ago by doctor Willy Gepts and myself. For the greater part of this talk I shall only be the spokesman of the distinguished participants of this Colloquium. However, I shall have to accept the responsibility for most of the conclusions and final considerations.

This review will be divided into 3 parts:

1. The initial, already 10 years old, concept of autoimmunity, on which the assumption is based that diabetes mellitus in itself is an autoimmune disease.
2. The present concepts of autoimmunity.
3. The discussion, on the basis of these concepts, of the data which argue in favour of autoimmune processes playing a part in the pathology of diabetes mellitus.

THE INITIAL CONCEPTS OF AUTOIMMUNITY

Autoimmune diseases entered medical thinking only 17 years ago when Roitt et al. (1956) discovered the presence of thyroid antibodies in the serum of patients affected with Hashimoto's goiter while Rose and Witebsky (1956) produced lymphocytic thyroiditis in the experimental animal by injecting homologous thyroglobulin. Some time afterwards autoimmunity was defined as a condition in which 'structural or functional damage is produced by the action of immunologically competent cells or antibodies against normal components of the body' (Mackay and Burnet, 1963).

It was then proposed to separate such conditions into 2 distinct groups: the acquired

* Presidential Adress.

TABLE 1
Secondary, acquired or transient autoimmune diseases

Syndrome	Tissue affected	Induced by
Dressler	Pericardium Myocardium	Necrosis Virus Trauma
Endophthalmia	Lens	Infection Trauma
Hemolytic anemia	Red cells	Ovarian tumor Lymphoma Mononucleosis infection
Male sterility	Testicles	Stasis in vas deferens

or transient and the idiopathic diseases. In the first group (Table 1) the pathological process was recognized as the result of a primary anomaly arising in the tissues. Despite the normal working of the immune system, assuring tolerance of all the body components, the abnormal antigen admittedly induced an autoimmune reaction, i.e., an immune reaction against a tissue component of the body itself. Peri- and myocarditis, arising after myocardial damage by viral attack, surgery or infarct, is the best example of such a condition.

The second group, that of idiopathic diseases (Table 2), includes several conditions which are often associated. Here organ-specific autoimmunity and tissue damage are supposed to be related to the spontaneous loss of the normal tolerance of these tissue components. This loss of tolerance was thought to be due to a sudden abnormality arising within the immune system, i.e., proliferations of immunologically competent cells active against certain constituents of the body. Normally such forbidden clones should have been eliminated once and for all during fetal life (Burnet, 1959a, b; Mackay and Burnet, 1963).

According to criteria set forth by Witebsky, Feltkamp (1966) and others, idiopathic autoimmune diseases have a series of common characteristics (Table 3): the serum contains specific antibodies and thus increased levels of IgG. In the tissues one finds destructive cell lesions and characteristic lymphocytes and plasma cells infiltrations. Moreover, similar lesions can be reproduced by injecting in the experimental animal extracts of its own tissues. On the clinical side, one finds that these autoimmune diseases are more frequent in women than in men and that their incidence increases with age.

Another important trait is the occurrence in the patients thus affected of other so-called autoimmune diseases or at least of antibodies pertaining to these diseases. Among

TABLE 2
Idiopathic autoimmune diseases

Tissue or organ affected	Disease
Blood	Idiopathic hemolytic anemia Idiopathic thrombocytopenia
Collagen	Rheumatoid arthritis Lupus erythematosus diffusus etc.
Thymus	Myasthenia gravis
Gastric mucosa	*Pernicious anemia*
Adrenal cortex	*Addison's disease*
Thyroid	*Thyroiditis and hypothyroidism*

the close relatives of such patients one finds a high incidence of: (1) the same disease; (2) antibodies characteristic of that disease; and (3) other autoimmune diseases. The best example of this pathology is autoimmune thyroiditis, leading to spontaneous myxedema. All stages have now been studied from normality to asymptomatic thyroiditis and to clinical hypothyroidism (Bastenie et al., 1972a). Not only the morphological changes in the thyroid and in the pituitary induced by the progressive thyroiditis and parenchyme destruction, but all criteria set forth for idiopathic autoimmune diseases are fulfilled (Feltkamp, 1966).

How far does diabetes mellitus conform to these criteria? Admittedly the overall incidence of diabetes is highest in women and increases with age (Table 4). Insulitis has been described, albeit almost exclusively in juvenile diabetics. Insulitis has been reproduced experimentally and in a few animals has led to the development of diabetes. Moreover, the association of diabetes with other autoimmune diseases has repeatedly been shown, mostly with thyroiditis. Diabetes itself runs in families.

It is easy to understand that many are inclined to include diabetes among the idiopathic autoimmune diseases.

THE PRESENT CONCEPTS OF IMMUNITY AND AUTOIMMUNITY

The new knowledge which has upset this assumption results from the shifting of the study of antibodies to that of the cellular reactions to the antigens. Only recently was delayed hypersensitivity (i.e. specific cell-mediated immunity) considered in the pathogenesis of human autoimmune disorders. The mobilization of cellular defenses was first studied in experimental autoimmunity in the rejection of homografts. These studies have led to the already classical knowledge concerning the cellular basis of immune responses as reviewed by Roitt et al. (1969).

TABLE 3
Characteristics of idiopathic autoimmune diseases (AID)

Serum
Antibodies: IgG ↗
Complement ↘

Tissues
Parenchyme destruction
Lymphoblasmatocytes infiltrations

Experimental reproduction
Tissue lesions
Antibodies

Clinic
F > M

↗ with age

Associations
In patient: other AID antibodies
In close relatives: same AID
other AID antibodies

TABLE 4
Diabetes mellitus as an idiopathic AID

Criteria of AID	Diabetes
F > M	+
Incidence ↗ with age	+
Lymphocytic infiltrates	+ (Only juvenile diabetes)
Experimental production	+
Association-other AID	+
Association in close relatives	
Diabetes; other AID	+
Antibodies in serum IgG ↗	0?

The small lymphocytes belong to 2 distinct populations: one dependent on the presence of the thymus and therefore called T lymphocytes and the other independent of the thymus and originating from the bone marrow, called B lymphocytes. The T lymphocytes constitute the greater part of the small lymphocytes circulating in the blood. B lymphocytes appear to be more restricted to lymphoid tissue.

The T lymphocytes can be non-specifically stimulated in cultures by phytohemagglutinin, a non-specific mitogen which acts on other receptor sites besides those which are antigen-sensitive. This response to non-specific mitogens provides some measure of the overall activity of T lymphocytes in the blood but need not necessarily reflect their ability to react to specific antigens (Fudenberg et al., 1971).

The small T lymphocytes can also be stimulated into blast cells by the action of an antigen, which has been processed by the action of macrophages and thereby transformed into an immunogen. The clones of cells which stem from the immunogen-stimulated T lymphocytes will differentiate into 'killer cells'. The effects of these cells have been well studied in graft rejections, in human thyroiditis and in tissue cultures of Hashimoto goitre.

The immunogen-stimulated T lymphocytes further secrete soluble factors which have several properties: distention and increased permeability of the capillaries, and stimulation or inhibition of macrophages. There is further evidence that the immunogen-stimulated T cells can cooperate with the B cells and control their activity (Allison et al., 1971).

The B cells, stimulated by an immunogen (transformed antigen) can also differentiate into blast cells which will produce cell clones resulting in the formation of plasmatocytes. These specialized cells are the seat of synthesis and secretion of specific antibodies. It is now clear that the production of antibodies, important as it may be in certain cases (as for instance in hemolytic anemia), is in many cases of little direct pathogenic significance. The antibodies may, however, stimulate inactive tolerant T lymphocytes into immune reactions (cf. Heremans, 1973).

Research has now turned to the study of the action of antigens in calling forth the reactions of immunocytes and to the study of these reactions in order to explain the disappearance of the normal state of tolerance, which the immunocytes normally display

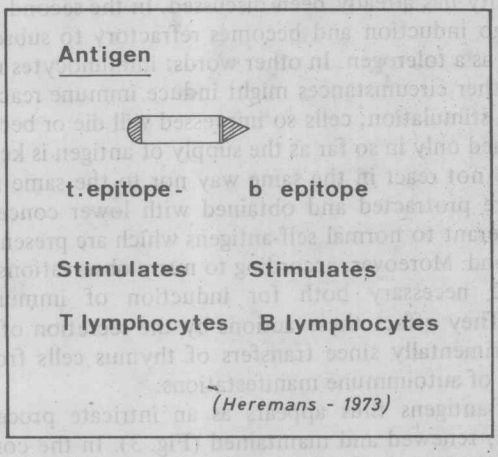

FIG. 1. Epitopes of antigens (after Heremans, 1973).

towards the various components of 'self'. Antigens are considered as possessing epitopes (Fig. 1), which are the immunogenic sites directed against T or B lymphocytes and are therefore called t or b epitopes. These epitopes are composed of proteins, polysaccharides or nucleic acids. Many epitopes are normally hidden and are brought to light only after partial enzymatic breakdown or denaturation (i.e. uncoiling) of the macromolecule.

Even resting immunocytes exhibit a low level of immunoglobulin synthesis. A fraction of this immunoglobulin is incorporated into their cell membrane, where it stays for a period of hours before being lost (Fig. 2). The combination on these receptor sites of the

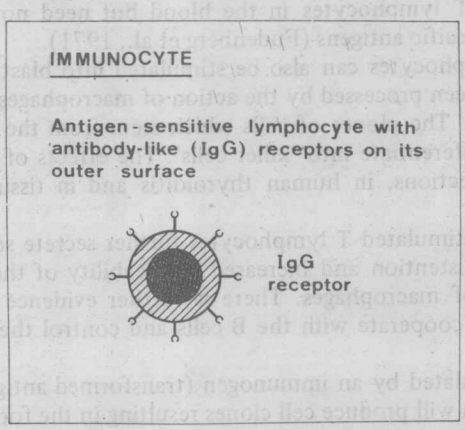

FIG. 2. Schematic representation of an immunocyte.

immunoglobulin with the antigen can be observed in autoradiographic preparations seen in electron microscopy: when the antigen is marked with radioiodine, it impresses a silver reagent. The black dots which then appear on the cellular membrane are the results of the combination with the Ag on the receptor sites. Such a combination may result in: (1) induction of immune reactions; and (2) inhibition of immune reactions.

The first possibility has already been discussed. In the second possibility the immunocyte fails to undergo induction and becomes refractory to subsequent stimulation: the antigen has behaved as a tolerogen. In other words: immunocytes may become tolerant to antigens which in other circumstances might induce immune reactions. They do so when submitted to excess stimulation; cells so impressed will die or become inactive. However, tolerance is maintained only in so far as the supply of antigen is kept up.

T and B cells do not react in the same way nor to the same antigens. T tolerance is faster in onset, more protracted and obtained with lower concentrations. Therefore T lymphocytes are tolerant to normal self-antigens which are present in low concentrations in the circulating blood. Moreover, according to many observations in vivo (see Heremans. 1973), T cells are necessary both for induction of immune responses and for immune tolerance. They effect these actions by the secretion of associative antibodies. This is shown experimentally since transfers of thymus cells from young to old mice reduce the incidence of autoimmune manifestations.

Tolerance to self-antigens thus appears as an intricate process, which must continuously be acquired, renewed and maintained (Fig. 3). In the concept of balanced self-tolerance, proposed by Heremans, self-antigens present in small quantities in the serum

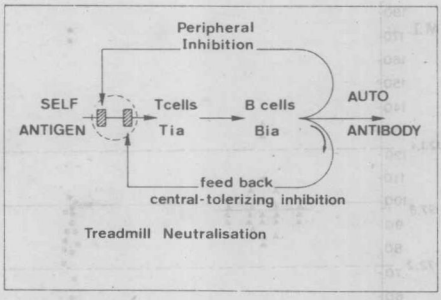

FIG. 3. The concept of balanced self-tolerance in the normal state (Heremans, 1973).

would partly activate the inactive T cells (Ti) and B cells (Bi) so that some slight degree of antibody formation would be produced. However, this autoimmune reaction eliminates part of antigen, and on the other hand the auto-antibodies exert a negative feed-back on the production of antibodies. Thus in the normal state self-antigens and autoimmune reactions at every moment neutralize each other as in a kind of treadmill. Autoimmunity results from the break-down of this balance, which can be due to changes in the quality or the quantity of the self-antigens – or to an alteration in the immune system – often an immune deficiency, cellular or humoral (Table 5).

TABLE 5
Breakdown of tolerance of self-antigens

Factors affecting	– antibody forming cells
	– cell mediated immune reactions
Defect of *control mechanism*	
	– genetic
	– acquired
Altered *antigens*	– chemical drugs
	– infections (viral)
	– metabolic processes
	– cell death

An important byproduct of the experimental work just reviewed consists of a series of tests of cell-mediated immunity that can now be applied to clinical studies. The rosettes formation test only detects the presence of sensitized cells. These cells have immunoglobulins at their surface. When brought into contact with red cells coated with the antigen, the red cells will form a ringlet or rosette around the sensitized lymphocytes.

A more specific test of cellular immunity is the leukocyte migration test (LMT) based on the principle that sensitized T lymphocytes, when in contact with the sensitizing factor, secrete a polypeptide that inhibits the migration of guinea pig macrophages and also of human leukocytes.

Normal migration of leukocytes in a migration chamber is markedly reduced when

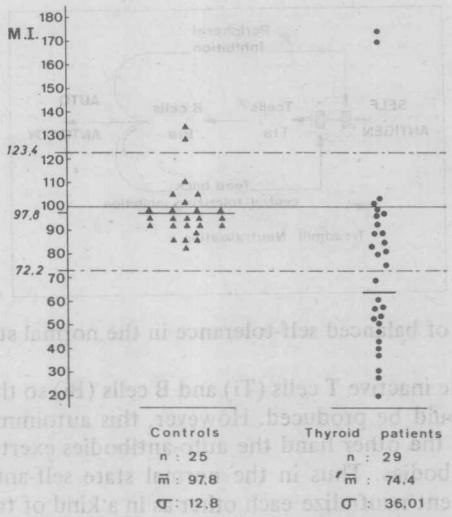

FIG. 4. Migration index (surface with antigen/surface without antigen) in normal and thyroid patients; the antigen used is thyroglobulin (From Delespesse et al., 1973, by courtesy).

antigen to which the cells are sensitized is added to the medium. Results of the test are easily expressed as a migration index, as seen in Figure 4 comparing results of normal controls and patients affected with various thyroid diseases: a group of the latter display definite reactions against thyroglobulin.

The third test, admitted as specific of cell-mediated immunity, is the lymphoblast transformation test (LTT). When lymphocytes of a subject sensitized to a given antigen are cultured in the presence of this antigen, lymphoblastic transformation is induced. This can be studied by the morphological changes, for instance the number of mitoses, or by the measurement of the tritiated thymidine incorporated into the lymphocytes culture.

With these new concepts in mind and techniques at hand we can turn back to our problem, the study of autoimmunity in diabetes.

THE STUDY OF AUTOIMMUNITY IN DIABETES

As recalled earlier, clear-cut autoimmune diseases like lymphocytic thyroiditis have definite clinical, biological, serological and epidemiological characteristics. At first sight diabetes mellitus responds to these criteria. However, on a closer look, discrepancies become apparent (Table 6).

The clinical characteristics of autoimmune diseases (age and sex incidence) are found only in the adult-onset type: no other signs of immune abnormality can be detected in this form of diabetes except for the possibility of an immune complex disease and the association with other so-called idiopathic autoimmune conditions. It is clear that the immune complexes formed in insulin-treated patients are related to exogenous insulin and that deposition of this material in the capillary walls is by no means a true autoimmune process. As to the association of diabetes with other autoimmune conditions, we shall