IMMUNOLOGICAL ASPECTS OF GRAVES' DISEASE

Immunologische aspecten van
de ziekte van Graves

PROEFSCHRIFT

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Bastiaan Marcelles van Ouwerkerk
geboren te Hilversum

PROMOTIECOMMISSIE

PROMOTOR	: Prof. Dr. G. Hennemann
PROMOTOR	: Prof. Dr. R. Benner
OVERIGE LEDEN	: Prof. Dr. J.C. Birkenhäger Prof. Dr. J. Jeekel

Voor Charlotte

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ABBREVIATIONS

AT	autoimmune thyroiditis
cAMP	cyclic adenosine 3',5'-monophosphate
DNA	deoxyribonucleic acid
ECFMG	educational council for foreign medical graduates
ELISA	enzyme linked immunosorbent assay
Fab	antibody binding region of immunoglobulin
Fc	constant region of immunoglobulin
FACS	fluorescence activated cell sorter
FSH	follicle stimulating hormone
FT_4I	free thyroxine index
Gm	immunoglobulin gene
GD	Graves' disease
HCG	human chorion gonadotropin
HLA	human leucocyte antigen
HT	Hashimoto's thyroiditis
Ig	immunoglobulin
IL	interleukin
LATS	long acting thyroid stimulator
LH	luteinizing hormone
M-Ab	microsomal antibody
MHC	major histocompatibility complex
MIF	migration inhibition factor
MMI	methimazole
NK	natural killer
OGD	ophthalmic Graves' disease
PM	primary myxoedema
PTU	6-propyl 2-thiouracil
RES	reticulo endothelial system
SD	standard deviation
SEM	standard error of the mean
SLE	systemic lupus erythematosus
T_3	3,3',5-triiodothyronine
T_4	3,3',5,5'-tetraiodothyronine (thyroxine)
^{99m}Tc	technetium
Tg-Ab	thyroglobulin antibody
Th	helper T cell
Ts	suppressor T cell
TBII	thyrotropin binding inhibiting immunoglobulin
TDA	thyrotropin displacing activity
TGI	thyroid growth immunoglobulin
TRA	thyrotropin receptor antibody
TRH	thyrotropin releasing hormone
TSAb	thyroid stimulating antibody
TSH	thyroid stimulating hormone (thyrotropin)
TSI	thyroid stimulating immunoglobulin
VEP	visual evoked potential

CHAPTER I

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INTRODUCTION AND SCOPE OF THE STUDY

1. General considerations

Graves' disease is a disease of the thyroid gland, often occurring in young adults (15-40 years) and is four times more frequent in women than in men.

The thyroid gland is a small organ, located in the lower portion of the neck. It produces thyroid hormones (thyroxine = T4 and 3,3',5-triiodothyronine = T3), which exert metabolic functions in the body.

Hyperfunction of the thyroid leads to psychological and physical restlessness. The patients are stressed, talk unceasingly and are overactive. They suffer from palpitations, increased perspiration, heat intolerance, loss of weight despite increased appetite and sometimes diarrhoea. Ocular complaints include burning sensations, tears or pain in the orbit, and visual disturbances. Part of the patients develop goiter and/or globus sensations. Frequently they feel tired, dyspnoic, and have proximal muscle weakness. On physical examination a warm moist skin, tachycardia, and tremor of the hands are observed. A diffuse goiter and typical ocular signs and sometimes pretibial myxoedema may occur. Rarely cervical lymph node enlargement and splenomegaly are associated with Graves' disease. The ophthalmopathy may precede or even develop after the treatment of hyperthyroidism and sometimes occurs without hyperthyroidism. Pretibial myxoedema nearly always occurs in association with ophthalmopathy.

Graves' disease is considered to be an autoimmune disease. In the following sections autoimmune diseases will be discussed in general and subsequently the pathophysiology of autoimmune diseases of the thyroid as a whole and of Graves' disease in particular will be described.

2. General aspects of autoimmune disease.

Human autoimmune diseases can be regarded as a spectrum with organ-specific disorders such as Hashimoto's thyroiditis at one pole, and non-organ-specific disorders like systemic lupus erythematosus (SLE) at the other (Roitt, 1984) (Table I). In organ specific disorders an immune response is directed against an antigen within that particular organ often leading to dysfunction of the organ. In non-organ-specific disorders autoantibodies directed against antigens, which are common to most cells in the body, may cause lesions throughout the body. There may be overlap within diseases and familial aggregation at each pole of the spectrum.

TABLE I: SPECTRUM OF AUTOIMMUNE DISEASE

ORGAN-SPECIFIC ⟷				NON ORGAN SPECIFIC
Hashimoto's thyroiditis	Myasthenia gravis	Autoimmune hemolytic anemia	Primary biliary cirrhosis	Systemic lupus erythematosus
Primary myxoedema	Juvenile diabetes	Idiopathic thrombocytopenic purpura	Active chronic hepatitis	Discoid LE
Graves' disease	Goodpasture syndrome	Idiopathic leucopenia	Cryptogenic cirrhosis (some cases)	Dermatomyositis
Pernicious anemia	Pemphigus vulgaris		Ulcerative colitis	Scleroderma
Autoimmune atrophic gastritis	Pemphigoid		Sjögren syndrome	Rheumatoid arthritis
Premature menopause (few cases)	Sympathetic ophthalmia			
Male infertility (few cases)	Phacogenic uveitis			
Adrenal insufficiency	Multiple sclerosis ?			
Idiopathic Hypoparathyroidism				

In 1900 Ehrlich postulated the idea, that normally the immune system does not react or produce antibodies against the body's own components (horror autotoxicus). This phenomenon was originally described as immunologic tolerance to self components. When this tolerance to self terminates and the immune system starts to react against self components, autoimmunity develops. As a result, antibodies or cells react with self constituents, thereby causing disease. In 1944 Burnet formulated the theory of immunological self-tolerance acquired during the prenatal period. This self-tolerance might be changed or broken down by many causes such as immunologic, genetic, virologic, hormonal and other factors, acting singly or synchronously with the others. However, not all autoimmune responses are harmful, because certain forms of autoimmune responses are essential for the diversification and functioning of the immune system. For instance the recognition of cell surface antigens encoded by the major histocompatibility complex (MHC) and of idiotypes of antibodies are characteristics of a normally responsive immune system. The formation of autoantibodies not always leads to disease. The development of autoimmune disease depends on many factors, such as balance in cellular interactions and idiotype-network, which is described in the following section. The presence of autoantibodies can be the cause of autoimmune damage, but sometimes is the result of tissue damage. An example of this latter phenomenon is the transient appearance of antibodies to thyroidal microsomes in virus-induced subacute thyroiditis. Thus, autoantibodies may also represent epiphenomena of immune reactivity.

Some criteria for the establishment of an autoimmune disease are listed in Table II.

Table II Criteria for establishing autoimmune disease

1. Demonstration of circulating autoantibodies, cell bound antibodies or cell-mediated phenomena in the serum of patients with the disease.
2. Characterisation (and isolation) of the antigen against which antibodies or cells are directed.
3. Production of antibodies and/or cell mediated immunity in animals by using the same antigen.
4. Actively sensitized animals develop pathological changes in the corresponding tissue similar to those in the human disease.

2.1. Theories of the underlying causes of autoimmunity.

Many theories and mechanisms have been proposed for the generation of autoimmune responses (summarized in Table III, from Theofilopoulos, 1982).

Table III Possible factors contributing to the etiology and/or pathogenesis of autoimmune diseases.

Release of sequestered antigens
Diminished suppressor T cell function
Enhanced helper T cell activity (T cell bypass)
Thymic defects
Presence of abnormal clones, defects in tolerance induction
Polyclonal B cell activation
Refractoriness of B cells to suppressor messages
Defects in antigen-presenting cells
Stem cell defects
Defects in the idiotype-anti-idiotype network
Abnormal genes: immune response genes, immunoglobulin genes
Viral factors
Hormonal factors

There are several instances in which such autoimmune responses are not initiated by normal self-antigens, but by exogenous antigens that crossreact with self-antigens or by (chemically or virally) modified self-antigens. These theories will be described briefly.

a. Release of sequestered antigens.

If an antigen is sequestered in an organ, no immunologic tolerance at the T or B cell level can be established. When later in life tissue damage causes liberation of these antigens into the circulation, contact with T and B cells may lead to autoantibody production. Examples are formation of autoantibodies against sperm after vasectomy, against the crystalline lens after eye injury, against heart muscle antigens after myocardial infarction etc. In most of these instances the autoimmune response is transient. Once antigen has been liberated by nonspecific injury, persistent antigen presentation may occur due to autoantibody mediated cell damage. This may lead to progressive autoimmune disease.

b. Defects in induction and maintenance of tolerance.

Although autoreactive lymphocytes are regularly eliminated, inactivated or suppressed, it can be envisaged that this process might be inadequate at a specific level. Such an "escaped" clone of autoreactive lymphocytes might cause autoimmunity.

c. Enhanced helper T cell activity.

For most immune responses to antigens, collaboration between helper T cells and B cells is required. Unresponsiveness to self antigens may be maintained at the helper T cell level. However, when helper T cells are activated by exogenous antigens, virally or chemically modified self-antigens that cross-react with self, self-directed cellular and humoral immunity can be envisaged (Weigle, 1980).

d. Diminished suppressor T cell function.

Alterations in the function and number of immunoregulatory T cells are common finding in many autoimmune diseases. These abnormalities gain clinical importance from the observation that in multiple sclerosis fluctuations in suppressor T cells parallel exacerbations and remissions (Huddlestone and Oldstone, 1979). These observations may be relevant, as immune responses are normally down-regulated by complex interactions between helper and suppressor T lymphocytes and their soluble products. B cells with the potential to produce autoantibodies are held in a dormant state by action of suppressor cells, a lack of "help" from appropriate helper cells, or both. When number or function of an autoantigen-specific clone of suppressor T lymphocytes decreases, the dormant B cells may be activated and autoantibodies may appear.

e. Another possibility could be the triggering of autoreactive B lymphocytes through their receptor for a particular polyclonal activator (such as bacterial lipopolysaccharides, dextran sulphate and Epstein Barr virus). The production of autoantibodies by B cells might also be caused by helper T cells recognizing a receptor idiotype or membrane determinant of B cells.

f. Defects in antigen-presenting cells.

Antigen-presenting cells may play an essential role in the cellular and molecular events that underly immune competence by processing and presenting antigen to lymphocytes and by secreting humoral factors that influence lymphocyte activities. Different antigen-presenting cells can preferentially activate helper and suppressor T cells. Thus, deletion or functional inhibition of a particular subpopulation of antigen-presenting cells may account for the induction of autoimmunity. Functional inhibition might be due to diminished phagocytosing capacity, e.g. of immune complexes. Theoretically, diminished clearance of immune complexes could lead to a greater deposition in tissues with subsequent damage.

g. Defects in the idiotype-anti-idiotype network.

Regulation of immune responses can operate via idiotypic and anti-idiotypic determinants. Anti-idiotypic antibodies may suppress as well as enhance immune responses, dependent on the conditions. One can speculate that autoimmune responses may be the result of defects in immunoregulation that allow under/over-production of anti-idiotypic

antibodies. Such defects would permit either unchecked production of autoantibodies or cyclic stimulation of idiotypes in the absence of the inciting antigen.

2.2. Genetic factors in autoimmune disease.

Familial tendency of autoimmune disease supports the view that genetic predisposition may occur. The role of genetic factors in determining the incidence, onset and nature of autoimmune processes has become evident. The genes that code for the magnitude and nature of immune responses to antigens predominantly are the genes coding for the HLA-complex and the immunoglobulin genes. Several of these HLA-antigens are clearly correlated with some autoimmune diseases. Table IV gives some examples of associations between HLA-DR and autoimmune diseases. Graves' disease for example, is closely related to HLA-B8 and HLA-DR3 in Caucasians, but in Japanese populations to HLA-DR5 and HLA-DR8.

Table IV HLA-DR and autoimmune diseases.

	Antigen	Relative Risk
Multiple sclerosis	DR2	4.1
Goodpasture's syndrome	DR2	2.4
Celiac disease	DR3(DR7)	10.8
Sicca syndrome	DR3	9.7
Addison's disease	DR3	6.3
Graves' disease	DR3(B8)	3.7
Juvenile diabetes	DR3(DR4)	5.6
Myasthenia gravis	DR3	2.5
Idiop. membr. nefropathy	DR3	12
Rheumatoid arthritis	DR4	4.2
Pemphigus	DR4	14.4
IgA nephropathy	DR4	4
Hydralazine-induced SLE	DR4	5.6
Hashimoto's disease	DR5	3.2
Pernicious anemia	DR5	5.4
Juvenile rheumatoid arthritis	DR5	5.2

Although frequently simultaneous expression of multiple organ-specific autoimmune diseases are observed in a given individual, no uniform associations between HLA-type and a combination of autoimmune diseases can be found. This suggests a distinct genetic background at least in part for each autoimmune disease. An example of the simultaneous occurrence of organ specific autoimmune diseases is the Schmidt syndrome, in which autoimmune thyroid disease, insulin dependent diabetes mellitus and sometimes autoimmune Addisons disease are associated (type II polyendocrine autoimmune disease). These patients sometimes also suffer from pernicious anemia, vitiligo,

ovarian failure and alopecia. This syndrome is believed to have a recessive inheritance with greater penetrance in females. Another example is the type I polyendocrine glandular failure consisting of hypoparathyroidism, Addisons disease and frequently moniliasis, with or without other organ specific autoimmune disease. This syndrome occurs in children and more frequently in females. A third example is the frequent occurrence (>30%) of parietal cell antibodies in patients with autoimmune thyroid disease (thyro-gastric antibody). Of these individuals 5-10% will develop pernicious anemia. The genetic, environmental and immunologic factors predisposing for these combined abnormalities are unclear. The way in which HLA genes affect the susceptibility to autoimmune diseases is not known. Theoretically, there may be effects on the magnitude on immune responses, on regulatory T cells, the metabolism of steroid hormones or on the handling of antigens by phagocytes.

Other genetic markers are the genes that specify phenotypic markers of immunoglobulins. One of these phenotypes, termed Gm, is a polymorphic serologic marker in the constant region (Fc) of immunoglobulins (Fig.1). Its variants are associated with Graves' disease, myasthenia gravis, and type I diabetes mellitus (Nakao et al., 1980).

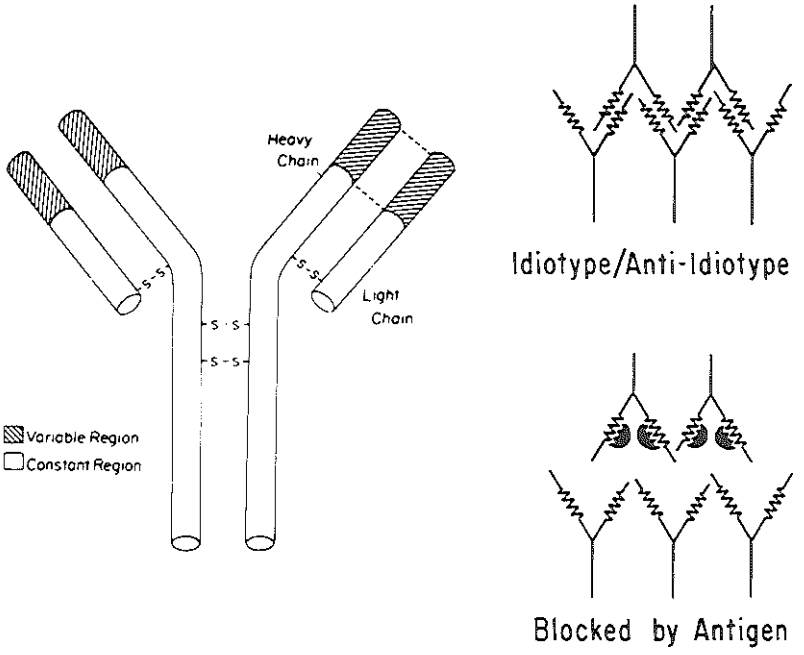

Fig.1 Immunoglobulin molecule (left) and blocking of idiotype-anti-idiotype reaction by antigen (right).
The variable region of the molecule forms a cavity that accommodates the antigen; hence, it is also called the antigen-binding region. This region contains the serologically defined structures termed idiotypes.

In conclusion, it seems that genetic markers, like HLA DR, Gm, and probably other factors are important elements in susceptibility to autoimmune diseases. A combination of several factors might give stronger clinical correlation than a single parameter. In Graves' disease for instance, the association with HLA B8/DR3 becomes stronger when combined with Gm typing. The genes that are related to susceptibility of an individual to develop autoimmune disease, might permit already formed autoantibodies to develop lesions. Thus, the expression of the disease through tissue lesions depends on immunoregulatory disturbances which might be caused by or operate in combination with genetic markers.

The fact that autoimmune diseases predominantly affect females, indicates another genetically based cause of susceptibility yet to be clarified. Probably female sex hormones rather than X-chromosome-associated genes influence the formation of autoantibodies and the expression of autoimmune disease.

2.3. Drug induced autoimmunity

Some chemicals and drugs are known for their potential to induce autoimmune phenomena in persons with certain HLA types. Some examples of the association between drugs and class II-MHC alleles are:

a. Hydralazine-induced SLE syndrome and HLA-DR4 (Batchelor et al., 1980).
b. Glomerular nefropathy induced by gold or D-penicillamine and HLA-DR3 (Wooley et al., 1980).
c. Vinylchloride-induced scleroderma-like lesions and HLA-DR5 (Gleichmann et al., 1983).
d. Symptoms of collagen vascular disease induced by spanish toxic oil and HLA-DR3 or -DR4 (Gleichmann et al., 1983).

2.4. Viral factors in autoimmunity.

Virus may induce aberrant responses and autoimmune phenomena. Viral infection can generate a population of killer T cells, which are specifically cytotoxic for host cells infected with that virus.

Damage of the infected cell may lead to release of autoantigens, which induce autoantibody formation. As stated before, this autoantibody-formation may be an epiphenomenon and not a cause of autoimmune mediated damage. An example of this virus induced autoimmunity might be polyarthritis after Hepatitis B infection.

2.5. Immunopathologic mechanisms leading to tissue damage and/or functional disturbances.

Four immunologic reaction types are known:

1. type 1. IgE mediated degranulation of mast cells (anaphylactic sensitivity).
2. type 2. antibody dependent cytotoxic hypersensitivity.
3. type 3. immune complex mediated hypersensitivity.
4. type 4. cell-mediated (delayed type) hypersensitivity.

In autoimmunity these four types of immune reaction may operate apart and together. Today strong indications exist for the participation of type 2, 3 and 4 in the induction of autoimmune diseases. A more detailed description follows below in a, b and c respectively:

a. Antibody dependent cytotoxic hypersensitivity

Type II hypersensitivity reactions involve the action of IgG or IgM antibodies on structures on cell membranes. These may be normal or modified cell membrane structures (e.g. receptors for hormones) or absorbed free antigens. The combination of antibody with the cell membrane structure may lead to cell damage via binding of the Fc portions of the antibodies to complement ('complement-dependent antibody lysis'), to phagocytes (phagocytosis) or to other, poorly defined cytotoxic cells with receptors for the Fc portions ('antibody-dependent cell-mediated cytotoxicity'). Combination of antibody with receptors may also lead to stimulation or blockage of the receptor. Examples of complement-dependent antibody lysis of autoimmune origin are autoimmune hemolytic anemia, neutropenia, lymphopenia, thrombocytopenia in which (auto)antibody to the particular cell type induces the lysis of the cell by complement. Other examples are diseases such as pemphigoid and Goodpasture's syndrome supposed to be due to the deposition of anti-basement membrane antibodies along the basement membranes of the dermal-epidermal junction or the glomeruli and alveoli, respectively. Examples of diseases in which anti-receptor antibodies may play a pathogenetic role include type I diabetes mellitus, Graves' disease and myasthenia gravis.

b. Immune complex mediated hypersensitivity.

Antigen-antibody immune complexes may cause tissue damage, when deposited in tissues, activate complement factors and attract granulocytic or monocytic cells. This type of autoimmune reaction often is non-organ specific and a typical example is systemic lupus erythematosus (SLE) with immune complex disease through vasculitis. Deposition of immune complexes in blood vessel walls leads to increased vascular permeability, induced by vasoactive amines released from basophils and platelets. As a consequence complement is activated and platelets are aggregated. Then polymorphonuclear phagocytes are attracted by chemotactic complement splitting products, and they will release enzymes causing damage to vessel walls.

c. Cell mediated (delayed type) hypersensitivity.

Sensitized T lymphocytes may cause tissue lesions by releasing lymphokines and/or by attraction of inflammatory cells such as granulocytes and monocytes. Cytotoxic T cells may also be involved. The cellular composition of the infiltrate is often dependent on the nature of the eliciting antigen. Peak reactions are usually obtained one or several days after contact of the sensitized T lymphocytes with the relevant antigen. An example of this type of immune reaction is Hashimoto's thyroiditis (Theofilopoulos, 1984).

2.6. Conclusion.

The induction of autoimmunity is probably multifactorial and comprises immunologic, genetic, endocrinologic and virologic aspects.

Since autorecognition is apparently a normal event in a functioning immune system, immune reactivity to self determinants may result from imbalanced homeostasis of immunity including the idiotypic-anti-idiotypic responses in a genetically predisposed individual. Probably viral and other factors play a precipitating role in these predisposed individuals.

Before T cells can differentiate to become effector cells, they must recognize both autoantigen and MHC determinants. If self recognition occurs and for several reasons suppression of T lymphocyte activation is impaired, autoreactive T cells will help or activate autoreactive B cells to synthesize specific autoantibodies. These autoantibodies are not always harmful and sometimes do not produce tissue lesions. When however, expression of these autoantibodies is enhanced (by hormonal, viral or environmental factors ?) ,autoimmune manifestations may develop.

3. Immunological aspects of autoimmune thyroid disease.

In 1956 Witebsky and Rose succeeded to induce experimental autoimmunity and Roitt et al identified thyroid autoantibodies in the serum of Hashimoto's thyroiditis patients.

Autoimmune thyroiditis (AT) in men can be devided into Hashimoto's Thyroiditis (HT) and primary myxoedema (PM). HT is characterised by hypothyroidism and goiter, due to antibodies against thyroglobulin and thyroid microsomes. PM is characterised by hypothyroidism without goiter (atrophy of the thyroid gland) and is considered to be due to antibodies that block the TSH receptor (hormone synthesis and growth blocking). Graves' disease at the other pole of the spectrum of autoimmune thyroid disorders, is characterised by hyperthyroidism and diffuse goiter, with or without ocular signs. The hyperfunction and goiter development in GD is due to antibodies directed to the TSH receptor, which stimulate the receptor (like TSH) to hormone synthesis (thyroid stimulating immunoglobulins, TSI) and thyroid growth. Goiter formation may also be caused by thyroid growth promoting immunoglobulins (TGI), as described in patients with simple goiter.

Hypothyroidism due to AT may precede the development of Graves' disease (GD), but the reverse sequence is more often observed. The development of AT after GD usually follows surgery, radioiodine therapy or antithyroid drug therapy, which may alter the immune response from TSI production to the destructive antibodies against microsomal antigen or thyroglobulin (Fig.2). An alternative possibility however is that both types of autoantibodies are present, but that the autoantibodies leading to irreversible destruction ultimately supervene and that the stimulating antibodies therefore cannot exert an effect anymore. This process may be accelerated by treatment with surgery or radioactive iodine.

In HT goiter formation and hypothyroidism is usually caused by lymphocytic infiltration of the thyroid gland. Development of hypothyroidism is enhanced by antibody-dependent hypersensitivity, which leads to destruction of thyroid cells (Fig.2). Hypothyroidism in turn leads to diminished negative feed-back of pituitary TSH production. The enhanced TSH production causes stimulation of thyroid

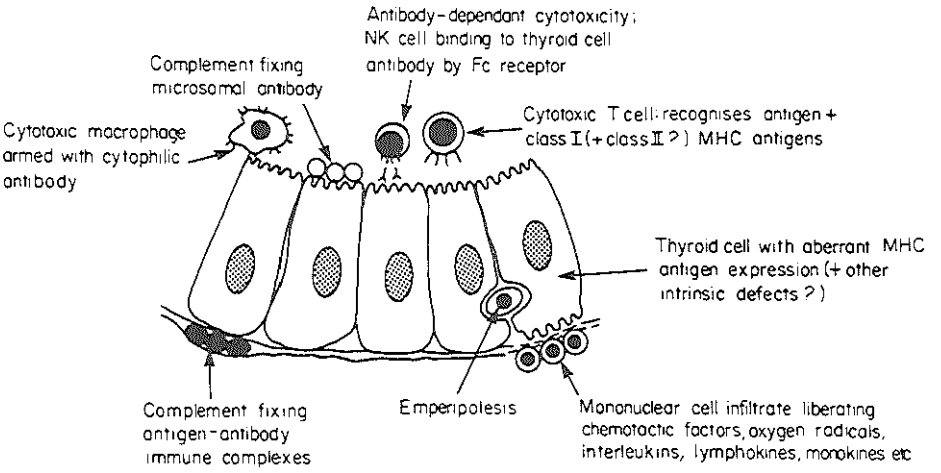

Fig.2 Representation of autoimmune mechanisms that may lead to destruction and/or hypofunction of the thyroid gland. (From Weetman and McGregor, Endocrine reviews 1984;5:326).

follicular cell growth and increase in goiter size. Thyroxine treatment usually diminishes goiter formation in these cases.

In PM, apart from TSH blocking antibodies, humoral immune mechanisms (cytotoxicity of microsomal antibodies, Fig.2) may also be present, thereby causing thyroid gland destruction.

The mechanisms contributing to autoimmune disease of the thyroid are discussed in the following sections.

3.1. Antigen dependent mechanisms.

a. Somatic mutation or chemical alteration of thyroid follicular cell antigens (microsomes, thyroglobulin, cell surface components) during life may lead to an altered antigen, that induces an immune reaction. This may lead to an attack on the thyroid cell, that may cause atrophy of the thyroid leading to hypothyroidism or to lymphocytic infiltration leading to goiter formation or to both. For instance, an immunochemically abnormal thyroglobulin causes autoimmunity in spontaneous autoimmune thyroiditis.

b. Autoantigens as e.g. thyroglobulin occur normally in the systemic circulation and may as a consequence induce autoimmune reactions after disturbance of the control mechanisms normally preventing autoimmunity.

3.2. Breakdown of tolerance.

Tolerance may be broken by incorporation of viral proteins or drugs in the cell membrane or modification of other autoantigens. Such a mechanism may modify thyroglobulin as to become immunogenic (Weigle,1965), similarly as heterologous thyroglobulin can induce autoimmunity. Probably the maintenance of tolerance by circulating autoantigens is dependent on their concentration (Weigle, 1971).

3.3. Disordered immunoregulation.

The two major pathways for immunoregulation of the B cell are modulation by cellular interactions, particularly by suppressor T cells (a), and influences of the idiotype-anti-idiotype network as proposed by Jerne (1971) (b).

a. The autoantibody producing B lymphocyte normally should be inhibited by suppressor T cells and by factors that they produce. When a selective deficiency of antigen-specific suppressor T cells occurs, autoreactive T and B cell clones might proliferate. Simultaneously, the autoreactive B cells may start to produce autoantibodies (Fig.3A). This might be true for the production of thyroid autoantibodies in individuals with an inherited disorder related to HLA markers (Fig.3B).

b. The antigen recognition site of an antibody is determined by the variable-region amino acids of the heavy and light chains of the immunoglobulin (Fab). The variable regions can evoke the generation of specific antibodies binding to these sites. Such sites are called idiotypes. This is a serologically identifiable configuration in the antigen-binding region (Fab) of an antibody (Fig.1). Because the variable region of an antibody has a unique structure, it can be immunogenic. As a result, anti-idiotypic antibodies can be formed and these immunoglobulins can be measured in serum. For example antibodies to human thyrotropin (TSH) can elicit anti-idiotypic antibodies when injected in a rabbit. These anti-idiotypic antibodies bind to thyrotropin receptors (as do the antibodies directed to thyrotropin receptors !) and stimulate cAMP synthesis in cultured thyroid cells (Farid et al., 1982), thus behaving like thyroid stimulating immunoglobulins in Graves' disease (Fig.4).

The network theory of immunoregulation predicts that in autoimmunity anti-idiotypic antibodies are produced that are directed against the idiotype of autoantibodies (Fig. 3C). The idiotypic determinants of the anti-idiotypic antibodies can in turn elicit the production of other anti-idiotypic antibodies. The regulatory pattern of the network is based on suppression. Before antigenic stimulation occurs, a steady state exists in which low concentrations of idiotypic antibodies are maintained. Antigenic challenge upsets idiotype equilibrium and perturbs the steady state leading to increased production of antibodies. The targets of immunosuppression by anti-idiotypic antibodies are both B and T cells. Breakdown of this network could lead to autoimmune disease. For instance a point mutation could allow unbalanced production of autoantibodies. A hormone could also act as antigen and stimulate antibody production (Fig. 4), against which anti-idiotypes would act as anti-receptor antibodies, for instance antibodies against insulin receptors in diabetes mellitus or antibodies against anti-DNA idiotypes in patients with SLE in remission.

3.4. HLA system.

Immune reactivity is dependent upon antigen presentation by antigen-presenting cells in combination with HLA class I or II

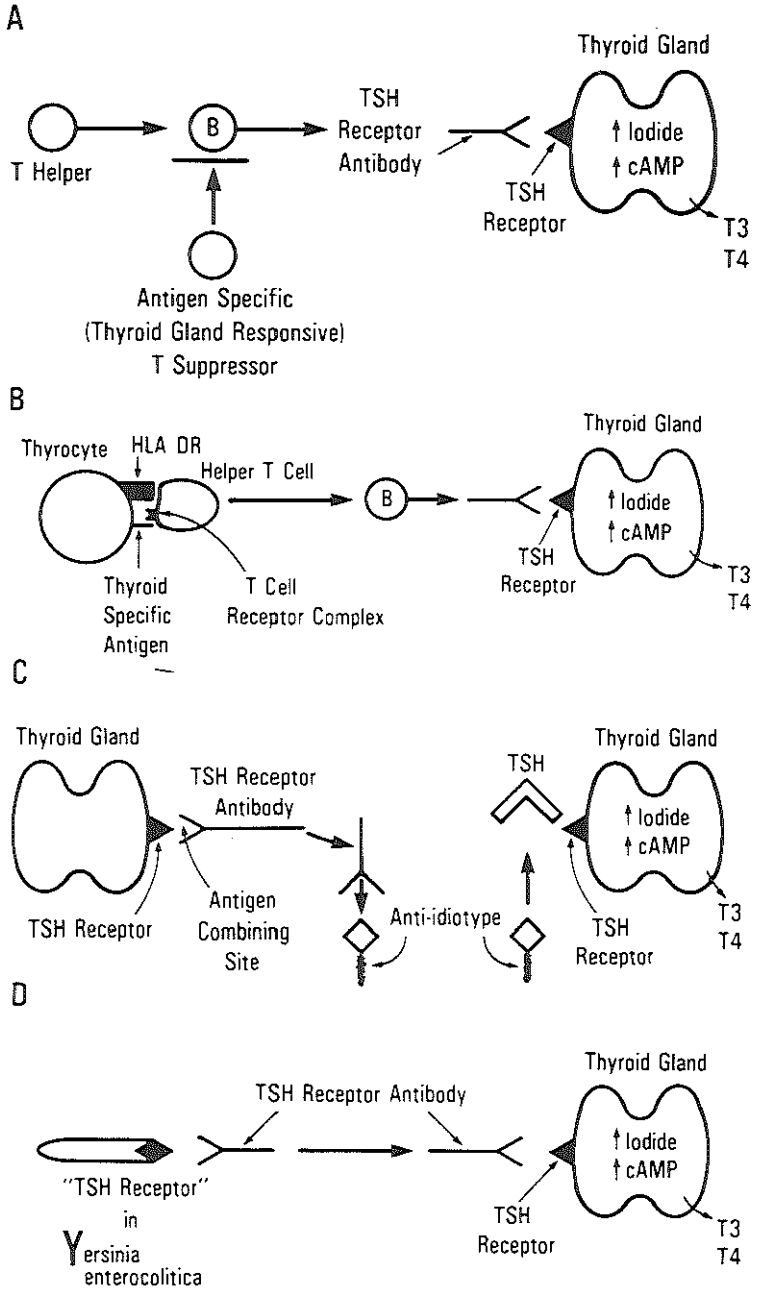

Fig.3 Possible explanations for the etiology of Graves' disease. (From Burman and Baker, Endocrine reviews 1985;6:217).

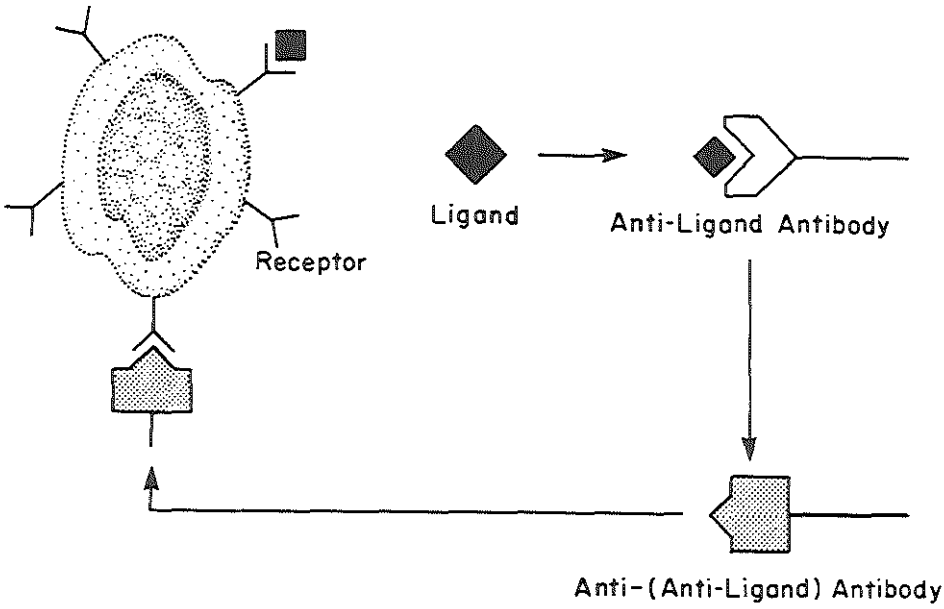

Fig.4 Anti-idiotypes also acting as anti-receptor antibodies. The receptor and the anti-ligand antibody have homologous structures that bind to the ligand. An anti-idiotype, or anti-(anti-ligand) antibody, combines with the homologous ligand-binding structures in both the antibody and the receptor. The anti-idiotype may stimulate the receptor by mimicking the physiologic effects of the ligand, or it may block the receptor. (From Shoenfeld and Schwartz, N Engl J Med 1984; 311:1021).

determinants. When these determinants are present on the cell surface of the involved organ, i.e. the thyroid, an enhanced HLA antigen presentation could be the trigger to autoimmune reactions. Such an enhanced HLA presentation on the cell surface might be induced by viral infection through gamma interferon stimulation (Botazzo et al, 1983). Correlation between HLA-DR5 and Hashimoto's disease, and between HLA-DR3 and Graves' disease has been described.

3.5. Conclusive remarks.

It has become possible to induce experimental autoimmune thyroiditis (EAT) in mice, chicken and guinea pigs. This EAT resembles that in the human, providing evidence that Hashimoto's thyroiditis in man is of autoimmune nature. Unfortunately no such animal model for anti-TSH receptor antibodies leading to Graves' disease has been developed until now.

4. Graves' disease (GD)

Graves' disease is characterised by hyperthyroidism, often diffuse goiter and ocular manifestations and sometimes acropachy.

Stimulation of thyroid cell function normally occurs when TSH, released from the pituitary, binds to the TSH-receptor. There are about 1000 TSH receptors on each thyroid cell. A current concept of the interaction of TSH with its thyroid receptor is shown in Fig. 5. TSH binding to the glycoprotein component of the receptor is the initial high affinity interaction. The ganglioside component of the TSH receptor is postulated to confer additional receptor specificity by distinguishing between TSH and related glycoprotein hormones (LH, FSH, HCG). Interaction of the ganglioside component with TSH leads to conformational change of the TSH beta-subunit and the lipid bilayer to facilitate signal transduction across the membrane.

Monoclonal antibodies to the ganglioside component (Fig.5) of the TSH receptor do not need the glycoprotein portion of the receptor to

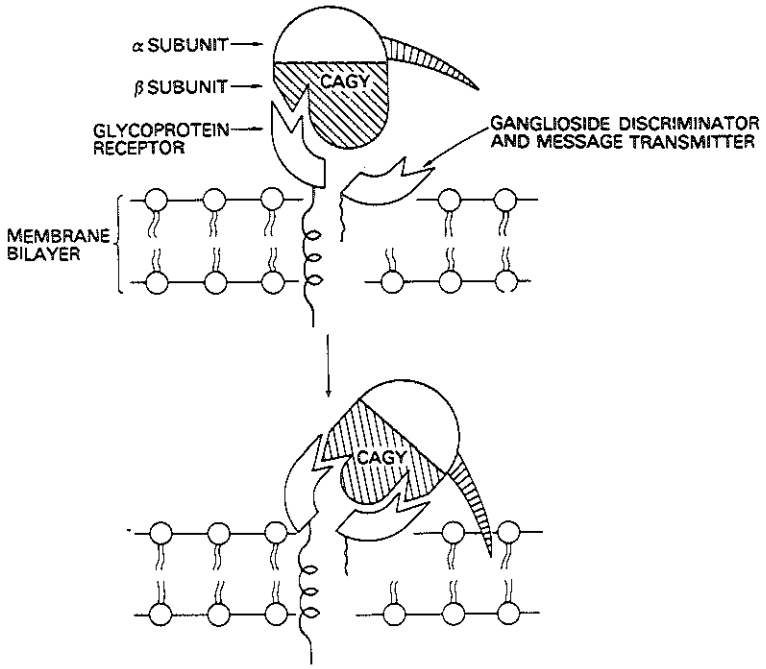

Fig.5 Proposed model of TSH receptor composed of glycoprotein and ganglioside component. After the TSH beta subunit interacts with the receptor, the hormone changes its conformation, and the alpha subunit is brought into the bilayer where it interacts with other components. The end result includes a change in organisation of the membrane bilayer, a change in the transmembrane electrochemical ion gradient, changes in the lipid turnover, and the initiation of growth and cyclic AMP signals. (From Fraser and Venter, J Allergy Clin Immunol 1984; 74:662).

stimulate cAMP. Direct interaction with the ganglioside component is sufficient. Monoclonal antibodies raised to the glycoprotein component of the TSH receptor can behave as blocking antibodies (Valente et al 1982), and might act like the immunoglobulins that block the action of TSH resulting in hypofunction and atrophy of the thyroid gland.

GD is due to autoantibodies, which by binding to (or in the region of) the TSH receptor of the thyroid follicular cell stimulate thyroid function. This results in enhanced thyroxine (T4) and 3,3',5-triiodothyronine (T3) synthesis. These autoantibodies are currently called thyroid stimulating immunoglobulins (TSI).

Other antibodies may compete for TSH binding sites (TSH blocking) but lack the ability to stimulate the thyroid cell. The general term, thyrotropin receptor antibodies (TRA) for both types of antibodies has been proposed by Volpe (1977).

It is unclear how predisposing and/or environmental factors (stress and infectious agents) result in the immunologic phenomena leading to the production of TSI. Several models of perturbations of the normal immune response leading to GD have been described but all of them are inadequate as to completely explain the generation of this autoimmune disease. One concept focusses on the antigen specific suppressor T cell defect, but probably much more factors play a role (Weetman and McGregor, 1984). There are probably at least six ways by which TSH receptor antibodies could interact with the TSH receptor, each of which would influence the end result of thyroid hormone release. The interaction between TSH receptor and antibodies might be modulated by other antigens on the thyroid membrane, such as receptors for the Fc portion of immunoglobulin molecules, prostaglandin receptors, complement receptors, viral or bacterial receptors, thyroglobulin or microsomal determinants (Fig. 6).

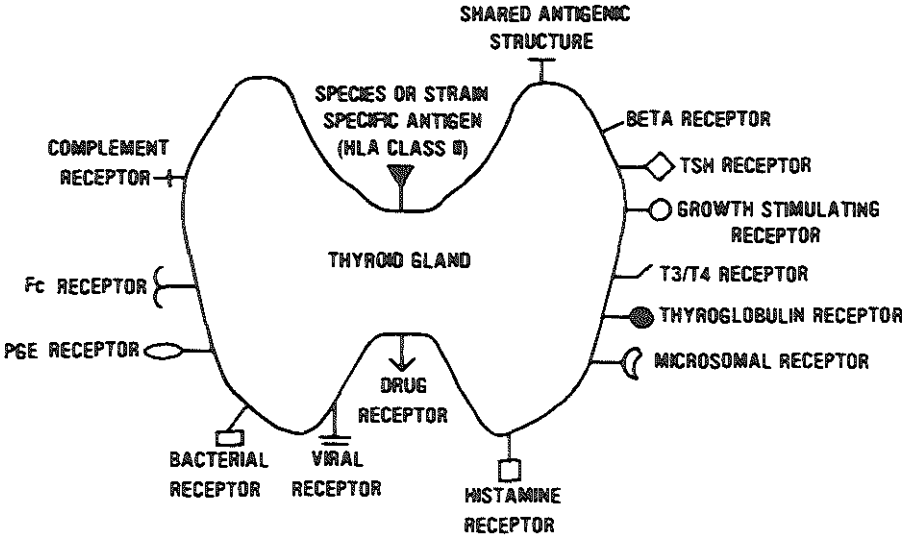

Fig.6 Different antigens on the thyroid cell membrane involved in possible autoimmune reaction. (From Burman and Baker, Endocrine reviews 1985; 6:218).

There is no consensus regarding the binding characteristics and the molecular weight of the TSH receptor. As determined by different techniques, the TSH receptor is capable of binding TSH with an approximate Ka value of 10^{10} M^{-1}.

4.1. Antibodies involved in GD.

GD is an autoimmune disorder, characterised by circulating TSH receptor antibodies. These TSH receptor antibodies are believed to be a heterogeneous population of stimulatory and inhibitory immunoglobulins. When stimulatory IgG predominate, thyrotoxicosis evalues. Hypothyroidism would be caused if predominantly inhibitory acting immunoglobulins were present, as is the case for instance in primary hypothyroidism (see above).

Characterisation of TSH receptor antibodies started with the studies of Adams and Purves in 1956, describing the long acting thyroid stimulator (LATS) in human sera as they investigated newer assays for the detection of human TSH. Adams and Purves noted delayed and prolonged (as compared to the effect of TSH) release of thyroidal radioactive iodine in guinea pigs, when injected with sera from thyrotoxic patients. This LATS acted very similarly to TSH in generating thyroid cell activity in the mouse (Adams, 1958) and was later shown to be an immunoglobulin.

In 1967 Adams and Kennedy described an immunoglobulin competing with LATS in its ability to bind to human thyroid membrane in vitro. These immunoglobulins were called LATS protector and were believed to be human thyroid stimulating antibody.

Table V
TSH receptor antibody detection

Functional assays	Binding assays
- Release 131J from mouse thyroid (LATS)	- LATS protector assay
- Stimulation of human thyroid cAMP in vitro (TSI, TSAb)	- inhibition of ^{125}I-TSH binding to human thyroid membranes (TDA)
- Cytochemical assay	- TSH binding inhibitory immunoglobulin (TBII)
- Colloid droplet formation in human thyroid slices	- Fat cell membrane radio-ligand assays
	- Fat cell ELISA

Smith and Hall (1974) described inhibition of ^{125}I-TSH binding to human thyroid membranes by Graves' sera. Since that time apart from assays demonstrating directly or indirectly binding of antibodies to membranes (binding assays), assays detecting stimulation of cAMP production, iodide uptake or T_3/T_4 production (functional assays) have been developed (Table V). Stimulation of cAMP initiates a cascade of cAMP-kinase regulated processes that lead to iodide uptake, thyroglobulin biosynthesis, iodination and degradation of/to T3 and T4, and release of T3/T4 into the bloodstream.

The TSH receptor antibodies are mainly from the IgG class, but

also IgM, IgA and IgE antibodies have been detected in thyroid glands of Graves' patients. It is not known if IgM, IgA or IgE antibodies alone can mediate or modulate stimulatory events by the TSH receptor (Burman and Baker, 1985). As several IgG subclasses are found in Graves' sera, it is believed that TSI are produced by more than one clone of B lymphocytes and represent a heterogeneous polyclonal response. Such a heterogeneous response might explain the nonthyroidal abnormalities occasionally observed in Graves' disease: ophthalmic Graves' disease (OGD), pretibial myxoedema, myasthenia gravis, diabetes mellitus, vitiligo and adrenal insufficiency.

The prevalence of TSH receptor antibodies in untreated Graves' disease depends on sensitivity of the assay used. The LATS and LATS protector assays are positive in 30-70% of GD patients. The TBII assays are positive in 70-80% of active GD patients. The stimulatory assays (cAMP response, iodide uptake) are reported to be positive in 80-100% of active GD patients. The most sensitive assay to detect TSI is the cytochemical bioassay quantitating lysosomal changes. Comparing the sensitivity of the several assays of TSH receptor antibodies (apart from the LATS assay), Loeffler et al (1985) concluded that the least sensitive assay was the TBII procedure with human thyroid membranes.

Presence of autoantibodies is not unusual in normal people. They may play a physiological role in clearing the undigested autoantigens. The ability to modulate or regulate the formation of these autoantibodies thus might discriminate normal persons from subjects with autoimmune diseases like that of the thyroid. Anti-idiotypic antibodies could play an important role in inactivating TSH receptor antibodies. TSH receptor antibodies are rarely found in patients with multinodular goiters, toxic adenoma or (sub)acute thyroiditis. Relatives of GD patients are reported to have positive TSI activity in about 25%. It is uncertain whether these subjects will develop overt GD or not.

Besides TSI, thyroid growth stimulating immunoglobulins (TGI) have been detected in Graves' disease. TGI are associated with goiter formation without increased production of thyroid hormone. They can be measured with the sensitive cytochemical bioassay (Drexhage et al, 1980) and are found in 70% rather than all goitrous Graves' patients. With the FRTL-5 rat thyroid cells, TGI have been demonstrated in 85% of untreated Graves' patients (Valente et al, 1982).

In conclusion, in patients with active GD there may be (theoretically) three distinct populations of autoantibodies:

1. TSI, with little or no growth promoting activity, but with hormone stimulation.

2. TGI, with little or no effect on cAMP release, but with growth stimulation.

3. cAMP and cell growth stimulating antibodies.

This heterogeneity in specificity of autoantibodies to the TSH receptor might explain the different clinical manifestations of GD. Patients with predominantly type 1 antibodies develop hyperthyroidism, those with type 2 antibodies develop goiter and those with type 3

both goiter and hyperthyroidism. The different possible interactions of TSI and the TSH receptor are depicted in Fig.7.

4.2.Cellular aspects of immune mechanisms in GD

Circulating B cells which bind the TSH receptor can be demonstrated in normals and are reported to be increased in Graves' disease. Whether this phenomenon is of pathological relevance remains to be discussed.

Defective suppressor T cell activity (due to decreased number and/or function of antigen specific lymphocytes) has been proposed in Graves' disease. Investigations of lymphocyte numbers and function in Graves' patients have attempted to delineate defective suppression. Some investigations describe a decreased number of suppressor cells in GD, others found normal numbers, the results thus being conflicting (van Ouwerkerk et al, 1986). A decrease in the total number of peripheral blood suppressor T cells however is difficult to envisage, because only a fraction of the total suppressor T cell number can be

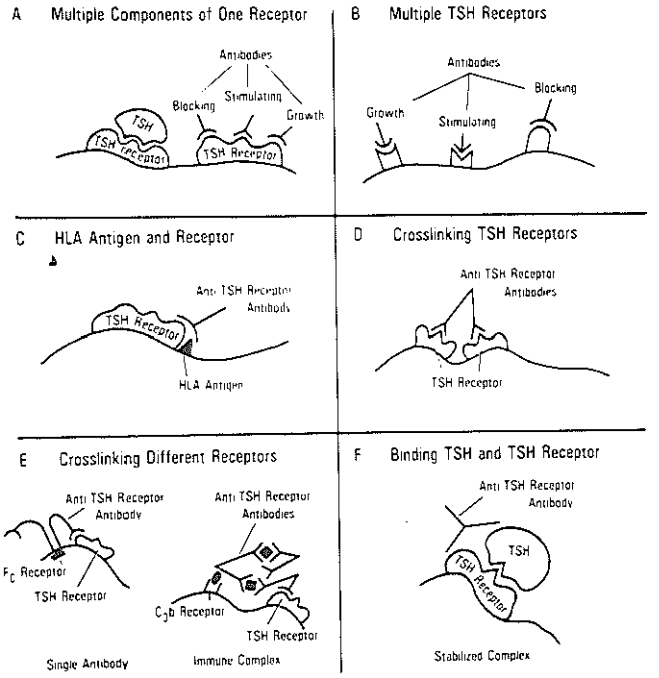

Fig.7 Possible interactions of thyroid stimulating immunoglobulins and the TSH receptor. (From Burman and Baker, Endocrine reviews 1985; 6:219).

specific for the autoantigens involved in this organ specific autoimmune disease. It remains uncertain whether a suppressor T cell defect exists in peripheral blood. Studies concerning a suppressor defect in the thyroid gland itself have been performed. Technical difficulties arise however in separating thyroid cells from blood, that circulated in the thyroid gland . Decreased functional activity of circulating suppressor T cells in patients with autoimmune thyroid diseases has also been described (Aoki et al, 1979). Antithyroid drugs have been shown to directly restore suppressor cell activity (Balazs et al, 1973, Goldrath et al, 1982) thus probably affecting intrathyroidal autoimmunity.

The site of anti-thyroid antibody production appears to be the thyroid itself as lymphocytes derived from thyroid tissue of Graves' patients produce thyroglobulin antibodies in vitro (Mori et al, 1985). TSH receptor antibodies are probably produced in the thyroid as well as in several lymphoid tissues, bone marrow and cervical lymph nodes. Other lymph nodes and peripheral blood may also contribute to some extent (Weetman et al,1984).

An antigen specific defect in suppressor T cell function can be investigated with direct or indirect migration inhibition factor (MIF) assays. The group of Volpe (Strakosch et al, 1982) was the first to attempt to measure suppressor lymphokine production using a bioassay in which these lymphokines inhibit the migration of mononuclear cells from a capillary tube. They demonstrated using this MIF assay a thyroid antigen specific cell mediated immunity in GD patients, but others (Ludgate et al, 1985) have been unable to reproduce these findings.

Other cellular events involving lymphocyte activity and lymphokine function might be applied to the study of GD. T cell development can be stimulated by interleukin 1 (IL-1), a peptide produced by activated macrophages. Stimulation of helper T cells by IL-1 and antigen causes the release of interleukin-2 (IL-2), which by binding to IL-2 receptors on helper and cytotoxic/suppressor T cells stimulate their proliferation and probably gamma interferon production. IL-2 might also enhance natural killer cell (NK) activity. Enhanced NK cell activity has been described in HT and GD patients, suggesting that the T helper cell activity is enhanced in Graves' disease.

4.3. Genetic factors in GD.

The genetic factors leading to the expression of Graves' autoantibodies (TSI) have been partially discussed in chapter 2.2. Less than 50% of GD patients have a positive family history. A significant correlation has been shown between the haplotype HLA-B8/DR3 and Graves' disease (Farid et al, 1980), the relative risk being 3.7. Conflicting results have been found in studies investigating the association of GD with Gm allotypes (immunoglobulin genes not linked with HLA loci).

The effect of HLA status on outcome of therapy of GD (partial thyroidectomy, radioiodine and antithyroid drugs, all modulating immune mechanisms) has been investigated by several groups. Patients with HLA-DR3, who remain strongly positive for TSI tend to relapse after antithyroid drug treatment (McGregor et al, 1980). This has not

been confirmed by others (Allannic et al, 1983). Probably the duration of therapy may play a role. Perhaps subgroups of GD patients with different prognosis based upon HLA status will be made in the future.

In search for the explanation of the female preponderance of autoimmune diseases, many theories have been proposed. A protective effect of testosterone has been proposed (Theofilopoulos, 1984). Probably female sex hormones play a role as well. Recently discovered B cell genes on the X chromosome could explain, why women more frequently show active autoimmune disease.

4.4. Etiology and pathogenesis of Graves' disease

The cause of GD is still unclear, but recent investigations (discussed in Chapter 4.1., 4.2., 4.3.) suggest four possible theoretical explanations, some of which may be operative simultaneously.

a. An inherited defect in specific suppressor cell function allows helper T cells to stimulate B cells. This results in production of TSH receptor antibodies.
b. The initial event in GD might be the expression of HLA-DR on the thyroid cell membranes after a bacterial or viral infection through interferons (Botazzo et al,1983), causing T cell proliferation and TSH receptor antibody production by B cells.
c. Disturbed idiotype-anti-idiotype interactions.
Normally present TSH receptor antibodies might be "suppressed" by anti-idiotype antibodies against these TSI. Disturbance of the idiotype anti-idiotype network might enable the increased production of TSH receptor antibodies, and the stimulation of the thyroid gland by these antibodies.
d. Recently specific structures have been documented in Yersinia enterocolitica and Escherichia coli that bind TSH in vitro with high affinity (Fig 3d). The presentation of these components by antigen presenting cells might be sufficient to induce an anti-TSH receptor immune response, whereas the TSH receptor presented by thyroid cells might be inappropriate for activation of similar lymphocytes. They might even induce TSH binding antiidiotypic antibodies, the former causing hyperthyroid GD.

4.5. Therapy of GD with antithyroid drugs and impact on immune mechanisms

Remission of Graves' disease without therapeutic intervention was reported to occur in 30% of cases (Wilson, 1967). Propranolol treated patients show remission rates of 14-36% (Weetman, 1984). However treatment of GD with partial thyroidectomy, radioiodine or antithyroid drugs usually leads to remission rates higher than 30%. The mechanisms of action of the antithyroid drugs belonging to the thiocarbamides are listed in Table VI.

Table VI
Mechanisms of action of thiocarbamides

Intrathyroidal

1. Inhibit thyroglobulin iodination by diverting oxidized iodide away from thyrosine residues.

2. Inhibit coupling reaction between iodotyrosines (T_1 and T_2) to form iodothyronines (T3 and T4).

3. Bind to thyroglobulin and possibly alter its structure (effect in vivo?).

4. Inhibit thyroglobulin biosynthesis (effect in vivo?).

Extrathyroidal

1. Inhibit peripheral conversion of T4 to T3 (propylthiouracil only).

2. Affect immune system
   a. decrease lymphocyte responsiveness in vitro
   b. decrease circulating thyroid autoantibody titers
   c. restore normal suppressor cell activity
   d. may directly affect intrathyroidal autoimmunity

Many authors described a fall in thyrotropin receptor antibody levels during methimazole (MMI) treatment. Remission of Graves' hyperthyroidism may be related to the fall in TSI levels dependent on the dose and duration of MMI treatment. The remission rate with antithyroid drugs is reported to be 41-55%. Treatment with high-dose antithyroid drugs combined with thyroid hormones has been reported to be superior, giving a remission rate of 75% compared with 42% after fixed low-dose treatment without thyroid hormone supplementation (Romaldini et al, 1983).

The duration of MMI treatment usually is 6-18 months. As stated above high dose regimens are preferable assuming that MMI exerts a direct immunosuppressive effect. The required/optimal duration of therapy remains a controversial point. Similar remission rates (30-40%) have been described after short term treatment (2-6 months) and longer periods of drug administration (12-24 months) (Cooper, 1984). However, more recent studies suggest that prolonged treatment (or high dose regimen) does increase the frequency of remissions (Romaldini et al, 1983).

Impact of MMI on immunologic parameters.

Excessive thyroid hormone is reported to influence lymphocyte function including TSI production (Alquist, 1976), but many recent studies suggest the absence of an effect of thyroid function on immune status (Weetman and McGregor, 1984). The immunosuppressive effect of MMI has been shown in vitro to be effected by interference with oxidative reactions within the antigen-presenting macrophages and

monocytes from the affected thyroid gland (Weetman et al, 1984). The fall in ^{99m}Tc uptake as often described in GD patients treated with MMI seems to be related to a decline in TSI levels, suggesting an immunosuppressive effect of MMI. The fall in TSI levels during MMI treatment is reported to be related to an effect of MMI on lymphocytes within the thyroid gland. A diminution of lymphocytic infiltration in thyroid and thymus of Graves' patients after MMI therapy has been described (Simpson et al, 1975; Young et al, 1976), but conflicting data are reported concerning the changes in lymphocyte subpopulations in peripheral blood of GD patients (see 4.2).

Many studies have been performed in order to elucidate why about 50% of the MMI treated GD patients relapse after discontinuation of therapy. The following explanations have been proposed:

a. In the relapse group extrathyroidal sites of TSI synthesis might be more important than in those patients who stay in remission. MMI is predominantly concentrated in the thyroid gland and will therefore exert its immunosuppressive activity principally there (McGregor et al, 1980).
b. Relapse occurs when antigen-presenting cells are more resistant to the effects of MMI. This might be related to HLA-DR3, because DR3 positive patients tend to relapse more frequently (Schleusener et al, 1983; McGregor et al, 1980; Teng et al, 1981). This might be associated with persistence of antigen presentation by macrophages as described in normal subjects (Legrand et al, 1982).
c. Relapse might be related to the dosage and duration of therapy. Prolonged treatment and high dose regimen would increase remissions (Romaldini et al, 1983).

5. Ophthalmic Graves Disease (O.G.D.), also called Graves' ophthalmopathy.

In OGD the extraocular muscles are infiltrated by lymphoytes leading to activation of fibroblasts to produce collagen and mucopolysaccharides. The muscles become oedematous and fibrotic causing muscle enlargement and deformity. Also lacrimal glands may be infiltrated. The muscle enlargement and retrobulbar fat accumulation may lead to proptosis, edema, diplopia and visual failure by venous drainage obstruction and compression of the optic nerve.

5.1. OGD and thyroid function.

The clinical expression of OGD, pretibial myxoedema and acropachy (a form of osteoarthropathy) are probably immune-mediated. The majority of patients with OGD have had or will develop hyperthyroidism. A minority remains euthyroid or becomes hypothyroid, but virtually all subjects with OGD can be shown to manifest some abnormality of thyroid function (Tamai et al, 1980). A link between eye signs and thyroid autoimmunity has been suggested. Eye muscle cell cytotoxicity by lymphocytes of a patient with OGD has been demonstrated (Blau et al, 1983), suggesting that cell-mediated

autoimmunity is involved. Moreover it has been described that monoclonal antibodies against human thyroid membranes cross react with eye muscle antigen (Tao et al, 1984), suggesting that humoral autoimmunity might also be involved.

5.2. OGD and autoimmunity.

No correlation has been found between OGD and TSH-receptor antibody activity. The association of eye signs with Graves' disease suggests a similar pathogenesis, but expression of both entities may be separate. Several studies have focussed on the binding of thyroglobulin or Tg-antiTg complexes to membranes of extraocular muscles (McDougall and Kriss, 1979), but recently cell mediated cytotoxicity by lymphocytes independent of Tg or Tg immune complexes was described (Blau et al, 1983). Also it has been shown that monoclonal antibodies against thyroglobulin react with membranes of human orbital connective tissue (OCT). This might indicate sharing of antigenic determinants by thyroglobulin and OCT (Kuroki et al, 1985). It might be useful to determine antibodies directed against eye muscle antigen in patients with OGD to investigate whether their presence relates to disease activity. In view of the clinical heterogeneity it might well be that the pathogenesis of OGD is multifactorial.

5.3. Treatment of OGD.

Treatment of OGD always starts with attempting to achieve euthyroidism (if not present). When eye signs nevertheless progress, corticosteroids or local supervoltage irradiation will diminish soft tissue involvement. Other modes of immunosuppression might improve more or less the congestive changes in extraocular muscles. Cyclophosphamide (Wall et al, 1979) and plasmapheresis (Kelly et al, 1983) have been employed with little success.

A new option for treatment of OGD might be Cyclosporin A. This is an immunosuppressant especially affecting helper T cells and effective in humoral and cell-mediated immunity and chronic inflammation. Cyclosporin inhibits the synthesis and release of lymphokines by helper T cells. It does not interfere with the effects of already released lymphokines. Suppressor cells would be insensitive to cyclosporin A. Thus cyclosporin A might exert beneficial effects in OGD, just as in other autoimmune diseases and it has been shown to be effective in experimental autoimmune diseases in animal models. Clinical trials are currently being done with this drug in patients with OGD. Orbital surgery is advocated as an alternative to immunosuppressive treatment in OGD.

6. Scope of the study.

Assuming an autoimmune nature of Graves' disease, we were interested to know whether treatment with methimazole (MMI) would, apart from restoring the euthyroid state, exert influences on immunologic parameters.

Freshly diagnosed hyperthyroid patients with Graves' disease were all similarly treated with MMI 10 mg t.i.d. and when euthyroidism was achieved, with substitution of thyroid hormone. At regular intervals measurement of thyroid status and immunologic parameters was done (chapter III).

We were interested to know whether thyroid stimulating immunoglobulins (TSI) would change during treatment and whether values of TSI would allow us to determine the prognosis of the disease after a one year course of MMI. In order to measure these TSI, a bioassay was developed measuring cAMP in cultured human thyroid cells in vitro.

Assuming an influence of T lymphocytes on autoantibody production, we were also interested to know whether these patients would have abnormal numbers of T helper- and/or T suppressor lymphocytes, which would explain the abnormal immune status. In order to measure these T lymphocyte subpopulations we used monoclonal antibodies specific for these subpopulations and counted the cells with a fluorescence-activated cell sorter (FACS).

A parallel study in the same patients was performed to investigate the sensitivity of two different methods for the measurement of thyroid stimulating immunoglobulins (chapter IV). The cAMP bioassay was compared with two commercially available kits measuring inhibition of binding of TSH to thyroid cell membranes. Also conventional thyroid autoantibodies (anti-thyroglobulin antibodies and anti-microsomal antibodies) were measured as another expression of autoimmunity in this disease.

Finally two studies were performed concerning OGD. One (chapter V) was on two patients with unusual clinical presentation of OGD. The other study (chapter VI) was performed in patients with severe ophthalmic Graves' disease (OGD) in order to determine the impact of several therapeutic approaches on the ocular signs with emphasis on irradiation of retrobulbar tissue of these patients.

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CHAPTER II

Autoimmunity of thyroid disease

WITH EMPHASIS ON GRAVES' DISEASE

B.M. VAN OUWERKERK*, E.P. KRENNING*,**, R. DOCTER*, R. BENNER*,** and G. HENNEMANN*

Introduction

Graves' disease (GD), Hashimoto's thyroiditis (HT) and primary myxoedema should be regarded as thyroid autoimmune diseases[1]. GD is estimated to occur in 0.4 per cent of caucasians and is due to autoantibodies that stimulate the thyroid follicular cell, causing hyperthyroidism and often diffuse goitre. GD occurs about 10 times as often in women as in men[2]. HT and primary myxoedema are found four times as often in women as in men and are characterized by antibodies against thyroglobulin (in 2-15 per cent of all women between 25 and 75 years old) and microsomes (in 7 per cent of all adults)[2], leading to failure of thyroid hormone production. Recently it has been suggested that thyroid antibodies which inhibit the action of thyrotropin may also play a role in the development of primary hypothyroidism[3]. The purpose of this review is to describe and discuss the different pathogenetic aspects of autoimmune thyroid disease.

General characteristics of thyroid autoimmune diseases

Thyroid autoimmune diseases share the following characteristics with many other autoimmune diseases: 1) Frequent presence of autoantibodies against antigens of the organ involved (the antibody titre does not always correlate with the severity of the disease). Sometimes autoantibodies reflect a normal immune response to tissue injury in order to enable the RES to remove the liberated cellular components. Examples are autoantibodies against cardiac muscle after myocardial infarction and transient appearance of thyroid autoantibodies during subacute (De Quervains') thyroiditis. 2) Lymphoplasmacellular infiltrates occur in the organ involved, with destruction and fibrosis. 3) 'Overlap' with clinical and serological symptoms of one or more other autoimmune diseases (for example Schmidt syndrome: Hashimoto's thyroiditis with autoimmune Addison's disease and sometimes diabetes mellitus). 4) Genetic predisposition. 5) Preponderance in women. 6) Frequency increasing with age. 7) Remissions and exacerbations.

Thyroid autoantibodies

Antibodies against thyroid microsomes and thyroglobulin may lead to destruction of the thyroid gland, eventually resulting in overt hypothyroidism[4]. In 22 per cent of healthy adults (2838 Australians) at least one of a variety of autoantibodies is found, the most frequent being thyroid microsomal antibody (in 6.6 per cent of all healthy adults). This prevalence is higher in females than in males and the persistence is the same in both sexes, progressively accumulating with increasing age[5]. On the other hand autoantibodies directed against the thyroid stimulating hormone (TSH) receptor may stimulate hormone production and/or follicular cell growth, leading to hyperthyroidism and/or goitre respectively. In 1956 Adams and Purves[6] described the long-acting thyroid stimulator (LATS). This is a specific IgG antibody that stimulates synthesis of thyroid hormones in the mouse thyroid. Subsequently, several other thyroid-stimulating immunoglobulins (TSI) have been described (for review see ref. 7) that can be roughly divided into two groups according to their laboratory assay (table 1).

From the functional point of view 4 types of TSH receptor autoantibodies are distinguished: 1) Thyroid-stimulating immunoglobulins, promoting thyroid hormone synthesis[15]. 2) TSI-blocking immunoglobulins, inhibiting TSI (or TSH) stimulation of thyroid hormone synthesis[16]. 3) Thyroid growth

*Department of Internal Medicine and Clinical Endocrinology, **Department of Nuclear Medicine, Hospital 'Dijkzigt', Erasmus University, Dr. Molewaterplein 40, 3015 GD Rotterdam; ***Department of Cell Biology and Genetics, Erasmus University, Rotterdam.
Present address B.M. van Ouwerkerk: Municipal Hospital, Bankastraat 57, 3312 GA Dordrecht

TABLE 1: THYROID-STIMULATING IMMUNOGLOBULINS

Mouse assay	*Human assay*
Mouse thyroid stimulator (MTS)	human thyroid stimulator (HTS)
Long-acting thyroid stimulator (LATS)[4]	human thyroid adenylcyclase stimulator (HTACS)[13]
LATS protector[4]	thyrotropin-displacing activity (TDA)[14]
Animal assay	
Thyrotropin binding inhibiting immunoglobulin (TBII)[10,11]	
Thyroid growth stimulating immunoglobulin (TGI)[12]	

The different thyroid-stimulating immunoglobulins mentioned in the table are measured using bioassay techniques mostly in mice, or by in-vitro use of animal or human thyroid tissue

immunoglobulins (TGI), especially inducing growth of the follicular cells (non-toxic goitre)[12]. 4) TGI-blocking immunoglobulins, preventing TGI (or TSH) from stimulating cellular growth (primary atrophic myxoedema)[17].

The proposed mechanism by which TSH and TSH receptor antibodies stimulate the thyroid cell is shown in fig. 1. Both TSH and TSH receptor antibodies (acting as TSH agonists) bind to the TSH receptor and stimulate adenylate cyclase by activating a regulatory protein within the thyroid follicular cell membrane. This leads to enhancement of cell function (thyroid hormone synthesis and release).

The heterogeneity of responses in different assay systems for TSI is consistent with interactions with different epitopic sites. Recent studies have suggested that the TSH receptor (its surface interaction site) is composed of a glycoprotein component and a ganglioside component[18]. One might speculate that autoantibodies which inhibit TSH binding to thyroid membranes, are not necessarily identical with antibodies which stimulate function[19]. Monoclonal antibodies to the glycoprotein component (the high affinity binding site of the TSH receptor) can behave as blocking antibodies, while monoclonal antibodies to the gangliosides (the low affinity binding site of the TSH receptor) stimulate thyroid function[20]. Thus, the presence of blocking antibodies could account for the negative results with regard to thyroid-stimulating activity obtained with some IgG's from untreated patients with thyrotoxic GD.

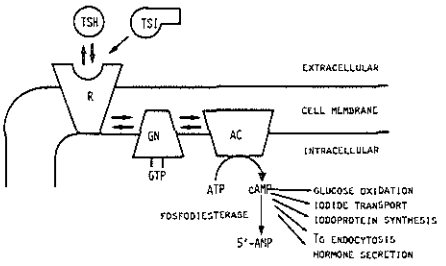

Fig. 1. Proposed mechanism by which TSH and TSH receptor antibodies stimulate the thyroid cell. Both TSH and TSH receptor antibodies (acting as TSH agonists) bind to the TSH receptor and, via a regulatory protein within the thyroid follicular cell membrane, induce stimulation of adenylate cyclase. This leads to stimulation of thyroid cell function. R = receptor, GN = regulatory protein, AC = adenylate cyclase.

In contrast to the stimulatory effect of TSI on thyroid cell function, most other autoantibodies related to other diseases act by blocking cell function (e.g. acetylcholine antibodies in myasthenia gravis, parietal antibodies in pernicious anaemia, insulin antibodies in insulin-resistant diabetes mellitus and beta-adrenergic receptor antibodies in some patients with allergic rhinitis and asthma[21]).

Pathology of thyroid autoimmune diseases

HT is characterized by diffuse destructive inflammatory lesions with enlargement of the thyroid gland, associated with loss and regeneration of thyroid follicular cells and high titres of antibodies to microsome-, thyroglobulin- and other thyroid-specific antigens[22]. In primary myxoedema on the other hand, thyroid atrophy is usually found with low titres of antibodies against microsomes and thyroglobulin. Recently it has been suggested that TGI-blocking[17] and/or TSI-blocking antibodies[3] may be responsible for this phenomenon. Both HT and primary myxoedema lead to hormonal failure.

In GD, foci of lymphocytic infiltration, typical of autoimmune thyroiditis, are usually found together with microsomal antibodies in the serum[1]. However, diffuse hyperplasia, overgrowth of acinar tissue and marked overproduction of thyroid hormones are the main characteristics, considered to be due to thyroid-stimulating antibodies which interact specifically with the TSH receptor and act similarly to the pituitary hormone TSH, but with a 'long-acting' effect.

Graves' disease

GD is a multisystem disease characterized by diffuse goitre and thyrotoxicosis, associated with infiltrative ophthalmopathy (in some 50 per cent of cases) and

infiltrative dermopathy (in some 5 per cent), and occasionally acropachy. GD, HT and primary atrophic myxoedema are closely interlinked autoimmune diseases, which often occur in the same family. One or the other may be seen in identical twins and the conditions may develop concurrently or sequentially in the same individual. In about 10 per cent of GD patients hyperthyroidism spontaneously passes into myxoedema[1]. Exacerbations and spontaneous remissions are classical features of GD. Permanent remissions of hyperthyroidism are observed in about 50 per cent of patients after one or more courses of antithyroid drug therapy[23]. Approximately one-third of patients treated at least 20 years earlier with antithyroid drugs were found to be hypothyroid[24]. Spontaneous fluctuations in the clinical activity of GD may be due to an anti-idiotypic network[25,26] of antibodies, which specifically attack the thyroid-stimulating antibodies.

At physical examination of GD patients, splenomegaly and lymphadenopathy may be observed as an indication of immunological stimulation. Laboratory studies may show lymphocytosis and hypergammaglobulinaemia.

The lack of correlation between thyroid size and thyroid function in GD (2-10 per cent of thyrotoxics do not have goitre) may be understood more easily after demonstration of TGI[12]. These TGI have been found in 85 per cent of patients with untreated GD[27] and may be important also in HT and in patients with euthyroid 'simple' goitre[12]. Parallel to these, TGI-blocking antibodies which block the trophic action of TSH have been demonstrated in patients with primary myxoedema[3,17]. The production of such blocking antibodies in addition to destructive immunological processes, may explain the case of spontaneous hypothyroidism preceding or following thyrotoxicosis[17]. The same may apply to the presence of euthyroidism in TSI-positive subjects.

Ophthalmic Graves' disease (OGD) is usually associated with either hyperthyroid GD or HT and its irregular exacerbations and remissions are in favour of an autoimmune pathogenesis[28]. Originally this clinical entity has been defined as typical ophthalmopathy in euthyroid patients without previous thyroid dysfunction. In the presence of Graves' hyperthyroidism, however, any eye sign present has been designated as Graves' ophthalmopathy. For convenience and since eye signs in both conditions form the same clincial entity, we only use the term OGD in this report. Immunological phenomena have also been proposed to explain the myositis involving periocular muscles which, in combination with fatty infiltration, causes the increased bulk of retro-ocular tissue. Exophthalmos-producing antibodies (EPAb's) from patients with OGD were shown to increase binding of TSH to retro-orbital tissue membranes[29]. These antibodies were considered to lack thyroid-stimulating properties, which is consistent with the clinical observation that OGD may change independently of hyperthyroidism. Murine monoclonal antibodies to a soluble human eye muscle antigen have been described, which lack thyroid-stimulating activity[30], favouring the hypothesis that OGD may be caused by an antibody different from TSI. Very recently the distinction between Graves' hyperthyroidism and OGD has been substantiated by the finding of an IgG which binds specifically to retro-orbital antigen and has no correlation with thyroid autoantibodies[31].

Pretibial myxoedema is seen in about 3-5 per cent of thyrotoxic GD patients and is nearly always associated with exophthalmos[32]. Patients with dermopathy nearly always show high values of TSI, although many TSI-positive patients do not have pretibial myxoedema. The stimulation of subcutaneous tissues to increased production of mucopolysaccharides and oedema may be due to a different type of antibody[33].

Immunoregulation

Several regulatory mechanisms can be envisaged as causing the naturally occurring non-responsiveness to self-antigens: 1) Regulatory T-cell circuits, especially suppressor T-cells. Suppressor T-cells may suppress autoreactive B- and/or helper T-cells (fig. 2). 2) Receptor blockade: saturation of antigen

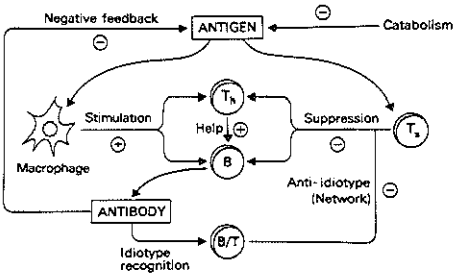

Fig. 2. Regulation of the humoral immune response. Th = helper T-cell, Ts = suppressor T-cell, B = B-cell. An (auto)antigen present on the surface of the macrophage may stimulate helper T-lymphocytes and B-lymphocytes. Activated helper T-cells may also stimulate B-cells to produce (auto)antibodies against the (auto)antigen. Antigen may also directly activate suppressor T-lymphoctyes which suppress both helper T-cells and B-cells, resulting in a decrase in antibody production.

receptors on the B-cell by high concentrations of antigen[34]. 3) Anti-idiotypic antibodies: antibody can by itself act as an antigen and elicit a second, anti-idiotypic antibody. Such anti-idiotypic antibodies may also inhibit autoimmune effects from autoantibodies. 4) Tolerance involving the functional deletion of autoreactive cells[35]. According to this view autoreactive B- and/or T-lymphocytes exist[36] but may be permanently blocked in their responsiveness, for instance by antigen-antibody complexes or suppressor T-cells. 5) Tolerance involving the clonal deletion of autoreactive B- and/or T-cells[37,38] during lymphocytopoiesis. According to this view autoreactive clones develop by somatic mutation, causing the generation of B- and/or T-cells that recognize self-antigens.

Tolerance involving the functional deletion of autoreactive lymphocytes and tolerance involving the clonal deletion of autoreactive lymphocytes are not mutually exclusive. Green et al.[39] have shown that suppressor T-cells capable of abolishing the functional activity of T-cells, can also cause clonal deletion of these T-cells. This clonal deletion could even be demonstrated after removal of suppressor T-cells.

In autoimmune thyroid disease, suppressor T-lymphocytes have been the primary subject of extensive studies. In the cellular mechanisms of tolerance to self-antigens, antigen-specific and antigen-non-specific suppressor T-cells are regarded as major regulatory mediators and have been demonstrated to be involved in several human autoimmune diseases[40]. Most authors believe that antigen-specific suppressor T-lymphocyte dysfunction also plays an important role in the defective immune control in GD. If regulation to induce non-responsiveness fails, then autoimmune processes start to develop. A current, simplified view of cellular cooperation in the immune response is shown in fig. 2. According to this view an (auto)antigen present on the surface of a macrophage may stimulate helper T-lymphocytes and B-lymphocytes. Activated helper T-cells also may stimulate B-lymphocytes to produce antibodies against the antigen. Antigen may also directly activate suppressor T-lymphocytes which suppress both helper T-cells and/or B-cells, resulting in a decrease in antibody production.

Autoimmunity may follow unopposed activation of the helper T-cell population or loss of suppressor T-cell influence. Abnormalities in the numbers and functional activity of suppressor T-cells reactive to self-antigen have been described in several autoimmune disorders[41]. However, in autoimmune disease other factors are also involved, in addition to failure of anti-self suppressor T-cells (alterations of helper cell function and humoral immune response may also play a role).

Evidence is accumulating that, besides humoral autoimmune mechanisms, cellular immunity plays an important role in the pathogenesis of GD. Several studies have shown a decreased number and/or impaired function of peripheral blood suppressor T-lymphocytes in hyperthyroid GD[42-51], although others have described the absence of T-lymphocyte abnormalities[52-56]. In these studies either the number of T-cells has been measured or general T-cell functions have been assessed. The macrophage migration inhibition factor (MIF) assay, however, more specifically tests dysregulation of organ-specific immune response. There is considerable evidence of an organ-specific defect in suppressor T-lymphocytes in GD and HT, as assessed with this test. This defective suppression allows a clone of thyroid-directed helper T-cells to proliferate and stimulate B-cells, which differentiate into plasma cells producing antibodies against thyroid antigens[57]. The same defect may allow certain effector T-lymphocytes to attack the thyroid cells, particularly killer cells that have receptors for the Fc region of IgG antibodies bound to target cells and, when attached to the antibody, may lyse the target cell independent of the presence of complement factors[52].

Defects in immune regulation in man may be genetically related to the structure of the human leucocyte antigen (HLA) complex. We therefore briefly comment on this cluster of genes and the histocompatibility antigens that they code for.

HLA complex

The HLA complex is located on the short arm of human chromosome 6 and consists of three classes of loci (fig. 3): Class I: code for HLA-A, -B and -C histocompatibility antigens. Class II: code for HLA-SB, -DR and -DC histocompatibility antigens. Class III: code for some of the complement proteins.

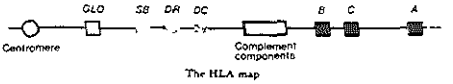

Fig. 3. The HLA complex on the short arm of human chromosome 6. HLA-A, -B and -C code for HLA class I histocompatibility antigens, and HLA-SB, -DR and -DC code for HLA class II histocompatibility antigens, which are intimately involved in regulation of the immune response. Class III code for some of the complement components. GLO = the gene for the erythrocyte enzyme glyoxalase.

HLA-A, -B and -C are surface glycoproteins occurring on virtually all human nucleated cells. Each of the loci coding for HLA-A, -B and -C or -D/DR may carry one of a large number of different alleles[58]. Class II antigens are thougt to be intimately involved in regulation of the immune response ('immune response genes') by somehow guiding the activation of helper T-cells of particular specificity[58]. Class II products have a complex set of immunological functions. They can be glycopeptides, primarily present on the surface of B-lymphocytes, on activated T-lymphocytes and on antigen-presenting macrophages. They have also been discovered on thyroid follicular cells, stimulated with phytohaemagglutinin in vitro[59]. The class II products may act in part by influencing the efficacy of interaction between T-cells and macrophages, between T- and B-lymphocytes and perhaps between B-cells and macrophages[60]. There is evidence that these gene products act in part by determining the manner in which macrophages present foreign antigens to T-cells (dual recognition), thus influencing the response of T-cells and determining whether the responding subset will consist of suppressor or helper T-cells.

A preferential chromosomal association between particular HLA-A,-B,-C and -DR alleles on the same chromosome is called linkage disequilibrium. In GD an association between HLA-B8, and DR3 has been described, probably reflecting a linkage disequilibrium[61]. Expression of HLA-DR on thyrocytes seems to be one of the earliest manifestations of autoimmune lymphocytic thyroiditis[62]. This finding is compatible with several other studies showing that cells which do not normally express class II antigens, do so under the influence of immune reactivity towards those cells[63-65]. Association of HLA-DR and susceptibility to certain diseases is generally characterized by: 1) Most of these diseases have a definite or suspected autoimmune pathogenesis. 2) Susceptibility is a dominant trait (homozygoty is not necessary). 3) The disease develops in only a minority of those carrying the allele associated with susceptibility.

The relative risk of HLA-DR3-carrying persons of developing GD is 3.7[66]. It should be noted, however, that not all patients with a particular disease possess the allele that is associated with susceptibility to that disease. In Graves' disease 64 per cent of the patients carry HLA-DR3[61]. The percentage of positive patients varies for different diseases. Examples of HLA-DR-associated diseases are: coeliac disease (nearly all patients carry HLA-DR3), Graves' disease, juvenile onset diabetes mellitus, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, Sjögren syndrome, systemic lupus erythematosus (SLE), autoimmune Addison's disease and chronic active hepatitis[60]. However, in all these diseases the event which activates autoimmune mechanisms has not yet been determined. A viral aetiology as precipitating factor of autoimmune disease has been hypothesized. In that case a possible explanation may be the following sequence of events: local virus infection → interferon production → induction of DR expression → presentation of autoantigens → autoimmune T-cell induction → activation of effector B- and T-cells[67]. A co-existing abnormality of suppressor T-cell function, often reported in autoimmunity, would be an important factor permitting autoreactive T-cells to evoke autoimmune disease[67]. The equilibrium between helper and suppressor T-cells may be modulated by sex hormones, with androgens favouring suppression[68]. The protective effect of androgens probably explains why autoimmune diseases in general occur more often in women that in men[69]. The finding of a significantly lower fluorescence intensity of monoclonal antibodies against suppressor T-cells in women than in men[70] may indicate defective suppressor cell function in women, favouring the induction of autoimmune disease.

Effect of treatment of Graves' disease

Graves' hyperthyroidism can be treated successfully with the thiocarbamide antithyroid drugs: methimazole (MMI), carbimazole through its bioactivated product MMI, and propylthiouracil. Thiocarbamides have a serum half-life of 3-6 hours and are concentrated in the thyroid gland[71]. The elimination time for MMI in the thyroid gland is much longer than that in blood[72]. MMI not only blocks the synthesis of thyroid hormone by impairing the conversion of iodine to organic compounds and the coupling of iodothyrosins in thyroglobulin, but also has an immunosuppressive effect by inhibiting the production of thyroid-directed antibodies[73] by lymphocytes within the gland, which usually are a major source of antibody production in autoimmune thyroid disease[74,75]. There is still some discussion about the direct influence of hyperthyroxinaemia itself on the defective suppressor T-cell function. Very recently it has been shown that TSI levels decreased similarly in hyperthyroid GD patients treated either with perchlorate or with thiocarbamides[76,77]. Since no immunosuppressive action is known from perchlorate, it was suggested that restoration to the euthyroid state caused the decrease in TSI levels

rather than any immunosuppressive activity of drugs administered. On the other hand, Okita et al.[45] found a generalized suppressor T-cell defect also in patients with Hashimoto's thyroiditis, either euthyroid or hypothyroid, thus suggesting that the thyroid function status did not play an important role in immunoregulation. These observations were confirmed by others[47]. Furthermore it has been shown that, many years after thyroid-destructive therapy with radioactive iodine, the majority of patients with a history of GD have a persistent defect in suppressor T-cell function[78]. Thus the hyperthyroid state per se in this study does not seem to play a decisive role in the defective suppressor cell function. Further studies are needed to solve this controversy about the direct effect of hyperthyroxinaemia and antithyroid drugs on immunoregulation.

Conclusion

Organ-specific autoimmune diseases, including thyroid autoimmune diseases, may be due to a specific defect in immunoregulation by suppressor T-lymphocytes that permits activation of organ-specific autoreactive 'forbidden' clones of helper T-cells. These helper T-cells may stimulate autoreactive B-cells to produce autoantibodies. A special HLA-DR type may be essential for the induction of this defective immunoregulation. When antibodies against thyroglobulin and thyroid microsomes are produced, destruction of the thyroid gland and hormonal failure may result. On the other hand, thyroid-stimulating immunoglobulins are related to hyperthyroidism and thyroid growth-stimulating immunoglobulins to goitre formation. The effects of TSI and TGI (and of TSH) may be prevented by blocking antibodies (TSI block and TGI block) that may evoke hypothyroidism and thyroid atrophy.

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CHAPTER III

CELLULAR AND HUMORAL IMMUNITY IN PATIENTS WITH HYPERTHYROID GRAVES' DISEASE BEFORE, DURING AND AFTER ANTITHYROID DRUG TREATMENT.

B.M. van Ouwerkerk[1], E.P. Krenning[1,2], R. Docter[1], G. Bos[1], A. van Oudenaren[3], R. Benner[3] and G. Hennemann[1].
Departments of Internal Medicine III[1], Nuclear Medicine[2] and Immunology[3], University Hospital "Dijkzigt", Rotterdam, The Netherlands.

Present address B.M. van Ouwerkerk: Department of Internal Medicine, Merwede Hospital Dordrecht, the Netherlands, to whom requests for reprints should be addressed.

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SUMMARY

Many reports of thyroid stimulating immunoglobulins (TSI) in relation to treatment of Graves' disease have been published and with variable results concerning prediction of permanent remission or relapse after therapy. A range of methods has been used and little has been published measuring TSI by using their ability to stimulate cyclic AMP production in human thyroid cells in monolayer culture. We therefore conducted a prospective study of the predictive value of such an assay in patients with hyperthyroid Graves' disease before, during and after treatment of one year with methimazole and thyroid hormone substitution. Furthermore, the possible relationship between activated suppressor T lymphocytes and TSI in patients followed before, during and after medical therapy has been studied.
Patients were divided into two groups; Group I, 15 patients, who stayed in remission and Group II, 14, who relapsed during the first year after discontinuation of therapy. Mean TSI activity did not differ between the two groups before and during the first half year of medication. In the second half year of treatment, however, mean TSI activity was significantly lower in Group I. TSI activity at the end of treatment appeared to have no value in predicting final outcome. Increased TSI activity in Group II during treatment was reflected in an increased pertechnetate thyroidal uptake as compared to that in Group I. There was no relationship between changes in TSI activity and T cell subsets (Leu 1, 2a, 3a). We found no differences in T lymphocytes between the two groups at any time during observation. Subsets of T lymphocytes in both patient groups were not different from normal. If changes in T cell subpopulations are present in Graves' disease, they are at least not measurable in peripheral blood.

INTRODUCTION.

Hyperthyroid Graves' disease is caused by autoantibodies which stimulate thyroid function by binding to the region of the TSH receptor on the thyroid follicular cell membrane (Smith, 1981). The underlying disorder in immunoregulation has not been clarified, but one theory stresses a defect in antigen specific suppressor T cells (Strakosch et al, 1982). There have been many reports about the decline in thyroid autoantibodies during antithyroid drug therapy (Fenzi et al, 1979; Docter et al, 1980; McGregor et al, 1980; McGregor et al, 1982; Gossage et al, 1983; Wenzel and Lente, 1984; Rapoport et al, 1984; Madec et al, 1984; Grant et al, 1985). In most thyroid stimulating immunoglobulins (TSI) were tested using TSH displacing or TSH binding inhibition assays (TBII), which test binding rather than biological activity of TSI. Furthermore not all use human thyroid tissue in their system. The results of these tests show disappointingly weak correlation with activity of disease and are poor predictors of outcome of treatment. TSI can also be measured and probably more appropriately by its ability to produce cAMP in cultured thyroid cells of human origin (Wenzel and Lente, 1984; Rapoport et al,

1984; Madec et al, 1984; Grant et al, 1985). There are to our knowledge only two publications (Rapoport et al, 1984; Madec et al, 1984), which show TSI results using this assay system during follow up of patients, albeit of limited duration. We here report a further, longer such study.
Reports about changes in function and number of suppressor T cells before and during drug treatment in Graves' disease have been conflicting (Okita et al, 1981; Sridama et al, 1982; Topliss et al, 1983; Pacini and DeGroot, 1983; Ludgate et al, 1984). We have also investigated whether numbers and ratios of circulating T cell subsets are affected in Graves' disease, change during therapy, and whether T cell subset-ratios correlated with TSI activity at any stage of the study.

PATIENTS AND METHODS

Forty new patients with hyperthyroidism and diffuse goitre, with or without ophthalmopathy were evaluated. Of them eleven were excluded because of poor compliance (8 patients) or language difficulties (3 patients), leaving 29 of whom 27 were women. At regular intervals clinical examination and laboratory tests were performed, including clinical status and NOSPECS classification (Werner, 1977), serum values of free thyroxine index (FT_4I), total 3,3',5-triiodothyronine (TT_3), TSH response to thyrotropin releasing hormone (TRH), thyroid stimulating immunoglobulins (TSI) and lymphocyte subpopulations. Furthermore thyroid scintigraphy and 20 min thyroidal uptake of ^{99m}Tc pertechnetate were evaluated.
The patients were treated with methimazole (MMI) 10 mg t.i.d. for one year. Six patients showed an allergic reaction and were subsequently treated with propylthiouracil (PTU) 400 mg/day, which they all tolerated well. When euthyroidism was achieved (usually after one month), l-triiodothyronine, 1 ug/kg body weight divided into three doses/day, was added (five patients received l-thyroxine instead in a dose of 2.2 ug/kg/day). Fifteen patients, all women (mean age $\pm$ SD: 33.2 $\pm$ 9 years, range 18–47) stayed in remission (group I) during the first year after cessation of medication and the remaining 14 (mean age $\pm$ SD 41.9 $\pm$ 15.8 years, range 18–72) had a relapse (group II) within a year after cessation of the drugs. The difference in age between the two groups is not significant. Remission was defined as a normal TSH response to TRH and a normal FT_4I and total T_3.
Compliance with medication was checked on the basis of the following parameters: FT_4I (must be near zero except in the 5 patients who received l-thyroxine); TT_3 (must be high to normal); TSH (suppressed 30 min after intravenous TRH).
Serum T_4 (normal values 60–140 nmol/l), serum T_3 (normal values 1.1–3.1 nmol/l) and TSH (normal values < 1.0–4.9 mU/l) were estimated by standard radioimmunoassay techniques. T_3 resin uptake was measured with the Triosorb kit (Abbott laboratories, Chicago, Illinois). FT_4I (normal values 18–41) was calculated by multiplying the concentration of T_4 by the T_3 resin uptake divided by 100. Goitre size was measured by planimetric measurement of 99mTc thyroid scans as described by Smeulers et al (1977).
TSI activity was assayed as published elsewhere (van der Gaag et al, 1985) in whole serum and with ammonium sulphate precipitated IgG. In

essence, thyroid cells were isolated by trypsin (0.2%) digestion of fragments of human thyroid glands obtained at thyroidectomy performed for benign nodular goiter or GD and were washed in buffer containing 1,58 mg/ml NaH_2PO_4, 6.8 mg/ml NaCl, 0.4 mg/ml KCl, 0.2 mg/ml $MgSO_4$, 1 mg/ml glucose, 10 ug/ml phenol and 1.3 mg/ml $NaHCO_3$ with 10% human serum and centrifugation. The suspension was filtered through a nylon stocking and washed with Medium 199, containing 10% newborn calf serum, 60 U/ml penicillin, 60 ug/ml streptomycin, 60 ug/ml neomycin sulfate and 2.2 mg/ml $NaHCO_3$. Cells were counted and plated in 24-well culture plates with Medium 199 (1-2x105 cells/well in 2 ml). Plates were incubated for 24-48 hrs at 37 C in a water saturated 5% CO_2-95% air mixture. To start measurements, the culture fluid was removed, and monolayers were incubated at 37 C with 0.25 ml test medium (Richmond Park Memorial Institute (RPMI) 1640, containing 0.5% fetal calf serum, 60 U/ml penicillin, 60 ug/ml streptomycin, 60 ug/ml neomycin sulfate, 5 ug/ml transferrin, 8 ug/ml insulin, 362 ng/ml cortisol, 110 ug/ml 3-isobutyl-1-methylxantine, 3.5 mg/ml $NaHCO_3$ and 20 ul (heated at 56 C) whole serum, or 200 ug of the immunoglobulin precipitate to be tested. Both preparations gave similar results. After 16 hrs the supernatants were removed and assayed for cAMP according to the method of Brown et al, 1971), using standards diluted in test medium. Sera from patients were systematically tested in three separate assays with cell preparations from different thyroids. For each patient all sequentially sampled sera were tested in the same assay. The amounts of cAMP measured in the wells after 24 hr incubation with patients sera were compared with those obtained with 20 ul of normal sera and with sera known for their TSI activity. Correction for interassay differences of cAMP values was performed by normalisation with respect to the mean values of the samples with known TSI activity. Values were expressed as pmol cAMP/well. The mean basal value of cAMP in 27 normal subjects was (mean $\pm$ SD) 6.6 $\pm$ 2.1 pmol/well for the various cell preparations. Mean values of cAMP in the samples with known TSI ranged from 26 to 186 pmol/well. The lowest concentration of added bovine TSH that stimulated cAMP production was 0.01 mU/ml.

Peripheral mononuclear cells were isolated by Ficoll-Isopaque gradient centrifugation by the method of Boyum (1967), washed twice, suspended in a medium consisting of 25% fetal calf serum, 65% RPMI and 10% dimethylsulfoxide and subsequently frozen to -80 C in a polystyrene box and stored in liquid nitrogen. Vials with frozen mononuclear cells were thawed at 37 C and subsequently washed and suspended in 5% bovine serum albumin in phosphate-buffered saline. Nucleated cells were counted with a Coulter counter.

Generally the yield of viable mononuclear cells after thawing was 75%, according to the scatter profile by flow cytometry. The cell suspension was diluted to a concentration of 10^7 cells/ml. One hundred ul of the suspension was incubated with 1 ug of fluorescein isothiocyanate labelled (FITC) anti-Leu antibodies (Beckton Dickinson Monoclonal Antibody Center, Sunnyvale, CA, USA). The following antibodies were used: Anti-Leu-1 (directed against a human common T cell antigen), anti-Leu-2a (against a human cytotoxic/suppressor T cell antigen), and anti-Leu-3a (against a human helper/inducer T cell antigen).

After incubation for 30 min and washing at 4 C, cells were analysed by flow cytometry. Flow cytometry analysis was performed with a fluorescence-activated cell sorter (FACS II, Becton Dickinson FACS

Systems, Sunnyvale, CA, USA). From each sample 10^4 cells were analysed, as described in detail by van Hattum et al (1984). Correction for variation between values of two different series of measurements on two different FACS systems has been performed. Statistical analysis was performed using Student's two tailed t-test (paired and unpaired).

RESULTS

The changes in FT_4I values of the two groups are shown in Figs. 1 and 2. No significant difference in mean values between the two groups was found before or while on treatment (Table). Relapse of hyperthyroidism (Group II) was found after a mean period of 3 months (range 1-12 months) (fig. 2). The TSI values of both groups are shown in the Table. After 6 and 12 months of MMI treatment the difference was significant ($p < 0.01$ and $p < 0.05$ resp.), with a lower TSI level in group I (remission) than in group II (relapse) . In group I TSI levels became normal in 8 out of 14 patients tested after 6 months of therapy and in 11 out of 15 patients tested after 12 months of therapy, but in group II in only 3 out of 12 patients after 6 months of therapy and in 6 out of 14 patients tested after 12 months of therapy.

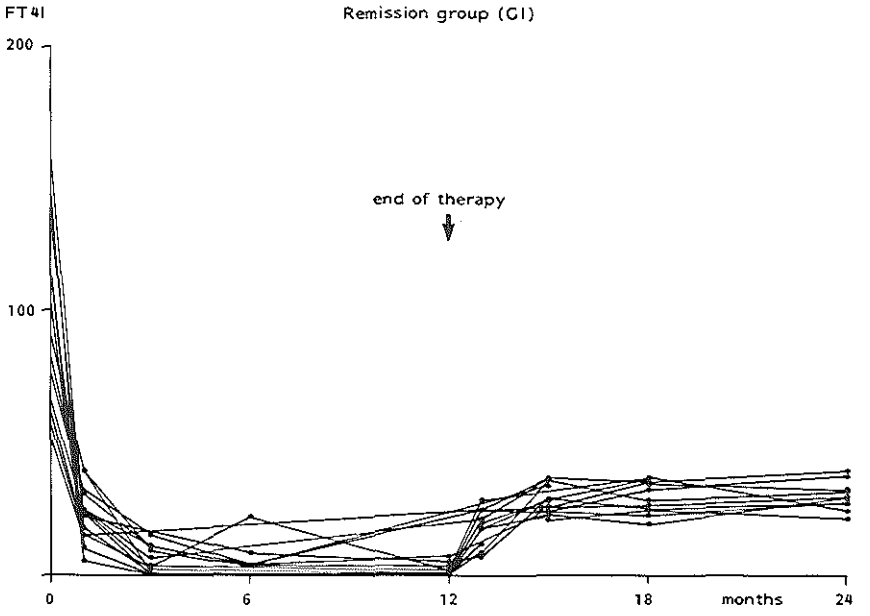

Fig.1: FT_4I in patients of Group I before, during and after treatment (normal values 18-41). The FT_4I values of one patient receiving thyroxine substitution, are not included.

TABLE: Studies before, during and after drug therapy for Graves'disease in 15 patients (group I, GI) who stayed in remission one year after treatment and 14 patients (group II, GII) who relapsed within one year after treatment.

	pretreatment		at 6 months of therapy		at 12 months of therapy	
	G I	G II	G I	G II	G I	G II
FT_4I	90.8 ± 8.5	108.8 ± 10.9	4.5 ± 1.6	3.6 ± 2.6	2.4 ± 0.7	3.3 ± 1.6
(N 18-41)	n=15	n=14	n=13	n=11	n=11	n=13
	NS					
TT_3	6.09 ± 0.9	6.45 ± 0.9	4.3 ± 0.5	3.5 ± 0.5	3.8 ± 0.5	3.6 ± 0.4
(N 1.1-3.1 nmol/l)	n=14	n=10	n=12	n=11	n=11	n=11
	NS					
^{99m}Tc-uptake%	13.4 ± 2.6	12.5 ± 2.1	8.3 ± 3.1	11.1 ± 2.1	3.0 ± 0.6	7.4 ± 1.8
	n=14	n=11	n=13	n=12	n=12	n=10
	NS		NS		p<0.05	
Goitre size cm^2	23.9 ± 1.3	28.2 ± 4.6				
	n=15	n=14				
	NS					
TSI c AMP	28.9 ± 6.0	37.6 ± 7.0	11.0 ± 2.9	32.2 ± 7.3	6.7 ± 1.2	20.6 ± 6.5
(N < 10.8	(3-68)	(8-84)	(0-35)	(7-85)	(0-15)	(4-100)
	n=14	n=12	n=14	n=12	n=15	n=14
pmol/well)	NS		p<0.01		p<0.05	
Th (x 10^9/l)	1.3 ± 0.1	1.4 ± 0.2	1.5 ± 0.2	1.7 ± 0.3	1.6 ± 0.3	1.5 ± 0.2
	n=14	n=12	n=13	n=12	n=14	n=13
	NS		NS		NS	
Ts (x10^9/l)	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	0.9 ± 0.1
	n=14	n=12	n=13	n=12	n=14	n=13
	NS		NS		NS	
Th/Ts	1.7 ± 0.2	1.7 ± 0.2	1.5 ± 0.2	1.5 ± 0.1	1.4 ± 0.1	1.7 ± 0.2
(N 0.9-2.1)	n=14	n=12	n=13	n=12	n=14	n=13

Values are expressed as mean ± SEM. Note: 3 patients entered the study after six months of therapy. One patient in Group I and 4 patients in Group II had T_4 substitution instead of T_3. The FT_4I values from these patients are not included in the treatment period.

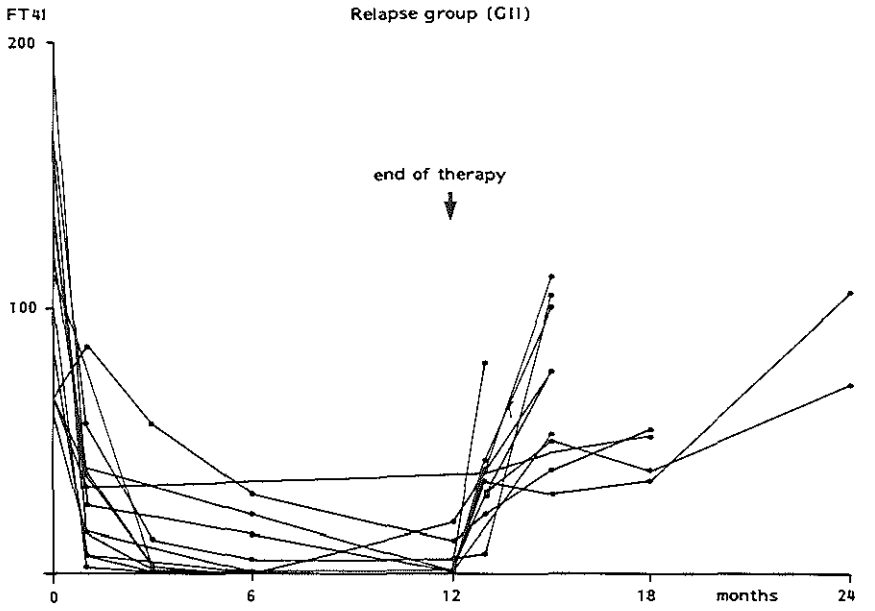

Fig. 2: FT_4I in patients of Group II before, during and after treatment (normal values 18-41). The FT_4 values of 4 patients receiving thyroxine substitution, are not included.

Peripheral blood suppressor and helper T cell content and the T helper/T suppressor (TH/TS) ratio before therapy in the 2 groups did not significantly differ from normal, or change during treatment. Between the 2 groups also no difference was found (Table). Furthermore no correlation between TSI activity and TH/TS ratio could be found in the two groups of patients.

To test compliance with treatment FT_4I has been measured during treatment. As patients were being treated with 30 mg MMI per day (or 400 mg PTU per day) and $l\text{-}T_3$, FT_4I values were indeed low to zero. Furthermore substitution with triiodothyronine resulted in high to supranormal values of TT_3 (Table). In group I one patient showed on one occasion (6 months) an inadequate suppression of FT_4I, whereas in group II two patients on several occasions had too high FT_4I values (Not included in the table are the 5 patients, who were treated with T_4 instead of T_3-substitution). Goitre size between the two groups was not significantly different (Table). The stimulatory effect of TSI on thyroid activity expressed as 20 min uptake of ^{99m}Tc showed no significant difference between the two groups, neither in the untreated state, nor at 6 months of therapy. At 12 months of therapy, however, we found a significant difference (3.0% vs 7.4% in group I and II respectively, Table). A poor correlation was found between TSI activity and pertechnetate uptake in both groups (r=0.36 and r=0.43), when considering all values. The correlation between pretreatment TSI activity and uptake of pertechnetate was r=0.60 in group I and r=0.45 in group II.

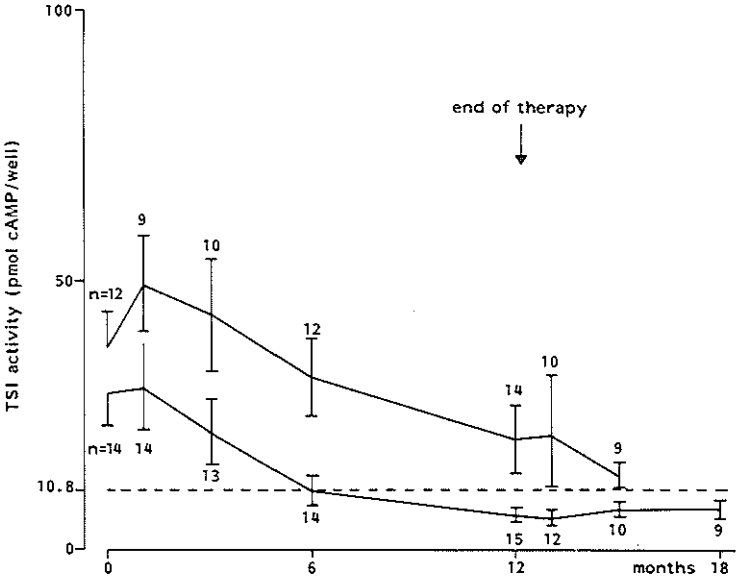

Fig.3: TSI activity (mean ± SEM) before, during and after drug treatment in patients who remained euthyroid in the first year after discontinuation of therapy (group I) (lower line) and who relapsed (group II) (upper line). Dotted line represents the upper normal value. Significant differences only at 6 and 12 months ($p < 0.01$ and $p < 0.05$ respectively).

The remission group (I) was further followed in the second and third year after cessation of therapy (mean follow up 28 months after therapy, range 17-42 months). In this period 5 more patients relapsed (at month 18, 20, 22, 24 and 30 respectively). Therefore the total relapse rate was 66% (19 of 29 patients) within 2.5 years after treatment. The final remission group (34%, 10 out of 29 patients) had a mean TSI value of 7.0 (range 0-15) pmol/well after 12 months of therapy.
Four of the 5 patients from group I who ultimately relapsed, had normal TSI activity at the end of therapy suggesting a temporary remission of the disease.In group II 6 out of 14 patients became TSI negative after 12 months of therapy despite subsequent relapse. On the other hand 4 out of 15 serum TSI values in group I remained elevated (although moderately) despite sustained remission.

DISCUSSION

In the present study TSI has been measured with a biological test using human thyroid cells. Again, as in a previous study from our laboratory (Docter et al 1980) a poor correlation was found between

TSI activity and uptake of pertechnetate, as has been described earlier (Gossage et al, 1983[b]) for patients with untreated Graves' disease. This might be due to the fact, that TSI are a mixture of immunoglobulins which are of variable effectiveness in activating the TSH receptor (Gossage et al, 1983[b]).
Comparison of TSI levels in the 2 groups of GD patients in our study did not reveal a significant difference in the hyperthyroid (untreated) state, but significant differences at 6 and 12 months of antithyroid drug therapy. This has been mentioned in earlier reports for the same TSI assay (Rapoport et al, 1984; Madec et al, 1984) and for other types of assays (McGregor et al, 1980; McGregor et al, 1982; Gossage et al, 1983[a]; Wenzel and Lente, 1984; Grant et al, 1985). TSI levels in our hands apparently do not predict prognosis of final outcome of the disease. This is in contrast with most of the studies in this field. We conclude that in case of untreated Graves' disease no single parameter that is normally used on a large scale to diagnose Graves' disease, can predict the outcome after antithyroid drug treatment. However, when TSI is used in combination with HLA-DR3 a more precise prediction might be made (Irvine et al, 1977; Bech et al, 1977; McGregor et al, 1980[b]), although this has not been confirmed by others (Allannic et al, 1983).
Although signs of hyperthyroidism disappeared during drug treatment, our patients were still biochemically thyrotoxic (blunted TRH test, not illustrated). Nevertheless TSI levels declined in both patient groups. This fact is not in favour of the theory of some (Wenzel and Lente, 1984), that restoration to euthyroidism per se (induced by MMI) causes diminution of autoantibody production. Others consider the thyroid hormone status irrelevant to immunoregulation (see also Weetman et al, 1984).
The compliance to drug treatment in both groups was acceptably good. In the relapse group 2 patients showed occasionally too high FT_4I values during treatment. This may be either due to insufficient compliance or to the large goitre of these patients. Probably a larger dose of MMI should have been given in these cases ,as advocated by Romaldini et al (1983).
The cause of production of autoantibodies directed against the TSH receptor of the thyroid follicular cell in Graves' disease has been subject of many studies. Several theories concerning a disordered immunoregulation in organ specific autoimmune disease have been put forward (Reinherz and Schlossman, 1980; Fauci, 1980). Antigen specific and antigen-non-specific suppressor T cells are regarded as major regulatory mediators in several human autoimmune diseases (Reinherz and Schlossman, 1980).
Since, in addition to humoral autoimmune mechanisms (Adams and Kennedy, 1967; Smith and Hall, 1974; Docter et al, 1980) cellular autoimmunity plays an important role (Okita et al, 1981; Sridama et al, 1982; Topliss et al, 1983; Pacini and DeGroot, 1983; Ludgate et al, 1984), we attempted to demonstrate changes in T lymphocyte subsets in peripheral blood using monoclonal antibodies against the characteristic glycoproteins of these cells. Unfortunately, these monoclonal antibodies do not identify clones of specifically activated T lymphocytes, but give an indication of the size of the whole population of the subsets. We could not demonstrate a substantial decrease (in peripheral blood) in suppressor T lymphocyte numbers nor an increase in the helper/suppressor ratios in untreated hyperthyroid

patients, neither during nor after treatment. This is in contrast to suggestions of several of the above mentioned studies (Okita et al, 1981; Sridama et al, 1982; Topliss et al, 1983; Pacini and DeGroot, 1983; Ludgate et al, 1984). We did not find T lymphocyte abnormalities in GD patients which is in agreement with others (Allison, 1976; Beall and Kruger, 1980; MacLean et al, 1981; Wall and Chartier, 1981; Ludgate et al, 1985), nor a significant effect of MMI treatment on these T cell subsets. Ratios of helper to suppressor T cells in normal controls ranged from 0.9 to 2.1 as reported in other studies (Iwatani et al, 1983; Wall et al, 1983). It is difficult to demonstrate any defective immunoregulation by examining peripheral blood lymphocytes, especially when one does not differentiate between specifically activated lymphocytes and other lymphocytes. The number of suppressor T lymphocytes, which specifically suppress the helper T lymphocytes specific for thyroid antigens probably represents only a small percentage of the total T lymphocyte population. This might be the reason for our finding and that of others (Wall et al, 1983), of normal numbers of total suppressor T lymphocytes in the circulation. Another explanation of our negative findings might be that the disordered immunoregulation is exclusively located in the involved organ. A decrease in the percentage of suppressor T cells in thyroid tissue together with a normal incidence of suppressor T cells in peripheral blood has been described (Wall et al, 1983).
In conclusion; although the mean TSI level as measured by cAMP response in human thyroid monolayers at 6 and 12 months during therapy differed in the two groups, a single serum TSI value had no predictive significance regarding the final prognosis after medical therapy. Furthermore, we were unable to demonstrate a decreased number of T suppressor cells or an increased TH/TS ratio in peripheral blood of GD patients.

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CHAPTER IV

Measurement of thyroid stimulating immunoglobulins: Comparison of the method measuring cAMP production in human thyrocytes in culture and two different kits (THYBIA and TRAK), measuring thyrotropin binding inhibiting immunoglobulins.

B.M. van Ouwerkerk, R. Docter, G. Bos and G. Hennemann.

Department of Internal Medicine III and Clinical Endocrinology, University Hospital Rotterdam Dijkzigt, The Netherlands.

Present adress BMvO: Department of Internal Medicine, Merwede Hospital Dordrecht, The Netherlands, to whom requests for reprints should be adressed.

submitted for publication

Abstract

Sera of patients with hyperthyroid Graves' disease were tested for the presence of thyroid stimulating immunoglobulins (TSI) in a system measuring cAMP production by cultured human thyrocytes. These sera were also tested for thyrotropin binding inhibiting immunoglobulins (TBII) in two commercial kits (THYBIA and TRAK). In the TSI assay positive results were found in 20 out of 26 untreated patients with Graves' hyperthyroidism (77%), whereas the THYBIA assay was positive in 10 out of 26 patients (38%) and the TRAK assay in 9 out of 26 patients (35%). In the three assays a significant decline of activity was observed during treatment with methimazole. There was a moderate correlation between the cAMP assay and the THYBIA and TRAK assay ($r=0.67$ and 0.66 respectively). The correlation between the two TBII assays was $r=0.68$.
No predictive value could be attributed to a positive or a negative result in any single test after one year of antithyroid drug treatment. However, a relapse was seen in all (six) patients in whom both the TSI and one TBII test was positive at the end of drug therapy. No correlation could be found between TSI or TBII and antibodies against thyroglobulin (Tg-Ab) and microsomes (M-Ab). No significant decline in Tg-Ab was found during antithyroid drug treatment but the decline in M-Ab was significant ($p<0.025$), suggesting a drug-induced change in autoimmune reaction.It is concluded, that the cAMP assay for estimating TSI is more sensitive than the assays measuring inhibition of TSH binding. After one year of antithyroid drug treatment both types of tests (when used single) do not have predictive value towards the final outcome of treatment.

INTRODUCTION

Graves' disease (GD) is a multisystem disease characterised by diffuse goiter and thyrotoxicosis associated with ophthalmopathy, dermopathy and occasionally acropachy. Hyperthyroidism in GD is due to autoantibodies which stimulate thyroid function by binding to or in the region of the thyroid stimulating hormone (TSH) receptor (1). These antibodies can be measured in various ways: as longacting thyroid stimulator (LATS) (2,3) or LATS protector (4) in mice, or by their activity of inhibition of binding of TSH to the receptor: thyrotropin binding inhibiting immunoglobulins (TBII) assay (5,6). Other assays are those based on the cyclic AMP response of thyroid tissue to TSH or thyroid stimulating immunoglobulins (TSI), using thyroid cell membranes (7,8), thyroid slices (9,10) or human thyroid cells in monolayer culture (11-16), and the cytochemical bioassay (17).
There is considerable difference in reported percentages of positive sera in patients with hyperthyroid Graves' disease (30-95%). This may be, apart from differences in sensitivity, due to the fact that different assays may measure different immunoglobulins. For example, the TBII assay measures immunoglobulins in their capacity to prevent TSH from binding to the TSH receptor. These immunoglobulins may thus be as well thyroid stimulating as blocking immunoglobulins. The cyclic AMP response assay however measures only stimulating activity. This latter assay is therefore at least theoretically more

specific towards the measurement of stimulating immunoglobulins than the TBII assay. To test the merits of these two types of assay-systems in active Graves' disease, we evaluated sera of patients with unaequivocal hyperthyroid Graves' disease in the biological assay using human thyrocytes in monolayer culture and compared the results with that of the TBII assay as available in two commercial kits. Also the clinical relevance of the different types of assays with regard to prognostic significance after antithyroid drug treatment was assessed.

PATIENTS AND METHODS

Twentynine patients with hyperthyroidism and diffuse goiter (with or without OGD) were included in this study. The diagnosis was made on the basis of clinical assessment, elevated serum free thyroxine index (FT_4I), failure of serum TSH to respond to thyrotropin-releasing hormone (TRH), and diffuse thyroidal uptake on 99mTc scintigraphy. Patients were all treated with methimazole 30 mg/day and when euthyroidism was achieved (usually after one month) triiodothyronine was added in doses between 50 and 100 ug daily (depending on body weight). This regimen was maintained during one year. Ten patients (mean age $\pm$ SD, 34.9 $\pm$ 9.4 years) were in remission (group I) during a mean follow up of 30.9 months (range 17-42) after discontinuation of therapy. Nineteen patients (38.7 $\pm$ 15 years) relapsed (group II); patients 16 to 29 (Table I) within one year after discontinuation of medication and patients 11 to 15 between 18 and 30 months after therapy.

At month 0,6 and 12 during drug therapy serum samples were drawn for estimation of FT_4I, TT_3, antibodies against thyroglobulin and microsomes, TSI (cyclic AMP assay) and TBII (THYBIA and TRAK assay). Also at these times basal TSH and 30 min after TRH stimulation was measured.TSI activity was assayed in whole serum and in IgG's precipitated with ammonium sulphate, as published elsewhere (18,19). The upper limit of normal is 10.8 pmol cAMP/well.

TBII was assayed with the THYBIA-assay (Byk-Mallinckrodt Radiodiagnostica, Dietzenbach 2, Germany) and the TRAK-assay (Henning Berlin GMBH, Berlin 2, Germany). The upper limit of normal, estimated in our laboratory in 20 controls, was 20 U/L for the THYBIA assay and 16 % for the TRAK assay.

Antibodies against thyroglobulin (Tg-Ab) were assayed with the SB-AB-HTG-2 assay (International-CIS, St-Quentin-Yvelines CEDEX, France).

Antibodies against microsomes (M-Ab) were assayed with the Thymune-M assay (Wellcome reagents limited, Beckenham BR3 3BS, England). Titres from negative to strongly positive were expressed as 0 to 6 respectively.

Goitre size was measured by planimetry of the thyroid scintigram, as described by Smeulers et al (20).

Statistical analysis was performed using Students t-test and X^2-test.

RESULTS

The data of the patients subdivided in group I and II are summarized in Table I.

Table I: Measurements of FT_4I, goiter size and different antibodies directed to the thyroid before and during (6 and 12 months) medical therapy in 29 patients with hyperthyroid Graves' disease. Patients 1-10 had remission of the disease (group I) and patients 11-29 relapsed (group II).

PATIENT no.	SEX F/M	AGE yrs	FT_4I start	GOITER size cm^2	TSI (pmol cAMP/well) 0	6 months	12	THYBIA (U/l) 0	6 months	12	TRAK (%) 0	6 months	12	Tg-Ab (ug/ul) 0	6 months	12	M-Ab 0	6 months	12
1	F	28	52	30			6.3			0			1			3.6			3
2	F	45	134	25	66.4	29.2	9.2	24.9	0.7	5.6	37	9	8	7.8	4.6	2.9	0	0	0
3	F	25	102	17	49.8	15.6	6.6	13.6	10.2	9.5	2	0	0	3.9	2.4	2.3	4	2	3
4	F	43	64	23	8.9	7.0	6.3	12.4	11.8	11.4	1	1	0	23.7	9.3	8.6	3	3	2
5	F	33	77	15	8.2	9.7	4.4	9.8	0	0.4	2	5	0	3.2	3.2	3.9	3	2	2
6	F	18	83	22	15.2	4.0	11.2	5.4	0	0	9	0	0	4.0	2.8	3.1	3	1	1
7	F	42	61	22	30.2	20.4	11.3	9.3	8.5	11.4	1	0	0	2.8	2.7	3.0	0	0	0
8	F	34	66	25	50.0	12.6	15.0	18.0	3.6	14.0	5	0	0		74.2	83.5		3	3
9	F	47	112	31	10.3	0	0	2.0	9.1	8.6	2	0	0	6.9	4.5	3.5	3	2	2
10	F	34	57	25	3.0	0	0	11.5	5.8	8.2	0	0	0	3.3	3.8	2.3	3	2	3
11	F	30	56	19	13.2	11.6	9.0	12.2	12.5	2.9	1	0	0	3.0	2.6	5.0	2	1	1
12	F	26	115	23	39.5	7.5	7.4	18.6	11.7	10.7	1	0	0	7.5	3.8	4.5	4	1	0
13	F	22	91	27	10.3	0	0	11.4	10.1	8.9	0	0	0	4.1	4.8	8.3	2	2	2
14	F	43	138	33	68.1	34.7	11.8	35.1	20.3	13.7	172	28	14	4.4	1.6	1.4	3	1	1
15	F	28	181	25	31.5	2.0	1.4	11.2	15.5	12.3	14	3	1	4.0	2.1	2.7	3	2	2
16	F	72	65	13	8.4	7.8	6.9	3.0	3.8	3.7	10	10	5	3.0	4.1	2.9	4	3	3
17	F	25	119	26	16.1	7.1	8.6	6.1	0	0	12	8	6	3.2	3.6	3.5	6	4	4
18	F	36	85	29	65.4	51.0	26.0	22.2	13.8	10.3	56	24	7	3.8	2.8	1.7	4	3	3
19	F	42	60	16	11.5	9.6	7.2	18.2	18.8	19.2	8	6	6	3.1	2.1	2.9	3	3	3
20	M	40	187	30	37.0	66.0	22.5	27.4	32.9	26.9	30	62	28	3.5	2.4	2.3	2	1	0
21	F	31	66	83	31.6	45.4	28.1	45.6	31.3	22.1	69	21	11	2.5	2.7	2.6	0	1	1
22	F	41	66	18			7.6			5.3			0			3.0			0
23	F	52	137	17			8.2			6.9			1			5.6			3
24	F	49	100	18	83.9	85.4	99.8	21.8	24.2	28.5	56	48	63	3.1	2.4	1.6	1	0	0
25	M	70	102	27	14.6	20.6	14.4	7.4	6.3	11.6	9	5	8	2.8	2.9	2.2	0	0	0
26	F	50	101	35	35.6	29.5	27.1	20.7	15.8	14.7	21	10	7	2.5	3.0	3.2	0	0	0
27	F	35	99	35	60.3	11.9	3.9	38.4	7.2	6.6	7	2	20	23.1	15.3	10.3	5	3	2
28	F	18	165	32	59.0	37.9	15.8	36.5	33.5	21.0	131	93	34	3.7	3.1	3.4	0	0	0
29	F	25	160	18	27.6	14.2	12.9	31.9	18.9	15.3	69	18	10	3.2	2.4	2.6	3	3	2
mean		37.4	95.7	23.7	32.9	20.8	13.4	18.3	12.6	10.7	27.9	13.6	7.9	5.4		6.4	2.44		1.48
sd		13.3	38.6	6.8	23.3	21.5	18.3	11.7	9.8	7.6	42.8	22.7	13.7	5.6		15.0	1.7		1.26

Goiter size between these 2 groups was not significantly different.
Positive results in the TSI assay were found in 20 out of 26 untreated hyperthyroid patients (77%). After 6 months of drug therapy positive TSI activity was present in 15 of 26 patients (58%) and after 12 months in 12 of 29 patients (41%).
Comparing the TSI assay with the THYBIA assay we found a correlation coefficient (r) of 0.67. The TSI versus TRAK correlation showed a r=0.66. TRAK versus THYBIA showed a r=0.68.
Comparison of the percentages of positive results in our patients (Table II) shows higher sensitivity of the TSI assay than of the THYBIA and TRAK assay.
A significant difference between TSI and THYBIA or TRAK results was found in the untreated patients ($p < 0.005$), and after 6 months ($p < 0.005$) and 12 months ($p < 0.005$).
At the end of treatment 6 patients were positive in both TSI and at least one TBII assay and a relapse was observed in all. A relapse within one year after treatment was observed in 5 out of 8 patients, who remained positive for only one assay (TBII or TSI). In 15 patients both types of assay became negative after MMI treatment, but 8 of these relapsed after discontinuation of therapy (Table III).

Table II: positive results in assays for TSI and TBII.

	untreated	after 6 months of therapy	after 12 months of therapy
TSI	20/26 (77%)	15/26 (58%)	12/29 (41%)
THYBIA	10/26 (38%) a	5/26 (19%) a	4/29 (14%) a
TRAK	9/26 (35%) a	7/26 (27%) a	4/29 (14%) a

a: $p < 0.005$ versus TSI.

Table III: Results of TSI assay in combination with TBII assay, at the end of one year of MMI treatment in 29 patients and relapse frequency within one year after discontinuation of MMI.

	Positive TSI and TBII	Only one assay positive	Negative TSI and TBII
No. of patients after one year of MMI therapy	6	8	15
Relapse of hyperthyroidism	6	5	8

No relationship was found between TSI or TBII and thyroid microsomal and thyroglobulin antibodies.
A significant decline in activity of TSI ($p < 0.01$), THYBIA ($p<0.01$) and TRAK ($p < 0.025$) during treatment was found as an indication of immunological change during MMI treatment. This change was also found in M-Ab ($p<0.025$).

DISCUSSION

Investigations using TSI or TBII assays give variable results with regard to predictability of outcome of medical treatment of hyperthyroid Graves' disease (6, 14, 15,21).

We compared our own TSI bioassay with commercially available TBII kits. In 20 out of 26 hyperthyroid patients (77%) we could detect TSI activity. In the more widely used type of assay (TBII) we showed positive results in 10 out of 26 (38%) in the THYBIA kit and in 9 of 26 (35%) in the TRAK kit. These findings indicate higher sensitivity of the TSI bioassay as compared to the TBII assays (Table II).
Some authors describe a positive correlation between TSI and TBII (22), but others did not find any significant relationship (16,23,24). We found a moderate correlation between TSI and both kits, which may indicate in that TBII activity is not necessarily synonymus to thyroid stimulating activity (25). This underlines the heterogenity of anti-TSH receptor antibodies (6).
In our study using the TSI assay (18,19), prediction of relapse appeared to be difficult. In the remission group 3 out of 10 patients remained TSI positive and in the relapse group 10 out of 19 patients became TSI negative after a MMI course of one year. This is in contrast to a more positive report concerning predictability of prognosis with regard to TSI (14). Others (15) however also could not attribute prognostic significance to a negative TSI result at the end of treatment.
When a combination of TBII and TSI assays are used, a better prognostic significance in predicting relapse has been described (26). In our hands relapse was found in all 6 patients (out of 29) who remained positive for both TSI and one TBII assay at the end of treatment. However, in 15 patients who became negative for both TSI and TBII after one year of MMI treatment, 8 patients (53%) still relapsed. A change in thyroglobulin- and microsomal-antibodies , which are related to thyroid destruction leading to hypothyroidism (27) might also be found during MMI treatment (28,29). In our study no significant change in Tg-Ab during treatment could be found, but the difference in M-Ab before and one year after MMI treatment was significant. This latter phenomenon may be an indirect indication of the immuno-suppressive effect of MMI and confirms earlier reports (28,29).
The high relapse frequency (50%) after one year MMI course, which is even higher (67%) after 30 months of follow up, poses the question of the usefulness of antithyroid drug treatment as a definite form of therapy of Graves' thyrotoxicosis. Probably more patients should be treated with radioactive iodine (30) despite the disadvantage of a higher incidence of hypothyroidism as time progresses (31,32).

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CHAPTER V

Severe ophthalmic Graves' disease and autoimmune thyroid disorders with different clinical expression

B. M. VAN OUWERKERK, E. N. W. JANSSENS, R. WIJNGAARDE* and E. P. KRENNING

SUMMARY

Ophthalmic Graves' disease (OGD) may be associated with autoimmune thyroid disease in various ways. We present the medical histories of two patients who developed OGD in the course of autoimmune thyroid disease with varying clinical expression, which needed different therapeutic approaches. The possible pathogenesis and the therapy of OGD are reviewed. Neth J Med 1983;26:176.

INTRODUCTION

In the general population Graves' disease (GD) occurs in 0.3 per cent of females and in 0.05 per cent of males[1]; 70 per cent of these patients have eye signs (NOSPECS class I; table I). At present the pathogenesis of ophthalmic Graves' disease (OGD) is unknown, but all hypotheses suggest or underline an autoimmune disorder[4], in which humoral and cellular immunity initiates the development of lymphocytic infiltration and oedema of orbital tissues. Extraocular muscles in particular are affected, but lacrimal glands, orbital fat and the optic nerve may also be involved.

TABLE I: ABRIDGED CLASSIFICATION OF EYE CHANGES OF OGD: NOSPECS[2,3]

*Class**	*Symptoms*
0	no signs or symptoms
1	only signs, no symptoms (signs of lid retraction and stare)
2	soft tissue involvement (symptoms and signs)
3	proptosis
4	extraocular muscle involvement (diplopia, etc.)
5	corneal involvement (keratitis)
6	loss of visual acuity (optic nerve involvement)

* For one patient it is not necessary to pass the successive classes to reach class 6. For instance, loss of visual acuity without other eye signs may be encountered in OGD.

The finding of thyroglobulin receptors in orbital tissues has led to the hypothesis that antibodies against thyroglobulin bound to these receptors cause the formation of immune complexes with subsequent intraorbital cellular damage and lymphocytic infiltration[5]. When cellular damage occurs, a release of muscle protein and histamine may sensitize lymphocytes, with a subsequent cellular immune reaction against muscle cell membranes. Increased vascular permeability may lead to oedema and thickening of extraocular muscle. An increased retrobulbar content can also result from production of mucopolysaccharides by mast cells and fibroblasts with subsequent fluid retention, and from oedema due to venous and lymphatic obstruction[5]. Another hypothesis[6] postulates that subunits of thyrotropin (TSH) in combination with a specific IgG cause OGD. In this respect one could understand the clinical observation (never well-analysed) that patients with Graves' disease who are overtreated with antithyroid drugs and thus develop hypothyroidism with high levels of TSH, are prone to develop OGD or show exacerbation of eye signs.

Circulating autoantibodies against a soluble eye muscle antigen have recently been demonstrated in patients with OGD[4]. Mouse monoclonal antibodies against a specific eye muscle antigen appeared to crossreact with thyroidal microsomes, underlining the relationship between thyroid autoimmune disease and OGD. The prevalence of thyroglobulin antibodies in Hashimoto's thyroiditis (HT) is 85 per cent; in Graves' disease it is 50 per cent[7] and in euthyroid OGD 24-36 per cent[8,9]. Thyroid-stimulating immunoglobulins (TSI) have been detected in patients with untreated GD in 60-80 per cent, in HT in 22-51 per cent[10] and in euthyroid OGD in 43 per cent[11]. This indicates the relationship between HT, GD and OGD as illustrated in fig. 1 (adapted from Brown[12]).

Departments of Internal Medicine III and Clinical Endocrinology; *Department of Ophthalmology, Hospital 'Dijkzigt', Erasmus University, Dr. Molewaterplein 40, 3015 GD Rotterdam.

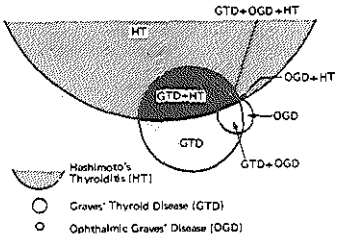

Fig. 1. Relationship between Hashimoto's thyroiditis, Graves' disease and ophthalmic Graves' disease (adapted from Brown[12]).

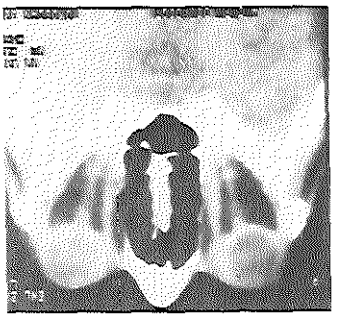

Fig. 2. Orbital CT-scan of patient A with severe OGD, showing bilateral proptosis and symmetrical enlargement of medial rectus muscles.

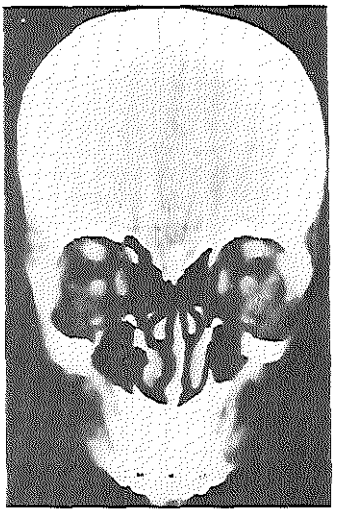

Fig. 3. Coronal CT-scan of patient A with severe OGD, showing enlargement of the superior complexes (superior rectus muscle and superior oblique muscle) and of the medial rectus muscles.

METHODS

Thyroxine (T_4; normal values 60-140 nmol/l) and thyrotropin (normal values < 1.0 – 5 mU/l) were estimated by radio-immunoassay[13,14]. Triiodothyronine (T_3) resin uptake was measured with the Triosorb kit from Abbott Laboratories. The free T_4 index (FT_4I) was calculated by multiplying the concentration of T_4 by the T_3 resin uptake divided by 100. Normal values for our laboratory are 18-38. The presence of thyroid-stimulating immunoglobulins was tested by measuring the cyclic AMP response in human thyroid cell cultures after addition of patient's serum IgG (unpublished, R. Docter and G. Bos). Thyroglobulin and microsomal autoantibodies were determined using an indirect immunofluorescence test.

CASE REPORTS

Patient A

In 1975 a 44-year-old male patient with a 15-year history of diabetes mellitus, well controlled with 28 U long-acting insulin each day, presented at the out-patient clinic with fatigue, rapid increase in weight, palpebral swelling and chilliness. He appeared hypothyroid. The pulse rate was 54/min and a nodular goitre (twice the normal size) was present. No signs of OGD other than palpebral oedema were noted (NOSPECS class 0-1). Laboratory tests revealed a total serum thyroxine level of 2 nmol/l and a TSH level of 226 mU/l. The ^{131}I thyroid uptake showed a 1% dose after 3 h. Circulating thyroid microsomal and thyroglobulin antibodies were weakly positive. Primary hypothyroidism due to autoimmune thyroiditis was diagnosed at that time. Thyranon (desiccated thyroid containing T_4 and T_3) was given up to 100 mg daily. The patient felt well with T_4 levels of about 60 nmol/l until two years later, when he started complaining of lacrimation, fatigue, hyperhidrosis and weight loss. Examination revealed marked palpebral oedema, increased conjunctival injection, lacrimation and chemosis. Hertel values (measurement of proptosis of the eye) were 19 mm for both eyes (normal value 12-22 mm; NOSPECS class 2). The size of the goitre had not changed.

The total serum T_4 level was 185 nmol/l. This value is too high during substitution with desiccated thyroid: T_4 levels of about 60 nmol/l are normally achieved for euthyroidism[15]. After discontinuation of substitution therapy, serum T_4 levels did not fall (T_4 184 nmol/l, FT_4I 59). Circulating microsomal and thyroglobulin antibodies were absent at this time. ^{131}I thyroid scanning gave a somewhat enlarged thyroid image with diffuse distribution of the isotope. The patient had therefore developed Graves' hyperthyroidism with OGD. Treatment was started with carbimazole (4 × 10 mg daily) in combination with levothyroxine replacement. This regimen led to euthyroidism. The patient's condition improved but eye signs gradually exacerbated in the absence of increased TSH levels (serum TSH < 1 mU/l). In view of severe exophthalmos with 25-27 mm bilateral proptosis and develop-

ment of diplopia (NOSPECS class 4), 60 mg prednisone daily was given and led to rapid improvement. Diplopia disappeared and chemosis, palpebral oedema, conjunctival injection and proptosis (Hertel values of 22 mm) diminished. Prednisone was gradually reduced until, after one year, at a dosage of 7.5 mg daily the eye signs exacerbated again. Resumption of prednisone treatment in even larger doses gave no improvement. Diplopia recurred with a 25 mm proptosis of both eyes and marked palpebral oedema. CT-scanning of the orbit revealed swelling of the superior and medial rectus muscles of both eyes, consistent with OGD (figs 2 and 3). Retro-orbital irradiation with 2000 rad (20 Gray) was given in ten sessions[16]. The diplopia disappeared and eye signs gradually improved. The patient is doing well one year after irradiation. Proptosis of both eyes is 22 mm (NOSPECS class 2).

Patient B

In 1981 an 81-year-old woman with hypertension and coronary insufficiency visited the ophthalmologist with palpebral oedema and progressive loss of visual acuity. There was fatigability and in the past two months she had not been able to read newspapers. Clinically she was hypothyroid, but otherwise healthy. The pulse rate was 64/min and the tendon reflexes were low. Palpebral oedema was prominent. The thyroid gland was not palpable. VOD was 1/300 and VOS 1/10 (NOSPECS class 6). The right eye showed cataract. The free T_4 index was 16. The TSH level was 26.7 mU/l with a rise to 84.3 mU/l 30 min after stimulation with 200 µg TRH i.v. Circulating thyroglobulin antibodies (1:320), microsomal antibodies (1:320) and thyroid-stimulating immunoglobulins were present. Orbital CT-scanning revealed thickening of the superior and medial rectus muscles of both eyes (fig. 4). The latency time of visual evoked potentials[17] was increased. Primary hypothyroidism (Hashimoto's disease, atrophic variant) and OGD were diagnosed.

Treatment with thyroxine and prednisone was started. This resulted in dramatic improvement of VOS to 10/10 (NOSPECS class 2). Probably due to cataract of the right eye VOD remained 2/300. One year later she appeared to be in good health without prednisone and with thyroxine replacement. Orbital CT-scanning after one month of prednisone therapy showed only a moderate decrease in the thickening of the extraocular muscles (fig. 5). The latency time of visual evoked potentials showed normalization parallel to the improvement in visual acuity.

DISCUSSION

Both patients are likely to have had an autoimmune thyroid disorder, with development of OGD. Patient A had primary hypothyroidism, but frank hyperthyroidism developed after a three-year course of replacement therapy with desiccated thyroid, which is a very unusual sequence of events. In view of the associated distinct eye signs, Graves' disease was diagnosed. Patient B had primary hypothyroidism (Hashimoto's disease, as assessed by the high titres of antibodies against thyroglobulin and microsomes) and OGD.

The diagnosis of Hashimoto's thyroiditis is unlikely in patient A, because initially he showed low titres of antibodies against thyroglobulins and microsomes. Although seronegative HT has been described[18], an alternative explanation of the hypothyroidism may be the presence of circulating thyroid-blocking antibodies, which block the stimulating action of TSH or TSI on the thyroid gland. These antibodies have been demonstrated in

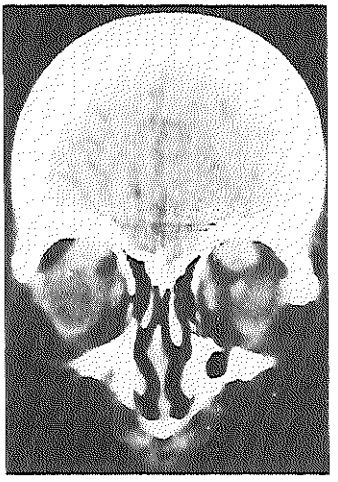

Fig. 4. Coronal CT-scan of patient B with primary hypothyroidism and severe OGD, showing massive bilateral enlargement of the superior complexes.

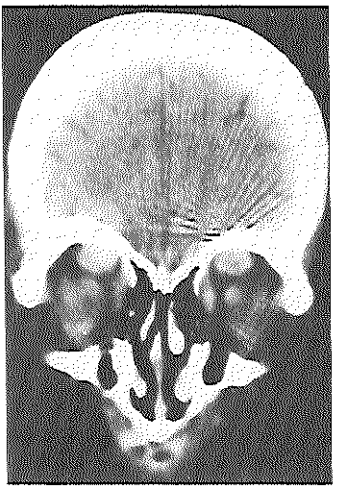

Fig. 5. Coronal CT-scan of patient B after one month of corticosteroid treatment, showing a moderate decrease in the dimensions of the superior complexes.

thyroid autoimmune disease[19]. Monoclonal antibodies to the thyrotropin receptor, which can block thyroid stimulation, have been recently obtained[20]. Subsequently patient A became hyperthyroid. One could speculate that, during the hypothyroid phase, thyroid-blocking IgG activity prevailed in the serum, whereas in the following hyperthyroid phase TSI activity predominated. This unusual sequence of clinical events (hyperthyroidism following hypothyroidism) has been mentioned only a few times in the literature[21–26]. With regard to GD the sequence of events is usually the reverse, i.e. hyperthyroidism followed by hypothyroidism. The latter is thought to be caused by humoral and cellular immune-mediated thyroid destruction[12]. This type of thyroid destruction is probably also the cause of the primary hypothyroidism in patient B.

The symptoms of OGD are commonly classified according to 'NOSPECS' (table I). Goitre, hyper- and hypothyroidism are important signs to support this diagnosis. Usually the thyroid status is analysed by measuring serum FT_4I and, when hypothyroidism is suspected, TSH has to be determined. When FT_4I and TSH are normal, additional endocrinological studies (TRH test and/or T_4 or T_3 suppression test) are warranted, e.g. to establish thyroid autonomous function as might be found in so-called euthyroid OGD (fig. 1). In clinically euthyroid OGD, about 60 per cent of patients show an abnormal TSH response to TRH, 25 per cent show normal TRH and suppression tests, and the remainder show an abnormal suppression test only. The follow-up on euthyroid OGD patients in whom both tests are normal, may show alterations indicating development of hyper- or hypothyroidism[8]. Furthermore, the presence of circulating antibodies against thyroglobulin and microsomes, and/or TSI can confirm an autoimmune thyroid disorder associated with OGD. Increased latency times of visual evoked potentials (VEP) can be demonstrated in patients with hypothyroidism and in OGD[17]. In hypothyroidism this may be due to impaired nerve conduction. In OGD, pressure of enlarged retro-orbital muscles in the apex of the orbit may cause this phenomenon, as demonstrated in patient B. Other possible causes of VEP alterations are circulatory disturbances due to mechanical pressure and cellular infiltration of the vessel walls.

To exclude other disorders causing exophthalmos in euthyroid subjects without thyroid autonomy, especially when the eye signs are unilateral, a CT-scan of the orbit may be very helpful. In over 90 per cent of all OGD patients, orbital CT-scanning reveals thickening of the extraocular muscles, especially of the inferior and medial rectus muscles[27]. Common causes of unilateral proptosis, besides OGD, are orbital (primary and secondary) neoplasms, vascular anomalies and encephalocele. Axial myopia and inflammatory orbital pseudotumour must be considered as well[28,29]. Cooperation between internists, ophthalmologists, radiologists, neurosurgeons and otolaryngologists is therefore required in these cases.

The therapeutic approach to OGD depends on the severity of signs and symptoms. Above all, euthyroidism must be achieved. In cases of hypothyroidism this means simple substitution with synthetic thyroxine preparations. When hyperthyroidism exists, antithyroid drug treatment with carbimazole or propylthiouracil is indicated. It has been demonstrated[30] that carbimazole has an immunosuppressive effect as well. Theoretically this may have implications for the treatment of OGD. In our opinion it is very important to combine antithyroid drugs with thyroxine, in view of the clinical experience that overtreatment with antithyroid drugs with subsequent increase in TSH levels causes deterioration of eye signs. When OGD deteriorates despite optimal treatment, corticosteroids (e.g. 60 mg prednisone daily) during a short period usually give improvement. The prednisone dose should be reduced very gradually after improvement of OGD, because flare-up of eye signs is not uncommon when prednisone is discontinued too abruptly. In the case of progressive corneal involvement, impaired visual acuity or limitation of eye movements as a result of massive infiltration of retro-orbital tissue, a more aggressive approach is often needed. Two forms of treatment are then available: surgical orbital decompression[31] and supervoltage orbital radiotherapy[16]. The latter method has gained in popularity since side effects in the sense of damage to the nervous system, in particular to the optic nerve, have not been documented despite long-term follow-up (15 years). The disadvantages of decompression are the high frequency (about 25 per cent) of postoperative diplopia[31], transient numbness of the upper lip on the side of the operation and possible recurrence of the ophthalmopathy. Despite the absence of indications that orbital irradiation has significant side effects, one should probably be cautious to treat young patients in this way, at least until follow-up studies over longer periods confirm the absence of harmful effects. In patients under 40, orbital decompression should be seriously considered when aggressive therapy is needed. The average and maximal decreases in proptosis in both types of treatment are comparable. After irradiation, diminution of proptosis may continue for several months; but the (pos-

sible) visual improvement occurs rapidly. Recurrence of ophthalmopathy after orbital irradiation has not been described[16,32]. Improvement of severe diplopia with these therapies is generally poor, and an external eye muscle operation is indicated as soon as the eye signs are stabilized. Because most patients with severe OGD are over 40 (in contrast to the average age of all patients with Graves' disease), orbital irradiation has become the treatment of choice for most of our patients with severe eye signs of recent onset.

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CHAPTER VI

Radiotherapy of severe ophthalmic Graves'disease

B.M. van Ouwerkerk*[1], R. Wijngaarde**, G. Hennemann*, J.G. van Andel****, and E.P. Krenning*, ***
Departments of *Internal Medicine III and Clinical Endocrinology, **Ophthalmology, and ***Nuclear Medicine of the University Hospital Rotterdam, "Dijkzigt", Dr Molewaterplein 40, 30215 GD Rotterdam and ****Department of Radiotherapy of the Rotterdam Radio Therapeutic Institute (R.R.T.I.).

ABSTRACT. The effects of orbital irradiation on Ophthalmic Graves' Disease (OGD) were evaluated in 24 patients . All patients were irradiated after a median duration of eye symptoms of 12 months (range 3-36 months). Irradiation therapy was performed for loss of visual acuity, progressive diplopia and/or keratitis due to recent progression of eye symptoms. During a follow up period of 2-4.5 years, in 11 patients after irradiation , a mean decrease in proptosis oculi of 5.1 mm was found while 8 of these patients showed a mean increase in visual acuity of 0.26. Apparently, a decrease of activity of the inflammatory process in retrobulbar tissues can be achieved after irradiation, over a longer period of time in many patients. Furthermore concomitantly administered corticosteroids could be tapered off completely in all patients. Treatment of severe OGD of recent onset with irradiation, in combination with a short course of prednisone is very effective. This study also shows that prednisone treatment alone is not very successful because of the high recurrence rate of eye signs after decrease of the dose and the many side effects of the drug.

INTRODUCTION

At the present time the genesis of Ophthalmic Graves' Disease (OGD)[2] is not clarified, but all hypotheses suggest or underline an autoimmune genesis. Recently, circulating autoantibodies against a soluble eye-muscle antigen have been found in OGD (1). Humoral and cellular immune mechanisms initiate the development of lymphocytic infiltration and edema of orbital tissues (extra-ocular muscles, orbital fat, lacrimal glands and even the optic nerve).
For severe symptoms of OGD like diplopia, keratitis or loss of visual acuity (Nospecs class 4,5 or 6; Tabl 1), many therapeutical approaches are advocated (2), but no standard method of therapy has been generally accepted until now. Most authors describe improvement by systemic administration of corticosteroids in large quantities (60-120 mg prednisone daily) (2-4). Another mode of treatment consists of radiotherapy of retrobulbar tissues. In the late sixties Kriss et al. introduced supervoltage orbital radiotherapy. This group reported an efficacy in 21 of 23 patients (5). It was postulated that failure to respond to supervoltage radiotherapy was due to longstanding (more than 2 years) duration of symptoms (with subsequent fibrosis of the affected retroorbital muscles).
Here we report on 24 patients with severe OGD and the effect of corticosteroids and radiotherapy on proptosis, visual acuity and other ocular sings.

PATIENTS AND METHODS

Twenty-four patients (17 women age 32-74, mean 53.9 years and 7 men age 40-71, mean 59.5 years) with severe OGD were referred to the "Orbitateam" in our hospital because of progressive deterioration of eye sings during or after a history of thyroid disease. At the time of referral 3 subgroups of patients could be distinguished:
i) Fourteen patients treated with a combination of carbimazole and thyroxine for Graves' disease. Seven of these patients were hypothyroid due to undersubstitution with thyroxine during carbimazole administration as assessed by an elevated TSH and/or low serum thyroxine (T_4) level. The other seven patients were euthyroid on a combination of carbimazole 40 mg and thyroxine 2.2 ug/kg body weight daily.
ii) Eight patients treated with thyroxine. Five because of primary hypothyroidism due to previous treatment for hyperthyroid Graves' disease with radioactive iodine. Two patients, who underwent partial thyroidectomy for Graves' disease became hypothyroid. One patient be-

[1]Present address: Gemeente-Ziekenhuis, Dordrecht.
Authors are members of the "Orbitateam".

[2]Originally this clinical entity has been defined as typical ophthalmopathy in euthyroid patients without previous thyroid dysfunction. In the presence of Graves' hyperthyroidism however eye signs have been designated as Graves' ophthalmopathy. For convenience and since eye signs in both conditions constitute the same clinical entity, typical eye signs in this report are termed as OGD in patients with (previous or present) Graves' disease independent of the thyroid status at the time of referral.

Key-words: Ophthalmic Graves' disease, Graves' ophthalmopathy, exophthalmos, proptosis oculi, diplopia, keratitis, prednisone, radiotherapy.

Correspondence: Dr. E.P. Krenning, Department of Nuclear Medicine, University Hospital Rotterdam "Dijkzigt", Rotterdam, the Netherlands.

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came hypothyroid after a history of untreated hyperthyroid Graves' disease.
iii) Two patients without medication. They were clinically euthyroid, had normal thyroid function indices, but blunted TSH response to a bolus injection of 200 ug TRH.
After referral, the following medication was given to all patients of subgroup a and c: carbimazole 40 mg daily in combination with adequate thyroxine replacement (TSH levels <1 mU/l) (6). In the patients of subgroup b thyroxine substitution was also given in doses resulting in low serum TSH levels. Despite optimal TSH suppression and clinical euthyroidism, eye signs exacerbated in all 24 patients.
Eighteen of them were subsequently treated with prednisone. Six patients did not receive high doses of prednisone because of coexisting hypertension. The general scheme of steroid treatment was as follows: an initial daily dose of 30-60 mg prednisone was continued during 4 weeks and was subsequently tapered off by 5 mg every 2 weeks. All 18 patients showed rapid improvement of eye signs, but this appeared to be transient, because eye signs worsened when a dose of 20-15 mg daily was reached. Subsequently, retrobulbar irradiation was applied to these 18 patients and to the other 6 not treated with prednisone. In all but one patient (in 5 hypertensive patients blood pressure was by this time normalised by medical treatment) prednisone (15-30 mg daily) was given during a mean period of 1 month and tapered off completely in a mean period of 6 months (range 1-16 months). The indication for irradiation was based on: visual impairment (n = 12, Nospecs 6), diplopia (n = 12, Nospecs 4) or keratitis (n = 4, Nospecs 5) with lacrimation (see Table 1). The ophthalmopathy index was calculated as described by Donaldson et al. (5), with slight modification after Bartalena et al. (8). Three patients were irradiated unilaterally, all the others on both sides. Follow up of the first 11 patients extends from 2-4.5 years and of the whole group from 2 months to 4.5. years.
The median duration of eye signs of the 24 patients until irradiation was 12 months (range 3-36 months). Examination of all patients by the ophthalmologist included measurement of proptosis (with Hertel exophthalmometer: normal values 15-22 mm) (7), visual acuity, motility of the eyes, intraocular pressure and evaluation of conjunctival injection. Visual Evoked Potentials (VEP) were measured one or several times during treatment in 22 patients, because delayed evoked potentials, being a sensitive symptom not only of demyelination, but also of compression of the optic nerve (9), are found in patients with OGD (10). VEP were elicited with a checkerboard pattern reversal stimulus of 30° subtended visual angle and an individual check size of 1°. Contrast between the checks was 80 % and the reversal frequency 2 per second. Amplitudes and latencies of the main positive peak were measured, of which the latency-measurements are only reproduced, amplitudes being rather variable.
In 21 patients at one or more times, orbital computerized tomograms (CT scans) were made in order to evaluate ocular muscle enlargement consistent with OGD and to rule out other anomalies causing proptosis or other signs mimicking OGD.
Irradiation was performed with a 4 meV collimated photon beam. The anterior border of the irradiation field was always 12 mm behind the front of the cornea to avoid irradiation of the ipsilateral lens. As posterior border the anterior margin of the sella turcica was taken. The lower and upper border were the floor and the roof of the orbit respectively. The field was angled 5° posteriorly in order to avoid irradiation of the contralateral lens (5). The head of the patient was fixed by a perspex cast. The total administered dose on the 80 % isodose was 20 Gray delivered in 10 fractions of 2 Gray each, given over two periods of 5 days in two consecutive weeks (1 Gray = 100 Rad).
Statistical analysis of the results was performed using paired and unpaired Student t-tests.

Table 1 - *Abridged classification of eye changes of OGD: "Nospecs" American Thyroid Association (7)*

Class[1]	Symptoms
0	No signs or symptoms
1	Only signs, no symptoms (signs of lidretraction and stare)
2	Soft tissue involvement (symptoms and signs)
3	Proptosis
4	Extraocular muscle involvement (diplopia etc.)
5	Corneal involvement (keratitis)
6	Sight loss (optic nerve involvement)

[1] For one patient it is not necessary to pass the successive classes to reach class 6. For instance sight loss without other eye signs may be encountered in OGD. Radiotherapy is generally indicated in the severe forms of classes 2 and 3 and in classes 4-6.

RESULTS

The deterioration of eye signs before referral to our hospital is shown, when columns A and B of Figures 1 and 2 are compared. The data in column A were collected by the referring ophthalmologists and internists. Adjusting carbimazole and/or thyroxine therapy did not result in significant improvement of eye signs and corticosteroids were administered. The changes in proptosis and visual acuity after 4 weeks of high dose prednisone in 18 patients are shown in column C of Figures 1 and 2. All 18 patients with severe proptosis (>25 mm) responded to this therapy, but the decrease in proptosis of the whole group is not significant. The mean improvement of visual acuity of 0.21 after 4 weeks of high dose prednisone is significant ($p < 0.025$). High dose prednisone resulted in rapid (1-2 weeks) improvement of soft tissue involvement in nine patients. In the other nine patients the effect of corticosteroids was only partial in the sense that, the longstanding diplopia, a

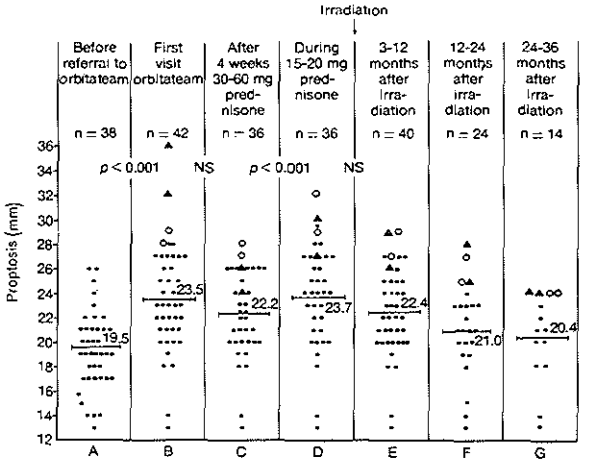

Fig. 1 - *Proptosis in patients with OGD during and after different forms of therapy. Each eye is considered separately (n = number of eyes measured). Patients with extremely abnormal values before or at referral are symbolized (o....) to enable the evaluation of treatment in these particular cases.*

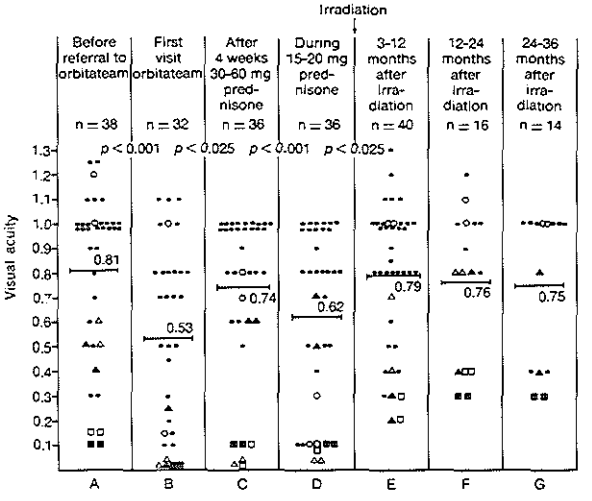

Fig. 2 - *Visual acuity in patients with OGD during and after different forms of therapy. Each eye is considered separately. (see Fig. 1).*

symptom that was not prominent in the other nine patients, did not disappear appreciably. Lowering the prednisone dose by 5 mg in 2 weeks always resulted in worsening of OGD signs, when a dose between 20 and 15 mg was reached. This is shown in Figures 1 and 2, column D. The changes in proptosis and visual acuity are significant ($p < 0.001$, paired t-test). Side effects of prednisone were frequently observed: appearance of a moon face in all patients, deep venous thrombosis (1 x), deterioration of diabetes mellitus requiring insulin treatment (3 x), depressive psychosis (1 x) and progressive intraocular hypertension (2 x). The results of irradiation in the 24 patients are shown in column E-G in Figures 1 and 2. Figure 1 shows the mean diminution of proptosis at different time intervals after irradiation. Three to 12 months after irradiation the mean decrease of proptosis was 1.3 mm (not significant). Statistical comparison of columns F and G with the others is not

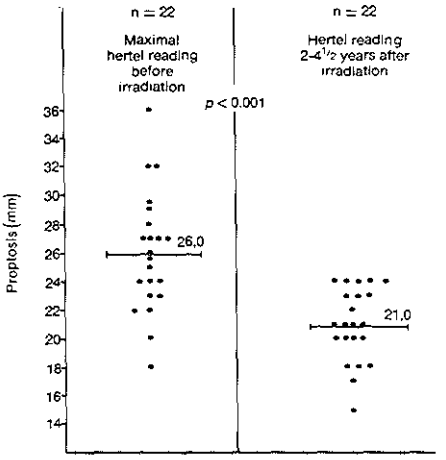

Fig. 3 - *Proptosis before and 2-4.5 years after irradiation in the first 11 patients of the study (p < 0.001). Each eye is considered separately. (see Fig. 1).*

justified because of the great difference in number of patients. However, separate analysis of the follow up of the first 11 patients for 2-4.5 years after irradiation shows a mean (± SD) decrease of proptosis of 5.0 ± 0,6 mm ($p < 0.001$, Fig. 3). The mean improvement of visual acuity of 0.17 ($p < 0.025$) 3-12 months after irradiation is shown in Figure 2, column E. The results in the second and third year after irradiation are not suitable for statistical comparison. VEP measurements were performed in 22 patients. In 12 patients a second or third VEP was made in order to follow the electrophysiological process of optic nerve compression. When visual acuity was below 0.4, delayed responses were always observed, as has been shown earlier (10). While, in older age (beyond 60 years), delayed latencies were also observed in 23 of 31 eyes, when visual acuity was above 0.4. These findings are interpreted as an early sign of optic nerve compression, since in most of these cases the CT scan showed obliteration of the orbital apex by enlarged muscles. Figure 4 shows the values of latencies of the patients examined before and during the several modes of treatments. A mean diminution of latencies of 9 msec. ($p < 0.025$) after prednisone therapy is shown. But also an increase of 11 msec. ($p < 0.025$) in the first year after irradiation is observed. The numbers of measurements in columns F and G are too small for comparison.

The orbital CT scans of 21 patients showed abnormalities consistent with OGD: rectus muscle enlargement, especially of the inferior and medial rectus muscles, as described earlier (11), uni- and bilateral proptosis and increase of retroorbital fat volume. In CT scans of 13 patients, apical orbital obliteration by enlarged muscles was observed. Nine of these 13 patients had decrease of visual acuity at referral, probably caused by compression of the optic nerve. In these cases rapid improvement of visual acuity could be obtained with corticosteroids. Four patients with apical obliteration on the CT scan did not have decrease of visual acuity. The orbital CT scans of 8 patients did not show apical obliteration. Four of these had visual impairment, which improved after corticosteroid treatment in 3 cases.

Motility disturbances of eyes, like upgaze diplopia responded well to prednisone, but severe diplopia, i.e. in all directions, did not improve. Irradiation failed to result in disapperance of longstanding diplopia in only two out of 9 patients. In these cases retroposition of the most affected muscle was performed.

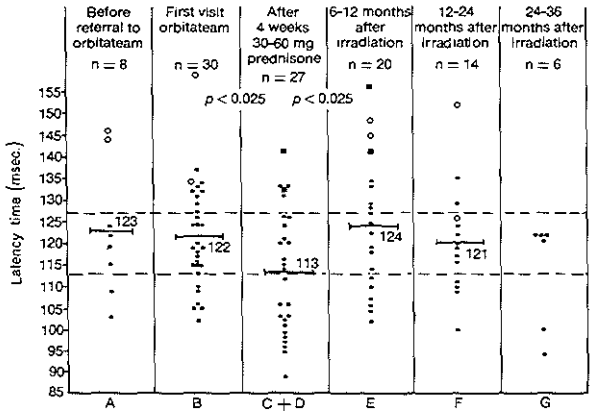

Fig. 4 - *Latencies (indicating conduction abnormalities of the optic nerve) of visual evoked potentials during and after different forms of treatment for OGD. Each eye is considered separately. Dotted lines represent the mean value and the mean + 2SD value (upper dotted line) of the normal population (9).*

Visual fields (Goldmann) were examined in 18 patients. In 9 patients no defects were seen. The other 9 patients however showed impairment of central sensitivity. Five of these patients showed improvement (or normalization) of visual fields after irradiation.

Secondary signs of OGD like chemosis and conjunctival hyperemia were observed in all 24 patients and always disappeared gradually.

Elevated intraocular pressures were frequently observed, especially on upgaze, as stretching of the often affected inferior rectus muscle causes elevation of pressure (12). In case of glaucoma, treatment with local beta-blocking agents was installed. Under these conditions no serious problems with regard to intraocular pressure were encountered during prednisone and/or irradiation. The effect of irradiation on elevated upgaze-pressure has not been systematically examined. The mean ophthalmopathy index before prednisone treatment was 6.4 and decreased to 2.56 after irradiation (mean follow up period 20 months, range 3-50 months), the mean difference being 3.85 ± 0.41 ($p < 0.001$).

DISCUSSION

Ophthalmic Graves' disease is often a self-limiting condition. When severe signs, such as keratitis, ophthalmoplegia and optic neuropathy occur, treatment is required in order to avoid permanent diplopia or blindness. Corticosteroids and orbital irradiation have been used in these cases. When hyperthyroidism is coexisting, "high" doses (40 mg daily) of carbimazole, (30 mg) methimazole (MMI) or (400 mg) propylthiouracil (PTU) in combination with adequate doses of thyroxine substitution is our choice of treatment, instead of treatment with antithyroid drugs alone in decreasing amounts to a (low) continuing dose. To our feeling (admittedly never properly investigated), eye signs less frequently deteriorate during the combined regimen, in which higher doses of thiocarbamides are used, as compared when antithyroid drugs are given alone. An additional advantage of administration of higher doses of these antithyroid drugs might be the immunosuppressive action of MMI (carbimazole is directly converted to MMI, which is the active compound) and PTU, which has been described in hyperthyroid Graves' disease and which is dose dependent (13). It might be possible that these drugs also act as immunosuppressants in OGD. That we have not observed a beneficial effect on OGD during optimal medical treatment in the patients reported here, may be explained by the fact that OGD had deteriorated already too far and/or that medical treatment before institution of prednisone was given for relatively too short a period to show any possible favorable effect.

In 18 of the 24 patients corticosteroids were given as advocated by Werner (4), with good results in 9 and with partial effect in the other nine cases. As longterm treatment with high dose of corticosteroids is undesirable and lowering the dose to 20-15 mg prednisone daily always resulted in flare up of eye sings in all our patients, radiotherapy was instituted in combination with prednisone. The group of Kriss was the first to describe the effects of supervoltage orbital irradiation in 23 patients, of whom fourteen had shown poor results with corticosteroid treatment (5). Fifteen of these 23 patients showed excellent response to irradiation (without concomitant corticosteroid-administration) and 6 patients showed fair response. Two patients with ophthalmoplegia did not respond. In 1977 Covington et al. described improvement in 5 of 7 patients after supervoltage orbital irradiation with 2000 Rad (14), These patients had failed to respond to corticosteroids. The results were poor in an almost blind patient and in one patient recurrence after irradiation was described. Disappositing results were described in 20 patients, irradiated without concomitant corticosteroid treatment (15). Of these patients with moderately severe OGD only 7 patients showed some response and 4 patients improved minimally, while symptoms in 9 patients remained unchanged. Probably the long duration of eye symptoms (34 months on the average) was responsible for these poor results.

Better results are obtained when a combination of irradiation and corticosteroids is applied in patients with OGD of recent onset. Good response after this combination therapy has been described, especially when eye signs recently developed (8, 16). Irradiation plus corticosteroid treatment is also superior to treatment with corticosteroids alone (8), because relapse after discontinuation of steroids is frequently observed. Improvement of eye symptoms has been described in 13 of 16 patients treated with corticosteroids, but 8 relapsed after discontinuation of corticosteroids (17). Even a relapse rate of 100 % has been described (18). This is also our experience and a major reason to institute irradiation in our patients with OGD.

In the follow up for 2-4.5 years of the first 11 patients treated with radiotherapy in combination with corticosteroids we noticed a response in all patients with regard to proptosis. The mean decrease was 5.0 mm (Fig. 3) and this seems to be better than previously described (8, 14, 16). The important factor, which may explain the better results in our patients is most probably the fact that the duration of eye sings before irradiation in our patients is much shorter. This aspect is stressed by Bartalena et al. (8), when they consider a subgroup of 7 patients with a short period of eye symptoms who show a mean decrease of proptosis of 4 mm after irradiation. At this stage it is not feasible to evaluate the ultimate beneficial effects of irradiation on OGD in our patients, because it is not known how long therapeutical effects continue after irradiation.

A significant decrease of latencies of the VEP was observed after treatment with prednisone ($p < 0.025$, Fig. 4), in association with an increase of visual acuity (Fig. 2). After irradiation, however, an increase of laten-

cies is shown 6-12 months later ($p < 0.025$, Fig. 4, column E). Since the longer follow up groups are not suitable for comparison (because of the low numbers in the latter), it is not known if this increase is only temporarily. The increase of latencies after irradiation may be suggestive of a side effect of irradiation. Longer follow up of more patients is obviously needed to investigate the exact permanent effects of irradiation on VEP latencies.

Other side effects during irradiation were not observed. Probably because most of our patients were protected from temporary increase of edema by a moderate dose of prednisone (15-30 mg). In all cases corticosteroids could be tapered off in a mean period of 6 months after irradiation.

In conclusion we like to stress that when eye signs prove to be progressive (Nospecs 4-6) it is important to start early (at least within 2 years after development of OGD) with retroorbital irradiation in combination with a short course of prednisone. In this respect it is noteworthy that no harmful effects in a longterm (15 years) follow up after irradiation have been detected (19). Surgical orbital decompression does not lead to better average results (20), as compared to the results in our patients and often has the disadvantage of side effects like diplopia and (temporary) numbness of the upper lip. Irradiation is believed to cause a diminution of lymphocytic infiltration in retroorbital tissue (21), but not to influence the postulated autoimmune disorder causing OGD. In this respect systemic administration of cyclosporin, an immunosuppressant selectively acting against proliferating helper - or cytotoxic T lymphocytes, might be a promising treatment in the near future in patients with progressive OGD of recent onset (22).

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CHAPTER VII

SUMMARY AND CONCLUSIONS

Graves' disease (GD) is characterized by hyperthyroidism, often with diffuse goitre and ocular manifestations (ophthalmic Graves' disease, OGD) and sometimes with pretibial myxoedema and acropachy. GD is an autoimmune disease, in which antibodies directed to the TSH-receptor of the follicle cell stimulate the thyroid gland to excessive production of thyroid hormone (thyroxine) via the adenylate cyclase system.

In chapter I a review of the literature on theories of the pathogenesis of autoimmune diseases is given. Using these theories an attempt is made to explain the development of autoimmune thyroid diseases. These diseases can be divided into thyroid diseases with normal hormone production (euthyroidism) and thyroid diseases with insufficient hormone production (hypothyroidism) or with excessive hormone production (hyperthyroidism). In GD hyperthyroidism is often associated with goiter. The various immunological mechanisms leading to GD are divided into cellular- and humoral reactions. There is a genetic predisposition to the development of these reactions. However, it remains unclear, why in a genetically predisposed individual at a certain moment this immune reaction leading to overt disease starts to occur. Several theories about this phenomenon are described. Then, treatment of GD with the antithyroid drug methimazole (MMI) is discussed. This MMI inhibits excessive production of thyroid hormone and also influences immunological disturbance. Finally OGD is described as a separate entity, often occurring in association with hyperthyroidism due to GD, but also with Hashimoto's thyroiditis (HT) or with a normal thyroid status. The autoimmune aspects of OGD and the effects of a number of treatment modalities are described.

In chapter II a review of the literature is given concerning autoimmune thyroid diseases such as GD, HT and primary myxoedema. These diseases are characterised by the frequent occurrence of autoantibodies directed against thyroid antigens. At the same time plasmocellular infiltrates with destruction and fibrosis are found. Overlap with other autoimmune endocrine diseases may occur (e.g. Schmidt syndrome). Also a genetic predisposition related to the HLA-DR antigen may be found and the diseases occur mainly in women. Spontaneous exacerbations and remissions occur and the various thyroid diseases may be found in the same individual together or sequentially. The TSH-receptor and anti-TSH-receptor antibodies are discussed in detail. These antibodies may be divided into four subtypes:

1. Thyroid stimulating immunoglobulins (TSI), which stimulate thyroxine synthesis.

2. TSI blocking antibodies, which inhibit thyroxine synthesis.

3. Thyroid growth stimulating immunoglobulins (TGI), which may cause euthyroid goitre.

4. TGI blocking antibodies, which inhibit thyroid growth, causing thyroid atrophy and hypothyroidism (primary myxoedema).

The blocking antibodies may also block TSH with regard to its thyroxine synthesis and/or growth stimulating properties.
A disordered immune regulation might be the cause of autoimmune thyroid disorders. Helper- and suppressor T lymphocytes and also antibodies directed against epitopes of other antibodies (anti-idiotypic antibodies) may play a role in immune regulation.
In chapter III a clinical study concerning cellular (T and B lymphocytes) and humoral (TSI) immunity in patients with GD is described. This study, started in 1981, concerned analysis of numbers of helper- and suppressor T lymphocytes in peripheral blood of 29 patients with unequivocal GD. We were interested to know whether a decreased number of suppressor T lymphocytes could be found. This would theoretically explain an increased activity of helper T cells leading to stimulation of B lymphocytes to produce TSI. Moreover a TSI bioassay (measuring stimulation of cAMP production in human thyroid cells in vitro) was developed. Using this assay, TSI could be measured before, during and after medical treatment with MMI. Patients with hyperthyroidism and mostly diffuse goitre were being treated during one year with MMI. At regular intervals blood was drawn in order to measure thyroid hormone status and immunological parameters. No abnormal numbers of helper- and suppressor T lymphocytes could be found in the peripheral blood before, during or after treatment. It is speculated, that a possible decreased number and activity of suppressor T cells, if any, might only be found in the thyroid gland itself, this being the main site of TSI production.
In 77% of the untreated patients an elevated TSI activity could be found and a significant decrease of mean TSI activity could be found during treatment in both groups of patients (those who stayed euthyroid and those who relapsed after cessation of the drugs). This was considered to be an indication of a decrease of autoimmune reactivity due to MMI. Before treatment no significant difference in mean TSI activity between the relapse- and the remission group could be demonstrated, but after 6 and 12 months of MMI treatment a significant difference was found between the two groups. The relapse group showed significantly higher TSI activity than the remission group at both times. TSI values, however, did not appear to have prognostic significance towards the final outcome of the disease after MMI treatment.
Chapter IV focusses on different assays for thyroid stimulating antibodies. The biological assay for TSI in human thyroid cells is compared with commercially available kits measuring inhibition of binding of TSH (TBII). The correlation between these two types of assays was moderate, suggesting that the different assays measure different antibodies. TSI bind to or in the region of the TSH-receptor on the thyroid cell membrane and stimulate thyroxine production. TBII also bind to or in the region of the TSH-receptor but these antibodies are measured by their inhibitory effect on binding of TSH to its receptor. The latter is not necessarily related to biological activity. Our findings with these two types of assays may be

considered as support for the heterogeneity of TSH-receptor antibodies as described in chapter I and II.
In chapter V, 2 patients with severe OGD and autoimmune thyroid disease with different clinical expression are described. Patient A suffered, apart from diabetes mellitus type I, from a primary hypothyroidism for which he had been treated during 3 years with thyroxine. He developed hyperthyroidism (type GD) and ocular signs. The eye symptoms deteriorated despite antithyroid drug treatment. Prednisone induced temporarily improvement, i.e. a decrease of the prednisone dose below 10 mg per day caused flare up of eye signs. Finally, retrobulbar supervoltage irradiation induced permanent improvement. Patient B presented with loss of visual acuity and signs of hypothyroidism. Primary hypothyroidism and OGD were confirmed. Imminent blindness necessitated treatment of this patient with prednisone (apart from thyroxine substitution) resulting in rapid improvement. Both patients had OGD of comparable severity, but interestingly a different expression of thyroid autoimmune disease. They both had a favorable reaction to corticosteroids, but finally patient A needed retrobulbar irradiation in order to inhibit the intraorbital inflammatory process.
In chapter VI 24 patients with severe OGD are described. They all had signs of autoimmune thyroid disease and, despite adequate treatment of the thyroid disorder, developed deterioration of eye signs (NOSPECS class 4,5 and 6, corresponding respectively with diplopia, keratitis and visual loss). Patients treated with prednisone showed a significant improvement of ocular signs, which, however, worsened after tapering off the dose. Finally all patients were irradiated on the retrobulbar tissue causing a significant permanent improvement of the mean values of proptosis and visual acuity. Retrobulbar irradiation is recommended as a safe treatment in severe OGD.
As this thesis is based on the above mentioned five articles, discussion on the results in relation to results of similar studies as described in the literature, can be found in chapter 2-6. Humoral immune mechanisms, as measured by TSI activity are considered to cause hyperthyroidism in patients with Graves' disease. The cellular immune mechanisms leading to the development of TSI can not be measured in peripheral blood, as stated in this study. Studies on lymphocytes and other immunologically active cells in the involved organ might probably disclose abnormalities that are not measurable in peripheral blood.

SAMENVATTING EN CONCLUSIES

De ziekte van Graves is een ziekte, die gekarakteriseerd wordt door hyperthyreoidie met vaak een diffuus struma en oogverschijnselen (ophthalmic Graves' disease, OGD) en soms met pretibiaal myxoedeem en acropachie. Het is een autoimmuun ziekte, waarbij antistoffen gericht tegen de TSH-receptor van de follikelcel, de schildklier aanzetten tot een overproductie van schildklier hormoon (thyroxine) via stimulatie van adenylate cyclase.
In hoofdstuk I worden, uit literatuurstudie, theorieen over de ontstaanswijze van autoimmuun ziekten beschreven. Met behulp van deze theorieen wordt getracht een verklaring te vinden voor de ontwikkeling van autoimmuun ziekten van de schildklier. Deze zijn onder te verdelen in schildklier ziekten, waarbij een normale hormoon productie (euthyreoidie) bestaat en ziekten, waarbij te weinig hormoon productie (hypothyreoidie) of teveel hormoon productie (hyperthyreoidie) voorkomt.
Bij de ziekte van Graves komt meestal naast hyperthyreoidie ook een struma voor. De verschillende immunologische mechanismen, die hiertoe leiden worden onderverdeeld in cellulaire- en humorale. Genetische factoren predisponeren tot het ontstaan van deze reacties. Het is echter nog niet duidelijk, waarom op een bepaald moment bij een genetisch gepredisponeerd individu een immunologische reactie in gang gezet wordt, waardoor uiteindelijk de ziekte manifest wordt.
Verschillende theorieen hieromtrend worden beschreven. Vervolgens wordt de behandeling van de ziekte van Graves met het schildklier remmende medicament methimazol besproken. Dit middel remt de overmatige thyroxine productie, maar heeft ook effecten op het immunologische deraillement. De OGD wordt tenslotte beschreven als een aparte entiteit, die vaak in associatie met hyperthyreoidie ten gevolge van de ziekte van Graves, maar ook met Hashimoto's thyreoiditis voorkomt. De autoimmuun aspecten van OGD worden belicht en ook de effecten van verschillende behandelingsmogelijkheden.
In hoofdstuk II wordt aan de hand van literatuurstudie ingegaan op autoimmuun schildklier ziekten zoals de ziekte van Graves, Hashimoto's thyreoiditis (HT) en primair myxoedeem. Deze ziekten worden alle gekenmerkt door het frequent voorkomen van autoantistoffen gericht tegen antigenen in de schildklier. Tevens vindt men plasmacellulaire infiltraten in de schildklier, met destructie en fibrose. Er kan een overlap zijn met andere autoimmuun endocriene ziekten (b.v. Schmidt syndroom). Men vindt verder een genetische predispositie gerelateerd aan het HLA-DR antigeen en een overwegend voorkomen van de ziekte bij vrouwen. Spontane exacerbaties en remissies komen voor en een individu kan de verschillende schildklier ziekten tegelijk of na elkaar krijgen.
Meer in detail wordt ingegaan op de TSH-receptor en ertegen gerichte antistoffen. Deze antistoffen kunnen in vier subtypen onderverdeeld worden:

1. Schildklier stimulerende immuunglobulinen, TSI, die de thyroxine synthese bevorderen.

2. Immuunglobulinen, die de werking van TSH of van TSI blokkeren en de thyroxine synthese remmen.

3. Schildklier groei bevorderende immuunglobulinen, TGI, die euthyreotisch struma veroorzaken.

4. Immuunglobulinen, die TSH of TGI blokkeren en hypothyreoidie en schildklier verschrompeling geven (primair myxoedeem).

Bij autoimmuun schildklier aandoeningen wordt een gestoorde immuunregulatie als mogelijke oorzaak aangewezen. Bij immuun regulatie spelen helper- en suppressor T lymphocyten een rol en hebben antistoffen gericht tegen andere antistoffen (anti-idiotypische antistoffen) mogelijk een plaats.
De cellulaire (T en B lymphocyten) en humorale (TSI) immuniteit bij de ziekte van Graves was onderwerp van de klinische studie, die in hoofdstuk III beschreven wordt. Wij startten in 1981 een onderzoek naar het aantal helper- en suppressor T lymphocyten in het perifere bloed van 29 patienten met de ziekte van Graves. Wij wilden weten of er een verlaagd aantal aan suppressor T cellen gevonden kon worden, die theoretisch verantwoordelijk zou kunnen zijn voor de ontwikkeling van meer helper T cel activiteit. Dit zou op haar beurt B lymphocyten kunnen stimuleren tot de productie van TSI. Tevens werd een bioassay voor meting van TSI (stimulering van cAMP productie in humane schildklier cellen in vitro) opgezet. Hiermee kon de TSI voor, gedurende en na behandeling met methimazol gemeten worden. Patienten met hyperthyreoidie en een diffuse soms vergrote schildklier werden gedurende 1 jaar behandeld met methimazol en op vaste tijdstippen werd bloed afgenomen voor bepaling van hormoonspiegels en immunologische parameters. Er werd geen abnormaal aantal helper- of suppressor T lymphocyten in het perifere bloed gevonden, voor, tijdens of na behandeling. Het vermoeden wordt geuit dat, zo er een verminderde suppressor T lymphocyt-activiteit en -hoeveelheid bestaat bij de ziekte van Graves, deze mogelijk alleen in de schildklier zelf bestaat. Een verhoogde TSI activiteit kon bij 77% van de onbehandelde patienten gevonden worden en een significante daling van de gemiddelde TSI, werd tijdens de behandeling gevonden. Dit wordt beschouwd als een aanwijzing voor een daling van autoimmuun reactiviteit onder invloed van methimazol. In de onbehandelde fase werd er geen significant verschil in TSI activiteit gevonden tussen de groep patienten, die na behandeling euthyreoot (remissies) bleven en de groep, die recidiveerde. Na 6 en 12 maanden behandeling werd er echter wel een significant verschil tussen de beide groepen gevonden. De recidief groep had een significant hogere TSI activiteit na 6 en 12 maanden behandeling. De TSI bepaling bleek overigens geen prognostische waarde te hebben voor het uiteindelijke beloop na behandeling met methimazol, zoals inmiddels ook door enkele auteurs beschreven werd.
In hoofdstuk IV wordt nader ingegaan op de technieken ter bepaling van schildklier stimulerende antistoffen. De biologische assay voor TSI met humane schildkliercellen wordt vergeleken met de commercieel verkrijgbare bepalings methoden voor antistoffen, die de binding van TSH aan de TSH receptor remmen (TBII). De correlatie tussen deze 2 technieken was matig en dit wordt beschouwd als een uiting van het feit dat mogelijk 2 verschillende antistoffen gemeten worden. De TSI bindt zich op of in de buurt van de TSH receptor op de schildklier

celmembraan en stimuleert thyroxine productie en is dus een goede maat voor biologische activiteit. De TBII bindt zich ook op of in de buurt van de TSH receptor en voorkomt daardoor binding van TSH aan zijn specifieke bindingsplaats. TBII bepaling hoeft nog geen biologische activiteit te betekenen. Onze bevindingen met deze twee verschillende technieken bevestigen de heterogeniteit van TSH receptor antistoffen, zoals beschreven in hoofdstuk I en II.
In hoofdstuk V worden 2 patienten beschreven met ernstige OGD en autoimmuun schildklierziekten, die zich op verschillende wijze klinisch presenteerden. Patient A is een man, die naast diabetes mellitus type I een primaire hypothyreoidie had en die gedurende 3 jaar met thyroxine gesubstitueerd was. Vervolgens ontwikkelde hij hyperthyreoidie (type ziekte van Graves) en oogverschijnselen, die ondanks thyreostatische behandeling toenamen. Prednison gaf tijdelijk verbetering. Dalen van de dosis prednison onder de 10 mg per dag veroorzaakte opvlammen van de oogverschijnselen. Uiteindelijk gaf retroorbitale bestraling blijvende verbetering. Patient B presenteerde zich met verlies van gezichts vermogen en hypothyreoidie. De diagnose primaire hypothyreoidie en OGD werd bevestigd. Wegens dreigende blindheid werd naast thyroxine substitutie ook prednison gegeven met goed resultaat. Beide patienten hadden dus OGD, maar elk met een andere uiting van autoimmuun schildklier ziekte. Beiden reageerden op corticosteroid behandeling, maar uiteindelijk moest patient A toch bestraald worden om het immunologische proces in de orbita te remmen.
In hoofdstuk VI worden 24 patienten met ernstige OGD beschreven. Zij hadden allen tekenen van autoimmuun schildklierziekte en ontwikkelden, ondanks adequate behandeling van de schildklier en meestal ook prednison toediening, toename van oogverschijnselen (NOSPECS klasse 4,5 en 6, respectivelijk corresponderend met dubbelzien, hoornvlies ontsteking en verlies van gezichtsvermogen). De patienten die met prednison werden behandeld vertoonden gemiddeld een significante verbetering van oogverschijnselen, die echter weer verslechterden bij dalen van de dosis. Uiteindelijk werden alle patienten op het retrobulbaire weefsel bestraald, waardoor een gemiddeld significante blijvende verbetering van proptosis en gezichtsvermogen optrad. Retrobulbaire bestraling wordt bij ernstige OGD als een veilige behandeling aanbevolen.
Daar dit proefschrift gebaseerd is op de bovenvermelde vijf artikelen, kan de discussie over de resultaten mede in het licht van resultaten, die beschreven zijn in vergelijkbare studies, gevonden worden in hoofdstuk 2-6. Humorale immuun mechanismen, zoals gemeten met TSI activiteit, worden beschouwd als oorzakelijke factoren voor hyperthyreoidie bij patienten met de ziekte van Graves. De cellulaire immuun mechanismen, die leiden tot de ontwikkeling van TSI, kunnen niet gemeten worden in perifeer bloed, zoals gesteld wordt in deze studie. Onderzoek naar lymfocyten en andere immunologisch actieve cellen in het aangedane orgaan zouden misschien afwijkingen aan het licht brengen, die niet meetbaar zijn in perifeer bloed.

NAWOORD

De totstandkoming van dit proefschrift is het resultaat van inspanning van een geheel team van onderzoekers. Allen, die bij dit proefschrift betrokken waren, ben ik veel dank verschuldigd.

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Jorg Hennemann heeft het mij mogelijk gemaakt om de door mij gewenste klinische studie te doen bij patienten met de ziekte van Graves. De vele gesprekken over dit onderwerp, die ik, vaak 's avonds laat per telefoon, met hem voerde, waren steeds verhelderend en opbouwend. Zij laten een goede herinnering bij mij achter.

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Greetje Bos heeft met zeer veel inzet geholpen bij de uitvoering van de laboratorium proeven en wist altijd het hoofd koel te houden, als mij dat niet meer lukte. Bij afwezigheid van Greetje was Hans van Toor steeds bereid in te springen.

Vele chirurgen in het "Dijkzigt" hebben mij tijdig gewaarschuwd, wanneer ik schildklier weefsel kon krijgen voor de TSI assay.

Adri van Oudenaren ben ik ook zeer erkentelijk voor zijn inzet bij de bepalingen van T lymfocyten. Hij bediende regelmatig met koele rust de opwindende FACS.

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Joop van der Merwe heeft zeer waardevolle kritiek geleverd op de inleiding en was steeds bereid om nieuw theoretisch materiaal aan te voeren.

De contacten met Rinia Wijngaarde in het "orbitateam" heb ik altijd zeer op prijs gesteld. Zij heeft de artikelen over OGD mede mogelijk gemaakt.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 24 april 1950 te Hilversum geboren. Hij deed in 1969 eindexamen Gymnasium B aan het gemeentelijk Gymnasium te Hilversum.
Hij studeerde medicijnen aan de Rijksuniversiteit te Utrecht en deed doctoraal examen in 1975. In 1976 behaalde hij het ECFMG examen, en in 1977 het artsexamen.
De militaire dienst werd vervuld bij de geneeskundige dienst van de Koninklijke Marine, en wel bij het corps mariniers te Doorn en Rotterdam.
Op 1 september 1978 begon hij zijn opleiding tot internist op de afdeling Interne Geneeskunde III (Hoofd: Prof. Dr J.C. Birkenhäger) van het Academisch Ziekenhuis Rotterdam "Dijkzigt". Van 1 november 1979 tot 1 oktober 1980 werd de opleiding onderbroken met stages op de afdeling Interne Geneeskunde (Hoofd: Prof. A. Muller) van het Hôpital Cantonal te Genève.
In 1981 werd gestart met klinisch onderzoek bij patienten met de ziekte van Graves onder leiding van Prof. Dr G. Hennemann.
Op 1 september 1983 werd hij ingeschreven in het Specialisten Register als internist en sindsdien is hij als internist verbonden aan het Merwede Ziekenhuis te Dordrecht.