CLINICAL STUDY

Changes in expression of T-helper (Th) 1- and Th2-associated chemokine receptors on peripheral blood lymphocytes and plasma concentrations of their ligands, interferon-inducible protein-10 and thymus and activation-regulated chemokine, after antithyroid drug administration in hyperthyroid patients with Graves' disease

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Abstract

Objective: Although Graves' disease is considered an autoantibody-mediated, T-helper 2 (Th2)-dominant disease, Th1-dominance may prevail in its initial phase. We longitudinally investigated Th1/Th2 balance in untreated hyperthyroid patients with Graves' disease after treatment of methimazole (MMI), an antithyroid drug.

Design: University clinic outpatients were studied prospectively.

Patients: Subjects included 23 untreated hyperthyroid patients with Graves' disease and 17 age-matched control subjects.

Methods: Before and after treatment, we measured Th1- and Th2-associated chemokine receptors (CXCR3 and CCR4), on peripheral blood lymphocytes using flow cytometry, as well as plasma concentrations of their ligands, interferon-inducible protein (IP)-10 and thymus and activation-regulated chemokine (TARC).

Results: The percentage of CXCR3-expressing cells among CD4⁺T lymphocytes and plasma IP-10 was significantly higher in hyperthyroid Graves' disease patients than in controls. At 12 and 24 weeks after initiation of MMI, percentage of CXCR3-expressing CD4⁺T lymphocytes had decreased significantly, while the percentage of CCR4-expressing CD4⁺T lymphocytes had increased significantly at 24 weeks. The CXCR3/CCR4 ratio had decreased significantly at 24 weeks. Plasma concentrations of IP-10 had decreased significantly at 12 and 24 weeks. Plasma concentrations of TARC also had decreased significantly at 24 weeks.

Conclusions: In hyperthyroid patients with Graves' disease in the active phase, Th1 cells rather than Th2 cells predominated among peripheral blood lymphocytes. After initiation of MMI, an ongoing transition from Th1 to Th2 dominance occurred.

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Introduction

T-helper (Th) lymphocytes consist of two subpopulations, Th1 and Th2, based on distinctive patterns of cytokine production (1). Th1 lymphocytes, which secrete interferon (IFN)- γ and interleukin (IL)-2, are associated strongly with cell-mediated immune responses, while Th2 lymphocytes secrete IL-4 and IL-5 and are involved in humoral immunity (2). Th2 immune responses also down-regulate Th1 immune responses (2). Since these subpopulations tend to function antagonistically toward one another, the

balance between Th1 and Th2 lymphocytes may determine the outcome of autoimmune diseases (3).

Graves' disease, which causes hyperthyroidism, is an autoimmune disease involving the production of thyrotropin (TSH) receptor (TSHR) antibodies (TRAb), which overstimulate thyroid cells to result in hyperthyroidism (4). Graves' disease is characterized by persistent infiltration of the thyroid gland by lymphocytes composed mainly of CD4⁺ and CD8⁺ lymphocytes (4). A previous study demonstrated that thyroid glands affected by Graves' disease contained TSHR-specific T-cell clones that were characterized as Th2 on the basis of an

increased ratio of IL-4 to IFN- γ (5). Further, an *in vitro* study of cultured whole bloods from Graves' disease patients showed an increase in Th2-associated cytokine production (6). Graves' disease has long been thought to be an autoantibody-mediated, Th2-dominant autoimmune disease. However, using a model in which mice were injected with adenovirus expressing TSH receptor, Nagayama *et al.* demonstrated that an immune shift toward Th2 was accompanied by a decrease rather than an expected increase in production of thyroid stimulating antibody, which also suggested that predominant Th1 immune responses to TSH receptor are associated with induction of Graves' disease (7). More importantly, human TSH receptor stimulating antibodies (TSAbs) are predominantly immuno-globulin G (IgG)1, a Th1 type subclass in humans (8). Thus, the classification of Graves' disease as typical Th1 dominant disease is still under debate.

Chemokines are chemotactic cytokines controlling recruitment of leukocytes from the blood by regulating integrin adhesiveness (9). Based on structural motif – specifically, the position of cysteine residues near their N-termini – chemokines have been divided into four subfamilies: a CXC family with one other amino acid between two cysteines; a CC family with no interposed amino acids; a C family with only one cysteine; and a CX3C family with three other amino acids between two cysteines (9). The chemokines act upon their effectors via specific receptors that belong to a seven-transmembrane-domain G protein-coupled family. The chemokine receptors CXCR3 and CCR5 are expressed exclusively on Th1 lymphocytes (10), while Th2 lymphocytes preferentially express CCR4 and CCR3 (11). Recently, Romagnani *et al.* demonstrated recruitment of CXCR3-expressing Th1 lymphocytes and expression of the corresponding ligand, interferon-inducible protein (IP)-10, in thyroid glands of patients at the initial phase of Graves' disease; this suggested Th1 rather than Th2 dominance within the thyroid gland at the onset of illness (12). Several other studies also reported increased serum IP-10 in patients with autoimmune thyroid diseases including Graves' disease (13–15). Thus, the assertion that Graves' disease is a Th2-driven disease is becoming increasingly controversial. A longitudinal study would be required to determine Th1/Th2 balance in patients with Graves' disease during the clinical course. However, no reports have described changes in Th1/Th2 balance in prospective analyses of chemokine receptor expression after treatment in these patients. Moreover, no reported studies have examined changes in plasma concentrations of ligands at these receptors, such as thymus and activation-regulated chemokine (TARC; a ligand for CCR4), after the treatment of Graves' disease.

We presently carried out a longitudinal investigation of Th1/Th2 balance in newly diagnosed hyperthyroid patients with Graves' disease, who were then given an antithyroid drug, by measuring Th1- and Th2-associated chemokine receptors on peripheral blood

lymphocytes using flow cytometry as well as plasma concentrations of the receptor ligands.

Materials and methods

Subjects

We studied 23 untreated patients with Graves' disease (16 female and 7 male) and also 17 age-matched healthy control subjects. Subjects in the patient group had been referred to the outpatient clinic at the Dokkyo Medical University Hospital for treatment of hyperthyroidism. Patient age was 41.3 ± 14.9 years (21 to 63). Graves' disease was diagnosed based upon the presence of diffuse goiter, the elevated serum thyroid hormones (FT4 and FT3) and suppressed TSH, and the presence of TRAb. All patients with Graves' disease were treated with methimazole (MMI; 15–30 mg/day). The drug dose was decreased gradually whilst monitoring maintenance of normal thyroid function. At the end of the 24-week treatment period 21 were in a euthyroid state. Excluded from the study were patients with coexistent other organ-specific autoimmune disease; patients with medications that could affect the immune systems (such as corticosteroid) were also excluded, as were patients with allergic disease, such as those with atopic dermatitis or bronchial asthma. All patients gave informed consent. The study was approved by the local Ethics Committee of our institution.

Methods

Blood samples were obtained at study entry (baseline) after 12 weeks, and after 24 weeks of treatment. For plasma separation, venous blood was collected in a tube containing EDTA-2Na between 0800 and 0900 h after an overnight fast. After plasma samples were centrifuged at 2500 *g* for 15 min, the supernatant was stored at -30°C until use.

Analysis of chemokine receptors by flow cytometry

Heparinized whole blood (0.1 ml) was incubated with antibodies to CXCR3 (DAKO Cytomation, Glostrup, Denmark), CCR4 phycoerythrin (Becton Dickinson, San Jose, CA, USA), and to CD4 peridinin chlorophyll protein (Becton Dickinson) conjugated all with carboxyfluorescein succinimidyl ester for 15 min at room temperature. Red blood cells were lysed by treating blood samples with ammonium chloride; white cells and then were resuspended in phosphate buffered saline, for analysis by a FACScan (Becton Dickinson). Receptor expression data were analyzed with CellQuest software (Becton Dickinson). Results are expressed as percentages of CD4+ cells expressing CXCR3 and CCR4, as well as the ratio of CD4+ cells bearing

CXCR3 to those bearing CCR4. We regarded CXCR3 as a Th1-associated chemokine receptor, and CCR4 as a Th2-associated chemokine receptor.

Measurements of chemokines in plasma

Plasma concentrations of IP-10 were measured by ELISA (human IP-10 immunoassay, R&D Systems, Minneapolis, MN, USA). Intra- and interassay coefficients of variation (CV) were 2.43–4.82% and 5.45–8.26% respectively. Plasma concentrations of TARC also were measured by ELISA (human TARC immunoassay, R&D Systems). Intra- and inter-assay CV were 2.90–4.22% and 3.75–5.35% respectively.

TRAb was measured by a commercial RIA kit (TRAb CosmicIII, RSR, UK). Serum IgG was determined by an immunonephelometric assay (Dade Behring, Marburg, Germany). Serum IgE was measured by a fluoro-enzyme immunoassay (Phadia, Tokyo, Japan).

Statistical analysis

Data are expressed as the mean $\pm$ s.d. or the median and interquartile ranges. Differences between groups were analyzed by a Student's paired *t*-test or an unpaired *t*-test. For nonparametric data, differences between groups were analyzed by Wilcoxon's matched paired test or the Mann–Whitney test. Correlation was determined by linear regression analysis. Logarithmic transformation of serum IgE concentration was used to render the distribution normal. A *P* value below 0.05 was accepted as indicating statistical significance. Statistical analyses were carried out using SPSS 8.0 J software (SPSS, Tokyo, Japan).

Results

As shown in Table 1, both plasma IP-10 and TARC concentrations at the baseline were significantly higher in hyperthyroid patients with Graves' disease than in control subjects (154.1 ± 62.1 vs 107.7 ± 44.8 pg/ml, $P=0.0078$; and 57.6 ± 38.7 vs 25.7 ± 13.9 pg/ml, $P=0.0025$ respectively). The percentage of CXCR3-expressing CD4⁺T lymphocytes was significantly higher in hyperthyroid patients with Graves' disease than in control subjects (27.0 ± 7.0 vs $22.6 \pm 5.5\%$, $P=0.0378$), while we found no significant difference in CCR4-expressing cells among CD4⁺T lymphocytes or in the CXCR3/CCR4 ratio between patients with hyperthyroid, patients with Graves' disease and control subjects (Table 1). At baseline, neither FT3 nor FT4 correlated with the percentage of CXCR3-expressing cells among CD4⁺T lymphocytes, the percentage of CCR4-expressing cells among CD4⁺T lymphocytes, and plasma concentrations of their ligands, IP-10 and TARC (data not shown). We also found no significant relationship between TRAb and the expression of

Table 1 Characteristics and laboratory data of control subjects and untreated patients with hyperthyroid Graves' disease.

	Control	Hyperthyroid GD	<i>P</i> value
<i>N</i> (female/male)	17 (12/5)	23 (16/7)	
Age (years)	37.1 ± 12.8	41.3 ± 14.9	0.3560
Plasma IP-10 (pg/ml)	107.7 ± 44.8	154.1 ± 62.1	0.0078
Plasma TARC (pg/ml)	25.7 ± 13.9	57.6 ± 38.7	0.0025
CXCR3+/CD4+ (%)	22.6 ± 5.5	27.0 ± 7.0	0.0378
CCR4+/CD4+ (%)	12.9 ± 9.0	12.2 ± 5.4	0.7503
CXCR3+/CCR4+ ratio	2.28 ± 1.01	2.78 ± 1.70	0.2878

GD, Graves' disease; IP-10, interferon-inducible protein; TARC, thymus and activation-regulated chemokine. Data are mean $\pm$ s.d.

chemokine receptors or their ligands concentration at baseline.

As shown in Table 2, both serum FT4 and FT3 were significantly reduced at 12 and 24 weeks after beginning MMI treatment. TRAb also was reduced at 12 and 24 weeks after treatment initiation. TRAb was lower at 12 weeks than at 24 weeks. Serum total IgG was significantly increased at 12 and 24 weeks after initiating treatment, while no significant changes in serum IgE were found.

Figure 1 displays representative flow cytometric results concerning changes in the percentage of CXCR3-expressing or CCR4-expressing cells among peripheral CD4⁺T lymphocytes after treatment with MMI in a typical patient with hyperthyroid Graves' disease. We found a marked reduction in the percentage of CXCR3-expressing CD4⁺T lymphocytes and a marked increase in the percentage of CCR4-expressing CD4⁺T lymphocytes at 24 weeks compared with baseline.

After treatment with MMI, the percentage of CXCR3-expressing CD4⁺T lymphocytes decreased from $27.0 \pm 7.0\%$ at baseline to $24.0 \pm 7.0\%$ at 12 weeks and to $22.8 \pm 6.3\%$ at 24 weeks ($P=0.0132$ and $P=0.0026$ respectively; Fig. 2A). Furthermore, the percentage of CXCR3-expressing cells was significantly lower at 24 weeks than at 12 weeks ($P=0.0426$). On the other hand, the percentage of CCR4-expressing CD4⁺T lymphocytes increased significantly, from $12.2 \pm 5.4\%$ at baseline to $14.7 \pm 4.8\%$ at 24 weeks ($P=0.0218$) after beginning treatment (Fig. 2B). The percentage of CCR4-expressing cells was significantly higher at 24 weeks than at 12 weeks ($P=0.0147$). The CXCR3/CCR4 ratio decreased significantly from 2.08 (1.65, 3.50) at baseline to 1.59 (1.20, 1.83) at 24 weeks ($P=0.0058$) after beginning treatment (Fig. 3B). The ratio was also significantly lower at 24 weeks than at 12 weeks ($P=0.0288$).

After initiation of MMI treatment, plasma concentrations of IP-10 decreased significantly, from 154.1 ± 56.1 at baseline to 91.3 ± 34.1 pg/ml at 12 weeks and to 94.4 ± 30.3 pg/ml at 24 weeks ($P=0.0002$ and $P=0.0001$ respectively; Fig. 4A). On the other hand, plasma concentrations of TARC decreased significantly, from 52.0 (40.3, 62.1) at baseline to 34.0 (23.9, 49.2) pg/ml at 24 weeks ($P=0.0414$; Fig. 4B).

Table 2 Changes in thyroid hormones, TRAb, total IgG, and IgE after treatment with methimazole in hyperthyroid patients with Graves' disease.

	Duration of methimazole treatment		
	0 week	12 weeks	24 weeks
FT4 (ng/dl)	5.25 ± 1.90	1.45 ± 1.72 [§]	1.36 ± 0.53 [§]
FT3 (pg/ml)	18.2 ± 7.22	6.23 ± 5.66 [§]	4.20 ± 1.47 [§]
TSH (μU/ml)	0.006 (0.005, 0.008)	0.015 (0.007, 0.093)*	0.034 (0.012, 1.038) [†]
TRAb (%)	41.0 (21.9, 70.4)	32.1 (12.2, 39.3)*	18.6 (10.1, 41.4) ^{†‡}
Total IgG (mg/dl)	1247 ± 314.5	1311 ± 343.1*	1386 ± 356.5 [†]
IgE (log ₁₀ mg/dl)	1.99 ± 0.72	2.03 ± 0.71	2.07 ± 0.63

Data are mean ± s.d. or median and interquartile. **P* < 0.05, [†]*P* < 0.01, [‡]*P* < 0.001, [§]*P* < 0.0001 vs 0 weeks, [¶]*P* < 0.01 vs 12 weeks. TRAb, thyrotropin (TSH) receptor antibodies; FT4, free thyroxine; FT3, free triiodothyronine. The reference ranges for laboratory values are: FT4, 0.97–1.79 ng/dl; FT3, 2.73–4.50 pg/ml; TSH, 0.3–3.0 μU/ml; TRAb, 0–15.0%.

Discussion

The present study confirmed that plasma concentrations of IP-10, the ligand for CXCR3, were significantly higher in hyperthyroid patients with Graves' disease than in control subjects (13–16). Furthermore, plasma IP-10 decreased to concentrations resembling those in control subjects when a euthyroid state had been restored by MMI treatment. Antonelli *et al.* also reported that serum IP-10 was significantly higher in patients in the active phase of Graves' disease than in patients with Graves' disease rendered euthyroid by MMI treatment, suggesting that reduction in serum IP-10 in patients treated with MMI may be associated with immunomodulatory effects of MMI (14, 15). They also demonstrated that circulating IP-10 was significantly higher in patients with Graves' disease than in those with toxic nodular goiter (14, 15), suggesting that hyperthyroidism itself is not associated with increased circulating IP-10. Thus, increased circulating IP-10 in patients with Graves' disease may be specifically sustained by the autoimmune, inflammatory process.

Immunohistochemical studies demonstrated high expression of IP-10 in thyroid glands of patients with recent-onset Graves' disease (12, 13, 17). Thyroid follicular cells as well as infiltrating lymphocytes may be possible sources of IP-10 in sera from patients with newly diagnosed Graves' disease. Recently, Antonelli *et al.* have shown that in patients with Graves' disease, serum IP-10 is significantly reduced after thyroidectomy (18) or iodine-131 therapy (19). These results suggest that thyroid gland is the main source of circulating IP-10 in patients with Graves' disease. Increased plasma IP-10 would promote infiltration of activated lymphocytes into the thyroid gland, thus representing a potential indicator of disease activity in Graves' disease.

On the other hand, we found that plasma TRAC, a ligand for CCR4, was significantly higher in hyperthyroid patients with Graves' disease than in control subjects. To our knowledge, this report is the first to demonstrate elevated plasma TARC concentrations in hyperthyroid patients with Graves' disease. This result supports the hypothesis that Th2-associated chemokines may

Figure 1 Representative results showing changes in the percentage of CXCR3-expressing or CCR4-expressing cells among CD4+ peripheral blood T lymphocytes after treatment with methimazole in a typical patient with hyperthyroid Graves' disease, as shown by flow cytometry.

Figure 2 Changes in the percentage of CXCR3-expressing cells (A) or CCR4-expressing cells (B) among CD4+ peripheral blood T lymphocytes after treatment with methimazole in hyperthyroid patients with Graves' disease. Data are expressed as mean $\pm$ s.d.

participate in the pathogenesis of Graves' disease. The present study also demonstrated that plasma TARC significantly decreased after treatment with MMI. Since a control group of toxic nodular goiter was not included in the present study, we cannot determine whether elevated plasma TARC concentrations are associated with the excess of thyroid hormones or the autoimmune inflammatory process in patients with Graves' disease.

Figure 3 Changes in the CXCR3/CCR4 ratio after treatment with methimazole in hyperthyroid patients with Graves' disease. (A) Changes in the CXCR3/CCR4 ratio in individual patients. (B) Changes are expressed as median and interquartile. Boundaries of boxes, 25th and 75th percentiles; horizontal lines through boxes, median; whiskers, 10th and 90th percentiles; filled circles, outliers.

Similarly, a control group of toxic nodular goiter treated with MMI should be studied to investigate whether decreased plasma TARC after MMI is due to the transition from hyperthyroidism to euthyroidism or to the immunomodulatory effects of MMI. Several studies have reported that both plasma concentrations of TARC and expression of its receptor, CCR4, on peripheral blood CD4+ cells were increased in patients with atopic dermatitis, suggesting that elevated plasma TARC may reflect disease activity in atopic dermatitis (20–22). Furthermore, prevalence of CCR4+ cells among peripheral blood cells correlated positively with serum IgE in patients with atopic dermatitis (20), linking the pathogenesis of atopic dermatitis to Th2 dominance.

Figure 4 Changes in plasma concentrations of interferon-inducible protein (IP)-10 (panel A), and thymus and activation-regulated chemokine (TARC; panel B) in hyperthyroid patients with Graves' disease after treatment with methimazole. (A) Data are expressed as mean $\pm$ S.D. (B) Data are expressed as median and interquartile. Boundaries of boxes, 25th and 75th percentiles; horizontal lines through boxes, median; whiskers, 10th and 90th percentiles; filled circles, outliers.

However, the present study showed the discordance between plasma concentrations of TARC and the percentage of CCR4-expressing cells among peripheral blood CD4⁺ cells in hyperthyroid patients with Graves' disease. One possible explanation for the discordance is that unlike atopic dermatitis, Th2 cells may not play a significant role in the active phase of Graves' disease. Another possibility involves the relatively small number of subjects studied, i.e., a type II error, which could be minimized by modestly increasing sample sizes.

We found a higher percentage of CXCR3-expressing cells among peripheral blood CD4⁺ cells in patients with hyperthyroid Graves' disease than in control subjects. This suggests that Th1 dominance may play

an important role in disease induction. In essential disagreement, Aust *et al.* reported that the intrathyroidal lymphocytes from patients with Graves' disease showed higher expression of CXCR3 than autologous peripheral blood lymphocytes (23). No significant difference in percentage of CXCR3-positive cells was found between peripheral blood lymphocytes from their Graves' disease patients and those from their control subjects. However, their cross-sectional study compared the expression of CXCR3 on peripheral blood lymphocytes between Graves' disease patients in already rendered euthyroid after antithyroid drugs and control subjects (23). Thus, the discrepancy between the two studies may reflect a difference in timing of measurements with respect to treatment. In patients with Graves' ophthalmopathy, diffuse infiltration by lymphocytes is prominent in orbital fibroadipose tissues of Graves' as well as in the thyroid gland (24). Reflecting findings in the thyroid gland, a previous study in patients with recent-onset Graves' disease showed that cells cloned from the orbital tissues demonstrated a Th1 cell-mediated immune response predominating in the orbits, while Th2 immune response may predominate a later stage of disease such as after more than 2 years (24). A recent study has shown that serum concentrations of IP-10 are significantly higher in patients with active Graves' ophthalmopathy than in those with inactive Graves' ophthalmopathy, and that retrobulbar cells from patients with active Graves' ophthalmopathy can produce IP-10 under the influence of cytokine such as IFN- γ , while treatment of these cells with PPAR- γ agonists suppress IFN- γ -induced IP-10 release (25).

Recruitment of lymphocytes in Graves' disease is a multistep process involving adhesion to and migration across the endothelium, movement through the interstitium, and finally contact with thyroid follicular cells (26). Chemokines and chemokine receptors play an important role in regulating traffic of effector T lymphocytes into inflamed areas (26). As mentioned previously, the ligand for CXCR3, IP-10, was also increased in plasma from patients with hyperthyroid Graves' disease. Thus, both high CXCR3 expression and high plasma concentrations of the ligand IP-10 may contribute to migration of Th1 cells from peripheral blood to the affected thyroid gland in patients with hyperthyroid Graves' disease, which would result in accumulation of CXCR3-expressing cells in the thyroid gland.

The present study demonstrated for the first time that the percentage of CXCR3-expressing cells among peripheral blood CD4⁺ cells, representing Th1-associated lymphocytes, decreased significantly after MMI treatment in hyperthyroid patients with Graves' disease; on the other hand, the percentage of CCR4-expressing cells among peripheral blood CD4⁺ cells, representing Th2-associated lymphocytes, increased significantly after MMI treatment. In a cross-sectional study, Romagnani *et al.* immunohistochemically demonstrated

high expression of CXCR3 and IP-10 proteins in mononuclear cells surrounding follicular structures in thyroid glands from patients with recent-onset Graves' disease, suggesting the importance of a Th1-dominant response in the initial phase of inflammation in this disease (12). However, no previous reports prospectively identified changes in Th1/Th2 balance according to expression of chemokine receptors and their ligands after the patients began treatment. This is the first study to longitudinally evaluate both the expression of chemokine receptors on peripheral blood lymphocytes and the corresponding ligands concentration in plasma after MMI treatment. We found that both the percentage of cells expressing CXCR3 and the plasma concentrations of its ligand IP-10, which are Th1-associated, were significantly decreased after MMI treatment; this suggested that the Th1/Th2 balance favored Th1 rather than Th2 dominance in the initial phase of Graves' disease. On the other hand, the present study showed a significantly increased percentage of CCR4, a Th2-associated chemokine, after 24 weeks of treatment with MMI. The CXCR3/CCR4 ratio, indicating Th1/Th2 balance, also showed a significant decrease after MMI treatment compared with baseline. Furthermore, the ratio was significantly lower at 24 weeks of treatment than at 12 weeks. These results suggest that a progressive and gradual transition from Th1 dominance to Th2 dominance occurs during the clinical course of Graves' disease upon treatment with MMI, an antithyroid drug. Several studies also reported that peripheral lymphocytes reflect the autoimmunity in the thyroid gland as the expression of generalized activation of the immune system in patients with autoimmune thyroid disease (27–29). Thus, CXCR3-expressing peripheral blood lymphocytes correlate with activity of Graves' disease, suggesting that the prevalence of such cells among peripheral blood lymphocytes would be a useful surrogate marker for thyroid autoimmune activity in this disease.

Whether or not MMI can directly induce a shift from Th1 dominance to Th2 dominance in the clinical course of Graves' disease remains unclear. In addition to lowering circulating thyroid hormones, MMI may have immunosuppressive effects (30). In fact, serum TRAb, a pathologic autoantibody, was found to decrease with time after administration of MMI (30). A previous study also showed decreased numbers of peripheral blood helper T lymphocytes and activated intrathyroidal T lymphocytes during antithyroid drug therapy (31). Thus, MMI is likely to directly decrease the expression of Th1-associated chemokine receptors and increase expression of Th2-associated chemokine receptors on peripheral blood lymphocytes, bringing about a change from Th1 dominance to Th2 dominance in the latter part of the clinical course. However, a control group treated with radioiodine or subtotal thyroidectomy should be studied to confirm a direct nature of MMI

effects on the immune system in hyperthyroid patients with Graves' disease.

In conclusion, Th1 rather than Th2 cells were predominant among peripheral blood lymphocytes in hyperthyroid patients in the active phase of Graves' disease, suggesting that Th1 dominance is responsible for disease induction. After treatment with MMI, a progressive transition from Th1 to Th2 dominance was evidently found over time. Thus, CXCR3-expressing peripheral blood lymphocytes correlate with activity of Graves' disease, suggesting that the prevalence of such cells among peripheral blood lymphocytes would be a useful surrogate marker for thyroid autoimmune activity in this disease.

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