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THE IMMUNOLOGY OF AUTOIMMUNE THYROID DISEASE

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The importance of autoimmunity in the etiology of many endocrine disorders, particularly those of the thyroid, is now clearly recognized. While there is not yet full understanding of all elements of the autoimmune process, there has been a vast expansion of genetic and immunologic data that increasingly strengthens the case for a genetic disorder in immunoregulation as a basis for these conditions. In the following article, we shall first briefly review some important aspects of the immune system, and describe potential mechanisms which could result in *organ specific autoimmune disease*. We shall then address ourselves more specifically to the subject of *autoimmune thyroid disease*.

The cellular components of the immune system are lymphocytes and macrophages. All *lymphocytes* are derived from bone marrow stem cells (Figure 1). About 55% are then processed in the thymus and hence become thymus-dependent (T) lymphocytes. T-lymphocytes are incapable of secreting antibodies. However, they do control antibody production by secretion of substances (lymphokines) that act upon contiguous or nearby lymphocytes. T-lymphocytes have important cell subsets: the helper or inducer cells that

aid the antibody-producing cells, and the suppressor/cytotoxic cells that suppress antibody production, or exert a direct cell-damaging effect.

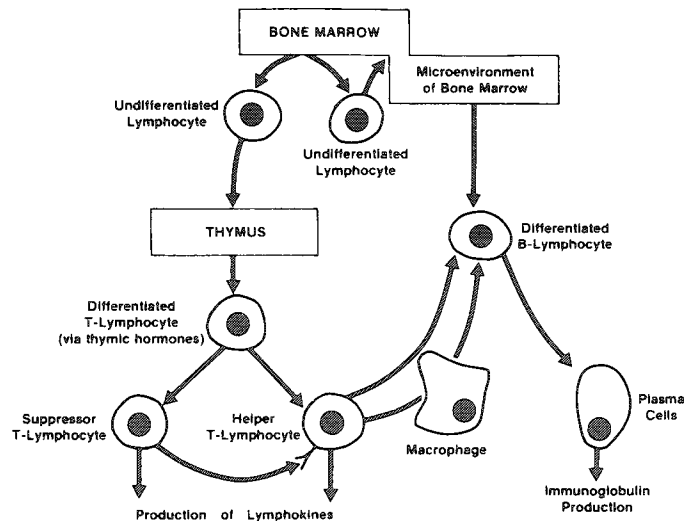


Figure 1
Simple overall scheme of lymphocyte differentiation and cellular immune reactions. Bone marrow stem cells differentiate to T- or B-lymphocytes, which interact with each other to modulate antibody production. (Topliss, DJ, et al: Clin Endocrinol 15:335-41, 1981, with permission.)

The remaining 45% of lymphocytes are the bursa-equivalent (B) lymphocytes and are so termed because they are analogous to the lymphocytes processed by the bursa of Fabricius in birds. These are not processed in the thymus but become differentiated within the bone marrow itself. B-lymphocytes are capable of differentiating into plasma cells and producing antibodies. B-lymphocyte activity is controlled by helper T-lymphocytes except under unusual circumstances, i.e., certain unusual antigens can act as polyclonal B-lymphocyte activators, although this mechanism is probably not of importance in autoimmune thyroid disease. Generally, helper T-lymphocytes are necessary to exert a direct effect on the production of immunoglobulin (Ig) by B-lymphocytes; indeed, no such antibody formation will occur in the absence of these antigen-specific helper T-lymphocytes. On the other hand, specific suppressor

T-lymphocytes appear to exert their main effect by suppressing specific helper T-lymphocytes. Some lymphocytes lack characteristics of T and B cells and are designated "null lymphocytes."

While *macrophages* are also necessary for reaction to antigen, the macrophage itself is not specifically sensitized and thus is a nonspecific, albeit essential part of the immune response.

The breakdown of self-tolerance in a general or specific manner is fundamental to the development of autoimmune disease. In fetal and postnatal life, the organism produces, by apparently random mutation or generation, a vast repertoire of new clones of lymphocytes which will be capable of interacting with any possible antigen with which the organism may come into contact during life. Thus occasionally, at random, self-reactive T- and B-lymphocytes are generated. Self-reactive lymphocytes are generally eliminated during fetal life because there is an overall lack of helper cell activity required for clonal expansion. This has been called "clonal abortion." In the child, and especially in the adult, three other control mechanisms become important in limiting the proliferation of such clones. These are idiotype network feedback, receptor blockade, and suppressor T-lymphocyte action.

"Idiotype" network feedback. The area of an antibody around and including the antigen-combining site is unique to that particular antibody and is called the idiotype. Because it is unique, it is perceived as immunologically "foreign," i.e., it is antigenic, and can provoke the formation of antibody to that unique area. This antibody is termed "antiidiotypic." By binding to the immunoglobulin-like cell surface receptors of the specific lymphocyte that has acted as the antigen, the antiidiotypic antibody suppresses the activity of that lymphocyte. This idiotypic-antiidiotypic network is an important control mechanism of immune response, probably predominantly suppressing reactions to antigen.

Receptor blockade. Immune paralysis induced by large amounts of antigen swamping the receptors, and perhaps preventing the cell-cell interactions required for immune response, is called receptor blockade. This mechanism has not been demonstrated in thyroid disease.

Suppressor T-lymphocyte action. To be discussed subsequently.

Target Cell Interactions

In autoimmune thyroid disease there is evidence of both thyroid stimulation (thyroid-stimulating antibody) and thyroid cell damage. The latter is primarily mediated by mechanisms that involve antibodies although direct cellular cytotoxicity by lymphocytes also occurs.

Antibody-dependent cell-mediated cytotoxicity (ADCC) requires the prior attachment of antibody to the target cell. Thereafter, null lymphocytes (lacking T- or B-lymphocyte surface antigens) and macrophages can attach themselves via the bound antibody and exert noncomplement-mediated cytotoxic effects. ADCC has been demonstrated in autoimmune thyroid disease.

Natural killer (NK) cells. Part of the null lymphocyte population are cytotoxic without the need for antibody coating of the target cells. Immune complexes (antibody-antigen combinations) can also attach themselves to target cells. The cells are then destroyed by macrophages or killer cells. The role of NK cells in thyroid disease is currently being explored.

Autoimmune Thyroid Disease and Genetics

It is evident that both Graves' disease and Hashimoto's thyroiditis aggregate in specific families and thus appear to be genetically induced.^{1,2} Indeed, these two disorders tend to occur in the same families, and may even coexist within the same thyroid gland.^{2,3,4} Moreover, homozygous twins have been reported where one twin has Graves' disease (GD) and the other has Hashimoto's thyroiditis (HT).^{5,6} An increased incidence of other autoimmune diseases in patients with GD or HT, as well as in their families, is now well known.^{7,8} These include diabetes mellitus, pernicious anemia, myasthenia gravis, rheumatoid arthritis, idiopathic thrombocytopenic purpura, Addison's disease, vitiligo, and chronic active hepatitis.^{9,10} In addition, functional thyroid disturbances and thyroid antibodies occur in about one half of the first-order relatives of patients with autoimmune thyroid disease.⁹

The occurrence of GD in both siblings of dizygotic twins is reported to be about 3% to 9%, and of monozygotic twins to be 30% to 60%. The fact that monozygotic twins have a higher concordance rate is strong evidence for a genetic basis of GD, but genetic factors alone do not explain why some develop the disease, while others do not. Thus, it would appear that other influences also are necessary before the disease is expressed.⁷

Studies of the age-specific incidence rates in both GD and HT have indicated that both disorders occur at random in genetically predisposed populations.⁷ In addition, both autoimmune thyroid diseases, as well as the presence of thyroid autoantibodies, have been observed more often than expected among patients with Down's syndrome and their maternal relatives. Some patients with gonadal dysgenesis and perhaps their mothers also have increased frequency of thyroid autoimmunity. The biological implications of these associations are unclear, but common pathogenetic mechanisms may be involved in the development of chromosomal aberrations and thyroid autoimmunity.

HLA, Graves' Disease, and Hashimoto's Thyroiditis

HLA is a region on chromosome 6 which includes several gene loci. The products of many of these genes are expressed as cell surface glycoprotein receptors and influence cell-cell interaction. HLA in man is structurally and functionally homologous to the major histocompatibility region in mice, dogs, Rhesus monkeys and other higher vertebrates. Many biologically important functions, including qualitative and quantitative control of the immune response to certain antigens, killing of virus-infected cells, and synthesis of several complement components have been associated with the major histocompatibility complexes of several species. However, these complicated effects are not directly assessed in testing for disease associations in man. Instead, the specific cell surface antigens are identified as markers of these effects. At least four loci have been clearly demonstrated within the HLA region. The A and B loci determine the classical transplantation antigens identified by serological methods. The C locus determines another series of serologically detected antigens, but the functions are unknown. D-locus antigens cannot be detected by conventional serological testing and are detected by mixed leukocyte cultures.¹¹ More recently, a D-related (DR) HLA antigen system has been studied by means of serological techniques.

In GD there have been several studies of HLA association. In Caucasians, an increased frequency of the B-locus antigen HLA-B8 has been found. The relative risk of GD in persons with HLA-B8 compared to persons lacking in this antigen is 2.4. In addition, HLA-Dw3 and HLA-DRw3 have been found to be increased in incidence in Graves' disease in Caucasians; the relative risk for GD is 5.2 in persons with this HLA antigen.¹¹ It has been shown that patients with GD who go into remission following treatment with antithyroid drugs are much less likely to have HLA-B8 or HLA-Dw3 (DRw3) than those who relapse.^{12,13,14} That is, the presence of HLA-Dw3 (DRw3) appears to be associated with an increased incidence of persistence of the disease without remissions. Thus, this form of typing has a predictive value in determining which patients will remit or relapse after antithyroid drug therapy.

In the Japanese, HLA-B8 is only rarely found; the same is true of HLA-Dw3. However, an increased incidence of HLA-Bw35 and DHO (now termed Dw12) in Graves' disease has been found in the same population. The latter results are of considerable interest since the association of Graves' disease and type I diabetes mellitus in Caucasians involves an increased incidence of HLA-Dw3 in both groups; conversely, in type I diabetes in Japanese, there is an increased incidence of HLA-DYT, rather than HLA-DHO (Dw12) indicating genetic dissimilarities between Japanese patients with Graves' disease as opposed to those with diabetes mellitus.^{15,16} In the Chinese, at least one study has shown an increase in HLA-B46¹⁷ in Graves' disease. Thus, Chinese, Japanese, and Caucasians have distinct HLA-disease linkages.

In Hashimoto's thyroiditis, a relationship between HLA-DRw3 and the atrophic form of this disorder has been reported,¹⁸ but in the goitrous form of HT an increased incidence of HLA-DRw5 has been described.¹⁹

Therefore, it seems probable that linkage of defined HLA loci to disease-predisposing genes is responsible for the HLA associations observed with GD and perhaps HT as well. It is possible that disease susceptibility is due to human homologues of the murine immune response (Ir) or immune suppression (Is) genes (more likely the latter), which modulate the strength and characteristics of the immune re-

sponse to certain specific antigens. Ir genes appear to be important in experimental autoimmune thyroiditis in mice; but as yet neither Ir nor Is genes have been unequivocally demonstrated in man. It is of interest that in the mouse various lymphokines, including suppressor factors, are coded for within the histocompatibility locus, in an area analogous to the HLA-D region of man.

Immune Nature of Graves' Disease and Hashimoto's Thyroiditis

While it seems evident that GD is closely related to HT, both genetically and pathogenetically, and that each

Table I

Immune stigmata associated with Graves' disease and Hashimoto's thyroiditis

Stigma	Graves' disease	Hashimoto's disease
Lymphocytic infiltration in thyroid	Frequently present	Almost invariable
Immunoglobulins in thyroid stroma	Yes	Yes
Type of infiltrating lymphocytes in thyroid	B- and T-lymphocytes, some unidentified lymphocytes	B- and T-lymphocytes, some unidentified lymphocytes
Immune complexes in circulation	Common	Common
Thymic enlargement	Common	Common
Lymphadenopathy and splenomegaly	Infrequent	—
Relative lymphocytosis	Common	—
Hypergamma-globulinaemia	—	Common
Benefit from corticosteroid therapy	Yes	Yes
Thyroid-stimulating immunoglobulin	Almost all	Infrequent
Exophthalmos	Common	Occasional
Evidence of cell-mediated immunity	Yes	Yes
Evidence for a defect in suppressor T-lymphocytes	Yes	Yes
Other autoimmune diseases in patients	e.g., pernicious anemia, diabetes mellitus, myasthenia gravis, Addison's disease, idiopathic thrombocytopenic purpura	e.g., pernicious anemia, diabetes mellitus, myasthenia gravis, Addison's disease, chronic active hepatitis
Thyroid antibodies in relatives	50%	50%
Thyroid and other autoimmune diseases in relatives	Common	Common
HLA genes (Caucasians)	HLA-B8-Dw3	Atrophic form: HLA-B8 and HLA-DRw3 Goitrous form: HLA-DR5
Animal models	—	Yes

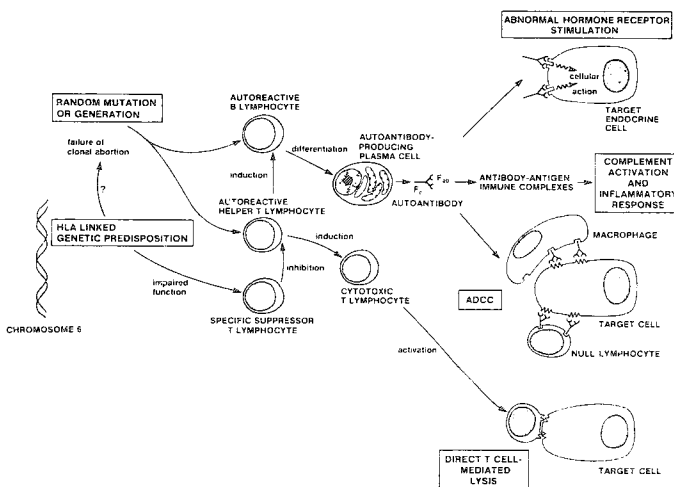


Figure 2
Immune mechanisms in organ-specific autoimmune disease. On the right are the effector mechanisms; ADCC is antibody-dependent cell-mediated cytotoxicity. In the center are the immunoregulatory interactions controlling the effector mechanisms. On the left are the fundamental genetically associated processes generating autoimmunity.

is caused by closely related immunological abnormalities (Table I), there are at least a few aspects of each condition that distinguish the two maladies. Consequently they cannot be construed merely as different expressions of a single entity. Although the pathogenesis of these two conditions has not been clarified, a working hypothesis, supported by evidence gained so far, may be proposed which can be tested in future studies. As shown in Figure 2, the major immune system actions in organ-specific autoimmune disease as illustrated by autoimmune thyroid disease are:

1. Autoantibodies that can bind at, or very close by, the receptor for thyroid-stimulating hormone (TSH), and that are generally capable of evoking a cellular response like that of hormone stimulation.
2. Antibody-antigen immune complexes, which if complement-fixing can trigger an inflammatory response.
3. Antibody-dependent cell-mediated cytotoxicity.
4. Direct T-cell-mediated cell damage.

The first three of these mechanisms require clones of autoantibody-producing B-cells; the fourth requires a clone or clones of cytotoxic T-lymphocytes. The unleashing of the activity of these cells requires the cooperation of autoreactive helper T-lymphocytes, which along with autoreactive B-lymphocytes arise throughout life by random generation. Clonal expansion is prevented by suppressor T-lymphocyte control. If there is a specific suppressor T-lymphocyte defect, qualitative or quantitative, then autoimmune reactions and persistent disease are permitted to occur. It seems probable that both disorders are due to specific genetic defects in immunological regulation. As will be discussed below, there is now evidence that abnormalities in specific populations of suppressor T-lymphocytes may be the basis of such a defect.

We suggest that with such a defect, a normally randomly mutating thyroid-directed self-reactive "forbidden" clone of thymus-dependent (T) "helper" lymphocytes is permitted to survive. It may well be that all normal persons have the capacity to produce "forbidden" clones of self-reactive lymphocytes directed towards normal body constituents. In normal persons, however, suppressor T-lymphocytes will exercise normal immunological control, thus suppressing these "forbidden" clones of "helper" T-lymphocytes, and preventing them from interacting with their complementary antigen⁹

In persons genetically predisposed to either GD or HT, it may only take the random appearance of the appropriate thyroid-directed "forbidden" clone of "helper" T-lymphocytes, which then escapes normal control because of the presumed genetic defect. While there have been suggestions that it is necessary to have some thyroidal antigenic alteration, possibly induced by viral interaction, it is at least equally possible that no such antigenic alteration is necessary. Certainly such antigenic alteration by itself is insufficient to sustain chronic autoimmunity. In any event, once the appropriate "forbidden" clone of "helper" T-lymphocytes has survived, we postulate that it then will interact with its complementary antigen, presumably on the thyroid cell membrane, setting up a localized cell-mediated immune (CMI) response. Subsequently, the same "helper" T-lymphocytes cooperate with and direct groups of appropriate bursa-equivalent (B) lymphocytes, which in consequence produce the thyroid autoantibodies. In the case of GD, these

take the form of thyroid-stimulating immunoglobulins (TSI), which appear to be antibodies directed against the TSH receptor or a contiguous cell membrane site. The TSI, after its interaction with the TSH receptor on the thyroid cell membrane, then appears to stimulate the thyroid follicular cells in a manner indistinguishable from TSH. (See *Thyroid Today*, Vol. 3, No. 5.) The other thyroid autoantibodies may exert deleterious effects on the thyroid cells, either alone, or as antigen-antibody immune complexes, or in cooperation with lymphocytes or "killer" cells⁹

Other reported means by which thyroid autoantibodies act on the thyroid include inhibition of TSH action with consequent hypothyroidism, thyroid growth promotion in goitrous autoimmune thyroid disease, and thyroid growth inhibition in atrophic myxedema. Lastly, such antibodies may be directed against thyroid hormones themselves and may be responsible for discrepant radioimmunoassay results.

For a full discussion of the evidence on which our hypothesis is based as well as speculations on the role of stress in the precipitation of hyperthyroidism, the nature of the remissions in Graves' disease, and the etiology of exophthalmos, the reader is referred to reviews published elsewhere.^{7,8} However, it is of interest to briefly discuss the results of migration inhibition factor studies and lymphocyte cultures, which indicate the importance of suppressor T-lymphocyte dysfunction.

Lymphocyte culture experiments. If lymphocytes from GD are cultured with either phytohaemagglutinin²⁰ (PHA) or with crude normal human thyroid antigen,²¹ or with a membrane-rich human thyroid fraction,²² the lymphocytes will secrete thyroid-stimulating immunoglobulin (TSI) into the medium. This can be detected by demonstrating that the immunoglobulin from the medium can stimulate human thyroid slices to produce increased quantities of cyclic AMP. Since PHA stimulates only T-lymphocytes, these results imply that PHA stimulated thyroid-directed "helper" T-lymphocytes, which then interacted with thyroid-directed B-lymphocytes; the B-lymphocytes then responded by releasing TSI into the culture medium. Lymphocytes from patients with non-immune thyroid disease, or from healthy controls, generally do not secrete TSI into the medium under these circumstances. The fact that normal human thyroid fractions can stimulate previously sensitized GD lymphocytes suggests that no antigenic alteration is necessary for lymphocytic interaction.

Studies of sensitization and suppressor function of T-lymphocytes in GD and HT. One procedure currently available for measuring cell-mediated immune responses *in vitro* is the migration inhibition factor (MIF) test. MIF is a lymphokine secreted by T-lymphocytes after exposure to an antigen to which they have been previously sensitized. This lymphokine will inhibit the migration of T-lymphocytes *in vitro*. Previous studies have employed whole leukocyte populations and have been subject to some criticism on this basis. Current studies in our laboratory have employed preparations of T-lymphocytes alone.²³ We have reported that T-lymphocytes from patients with GD or HT will manifest MIF production in response to human thyroid antigen. This response appears to be organ-specific. Moreover, studies in our laboratory have confirmed that using an indirect MIF test, the supernatants from cultures of lymphocytes from patients with Graves' disease or Hashimoto's thyroiditis (cultured in the presence of thyroid antigen), but not those from normal subjects, will cause inhibition of migration of

normal T-lymphocytes²⁴ It is now possible to conclude that there is indeed evidence of sensitization of T-lymphocytes against thyroid antigen in patients with GD or HT. Moreover, when a cell membrane preparation was compared as an antigen with crude thyroid homogenates, the former was much more active by mass.

When T-lymphocytes from normal persons were added to GD or HT lymphocytes in a ratio as low as 1:9, the ability of the GD or HT T-lymphocytes to produce MIF was abolished.^{25,26} This suggests that suppressor T-lymphocytes within the normal T-lymphocyte population inhibit the ability of the sensitized T-lymphocytes from GD or HT to produce MIF. When two populations of GD or HT T-lymphocytes were mixed together, there was no abolition of MIF production. Moreover, when normal lymphocytes were incubated with mitomycin C (which impairs suppressor lymphocyte function) before adding them to the GD or HT T-lymphocytes, then the ability of the normal T-lymphocytes to influence the GD or HT T-lymphocytes was lost. Similarly, in more recent studies, we have shown that (1) irradiation of the normal T-lymphocytes with 1000 rad, a dose designed to disturb only the function of the suppressor T-lymphocytes, will destroy their ability to suppress the Graves' or Hashimoto's T-lymphocytes, although the viability of the cells was not affected,²⁷ and (2) treatment of the normal T-lymphocytes with cimetidine (an H-2 receptor blocker which impairs suppressor function) also completely vitiated the suppressing ability of the normal T-lymphocyte population. Whereas this latter result does not establish that the defect in Graves' and Hashimoto's diseases involves the H-2 receptor on specific suppressor T-lymphocyte populations, the possibility at least deserves further investigation.²⁸ On the basis of these results we propose that the lack of at least one population of suppressor T-lymphocytes in GD and HT had permitted the sensitized (presumably "helper") T-lymphocytes from the patients to produce MIF when exposed alone to the antigen.

In our most recent studies, we have shown that the suppressor T-lymphocyte defect persists for decades after treatment of Graves' disease, and thus would appear to be unrelated to the hyperthyroidism *per se*, or its treatment.²⁹

Summary

We propose that both in GD and HT a genetic abnormality results in a specific defect in immune mechanisms which is almost certainly a dysfunction of specific suppressor T-lymphocytes. Such a defect permits a randomly appearing, thyroid-directed "forbidden" clone of "helper" T-lymphocytes to survive, interact with previously normal

thyroid cell membranes, and initiate either GD or HT by cooperating with groups of already present appropriate B-lymphocytes. In consequence, the latter produce thyroid antibodies (in the case of GD, TSI) to complete the pathophysiological expression of these conditions.

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