

An Elevation of Serum Immunoglobulin E Provides a New Aspect of Hyperthyroid Graves' Disease

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ABSTRACT

In hyperthyroid Graves' disease, short-term methimazole is sufficient to induce lasting remission in some patients, but even long-term treatment fails to do so in others. We have evaluated the role of autoimmune abnormalities in the helper T cell type 2 (TH2)-interleukin-13 (IL-13)-TSH receptor system in maintaining hyperthyroidism by comparing IgE levels in patients with various thyroid diseases.

One hundred and ninety-three patients with hyperthyroid Graves' disease were treated with methimazole, and blood samples were obtained to measure serum levels of T_4 , T_3 , TSH, thyroglobulin, antimicrosomal antibody, TSH binding inhibitory Ig (TBII), thyroid-stimulating antibody, thyroid stimulation-blocking antibody, IgE, interferon- γ , IL-4, and IL-13. Elevation of serum IgE (≥ 170 U/mL) was found in 35.5% of patients with hyperthyroid Graves' disease, and serum levels of T_4 , T_3 , antimicrosomal antibody, and TBII were significantly greater in patients with IgE elevation than in those with

normal serum IgE. During methimazole treatment, there was a parallel decrease in the serum T_4 concentration in the presence or absence of an IgE elevation. However, there was a significantly smaller decrease in TBII in patients with elevated IgE than in those with normal IgE. As a result, the remission rate was significantly greater in patients with normal IgE than in those with IgE elevation. Serum levels of IL-13 were elevated in 64.7% of patients with IgE elevation in the absence of detectable TH1 marker, interferon- γ .

These findings suggest that in one third of patients with hyperthyroid Graves' disease, TH2 cells are stimulated and secrete excess amounts of IL-13, which subsequently stimulates B cells to synthesize more TSH receptor antibody and IgE, so that during methimazole treatment TBII decreases less in patients with IgE elevation, producing a lower remission rate. (*J Clin Endocrinol Metab* 85: 2775–2778, 2000)

GRAVES' DISEASE is an autoimmune disorder of the thyroid characterized by the production of autoantibodies against TSH receptor (TRAb) (1–3). TRAb, composed of thyroid-stimulating antibody (TSAb), TSH binding inhibitory Ig (TBII), and thyroid stimulation-blocking antibody (TSBAb), is in the IgG class (4, 5) and is pathogenic for the disease, with stimulating effect, where hyperthyroidism is the result of TSH receptor stimulation (1–3). The etiology of Graves' disease as an autoimmune disorder, however, is multifactorial on the basis of genetic susceptibility interacting with environmental and endogenous factors (3). Several lines of evidence suggest a relationship between thyroid autoimmunity and the allergic responses. First, IgE as well as IgG and IgM deposit in some Graves' thyroid tissue (6) and ocular muscles of hyperthyroid Graves' disease (7, 8). Second, associations have recently been reported between recurrence of hyperthyroid Graves' disease and allergic rhinitis of pollen allergy (9, 10), one of the most common diseases associated with the synthesis of IgE (11). Third, the presence of IgE class thyroid peroxidase autoantibodies has been reported in some patients with Graves' disease and

Hashimoto's thyroiditis (12). Finally, we reported the high incidence of IgE elevation in hyperthyroid Graves' disease and a lesser reduction of TRAb in association with IgE elevation (13). IgE, therefore, might play a role in the induction and maintenance of hyperthyroid Graves' disease. Less is known, however, about the role of IgE, because limitation in obtaining the sufficient amount of IgE has hampered examination of the nature of IgE class autoantibodies in TRAb activity.

We thus searched for the circulating levels of cytokines, including interferon- γ (IFN γ), interleukin-4 (IL-4), and IL-13, that participate in the production and secretion of Igs. We also studied the decay of TBII and remission rate from hyperthyroid Graves' disease with or without elevated levels of IgE during methimazole treatment. Here we report the association of Graves' disease with IgE and IL-13, suggesting the link between autoimmune thyroid disease and immune responses involving TH2 cells.

Materials and Methods

One hundred ninety-three patients (29 males and 164 females, aged 15–69 yr) with hyperthyroid Graves' disease, 72 patients (5 males and 67 females, aged 22–69 yr) with Hashimoto's thyroiditis, 40 patients (2 males and 38 females, aged 13–69 yr) with simple goiter, and 39 patients (1 male and 38 females, aged 20–81 yr) with nodular goiter were in this study. Forty-two normal subjects (2 males and 40 females, aged 18–63 yr) served as controls. The diagnosis of Graves' disease was based on clinical features such as diffuse goiter, exophthalmos, tachycardia, tremor, and sweating and laboratory data such as undetectable serum

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TSH and elevated levels of serum T_4 , T_3 , and TRAb (TSAb and TBII). Hyperthyroid patients were treated with methimazole (20–30 mg/day) (14–17) and visited the out-patient clinic once a month for laboratory examinations [serum levels of TSH, T_4 , T_3 , TSAb, TBII, thyroglobulin (Tg), and IgE]. Three to 4 months after the initiation of methimazole, blood was obtained to measure TSBAb, IFN γ , IL-4, and IL-13.

The diagnosis of Hashimoto's thyroiditis was made on clinical features such as diffuse and firm goiter and laboratory data such as the presence of thyroid antimicrosomal antibody (MCHA), normal or reduced levels of serum T_4 and T_3 , and normal or elevated levels of serum TSH. MCHA was measured by the tanned red cell hemagglutination technique using a commercially available kit (Fiji Zoki, Tokyo, Japan). MCHA was considered positive when a positive reaction was obtained in the presence of dilute serum (dilution, $\times 100$ or more).

The diagnosis of simple goiter was made on clinical features such as small, soft, and diffuse goiter and laboratory data such as normal serum levels of T_4 , T_3 , and TSH and the absence of MCHA. The diagnosis of nodular goiter was made on clinical and laboratory findings such as nontoxic goiter in the absence of circulating autoantibodies and non-malignant histology on aspiration biopsy.

Serum levels of T_4 , T_3 , TSH, Tg, and TBII were measured as reported previously (14–17). TSAb (18) and IgE (19) were measured by Mitsubishi Kagaku Bio-Clinical Laboratory, Inc. (Tokyo, Japan). TSBAb was measured by Yamasa Shoyu Laboratory (Choshi, Chiba, Japan) (18). The normal ranges were 5–11 $\mu\text{g/dL}$ for T_4 , 78–190 ng/dL for T_3 , 0.1–5 $\mu\text{U/mL}$ for TSH, 3–42 $\mu\text{g/mL}$ for Tg, less than 15% for TBII, less than 180% for TSAb, and less than 30% for TSBAb, respectively. The data for IgE and MCHA were analyzed statistically with logarithmically transformed values and then converted to antilogarithmic values. Normal values for IgE were below 170 U/mL as reported previously (13). IFN γ (enzyme-linked immunosorbent assay; Bender MedSystems, Vienna, Austria), IL-4 (Cytoscreen US, ultrasensitive for human IL-4, Biosource Technologies, Inc., Camarillo, CA), and IL-13 (IL-13 enzyme-linked immunosorbent assay, Bender MedSystems) were also measured by Mitsubishi Kagaku Bio-Clinical Laboratories, Inc.

Statistical analyses were made using two-tailed unpaired Student's *t* test and Fisher's exact test where appropriate. $P < 0.05$ was considered statistically significant.

Results

Incidence of IgE elevation in various thyroid diseases

As shown in Table 1, 44 of 124 patients with Graves' disease (35.5%) had an elevation of IgE (≥ 170 U/mL), and this incidence was significantly higher than that of other thyroid disorders. IgE elevation was also found in 15 of 72 patients with Hashimoto's thyroiditis (20.8%). The difference

in incidence between Graves' disease and Hashimoto's thyroiditis was statistically significant ($P < 0.05$). In patients with simple goiter and nodular goiter, the incidence of IgE elevation was low (5.0% and 5.1%, respectively) and comparable to that in normal subjects (2.4%).

Thyroid parameters in patients with or without IgE elevation

As expected, serum levels of T_4 and T_3 were significantly elevated, and TSH was undetectable in patients with untreated hyperthyroid Graves' disease (Table 1). TBII, TSAb, and MCHA were higher in hyperthyroid patients with than in those without IgE elevation; the differences, except for TSAb, were statistically significant. In patients with simple goiter, Hashimoto's thyroiditis, and nodular goiter, there was no significant difference in any of the parameters (TBII, TSAb, MCHA, T_4 , T_3 , and TSH) between IgE elevated and IgE normal groups.

Incidence of TSBAb in Graves' disease with or without IgE elevation

Three to 4 months after the initiation of methimazole treatment, the presence or absence of TSBAb was examined in 59 patients with Graves' disease. TSBAb was detected in 4 of 15 patients with IgE elevation (26.7%), whereas it was detected in 3 of 44 patients without IgE elevation (6.8%). However, the difference was not statistically significant ($P = 0.0985$).

Detection of IL-13 in sera of hyperthyroid patients with IgE elevation

The presence of circulating cytokines (IFN γ , IL-4, and IL-13) was evaluated in 25 patients without and 17 patients with IgE elevation 3–4 months after methimazole treatment (Table 2). In the 25 patients without IgE elevation, IFN γ and IL-4 were undetectable, whereas IL-13 was detected in 3. In contrast, IL-13 was detected in 11 of 17 patients with IgE elevation, but there was no correlation between the levels of

TABLE 1. Various thyroid parameters in patients with or without IgE elevation

Group	No. of patients	Age (yr)	IgE (U/mL)	TBII (%)	TSAb (%)	MCHA	T_4 ($\mu\text{g/dL}$)	T_3 (ng/dL)	TSH ($\mu\text{U/mL}$)
1. Graves' disease									
IgE $\rightarrow$	80	43.4 ± 14.5	33 (12–90)	40.2 ± 21.2	626.4 ± 572.4	2522 (368–17267)	21.6 ± 5.7	426.1 ± 144.3	<0.1
IgE $\uparrow$	44	37.3 ± 14.4	564 (223–1427)	53.9 ± 21.1	658.8 ± 489.3	4934 (512–47580)	24.3 ± 5.3	489.6 ± 155.4	<0.1
<i>P</i> value		<0.03	<0.0001	<0.001	NS	<0.01	<0.02	<0.03	
2. Hashimoto's thyroiditis									
IgE $\rightarrow$	57	51.5 ± 12.8	35 (14–93)	4.2 ± 3.6		4642 (699–30813)	7.3 ± 2.8	115.9 ± 42.5	25.4 ± 51.8
IgE $\uparrow$	15	44.2 ± 18.2	392 (181–851)	4.3 ± 3.3		4756 (772–29090)	7.4 ± 2.3	114.9 ± 26.9	6.7 ± 11.0
<i>P</i> value		NS	<0.0001	NS		NS	NS	NS	NS
3. Simple goiter									
IgE $\rightarrow$	38	40.7 ± 16.4	46 (21–103)	3.5 ± 2.9			8.4 ± 1.4	129.6 ± 25.8	1.8 ± 2.0
IgE $\uparrow$	2	33.0^a	548 ^a	3.3^a			8.6^a	144.0^a	1.9^a
4. Nodular goiter									
IgE $\rightarrow$	37	49.9 ± 15.0	48 (24–98)	4.8 ± 1.9			8.6 ± 1.6	133.2 ± 29.8	1.8 ± 1.1
IgE $\uparrow$	2	40.0^a	466 ^a	4.6^a			8.6^a	128.0^a	1.3^a
5. Control									
	42	47.1 ± 15.2	39 (17–89)	4.6 ± 2.0	150.5 ± 44.3		8.8 ± 1.6	133.6 ± 29.2	1.9 ± 1.2

Data are the mean $\pm$ SD. Data for IgE and MCHA are means (mean $\pm$ 1 SD). Data for IgE and MCHA were analyzed with log-transformed values. *P* values were calculated by two-tailed unpaired Student's *t* test. MCHA, Antimicrosomal antibody.

^a Data are mean values.

TABLE 2. Circulatory cytokines in patients with hyperthyroid Graves' disease with or without IgE elevation

	Normal IgE group (% detected) ^a	Elevated IgE group (% detected)	P value
No. of patients	25	17	
Interferon- γ	0/25 (0.0)	3/17 (17.6)	NS
		[positive values (pg/mL): 3.18, 2.25, 2.30]	
Interleukin-4	0/25 (0.0)	0/17 (0.0)	Cannot compare
Interleukin-13	3/25 (12.0)	11/17 (64.7)	<0.001
	[positive values (pg/mL): 5.84, 12.50, 7.28]	[positive values (pg/mL): 101.00, 14.60, 5.54, 18.80, 6.79, 14.20, 4.71, 6.37, 12.80, 8.33, 13.50]	

Minimal detectable dose for serum interferon- γ , 1.56 pg/mL; minimal detectable dose for serum interleukin-4, 0.50 pg/mL; minimal detectable dose for serum interleukin-13, 3.12 pg/mL. *P* values were calculated by two-tailed Fisher's exact test.

^a Normal serum IgE concentration is less than 169 U/mL.

TABLE 3. Changes in serum levels of T₄, TBII, and IgE during methimazole treatment

Group	No. of patients	Duration of treatment with methimazole					
		0 month	2 months	4 months	6 months	8 months	10 months
T ₄ (mg/dL)							
IgE →	61	21.7 ± 5.8	10.8 ± 3.6	8.0 ± 2.2	7.9 ± 1.6	7.7 ± 1.4	7.8 ± 1.8
IgE ↑	28	23.0 ± 5.6	12.5 ± 4.8	9.6 ± 3.9	8.2 ± 2.9	8.2 ± 2.6	8.1 ± 1.4
<i>P</i>		NS	NS	<0.02	NS	NS	NS
TBII (%)							
IgE →	61	47.0 ± 20.6	36.5 ± 21.3	28.5 ± 20.1	21.9 ± 17.9	18.2 ± 15.7	20.7 ± 15.5
IgE ↑	28	54.7 ± 21.4	50.2 ± 23.9	45.9 ± 23.8	39.1 ± 22.9	34.2 ± 21.2	34.4 ± 24.3
<i>P</i>		NS	<0.01	<0.0006	<0.0002	<0.0001	<0.002
IgE (U/mL)							
IgE →	61	38 (15–100)	36 (14–90)	34 (13–89)	36 (15–89)	35 (15–82)	29 (15–55)
IgE ↑	28	501 (181–1387)	509 (185–1399)	466 (171–1274)	413 (147–1162)	407 (153–1087)	406 (141–1168)
<i>P</i>		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Data are the mean ± SD. Data for IgE are means (mean ± SD). Data for IgE were analyzed with log-transformed values. *P* values were calculated by two-tailed unpaired Student's *t* test.

IL-13 and IgE. IFN γ was detected in 3 of 17 patients with IgE elevation, but IL-4 was not found in these 17 patients.

Change in TBII and IgE during methimazole treatment in patients with hyperthyroid Graves' disease

In 61 patients with normal IgE and 28 patients with IgE elevation, serum levels of T₄, TBII, and IgE were followed for 8–10 months during methimazole treatment. As shown in Table 3, T₄ decreased similarly in both groups. TBII also decreased progressively during methimazole treatment, but the decrease was significantly greater in those with normal than in those with elevated IgE. IgE decreased slightly during methimazole treatment in the IgE elevated group, but was stable in the IgE normal group.

Remission rate in hyperthyroid patients with IgE elevation in response to methimazole treatment

Ninety patients with hyperthyroid Graves' disease were treated with methimazole for 1.5 yr, and the treatment was discontinued when serum levels of T₄, T₃, TBII, and Tg were normalized (16). We designated these patients as the remission group. As shown in Fig. 1, 31 of the 90 patients had elevated levels of serum IgE at the initiation of methimazole treatment; 11 of these patients (35.5%) underwent remission. In contrast, 38 of 59 patients with normal IgE levels (64.4%) had a remission, a significantly greater remission rate than that in the IgE elevated group (*P* < 0.002).

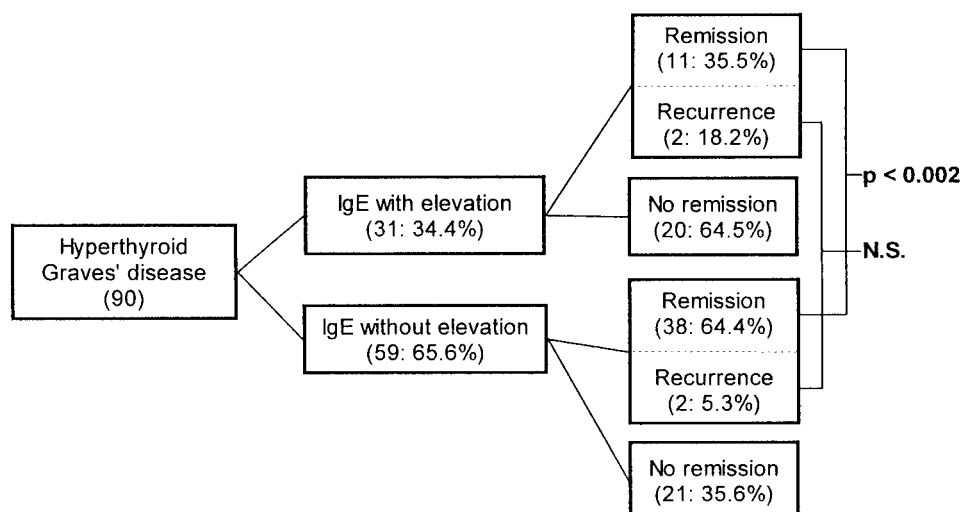
Discussion

With the increase in numbers of patients with Graves' disease, we reached the same results as those from the previous report that found that serum levels of IgE were significantly elevated in one third of patients with Graves' disease and a lesser reduction in TRAb in patients with elevated IgE levels than in patients with normal IgE (13). The incidence of IgE elevation in Hashimoto's thyroiditis (20.7%) is smaller than that in Graves' disease (35.5%), and this difference is significant, suggesting that the autoantibody system of Graves' disease is more affected by IgE elevation than that of Hashimoto's thyroiditis. The decrease in TRAb in Graves' disease during methimazole treatment was significantly smaller in the elevated IgE group than in normal IgE group.

If it were the case that TRAb synthesis is increased when IgE synthesis is stimulated, the remission rate should be lower in the elevated IgE than in the normal IgE group. Supporting this hypothesis is the significant difference in remission rate between IgE elevated and IgE normal groups of Graves' disease; with the criteria for remission (normal serum levels of T₄, T₃, TSH, TBII, and Tg) reported previously (17), patients with IgE elevation had a significantly lower remission rate (35.5%) than those with normal IgE (64.4%). The elevated levels of IgE remained relatively stable during this study.

We then next made a study of cytokines relevant to T helper cell function because T helper cells have been con-

FIG. 1. Two-year follow-up of methimazole-treated patients with hyperthyroid Graves' disease after discontinuation of methimazole. The *P* value was calculated by two-tailed Fisher's exact test.



sidered to play an important role in autoimmune thyroid disease (3). IL-13 enhances the production of IgM, IgG, and IgA (20) and is the dominant IgE-inducing cytokine that is produced early and for a prolonged time by T cells in situations where IL-4 is absent or present at low levels (21, 22). In this study there was an elevation of IL-13, but not of IL-4, in 64.7% of patients with IgE elevation, but not in patients without IgE elevation. There was no elevation of IL-10 or differences between patients with and without IgE elevation (data not shown). The TH1 cell marker, IFN γ (22), was not increased in the presence of a significant elevation of IL-13. It thus seems that TH2 cells are stimulated in hyperthyroid Graves' disease (23, 24) to secrete excess IL-13. One possibility is that this IL-13, in turn, stimulates B cells to secrete MCHA, TSAb, TSBAb, and IgE (21). The finding, however, that one third of patients with Graves' disease and one fifth of patients with Hashimoto's thyroiditis have elevated IgE raises the possibility that many of the phenomena described here may be epiphenomena of other immunoregulatory and inflammatory defects and that an underlying state of autoimmune thyroid disease may be a permissive factor for IgE elevation and excess IL-13. Although there is an association between IgE elevation and thyroid autoantibodies, the exact mechanism underlying the relationship is not apparent with this study and remains to be determined.

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