

Etiopathogenesis of Graves' disease

Michał Karasek^{1,2} & Andrzej Lewinski¹

1. Department of Endocrinology and Isotope Therapy, Medical University of Lodz – Polish Mother's Memorial Hospital – Research Institute, Lodz, POLAND.

2. Department of Electron Microscopy, Chair of Pathomorphology, Medical University of Lodz, POLAND.

Correspondence to: Prof. Dr. Michał Karasek,
Department of Endocrinology and Isotope Therapy,
Medical University of Lodz
Polish Mother's Memorial Hospital – Research Institute,
93-338 Lodz, Rzgowska 281/289, POLAND;
TEL: +48 42 271 1715
FAX: +48 42 271 1343
E-MAIL: karasek@csk.am.lodz.pl

Submitted: December 18, 2002

Accepted: February 24, 2003

Key words: **Graves' disease; autoimmune diseases; hyperthyroidism; ophthalmopathy; dermatopathy**

Neuroendocrinol Lett 2003; 24(3/4):161-166 pii: NEL243403R03 Copyright © Neuroendocrinology Letters www.nel.edu

Abstract

Graves' disease is an autoimmune disorder, caused by thyroid-stimulating antibodies, which bind to and activate the thyrotropin receptor on thyroid cells, inducing the synthesis and release of thyroid hormones. It is a polygenic and multifactorial disease that develops as a result of complex interaction between genetic susceptibility and environmental and/or endogenous factors. Graves' disease differs from other autoimmune diseases of the thyroid by specific clinical features, including hyperthyroidism, vascular goitre, ophthalmopathy and – less commonly – infiltrative dermatopathy. This article discusses current theories, regarding the etiology and pathogenesis of Graves' disease, including possible predisposing factors, autoimmune aspects of Graves' disease, ophthalmopathy, and dermatopathy.

Introduction

Graves' disease is an autoimmune disorder, caused by thyroid-stimulating antibodies (TSAb), which bind to and activate the thyrotropin receptor (TSHR) on thyroid cells, inducing the synthesis and release of thyroid hormones [1–3]. It is polygenic and multifactorial disease that develops as a result of complex interaction between genetic susceptibility and environmental and/or endogenous factors [1–3]. Graves' disease differs from other autoimmune diseases of the thyroid by specific clinical features, including hyperthyroidism, vascular goitre, ophthalmopathy and – less

commonly – infiltrative dermatopathy [2–4]. Women are affected 4 to 10 times more often than men, but, before the 10th, and after the 65th year of age, sex difference is significantly less pronounced. The disease may occur in every age, although it is 12 times more frequent in adults than in children [1, 4, 5]. It is estimated that in the world's population, 2% women and 0.2% men are affected by autoimmune thyroid disorders, whereas subclinical disease, reflected by the presence of thyroid antibodies, is 10-fold higher [6]. On the basis of epidemiological studies, performed in Poland in

1987–1990, it appears that about 0.2% of population (women – 0.26%, men – 0.09%) is affected by the disease and the incidence rate is about 4/100.000/year [1, 7]. In Sweden, during the years 1988–1990, the incidence of Graves' disease was 22.3/100.000/year [8], and it is especially high in Japan [9].

Predisposing factors

Genetic, environmental, and endogenous factors are considered to be responsible for the emergence of autoreactivity of T and B cells to the TSHR, leading to the development of Graves' disease [1–3, 6].

Genetic factors

Many authors point to a strong genetic component in the pathogenesis of Graves' disease [1–3, 6, 10–12]. The importance of genetic factors is suggested by the clustering of the disease within families and by a higher concordance rate for disease in monozygotic (20–60%) than dizygotic (3–9%) twins [10]. Moreover, thyroid antibodies have been reported in up to 50% of the siblings from patients suffering from Graves' disease [10]. The familial incidence of Graves' disease is 15-times higher in general population [13]. However, no single gene is known to cause the disease or to be responsible for its development.

Human leukocyte antigen (HLA) alleles have for many years been considered to be well-established risk factors in Graves' disease [1–3, 6]. The complex of HLA genes is located on chromosome 6 (6p21.3) [2, 12]. HLA gene expression can influence the development of autoimmune disorders in various ways. HLA molecules take part in the clonal selection of T lymphocytes in the thymus. HLA class II modulate the scale of immunological response through inconsistent ability to react with T lymphocyte receptor (TcR) during antigen presentation. This interaction is of great importance in the activation of T helper lymphocytes (Th) [14, 15]. During human life, T helper lymphocyte clones are quite often generated and directed against host cells. If the system of immunological superintendence is efficient, such clones are identified and destroyed. However, if the function of T suppressor lymphocytes is discriminated, then the autoreactive T helper lymphocyte clone persists. A particular set of HLA class II alleles may cause a stronger activation of T-cell receptors on autoreactive lymphocytes, leading to the development of autoimmunization [14, 16].

It is suggested that particular HLA haplotypes may be the primary factor, predisposing to the disease development. It may be associated with low activity of suppressor T lymphocytes or low production of unspecific suppressing factors, such as interleukin-10 (IL-10) or transforming growth factor β (TGF- β) [17]. In patients suffering from Graves' disease, an increased frequency of the following HLA class II have been shown: DQA1*0501, DR-3, DR-8, DQB1*0302, DRB1*02, DRB1*0304, and DRB1*0301/4 [12, 18, 19, 20, 21]. HLA haplotype DRB1*0304-DRB1*02-DQA1*0501

is associated with the maximal risk of autoimmune thyrotoxicosis [21].

Although most attention has been paid to HLA genes, there are also some other candidate susceptibility genes, including those encoding immunoglobulins and T-cell receptors, cytokines and their modulators, as well as other immunologically important molecules and autoantigens [6]. Three candidates have recently focused attention, namely thyroid stimulating hormone receptor (TSHR; codone 52), cytotoxic T-lymphocyte antigen 4 (CTLA-4; 106bp allele), and the gene encoding interleukin (IL)-1 receptor antagonist [3, 6, 18, 22–24]. The restricted nature of TSHR, stimulating antibody in Graves' disease, possibly provides the best evidence that crucial immunoglobulin genes could determine whether or not a patient develops these antibodies [25].

It should also be mentioned that the *loci* associated with susceptibility to Graves' disease (designated GD-1, GD-2, and GD-3) have been identified by linkage analysis on 14q31, 20q11.2, and Xq21.33–22 chromosomes [18, 26–28]. Recently, an evidence for new Graves' disease susceptibility locus at chromosome 18q21 has been provided [29]. Therefore, Graves' disease, like most autoimmune diseases, is inherited as a complex multigenic trait.

Environmental and endogenous factors

The following environmental factors that damage thyroid cell may be associated with the risk of development of Graves' disease: environmental toxins, bacteria, viruses, humoral factors, stress or excessive iodine uptake [1–3, 5, 6]. Infections have for many years been an attractive susceptibility factor but there is no clear evidence that infection directly induces Graves' disease. It seems that, except of retroviruses and foamy viruses, due to a direct influence on genome and an indirect effect through interferon- γ (IFN- γ), and *Yersinia enterocolitica*, the bacteria in which antigen protein in cellular membrane shows similarity to TSHR of thyroid follicular cell, and therefore, may be etiological factors of some importance, the remaining above mentioned factors may only promote development of the disease in persons with genetic susceptibility [1–3, 6, 30]. However, it should be mentioned that the role of *Yersinia enterocolitica* in the etiology of Graves disease has been recently neglected [5]. The similarity of some bacterial and viral antigens to TSHR [31] and changes in the receptor structure (especially TSHR) in thyroid follicular cells, caused by its mutation or bio modification by drugs, chemical compounds or inflammatory mediators [32–37], may underlie the origin of thyroid autoantibodies and the development of Graves' disease.

It has already been mentioned that the risk factor for Graves' disease is higher in females than in males, probably in result of the modulation of autoimmune response by estrogens [3]. Nutritional factors in early stages of life seem to be also of some importance because low birth weight is associated with an increased prevalence of thyroid antibodies [38].

Autoimmune aspects of Graves' disease

The role of TSH receptor and antibodies against this receptor

TSHR is the main antigen, inducing the generation of autoantibodies. TSHR is a G protein-coupled receptor with the characteristic seven membrane spanning regions. Along with FSH and LH receptors, TSHR molecule is a membrane-bound glycoprotein and has a similar structure of transmembrane domain and, what is more important, of a large extracellular domain which confers the ligand binding specificity. Particular extracellular epitopes of TSHR in humans are homologous in 7–85% with the responding fragments of LH receptor and, in 20–85%, with that of FSH receptor [39, 40]. TSHR has two unique insertions. The first insert of 8 residues is required for mature receptor to be expressed at the cell surface, the second insert of 50 residues is the probable site of TSHR cleavage, and is highly immunogenic [41]. Antibodies directed against TSHR mimic the effects of TSH, stimulating the function of thyroid follicular cells and causing hyperthyroidism. These TSAb are detected in circulating plasma of all patients suffering from Graves' disease. TSAb bind to conformational epitopes in the extracellular domain of the TSHR, which make up discontinuous segments that overlap the binding site for TSH [3, 42, 43]. The production of thyroid stimulating antibodies is dependent on T cells and circulating T cells recognize multiple epitopes of the TSHR [3, 44]. Hyperthyroidism is caused by the ability of TSAb to increase the production of intracellular cyclic AMP, leading in consequence, an excessive production of thyroxine and triiodothyronine [3].

The role of lymphocytic infiltration

Activated T cells, directed against thyroidal and nonthyroidal antigens, are present in peripheral blood of patients with Graves' disease [5, 45]. However, lymphocytic infiltrate is also present in the thyroid, consisting predominantly of activated T lymphocytes, but also in a smaller number of B lymphocytes, dendritic cells, and macrophages, occasionally in association with germinal centers [2, 3, 5, 46]. The degree of infiltration is highly variable [5]. Many activated T lymphocytes contains IFN- γ , a strong stimulator of the inflammatory process and immunological response [2, 45]. However, other cytokines and growth factors are also present and give impetus for the local proliferation of glandular tissue [3, 5, 47].

The role of thyroid cells

Thyroid cells play a very important role in the initiation of Graves' disease. As already mentioned, thyroid follicular cells are the source of thyroid antigens and target of TSAb but also, in response to T cell-derived cytokine – IFN- γ , they express HLA class II molecules and therefore, allow the cell to present its antigens to activated T lymphocytes, what results in the development of autoimmunization [3, 6, 35, 48, 49]. It seems that in the initiation of Graves' disease, B lymphocytes and dendritic cells play also some role because these

cells express costimulatory molecules CD80 and CD86 that are of crucial importance for naive T lymphocytes [3, 50]. The autoimmune process may further be exacerbated by the expression of other molecules by thyrocytes, such as IL-1, IL-6, CD40, and CD54 [3].

Goitrogenesis in Graves' disease

Goitre in Graves' disease may be induced by TSAb, as well as by activated lymphocytes infiltrating the thyroid gland [2, 3, 36, 51, 52]. TSAb probably stimulate the proliferation of thyroid follicular cells, although the precise mechanism of this proliferation is not clear. It could be a result of the activation of phospholipase A₂ (and C) and/or adenylate cyclase which is the second signal necessary for TSH-stimulated thyrocyte proliferation [32, 51, 52]. Cyclic AMP is recognized as the basic regulator of thyroid cell proliferation [53]. The proliferation of thyroid cells might also be induced by activated lymphocytes, infiltrating the thyroid because they are the source of various cytokines and growth factors which stimulate cell proliferation [54].

Thyroid-associated ophthalmopathy

Thyroid-associated ophthalmopathy, usually called Graves' ophthalmopathy, is a well-described but poorly understood component of Graves' disease [55–58]. The eye complications in Graves' ophthalmopathy range from discomfort and lid distraction to proptosis (the main clinical manifestation of the disease), dislopia, and sight loss, and are clinically evident in 20–50% of patients with Graves' disease, although only in 5–15% with moderate to severe manifestations [55, 56, 58]. Graves' ophthalmopathy is characterized by an enlargement of the extraocular muscles and an increase in the retrobulbar fat, and can be classified as non-infiltrative, called congestive subtype (including oedema of lids, periorbital tissues and conjunctiva, conjunctival injection and eye pain, irritation and a sensation of grittiness) and infiltrative, called ocular myopathy subtype (including infiltration, inflammation and enlargement of extraocular muscles) [56, 59, 60]. Although the pathogenetic mechanism of Graves' ophthalmopathy is still unclear and the nature of the involved autoantigens is not known (though a number of candidates have been suggested), recent studies support the hypothesis of autoimmune response against one or more shared or cross-reactive thyroid-orbit autoantigens [61, 62].

Some authors believe that extraocular connective tissue is the main location of the initial reaction [63, 64, 65]. Infiltrative process, caused by autoantibodies, leads to a local accumulation of stimulated T lymphocytes which may secrete cytokines (such as IL-1 α , IL-2, IFN- γ , tumor necrosis factor- α and - β (TNF- α and - β), and leukoregulin) which activate orbital fibroblasts and cause overproduction of collagen and glycosaminoglycans. In consequence, the accumulation of glycosaminoglycans causes tissue oedema, leading to a disproportion between orbital capacity and orbital tissue volume.

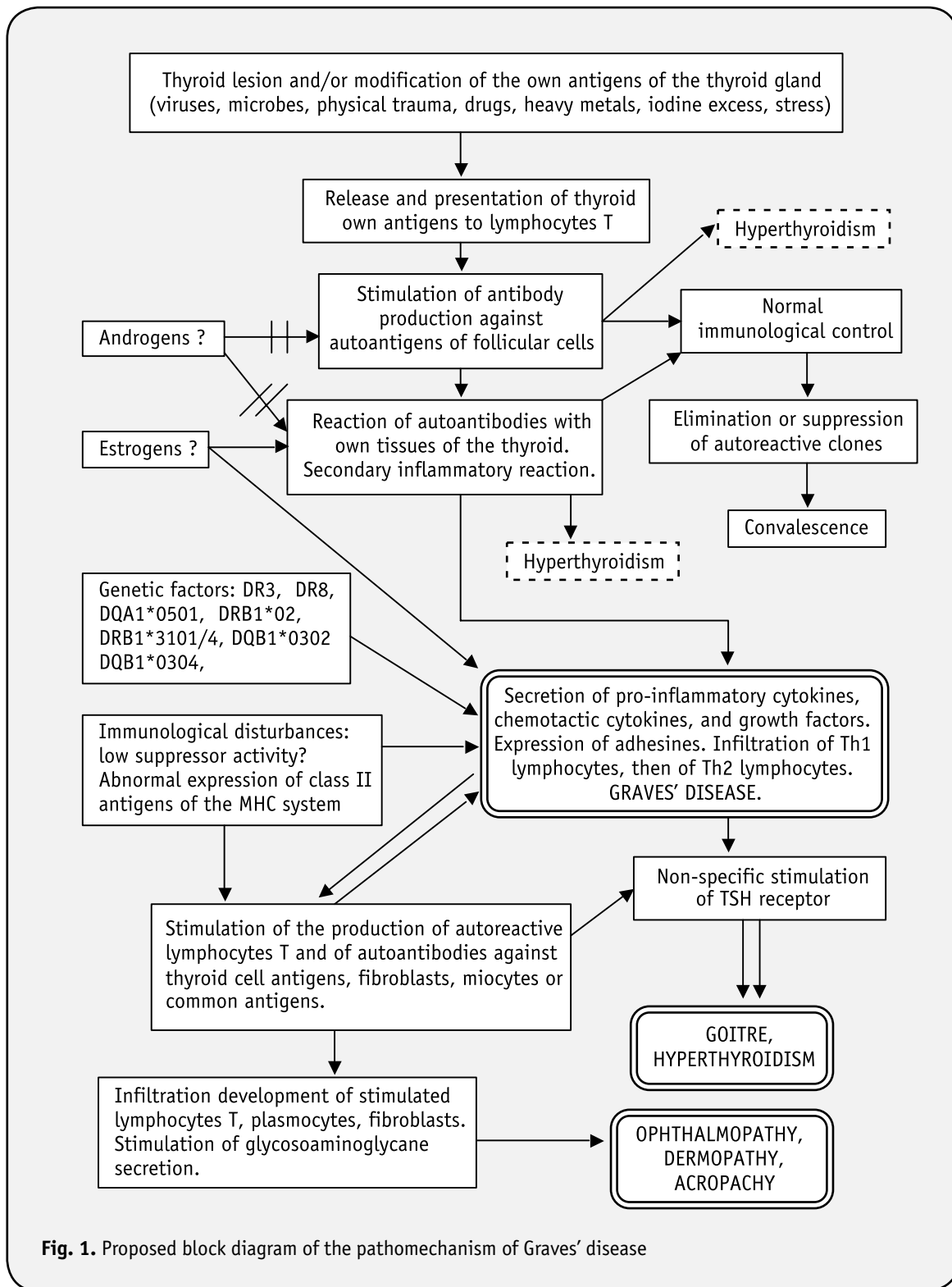


Fig. 1. Proposed block diagram of the pathomechanism of Graves' disease

Others propose that the eye muscle is the primary target of the autoimmune reaction, while orbital fibroblast stimulation and congestive changes are of secondary importance [65, 66, 67].

Early, the active phase of Graves' ophthalmopathy is associated with a cell-mediated immune response (Th1-like cytokines: IL-2, INF- γ , and TNF- β), inducing an expression of adhesion molecules on endothelial cells leading to the recruitment of activated leukocytes from blood vessels. The migration of leukocytes into

the retrobulbar tissue is intensified by the expression of leukocyte function-associated antigen-1/intercellular adhesion molecule-1 (LFA1-/ICAM-1) and very late antigen-4/vascular cell adhesion molecule-1 (VLA-4/VCAM-1). In addition, Th1-like cytokines induce the expression of certain immunomodulatory proteins (HLA-DR, adhesion molecules, heat-shock proteins) on orbital fibroblasts, adipocytes, and eye muscle cells. The late inactive phase is associated with a stimulation of

humoral reaction and secretion of IgG, IgM, and IgE by Th2-like cytokines (IL-4, IL-5, IL-10, IL-13) [61, 68].

Proinflammatory Th1-like cytokines may play a role in the development of eye muscle component of Graves' ophthalmopathy in the acute stage, whereas anti-inflammatory Th2-like cytokines may be protective in the chronic stage of the disease [69].

Dermopathy and acropachy

An uncommon manifestation in Graves' disease is a local pretibial oedema – a dermopathy which occurs in 1–2% of patients and is, sometimes, associated with acropachy [2, 3]. Dermopathy is characterized by lymphocytic infiltration of the dermis, the accumulation of glycosaminoglycans, and oedema. The overproduction of glycosaminoglycans (mainly hyaluronic acid) by fibroblasts is the primary cause of pretibial oedema and acropachy [56, 70, 71]. Although the presence of distinct pretibial oedema and acropachy is rarely observed in patients with Graves' disease (1% and 4%, respectively), detailed morphological studies of forearm skin sections reveal discrete dermopathy also in patients without oedema [56, 72]. The intensity of lymphocytic infiltrations in subcutaneous tissue is, however, significantly lower than that observed in retroocular tissues, although the histological patterns of both tissues are very similar [73].

Concluding remarks

Despite a significant progress in the understanding of Graves' disease, especially of its etiology and pathogenesis as well as of underlying immune system abnormalities, many problems are still to be solved; the current mechanisms, involved in the pathogenesis of Graves' disease, are presented in *Figure 1*.

REFERENCES

- Nauman J, Nauman A. Choroba Gravesa-Basedowa; etiopatogeneza, klinika, leczenie [(Graves' Basedow disease; etiopathogenesis, clinic, and treatment.) (In Polish with English abstract.)]. *Endokrynol Pol – Polish J Endocrinol* 1995; **46**(suppl 1/1):3–8.
- Baj Z, Ferenc T, Lewinski A. (1998) Immunopatologia choroby Gravesa-Basedowa [(Immunopathology of Graves' Basedow disease.) (In Polish with English abstract.)]. *Post Hig Med Dosw* 1998; **51**:531–46.
- Weetman AP. (2000) Graves' disease. *N Eng J Med* 2000; **343**:1236–48.
- Wiersinga WM, Prummel MF. An evidence-based approach to the treatment of Graves' ophthalmopathy. *Endocrinol Metab Clin North Am* 2000; **29**:297–319.
- McIver B, Morris JC. The pathogenesis of Graves' disease. *Endocrinol Metab Clin North Am* 1998; **77**:73–89.
- Weetman AP. New aspects of thyroid immunity. *Horm Res* 1997; **48**(suppl 4):51–4.
- Nauman J. Wyniki badan programu MZ-XVII prowadzonych w skali kraju; podsumowanie i wnioski [(Results of studies performed within MZ-XVII programme; most important observations and conclusions of country-wide studies.) (In Polish with English abstract.)]. *Endokrynol Pol – Polish J Endocrinol* 1991; **42**:359–67.
- Berglund J, Ericsson UB, Hallengren B. (1996) Increased incidence of thyrotoxicosis in Malmo during the years 1988–1990 as compared to the years 1970–1974. *J Intern Med* 1996; **239**:57–62.
- Okamura K, Nakashima T, Ueda K, Inoue K, Omae T, Fujishima M. Thyroid disorders in the general population of Hisayama Japan, with special reference to prevalence and sex differences. *Int J Epidemiol* 1987; **16**:545–9.
- Tomer Y, Davies TF. The genetic susceptibility to Graves' disease. *Bailliere's Clin Endocrinol Metab* 1997; **11**:431–50.
- Brix TH, Kyvik KO, Hedegus L. What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. *Thyroid* 1998; **8**:727–34.
- Gough SC. The genetics of Graves' disease. *Endocrinol Metab Clin North Am* 2000; **29**:255–66.
- Vyse TJ, Todd JA. Genetic analysis of autoimmune disease. *Cell* 1996; **85**:311–8.
- Luppi P, Rosiello MR, Faas S, Trucco M. Genetic background and environment contribute synergistically to the onset of autoimmune diseases. *J Mol Med* 1995; **73**:381–93.
- Steinman L. Escape from "horror autotoxicus" pathogenesis and treatment of autoimmune disease. *Cell* 1995; **80**:7–10.
- Anderson G, Anderson L, Larhmmar D, Rask L, Siguardardottir S. Simplifying genetic locus assignment of HLA-DRB genes. *Immunol Today* 1994; **15**:58–62.
- Volpe R, Row VV. (1985) Role for an antigen-specific defect in suppressor T lymphocyte function in autoimmune thyroid diseases. In: Walfish P, Wall JR, Volpe R, editors. *Autoimmunity in thyroid diseases*. New York: Academic Press; 1985. p. 79–93.
- Chistyakov DA, Savost'anov KV, Turakulav RI, Nosikov VV. (2000) Genetic determinants of Graves disease. *Mol Genet Metab* 2000; **71**:66–9.
- Boehm BO, Kuhn P, Manfras BJ, Chen M, Lee JC, Holzberger G et al. HLA-DRB3 gene alleles in Caucasian patients with Graves disease. *Clin Invest* 1992; **70**:956–60.
- Badenhoop K, Walfish PG, Rau H, Fisher S, Nicolay A, Bogner U et al. Susceptibility and resistance alleles of human leukocyte antigen (HLA) DQA1 and HLA DQB1 are shared in endocrine autoimmune disease. *J Clin Endocrinol Metab* 1995; **80**:2112–2117.
- Heward JM, Allahabadi A, Daykin J, Carr-Smith J, Daly A, Armitage M et al. Linkage disequilibrium between the human leukocyte antigen class II region of the major histocompatibility complex and Graves disease: replication using a population case control and family-based study. *J Clin Endocrinol Metab* 1998; **83**:3394–7.
- Blakemore AIF, Watson PF, Weetman AP, Duff GW. Association of Graves' disease with an allele of the interleukin-1 receptor antagonist gene. *J Clin Endocrinol Metab* 1995; **80**:111–5.
- Yanagawa T, Hidaka Y, Guimaracs V, Soliman M, Degroot IJ. CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. *J Clin Endocrinol Metab* 1995; **80**:41–5.
- Watson PF, French A, Pickerill AP, McIntosh RS, Weetman AP. Lack of association between a point mutation in the coding region of the TSH receptor gene and Graves' disease. *J Clin Endocrinol Metab* 1995; **80**:1032–5.
- Weetman AP, Yateman ME, Ealey PA, Black CM, Reimer CB, Williams RC et al. An investigation of thyroid stimulating antibody activity between different IgG subclasses. *J Clin Invest* 1990; **86**:723–7.
- Tomer Y, Barbesino G, Greenberg DA, Concepcion E, Davies TE. Linkage analysis of candidate genes in autoimmune thyroid disease. III. Detailed analysis of chromosome 14 localizes Graves' disease-1 (GD-1) close to multinodular goiter-1 (MNG-1). *J Clin Endocrinol Metab* 1998; **83**:4321–7.
- Tomer Y, Barbesino G, Greenberg DA, Concepcion E, Davies TE. A new Graves disease-susceptibility locus maps to chromosome 20q11.2. *Am J Hum Genet* 1998; **63**:1749–56.
- Barbesino G, Tomer Y, Concepcion ES, Davies TE, Greenberg DA. (1998) Linkage analysis of candidate genes in autoimmune thyroid disease. II. Selected gender-related genes and the X-chromosome. *J Clin Endocrinol Metab* 1998; **83**:3290–5.
- Vaidya B, Imrie H, Perros P, Young ET, Kelly WF, Carr D et al. Evidence for a new Graves disease susceptibility locus at chromosome 18q21. *Am J Hum Gene* 2000; **66**:1710–1714.

- 30 Tomer Y, Davies TF. Infection, thyroid disease and autoimmunity. *Endocrine Rev* 1993; **14**:107–19.
- 31 Wenzel BE, Franke TF, Heufelder AE, Hessemann J. Autoimmune thyroid diseases and enteropathogenic *Yersinia enterocolitica*. *Autoimmunity* 1990; **7**:295–303.
- 32 Furmaniak J, Smith BR. Immunity to the thyroid-stimulating hormone receptor. *Immunopathol* 1993; **14**:309–21.
- 33 Bahn RS, Dutton CHN, Heufelder AE, Sarkar G. A genomic point mutation in the extracellular domain of the thyrotropin receptor in patients with Graves' ophthalmopathy. *J Clin Endocrinol Metab* 1994; **78**:256–60.
- 34 Koop P, van Sande J, Parma J, Duprez L, Gerber H, Joss E et al. Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. *N Engl J Med* 1995; **332**:150–4.
- 35 Tokorabet L, Ropars A, Raby C, Charreire J. (1995) Phenothiazine induces *de novo* MHC class II antigen expression on thyroid epithelial cells. *J Immunol* 1995; **154**:3593–602.
- 36 Delamarre FGA, Drexhage HA. (1996) Leukocyte migration into autoimmune diseased endocrine tissues. *Eur J Endocrinol* 1996; **34**:9–11.
- 37 Drexhage HA. Autoimmunity and thyroid growth. Where do we stand? *Eur J Endocrinol* 1996; **135**:39–45.
- 38 Phillips DIW, Cooper C, Fall C, Prentice L, Osmond C, Barker DJP et al. Fetal growth and autoimmune thyroid disease. *Q J Med* 1993; **86**:247–53.
- 39 Misrahi M, Loosefelt H, Atger M, Sar S, Goiochou-Mantel A, Milgrom E. Cloning sequencing and expression of human TSH receptor. *Biochem Biophys Res Commun* 1990; **166**:394–9.
- 40 Paschke R, Ludgate M. The thyrotropin receptor and thyroid disease. *New Engl J Med* 1997; **337**:1675–81.
- 41 Rapoport B, Chazenbalk GD, Juarne JC, McLachlan SM. The thyrotropin receptor: interaction with TSH and autoantibodies. *Endocr Rev* 1998; **19**:673–716.
- 42 Chazelbalk GD, Wang Y, Guo J, Hutchison JS, Segal D, Jaume JC et al. A mouse monoclonal antibody to a thyrotropin receptor ectodomain variant provides insight into the exquisite antigenic conformational requirement, epitopes and *in vivo* concentrations in human autoantibodies. *J Clin Endocrinol Metab* 1999; **84**:702–10.
- 43 Kosugi S, Ban T, Akamizu T, Valente W, Kohn LD. Use of thyrotropin receptor (TSHR) mutants to detect stimulating TSHR antibodies in hypothyroid patients with idiopathic myxedema who have blocking TSHR antibodies. *J Clin Endocrinol Metab* 1993; **77**:19–24.
- 44 Martin A, Nakashima M, Zhou A, Aronson D, Werner AJ, Davies TE. (1997) Detection of major T cell epitopes on human thyroid stimulating hormone receptor by overriding immune heterogeneity in patients with Graves' disease. *J Clin Endocrinol Metab* 1997; **82**:3361–6.
- 45 Aust G, Lehmann I, Laue S, Scherbaum WA. Activated and interferon-gamma producing thyroid-derived T cells are detected in Graves' disease, thyroid autonomy as well as in non-toxic multinodular goiter. *Eur J Endocrinol* 1996; **135**:60–8.
- 46 Kabel PJ, Voorbij HA, de Haan-Meulman M, Pals ST, Drexhage HA. High endothelial venules present in lymphoid cell accumulation in thyroids affected by autoimmune disease: a study in men and BB rats of functional activity and development. *J Clin Endocrinol Metab* 1989; **68**:744–51.
- 47 Aust G, Scherbaum WA. Expression of cytokines in the thyroid: thyrocytes as potential cytokine producers. *Exp Clin Endocrinol Diabetes* 1996; **104**(suppl 4):64–7.
- 48 Bottazzo GF, Pujol-Borrell R, Hanafusa T, Feldmann M. (1983) Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet* 1983; **2**:1115–9.
- 49 Heufelder AE, Smith TJ, Gorman CA, Bahn RS. Increased induction of HLA-DR by interferon-gamma in cultured fibroblasts derived from patients with Graves' ophthalmopathy and pretibial dermopathy. *J Clin Endocrinol Metab* 1991; **73**:307–13.
- 50 Marelli-Berg FM, Weetman A, Frasca L, Deacock SJ, Imami N, Lombardi G et al. Antigen presentation by epithelial cells induces anergic immunoregulatory CD45RO+ T cells and deletion of CD451 T cells. *J Immunol* 1997; **59**:5853–5861.
- 51 Di Cerbo A, Di Paola R, Bonati M, Zingrillo M, De Filippis V, Corda D. Subgroups of Graves' patients identified on the basis of the biochemical activities of their immunoglobulins. *J Clin Endocrinol Metab* 1995; **80**:2785–90.
- 52 Di Paola R, Menzaghi C, De Filippis V, Corda D, Di Cerbo A. Cyclooxygenase-dependent thyroid cell proliferation induced by immunoglobulins from patients with Graves' disease. *J Clin Endocrinol Metab* 1997; **82**:670–3.
- 53 Lewinski A, Pawlikowski M, Cardinali DP. Thyroid growth-stimulating and growth-inhibiting factors. *Biol Signals* 1993; **2**:313–51.
- 54 Lewinski A, Klencki M. (1995) Obecny stan wiedzy na temat czynników regulujących procesy wzrostowe gruczolu tarczowego [(Factors involved in the regulation of thyroid growth processes – current status of research) (In Polish with English abstract.)]. *Endokr Pol – Pol J Endocrinol* 1995; **46**(suppl 1/1):9–23.
- 55 Bahn RS, Heufelder AE. Pathogenesis of Graves' ophthalmopathy. *N Engl J Med* 1993; **329**:1468–75.
- 56 Burch HB, Wartofsky L. Graves' ophthalmopathy: current concepts regarding pathogenesis and management. *Endocrine Rev* 1993; **14**:747–93.
- 57 Perros P, Kendall-Taylor P. Thyroid associated ophthalmopathy: pathogenesis and clinical management. *Baillieres Clin Endocrinol Metab* 1995; **9**:115–35.
- 58 Perros P, Crombie AL, Kendall-Taylor P. Natural history of thyroid associated ophthalmopathy. *Clin Endocrinol (Oxford)* 1995; **42**:45–50.
- 59 Wall JR, Salvi M, Bernard NF. Thyroid associated ophthalmopathy: a model for the association of organ-specific autoimmune disorders. *Immunology Today* 1991; **12**:150–3.
- 60 Weetman AP. Thyroid-associated eye disease: pathophysiology. *Lancet* 1991; **338**:25–8.
- 61 Bednarczuk T, Hiromatsu Y, Wall JR, Nauman J. T cell mediated immunity in thyroid-associated ophthalmopathy. *Progress in Thyroid Associated Ophthalmopathy, Satellite Symposium 12th International Thyroid Congress, Kyoto, Abstract Book, 2000.* p. 33–40.
- 62 Rasmussen AK, Nygaard B, Feld-Rasmussen U. ¹³¹I and thyroid-associated ophthalmopathy. *Eur J Endocrinol* 2000; **155**:60.
- 63 Heufelder AE, Dutton CM, Sarkar G, Donnonvan KA, Bahn RS. Detection of THS receptor DNA in cultured fibroblasts from patients with Graves' ophthalmopathy and pretibial dermopathy. *Thyroid* 1993; **3**:297–330.
- 64 Bahn R, Dutton C, Natt N, Jobs W, Spitzweg C, Heufelder AE. Thyrotropin receptor expression in Graves' orbital adipose tissue/connective tissue: potential autoantigen in Graves' ophthalmopathy. *J Clin Endocrinol Metab* 1998; **83**:998–1002.
- 65 Kasper M, Archibald C, De Bellis AM, Li AW, Yamada M, Chang HC et al. Eye muscle antibodies and subtype of thyroid-associated ophthalmopathy. *Progress in Thyroid Associated Ophthalmopathy, Satellite Symposium 12th International Thyroid Congress, Kyoto, Abstract Book, 2000.* p. 13–8.
- 66 Kiljanski J, Nebes V, Wall JR. The ocular muscle is the target of the immune system in endocrine ophthalmopathy. *Intl Arch Allerg Immunol* 1995; **106**:206–212.
- 67 Wall JR. Extra thyroidal manifestations of Graves' disease. *J Clin Endocrinol Metab* 1995; **80**:3427–9.
- 68 Heufelder AE. Pathogenesis of Graves' ophthalmopathy: recent controversies and progress. *Eur J Endocrinol* 1995; **132**:532–41.
- 69 Hiromatsu Y. Role of cytokines in the pathogenesis of thyroid-associated ophthalmopathy. *Progress in Thyroid Associated Ophthalmopathy, Satellite Symposium 12th International Thyroid Congress, Kyoto, Abstract Book, 2000.* p. 41–5.
- 70 Somach SC, Helm TN, Lawor KB, Bergfeld WF, Bass J. Pretibial mucin: histologic patterns and clinical correlation. *Arch Dermatol* 1993; **129**:1152–6.
- 71 Peacey SR, Flemming L, Messenger A, Weetman AP. Is Graves' dermopathy a generalized disorder? *Thyroid* 1996; **6**:41–5.
- 72 Wortsman J, Dietrich J, Traycoff RB, Stone S. Preradial myxedema in the thyroid disease. *Arch Dermatol* 1981; **117**:635–8.
- 73 Smith TJ, Bahn RS, Gorman CA. (1989) Connective tissue, glycosaminoglycans, and diseases of the thyroid. *Endocr Rev* 1989; **10**:366–91.