Interleukin-1- β Gene, But Not the Interleukin-1 Receptor Antagonist Gene, Is Associated With Graves' Disease

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Interleukin-1 (IL-1) is considered to be involved in the pathogenesis of Graves' disease. The aim of this study was to test whether the IL-1- β gene promoter region and exon 5 and IL-1 receptor antagonist (IL-1Ra) gene intron 2 polymorphisms could be useful genetic markers for susceptibility to Graves' disease. A normal control group of 163 healthy people and another group of 95 patients with Graves' disease were examined. Polymerase chain reaction (PCR) was used to analyze the variable number of tandem repeats (VNTRs) at intron 2 of the IL-1Ra gene for the polymorphism. PCR-based restriction analysis was done for the IL-1- β gene polymorphisms of the promoter region and exon 5 using endonucleases *Ava*I and *Taq*I, respectively. We found significantly increased frequencies of the C/C homozygous genotype (χ^2 test, $P=0.038$; odds

ratio (OR) = 2.558, 95% confidence interval (CI) = 1.205–5.430) and the C allele (χ^2 test, $P=0.011$; OR = 1.589, 95% CI = 1.094–2.309) in the IL-1- β gene promoter (–511 C/T polymorphism) in Graves' disease patients compared to normal controls. There were no significant differences in polymorphisms of IL-1- β gene exon 5 and IL-1Ra gene intron 2 between the patient and normal control groups. A subgroup analysis also demonstrated no association between the severity of the disease and any polymorphism of IL-1-related genes. We suggest that the IL-1- β gene promoter polymorphism can be used as a genetic marker for susceptibility to Graves' disease. It is worthwhile to study the cytokine genes further because of the association between cytokines and Graves' disease. *J. Clin. Lab. Anal.* 19:133–138, 2005. © 2005 Wiley-Liss, Inc.

Key words: interleukin-1- β ; interleukin-1 receptor antagonist; Graves' disease; single nucleotide polymorphisms (SNPs)

INTRODUCTION

Graves' disease is a common autoimmune thyroid disease and is the major cause of hyperthyroidism. Clinically, Graves' disease is characterized by diffuse goiter, thyrotoxicosis, and infiltrative ophthalmopathy. The pathogenesis of Graves' disease involves the action on the thyroid gland by thyrotropin receptor antibodies (TRAb) against thyrotropin (thyroid stimulating hormone (TSH)) receptors (1). These antibodies have both TSH-displacing and thyroid-stimulating activities. TRAb binds to the TSH receptors and activates adenylate cyclase, thereby initiating a chain of reactions that leads to thyroid growth and hyperse-

cretion of hormones (2). Therefore, immune response plays a central role in the pathogenesis of Graves' disease.

Cytokines, a large group of non-enzymatic protein hormones, participate in the induction and effector phases of all inflammatory and immune responses, and are therefore likely to play a critical role in the

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development of autoimmune diseases (3). Interleukin-1 (IL-1), which is primarily synthesized by activated mononuclear phagocytes, is a potent proinflammatory cytokine that plays a central role in the induction of fever, acute phase protein synthesis, and cachexia (4). It also exerts an influence on immunological properties, including T-cell activation, B-cell proliferation, and immunoglobulin synthesis (4,5). Therefore, IL-1 is considered to be involved in the development of various inflammatory and immune diseases, including Graves' disease (6,7).

IL-1 is actually a family of three proteins: IL-1- α , IL-1- β , and IL-1 receptor antagonist (IL-1Ra). IL-1- β has a similar structure and biological activity to IL-1- α and is the predominant species of human IL-1, whereas IL-1Ra antagonizes the activities of the former two proteins (4,5). The three proteins are encoded by different genes located on the long arm of chromosome 2 (5). These IL-1 related genes have been used as genetic markers to predict the development of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis, and alopecia areata (8–11).

Single nucleotide polymorphisms (SNPs) of the IL-1- β gene promoter and exon 5, and variable number tandem repeat (VNTR) polymorphisms of IL-1Ra gene intron 2 have been identified and used to screen for the relationship between the occurrence and severity of rheumatoid arthritis and osteoporosis (12,13). However, the results of studies that examined the association of these gene polymorphisms with susceptibility to Graves' disease are limited and contradictory (14–16). To test whether these polymorphisms could be markers of susceptibility for Graves' disease, we used PCR analysis to compare genotypes and allelic frequencies between a normal population and patients with Graves' disease by screening for the IL-1 gene promoter and exon 5 regions and IL-1Ra gene intron 2 polymorphisms.

MATERIALS AND METHODS

Patient Selection

A total of 95 unrelated patients (21 males and 74 females) between the ages of 17 and 71 years (average = 35.3 ± 10.5 years) with Graves' disease were enrolled in this study from January 2003 to June 2003. Graves' disease was defined by the presence of hyperthyroidism and diffuse goiter, supported by thyroid-related eye disease and the presence of serum antibodies to thyroid components (TSH receptor, microsome, and/or thyroglobulin). Information was also collected on the patient's age at onset, serum thyroid hormone levels and antibody titers, size of goiter, presence of exophthalmos, and history of hyperthyroidism.

A group of 163 healthy volunteers over the age of 40 years, who were euthyroid and had no history or family history of Graves' disease or other autoimmune diseases, served as controls. Informed consent was obtained from each subject that participated in the study.

Genomic DNA was prepared from peripheral blood using the Genomaker reagent kit (Blossom Inc., Taipei, Taiwan).

PCR

PCR was used to identify the genotypes of all IL-1 related genes. PCR of the polymorphisms was performed to a total volume of 50 μ L, containing genomic DNA (2–6 pmol of each primer), $1 \times$ Taq polymerase buffer (1.5 mM $MgCl_2$), and 0.25 U of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). The primers for the IL-1- β gene promoter region, IL-1- β gene exon 5, and IL-1Ra gene polymorphisms are listed in Table 1, according to a report by Cantagrel and coworkers (12). PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System

TABLE 1. Amplification primer sequences (5' to 3' orientation)

Set	Position	Primer and PCR conditions ^a	PCR product (bp)	Restriction site
IL-1 beta promoter	-511 C/T	U 5'-TGGCATTGATCTGGTTCATC-3'; D 5'-GTTTAGGAATCTGGACCAGA-3'; 95°C $\times$ 30 sec, 55°C $\times$ 30 sec and 72°C $\times$ 30 sec	190 + 114 or 304	<i>Ava</i> I
IL-1 beta exon 5	Exon 5	U 5'-GTTGTCATCAGACTTTGACC-3'; D 5'-TTCAGTTCATATGGACCAGA-3'; 95°C $\times$ 30 sec, 55°C $\times$ 30 sec and 72°C $\times$ 30 sec	135 + 114 or 249	<i>Taq</i> I
IL-1Ra	Intron 2	U 5'-CTCAGCAACACTCCTAT-3'; D 5'-TCCTGGTCTGCAGGTAA-3'; 95°C $\times$ 30 sec, 58°C $\times$ 30 sec and 72°C $\times$ 30 sec	I: 410 II: 240 IV: 325	VNTRs

^aU and D, respectively, indicate upstream and downstream primers.

VNTR, variable number tandem repeats.

2400 (Perkin Elmer). The cycling conditions are given in Table 1.

For IL-1Ra gene intron 2, 10 μ L of the products were loaded onto 3% agarose gel containing ethidium bromide for electrophoresis, and each allele was recognized according to its size. The 86-bp VNTRs of the IL-1Ra gene were classified as follows: I for 410-bp, II for 240-bp, III for 500-bp, IV for 325-bp, and V allele for 595-bp. The IL-1- β gene promoter polymorphism at position -511 was analyzed by PCR amplification followed by restriction analysis using *Ava*I (New England Biolabs, Beverly, CA) digestion. The C allele at position -511 was categorized as C, which showed up as 190-bp and 114-bp fragments on agarose electrophoresis. The T allele was 304-bp and was encoded at position -511. The region containing the polymorphic site within exon 5 of the IL-1- β gene was amplified and then digested by *Taq*I (New England Biolabs). Class E1 was 135 bp + 114 bp, and E2 was 249 bp, as shown on electrophoresis.

For statistical analysis, the genotype and allelic frequency distributions of polymorphisms in the control and Graves' disease patient groups were compared using the χ^2 test. When the assumption of the χ^2 test was violated (i.e., when one cell had an expected count of <1, or >20% of the cells had an expected count of <5), Fisher's exact test was used. Odds ratios (ORs) with 95% confidence intervals (CIs) were determined for the disease susceptibility of specific genotypes and alleles. Results were considered statistically significant when the probability of findings occurring by chance was less than 5% ($P < 0.05$).

RESULTS

In the genotype analysis, Graves' disease patients had a higher ratio of the C/C homozygous genotype (30.5%) and a lower ratio of the T/T homozygous genotype (17.9%) for the IL-1- β gene promoter compared to normal controls (C/C: 20.8% and T/T: 31.3%, respectively; χ^2 test, $P = 0.038$; Table 2). The OR for the risk of the C/C homozygous genotype in the Graves' disease patient group was 2.558, with a 95% CI of 1.205–5.430. However, the genotype distributions of IL-1- β gene exon 5 ($P = 0.851$) and IL-1 Ra gene intron 2 ($P = 0.897$) were almost identical in both groups.

In the allelic analysis, there were significantly increased C allele (56.3%) and decreased T allele (43.7%) frequencies for the IL-1- β gene promoter in Graves' disease patients compared to normal controls (C: 44.8% and T: 55.2%, respectively; χ^2 test, $P = 0.011$; Table 3). The OR for the risk of the C allele in the Graves' disease patient group was 1.589, with a 95% CI of 1.094–2.309. As in the genotype analysis, there were also no differences in allelic distribution for IL-1- β gene exon 5 ($P = 0.930$) and IL-1Ra gene intron 2 polymorphisms ($P = 0.849$) between the patient and control groups.

A further subgroup analysis was performed, and there were no significant associations of IL-1-related gene polymorphisms (including IL-1- β promoter polymorphism) with age at onset, thyroid hormone levels, TRAb titer, size of goiter, presence of exophthalmos, or history of hyperthyroidism (data not shown).

TABLE 2. Genotype frequencies of interleukin (IL)-1 beta gene promoter and exon 5 regions and IL-1Ra gene intron 2 between healthy control subjects and Graves' disease patients

Genotype	Graves' disease patients n = 95 (%)	Controls n = 163 (%)	P
IL-1 beta promoter ^a			0.038 ^b
-511*T/-511*T	17 (17.9)	51 (31.3)	
-511*C/-511*T	49 (51.6)	78 (47.9)	
-511*C/-511*C	29 (30.5)	34 (20.8)	
IL-1 beta exon 5			0.851 ^c
E1/E1	90 (94.7)	155 (95.1)	
E1/E2	5 (5.3)	7 (4.3)	
E2/E2	0 (0.0)	1 (0.6)	
IL-1Ra			0.897 ^c
I/I (410/410)	87 (91.6)	146 (89.6)	
I/II (410/240)	7 (7.4)	15 (9.2)	
II/II (240/240)	1 (1.0)	1 (0.6)	
IV/IV (325/325)	0 (0.0)	1 (0.6)	

^aOR for C/T = 1.884 (95% CI = 0.957–3.708), for C/C = 2.558 (95% CI = 1.205–5.430).

^bChi-square test.

^cFisher's exact test.

TABLE 3. Allelic frequencies of interleukin (IL)-1 beta gene promoter and exon 5 regions and IL-1Ra gene intron 2 between healthy control subjects and Graves' disease patients

Allelic frequency	Graves' disease patients n = 190 (%)	Controls n = 326 (%)	P
IL-1 beta promoter ^a			0.011 ^b
Allele T	83 (43.7)	180 (55.2)	
Allele C	107 (56.3)	146 (44.8)	
IL-1 beta exon 5			0.930 ^b
E1	185 (97.4)	317 (97.2)	
E2	5 (2.6)	9 (2.8)	
IL-1Ra			0.849 ^c
I (410 bp)	181 (95.3)	307 (94.2)	
II (240 bp)	9 (4.7)	17 (5.2)	
IV (325 bp)	0 (0.0)	2 (0.6)	

^aOR for C = 1.589 (95% CI = 1.094–2.309).^bChi-square test.^cFisher's exact test.

DISCUSSION

We report a novel finding that demonstrates an association between the IL-1- β gene and susceptibility to Graves' disease. Our results show increased frequencies of C/C homozygous genotype and C allele of the IL-1- β gene promoter (–511) in Graves' disease patients. In theory, the difference of a single nucleotide in this region may interfere with the expression of the IL-1- β gene, the resulting production of IL-1- β , and the subsequent immune reactions, and thus contribute to a person's susceptibility to Graves' disease. Therefore, the IL-1- β gene could be useful as a genetic marker to predict the development of Graves' disease.

The etiology of Graves' disease is complex and involves multiple genetic and environmental influences. The well-known genes that contribute to susceptibility for Graves' disease are the human leukocyte antigen (HLA) class II genes and the cytotoxic T lymphocyte antigen-4 (CTLA-4) gene (17–19). However, each of these genes contributes no more than 5% to the overall genetic susceptibility, indicating the involvement of other genes (20). Linkage analysis has identified loci on chromosomes 14q31, 20q11.2, and Xq21 (GD1, GD2, and GD3) that are associated with susceptibility to Graves' disease (21–23). Other candidate genes, such as TSH receptor (TSHr) and Fas ligand genes have also been examined, but no consistent association has been found (24–26). The gene encoding IL-1Ra has been reported to be associated with Graves' disease (14). However, to our knowledge, no studies to date have defined the relationship between the IL-1- β gene and Graves' disease. Our findings provide preliminary

evidence that the IL-1- β gene contributes to susceptibility for Graves' disease.

Graves' disease is caused by the stimulatory effect on the thyroid gland by TRAb, which is an IgG antibody (27). In general, stimulation of human CD4⁺ T helper (Th) lymphocytes by bacterial toxins, tissue injury, or various exogenous or endogenous antigens results in the development of the Th1 and Th2 patterns of cytokine production. Th1 cells produce interferon gamma, IL-2, and tumor necrosis factor- β , and promote cellular immune activity. Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, and provide optimal help for the humoral response, which is responsible for the production of antibodies. The Th1 cytokines inhibit the development of Th2-mediated responses, while Th2 cytokines cause reciprocal inhibition of Th1 responses (28,29). IL-1 is primarily a proinflammatory protein, but it also has immunoregulatory effects. It exerts its activity as a lymphocyte activating factor to enhance the production of IL-2, IL-4, IL-6, and interferon gamma (4,5), and therefore has potential effects on both Th1 and Th2 cell reactions.

The relationship between the activities of IL-1 and the changes in Th1/Th2 cytokine balance has been well studied by some authors. For example, Manetti and coworkers (30) found that blocking or neutralizing IL-1 activities may induce reduced production of IL-4, and that increased production of interferon gamma by T cells and IL-1Ra can make CD4⁺ T cells present the Th1-like cytokine profile in vitro. In addition, an in vivo study by Chensue et al. (31) showed that depletion of IL-1Ra resulted in a shift of response from the Th1 to the Th2-like profile. These results reveal that IL-1 favors

the development of Th2 and the inhibition of Th1 pathway reactions, and leads antibody formation, which is critical in the development of Graves' disease. Furthermore, Miyazaki et al. (7) demonstrated a positive correlation between the presence of IL-1- β on perifollicular endothelial cells and the presence of serum antimicrosomal antibodies, which may reflect the immune response of the thyroid gland and provide additional evidence for the role of IL-1- β in the occurrence of Graves' disease.

Our study showed that polymorphism of the IL-1- β gene promoter region, but not of IL-1- β gene exon 5, was significantly associated with susceptibility to Graves' disease. Therefore, we propose that a change in the regulation of IL-1 gene expression may modulate immune properties and autoantibody formation, resulting in the development of Graves' disease. This hypothesis should be further examined to elucidate the role of the IL-1- β gene in the network of IL-1- β production, the immune responses, and susceptibility to Graves' disease. However, there are two other mechanisms by which IL-1- β gene contributes to susceptibility to Graves' disease. First, the possible role of gene recombination linkage disequilibrium in the development of Graves' disease should not be excluded. For example, the CTLA-4 gene, another candidate gene for Graves' disease, is also located on chromosome 2 (2q33) and is very close to the IL-1- β gene site, 2q12-14 (19,32). Second, in addition to immunoregulatory activities, IL-1 also has direct biological effects on the thyroid gland. IL-1 can act as a chemical or hormone-like factor to regulate the proliferation and modulate the function of thyrocytes (6,33), and may therefore be involved in the development of Graves' disease.

Whether IL-1Ra gene polymorphism is associated with Graves' disease remains a matter of controversy. Using PCR for a VNTR analysis of IL-1Ra gene intron 2, Blakemore and coworkers (14) found that there was a significant increase in IL-1Ra II allele frequency and carriage rate in Graves' disease patients in the United Kingdom. However, studies in the United States and Germany were unable to corroborate this finding (15,16). Our data also showed no significant difference in genotype and allelic distributions for IL-1Ra gene intron 2 polymorphism between the patient and control groups. The reasons for the discrepancy between the results of our study and those of Blakemore et al. (14) are unknown, but may reflect the different genetic pools represented in the different racial populations. Because IL-1Ra antagonizes the activities of IL-1, it should be further investigated to define the association between the IL-1Ra gene and Graves' disease.

The relationships between polymorphisms of IL-1 related genes and the clinical severity of Graves' disease

were also evaluated in our study. Our results demonstrated no difference between the clinical manifestations and severity of Graves' disease and IL-1-related gene polymorphisms in Graves' disease patient subgroups. However, because IL-1- β gene polymorphism is associated with extrathyroidal manifestations of Graves' disease (15), and IL-1Ra gene polymorphism is a disease severity factor in other autoimmune diseases, such as systemic lupus erythematosus, alopecia areata, and type 1 DM (9,11,34), it is necessary to study these IL-1-related gene polymorphisms in a larger number of patients to determine the association between disease severity and gene polymorphisms.

Because of the lack of predictive markers for Graves' disease, most patients are diagnosed after symptoms develop. Therefore, a reliable marker for Graves' disease could lead to earlier diagnosis and treatment, and have a significant impact on improved patient care and outcomes. Our data indicate that IL-1 gene promoter polymorphism is a candidate genetic marker to screen for Graves' disease. The C/C homozygous genotype and C allele were prominent in the Graves' disease patients. Individuals with the C/C genotype and C allele in the IL-1 gene promoter (-511) might be expected to have a high risk of Graves' disease. Since the cytokines are a large group of proteins, and many of them participate in the modulation of immune reactions, other cytokine genes may also contribute to the development of Graves' disease. Therefore, it is necessary to investigate the other cytokine genes to predict the development of Graves' disease earlier.

REFERENCES

1. Larsen PR, Alexander NM, Chopra IJ, et al. Revised nomenclature for tests of thyroid hormones and thyroid-related proteins in serum. *J Clin Endocrinol Metab* 1987;64:1089-1092.
2. 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;82:670-673.
3. Kelso A. Cytokines: principles and prospects. *Immunol Cell Biol* 1998;76:300-317.
4. Dinarello CA, Wolff SM. The role of interleukin-1 in disease. *N Engl J Med* 1993;328:106-113.
5. Rosenwasser LJ. Biologic activities of IL-1 and its role in human disease. *J Allergy Clin Immunol* 1998;102:344-350.
6. Kawabe Y, Eguchi K, Shimomura C, et al. Interleukin-1 production and action in thyroid tissue. *J Clin Endocrinol Metab* 1989;68:1174-1183.
7. Miyazaki A, Hanahusa T, Itoh N, et al. Demonstration of interleukin-1 β on perifollicular endothelial cells in the thyroid glands of patients with Graves' disease. *J Clin Endocrinol Metab* 1989;69:738-744.
8. McDowell TL, Symons JA, Ploski R, Forre O, Duff GW. A genetic association between juvenile rheumatoid arthritis and a novel interleukin-1 α polymorphism. *Arthritis Rheum* 1995;38:221-228.

9. Blakemore AIF, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW. Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. *Arthritis Rheum* 1994;37:1380–1385.
10. Mansfield JC, Holden H, Tarlow JK, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. *Gastroenterology* 1994;106:637–642.
11. Tarlow JK, Clay FE, Cork MJ, Blakemore AIF, McDonagh AJG, Messenger AG. Severity of alopecia areata is associated with a polymorphism in the interleukin-1 receptor antagonist gene. *J Invest Dermatol* 1994;103:387–390.
12. Cantagrel A, Navaux F, Loubet-Lescoulie P, Nourhashemi F, Enault G, Abbal M. Interleukin-1 β , interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. *Arthritis Rheum* 1999;42:1093–1100.
13. Langdahl BL, Lokke E, Carstens M, Stenkjaer LL, Eriksen EF. Osteoporotic fractures are associated with an 86-base pair repeat polymorphism in the interleukin-1 receptor antagonist gene but not with polymorphisms in the interleukin-1 β gene. *J Bone Miner Res* 2000;15:402–414.
14. 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;80:111–115.
15. Cuddihy RM, Bahn RS. Lack of an association between alleles of interleukin-1 α and interleukin receptor antagonist genes and Graves' disease in a North American Caucasian population. *J Clin Endocrinol Metab* 1996;81:4476–4478.
16. Muhlberg T, Kirchberger M, Spitzwig C, Herrmann F, Heberling HJ, Heufelder AE. Lack of association of Graves' disease with the A2 allele of the interleukin-1 receptor antagonist gene in a white Europe population. *Eur J Endocrinol* 1998;138:686–690.
17. Heward JM, Allahabadia A, Daykin J, 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;83:3394–3397.
18. Chen QY, Huang W, She JX, Baxter F, Volpe R, Maclaren MK. HLA-DRB1*08, DRB1*03/DRB3*0101, and DRB3*0202 are susceptibility genes for Graves' disease in North American Caucasians, whereas DRB1*07 is protective. *J Clin Endocrinol Metab* 1999;84:3182–3186.
19. Yanagawa T, Hidaka Y, Guimaraes V, Soliman M, DeGroot LJ. CTLA-4 gene polymorphism associated with Graves' disease in Caucasian population. *J Clin Endocrinol Metab* 1995;80:41–45.
20. Davies TF. Editorial: autoimmune thyroid disease genes come in many styles and colors. *J Clin Endocrinol Metab* 1998;83:3391–3393.
21. Tomer Y, Barbesino G, Greenberg DA, Concepcion E, Davis TF. 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;83:4321–4327.
22. Tomer Y, Barbesino G, Greenberg DA, Concepcion ES, Davis TF. A new Graves' disease-susceptibility locus maps to chromosome 20q11.2. *Am J Hum Genet* 1998;63:1749–1756.
23. Barbesino G, Tomer Y, Concepcion ES, Davis TF, Greenberg DA. Linkage analysis of candidate genes in autoimmune thyroid disease. II. Selected gender-related genes and the X-chromosome. *J Clin Endocrinol Metab* 1998;83:3290–3295.
24. Bahn RS, Dutton CM, Heufelder AE, Sarker G. A genomic point mutation in the extracellular domain of the thyrotropin receptor in patients with Graves' ophthalmopathy. *J Clin Endocrinol Metab* 1994;78:256–260.
25. Bluher M, Krohn K, Wallaschofski H, Braverman LE, Paschke R. Fas and Fas ligand gene expression in autoimmune thyroiditis in BB/W rats. *Eur J Endocrinol* 1999;141:506–511.
26. Stuck BJ, Pani MA, Besrouer F, et al. Fas ligand gene polymorphisms are not associated with Hashimoto's thyroiditis and Graves' disease. *Hum Immunol* 2003;64:285–289.
27. Kriss JP, Pleshakov V, Chien JR. Isolation and identification of the long-acting thyroid stimulator and its relation to hyperthyroidism and circumscribed pretibial myxedema. *J Clin Endocrinol* 1964;24:1005–1028.
28. Romagnani S. Th1 and Th2 in human diseases. Short analytic review. *Clin Immunol Immunopathol* 1996;80:225–235.
29. Kocjan T, Wraber B, Repnik U, Hojker S. Changes in Th1/Th2 cytokine balance in Graves' disease. *Eur J Physiol* 2000;400(suppl):94–95.
30. Manetti R, Barak V, Piccinni MP, et al. Interleukin-1 favours the *in vitro* development of the type 2 helper (Th2) human T-cell clones. *Res Immunol* 1994;145:93–100.
31. Chensue SW, Bienkowski M, Eessalu TE, et al. Endogenous IL-1 receptor antagonist protein (IRAP) regulates Schistosoma egg granuloma formation and the regional lymphoid response. *J Immunol* 1993;151:3654–3662.
32. Bensen JT, Dawson PA, Mychaleckyj JC, Bowden DW. Identification of a novel human cytokine gene in the interleukin gene cluster on chromosome 2q12-14. *J Interferon Cytokine Res* 2001;21:899–904.
33. Rasmussen AK, Bech K, Feldt-Rasmussen U, et al. Interleukin-1 affects the function of cultured human thyroid cells. *Allergy* 1988;435–441.
34. Mandrup-Poulsen T, Pociot F, Molvig J, et al. Monokine antagonism is reduced in patients with IDDM. *Diabetes* 1994;43:1242–1247.