CHAPTER 7- AUTOIMMUNITY TO THE THYROID GLAND

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ABSTRACT

SUMMARY

This discussion stresses the normal occurrence of immune self-reactivity, the genetic and environmental forces that may amplify such responses, the role of the antigen-driven immune attack, secondary disease-enhancing factors, and the important contributory role of antigen-independent immune reactivity. Research on thyroid autoimmunity has benefited greatly by knowledge of the specific target antigens and easy access to blood cells and involved target tissue. As research moves apace in realm of molecular genetics and investigation of environmental factors that cause disease, we may look for rapid progress in understanding and controlling these common illnesses.

A BRIEF REVIEW OF IMMUNOLOGIC REACTIONS

The human immune system is comprised of about 2 X 10^{12} lymphocytes containing approximately equal ratios of T and B cells. B lymphocytes synthesize immunoglobulins that are first expressed on their membranes as clonally distributed antigen-specific receptors and then secreted as antibodies following antigenic stimulation. The ability of the immune system to recognize antigens is remarkable. A human being can produce more than 10^7 antibodies with different specificities. The concentration of antibodies in human serum is 15 mg/ml, which represents about 3 x 10^{20} immunoglobulin molecules per person! Since each B cell has approximately 10^5 antibody molecules of identical specificity on its surface, the human humoral immune system scans the antigenic universe with about 10^{17} cell bound receptors. To maximize the chances of encountering antigen, lymphocytes recirculate from blood to lymphoid tissues and back

to the blood. The 10^{10} lymphocytes in human blood have a mean residence time of approximately 30 minutes, thus an exchange rate of almost 50 times per day.

T lymphocytes develop from precursor stem cells in fetal liver and bone marrow and differentiate into mature cell types during residence in the thymus. Mature T lymphocytes are present in thymus, spleen, lymph nodes, throughout skin and other lymphatic organs, and in the bloodstream. B lymphocytes (immunoglobulin producing cells) develop from precursor cells in fetal liver and bone marrow and are found in all lymphoid organs and in the bloodstream. The ontogeny and functions of these cells have been identified in a variety of ways, including morphologic and functional criteria, and by antibodies identifying surface proteins which correlate to a varying extent with specific functions. Lymphocytes develop through stages leading to pools of cells which can be operationally defined, and be recognized by acquisition of specific antigenic determinants (1) (Fig. 7-1, Table 7-1). Human B and T cells normally express class I (HLA-A, B, C) major histocompatibility complex (MHC) antigens on their surface, and B cells express class II antigens (HLA-DR, DP, DQ). Activated T cells also express class II antigens on their surface, and are then described as DR+.

TABLE 7-1

KEY DIFFERENTIATION ANTIGENS WHICH CHARACTERIZE SPECIFIC LYMPHOCYTE SUBSETS

Primary			
Antigen	Synonyms	Distribution	Comment
CD2	LFA-2 Cells	T cells	Cytoadhesion molecule; NK Cells cognate to LFA-3
CD3	T3, Leu 4	All peripheral T Cells	T Cell reseptor complex cells
CD4	T4, Leu 3 (L3T4 in mice)	Class II restricted T Cells	CD4 binkds to MHC clas II (55-70% of peripheral T cells)
CD8	T8, Leu 2 Lyt 2	Class I restricted T Cells	CD8 binds to MHC class I (25-40% of peripheral T cells)
CD11a	LFA-1 chain	Leukocytes	LFA-1 chain adhesion molecule, binds to ICAM-1
CD14	LPS Receptor	Monocytes	Marker for monocytes
CD16	Fc R111	NK cells, Granulocytes	Low affinity Fc receptor
CD20	B1	B cells	Marker for B cells
CD25	TAC, IL2	Activated T and B cells and monocytes	Complexes with chain; T cell growth
CD28	Tp44	Most T cells	T cell receptor for B7-1
CD29	–	40-45% of CD4+ and CD8+	1 chain of VLA protein, an

		cells	“integrin” type of adhesion molecule
CD40	–	B Cells	B cell activation
CD45RO	–	25-40% of peripheral T cell subsets	Expressed on naive T cells
CD54	ICAM-1	T and B Cells	Cognate to LFA-1
CD56	NKH1	NK Cells, some T cells	Neural cell adhesion molecule; NK marker

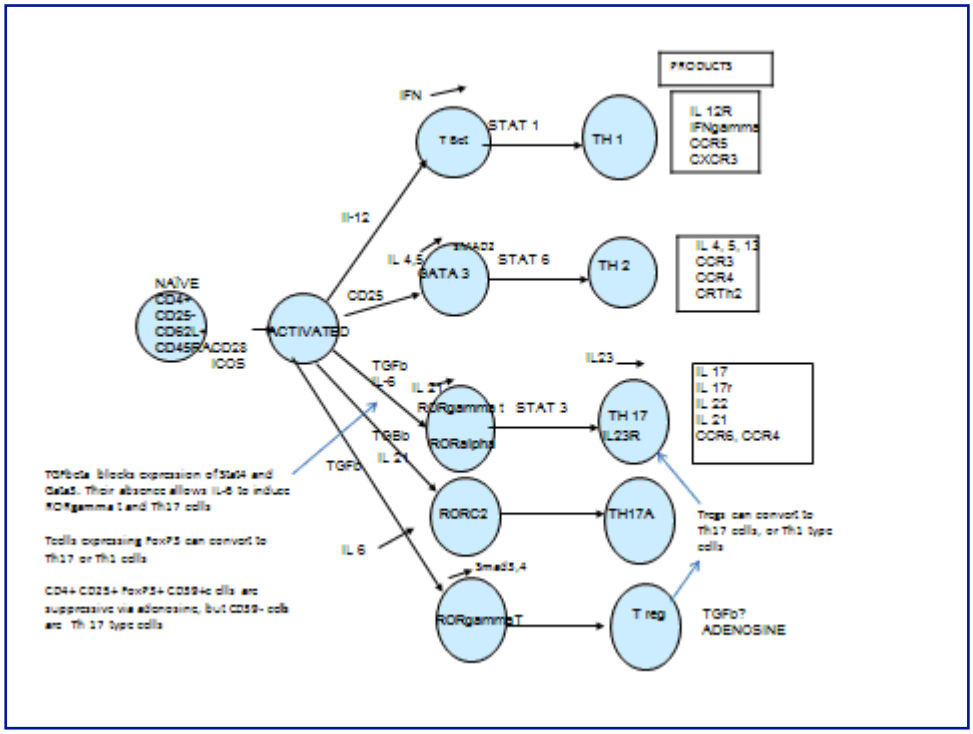

Figure 7-1: Development of T Cell Subsets. In the thymus, undifferentiated precursors give rise to CD4+ and CD8+ cells. In the peripheral lymphoid tissues CD4+ cells (CHO) differentiate following activation by exposure to cognate antigen into two subsets (TH1 and TH2), which are well characterized in the mouse, less so in man. Development of these cells is to some extent reciprocally controlled by cytokines, and the cytokines secreted are also distinct. CD8+ cells similarly mature after antigenic stimulation into less well defined subsets. or = effect on subset proliferation. = cytokines produced.

Lymphocyte Surface Molecules

T cells have on their surface T cell antigen receptors (TCR) which recognize an antigen/HLA complex, accessory molecules which recognize HLA determinants, and adhesion molecules which recognize their counterpart ligands on antigen presenting

cells (APCs). After activation, T cells also have new receptors for cytokines, the hormone products mainly produced by macrophages, T cells and B cells, which control other T or B cells (2) (Table 7-2). The T cell antigen recognition complex consists of disulfide-linked TCR heterodimers, usually the TCR-α and TCR-β chains, plus five or more associated peptides making up the CD3 complex (3). A small proportion of T cells have TCRγ and TCRδ chain instead of α and β chains. TCR-α and β peptides and $\gamma\delta$ peptides are derived from rearranged genes coding for proteins which are unique in each cell clone. The germline TCR genes are very large, containing 40 - 100 different V (variable) segments, D (diversity) segments (in genes), many J (junctional) segments, and one or two C (constant) segments (Fig. 7-2).

TABLE 7-2

CYTOKINES

Cytokine	Cell Source	Targets	Primary Effects On Targets
Type 1 IFN (IFN-α, B)	Mononuclear phagocyte, fibroblast	All	Antiviral, antiproliferative, increased class I MHC expression
Tumor necrosis factor	Mononuclear phagocyte, T cell	Neutrophil Liver Muscle Hypothalamus	Inflammation, Acute phase reactants, Catabolism, Fever
Interleukin-1	Mononuclear phagocyte	Thymocyte Endothelial cell Hypothalamus Liver Muscle fat	Costimulator Inflammation Fever Acute phase reactants Catabolism (cachexia)
Interleukin-6	Mononuclear phagocyte, endothelial cell, T cell	Thymocyte Mature B cell Liver	Costimulator, Growth, Acute phase reactants
Interleukin-2	T cells	T cell NK cell B cell	Growth; cytokine production, Growth, activation, Growth, antibody synthesis
Interleukin-4	CD4+ T cell, mast cell	B cell Mononuclear phagocyte T cell	Isotype switching, Inhibit activation, Growth
Transforming growth factor- β	T cells, mononuclear phagocyte, other	T cell Mononuclear phagocyte Other cell types	Inhibit activation, Inhibit activation Growth regulation
Interferon-γ	T cell, NK cell	Mononuclear phagocyte	Activation Activation

		Endothelial cell All cells	Activation. Increased class I and class II MHC
Cytokine	Cell Source		Primary Effects On Targets
Lymphotoxin	T cell	Neutrophil Endothelial cell NK cell	Activation Activation Activation
Interleukin- 10	T cell	Mononuclear phagocyte B cell	Inhibition Activation
Interleukin-5	T cell	Eosinophil B cell	Activation Growth and activation
Interleukin- 12	Macrophages	NK cells T cells	Activation Activation

Adapted from tables in Cellular and Molecular Immunology, Edition II by AK Abbas, AH Lichtman, and JS Pober, WB Saunders Company, Philadelphia

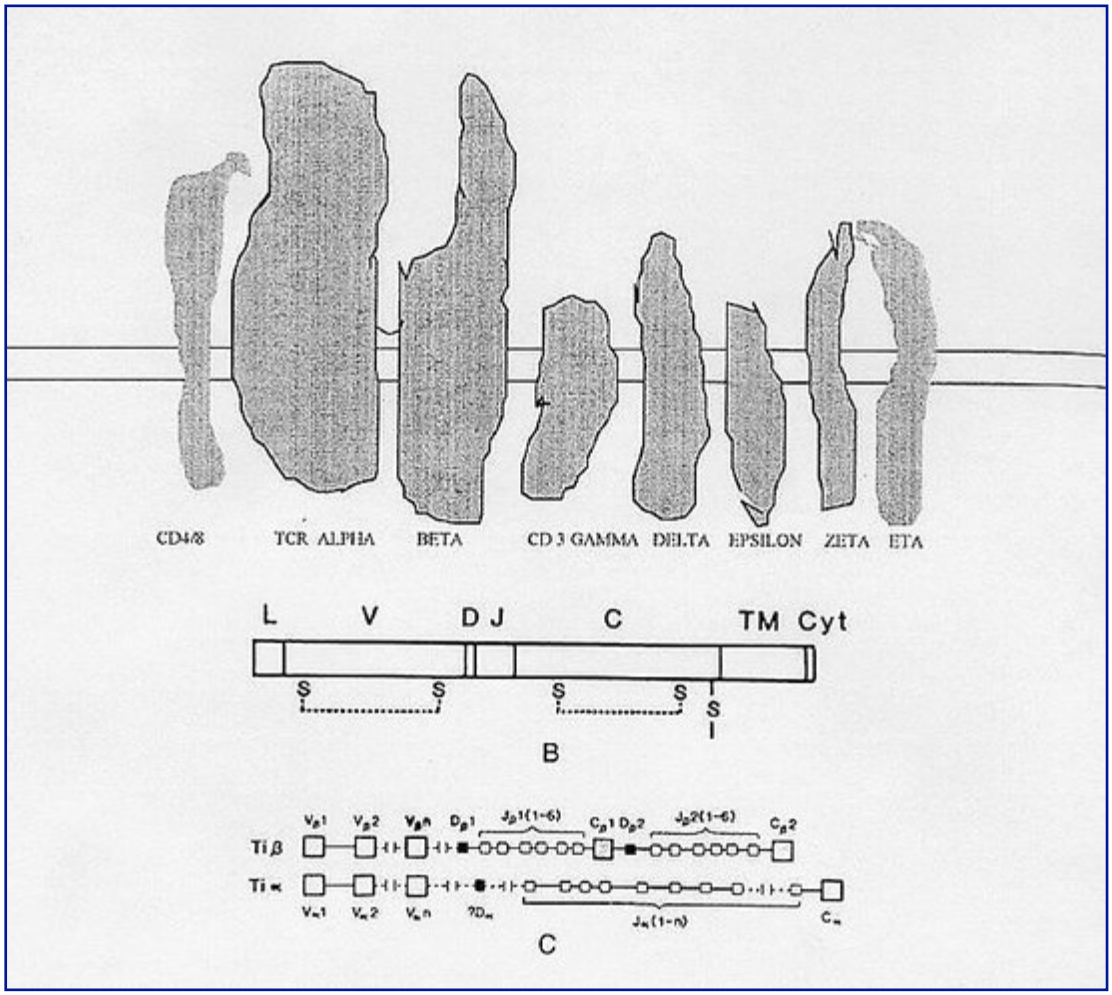

Figure 7-2: Cartoon of the human T cell receptor and its subunits. Part A shows subunit composition of the human T cell receptor. The TCR subunits are held together by S-S bonds and are closely associated with either the CD4 or CD8 molecule and chains of the CD3

complex. The subunits are anchored in the cell membrane. The CD3 complex consists of three subunits referred to as gamma, delta, and epsilon. Associated in the TCR complex is another pair of 16 kD homodimer (32 kD nonreduced), subunits existing as homodimers of zeta or heterodimers of zeta and eta. Part B shows the structure of the Ti subunits. The predicted primary structure of the -chain subunit after translation from the cDNA sequence is depicted, as are the variable region leader (L), V, D, and J segments, a hydrophobic transmembrane segment (TM) and cytoplasmic part (Cyt) in the C region, potential intrachain sulfhydryl bonds (S-S), and the single SH group (S) that can form a sulfhydryl bondwith the subunit. Part C shows a scheme of the genomic organization of human -and - chain genes. In the locus, V indicates the V gene pool located 5′, at an unknown distance from the D 1 element, the J 1 cluster, and the C 1 constant-region gene. Further downstream, a second D 2 element, J 2 cluster, and C 2 constant-region gene are indicated. A similar nomenclature is used for the Ti locus, in which only a single constant region is found. ?D indicates the uncertainly about the existence of a putative Ti -diversity element. (From Reference 1).

During development of each T cell, segments of the germline gene are rearranged so that one TCR gene V segment becomes associated with one D (in the case of TCR-β), one J, and one C segment to produce a unique gene sequence. This random combination of different V, D, and J and C segments, and additional variations in DNA sequence introduced in the J and D region during recombination, provides the enormous diversity of specific TCRs required to recognize the entire universe of T cell antigens. This process also means that all individuals have (before clonal deletion) preformed TCRs able to recognize thyroid autoantigens as well as thousands of other autoantigens.

Each TCR recognizes one specific antigenic peptide sequence termed an epitope (5), which consists of 8 - 9 amino acids for class I restricted T cells, and 13 - 17 amino acids for class II restricted T cells. However, T cells respond to several portions epitopes of any one antigen; these may represent overlapping peptide segments of the epitope. Thus the response of each individual T (and B) cell is extremely specific, but the combined effect of many T (and B) cells acting together is observed in the typical final polyclonal response.

T cells recognize antigen presented by an MHC-molecule; CD4+ T cells (often functioning as helper cells) recognize MHC class II molecules plus antigenic epitope, and CD8+ T cells (often functioning as cytotoxic cells) recognize MHC class I molecules plus antigenic epitope. The epitope fits within a cleft in the HLA-DR molecule and the

TCR functions to recognize this complex (Fig. 7-3). The five associated peptides of the CD3 complex are believed to be signal-transducers and to initiate intracellular events following antigen recognition. The normal response proceeds via TCR antigen recognition, then activation of the T cell through the combined effect of antigen recognition and costimulatory signals (see below) leading to T cell IL-2 secretion and IL-2 receptor expression, followed by proliferation of the T cell into an active clone.

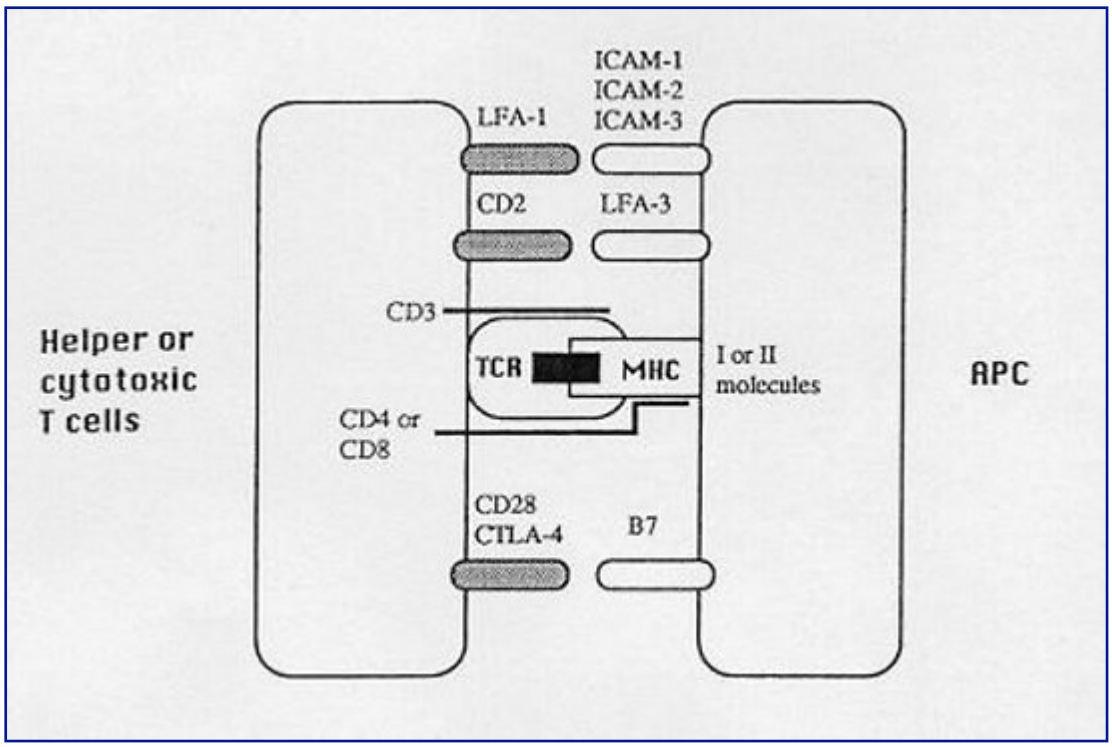

Figure 7-3: In this diagram the antigen is depicted in a cleft of the HLA-DR molecule on an APC, being recognized by the T cell TCR. "Adhesive" peptide segments may augment close contact. A CD4 molecule is associated with the TCR. Presumably the APC surface is normally covered with many DR molecules, each studded with an antigen. T cells must somehow scan these complexes in order to find the one that best fits their TCR.

Lymphocyte development is controlled by cytokines released by macrophages, dendritic cells, lymphocytes, and many other cells. Both T and B cells release a large array of cytokines which carry out their effector functions and alter the function of other cells (Fig. 7-1, Table 7-2). As lymphocytes mature in the thymus, and become activated on exposure to antigen, the types of cytokines to which they respond -- and produce -- become altered. In animals, and to a lesser extent in man, types of lymphocytes can be operationally defined by the cytokines produced. For example, Th1 T cells produce IL-2,

IFN-γ and TNF and are predominant in delayed hypersensitivity type reactions, whereas Th2 T cells produce IL-4 and IL-5, stimulate B cells, and are involved especially in antibody-mediated reactions. Cytokines produced by Th1 cells enhance the activity of this subset but inhibit Th2 cells, and vice versa. This type of regulation may be critical in determining an immune response and in suppressor phenomena. Additional Th subsets are now recognized, including Th17 cells which secrete IL-17 see below), as well as Th9 and Th22 cells which also have discrete pathological roles.

As well as cytokines and their receptors, T cells express a number of receptors for chemokines, integrins and selectins which are involved in the sequential stages of cell adhesion which leads to T cell homing to tissues (7). A word of caution is necessary however in terms of translating these findings into the human situation where boundaries between the subsets are less clear. It is also increasingly recognized that the simple dichotomy of T cells into two types is over-simple, with cytokines such as IL-12 being assigned to the Th1 subset although not being secreted by T cells, and production of this cytokine is stimulated by the Th2 cytokines IL-4 and IL-13, which will drive the immune response from Th2 towards Th1. The blurring of pattern that is seen in many autoimmune diseases challenges the dogma of an easy divide in the type of immune response.

Each B cell produces a unique immunoglobulin (Ig) programmed by an Ig gene which has also been rearranged from the germline V, D, J, and C segments (as for the TCR) (8). The TCR and Ig genes are, not surprisingly, members of one gene superfamily. Further diversity is provided by antigen-driven somatic mutations which occur during amplification of the progeny of a stimulated B cell, causing the production of a family of antibodies with slightly different sequences. B cells secrete their unique antibodies into surrounding fluids, and also express surface Ig which is therefore a B cell receptor for antigen (Fig. 7-4). The recognition process by antibodies involves the shape of the epitope - i.e. it is conformational and for B cells normally involves unprocessed antigen. Thus B cell and T cell epitopes for the same antigen are usually different segments or forms of the molecule.

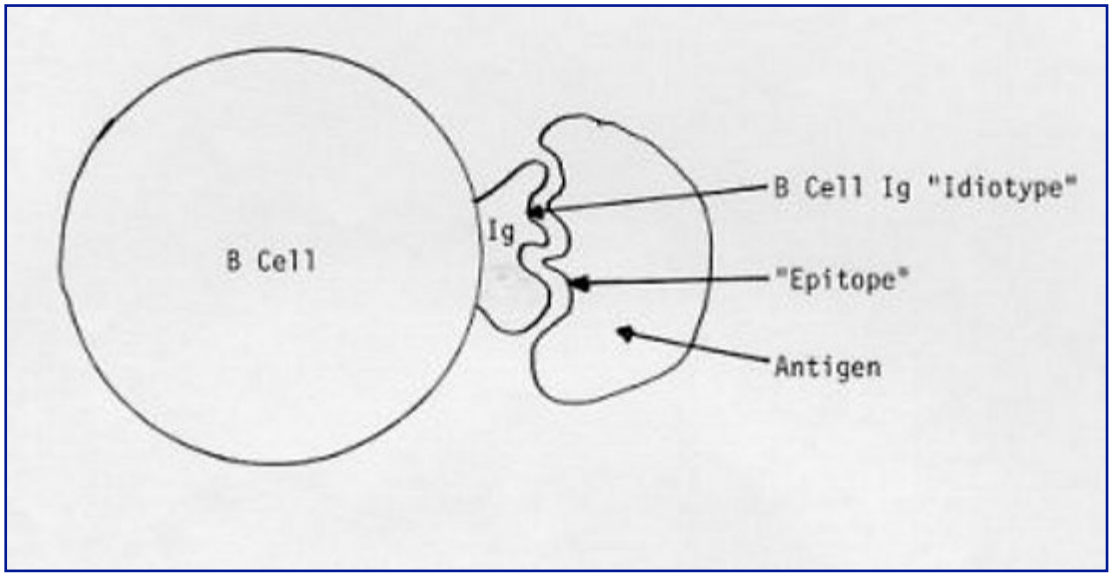

Figure 7-4: The B cell surface is studded with specific Ig molecules which function as high affinity receptors for specific antigen epitopes which match the shape of the Ig recognition idiotype.

Antigen Presentation On Mhc Molecules

The genes for the HLA-A, B, C and HLA-DR, DP, DQ molecules are on chromosome 6, and comprise some of the genes in a large immune response control complex (Fig. 7-5). Each cell surface HLA molecule is made up of 2 peptide chains; an α chain and β2 microglobulin for class I molecules, and α and β chains for class II. Each individual inherits from each parent one HLA-A, B, and C, one DRα and 3 DRβ genes, a pair each of DP and DQα and β genes, and other related genes which are not expressed, including DX and DO (Fig. 7-5). The genes are expressed in a co-dominant manner, and (in contrast to TCR and Ig molecules) are invariant in individuals. However, the genes are all highly polymorphic, that is, many alleles may exist for each gene. The actual evolutionary drive for this diversity is unknown. While TCR gene rearrangement provides the T cell repertoire to respond to individual antigens, HLA diversity guarantees that different individuals will have different T cell repertoires, which confers evolutionary advantage to the species in terms of responding to new pathogens.

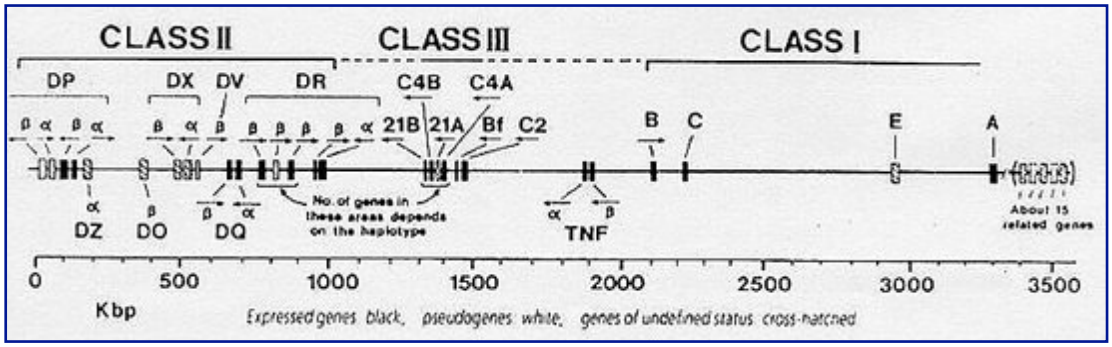

Figure 7-5: Partial map of the short arm of human chromosome 6 showing the molecular organization of the area containing the MHC loci, with details of the HLA Class I, II, and III genes. Map distances in kilobases were determined by pulsed-field gel electrophoresis. Genes are not drawn to scale. Expressed genes are designated by filled boxes _ (|_|). (From Trowsdale, J. and Campbell, R.D. Physical map of the human HLA region. Immunology Today, 9:34, 1988.)

The HLA molecules play a central role in T cell clonal selection during fetal development, in normal immune responses, and in presentation of self-antigens. In many instances -- including autoimmune thyroid disease (AITD) as detailed below -- inheritance of a specific HLA gene correlates with increased susceptibility to disease. In some cases this can be related to a gene coding for a specific amino acid in the HLA molecule which is believed to control epitope selection (often called determinant selection) and thus to be associated with disease susceptibility.

Antigen can be presented to $CD4^+$ T cells by conventional (or "professional") APCs, particularly dendritic cells (9), and also by B cells and activated T cells, and less effectively by a variety of other cells (fibroblasts, glial cells, thyrocytes), when these normally HLA-DR-negative cells are altered and express HLA class II molecules on their surface. This is because non-classical APCs cannot provide the necessary costimulatory signals, including the B7-1 (CD80) and B7-2 (CD86) molecules, which bind to CD28 on the T cell and are necessary for activation of certain T cells. If B7 molecules bind instead to CD152 (CTLA-4) on the T cell, the immune response is terminated. The individual roles of CD80 and CD86 are not clearly established, although some functions appear to be distinct (e.g. CD80 appears to stimulate CD152) and some overlapping (e.g. both stimulate CD28), and the tempo of their involvement at different times of the immune response is likely to be critical to the type of response produced. The maturation state of the dendritic cell is another determinant of immune homeostasis.

Semi-maturation, induced by proinflammatory cytokines like TNF-α, allows the development of a tolerogenic stage for these cells. Full maturation, induced by signaling through toll-like receptors, complement receptors or antibody Fc receptors, induces proinflammatory cytokine production by the dendritic cell and allows them to generate T cell immunity.

T And B Cell Responses

Antigen presentation to T cells leads to a variety of responses which include proliferative or suppressive functions, development of cell cytotoxic responses, control of Ig secretion, and many more. In addition, under specific circumstances, antigen presentation may cause the T cell to become non-responsive or anergic (10). Presentation of antigen and the accompanying second signal are required to activate a naive T cell and initiate an immune response; previously activated T cells are much less dependent on B7-mediated costimulation. Antigen recognition and APC-produced cytokines (Table 7-2) together cause T cell stimulation. This activates the T cell to express IL-2 receptors and to secrete IL-2 itself. Increased T-cell secreted IL-2 induces the responding T cell, and nearby ("bystander") T cells to proliferate. T-cell secreted IL-2, IL-6 and other cytokines and IL-4 cause B cells to be stimulated and proliferate and cell surface receptors such as CD40 on B cells and its ligand on T cells are also involved in B cell activation (11).

B cells themselves secrete distinct profiles of cytokines, in response to the engagement on CD40, and these cytokines can upregulate or downgregulate an immune response in a manner which depends on whether the B cell is simultaneously stimulated by antigen (12). Intimate T-cell to B-cell contact may account for antigen-specific help for T cell and B cell responses, whereas the effect of T cell-secreted cytokines on bystander T or B cells may account for stimulation of non-antigen-specific responses by these lymphocytes. The beneficial effects of rituximab, a CD20 specific, B depleting monoclonal antibody, in autoimmune conditions including Graves' disease (13) is related to its effects on inhibiting this interaction between T and B cells.

Th1 cells function as inflammatory cells, typical of a delayed hypersensitivity type reaction, while Th2 cells are more specifically helper cells for B cell immunoglobulin synthesis. A number of factors including TCR affinity and ligand density, and non-T cell-

derived cytokines such as IL-4 and IL-12, determine whether the outcome of an immune response is predominantly by Th1 or Th2 cells. A third population of T helper cells has been defined recently, based on their secretion of the pleiotropic proinflammatory cytokine IL-17, and are so called Th17 cells. The differentiation and expansion of these cells depends on the coordinate effects of IL-6, transforming growth factor beta (TGFβ) and IL-23 (14). These Th17 cells are responsible for defense against certain micro-organisms such as *Klebsiella*, *Borrelia* and fungi. Of relevance to this discussion, they also have important roles in tissue inflammation and organ-specific autoimmunity.

Although the concept of suppressor cells fell into disrepute during the late 1980s, there has been resurgence in interest with the recognition that $CD4^+$ cells expressing high levels of the IL-2 receptor, CD25, act in a way entirely in keeping with the previously defined suppressor population. These $CD4^+$, $CD25^+$ T cells have been termed regulatory or Treg cells. Such cells can prevent autoimmunity when transferred from healthy, naïve animals and their depletion results in autoimmune disease. Such cells express Foxp3 which encodes a critical transcription factor for their function: mutation of this gene in man results in the lethal immunological disorder IPEX syndrome that includes autoimmune hypothyroidism amongst its manifestations (15).

APCs have a central role in controlling Treg cells, with resting APCs (including thymic epithelial cells) promoting their development through the induction of the transcription factor Foxp3 (16). Activation of APCs, for instance through their T cell-like receptors, has the opposite effect, and at least one component responsible for the suppression of Tregs then is the cytokine IL-6; this pathway allows effector T cells to predominate over Tregs, thereby shifting the dynamic equilibrium in favor of an immune (or autoimmune) response. Another critical molecule in the Treg cell pathway is the costimulatory signal receptor, CD28, which is required for both development and maintenance of Treg function. TGFβ exposure induces Tregs, but when combined with IL-6, Th17 effector cells are generated. The absence or presence of IL-6 is thus critical to determining whether there is a regulatory milieu or a proinflammatory response mounted by Th17 cells. Both Th1 and Th17 cells are potent inducers of organ-specific autoimmunity, but their relative roles in each type of disease remain to be clarified.

It is increasingly clear that Treg are more complex a group of cells than originally clear. T regulatory cells can be classified as those which arise within the thymus and express Foxp3, and a Th3-like population which probably does not express this molecule and which develops in the periphery. More recently described regulatory cells have been phenotyped as CD4+CD69+ and CD4+NKG2D+ T cells. The glucocorticoid inducible tumor necrosis factor receptor (GITR) is expressed by both populations but $CD25^{bright}$ expression is not a requirement for regulatory T cell function.

The reciprocal relationship between Th1 and Th2 cells, exerted through secretion of cytokines, serves as another model of suppressor function. This paradigm is conceptually useful but is almost certainly too simplistic, not least because there may exist within the Th2 compartment different types of T cells, some with pathological effector function and others which act as physiological regulators of Th1 responses. Endeavors to manipulate the entire Th2 population, to deviate an immune response away from Th1 cells, may therefore lead to exacerbation of the immune response, and may explain the reciprocal relation between the prevalence of infectious disease and autoimmunity (18).

Killer (K) And Natural Killer (Nk) Cells

In addition to the standard T cell function described above, other cells participate in immune responses. Macrophages may destroy cells having immune complexes on their surface through recognition of the Fc portion of bound Ig. Other cells which do not bear the CD3 marker of T cell lineage exist (K and NK cells) and have the ability to spontaneously kill other cells (especially those expressing HLA antigens). NK cells can be detected by specific monoclonal antibodies such as anti-CD16, and are recognized phenotypically as large granular lymphocytes. Like T cells, NK cells can have a type 1 or 2 pattern of cytokine release. Macrophages, T, K, NK, or other cells also kill cells coated with immune complexes in the process of antibody-dependent-cell-cytotoxicity (ADCC) (Fig. 7-6).

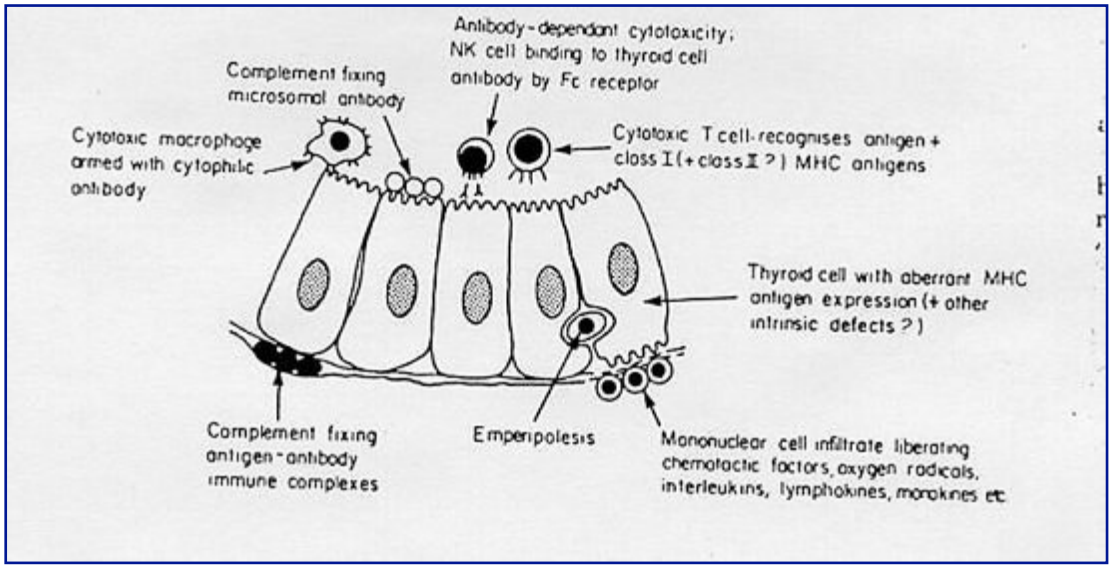

Figure 7-6: Some of the proposed mechanisms which could produce thyroid damage in AITD. Emperipolesis is the movement of lymphocytes and macrophages between epithelial cells and occurs in many organs such as gut, bronchus, and thyroid. The existence of interepithelial cells with immunoreactive potential is obviously relevant to an understanding of how autoantigens at the luminal surface of the thyroid cells may be exposed to

Self-Non-Self Discrimination

The immune system, which evolved to defend us from invading foreign proteins, normally tolerates (i.e. does not develop recognizable responses to) self-antigens. The level of this control is variable. For example, self-reactivity to serum albumin is not seen. However, antibodies to thyroid antigens exist in up to 20% of adult women, and their presence must be considered effectively normal. The development of tolerance is closely associated with the restriction of TCRs to recognizing an antigen only when presented by an HLA molecule. The process, which for T cells occurs in the fetal thymus, leads to elimination of some T cells, and retention of others with TCRs having desirable features. Self-antigens are believed to be presented on HLA molecules to T cells developing in the thymus. This implies that antigen must be in the thymus or in the circulation for tolerance to develop and indeed we now know that specialized cells in the thymus can express a panoply of autoantigens during development. T cells bearing autoreative TCRs are largely inactivated or destroyed. T cells which have the capacity to react with foreign antigens presented by self MHC molecules are allowed and retained (Fig. 7-7). This system is imperfect however and some T cells which react with MHC

molecules plus self-antigen are not deleted, which is the fundamental explanation for autoimmunity.

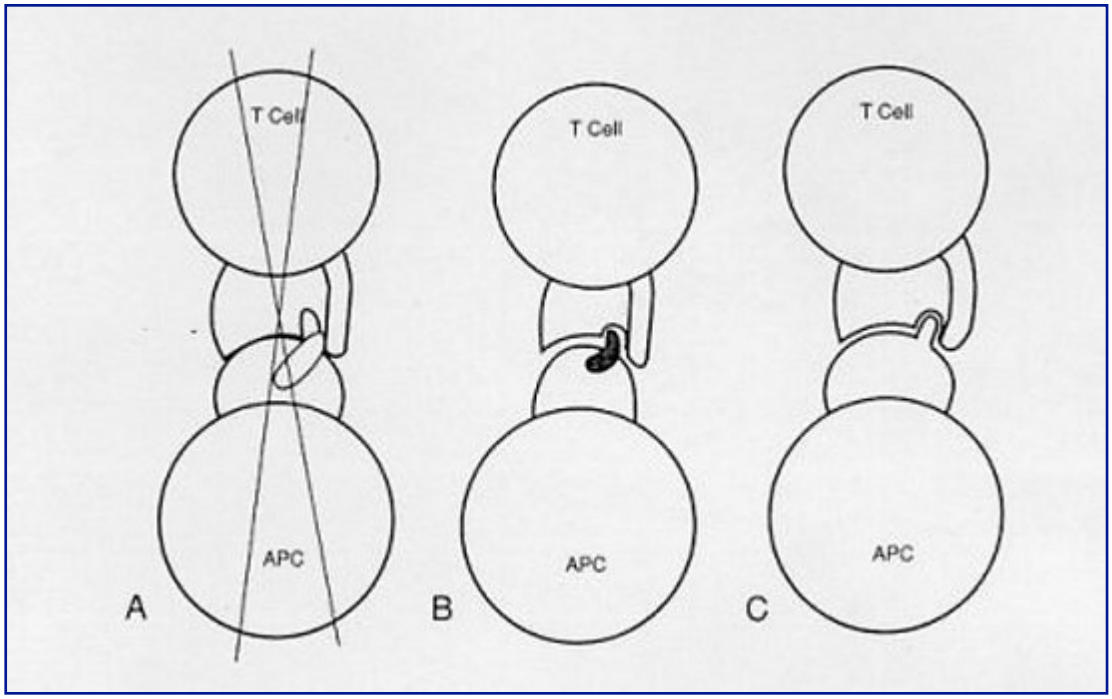

Figure 7-7: Left: Fetal Thymus; T cells strongly activated by DR alone, or strongly reactive to self-antigen presented by HLA molecules, are selectively destroyed. T cells, with a weak or absent response to DR alone, or to DR+ self-antigen, survive. Center: Normal Adult Immune Reaction; T cell TCR and APC-DR interaction is normally a weak or neutral signal. The presence of allo-antigen serves to switch the signal to positive. Right: Allo-MLR; Allogeneic DR is sufficiently different from autologous DR to act as a positive signal with or without antigen present.

The best evidence that thymic T cell deletion prevents autoimmunity in man comes from autoimmune polyglandular syndrome (APS) type 1, which is the result of an autosomal recessive mutation in the *AIRE* (AutoImmune REgulator) gene. Such patients have multiple autoimmune disorders, principally Addison's disease and hypoparathyroidism but including thyroid autoimmunity. The AIRE protein is expressed in the thymus by medullary epithelial cells and regulates the surprising expression of an array of self proteins (normally confined to extrathymic tissues) by these cells during fetal development. When through the *AIRE* mutation such self-antigens cannot be expressed to allow clonal deletion, autoimmunity ensues and this accounts for the early onset multiple autoimmunity found in this syndrome (reviewed in 19). Recently, dominant

mutations in *AIRE* have been identified and such patients have later-onset, milder phenotypes (19b). During maturation in the thymus, probably 95% or more of the lymphocytes produced are negatively selected, and die through a process described as programmed cell death or apoptosis. This process involves several genes including those required for apoptosis, such as Fas. A similar process is thought to ensue whenever a T cell is stimulated by its cognate antigen but does not receive a "second signal", and during induction of anergy by other mechanisms. Defects in Fas lead to preservation of autoreactive T cells in some models of animal autoimmune disease.

This trade-off between perfection in clonal deletion and repertoire maintenance allows a limited number of autoreactive T cells to survive, and thus sets the stage for autoimmune disease. The main mechanism to prevent autoimmunity by these escaped cells and also to induce tolerance to autoantigens not present in the fetal thymus or circulation is termed peripheral (i.e. non-thymic or central) tolerance, and is mainly effected by the Tregs described above.

B cells undergo a similar selection process in fetal bone marrow or liver, except for the participation of MHC molecules. If exposed to antigen during this early stage of development, B cells are permanently inactivated. As for T cells, the selection process is not perfect, and leaves some B cells having the ability to make antibodies directed to self-antigens in the adult. However, B cells require T cell help in order to proliferate and differentiate into mature Ig secreting cells. In the absence of self-reactive T helper cells, these B cells remain dormant and expanding clones do not develop. Although such clonal ignorance may be an important pathway in preventing B cell autoreactivity, it is not the only mechanism, and physiological concentrations of autoantigen may induce anergy of B cells, even when their affinity for autoantigen is low.

Tolerance to self-antigen can be overcome ("broken") in animals by injecting the antigen in an unusual site on the body, especially in the presence of adjuvant compounds such as mycobacterial fragments and oil, or alum, or by slightly altering the antigen structure, or by altering the responding immune system (for example, by whole body irradiation, or depletion of suppressor T cells). An additional mechanism for the inflammatory component of many autoimmune disorders has recently been proposed based on the evolutionary origins of mitochondria from bacteria. Given that the prime function of the

immune system is to defend the organism from microbes, it is possible that the immune system may mistake mitochondria released from damaged tissue through pattern-recognition receptors and thereby induce a ‘mistaken’ inflammatory response (20).

THE SYNDROMES OF THYROID AUTOIMMUNITY

The three syndromes classically comprising autoimmune thyroid disease are (1) Graves' disease with goiter, hyperthyroidism and, in many patients, associated ophthalmopathy (2) Hashimoto's thyroiditis with goiter and euthyroidism or hypothyroidism; and (3) primary thyroid failure or myxedema. Many variations of these syndromes are also recognized, including transient thyroid dysfunction occurring independently of pregnancy and in 5 - 6% of postpartum women, neonatal hyperthyroidism, and neonatal hypothyroidism. The syndromes are bound together by their similar thyroid pathology, similar immune mechanisms, co-occurrence in family groups, and transition from one clinical picture to the other within the same individual over time. The immunological mechanisms involved in these three diseases must be closely related, while the phenotypes probably differ because of the specific type of immunological response that occurs. For example, if immunity against the TSH receptor leads to production of thyroid stimulating antibodies, Graves' disease is produced, whereas if TSH blocking antibodies are formed or a cell destructive process occurs, the result is Hashimoto's thyroiditis or primary myxedema.

Associated with autoimmune thyroid disease in some patients are other organ specific autoimmune syndromes including pernicious anemia, vitiligo, myasthenia gravis, primary adrenal autoimmune disease, ovarian insufficiency, rarely pituitary insufficiency, alopecia, and sometimes Sjögren's syndrome or rheumatoid arthritis or lupus, as manifestations of non-organ specific autoimmunity. There has also been a description of pituitary antibodies and growth hormone deficiency in around a third of patients with autoimmune hypothyroidism, implying the existence of a substantial reservoir of pituitary autoimmunity in these patients but further work is needed to confirm these findings and to understand the basis for the autoimmune response against the pituitary (21).

THE ANTIGENS IN AUTOIMMUNE THYROID DISEASE

Thyroglobulin

The three most important antigens involved in thyroid autoimmunity are clearly defined. First to be recognized was thyroglobulin (TG), the 670 kD protein synthesized in thyroid cells and in which T3 and T4 are produced. Four to six B cell epitopes of TG are known to be involved in the human autoimmune responses and epitope recognition is similar in both Graves' disease and Hashimoto's thyroiditis (22). Animal studies suggest that antigenicity of the molecule is related to iodine content, but studies on human antisera do not consistently bear this out: these species differences and the role of measuring TG antibodies in thyroid disease are reviewed elsewhere (23).

Mouse experiments suggest that, to induce autoimmunity to TG, initial tolerance to dominant epitopes must be overcome, and the immune response then spreads to cryptic epitopes that are the major inducers of thyroidal T cell infiltration (24). One particular TG T cell epitope, Tg.2098, has been identified which is a strong and specific binder to the MHC class II disease susceptibility HLA-DRβ1-Arg74 molecule, and stimulates T cells from both mice and humans that develop AITD (25). This could be a major T cell epitope which might be involved in pathogenesis through initiating an immune response that then spreads to involve other autoantigens. Furthermore, screening a diverse library of small molecules has identified one, cepharanthine, which blocked Tg.2098 peptide binding and presentation to T cells in mice with experimental autoimmune thyroiditis; such an approach has obvious therapeutic potential (25a).

Tsh Receptor

The second antigen to be identified was the TSH receptor (TSH-R), a 764 aa glycoprotein. Antibodies to TSH-R mimic the function of TSH, and cause disease by binding to the TSH-R and stimulating (or inhibiting) thyroid cells, as described later. The human TSH-R is a member of a family of cell surface hormone receptors which are characterized by an extra-membranous portion, seven transmembrane loops, and an intracellular domain which binds the G_S subunit of adenyl cyclase (26, 27). Uniquely among G-protein-coupled receptors TSH-R undergoes post-translational cleavage to comprise a 53kD extracellular A subunit (53 kDa) and transmembrane and intracellular B subunit coupled by disulfide bridges. The A subunit may be shed provoking speculation on the role of this in stimulating autoimmunity. Recent evidence indicates that in Graves' disease TSHR antibody affinity maturation is driven by A-subunit multimers rather than

monomers (27a). Human TSH-R B cell epitopes are conformational and composed of several segments of the protein.

The initial description of mouse and hamster monoclonal TSH-R antibodies was significant for several reasons (28-30). Firstly, these antibodies confirmed that a single antibody was sufficient to activate the receptor, rather than two or more simultaneously. Secondly, they have permitted epitope mapping. One antibody preferentially recognized the free A subunit, not the holoreceptor, suggesting that free A subunit, shed from thyroid cells, may initiate or amplify the autoimmune response. Another antibody, in contrast to TSH, did not enhance post-translational TSH-R cleavage, which may extend the receptor half-life and thus account for the prolonged thyroid stimulation seen following antibody binding. Finally, these antibodies paved the way for the development of human monoclonal antibodies which have allowed a greatly improved understanding of the mechanisms involved in Graves' disease.

The first human monoclonal TSH-R stimulating antibody bound to the TSH-R with high affinity, either as IgG or as Fab fragment, and the monoclonal had similar features in all respects to known TSAb (thyroid stimulating antibodies) (31). This observation indicated that only a single species of antibody is needed to stimulate the receptor. More conventional approaches based on different methods of expressing the TSH-R have shown that TSAb preferentially recognize the free A subunit rather than the holoreceptor, either because of steric hindrance from the plasma membrane or membrane spanning region of the receptor or because of TSH-R dimerization (32). The epitopes for TBAb overlap with those for TSAb but are more focused on the C terminus and are able to recognize holoreceptor more efficiently. These observations have provided support from the hypothesis that shedding of free TSH-R A subunits may be critical in initiating or amplifying the autoimmune response in Graves' disease. Further evidence comes from immunization of mice with adenoviruses expressing different structural forms of the TSH-R: goiter and hyperthyroidism occur more frequently when mice are given virus that expresses the free A subunit rather than a receptor with minimal cleavage into subunits (33).

Patients with autoimmune thyroid disease may have both stimulating and blocking antibodies in their sera, the clinical picture being the result of the relative potency of

each species. Switching between one type of antibody and another in unusual patients, involving changes in concentration, potency and affinity, may be caused by a number of factors including levothyroxine treatment, antithyroid drug treatment and pregnancy, and can lead to difficulties in clinical care (35). TSH-R neutral antibodies have also been identified which do not block TSH binding and are unable to stimulate cAMP production; these antibodies are capable of inducing thyroid cell apoptosis in vitro and therefore could conceivably play a role in pathogenesis by inducing release of thyroid autoantigens (36).

Identification of the critical T cell epitopes has proved elusive although peptides 132-150 do appear to constitute one key epitope; there is poor correlation between binding affinity and T cell immunogenicity in experiments to attempt such localization (37). In animal studies, however, there is clear evidence of epitope spreading when mice are immunized with TSH-R peptide epitopes or TSH-R cDNA, indicating that dominant TSH-R epitopes are, at best, elusive (38). TSH-R mRNA transcripts and protein have been identified in retrobulbar ocular tissue, particularly the preadipocyte fibroblast, suggesting that TSH-R expression in the orbit could well be involved in the development of autoimmunity and ophthalmopathy, and similar TSH-R-expressing fibroblasts have also been found in the thyroid gland itself (39). Further support for involvement of the TSH-R comes from experiments showing that activation of the TSH-R stimulates early differentiation of preadipocytes, but terminal differentiation is not induced (40). An animal model with some features of similarity to human ophthalmopathy has been induced in mice by immunization with TSH-R A subunit plasmid given by a specific electroporation protocol (41). Oddly there was no thyroid lymphocytic infiltrate to accompany these orbital changes, which were very heterogeneous between immunized animals. It should also be noted parenthetically that an alternative pathway for fibroblast involvement in ophthalmopathy has been proposed which depends on the production of insulin-like growth factor antibodies in these patients but it is difficult to reconcile these findings with the orbital specificity of the autoimmune process in thyroid eye disease (42). Most recently, TSH-R has been identified in immature thymocytes, which can be stimulated by TSAb. This could in turn explain why thymic hyperplasia is seen in occasional cases of Graves' disease (42a).

Thyroid Peroxidase

The third thyroid antigen was described as "microsomal antigen" was identified as thyroid peroxidase (TPO) in 1985 (43) (Fig. 7-8). DeGroot's laboratory demonstrated that human antisera reacting to "microsomal antigen" precipitated human thyroid peroxidase (TPO) prepared from Graves' disease thyroid tissue () (Fig. 7-8) and at the same time Czarnocka et al. purified human TPO and confirmed identity with the microsomal antigen (44). The cDNA was cloned and sequenced in several laboratories (45-48). The interaction of human anti-TPO antisera and monoclonal antibodies also indicate the presence of several B cell epitopes which map to two main domains, A and B (reviewed in 49). Further experiments with monoclonal antibodies have defined individual amino acid residues that are critical for the two immunodominant regions (50). The epitopes recognized by antibodies are stable within a patient and may be genetically determined (51). Investigation of TPO epitopes recognized by T cells from patients with AITD has produced conflicting results but certain sequences are beginning to emerge which are shared between reports on various patients (52, 53). There is also debate as to whether patients with autoimmune hypothyroidism differ in their pattern of epitope recognition from healthy controls who are TPO antibody positive, and further work is required to analyze this in detail, as it might allow better prediction of those antibody positive individuals who will progress to overt hypothyroidism (54)

TPO is expressed on the thyroid cell surface as well as in the cytoplasm, and likely represents the cell-surface antigen involved in complement-mediated cytotoxicity as well as antibody-dependent cell mediated cytotoxicity (55). Intracytoplasmic binding of antibodies to TPO indicates that there is access to this compartment, but the consequences in vivo are unclear.

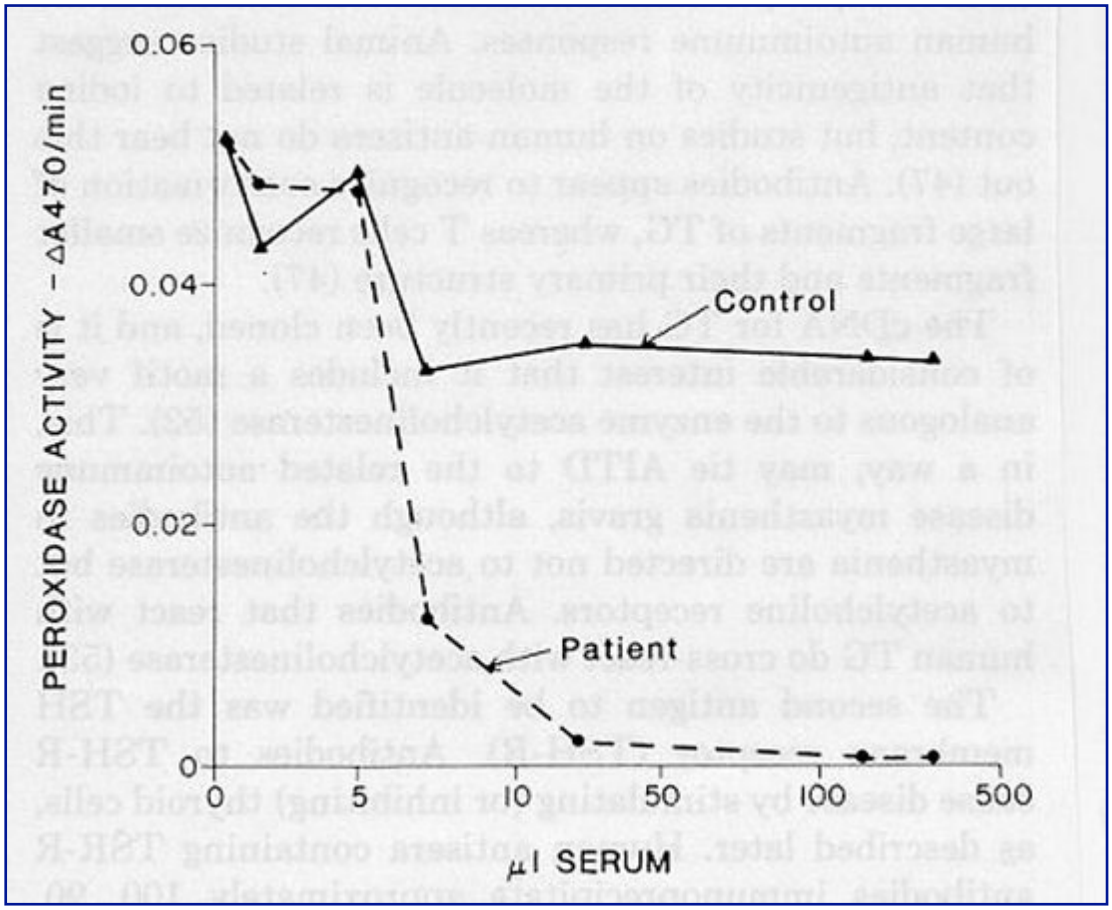

Figure 7-8: Precipitation of peroxidase activity by sera from a patient with autoimmune thyroid disease and positive "microsomal" antibodies, and from a control subject without circulating antibodies. TPO was precipitated by primary incubation with human sera, and removal of TPO-Ig complexes was achieved by addition of Protein H-Sepharose CL-4B. Residual hTPO activity in the supernatant was assayed in a guaiacol assay.

Other Antigens

Antibodies against the sodium/iodide symporter (NIS) were first shown functionally in cultured dog thyroid cells (56). Up to a third of Graves' disease sera contain antibodies capable of blocking NIS-mediated iodide uptake in cells transfected with the human NIS but the relevance of this for thyroid function is unclear (57). The same antibodies have also been detected using an immunoprecipitation assay (58). Others have found no such blocking activity using assays with cell lines displaying much higher ^{131}I uptake, in turn suggesting that any NIS blocking activity only occurs at limiting conditions (59). This implies that NIS autoantibodies probably have no effect in vivo. NIS expression on TECs is upregulated by TSH and downregulated by cytokines and the latter could impair

thyroid function in the setting of AITD when such cytokines are synthesized in the thyroid (60). Pendrin, an apical protein responsible for mediating iodide efflux from thyroid cells into the follicular lumen, has also been identified as an autoantigen. Autoantibodies were initially found in 81% of patients with AITD by immunoblotting (more frequently and at higher titer in Hashimoto's than Graves' patients) and also in 9% of controls (61), but the frequency of these autoantibodies detected using a radioligand binding assay is rather low at around 10% of patients and no controls (62).

Antibodies to a variety of other thyroid cell components are also occasionally present in AITD, including antibodies that react with thyroxine or triiodothyronine (63). The insulin-like growth factor receptor has also emerged as a possible autoantigen involved in ophthalmopathy, with antibodies being detected in patients with this complication, and this receptor co-localizes with the TSH-R on both fibroblasts and thyrocytes (64).

IMMUNE REACTIONS IN AUTOIMMUNE THYROID DISEASE

Humoral Immunity

The principal autoantibodies identified in AITD and the methods for detecting them are listed in Table 7-3. Antibodies to the TSH receptor are discussed in detail in Chapter 10, but, in brief, observation of a factor in serum of patients with Graves' disease causing long acting stimulation of thyroid hormone release from an animal's thyroid, in contrast to the short acting stimulation produced by TSH, led directly to our knowledge of TSH-R antibodies. We summarize here a huge amount of clinical and laboratory research. The antibodies directed to the TSH-R are currently separated into three types. Some antibodies bind to an important epitope in TSH-R and activate the receptor, producing the same effects as TSH, in particular causing generation of cyclic AMP. These antibodies may be referred to as TSI or TSAb -- thyroid stimulating immunoglobulins or thyroid stimulating antibodies. Other antibodies bind to different, or the same epitopes and interfere with radiolabelled TSH binding in certain assays -- thus they are known as thyrotropin binding inhibitory immunoglobulins or TBII. Still others bind and prevent the action of TSH -- thus blocking antibodies. These may either interfere directly with TSH binding or have less well characterized inhibitory effects. Numerous other names have also been used historically.

TABLE 7-3

ANTIBODIES REACTING WITH THYROID AUTOANTIGENS IN AITD AND TECHNIQUES FOR DETECTION

Antigen	Test Used To Identify Antibody
TG	Precipitin Hemagglutination assay Immunofluorescence on fixed sections of thyroid tissue: colloid localization
localization	Solid-phase RIA Immunoradiometric assay Hemolytic plaque assay
Colloid component other than TG	Immunofluorescence on fixed sections: colloid localization
Microsomal antigen/ TPO	Complement fixation Immunofluorescence on unfixed sections; thyroid tissue cell localization Cytotoxic effect on cultured thyroid cells Hemagglutination assay ELISA Solid-phase RIA TPO activity inhibition
TSH-R	Bioassay in mice cAMP production by thyroid cells, TSH- R transfected cells or membranes Iodide uptake by thyroid cells Thymidine incorporation by thyroid cells Inhibition of TSH action on thyroid cells Inhibition of TSH binding to cells or membranes Immunoprecipitation
Sodium/iodide symporter	Western blotting Immunoprecipitation Bioassay using cultured thyroid cells or cells transfected with the symporter

TSI cause non-TSH dependent stimulation of thyroid function, which, if of sufficient intensity, is hyperthyroidism. TBII comprise the mixture of TSI and TSH blocking antibodies, and therefore function cannot be predicted from the TBII level. Predominance of TSI characterizes Graves' disease, and TSH blocking antibodies are present in a small proportion of patients with Hashimoto's disease and primary myxedema. Probably a combination is present in most patients with AITD. Recent work indicates that both types of TSH-R antibody are present in Graves' sera at low concentration with high affinity and similar (but nonetheless subtly distinct) binding epitopes (65). TSI directly cause thyroid overactivity, their level correlates roughly with disease intensity, and a drop in levels correlates loosely with disease remission. Unlike TG and TPO antibodies which are polyclonal and not restricted by immunoglobulin subclass (reviewed in 66), there is evidence that some TSH-R are restricted to particular heavy and light chain subclasses, which may indicate an oligoclonal origin (67), and TSH-R stimulating antibodies are present at much lower concentration than TG and TPO antibodies.

Normal subjects can have TSH-R antibodies that bind to but do not activate the TSH-R and that generally have low affinity. These natural autoantibodies may be the precursors of the TSI that cause Graves' disease and it is possible that affinity maturation, with class switching of immunoglobulin isotype, is critical in determining the clinical consequences of TSH-R antibody production. Conversely, using the most sensitive binding assays, there are still a very small number of patients with Graves' disease who are apparently negative for these antibodies when their serum is tested; it is likely that the explanation lies in either assay sensitivity or exclusively intrathyroidal production of these antibodies (68).

Precipitating antibodies to TG were first detected by mixing antibody and antigen in equivalent concentrations, or by agar gel diffusion, as in the Ouchterlony plate technique. Subsequently, much more sensitive methods were developed, such as solid phase ELISA (69) and RIA (70), although for many years the tanned red cell hemagglutination test remained the assay of choice (71). Immunoradiometric assays (IRMA) used currently involve binding of serum antibodies to solid phase antigen, and secondary quantitation of antibody by binding labelled monoclonal anti-human Ig

antibody. These tests are very sensitive but lack specificity as so many healthy individuals are positive, albeit with a future risk of developing AITD.

Antibodies directed against TG are rarely present in children without evidence of thyroid disease. The prevalence in healthy persons increases with age, and low levels are frequently present in normal adults (72). The greatest frequency occurs in women aged 40-60 years. The frequency of antibodies in well persons correlates with the incidence of focal lymphocytic infiltration found on microscopic examination of thyroid tissue form healthy individuals (73). Over 90% of patients with Hashimoto's thyroiditis and primary myxedema have these antibodies. Low to moderate titers are found in half of patients with Graves' disease. TG antibodies are either absent or low in patients with subacute (De Quervain's) thyroiditis, who may present clinically like patients with Hashimoto's thyroiditis. In general human TG and its autoantibody bind complement weakly due to the widely scattered epitopes which are unable to allow antibody cross-linking.

The second important antigen-antibody system was originally recognized by antibodies which, by immunofluorescence, were observed to bind to non-denatured thyroid cytoplasm, to fix complement in the presence of human thyroid membranes (microsomes), or to bind to microsome-coated red cells (the MCHA assay). We now know this antigen is TPO (see previous Section 3) (Fig. 7-8). Almost all patients with Hashimoto's thyroiditis have TPO antibodies. They also occur in the normal population in the absence of clinically significant thyroid disease: in a recent survey of a population followed for 20 years, 26% of adult women and 9% of adult men had TPO and/or TG antibodies (74). However, the presence of such antibodies was shown to be associated with an increased risk of future hypothyroidism, especially if the TSH was also raised (subclinical hypothyroidism). Few sera from AITD contain TG antibodies in the absence of TPO antibodies, but the converse is not always true, so it has been proposed that screening for AITD could be undertaken initially with assays for TPO antibodies (75). This is particularly the case if the hemagglutination assay is used for TG antibodies; sensitive RIAs may detect a very high frequency of TG antibodies in individuals with autoimmune thyroid disease, even more than TPO antibodies (76). Using modern types of assay, TG antibodies occurring in isolation from TPO antibodies are more commonly found, and thus measurement of both antibodies might have clinical utility in certain situations, for instance in diagnosing possible causes for impaired fertility in women (77).

Antibodies detected by these techniques are believed to be similar to antibodies first described in the 1950s that fix complement in the presence of extracts from a thyrotoxic gland (78) and that have cytotoxic effects on thyroid cells (79). Sera from patients with Hashimoto's thyroiditis usually have high cytotoxic activity (80). Complement-mediated sublethal injury probably occurs in vivo since complement containing complexes have been identified in thyroid tissue of patients with Graves' disease and Hashimoto's thyroiditis (81). Thyroid cell expression of membrane proteins, especially CD59, helps prevent complement-mediated lysis (82), and this protein is upregulated by IL-1 and IFN-γ.

The cytotoxicity of circulating antibodies has also been explored using systems to detect antibody-dependent cell-mediated cytotoxicity (ADCC) in which nonimmunized lymphocytes (NK cells) or macrophages act as effector cells and kill antigen-coated target cells, following incubation of the targets with antibody (83, 84). This reaction does not require complement, instead depending on the interaction of antibody on the target cell with Fc receptors on the effector cells. The exact role of ADCC in the pathogenesis of autoimmune thyroid disease is unclear, as it has been investigated only as an in vitro phenomenon. Antibodies capable of mediating ADCC on target cells include those against TG and TPO, but other antigens may also be targets, and sera from patients with Hashimoto's thyroiditis, primary myxedema and Graves' disease cause ADCC, although the frequency is lower in Graves' disease (85). A further possible role for TPO antibodies has been suggested by the finding that these bind to cultured astrocytes and it is therefore possible that the controversial entity of Hashimoto's encephalopathy is the result of some autoimmune cross-reaction between thyroid and central nervous system (86).

Titers for all types of thyroid autoantibody obviously increase during the process of development of AITD. It is possible that one critical step in the production of TG autoimmune responsiveness is the generation of immunoreactive C-terminal fragments during hormone synthesis (which results in oxidative stress); these fragments may also lead to preferential presentation of TG epitopes by thyroid cells (87). Natural autoantibodies against TG may be more important in the initiation of the response than previously thought. These low affinity, mainly IgM antibodies, which are frequent in healthy individuals, can complex TG with complement and such opsonized complexes

can be taken up by B cells and presented to $CD4^+$ T cells (88). After first observation, antibody levels tend to be stable over months.

Radioactive iodine therapy in Graves' disease leads to a rise in thyroid antibody levels during the first few months after treatment (89), and exposure to high levels of IFN-α in those with pre-existing autoantibodies also does this (90, 91). With treatment of Graves' disease, or replacement therapy in Hashimoto's thyroiditis or myxedema, there is characteristically a gradual reduction in antibody levels over months or years, and some patients with total destruction of thyroid tissue eventually lose detectable antibody titers.

There are two major conformational epitopes on the TG molecule that are recognized differentially by sera from healthy subjects and those with AITD; linear epitopes are recognized by polyclonal antibodies from healthy individuals (92-94). Similar studies on TPO have indicated at least eight major domains for human autoantibodies which are probably conformational epitopes. Using recombinant proteins and synthetic peptides, human anti-TPO antibodies are found to recognize apparently linear epitopes in the area of amino acids 590-622 and 710-722 (95) but, again, the important B cell epitopes are conformational.

Peripheral blood mononuclear cells (PBMC) and thyroid lymphocytes from patients with AITD have among them activated cells that spontaneously secrete TG and TPO antibodies (96). B cell production of antibodies to TPO and TG is most easily shown using cells incubated with mitogens (97). Specific antibody secretion in response to PBMC stimulation by TG or purified TPO is more difficult to demonstrate (98). In patients with AITD, approximately 50 B cells secreting anti-TG antibodies are found per 10^6 PBMC (~2% of total Ig secreting cells) by using plaque-forming assays after stimulation of PBMC with pokeweed mitogen. B cells from AITD patients synthesize antibodies in response to insolubilized TG bound to Sepharose (98), which appears to function as an especially good antigen. There are reports of production of anti-TSH-R antibodies in vitro, but in general this response has been difficult to observe.

In fully developed AITD, the thyroid is clearly an important source of autoantibody and spontaneous autoantibody secretion by B cells is easily demonstrable (99). This is also supported by the histopathological features, including the demonstration of thyroid

antigen-specific B cells and the occurrence of secondary immunoglobulin gene rearrangement in intrathyroidal lymphoid follicles, together with a congruent pattern of adhesion molecule and chemokine expression (100). However, lymph nodes, bone marrow and possibly other organs also contribute to autoantibody production (101) and this explains why patients with apparently destroyed thyroid tissue, or those with resected thyroids, continue to have circulating thyroid auto-antibodies

Cell-Mediated Immunity In Autoimmune Thyroid Disease

Techniques for identification of T lymphocyte reactivity to foreign or autologous antigens depend on culturing mixed peripheral leukocytes or semi-purified thyroid or blood lymphocytes with an antigen to which the cells may have been pre-sensitized. Upon re-exposure to antigen, the sensitized cells change to a blast-like immature form, synthesize new protein, RNA, and DNA, and directly or through liberated effector molecules alter the function of target cells. Different endpoints characterize the various assays, including measurement of [^{3}H]-thymidine uptake, assay of migration inhibition factor (MIF), or leukocyte migration inhibition (LMI) (102), assessment of the mobility of lymphocytes, and cytokine assay, all after stimulation with antigen in culture.

Numerous reports have shown that T cell immunity can be detected in Graves' disease, Hashimoto's thyroiditis, and primary myxedema, although responsivity of T cells to thyroid antigens is much less than to exogenous antigens such as tetanus toxoid or tuberculin. Peripheral blood T cells respond to incubation with TG or TPO in the form of a microsomal preparation by thymidine incorporation, the so-called proliferation assay (103, 104). Responses by separated lymphocytes are generally weak; better responses are seen by adding IL-2 to thyroid antigen-stimulated cultures of diluted whole blood (105). Thyroid T cells responding to TG are of the CD4+ T helper type (106), or occasionally CD8+ cells (107). T cells also respond to crude thyroid antigen extracts in LMI assays (102). T cell lines and short term T cell clones (CD4+) are stimulated during co-culture with TECs to incorporate [^{3}H]-thymidine; DR+ TECs are especially effective stimulators (108 - 110). The identity of the antigen recognized on TECs is unknown but may well be TPO and/or TG.

The specific peptide epitope fragments of TPO recognized by lymphocytes of patients with HT were noted previously. T cell epitopes present within the extracellular domain of

the TSH-R are also heterogeneous with peptides bearing sequences of aa 158-176, 237-252, and 248-263 and 343-362 being especially important (111) but other epitopes (aa 57-71, 142-161, 202-221, 247-266) have been identified by others using different assay parameters (112). HLA-DR3 molecules bind TSH-R peptides with high affinity, which may explain the genetic association of this HLA specificity with Graves' disease (113).

T cell responses to an antigenic stimulus may use a wide variety of variable (V) TCR gene segments, or the response may be restricted to a few V segments. Restriction of autoreactive T cells to use of one or more V gene segments has been found in some experimental autoimmune models (4). Restricted Vα and Vβ usage in the whole intrathyroidal lymphocyte population has been reported (114, 115) but not confirmed by others (116, 117). However, intrathyroidal CD8+ T cells do display a degree of restriction although their autoreactive potential is at present not known (118). Presumably at an early stage of disease, the T cell response is clonally restricted, but as it advances, spreading of the immune response occurs, involving many more epitopes, leading to an unrestricted response as demonstrated in an animal model of AITD (119). Evidence has emerged of a combined TG and TPO epitope-specific cellular immunity, with CD8+ T cells reacting against these epitopes rising to 9% in the peripheral blood of patients with long-standing Hashimoto's thyroiditis (120).

While T cell immunization is conventionally recognized by a stimulatory effect of antigen, direct T cell cytotoxicity of thyroid cells has been recognized in a few studies. For example, Davies and co-workers developed a CD8+ T cell clone which was cytotoxic to autologous TEC and was appropriately class I restricted (121). Another potential consequence of T lymphocytic adherence to thyroid cells is the stimulation of thyroid cell proliferation via ICAM-1/LFA-3 interaction, rather than their destruction, which could lead to goiter formation (122).

Immune Complexes

In addition to the antibody and T cell responses, circulating immune complexes are found in patients with autoimmune thyroid disease as would be anticipated], although their pathogenic importance appears minimal. In a certain sense this is most fortuitous. Since many individuals have circulating TG antibodies and antigen, if the immune

complexes caused serious disease, it would be a catastrophe. Fortunately the immune complexes of TG and its antibody do not bind complement and do not cause serious illness such as immune-complex nephritis, except in rare instances (123, 124). Immune complexes, including complement terminal components, can however be recognized around the basement membrane of thyroid follicular cells (81) and may cause sublethal effects including release of proinflammatory mediators by TECs (125).

K And Nk Cell Responses

Many studies have been reported on natural killer (NK) cell activity and antibody dependent cell-mediated cytotoxicity (ADCC); their conclusions vary. Endo et al (126) found NK cells were decreased in Graves' disease and Hashimoto's thyroiditis, and presented evidence that this was due to saturation of their Fc receptors by immune complexes. Normal NK effector function was found in Hashimoto's thyroiditis PBMC (127) in one study, although by phenotyping, decreased NK cells in Graves' disease, and increased NK cells in Hashimoto's thyroiditis were reported in another (128). ADCC of thyroid cells, mediated by normal PBMC, was induced by TPO antibody positive sera (129) but other, unknown antibody-antigen systems may also contribute (85). Effector cell activity in ADCC was increased in Hashimoto's thyroiditis and in post-partum thyroiditis, and thought to be related to thyroid cell destruction (130). Other data have indicted that ADCC may be more important in primary myxedema than Hashimoto's thyroiditis explaining the difference in clinical presentation (131), but this has not been confirmed in studies showing equal ADCC activity in sera from both diseases (132).

Cytokines

Cytokines lie at the heart of the autoimmune response and can have a number of direct and indirect effects (Fig. 7-9). For example, IFN-γ is produced in the thyroid by infiltrating lymphocytes and causes HLA class I expression on the surface of TECs to increase and initiates class II expression. It also has a direct inhibitory function on TEC iodination and TG synthesis (133, 134). Caveolin-1 and TPO form part of the apical thyroxisome, responsible for thyroid hormone synthesis. Recent studies have shown that Th1 cytokines down-regulate caveolin-1, leading to intracytoplasmic thyroxine synthesis and mislocalization of the thyroxisome. Disruption of the thyroxisome in this manner may then lead to damage by reactive oxygen metabolites and apoptosis in Hashimoto's thyroiditis (135).

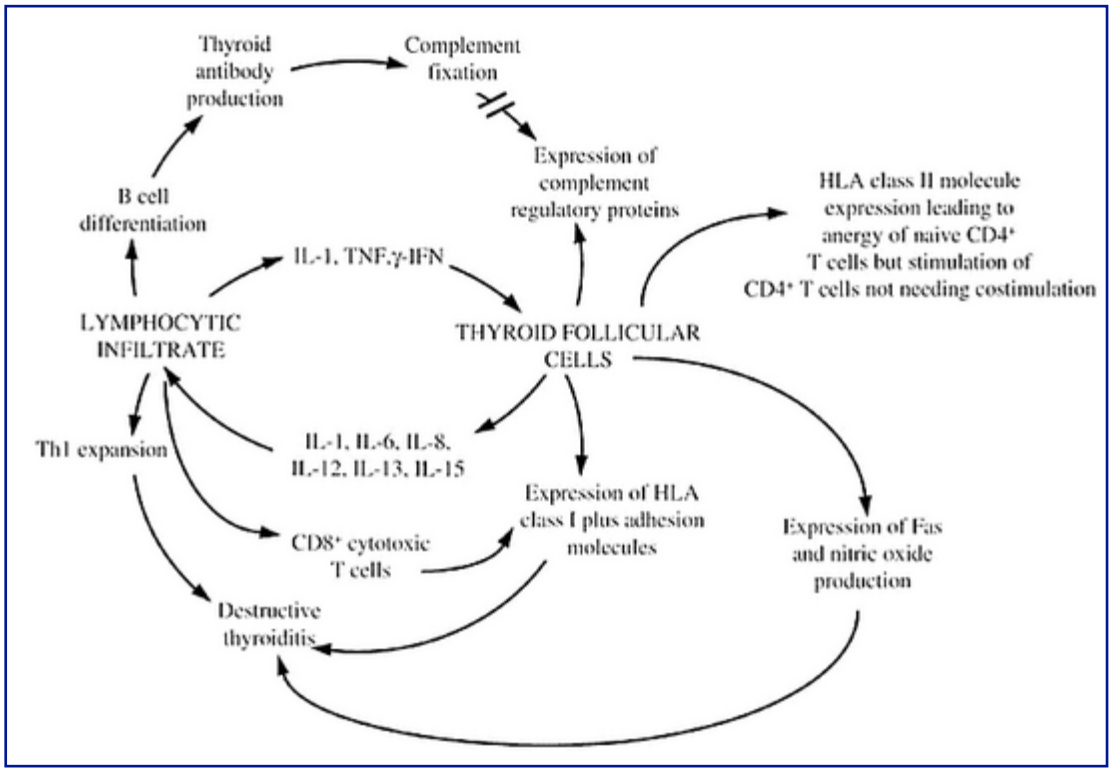

Figure 7-9: Interactions between thyroid follicular cells and the immune system in autoimmune thyroid disease. Reproduced from Weetman AP, Ajjan R, Watson PF. Bailliere's Clin Endocrinol Metab 11: 481-497, 1997 with permission.

IFN-γ is not essential for the development of AITD in mice but exacerbates disease activity (136). IL-2 can activate lymphocytes to produce IFN-γ, and activate NK cells. TNF is produced by infiltrating macrophages and is potentially cytotoxic to TEC. TEC can produce several cytokines, including IL-1, which may activate T cells, IL-6, which stimulates T and B cells and IL-8, a chemokine which attracts inflammatory cells (reviewed in 134). More recently IL-14 (taxilin) and IL-16 production by TECs has been described: the former regulates B cell growth and the latter is a chemoattractant for CD4+ cell (136a). Dendritic cells are important sources of IL-1β and IL-6 in the thyroid and can inhibit thyroid follicular cell growth (137). As an aside, plasmacytoid dendritic cell numbers are decreased in the blood in AITD, together with an alteration in their phenotype, but these cells increase in the thyroid gland, also suggesting that this cell type may be important in pathogenesis (138)

IL-1α causes dissociation of junctional complexes between thyroid cells which could expose hidden autoantigens (139). An ever wider array of factors besides the classical cytokines has been implicated in the pathogenesis of AITD, including the finding that thyroid cells can release angiopoietin-1 and -2 (140). These ligands serve as a chemoattractant for monocytes and the angiopoietin receptor, Tie-2, is increased in monocytes form AITD patients, suggesting a role for monocytes in thyroid damage. Vascular endothelial growth factor expression is increased in AITD and is important in angiogenesis in autoimmune goiters (141). Cytokines also seem to play a major role in the pathogenesis of thyroid-associated ophthalmopathy through their stimulatory actions on orbital fibroblasts (142). Exogenous cytokines given therapeutically can also precipitate autoimmune thyroid disease, probably in predisposed individuals. The best described such reaction is α interferon used in hepatitis C and cancer therapy (90). Destructive thyroiditis accounts for the majority of thyroid dysfunction after treatment with this cytokine, and risks are highest in white women, whereas smoking is protective (91).

6. SUMMARY

To summarize, augmented pools of activated and resting T and B cells reactive to thyroid antigen exist in patients with AITD. The time course of development of these reactive cells, before clinical disease is apparent, has not been established. The cells respond to biochemically normal antigen, and some reactive cells exist in otherwise healthy individuals. Immune complex formation appears to be of limited importance in the disease process. K and NK activity may be reduced in Graves' disease and increased in Hashimoto's thyroiditis and may contribute to the course of the disease: proliferative in Graves' disease and destructive in Hashimoto's thyroiditis. Cytokines have multiple actions in the thyroid in AITD and are likely to determine clinical manifestations such as ophthalmopathy. The role of the TEC in the autoimmune response is not simply passive and, as discussed below, the interaction between TECs and cells of the classical immune system may be critical in determining the outcome of an initially mild thyroiditis.

EXPERIMENTAL THYROIDITIS IN ANIMALS

Chronic thyroiditis histologically identical to that in Hashimoto's thyroiditis occurs spontaneously in Obese strain (OS) chickens (143), beagles (144), mice, and rats. It can be induced in dogs (145), mice, rats, hamsters, guinea pigs, rabbits, monkeys (146), and

baboons (147) by immunization with autologous or allogenic thyroid homogenate mixed with adjuvants, or by using heterologous TG , or TG that has been arsenylated or otherwise chemically modified. The need for modification of TG or adjuvant to break tolerance can also be overcome by immunization with cDNA (148). An important thyroiditogenic epitope includes a thyroxine residue (aa 2553) in human TG (149, 150) but the role of iodination at this site is unclear and may depend on the type of T cell assay system used, as well as other parameters (151). Mice have been the most frequently used model and have provided key insights into genetic susceptibility, pathogenesis and the development of Treg and autoreactive T cell repertoires (152).

Induced thyroiditis leads to formation of humoral antibodies and T cell- mediated immunity. Usually the histologic pattern conforms to that of T cell-mediated immunity (153). The role of TG antibodies is unclear but likely to be minor. An idiotype-anti-idiotype network exists for TG antibodies in mice but the induction of those antibodies does not lead to thyroiditis (154). Furthermore, the intensity of the thyroiditis correlates better with T cell-mediated immunity than with antibody levels, and can be transferred by T cells but not antibodies, and both CD4+ and CD8+ T cells are usually needed for transfer (155). In normal mice, thyroiditis can be produced by immunization with mouse TG in adjuvant, and transferred to isogenic animals by sensitized Ly-1+ T cells. The same cells, given before immunization, vaccinate against the development of thyroiditis during subsequent immunization (156).

However, a subpopulation of CD4+ T cells has an important regulatory role in tolerance to murine TG, keeping in check those TG-reactive T cells which escape thymic deletion and peripheral anergy-inducing mechanisms (157). Amelioration of thyroiditis by oral administration of TG (158) operates through enhancing the activity of these regulatory T cells although other mechanisms are possible. More recent studies have emphasized the importance of regulatory T cells in suppression of thyroiditis in animals immunized with TG. In particular, semi-mature dendritic cells, which can be induced with granulocyte-macrophage colony stimulating factor, can induce the function of TG-specific $CD4^+$, $CD25^+$ T cells which can suppress thyroiditis through the production of IL-10 (159, 160).

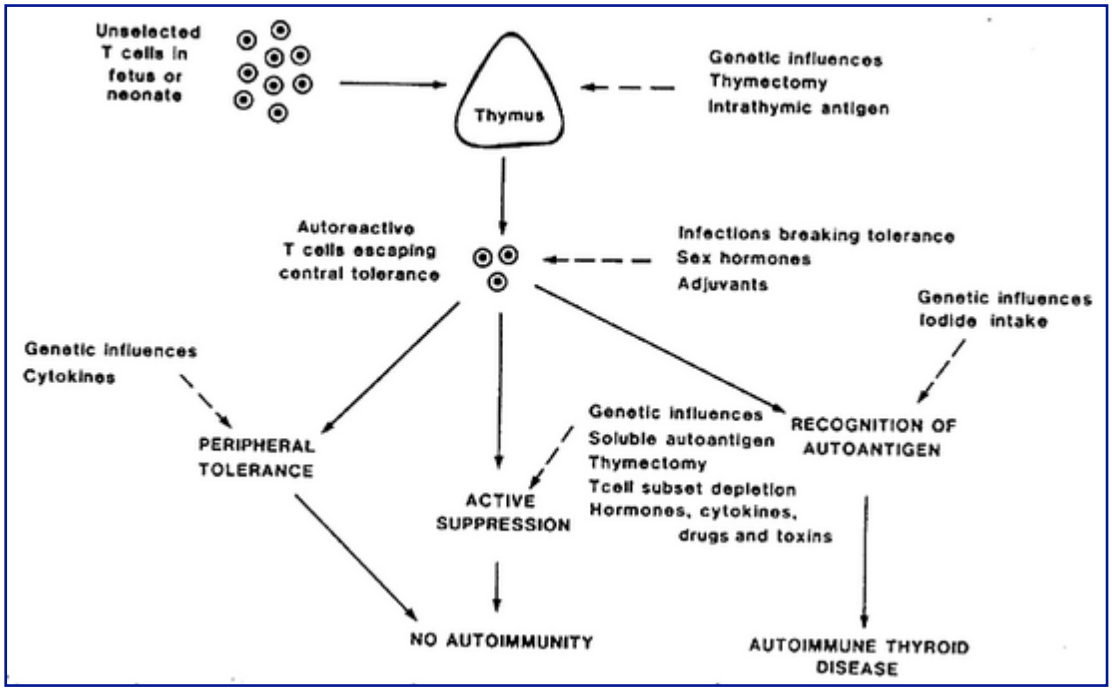

Figure 7-10: Control of thyroid antigen-specific T cells in experimental autoimmune thyroiditis. Development of disease depends on the balance of these factors, and their sites of operation are shown as dotted lines. Reproduced from (255) with permission.

Another model has used homologous (murine) TPO in an immunization protocol and this method established thyroiditis and TPO antibody production although none of the immunized mice developed hypothyroidism (161). HLA-DRB1*0301 (DR3) transgenic mice have been created which are susceptible to thyroiditis induced by TG immunization, unlike DR2 transgenics, thus confirming that HLA-DRB1 polymorphism determines susceptibility to autoimmune thyroiditis, and his model has been extended to study of the immune response to TSH-R, with results again showing the importance of the DR3 specificity (162). However, when modeling has attempted to reproduce Graves' disease by immunization of mice with adenovirus expressing the TSH-R, it is non-MHC genes which play a major role in controlling the development of hyperthyroidism (163). This concurs with the polygenic susceptibility and rather weak effect of HLA-DR3 in Graves' disease. The DR3-transgenic model has also been used to show that dietary iodide enhances the development of thyroid disease and depletion of CD4+, CD25+ Tregs exacerbates this iodide-induced thyroiditis (193a)

Spontaneous thyroiditis in OS chickens more closely resembles Hashimoto's thyroiditis than the immunization models just discussed, particularly as the birds develop

hypothyroidism as a consequence of the autoimmune process. Some evidence suggests that the thyroid of the newly hatched chick is intrinsically abnormal, since its function is partially non-suppressible by thyroid hormone and this constitutes an important element of the genetic susceptibility of these birds, together with genes controlling T cell responses and possibly glucocorticoid tonus. The MHC conversely has only a limited effect. Iodine plays a critical role in the induction of thyroid injury in OS chickens, most likely through the generation of reactive oxygen metabolites, and this injury is an early event, preceding lymphocytic infiltration (165). Iodination of TG is a second path by which iodine influences disease in OS chickens, as autoreactive T cells respond to the antigen only if it is iodinated (166).

Lymphocytic thyroiditis occurs spontaneously in the Buffalo and BB/W rat strains and the NOD (non-obese diabetic) mouse, especially the NOD.H-2h4 line (167). In both species, there are associated abnormalities in the animals' immune system. As in the OS chicken, administration of excess iodine augments the incidence of rat thyroiditis and iodine depletion reduces it (168). Iodine also enhances the susceptibility of NOD mice to thyroiditis, and further exploration of this model has demonstrated a key role for Th17 cells which accumulate within the thyroid (169). IL-17-deficient mice have a markedly reduced frequency of TG autoantibodies and thyroid lesions. Furthermore, selenium supplementation lowers serum TG antibody levels and decreases the prevalence of thyroiditis and the degree of infiltration of lymphocytes in iodine-treated NOD mice (169a). The susceptibility of NOD mice has also been exploited in a model in which the CCR7 gene was knocked out in this strain: such mice do not develop diabetes but do develop severe inflammation elsewhere including a severe thyroiditis with TG autoantibody formation and hypothyroidism (170). CCR7 is a chemokine receptor which is expressed by Tregs; the CC7-deficient mice had lower numbers of these cells. As well as this effect, it is possible that CCR7 deficiency impaired negative selection of thyroid reactive T cells.

Another intriguing aspect of this model comes from long-term observations in NOD.H-2h4 mice which have shown that TG antibodies occur initially and much later TPO antibodies appear, suggesting that tolerance at the B cell and presumably T cell level is broken first for TG and then by spreading (see above) for TPO (171). These results suggest a more important role for TG as an autoantigen in AITD than it is currently

assigned. When engineered through CD28 knockout to have a deficiency of Treg cells, NOD mice develop more severe thyroiditis than control animals, with thyroid fibrosis and hypothyroidism. Transferring healthy Treg cells reduces thyroiditis without increasing the total number of Treg cells, suggesting that endogenous Tregs in these mice are functionally defective (172).

The iodine-accelerated thyroid autoimmunity which occurs in NOD.H2(h4) mice is associated with TG and TPO but not TSH-R autoantibodies However transgenic animals expressing the human TSH-R A-subunit develop pathogenic TSH-R antibodies which can be detected in standard bioassays, and this is especially the case in female animals (172a). These antibodies only weakly cross-react with the murine TSH-R and so do not cause hyperthyroidism.

A third kind of model is produced by manipulation of T cells. The original description of thyroiditis in genetically susceptible rats by sublethal irradiation and thymectomy (173) has been followed by a number of more refined models in which T cell subsets can be perturbed more or less specifically to induce disease. For instance CD7/CD28 double-deficient mice have impaired Treg function and such animals develop spontaneous thyroiditis after 1 year of age (174). These experiments clearly demonstrate the recurrence of autoreactive T and B cells in normal animals and show that any of a number of factors which can perturb the regulation of these could result in autoimmune thyroiditis (Fig. 7-10). The most elegant model resulting from T cell manipulation is the generation of transgenic mice expressing a human T cell receptor specific for a TPO epitope, which resulted in a spontaneous destructive hypothyroidism and hypothyroidism (175). The CD8 T cells recognizing the epitope in these animals unconventionally were MHC class II rather than class I restricted and it is unclear whether this atypical behavior is significant to the creation of the model, nor is it yet clear what the mechanism is for thyroid cell destruction.

Another intriguing model is one in which necrotic thyroid cells can induce maturation of dendritic cells in vitro, and when injected back into autologous mice EAT is induced, with a lymphocytic thyroiditis and TG-specific IgG (176). It is not clear whether this protocol yields cryptic TG epitopes which can break tolerance. It is possible that such work could be reversed therapeutically to allow attenuation of EAT by pulsing tolerogenic dendritic cells.

Establishing an animal model of Graves' disease has been surprisingly difficult despite the cloning of the TSH-R. Spontaneous models are not obvious, suggesting that critical differences in the TSH-R receptor between man and other mammals (such as glycosylation) may be necessary to break tolerance (177). However, immunization of AKR/N mice (but not other strains sharing the same MHC haplotype) with murine fibroblasts doubly transfected with the human TSH-R and haploidentical MHC class II genes results in a syndrome similar to Graves' disease except that thyroid lymphocytic infiltration was not induced (178), whereas thyroiditis is a feature of immunization with the TSH-R (179). This is a promising model although its exact physiological parallel remains unclear, particularly as fibroblasts may behave differently to TECs in terms of antigen presentation. This is because the fibroblasts used express the critical costimulatory molecule B7-1 and also because the procedure causes generalized in vivo immune activation. This model is therefore not evidence that thyroid follicular cells (which do not normally express B7) could initiate thyroid autoimmunity.

More recent models include the use of transgenic mice expressing the A-subunit of the TSH-R, which develop lymphocytic infiltration of the thyroid, hypothyroidism and autoantibodies against TG and TPO as well as TSH-R following immunization with the TSH-R expressed in adenovirus and regulatory T cell depletion (180). Although obviously a contrived system, this model does clearly show that spreading of the immune response can occur to include the normal array of antibodies found in patients, and that this can result in a severe thyroiditis. Some of the difficulties in producing reliable animal models of Graves' disease are seen in the disparity between hyperthyroidism in the animal and the presence of TSH-R antibodies detected by bioassays using human TSH-R. This may be the result of loci in the immunoglobulin heavy chain variable region contributing in a strain-specific manner to the development of antibodies specific for the human or the mouse TSH-R (181). This novel finding of a role for immunoglobulin heavy chain variable region genes in TSI specificity indicates a possible role for them genetic susceptibility to human Graves' disease.

One unexpected finding has been the observation that mice with a TSH-R knockout do not differ in their response to immunization with TSH-R when compared to healthy animals, whereas the expectation was that such animals would have no tolerance to this

autoantigen (as it had been absent throughout development) and therefore a greater immune response would be predicted (182). This suggests that thymic (central) tolerance is not a critical step in self tolerance to this autoantigen. The same conclusions have been drawn from the finding of similar intrathymic transcript levels of thyroid autoantigens (TPO and TSH-R) in mice which are genetically susceptible or resistant to the development of EAT (183). However the situation may be more complex than originally imagined, as the same group has identified a role of the *Aire* gene in the response to TSH-R and in *Aire*-deficient mice, intrathymic transcripts of TSH-R and TG are reduced while the expression of TPO is nearly abolished (184). These results are compatible with the finding of an increase in AITD in autoimmune polyglandular syndrome type 1, but at a much lower frequency than the classical disorders of Addison's disease and hypoparathyroidism. It is also intriguing that TPO transcripts are so much more affected in the *Aire*-deficient murine thymus, perhaps explaining (via more rigorous tolerance) the rather weak response to this autoantigen, compared to TG, in the mouse.

Balb/c strain mice appeared to develop orbital changes suggestive of ophthalmopathy when given TSH-R primed T cells derived from donor mice immunized with TSH-R protein or cDNA but this model has not proved reproducible by the original authors, for reasons which are not yet clear, although complex histological artefacts may be part of the answer (185). A somewhat more convincing model of ophthalmopathy has been described recently in which deep injection of plasmid containing the TSH-R A subunit into the leg muscles of BALB/c mice followed by electroporation resulted in a wide variety of histological orbital changes and obvious eye signs (41). However the animals developed TSH-R blocking rather than stimulating antibodies and thyroiditis was absent. Nonetheless these finding support a pathogenic role for the TSH-R in the pathogenesis of thyroid eye disease.

The clear general concept to be derived from all of these studies is that a genetically controlled balance of helper and suppressor T cell function is needed to prevent autoimmunity, and that a variety of perturbations can lead to onset of the disease.

RELATION OF THE IMMUNE RESPONSE TO THE THYROID CELL: STIMULATION AND DESTRUCTION

For certain we know that the autoantibodies can stimulate the thyroid and cause overactivity in Graves' disease, and can in select circumstances inhibit thyroid function and cause hypothyroidism in neonates and some adults. Whether thyroid antibodies are primary cytotoxic agents in AITD remains an unsettled issue. TG antibodies are probably not normally cytotoxic, but TPO antibodies can certainly mediate complement-dependent thyroid cell cytotoxicity and ADCC. However, the frequently reproduced natural experiment of transplacental antibody passage from a mother with AITD to her fetus, without evidence of thyroid damage, clearly shows that antibodies alone are not destructive to the thyroid.

Cell-mediated immunity is thought to be important in thyroid cell destruction, and T cells have been shown to be reactive to TECs. T cell lines or clones have been shown to react to TECs (108-110), but the nature of the antigen recognized is unknown. One CD8+ T cell clone in man has been shown to be cytotoxic specifically to autologous TECs (121), suggesting that cell-mediated TEC destruction is an important process, and similar activity has been reported in CD8+ T cell lines and clones derived from mice with experimental autoimmune thyroiditis (186). A second type of T cell-mediated cytotoxicity is that mediated by $\gamma\delta$ TCR-bearing T cells and specific recognition of TECs by such cells has been reported in Graves' disease, but the exact autoantigen involved is unknown (187). In animals it is clearly shown that there can be a marked dissociation between the extent of histologic thyroiditis and the levels of antibodies, again suggesting that T cells rather than antibodies mediate cell destruction. However, it must be admitted that the hard evidence for direct T cell-mediated cytotoxicity in thyroid autoimmunity in man is meagre at present.

There are 3 mechanisms by which T cells might mediate TEC destruction and evidence for all 3 operating in AITD has accrued. Firstly, cell lysis might be effected via T cell-derived perforin, which leads to pore formation in target cell surfaces, and certainly the thyroid lymphocytic infiltrate contains perforin-expressing T cells in AITD (188). Secondly, T cells expressing Fas ligand, especially the CD8+ subset, can induce apoptosis in TECs expressing Fas (189). Fas is induced by IL-1β on TECs, whereas TSH-R stimulation inhibits Fas expression (190) and this may lead to the involvement of this pathway in Hashimoto's thyroiditis but not Graves' disease, as TSI would act like TSH in the latter to diminish Fas expression (and other regulatory molecules). It has

been suggested that T cells may not be necessary, as Hashimoto TEC may express Fas ligand, and autocrine/paracrine interaction with Fas may lead to TEC death (191). The mechanisms for this are unclear and as yet there is no consensus on the role this may have in AITD. The picture is complicated by the upregulation of molecules which protect against apoptosis such as Bcl-2. The pattern of expression of this molecule is different in Graves' and Hashimoto's diseases, suggesting that TECs are protected in the former and more sensitive to destruction in the latter (192). Whether these differences depend on cytokines, genetics or other factors is unknown (193). Finally, T cell-derived cytokines can injure the TECs directly, leading to functional impairment (133-135), and by triggering other phlogistic pathways such as nitric oxide synthesis (194).

POSSIBLE EXPLANATIONS FOR AUTOIMMUNITY

Many reasons for the development of autoimmunity have been advanced, and these are briefly catalogued below. Cross-reacting epitopes, aberrant T or B cell regulatory mechanisms, inheritance of specific immune response-related genes, and aberrant HLA-DR expression on TECs have all at some time been considered important for development and progression of thyroid autoimmunity.

1. Abnormal presentation of antigen could occur due to cell destruction, or viral invasion, so that large amounts of antigen or cell fragments are liberated locally into the lymphatics. Excessive levels of antigen are produced, thereby overwhelming the usual low dose tolerance mechanism.

2. Abnormal antigen could be produced by a malignancy, or damage to the cell by viral attack, or other means. This antigen could be a partially degraded or denatured normal antigen, for example.

3. Cross-reacting bacterial or viral epitopes e.g. *Yersinia enterocolitica* (195) could induce immune responses that happen to cross-react with a self-antigen having identical conformation. An extension of this concept is that the normal anti-idiotypic control response happens to produce an Ig or T cell that cross-reacts with self-antigen. For example, experimentally produced anti-idiotypic monoclonal antibodies directed to TSH antibodies bind to and stimulate the TSH-R (196).

4. Somatic mutation of a TCR gene could lead to a clone of self-reactive cells. However, somatic mutation of TCR genes is believed to occur very rarely if at all, and such monoclonal or oligoclonal activation has not been documented in autoimmune disease. Somatic mutation of B cell Ig genes is, as described above, a normal phenomenon during an antigen-driven proliferative response. Such an event could occur by chance during response to any antigen and this does not effectively introduce any new variable, since B cells capable of producing Igs that can react with self-antigens are already normally present. However, TSI seem to be clonally restricted and, until the V gene usage of these antibodies is documented, it remains possible that Graves' disease is due to the inheritance of a unique, etiologically critical V gene encoding TSI.

5. Inheritance of specific HLA, TCR, or other genes that code for proteins having especially effective ability to process or present antigen.

6. T cell or B cell feedback control mechanisms could be aberrant due to hereditary or environmental factors.

7. Failure of clonal deletion could leave self-reactive T cells present in the adult. In fact this is clearly normal, as described above.

8. Failure of normal maturation of immune system could allow fetal T and B cells that are autoreactive and of wide specificity to persist.

9. Polyclonal activation of T or B cells, by some unknown stimulus, could lead to B cells producing self-reactive Ig, in the apparent absence of antigenic stimulus. This theory is in a sense impossible to disprove but would need to co-exist with other abnormalities to explain disease remission, genetic associations, associated diseases, etc. Polyclonal activation is not typical of peripheral lymphocytes of patients with AITD (197).

10. TECs could express MHC class II molecules as a primary event and then could function as APCs, including antigens on their cell surface.

11. Environmental factors could distort normal control. For example, stress or steroids may alter immunoregulation, and the potential role of dietary iodine has been mentioned above.

Abnormal Exposure To Thyroid Antigens And The Effects Of Pregnancy

Damage to the thyroid might release normally sequestered antigens, inducing an immune response. Damage to thyroid cells does indeed occur in viral thyroiditis, such as in association with mumps or in subacute thyroiditis of unknown cause, but autoantibodies appear only transiently at low titer, and progressive lesions of the thyroid do not usually occur (reviewed in 198, 199). External irradiation to the thyroid, including that from nuclear fallout, can also lead to an increase in Graves' disease or thyroid antibody production (200, 201), but it is unclear if this is caused by autoantigen release or an effect on the lymphocytes which are radio-sensitive. Even occupational exposure to ionizing radiation appears to be a risk factor for the development of autoimmune thyroiditis (202). Another possible example where exposure to thyroid antigens released by gland injury leads to autoimmunity is the rare case of precipitation of Graves' disease and ophthalmopathy after ethanol injection of thyroid nodules (203).

A powerful argument against the hidden antigen hypothesis is that TG is a normal component of circulating plasma (204). One might turn the first argument around and suggest that thyroiditis results from a lack of exposure to TG at some period, an exposure that is necessary to depress continuously an otherwise inevitable immune response. This suggestion has no clinical or experimental support, and the available evidence indicates that TG is present in the plasma of patients with active immunity. It remains to be seen how sequestered TPO and TSH-R are, but the appearance of T cells capable of proliferating in response to these antigens, in apparently healthy individuals, also argues against any sequestration (205). What is clear is that availability of the thyroid autoantigen is essential to maintain the autoimmune response: complete removal of thyroid antigens following thyroidectomy and remnant ablation with radioiodine leads to disappearance of antibodies to TG, TPO and TSH-R (206). Although this is not surprising, it does suggest that extrathyroidal sources TSH-R are insufficient normally to maintain an autoimmune response.

A variant on this theme is that of microchimerism, the persistence of fetal cells in maternal tissues. Studies have found evidence of microchimerism in thyroid tissue from patients with and without AITD (207, 208). Could such sequestered fetal material make the thyroid prone to an alloimmune response, and be responsible for the exacerbation of AITD seen in the postpartum period? If so, this phenomenon would help to explain the high frequency of AITD in women. Twins from opposite sex pairs should have an increased risk of thyroid autoimmunity compared to monozygotic twins if microchimerism has a role, and indeed such twins have been found to have more frequent thyroid autoantibodies (209). However, although parity is associated with an 11% increase in the risk of all female-associated autoimmune disorders, there is no increase with multiple pregnancies, which rather argues against a microchimerism mechanism (210). During and after pregnancy, major changes in Treg function occur and direct effects on the cytokines produced by T cells can also be demonstrated (211). It is these alterations that are most probably the ultimate cause of the increase in autoimmunity after pregnancy.

It seems likely that sex steroids play a role in determining the autoimmune response. For instance, in a recent study of an animal model of Graves' disease, 5α-dihydrotestosterone was given to mice a week before immunization with TSH-R, and this reduced both the severity of the hyperthyroidism that developed and downregulated the Th1 response (211a). Another hypothetical reason for the unequal sex ratio is that skewed X chromosome inactivation could contribute through the failure of some autoantigens expressed on one X chromosome to be expressed at a critical point in the disease pathway. A recent survey of 309 patients with Graves' disease and 490 with Hashimoto's thyroiditis found skewed inactivation of the X chromosome in Graves' disease (odds ratio 2.2) but not Hashimoto's thyroiditis; when combined with 4 other studies in a meta-analysis, the results remained significant for Graves' disease and reached significance for Hashimoto's thyroiditis (odds ratio 2.4) (212).

Abnormal Antigens

An abnormal antigen might also serve to produce an immune reaction. The protein abnormality could be either congenital or acquired by an injury such as a virus infection. To date there is no evidence which indicates that TG, TPO, or other proteins of the thyroid of a patient with autoimmunity are abnormal. Minor allelic differences apparently

do occur but attempts to associate thyroid disease with polymorphisms of the TPO and TSH-R genes have been unsuccessful.

Cross-Reacting Antigens

The theory that immune reactivity to an environmental antigen could lead to antibodies that cross-react with thyroid antigens has been bolstered by studies which show a relationship between Graves' disease and antibodies to the common enteropathogen *Yersinia enterocolitica*. An increased incidence of antibodies to *Yersinia* is found by some, but not all authors, in patients who have Graves' disease, and there are saturable binding sites for TSH on Yersinia proteins (213). After infection by *Yersinia*, human sera contain Igs that bind to TEC cytoplasm (195), and IgGs which appear to compete with TSH for binding to thyroid membrane TSH receptors (214). The antigens involved may in fact include proteins encoded by plasmids present in the *Yersinia*, rather than intrinsic Yersinia proteins, but that does not alter the general concept (215). Arguing strongly against a role for *Yersinia* is the fact that there is no unique pattern of serological immunoreactivity to *Yersinia* antigens in patients with AITD (216), and most patients with this infection do not develop Graves' disease. Moreover, there was no association between *Yersinia* infection and autoimmune thyroid disease in a large prospective study of individuals developing AITD (217).

In theory an initial response to one antigen might proceed by reacting to the other antigen, and thereby spread and augment the autoimmune process. In the context of T cell autoreactivity there is much greater scope for molecular mimicry whereby a response to an exogenous epitope leads to a cross-reactive response to an endogenous autoantigenic epitope. Simple sequence homology is insufficient to predict this, as shown elegantly by the cross-reactivity of two TPO epitopes showing a similar surface but not amino acid sequence (218). This makes the prediction and study of molecular mimicry much more difficult than is generally appreciated (219). For these reasons, it may be naïve to believe that the putative orbital antigen responsible for ophthalmopathy has to be an identical protein to that expressed in the thyroid.

VIRUS INFECTION

Virus infection has for years been speculated to be an etiological factor in most autoimmune diseases, by causing cell destruction and liberating antigens, by forming

altered antigens or causing molecular mimicry, by inducing DR expression, or by inducing CD8+ T cell responses to viral antigens expressed on the cell surface. Thyroid autoantibodies are elevated transiently after subacute thyroiditis, which is thought to be a virus-associated syndrome, but no clear evidence of virus-induced autoimmune thyroiditis in humans has been presented. In this regard it is of interest that persistent, apparently benign virus infection of the thyroid can be induced in mice (220), and that infection of neonatal mice with reo virus induces a polyendocrine autoimmunity (Fig. 7-11). These agents could work by liberating thyroid antigens. Virus infection might also augment autoimmunity by causing non-specific secretion of IL-2, or by inducing MHC class II expression on TEC. Despite many attempts to implicate retroviruses in AITD, results to date remain inconclusive (221). Human T lymphotrophic virus-1 has been repeatedly associated with various autoimmune disorders, including Hashimoto's thyroiditis; presumably the virus alters immunoregulatory pathways allowing autoimmunity to emerge (222).

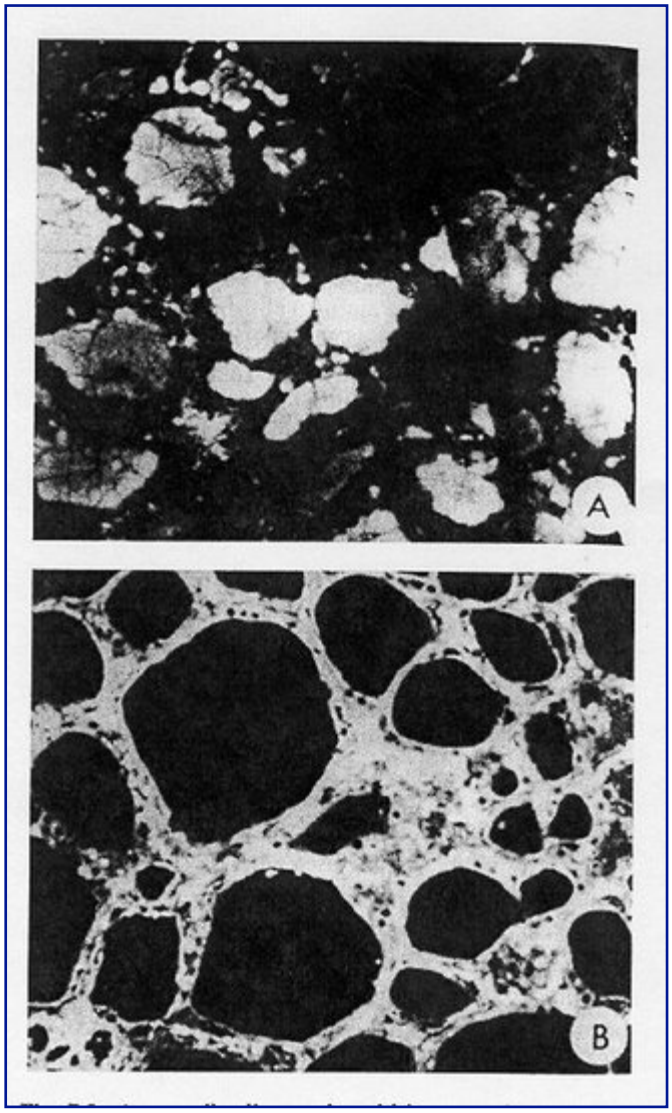

Figure 7-11: Autoantibodies to thyroid in sera of reovirus-infected mice detected by indirect immunofluorescence. (a) Frozen section of normal mouse thyroid incubated with sera obtained from mice 21 days after infection, showing staining of colloid characteristic of antithyroglobulin antibody (original magnification, X200). (b) Section of normal mouse thyroid (fixed in Bouin's solution) incubated with sera obtained from mice 21 days after infection, showing staining of thyroid acinar cells (original magnification, X 200). Reproduced with permission from I. Okayasu and S. Hatokeyama, Clin. Immunol. Immunopath., 31:334, 1984.

Lymphocyte Mutation And Oligoclonality

Apart from the evidence that some TSI may have an oligoclonal origin (67, 223), there is no evidence to support a clonal B cell abnormality in AITD. V gene usage by TSI will need to be analyzed to determine whether Graves' disease has a unique pathogenesis

determined by germ-line immunoglobulin genes. Thyroid-reactive T cells are present in healthy animals and man, as noted above, and therefore a defect at the clonal T cell level is less likely as a primary event in etiology than previously thought. A few autoreactive T cells can be expected to escape tolerance normally, particularly if the autoantigen in question is not available to delete T cells in thymus during fetal development. Stochastic events later in life affecting such undeleted T cells could readily explain the lack of complete concordance for AITD in genetically identical twins (224), and this lack of such concordance argues against an inherited pathogenic TCR as a primary event in AITD.

Genetic Predisposition

A role of heredity in AITD is clearly demonstrated by family studies (225, 226). The role of heredity in AITD is clear, since there is an increased frequency of AITD among family members, first degree relatives, and twins of patients with the illness (227). Indeed a detailed analysis of concordance in Danish twins with Graves' disease came up with the estimate that 79% of the liability for this disorder was attributable to genetic factors (228). Another strand of evidence is the variation of disease with race, although of course this is complicated by environmental influences too. Analyzing military personnel in the USA, it has been shown that HT is more frequent in white individuals, and lowest in black and Asian/Pacific Islander individuals (229). Despite some shared genetic susceptibility factors (see below), in Graves' disease the opposite is true. It is unknown why these ethnic differences occur and this is clearly an area that could be fruitfully explored further.

In an investigation of the relatives of a group of propositi with high circulating antibody levels and clinical thyroid disease, approximately half of the siblings and parents (first-order relatives) were found to have significant titers of thyroid antibodies, many being without clinical thyroid disease (230) but the transmission of thyroid autoantibodies is a more complex trait than the dominant inheritance originally thought (231, 232).

Together, such observations suggest that these diseases have a common genetic defect, although other genes are likely to be disease-specific in their effects, as reviewed extensively elsewhere (233). The most important susceptibility factor so far recognized is the inheritance of certain MHC class II genes. Inheritance of HLA-DR3 causes a 2 to

6-fold increased risk for the occurrence of Graves' disease or autoimmune thyroiditis in Caucasians, and inheritance of HLA-DR4 and DR5 has been found in some studies to increase the incidence of goitrous hypothyroidism (234). In post-partum painless thyroiditis an association with HLA-DR5 has been reported (235). HLA-DQA1*0501, which is often linked to DR3, may have an even more pronounced predisposing effect in Caucasians with Graves' disease (236), whereas HLA-DRB1*07 may be protective (227). A large series of 991 Japanese patients with AITD has been studied and the HLA susceptibility to Graves' disease differentiated from that to Hashimoto's thyroiditis, while 3 common haplotypes were identified which conferred protection against Graves' disease; one of these acted epistatically with the HLA-DP5 susceptibility molecule and another also conferred protection to Hashimoto's thyroiditis (238). It is noteworthy also that the relative risks conferred by HLA alleles is rather modest, borne out by the relatively low concordance for Graves' disease in HLA-identical siblings of patients with Graves' disease (239). This suggests the operation of other genetic susceptibility loci, also emphasized by the weak lod scores for linkage with the HLA region in family studies of AITD (240, 241).

The nature of these other loci is unclear and their identification is likely to require an extensive analysis involving thousands of families in studies using modern molecular techniques. Association studies have been the method of choice until recently, investigating various candidate genes, but with mixed success. Inconclusive results have been reported for associations of AITD with TCR polymorphisms, immunoglobulin allotype and TSH-R polymorphisms. The most consistent non-HLA association is between polymorphisms in the *CTLA-4* gene and both Graves' disease and Hashimoto's thyroiditis (242, 243). Despite claims to the contrary, there appears to be no additional risk conferred by *CTLA-4* (or HLA) polymorphisms in Graves' patients with clinical evidence of ophthalmopathy (244), but these *CTLA-4* polymorphisms may partially determine outcome after antithyroid drug (245, 246). Given the most important role of the interaction between CTLA-4 on T cells and the B7 family of molecules on APCs, it is possible that this association represents a genetic effect on immunoregulation, although, as with HLA-DR3, this is not specific for thyroid autoimmunity; the same polymorphism is also associated with type I diabetes mellitus and several other autoimmune disorders. Fine mapping of the *CTLA-4* region has confirmed that it is indeed this gene, rather than those in linkage disequilibrium, which is responsible for the associations, and the

polymorphisms may exert their effects by causing variation in levels of soluble CTLA-4, which in turn may after T cell activation, especially in Treg cells (247).

Polymorphism of the vitamin D receptor has been linked with Graves' disease, an association which has some biological plausibility as vitamin D has immunological effects (248). However a large survey comprising 768 patients with Graves' disease from the UK, compared to 864 controls, found no evidence of an association (249) and there is not yet any prospective evidence yet for vitamin D deficiency being associated with AITD (250). Polymorphisms in genes encoding molecules involved the NFkB inhibitor pathway modulating B cell function (*FCRL3* and *MAP3K7IP2*) are more likely to be involved in susceptibility to Graves' disease (251, 252).

Another genetic susceptibility locus in Graves' disease is polymorphism in the lymphoid tyrosine phosphatase *LYP/PTPN22* gene, which has been associated with functional changes in T cell receptor signaling. A study of 549 patients and 429 controls found that a codon 620 tryptophan allele conferred an odds ratio of 1.88 (253), although it should be noted that similar effects have been seen in many other autoimmune diseases. This result has recently been confirmed (254) and another likely locus is the IL-2 receptor alpha (CD25) gene region, which is also associated with other autoimmune diseases like type diabetes (255).

As well as genes controlling the immune response, genes that control the target organ susceptibility to autoimmunity are logical candidates for investigation. There is to be conclusive proof from both linkage disequilibrium and association studies, that polymorphisms in the *TSH-R* gene confer susceptibility to Graves' disease but not autoimmune hypothyroidism (256, 257). This is one of the few susceptibility factors that segregates with one rather than both types of thyroid autoimmunity, although polymorphisms in the *PDE10A* and *MAF* genes (which have many actions, including immune regulation) may also influence whether patients develop Graves' disease or Hashimoto's disease (257a). Although not thyroid-specific in tissue location, selenoproteins (SEP) are central to thyroid hormone deiodination and a significant association of HT with SNP in *SEPS1* (odds ratio 2.2) has been reported in a series of 481 Portuguese HT patients (258).

A different approach to chasing candidate genes has been genome scanning, although huge effort is required to undertake such studies. Based largely on this approach, other loci which may be important have been identified on chromosomes 14q31, 20q11 and Xq21 (241, 259), and the importance of a gene on the X chromosome is supported by the increased frequency of AITD in women with Turners syndrome, especially those with an isochromosome-X karyotype (260). However in a genome scan involving 1119 relative pairs, there was no replication of these findings (261). A more impressive genome wide scan of thousands of individuals with Graves' disease confirmed susceptibility loci in the major histocompatibility complex, *TSHR, CTLA4* and *FCRL3* and identified two new loci; the *RNASET2-FGFR1OP-CCR6* region at 6q27 and an intergenic region at 4p14 (262). Seven new loci for AITD, including *MMEL1, LPP, BACH2*, *FGFR1OP* and *PRICKLE1*, have been uncovered by using a custom made SNP array across 186 susceptibility loci known for immune-mediated diseases (263). In another study of almost 10000 Chinese patients with Graves' disease, five additional novel loci were identified and polymorphism in the TG gene was also confirmed to be associated with Graves' disease (264). Thus the genetic factors involved in AITD are increasingly more complex and their interactions with each other and with environmental factors in disease pathogenesis will be a major task to uncover.

Further developments in genetic analysis will no doubt bring even greater complexity to this area, albeit with the prospect of better defining patient subsets (265). It is now clear that to detect common, low-risk variants with reliability, huge sample sizes are essential facilitated by the haplotypic data available from the HapMap project, which means that genome wide variability can be detected using half a million single nucleotide polymorphisms (266). These studies present considerable logistical challenges, and many older studies of genetic associations in AITD have produced conflicting results as because of lack of power or population stratification issues. However a good example of the utility of such studies is a massive genome-wide association study in which a new set of SNPs, which includes polymorphism in *MAGI3*, has been associated with an increased risk of progression from TPO antibody positivity without hypothyroidism to the development of hypothyroidism (267).

As an aside, it should be noted that low birth weight, a known risk factor for several chronic disorders, has not associated with clinically overt thyroid disease or with the

production of thyroid autoantibodies in one study (268) but others have come to an opposite conclusion, with prematurity irrespective of birth size being another risk factor (269, 270).

Co-Occurrence Of Autoimmune Diseases

The co-existence of AITD and other diseases possibly of autoimmune cause has often been reported, and suggests some intrinsic abnormality in immune regulation. An extensive review of these associations has been published (271) and extensive population data bases have clarified the strength of the various associations (272). A striking association is with pernicious anemia. Perhaps 45% of patients with autoimmune thyroiditis have circulating gastric parietal cell antibodies (273), and the reverse association is almost as strong (274). Up to 14% of patients with pernicious anemia have primary myxedema, and pernicious anemia is increased in prevalence in patients with hypothyroidism (275).

Another strong association is with celiac disease, which is found 3 times more commonly in patients with AITD. Intriguingly the autoantibodies which are the hallmark of celiac disease, directed against transglutaminase, can bind to thyroid cells and thus could be implicated directly in thyroid disease pathogenesis (276). The association of Sjögren's syndrome and thyroiditis is not uncommon and both systemic lupus erythematosus (SLE) and rheumatoid arthritis are also significantly associated with AITD (277, 278). A high frequency of antibodies to nucleus, smooth muscle, and single-stranded DNA (26-36%) is found in AITD (279). Although multiple sclerosis has stood out as a putative autoimmune disease which is not obviously associated with AITD, meta-analysis has revealed there is an odds ratio of 1.7 for AITD in these patients (280).

Autoimmune Addison's disease and/or type I diabetes mellitus and AITD occasionally co-exist and this forms the autoimmune polyglandular syndrome (APS) type 2 (281). This is an autosomal dominant disorder with incomplete penetrance and is often associated with other disorders, such as vitiligo, celiac disease, myasthenia gravis, premature ovarian failure and chronic active hepatitis (282, 283). AITD is an infrequent feature of the much rarer APS type I (284) and there is no association between mutations in the *AIRE* gene, which causes APS type I, and sporadic AITD (285).

Together these data provide convincing proof of an association of other autoimmune phenomena with AITD. Most typically, this immunity is organ specific, but in one subset of patients, thyroid autoimmunity develops in association with the non-organic-specific collagen diseases. A syndrome of running together, of course, does not prove a causal association. Nevertheless, the plethora of associations and their familial occurrence indicates that a defect in the immune system may be more likely than primary defects in each organ. This in turn suggests a shared immunoregulatory defect, which is at least partly genetically determined, as these diseases often share similar genetic associations, including HLA, *CTLA-4*, *PTPN22* and *CD25* gene polymorphisms. Recently, analysis of HLA molecules has shown a pocket amino acid signature, DRβ-Tyr-26, DRβ-Leu-67, DRβ-Lys-71, and DRβ-Arg-74, that was strongly associated with type 1 diabetes and AITD (286). This could confer joint susceptibility to these diseases in the same individual by causing significant structural changes in the MHC II peptide binding pocket and influencing peptide binding and presentation. It is also clear however that there is a difference in the kind of clustering of other autoimmune disease in Hashimoto's thyroiditis and Graves' disease, presumably related to differences between these two types of thyroid disease in genetic predisposition (287).

Immunoregulation: Phenomena And Mechanisms

Possible abnormalities in immunoregulation have been addressed in hundreds of studies. The basic hypothesis of this work is that a deficiency of functional T suppressor cells, now termed regulatory cells, may allow uncontrolled T and B cell immune responses to thyroid (or other) antigens. As noted above, this concept is a major theme in experimentally induced or naturally occurring thyroiditis in animal models. Most of the studies to define immunoregulatory responses in AITD have relied on phenotyping (which may relate poorly to effector function in vivo) or in vitro assays done in unique conditions; as we have previously noted, T cell antigen expression and function can vary depending on source of cells, stage of disease, the use of any stimulating agent in vitro, culture conditions, etc.

Sridama and DeGroot found decreased suppressor cells, defined as CD8+ peripheral blood T cells in patients with Graves' disease (288, 289). These results have been challenged, and some investigators have reported depression of CD4+ cells in AITD

(290). However, overall, there is now agreement that, in thyrotoxic patients with Graves' disease, a decrease in CD8+ T cell number (291, 292) is characteristically present, and that a similar abnormality exists in the thyroid. CD8+ cells return gradually toward normal during therapy, and are usually but not always normal at the end of therapy (292) (Fig. 7-12). The phenomenon is present but less evident in Hashimoto's thyroiditis patients. It has been attributed by some workers to increased thyroid hormone levels (293), although this issue is clouded, since there are reports disproving the idea that hyperthyroidism per se induces suppressor cell abnormalities in humans, and reduced suppressor T cells (Ts) are found present long after thyrotoxicosis is cured (294). Our interpretation is that the abnormality is not due specifically to excess T4 in blood, but is a manifestation of ongoing active autoimmunity, for reasons which are unclear. Reduced nonspecific "suppressor" T cell function may be in part an inherited abnormality, and is probably also a manifestation of the augmented immune reactivity ongoing in AITD patients. It may be largely a secondary phenomenon, but one which augments and continues the immunological disease. The mechanism causing such reduced Ts number and function is unclear.

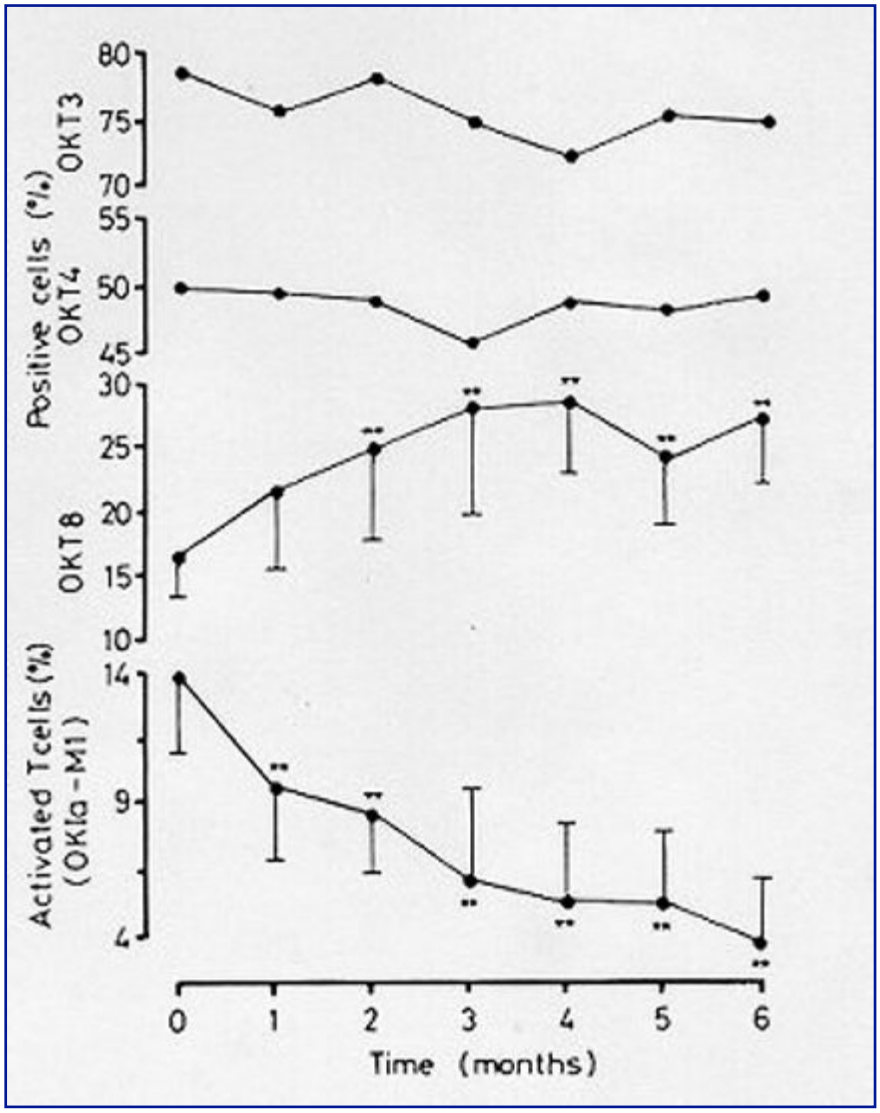

Figure 7-12: Influence of a 6 month course of carbimazole on peripheral blood T cell subsets of 29 patients with hyperthyroid Graves' disease (Mean SD). OKT4 = CD4+ OKT3 = CD3+ OKT8 = CD8+ ** = $p < .001$ vs. zero time value (From Reference 265)

These older findings need to be related to recent developments in understanding Treg function. One study has found that despite increased numbers of CD4+ T cells bearing the T regulatory cell markers CD25, Foxp3, GITR and CD69, in both thyroid and PBMC of patients with AITD, there is a non-specific defect in regulatory function in vitro, which in turn must explain somehow why the increased number of regulatory T cells are so patently ineffective (295). For example there is an increase in circulating $CD69^{+}$ regulatory lymphocytes in AITD, and numbers are even higher in the thyroid

glands of these patients and yet they are functionally deficient in vitro (295a). The existence of a functional rather than numerical deficiency in regulatory T cells has also been suggested in a study of AITD patients, in which the defect was found to be detectable only when optimal in vitro conditions were achieved (296). Analysis in the earliest phases of disease may of course yield different results and unlocking how T regulatory cells can be activated seems an obvious but at present unrealizable therapeutic strategy. The finding that many thyroid infiltrating lymphocytes, early on in the disease process, are in fact recent thymic emigrants does suggest that there is a problem with central tolerance that allows autoreactive T cells to accumulate in the gland where the strength of local immunoregulation could be critical in determining whether disease progresses (297).

Thyrotoxic Graves' disease patients and those with active Hashimoto's thyroiditis have a high proportion of DR+ T cells in their peripheral circulation (291, 298), which indicates the presence of activated T cells. It is unlikely that these cells (> 20% of circulating T cells) are all responsive related to thyroid antigens, so they must include DR+ T cells with TCRs for many other antigens. There is also a marked increase in circulating soluble IL-2 receptors in thyrotoxic Graves' disease, but this appears to be typical of thyrotoxicosis per se, and not specifically Graves' disease (299). Nevertheless, there is no evidence for a generalized ongoing immune hyper-responsiveness in thyrotoxic patients. Perhaps these T cells (for many different specificities) are stimulated by IL-2, but in the absence of the required second signal provided by antigen exposure, do not induce B cell proliferation or cytotoxic responses.

Diminished, non-specific suppressor cell function is also observed in many autoimmune diseases including lupus, and multiple sclerosis and the results in AITD are equally non-specific. The most likely explanation for many “suppressor” phenomena is the reciprocal inhibition of Th1 and Th2 cells by their cytokine products, and powerful evidence shows how important this regulatory mechanism is in exacerbating or inhibiting autoimmune disease, at least in animal models. However regulatory phenomena utilizing cytokines are much more complex, and include both Th17 cells and invariant NKT (iNKT) cells. The latter share receptors with T and NK cells, with the α chain of the T cell receptor being invariant gene segment-encoded, and are notable for releasing cytokines when stimulated by antigen, thus endowing them with regulatory properties which may be

either stimulatory or inhibitory. Recently iNKT cell lines have been identified that can be stimulated with TG to induce EAT (300).

In keeping with the importance of the Th17 subset in inflammatory autoimmune diseases discussed earlier, there is an increased differentiation of circulating Th17 lymphocytes and an enhanced synthesis of Th17 cytokines in AITD, mainly in those patients with Hashimoto thyroiditis (301). Nonetheless a recent study has found an increase in both Th22 and Th17 cells and the levels of plasma IL-22 and IL-17 in patients with Graves' disease; the magnitude of these increases correlated TSH-R antibody levels (302). Circulating platelet-derived microvesicles are significantly raised in AITD patients and these can inhibit the differentiation of Foxp3+ Treg cells and induce differentiation of Th17 cells (302a). Another newly recognized T cell subset involved in the regulation of antibody production, comprising follicular helper T cells, is increased in the circulation of patients with AITD and correlates with autoantibody levels (303).

Many studies have examined T cell subsets in thyroid tissue of patients with active AITD. For example, Margolick et al (304) found increased CD8+ cytotoxic/suppressor cells and also increased CD4+ T helper cells, and a normal Th/Ts ratio. Canonica et al (305) found increased proportions of cytotoxic/suppressor T cells in thyroids of Hashimoto's thyroiditis patients. Infiltrating cytotoxic/suppressor cells in Hashimoto's thyroiditis were found usually to be activated and to express DR antigen, whereas this response was not so obvious in Graves' disease (306). Canonica et al (305) reported an increased proportion of activated T helper/inducer cells in both Graves' disease and Hashimoto's thyroiditis, and increased cells thought to represent cytotoxic T cells in Hashimoto's thyroiditis. Chemokine expression within the thyroid is likely to be an important determinant of this infiltration (307).

Increased CD8+CD11B- cells, presumed to be cytotoxic cells, were found in Graves' disease thyroids (in comparison to PBMC of Graves' disease or normal subjects), whereas "dull" CD8+CD11B+ natural killer cells were diminished (308). Other studies have suggested a reduction in NK cells in Graves' disease and an increase in Hashimoto's thyroiditis. Tezuka et al found decreased NK cells in Graves' disease thyroid tissue, no differences in the NK activity of PBMC between Graves' and normal patients, and that the NK cells in Graves' disease did not kill autologous thyroid epithelial

cells (309). We have already indicated other reports of normal NK and ADCC in Hashimoto's PBMC, and of increased ADCC in Hashimoto's thyroiditis. Most studies that have looked at Graves' disease tissues also indicate an increased proportion of B cells compared to peripheral blood subsets.

Cell cloning has also been used to examine thyroid and peripheral blood lymphocyte subsets. Bagnasco et al (310) found a predominance of cytolytic clones, releasing IFN-γ, in Hashimoto's thyroiditis but not in Graves' disease. Del Prete et al (311) found a high proportion of cytolytic cells with the CD8+ phenotype in clones from thyroid tissue, and felt these results may relate to autoimmune destruction of TEC but the non-specific methods used to derive such cytotoxic T cells raises questions about any pathophysiological relevance.

There is no clear predominance of Th1 or Th2 cytokines in the thyroid of patients with Graves' disease or Hashimoto's thyroiditis (312), although Th1 clones seem to predominate in the retrobulbar tissues in ophthalmopathy (313). It might simplistically be thought that Graves' disease represents a Th2 response, but the fact that some patients end up with hypothyroidism itself indicates the likely presence of a Th1 response too. This is supported by evidence from an animal model of Graves' disease: immune deviation away from a Th1 response, in γ-IFN knockout mice, did not enhance the response to TSH-R cDNA vaccination (314).

One situation in which it is likely that perturbation the cytokine milieu is responsible for the emergence of Graves' disease is during reconstitution of the immune system following lymphopenia induced by alemtuzumab treatment for multiple sclerosis, bone marrow or stem cell transplantation or after highly active antiretroviral therapy for HIV infection (315, 316). In these situations there is an initial increase in the Th1 response flowed by a Th2 response at the time when Graves' disease becomes apparent. Defects in T regulatory cells may also contribute.

A general summary of these data is difficult. The results probably at least indicate there are increased B cells, increased DR+ T cells, increased CD4+DR+ T helper cells, decreased CD8+DR+ T suppressor/cytotoxic cells, and possibly lower NK cells in Graves' disease AITD tissue and in blood than among normal subjects' PBMCs. The

intrathyroidal T cells are a mix of Th1 and Th2. Such studies have been performed primarily on patients with well developed and often treated disease, and do not bear directly on early stages of the disease, or on whether the changes represent primary or secondary phenomena. To date there has been no certain indication that a non-specific or specific suppressor cell defect exists in patients who are genetically predisposed to have AITD, or in most patients who have recovered from the illness, although observations on Treg and other recently defined T cell subsets appear to indicate defects that are likely to be causal.

Anti-Idiotype Antibodies

Whereas anti-idiotypic antibodies are thought to play a physiological role in immunoregulation, there is little evidence for participation in, or abnormality of, this function in AITD. Immunoglobulins from some patients with Graves' disease bind TSH (317). This suggests that anti-idiotypes to TSH antibodies are present and might theoretically function as thyroid stimulating immunoglobulins; or conversely that anti-idiotypes to thyroid stimulating antibody exist and can bind TSH. Either possibility remains to be confirmed. Sikorska (318) demonstrated the presence of antibodies in sera of AITD patients which inhibit binding of TG to monoclonal TG antibodies, and interpreted these as anti-idiotypes. We have looked for anti-TG anti-idiotypes in patients with autoimmune thyroid disease and failed to find them (319). On the other hand, weak anti-idiotypes of the IgM class have been found which bind to TPO antibodies and are present in pooled normal immunoglobulins as well as certain patient sera (320). Although one could postulate that a failure to produce anti-idiotype antibodies could be a feature of AITD, a more likely hypothesis is that anti-idiotypic antibodies are simply rarely produced at a detectable level. Since anti-idiotype antibodies raised in animals will suppress in vitro TG antibody production, the theory that lack of anti-idiotype control is causal in AITD remains attractive, but data to support it are scant.

De Novo Expression Of Class Ii Antigens On Thyroid Cells

De novo expression of HLA-DR on thyroid epithelial cells, from patients with Graves' disease, was first reported by Hanafusa et al (321) and was proposed as the cause of autoimmunity by Bottazzo et al. (322) who suggested that de novo expression of MHC class II molecules on these cells, which are normally negative, allows them to function as APCs. Lymphocyte-produced IFN-γ augments the expression of HLA-DR (also DP

and DQ) on thyroid epithelial cells, and that TNF-α further increases the induction caused by IFN-γ (323, 324). HLA-DR+ TECs definitely can stimulate T cells (325, 326) but this is critically dependent on the requirements of the T cell for a costimulatory signal, as Graves' TECs do not express B7-1 or B7-2 (327, 328). In contrast B7.1 expression on Hashimoto TEC has been recorded, but how this is differentially regulated, compared to Graves' disease, is unknown (329). We have shown that TECs can present antigen to T cell clones which no longer require costimulation through B7, yet not only fail to stimulate B7-dependent T cells but also induce anergy in these cells by at least two mechanisms, one of which is Fas-dependent (330, 331). Perhaps the most conclusive proof that class II expression by thyroid cells cannot induce thyroiditis comes from the creation of transgenic mice expressing such molecules on TECs – such animals have no thyroiditis and have normal thyroid functionm (332). For reasons which remain unclear, thyroid follicular and papillary cancers may express B7.1 and B7.2, and B7.2 expression is associated with an unfavourable prognosis (333).

HLA-DR is also expressed on TECs in multinodular goiter and in many benign and malignant thyroid tumors, and this does not appear to induce thyroid autoimmunity (334). Aberrant DR expression has not been shown to develop before autoimmunity. Normal animal thyroids not expressing class II molecules can become the focus of induced thyroiditis, and then express class II molecules (335). Furthermore, HLA-DR expression on Graves' disease thyroid tissue is lost when tissue is transplanted to nude mice (336). Thus a consensus position is that class II expression could be important, but is a secondary phenomenon in AITD, dependent on the T cell-derived cytokine, γ-IFN, and only allowing TECs to become APCs for T cells which have already received B7-dependent costimulation elsewhere. This could clearly exacerbate AITD once initiated, but teleologically the role of class II expression seems to be as a peripheral tolerance mechanism, allowing the induction of anergy in potentially autoreactive but still naive (ie. B7-dependent) T cells (Fig. 7-13). The recent description of hyperinducibility of HLA class II expression by TECs from Graves' disease suggests that such patients may be genetically predisposed to display a more vigorous local class II response and this would increase the likelihood of disease progression (337). The genes controlling this response are therefore worthwhile candidates for future studies of genetic susceptibility.

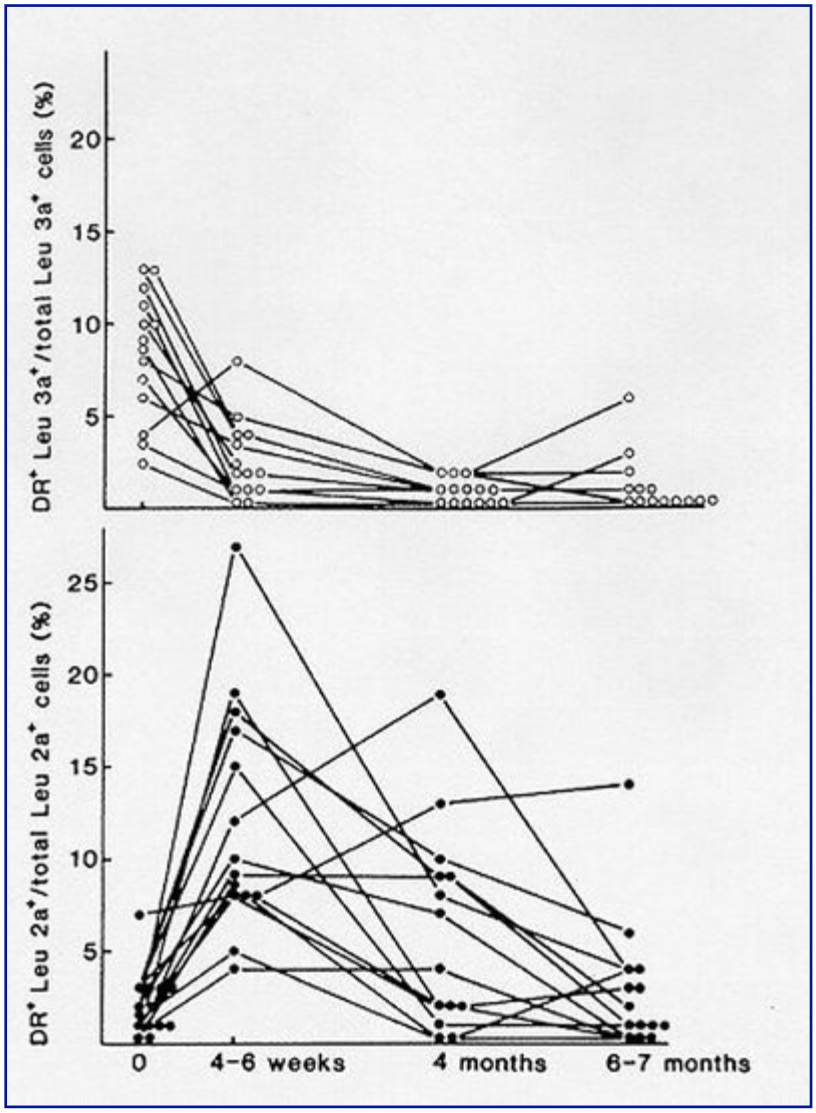

Figure 7-13: Alternative outcomes of MHC class II expression by thyroid follicular cells. Reproduced from Weetman (1997) New Aspects of Thyroid Autoimmunity. Hormone Research 48 (Suppl 4), 5154 with permission.

ENVIRONMENTAL FACTORS

Environmental factors include viral and other infections, discussed above. Strong evidence for an important role for environmental factors is provided by the incomplete concordance seen in the monozygotic twins or other siblings of individuals with AITD. Also, there are temporal changes in disease incidence that can only be the result of environmental influences, such as the rise in Graves' disease in children in Hong Kong, the steady rise in autoimmune thyroid disease in Calabria, Italy, the more than two-fold increase in lymphocytic thyroiditis over 31 years in Austria, and the changes in the rates

of histologically diagnosed Hashimoto's thyroiditis over a 124 year period (338, 339, 340, 341).

Such studies also show that environmental factors may change rapidly, making their ascertainment difficult and challenging. Epidemiological studies have also shown that there is a higher prevalence of thyroid autoimmunity in children raised in environments that have higher prosperity and standards of hygiene (342). This falls in line with the so-called hygiene hypothesis, that is, the idea that early exposure to infections may skew the immune system away from Th2 responses like allergy and also away from autoimmunity. IL-2 administration for treatment of cancer leads to the production of antithyroid antibodies, and hypothyroidism (and possibly a better tumor response) (343). IFN-α administration and other cytokines (91), as well as highly active antiretroviral therapy for HIV infection (344), have a similar effect, although interferon-β1b treatment has no significant adverse effect on AITD (345). However long-term follow up studies have shown that around a quarter of multiple sclerosis patients treated with this latter cytokine may develop autoimmune thyroid disease within the first year of treatment (346). It remains unclear how relevant any lessons from these observations are for AITD pathogenesis, as of course the doses of cytokines and drugs used therapeutically are vast. However, it has been reported that thyrotoxicosis tends to recur following attacks of allergic rhinitis (347). Possibly this is due to a rise in endogenous cytokines and the recent association of raised IgE levels with newly diagnosed Graves' disease indicates that this may be mediated by preferential Th2 activation (348).

Cigarette smoking is associated with Graves' disease, and with ophthalmopathy (reviewed in 349) although it seems to be that smoking is associated with a lower risk of autoimmune hypothyroidism (350). The mechanisms behind these complex changes uncertain and is doubtless more complex than a local irritative effect. Environmental tobacco smoke induces allergic sensitization in mice, associated with increased production of Th2 cytokines, but a reduction in Th1 cytokines, by the respiratory tract (351). It is therefore possible that modulation of cytokines contributes to the worsening of ophthalmopathy with smoking. On the other hand, as noted above, the opposite effect prevails in hypothyroidism and smoking exposure was associated with a lower prevalence of thyroid autoantibodies in a large population survey of over 15000 US citizens (352) and smoking cessation is known to induce a transient rise in AITD (353).

To explain this, investigations have been undertaken on anatabine, an alkaloid found in tobacco; this compound ameliorates EAT and reduces TG antibody levels in human subjects with Hashimoto's thyroiditis (354).

More general environmental pollutants have not been thoroughly explored for their possible effects (although there is some evidence from older experiments that methylcholanthracene can induce thyroiditis) but a recent study has demonstrated that polychlorinated biphenyls can induce the formation of TPO antibodies and lymphocytic thyroiditis in rats (355). A cross-sectional survey in Brazil has fond that Hashimoto's thyroiditis and thyroid autoantibodies are more frequent in individuals living near to a petrochemical complex than in controls (356). In addition pesticide use, especially of the fungicides benomyl and maneb/mancozeb, has been associated with an increased odds of developing thyroid dysfunction although the mechanism of action is unclear (357). it clear that this aspect of the environment warrants further study in human thyroid disease.

The role of dietary iodine is clearly established in animal models of AITD and circumstantial evidence exists for a similar role in man (358-360). The response is complex and recently it has been shown that iodide may exacerbate thyroiditis in NOD mice but not affect the production of TSH-R antibodies in the same strain (361). Such findings are intriguing as they raise the possibility that the thyroiditis which accompanies Graves' disease may not be due to the immune response to the TSH-R. Iodine may affect several aspects of the autoimmune response, as detailed in the section on experimental thyroiditis above. In addition, iodide stimulates thyroid follicular cells to produce the chemokines CCL2, CXCL8, and CXCL14 (362). These observations suggest that iodide at high concentrations could induce AITD through chemokine upregulation thus attracting lymphocytes into thyroid gland.

Dietary selenium has also been proposed as a contributor. A recent large epidemiological survey of two counties of Shaanxi Province, China, one with adequate and the other with low selenium intake, showed that higher serum selenium was associated with lower odds ratio of autoimmune thyroiditis (0.47) and hypothyroidism (0.75) (362a). However a recent Cochrane Systematic Review of trials of selenium supplementation has shown no clear beneficial clinical effect in HT, although TPO

autoantibody levels do fall over a 3 month period of supplementation (363). Vitamin D may be important in autoimmunity and many other disorders, as it is now recognized that individuals living in northerly latitudes may have suboptimal levels based on a fresh understanding of what normal levels of this vitamin should be. A significant inverse correlation has been observed between 25(OH)D levels and TPO antibody levels in Indian subjects, although the overall impact of this effect in terms of causality was low (364), and another prospective study has found no evidence for a role of vitamin D (250). A more recent large scale survey has found that for every 5 nmol/L increase in serum 25(OH)D there was an associated 1.5 to 1.6-fold reduction in the risk for developing Graves' disease, Hashimoto's thyroiditis or postpartum thyroiditis, but vitamin D was not strong associated with the level of thyroid autoantibodies (364a). These new results are also supported by a meta-analysis of all studies prior to this, indicating that low vitamin D levels, as well as frank deficiency, are indeed risk factors for AITD (364b).

A variety of lifestyle factors that are difficult to investigate may also be involved. It is otherwise difficult to account for the increase in AITD seen in same-sex marriages (365). Stress is likely to be important in the etiology of Graves' disease, although studies to date have had to rely on retrospective measures of this (reviewed in 366). Moreover stress does not appear to be asociated with the development of TPO antibodies in euthyroid women (369). Presumably stress acts on the immune system via pertubations in the neuroendocrine network, including alterations in glucocorticoids, but the complex interaction between the nervous, endocrine and immune systems includes the actions of neurotransmitters, CRF, leptin and melanocyte stimulating hormone as well and so unravelling the pathways whereby stress may alter the course of autoimmunity is difficult in the extreme (370). Indirect support for such a mechanism, mediated through norepinephrine, comes from experiments showing dramatic enhancement of delayed-type hypersensitivity by acute stress, the result of sympathetic nervous system activation on the migration of dendritic cells and subsequent enhanced T cell stimulation (371). Moderate consumption of alcohol appears to have a protective effect with regard to AIITD (371, 372). Given the diversity of these environmental factors, presumably operating on different genetic backgrounds, it will be difficult (if not impossible with current tools) to establish the relative importance of each in AITD.

NORMAL AUTOIMMUNITY

"Normal" people express antithyroid immunity, as previously described, and this must be important in understanding the overall mechanism of AITD. Antibodies to TG and TPO are present in both Graves' disease and Hashimoto's thyroiditis up to 7 years prior to diagnosis, increasing over time in the former and consistently elevated in the alter (374). Many people with low levels of antibodies but without clinical disease can be shown to have lymphocyte infiltrates in the thyroid at autopsy. B cells from normal individuals can be induced to secrete anti-TG antibody in vitro. These observations clearly show that incomplete deletion of clonal self-reactive T cells is indeed the normal (and indeed perhaps necessary) circumstance, and provide strong support for the idea that disordered control of this low level immunity may be important in the etiology of AITD.

EFFECT OF ANTITHYROID DRUGS ON THE IMMUNE RESPONSE

Antithyroid drugs are used in Graves' disease to decrease production of thyroid hormone, and also lead to diminution in TSI and other antibody levels. Clinical studies show that antithyroid drug administration also leads to a diminution in antibody production in thyroxine replaced Hashimoto's thyroiditis patients (375), proving that their effect is not simply due to control of hyperthyroidism in Graves' disease. Somewhat surprisingly (376), administration of $KClO_4$ to patients with Graves' disease leads to diminished serum antibodies, suggesting that the effect of treatment is not specific for thionamide drugs, but could be mimicked by this compound. Antithyroid drugs inhibit macrophage function, interfering with oxygen metabolite production (377).

Following antithyroid drug treatment of active Graves' disease, there is a prompt short-term increase of DR+CD8+ T cells in the bloodstream as described above. Antithyroid drugs inhibit the production of cytokines, reactive oxygen metabolites and prostaglandin E_2 by TECs and the reduction in these inflammatory mediators may explain the site-specificity of the immunomodulation produced by antithyroid drugs (125). Another pathway for an immunomodulatory action of these drugs is via the upregulation of Fas ligand expression, which may then attenuate the autoimmune response of Fas-expressing T cells (378). Only approximately 50% of patients enter remission after treatment with antithyroid drugs, a fact which must be accommodated in any hypothesis concerning an immunomodulatory action of these agents. Those patients with Graves' disease who have the highest IgE and IL-13 levels in the circulation are the most likely to

relapse (379). In turn, this suggests that antithyroid drugs only effect remission in individuals who do not have a strong Th2 response; those with the strongest such responses seem unlikely to be affected by the relatively weak action of such drugs.

AITD AS A CONSEQUENCE OF A MULTIFACTORAL PROCESS (TABLE 4) (FIG. 7-14)

TABLE 4

DEVELOPMENT OF AUTOIMMUNE THYROID DISEASE

Stage 1 –Basal State
Normal exposure to antigen such as TG and normal low levels of antibody response Inherited susceptibility via HLA-DR, DQ, or other genes
Stage 2a –Initial Thyroid Damage and Low Level Immune Response
Viral or other damage with release of normal or altered TG, TPO, or TSH-R Increased antibody levels in genetically susceptible host with high efficiency HLA-DR, DQ, TCR molecules Infection induced elevation of IL-2 or IFN-γIL-2 stimulation of antigen specific or nonspecific ThIFN-γ stimulation of DR expression and NK activation Glucocorticoid-induced alterations in lymphocyte function during stress
Stage 2b –Spontaneous Regression of Immune Response
Diminished antigen exposure Anti-idiotype feedback Antigen specific Ts induction
Stage 3 –Antigen Driven Thyroid Cell Damage (or Stimulation)
Complement dependent antibody mediated cytotoxicity Fc receptor+ cell ADCC by T, NK, or macrophage cells NK cell attack Direct CD4+ or CD8+ T cell cytotoxicity Antibody-mediated thyroid cell stimulation
Stage 4 –Secondary Disease Augmenting Factors
Thyroid cell DR, DQ expression –APC function Other molecules (cytokines, CD40, adhesion molecules) expressed by thyroid cell Immune complex binding and removal of Ts
Stage 5 –Antigen Independent Disease Progression
Recruitment of nonspecific Th or autoreactive Th Autoreactive Th bind DR+ TEC or B cells IL-2 activation of bystander Th
Stage 6 –Clonal Expansion with Development of Associated Diseases
Antigen release and new Th and B recruitment Cross reactivity with orbital antigen IL-2, IFN-γ augmentation of normal immune response to intrinsic factor, acetylcholine receptor, DNA, melanocytes, hair follicles, etc.

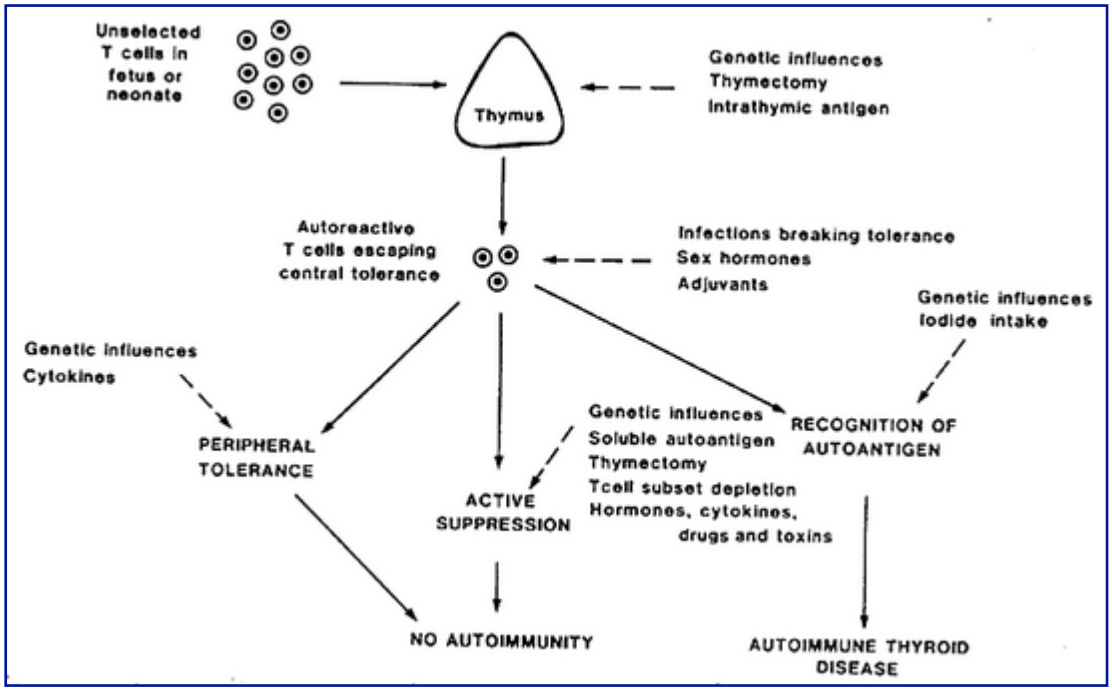

Figure 7-14: Theoretical Sequence of Development of AITD.

Thus one is led to the uncomfortable position that AITD is probably not caused by a single factor, but rather due to very many factors which interact. In terms of genetic and environmental factors, as well as factors that may be termed existential (such as age, being female and parity), these may all have to coincide in a favorable way for AITD to occur, in keeping with the Swiss-cheese model for accidents (Fig 7-15). We have divided the roles of these potential disease activity factors into a series of stages, emphasizing the predisposing events, antigen driven responses, and then the secondary and nonspecific amplification which ensues.

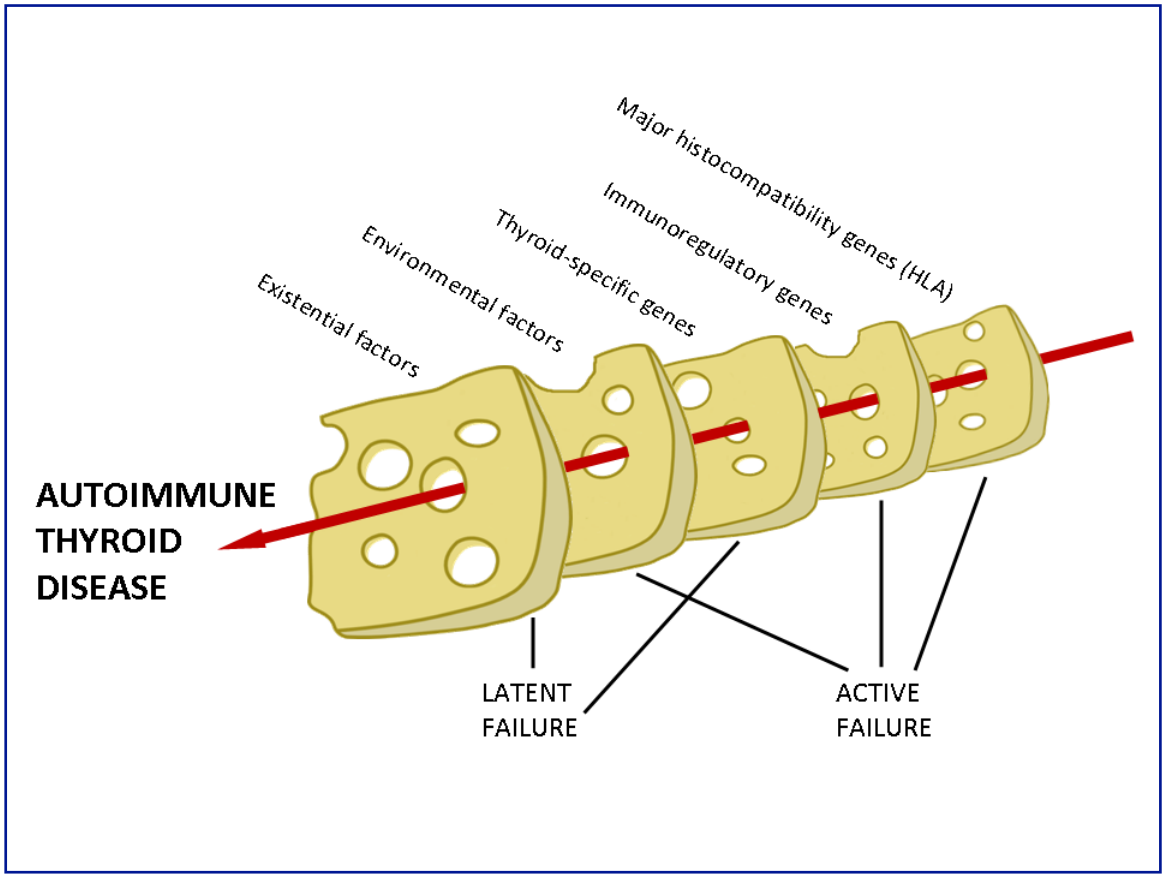

Figure 7-15: A Swiss cheese model for the causation of autoimmune thyroid disease, showing the effect of cumulative environmental, genetic and existential weaknesses lining up to allow AITD to occur, like the holes in the slices of cheese. In reality each of the slices depicted is composed of many individual components. The Swiss cheese model for accident causation, for instance an airplane crashing, incorporates active failures (e.g. pilot error) and latent failures (e.g. maintenance deficiency). Some factors contributing to the initiation of AITD are latent (e.g. ageing, growing up in a hygienic environment) and others are active (e.g. possession of an HLA allele which permits presentation of a thyroid autoantigen). Reproduced with permission from Weetman AP, Europ Thy J 2013 in press.

Stage 1 -- In the basal state, Stage 1, immune reactivity to autologous antigen occurs as a normal process. This probably exists at a physiologically insignificant level, since not all T or B cells reacting with TSH-R, TPO or TG are clonally deleted, and Ag is normally present in the circulation. If assays become sensitive enough, we probably will find some level of antibodies to TSH-R, TPO and TG present in most or even all healthy persons, increasing in prevalence and concentration with age, and especially in women, since being female somehow augments antithyroid immunity many-fold. Patients who have inherited certain susceptibility genes will be especially prone to develop AITD

because their T and B cell repertoire includes cells recognizing self-antigen, or their immunocytes are especially good at collecting, presenting, and responding to antigen.

Stage 2 -- Possibly viral infection, or other causes of cell damage, or cross-reacting antibodies present after *Yersinia* (or other) infection, leads to release of increased amounts of (possibly modified) thyroid antigens which, in genetically prone individuals, leads to an increased but still a low level immune response. Nonspecific production of TNF-α and IFN-γ, in response to any infection or immune response, may augment MHC class expression on TECs, allow these cells to function as APCs, and increase production of the already established, normally occurring low levels of antibodies. The process may be affected by stress, although the mechanism remains quite uncertain. The process may go on over years, and wax and wane, as it has been shown that thyroiditis can be clinically apparent and then disappear. Factors involved in temporary or permanent suppression of the autoimmune response may include diminished thyroidal release of antigen, peripheral tolerance induction by DR-positive TECs which cannot provide a costimulatory signal, B cell anti-idiotype feedback, or the induction of T cells with a regulatory function, including those engendered by the mutual regulation of Th1 and Th2 subsets. In some individuals, thyroid cells may be less able to express DR, or may secrete TGF-β and suppress immune responses. Glucocorticoid administration and other immunosuppressives can also temporarily prevent the expression of nascent autoimmunity.

Stage 3 -- If suppressive factors do not control the developing immune response, the disease progresses to a new intensity, now driven by specific antigens, inducing cell hyperfunction (TSI), or hypofunction (TSH blocking or NIS antibodies), or cell destruction. Direct T cell cytolysis and apoptosis, ADCC, and K or NK cell attack play an important role at this stage, and now the disease becomes clinically evident.

Stage 4 -- As the disease develops, a variety of secondary factors come into play, and augments antithyroid immunoreactivity. Any stimulus which causes increased DR expression on thyroid cells, such as T cell release of IFN-γ, combined with increased TSH stimulation, may allow TECs to function as APCs. Although perhaps poor in this function, they are large in number and localized in one area. The TECs may also participate in the autoimmune process by several other pathways, including the expression of adhesion molecules, Fas, Fas ligand, CD40 and complement regulatory proteins, and the production of a number of inflammatory mediators such as cytokines, reactive oxygen metabolites, nitric oxide and prostaglandins. These events are, like

class II expression, dependent on cytokines and other signals generated by the intrathyroidal lymphocytic infiltrate. Some patients may inherit diminished T regulatory cell function. The ongoing immune reaction itself, may lead to nonspecific suppressor dysfunction, further augmenting immunoreactivity.

Stage 5 -- T cell derived cytokines may non-specifically induce bystander antigen specific T and B cells to be activated and produce antibody. Autoreactive cells will now accumulate in thyroid tissue because of the many strongly DR+ positive lymphocytes and TECs, and augment the developing response by lymphokine secretion or cytolysis, in a manner independent of thyroid antigens. At this stage in the disease, non-specific autoreactive immune processes may dominate a disease process which no longer depends upon antigen for its continuation.

Stage 6 -- As the concentration of activated T and B cells builds in thyroid tissue, and autoreactive and antigen nonspecific T cells become progressively involved, cell destruction may lead to release of new antigens. Cross-reacting epitopes, and nonspecific stimulation of T cells in genetically prone individuals, may cause the addition of new immunologic syndromes (exophthalmos, pretibial myxedema, atrophic gastritis) typical of older patients with more long standing and florid disease.

THYROIDITIS, MYXEDEMA, AND GRAVES' DISEASE AS AUTOIMMUNE DISEASES

HASHIMOTO'S THYROIDITIS (FIG. 7-16)

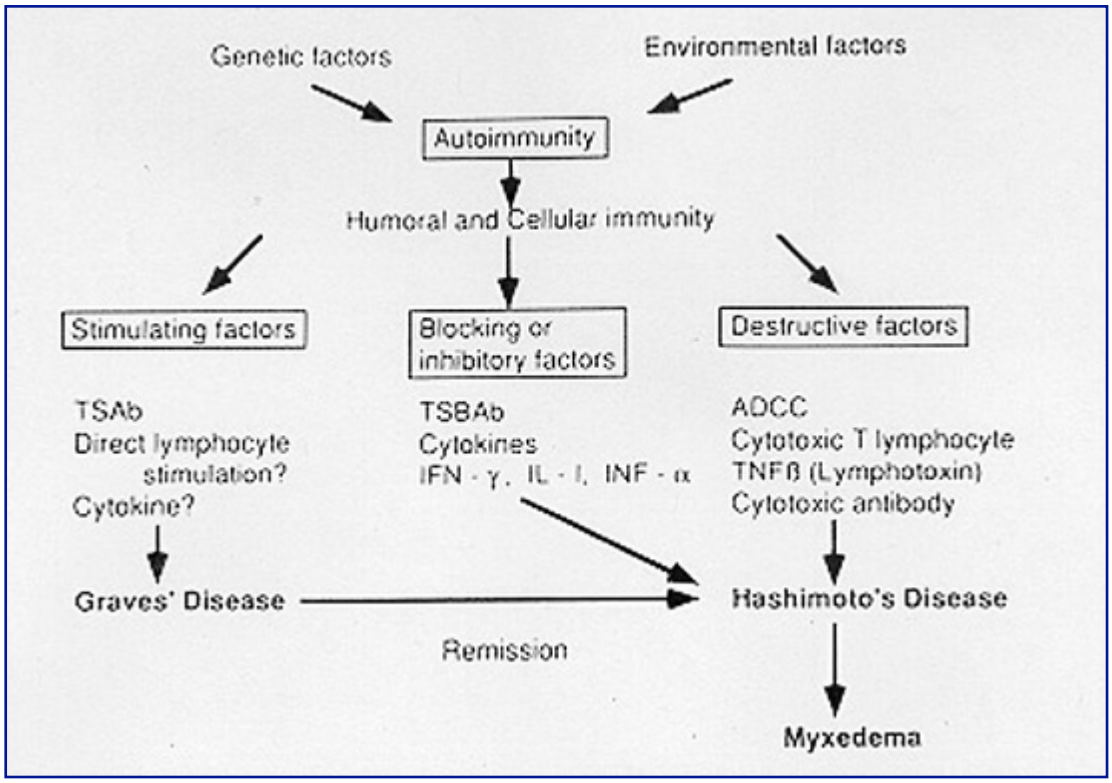

Figure 7-16: Balance of Immune Reactions Favoring Graves' or Hashimoto's Disease.

How well do the changes of Hashimoto's thyroiditis fulfill the criteria of an immunologic reaction? Neither the presence of autoantibodies in the serum of patients with Hashimoto's thyroiditis nor the demonstration in vitro of cytotoxicity of the serum constitutes definitive evidence that autoimmunity is the cause of the disease. Rarely, if ever, is there a well-defined initial immunizing event, and accordingly a shortened latent period after a secondary stimulus has not been observed. Further, experimental passive transfer of the immune state in normal recipients has not yet been attempted and has failed when human sera have been transfused into monkeys and other animals. This experiment is conducted by nature during pregnancy, since maternal antibodies cross the placenta. Transplacental passage of thyroid stimulating antibodies can produce neonatal thyrotoxicosis, and TSH blocking antibodies can produce transient neonatal hypothyroidism. Passage of TG antibody or TPO antibody has no detectable cytotoxic effect.

Assays for T cell reactivity in man, supplemented by data from animal models, provide compelling evidence of the autoimmune basis for Hashimoto's thyroiditis, but this does

not exclude an amplifying role for TG and TPO antibodies via ADCC, and, for TPO antibodies, via complement fixation. It may well be that T cell-mediated damage is required initially for all of these antibody-mediated events to take place, as this could be necessary for such access. Another striking feature of Hashimoto's thyroiditis is the development of Hürthle cells, with granular eosinophilic cytoplasm. This appears to be the result of a chronic inflammatory milieu, resulting in overexpression of immunoproteasomes (3680

The evidence is now overwhelming that an immune reaction mediated by T lymphocytes is involved in the development of experimental thyroiditis in animals and several mechanisms may operate singly or together in man to injure TECs. Lymphocytes presensitized to antigens of the thyroid are present in the circulation of most if not all patients and are believed to localize to the thyroid itself. Since T cell mediated immunity is frequently lethal to cells, it is logical to assume that the T cell mediated immune response in thyroiditis could cause first a goiter, with lymphocyte infiltration and compensatory thyroid cell hyperplasia, and then gradual cell death and gland atrophy. The circulating antibodies may also be a functional part of this reaction. We can accept the idea that T cell-mediated immunity is the major pathogenic factor in thyroiditis.

Idiopathic Myxedema

Even before the present era of immunologic study, the basic unity of Hashimoto's thyroiditis and myxedema was realized. To quote from Crile, writing in 1954 (381): "Struma lymphomatosa is responsible not only for large lymphadenoid goiters, but also for fibrosis and atrophy of the thyroid. The clinical spectrum of struma lymphomatosa extends from spontaneous myxedema with no palpable thyroid tissue to a rapidly growing goiter associated with no clinical evidence of thyroid failure."

Hubble (382) also drew attention to the occurrence of syndromes intermediate between those of myxedema and Hashimoto's thyroiditis, in which a small, firm thyroid gland can be felt on careful palpation. The histologic studies of Bastenie (383) and Douglass and Jacobson (384) revealed a close similarity in appearance of the thyroid remnant in myxedema and the Hashimoto gland. The immunologic studies of Owen and Smart (385), and the experience in most thyroid laboratories, indicate a similar incidence and titer of antibodies in myxedema and Hashimoto's thyroiditis. The familial association of

myxedema and thyroiditis was described earlier and so far no clear genetic susceptibility difference has been reported in the two diseases. Attempts to ascribe atrophy of the thyroid gland in myxedema to particular antibodies, such as those inhibiting growth or TSH (386), or which mediate ADCC have not been confirmed by other studies (reviewed in 234).

Thus, idiopathic myxedema is the end result of Hashimoto's thyroiditis, in which the phase of thyroid enlargement was minimal or was overlooked. We may assume that in idiopathic myxedema the cell-destructive T cell-mediated immune response is an important pathogenic factor in the illness, and that cytotoxic antibodies and TSH blocking antibodies contribute to the development of hypothyroidism, but perhaps in only a proportion.

Graves' Disease

Graves' disease is associated with a similar type of thyroid autoimmunity, since most hyperthyroid patients have circulating TG and TPO antibodies. High antibody levels are found in a small group of hyperthyroid patients and histologic examination of their glands show changes of both cell stimulation and focal thyroiditis (387). Some patients with clinical Graves' disease have tissue changes in the thyroid that are typical of thyroiditis (388). This type of patient with Graves' disease most often becomes hypothyroid after operation (389), or after ^{131}I therapy (390). It is also well known that some patients fluctuate from hyper- to hypothyroidism over a period of months and others behave in the converse fashion, and of course the familial association of Graves' disease with autoimmune hypothyroidism is well established.

The humoral response in Graves' disease leads to production of TG and microsomal TPO antibodies, but most importantly, as described in Chapter 10, B cells produce TSI, TBII and, in some, TSH blocking antibodies (35). TSI stimulate thyroid release of hormone primarily via cyclic AMP, although other pathways may also be activated by TSI in a proportion of patients. TSI are true cell stimulators and can even induce experimental goiter. However, the clinical picture in Graves' disease will be a balance between the stimulation produced by TSI and the opposing effects of any TSH blocking antibodies which may be present.

Evidence also supports a role for T cell mediated immunity to thyroid antigens in Graves' disease, and against orbital antigens in patients with associated ophthalmopathy. We speculate that Graves' disease may be a condition representing a semistable balance between stimulatory, blocking, and cell-lethal immune responses. Thus, TSI could cause thyroid hyperplasia and produce hyperthyroidism. Other antibodies might block the action of TSI either directly or, as in the case of NIS antibodies, indirectly, and prevent this hyperplastic response in some patients. Cytotoxic T cells will also gradually destroy cells and produce hypothyroidism either spontaneously or after therapy. It must be admitted that the etiology of ophthalmopathy still remains rather obscure, although the key role of cytokines in pathogenesis, causing fibroblast activation, seems firmly established.

RELATION TO OTHER DISEASES

Thyroid Cancer

Thyroid antibodies are present in increased prevalence (up to 32%) in patients with carcinoma of the thyroid, and usually are at low titer. Histologic evidence of thyroiditis is found in up to 26% of tumors. Histologic changes range from diffuse thyroiditis to focal collections of lymphocytes around the tumor or reactive lymphoid hyperplasia. Possibly release of antigens leads to increased thyroid autoimmunity. Some evidence suggests that patients who have thyroid antibodies have a better prognosis than antibody negative patients. Lymphoma and lymphosarcoma of the thyroid are associated with Hashimoto's thyroiditis (391), and there is compelling evidence that thyroiditis precedes development of the tumor. An increased frequency of carcinoma, especially of the papillary type, has been suggested in Hashimoto's thyroiditis but this relationship remains to be fully established (392).

ADOLESCENT GOITER Enlargement of the thyroid during the second decade, accompanied by normal results of function tests, usually is labeled adolescent goiter. If the examination includes needle biopsy, an appreciable incidence of Hashimoto's thyroiditis is found (393) - up to 65%. Eighty percent of these children with thyroiditis have a positive thyroid antibody test result. The parents of many of them have either overt thyroid disease or circulating thyroid antibodies. Hyperplasia, in response to an increased demand for thyroid hormone, and colloid involution are at the root of some of

these goiters, but Hashimoto's thyroiditis is the most frequent explanation of adolescent goiter in iodine sufficient areas.

Transient Thyrotoxicosis, Painless Thyroiditis, Postpartum Thyroiditis, And Related Syndromes

These illnesses, all similar, involve an acute exacerbation of thyroid autoimmunity occurring independent of, or following pregnancy in women, and in men. They are characterized by sequential inflammation-induced T4 and TG release, transient hypothyroidism, usually return to euthyroidism, and are discussed in Chapters 8 and 14. They are considered subtypes of Hashimoto's thyroiditis, and in the postpartum period, appear to result from release of the immunoregulatory effects of normal pregnancy (211).

Focal Thyroiditis

Focal lymphocytic infiltrations are frequently seen in Graves' disease, nodular goiter, nontoxic or colloid goiter, and thyroid carcinoma. The significance of these changes is not precisely known, but they correlate with positive antibody titers and may represent variations that do not differ qualitatively from thyroiditis.

Riedel's Thyroiditis And Ig4 Disease

This rare thyroid disorder is associated with both Hashimoto's thyroiditis and Graves' disease and in addition many patients have evidence of fibrosis elsewhere, such as the retroperitoneum, lung, biliary tract and orbit. In one large series, 12 of 15 patients had positive TPO antibodies (389) It is now recognized that some of these patients with multifocal fibrosclerosis have IgG4-related sclerosing disease in which lymphocytes and IgG4-positive plasma cells infiltrate the affected tissues, especially the lacrimal gland, biliary tree and pancreas but the exact relationship of this entity to Riedels' thyroiditis is unclear at present. There is a predominance of IgA rather than IgG4 in Riedel's thyroiditis, but the effects of steroids may obscure the few analyses that have been undertaken (395, 396). However it does appear that Hashimoto's thyroiditis can be divided into two discrete entities based on whether IgG4 plasma cells predominate in the thyroid infiltrate: in those individuals with IgG4 predominance, there is a greater male frequency, more rapid progression to hypothyroidism and more intense gland fibrosis (397). These studies have been predominantly undertaken in Japanese subjects. In a

recent survey from Europe, only 12.5% of Hashimoto thyroid glands showed this feature: there was an association with younger age and male sex, and fibrosis was identified in 96 % of the IgG4-related cases but also in 18 % of the non-IgG4-related cases (397a). The authors note that unlike other forms of IgG4-related disease, the fibrosis is not accompanied by intense eosinophilia or obliterative phlebitis. IgG4 subclass thyroid autoantibodies display heritability in individuals with high levels of both TPO and TG antibodies; it is possible that more sophisticated analysis of autoantibody subtypes could lead to new methods to predict the natural history of disease (398).

Other Problems

An association between the occurrence of maternal antithyroid antibodies and recurrent abortion has been reported (399) and although this association has been disputed, a recent study showed clear evidence that the presence of TPO antibodies was associated with a 3-4-fold increased risk of miscarriage in women having in vitro fertilization (400). There is also an association between breast cancer and thyroid autoimmunity (401, 402) and between depression in middle-aged women and the presence of TPO antibodies (403). The nature of these associations is unclear; does thyroid autoimmunity predispose to such adverse events, or is the presence of thyroid autoimmunity simply a marker of a non-specific disturbance in the immune system due to whatever has caused miscarriage, cancer or depression? Having thyroid autoimmunity is not all bad news. Community-dwelling older women who have TG and TPO antibodies are less likely to be frail than those who are antibody-negative (404). Again the reason for this unexpected finding is unclear but it certainly warrants follow-up.

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