

Increase in Serum Levels of Autoantibodies after Attack of Seasonal Allergic Rhinitis in Patients with Graves' Disease

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Key Words

Allergic rhinitis · Graves' disease · Autoantibodies ·
Pollinosis · Cedar pollen

Abstract

Background: The prevalence of allergic disease is increasing worldwide, but its influence on the clinical course of autoimmune diseases is unknown. **Objective:** The purpose of this study was to assess the effect of seasonal allergic rhinitis on the clinical course of Graves' disease, which has been considered a Th2-dominant autoimmune disease. **Methods:** Ten patients with Graves' disease, who were considered to be in a state of remission or near remission, were serially examined for 18 months starting from August. Five of them had seasonal allergic rhinitis due to Japanese cedar pollen, and the remaining patients had no such allergic disorders. Peripheral eosinophil counts, serum concentrations of cedar-pollen-specific IgE, anti-TSH-receptor antibody, anti-thyroid-peroxidase antibody and antithyroglobulin antibody were assessed at 2- to 4-month intervals. Serum thyroid hormones and TSH levels were also measured to evaluate disease activity. **Results:** All patients with pollinosis had attacks of allergic rhinitis caused by

cedar pollen in early March. Subsequently, peripheral eosinophil counts, pollen-specific IgE activity and serum levels of anti-thyroid-peroxidase and antithyroglobulin autoantibodies markedly increased. Serum levels of anti-TSH-receptor antibody increased in 3 patients in association with an increase in serum thyroid hormones but were always negative in 2 patients. The control patients without pollinosis showed no consistent change of these parameters. **Conclusions:** Seasonal allergic rhinitis aggravated the clinical course of Graves' disease and induced an increase in serum antithyroid autoantibody concentrations as well as an increase in pollen-specific IgE concentration. These data suggest that environmental antigens induce not only local allergic reactions, but also stimulate thyroid immune reactions toward Th2 proliferation, and finally aggravate Th2-dependent autoimmune thyroid disease.

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Introduction

Allergic rhinitis is a Th2-dependent disease and may influence the clinical course of other Th2-predominant diseases. Previously we had found that seasonal allergic

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rhinitis induced by Japanese cedar pollen aggravated Graves' disease and patients frequently suffered relapses of Graves' thyrotoxicosis [1]. Moreover, some patients develop a clinical onset of Graves' disease after an attack of allergic rhinitis [2].

Graves' thyrotoxicosis is induced by thyroid-stimulating anti-TSH-receptor antibodies, and thus humoral immunity is more important than cellular immunity [3]. In patients with Graves' disease, peripheral eosinophil counts were significantly increased [4]. Furthermore, serum concentrations of interleukin (IL) 5 [5], soluble CD30 [6], IgE [7] and eosinophil-derived neurotoxin were increased [8]. Moreover, it has recently been clarified that the association of seasonal allergic rhinitis was frequent in Graves' disease and rare in painless thyroiditis [9]. These data strongly suggest that Graves' disease is a Th2-predominant disorder, although there have been contradictory reports, i.e. that the Th1-driven response is important for the initial phase of Graves' disease [10–13].

Allergic diseases are often associated with Th2-dominant autoimmune disease, such as systemic lupus erythematosus [14–16]. However, little is known about the influence of seasonal allergic rhinitis on the clinical course of autoimmune diseases and on the production of autoantibodies. It is well known that Japanese cedar pollens are heavily disseminated at the end of February to early March and frequently induce cedar pollinosis [9, 17]. In this study, we examined the effects of Japanese cedar pollinosis on autoantibody production in order to clarify the mechanism of aggravation of Graves' disease.

Methods

Patients

We examined 10 female patients with Graves' disease who were considered to be in a state of near remission under a maintenance dose of 2.5 or 5 mg of methimazole per day or in remission after antithyroid drug therapy. Of these, 5 patients had their disease complicated by seasonal allergic rhinitis due to Japanese cedar pollen. None of these patients received any medications, including immunosuppressive drugs, for allergic rhinitis. The other 5 patients had no such allergic diseases. The clinical course was examined in these patients for 18 months starting from August 1999 or August 2000. Physical examination and laboratory tests were performed every 2–4 months. Informed consent was obtained from all patients.

Measurements

Peripheral eosinophil counts were made using an automated leukocyte differential system (Total Hematology Management System NE-7000; Sysmex Co., Kobe, Japan) [4]. The serum levels of IgE specific to Japanese cedar pollen were measured with UniCAP100 (Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden) using a fluorescence enzymatic immunoassay. The normal cutoff value was

0.34 UA/ml. Anti-TSH-receptor antibody was measured with a commercial radioreceptor assay kit (Dyno test TRAb Human Kit, Yamasa Corporation, Tokyo, Japan) [18]. This assay uses human recombinant receptors, and the cutoff value was 1.0 IU/l.

Antithyroglobulin antibody and anti-thyroid-peroxidase antibody were measured with commercial radioimmunoassay kits: TgAb (Cosmic Corporation, Tokyo, Japan) and TPO Antibody Kit 'Eiken' (Eiken Chemical Co. Ltd., Tokyo, Japan), respectively. Normal cutoff values were 0.3 U/ml and 0.1 IU/ml, respectively. Activities of anti-streptolysin-O antibody were measured by nephelometry for latex agglutination using LX-3000 (Eiken Chemical Co.). The lowest detection limit was 30 IU/ml.

Serum concentrations of IL-5 and IL-13 were measured with commercial enzyme immunoassay kits, Quantakine human IL-5 and IL-13 immunoassays (R&D Systems Inc., Minneapolis, Minn., USA), respectively. The minimum detectable doses of IL-5 and IL-13 were less than 3.0 and 32 pg/ml, respectively.

In order to examine the IgE-associated anti-TSH-receptor antibodies, 300 µl sera obtained from patients 1, 4 and 5 were incubated with anti-IgE-antibody-coated magnetic particles overnight at 4 °C. The mixture was centrifuged at 3,000 rpm for 15 min, and the obtained IgE-depleted sera were assayed for activities of anti-TSH-receptor antibodies, anti-thyroid-peroxidase antibodies and antithyroglobulin antibodies.

Statistical Analysis

Comparison of each parameter at different times was performed by the paired *t* test.

As for antithyroglobulin antibody, antibody values were transformed logarithmically, and the obtained values were compared between the two different times by the paired *t* test.

Results

Age, sex and clinical data of patients at various times are summarized in table 1. Patients with and without seasonal allergic rhinitis were categorized as groups A and B, respectively. Four patients had been receiving minimal maintenance doses of methimazole, and the remaining patients were followed without any medication. Even during attacks of allergic rhinitis, no patients received any antiallergic drugs. As expected, cedar-pollen-specific IgE was positive in all patients in group A and negative in group B patients. Anti-TSH-receptor antibodies were weakly positive in 2 patients in group A and in 4 patients in group B in November–January before an attack of allergic rhinitis (table 1). Anti-thyroid-peroxidase antibodies were positive in all patients except case 3. Antithyroglobulin antibodies were positive in all patients except case 1 in November–January. For each parameter, 'basal values' in November–January were compared to the values around the peak time of increase in group A. Peripheral eosinophil counts and serum levels of cedar-pollen-specific IgE were significantly increased in April–May and in

Table 1. Clinical data in patients with Graves' disease

Case	Age years	Peripheral eosinophil counts, n/mm ³		Cedar-pollen-specific IgE, UA/ml		Anti-TSH-receptor antibody, IU/l		Anti-thyroid-peroxi- dase antibody, IU/ml		Anti-thyroglobulin antibody, U/ml		Thyroid therapy in Apr–July
		Nov–Jan	Apr–May*	Nov–Jan	May–Jun*	Nov–Jan	Jun–Oct	Nov–Jan	Sept–Oct*	Nov–Jan	Sept–Oct*	
<i>Group A</i>												
1	34	211	583	8.9	27.0	1.3	3.2	154	386	<0.3	0.7	none methimazole 2.5 mg/day
2	19	127	358	14.5	26.4	<1.0	<1.0	83.5	965	0.7	2.7	
3	58	234	302	26.2	37.7	<1.0	<1.0	<0.1	<0.1	11.2	137	none
4	54	143	340	12.4	16.4	1.2	52.9	250	673	51.5	176	none
5	40	153	n.t.	15.9	17.8	<1.0	7.5	25.8	455	0.3	9.7	none
		Nov–Jan	Apr–May	Nov–Jan	May–Jan	Nov–Jan	Jun–Oct	Nov–Jan	Sept–Oct	Nov–Jan	Sept–Oct	
<i>Group B</i>												
6	27	30	68	<0.34	<0.34	<1.0	<1.0	272	1,110	86.4	73.3	none
7	58	225	231	<0.34	<0.34	2.3	1.5	158	82.3	10.2	7.5	methimazole 2.5 mg/day
8	36	133	151	<0.34	<0.34	1.2	2.1	5,678	6,747	20.1	28.3	none
9	25	70	24	<0.34	<0.34	1.2	<1.0	273	116	12.8	5.1	methimazole 2.5 mg/day
10	28	372	283	<0.34	<0.34	1.7	2.9	1,214	1,359	7.2	6.5	methimazole 5 mg/day

Group A = Patients with Graves' disease complicated by seasonal allergic rhinitis; group B = patients without seasonal allergic rhinitis; n.t. = not tested.

* $p < 0.05$: significant difference between values at two different time points.

May–June, respectively (table 1). Activities of anti-thyroid-peroxidase antibody were also significantly increased when an antibody-negative patient (case 3) was excluded. Activities of antithyroglobulin antibody were also significantly increased when statistical analysis was performed using logarithmically transformed antibody values.

Serial changes in peripheral eosinophil counts and serum levels of pollen-specific IgE, anti-TSH-receptor antibody, antithyroglobulin antibody and anti-thyroid-peroxidase antibody in case 4 are shown in figure 1. The increase in anti-TSH-receptor antibody was remarkable, and the peak was slightly delayed compared to that of eosinophil counts and pollen-specific IgE. Increases in antithyroglobulin and anti-thyroid-peroxidase antibodies were further delayed, and their peaks were found in October. This patient had a small goiter and the increase in thyroid hormones was mild, although anti-TSH-receptor antibody increased to 60 IU/l. Since the patient rejected the readministration of methimazole, she was followed without any drugs. Thyroid function returned to normal in association with a decrease in activities of anti-TSH-receptor antibody.

Peripheral eosinophil counts increased at the time of allergic rhinitis in most patients in group A, but no consistent change was found in group B patients (fig. 2). Two

patients in group A had a second slight increase in eosinophils in October. The cedar-pollen-specific IgE concentration clearly increased after attacks of seasonal allergic rhinitis in all patients (fig. 3), and a mild second increase in IgE was found around October in 3 patients in group A (fig. 3a). All patients in group B showed negative cedar-pollen-specific IgE during the observation period (fig. 3b).

Serum levels of anti-TSH-receptor antibody increased after an attack of allergic rhinitis in 3 patients in group A (fig. 4a). The remaining 2 patients showed a negative reaction during the observation period. In case 1, activities of anti-TSH-receptor antibodies increased gradually, and the serum TSH level became undetectable in association with a slight increase in serum free T₄ in September. Finally, methimazole was restarted 6 months later. In case 4, transient mild hyperthyroidism relapsed in April and May but resolved spontaneously in association with a decrease in anti-TSH-receptor antibodies. In case 5, antibody activity increased from less than 1.0 to 7.5 IU/l in June. In association with this change, thyrotoxicosis (free T₄, 2.7 ng/dl) relapsed and methimazole therapy (15 mg/day) was restarted in July. In group B, no consistent change was observed (fig. 4b).

The increase in the serum level of anti-thyroid-peroxidase antibody is shown in figure 5. All patients, except No. 3, showed a gradual increase in antibody activities in group A, although the peak was delayed from that of pollen-specific IgE or anti-TSH-receptor antibody. In group B, 2 patients (cases 6 and 8) had an increase in antibody activity in spring, but the antibody increase was observed before the attack of allergic rhinitis in case 6. The change in antithyroglobulin antibodies was more marked (fig. 6). Three patients in group A showed a marked increase, and the peak of activities was found around October. The other 2 patients also showed a gradual increase, but the change was slight. In group B, 1 patient (case 8) showed an increase in antithyroglobulin antibodies before the attack of allergic rhinitis, but 4 other patients showed no increase from December to September.

Compared to these changes, anti-streptolysin-O antibody levels did not change in any patient during the observation periods (fig. 7).

In order to examine the possibility of IgE-associated autoantibodies, sera with the highest anti-TSH-receptor antibody levels were obtained from cases 1, 4 and 5. Even after absorption of IgE, the activities of anti-TSH-receptor antibodies, anti-thyroid-peroxidase antibodies and antithyroglobulin antibodies were not changed (data not shown).

Serum levels of IL-5 and IL-13 were measured, but serum samples showed undetectable levels lower than the minimum detectable concentration.

Discussion

Little is known of the effect of allergic diseases on the clinical course of autoimmune disorders. It is often difficult to predict the time of association of a disease with an allergic disorder. Also many patients receive various kinds of immunosuppressive drugs. Therefore, the correct evaluation of spontaneous changes in disease activity is usually difficult. The prevalence of seasonal allergic rhinitis to Japanese cedar pollens is increasing in Japan, and more than 20% of subjects in the general population suffer from this allergy [19]. Japanese cedar pollen markedly disseminates at the end of February or early March every year, and thus the attacks of this pollinosis can be easily predicted. On the other hand, aggravation of Graves' disease is readily evaluated by changes in serum thyroid hormones and anti-thyroid antibody activities. For example, postpartum aggravation of autoimmune thyroid disease can be detected by postpartum development of thyroid dysfunction [20].

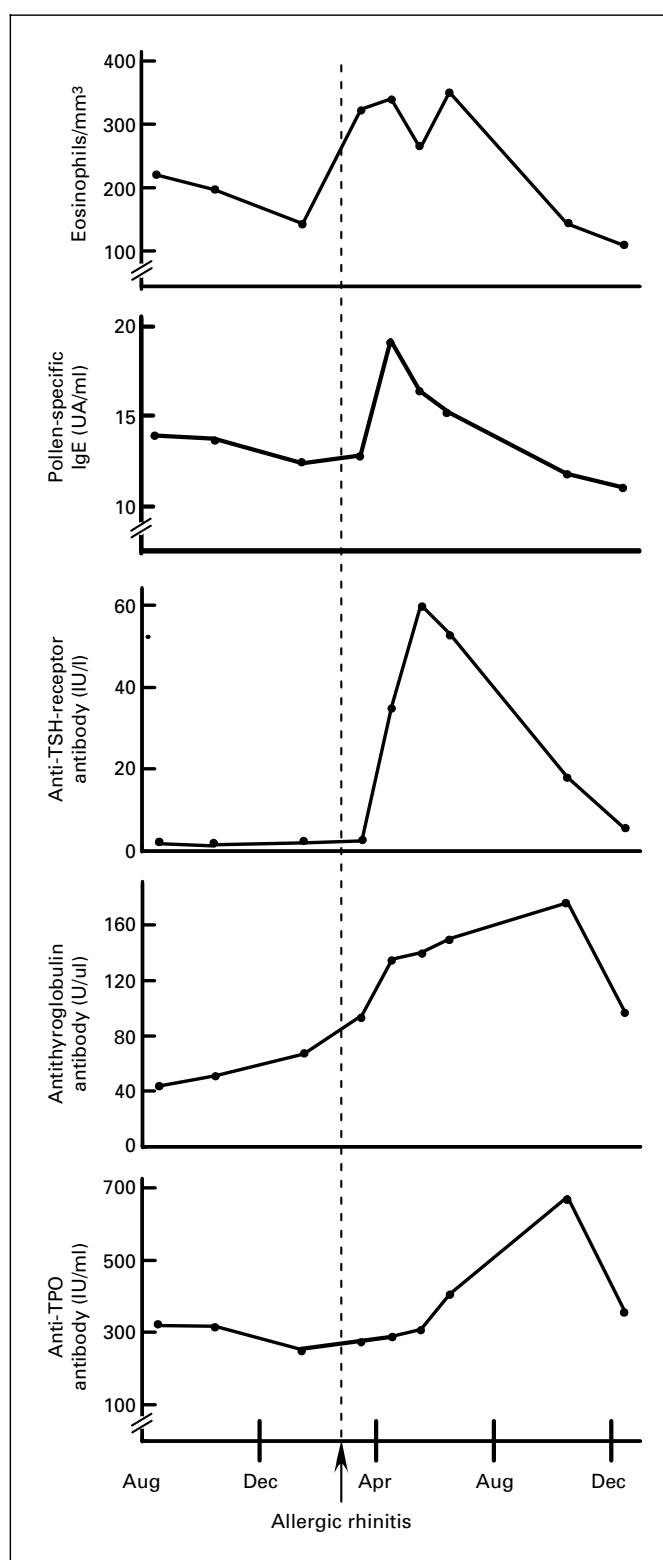
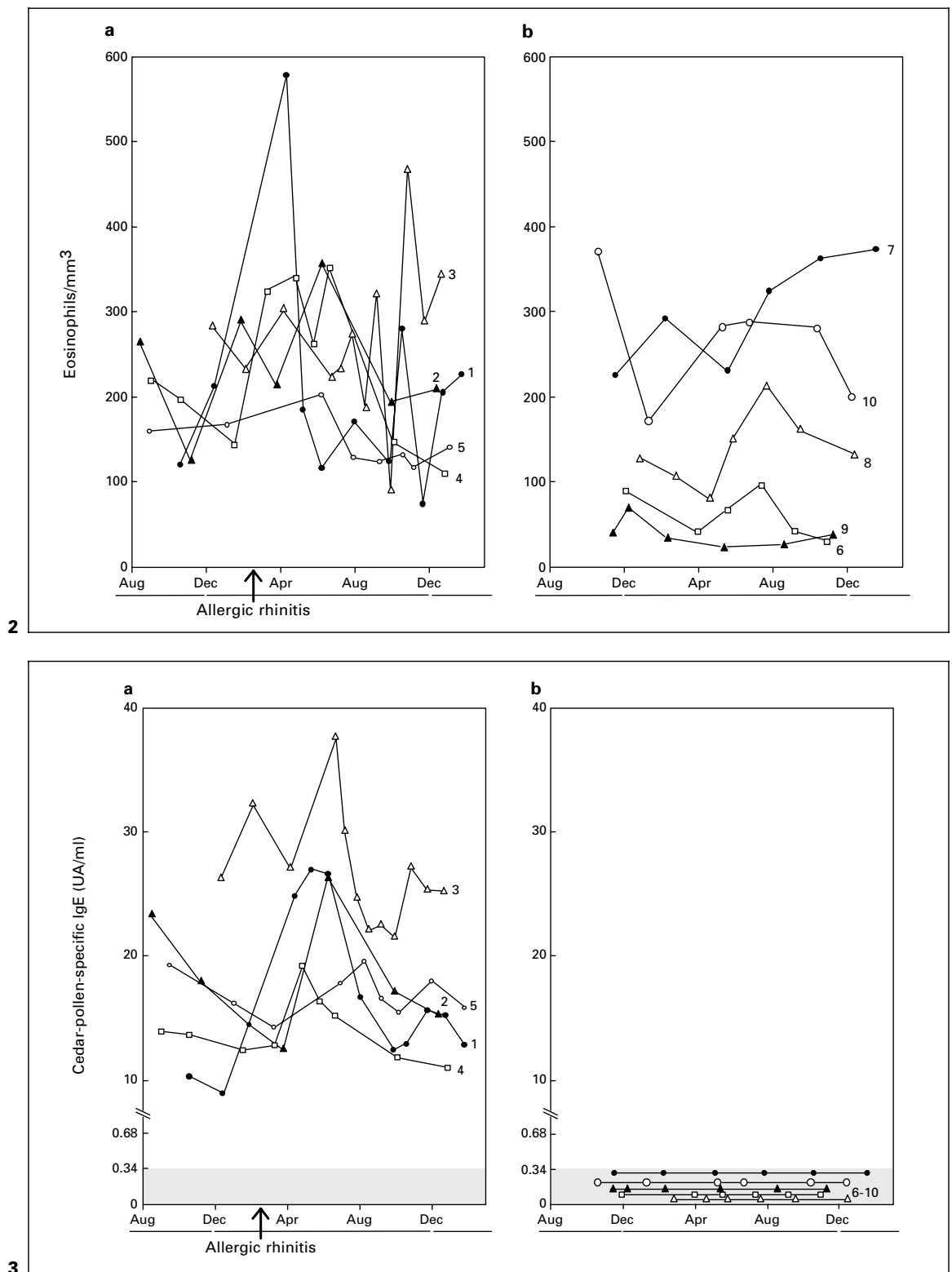
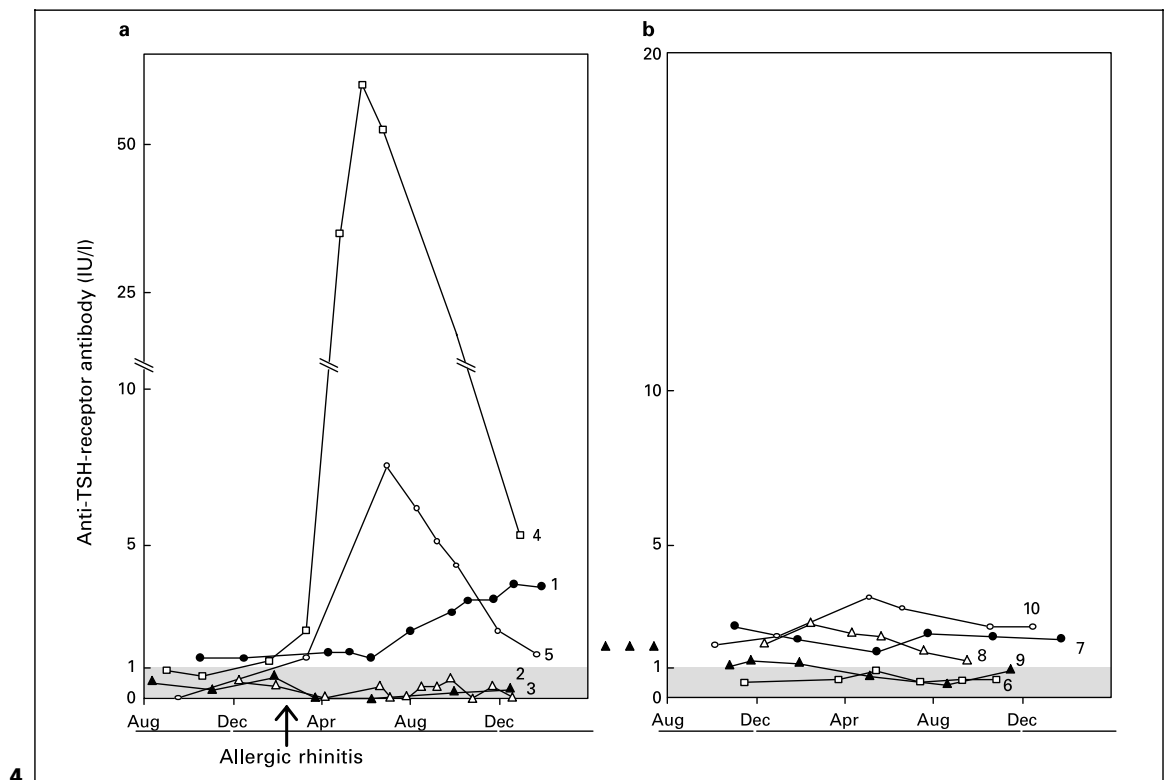
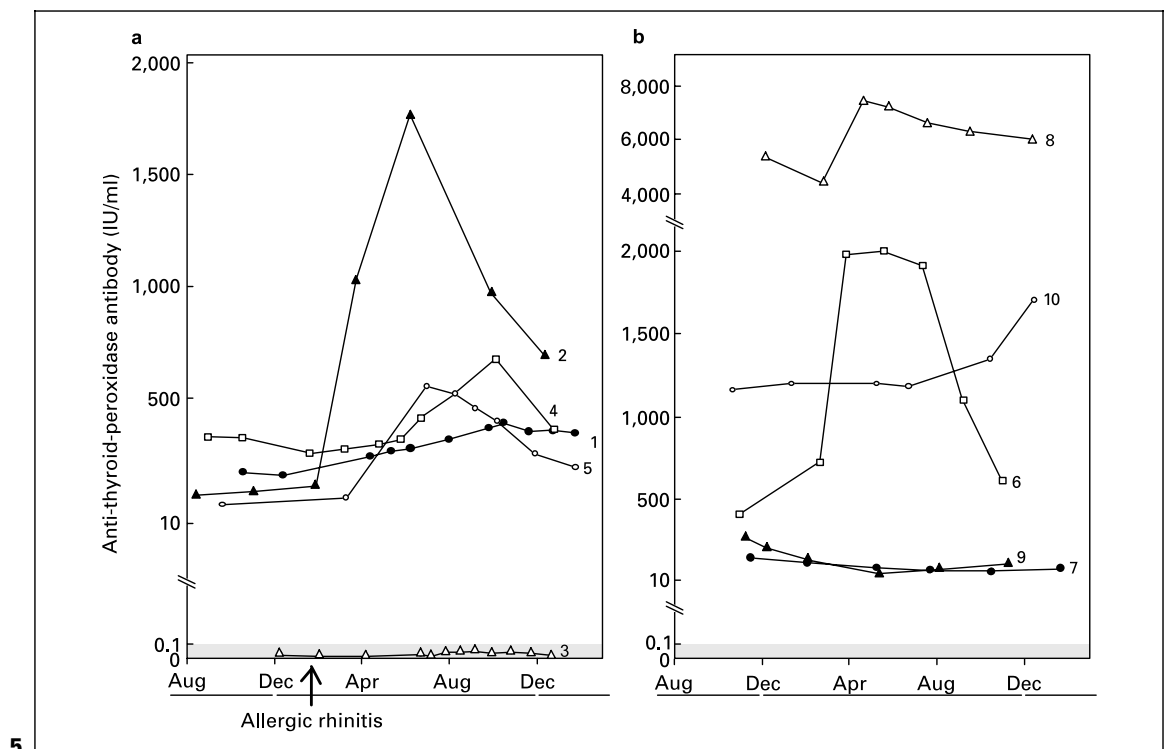


Fig. 1. Serial changes in peripheral eosinophil counts and serum levels of pollen-specific IgE, anti-TSH-receptor antibody, antithyroglobulin antibody and anti-thyroid-peroxidase (TPO) antibody in case 4.





4



5

Fig. 4. Serial changes in serum levels of anti-TSH-receptor antibody in patients with Graves' disease with (a) and without (b) seasonal allergic rhinitis.

Fig. 5. Serial changes in serum levels of anti-thyroid-peroxidase antibody in patients with Graves' disease with (a) and without (b) seasonal allergic rhinitis.

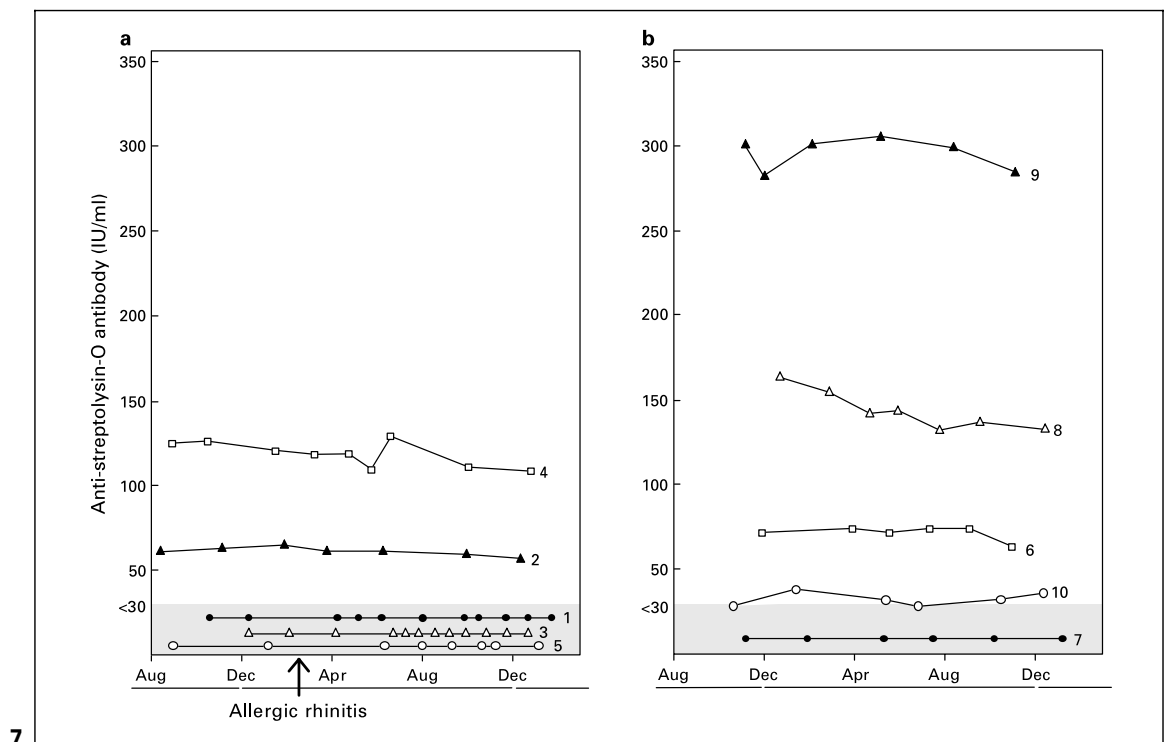
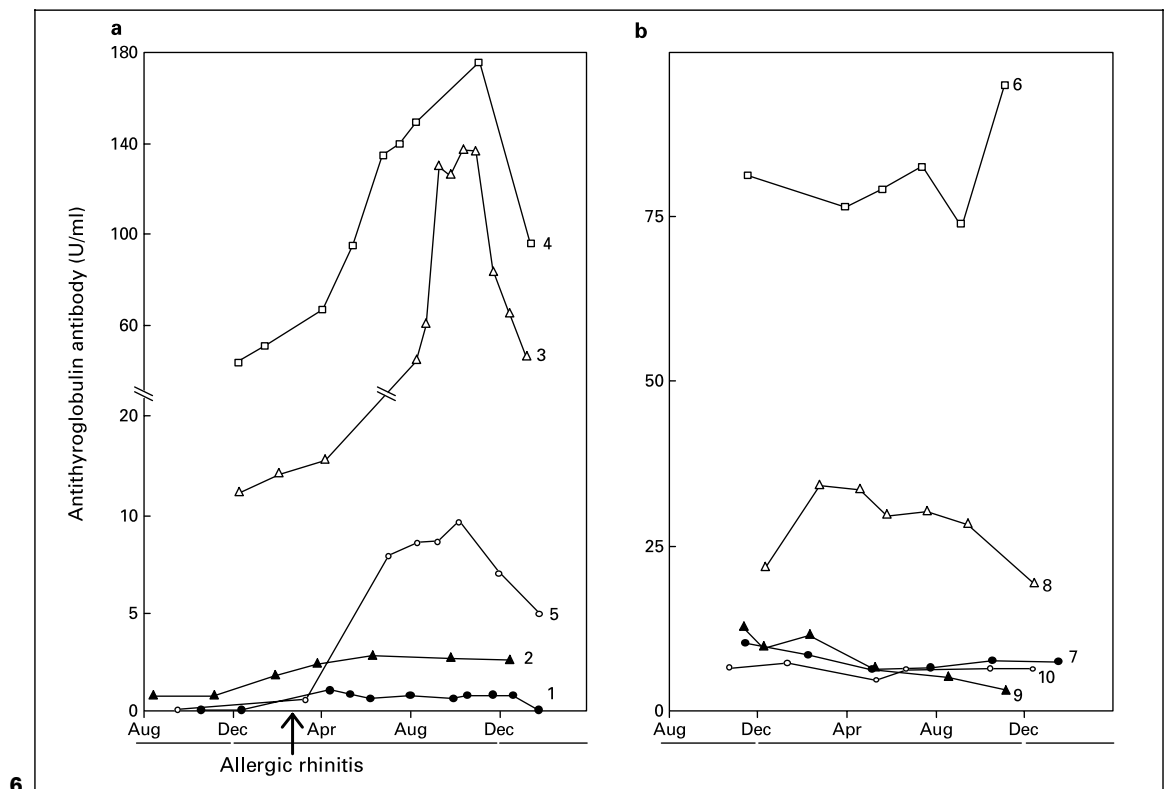


Fig. 6. Serial changes in serum levels of antithyroglobulin antibody in patients with Graves' disease with **(a)** and without **(b)** seasonal allergic rhinitis.

Fig. 7. Serial changes in serum levels of anti-streptolysin-O antibodies in patients with Graves' disease with **(a)** and without **(b)** seasonal allergic rhinitis.

In this study, all patients with cedar pollinosis showed a clear increase in autoantibody titers as well as an increase in cedar-pollen-specific IgE activity. The drug effects on these changes were negligible, since the patients were receiving only minimal doses of methimazole or no medication, except for patient 5 who received a second course of 15 mg of methimazole in July due to relapse of thyrotoxicosis. Large doses of methimazole may have an immunosuppressive effect [21], but a minimal maintenance dose may not have such an influence. The natural seasonal influence could also be ruled out, since the control patients without pollinosis showed no significant change in the titers of any antibodies during the period of 18 months.

The increases in antibody activities started soon after the attacks of rhinitis for every antibody, but the peaks of activity were slightly different among these. The pattern of increase was similar between cedar-pollen-specific IgE and anti-TSH-receptor antibody, although 2 patients were always negative. It is interesting to speculate that B lymphocyte clones to produce antireceptor antibodies would be inactivated completely and reproduction of antireceptor antibodies could not be induced by allergic stimulation in the latter 2 patients. The peak of IgE and antireceptor antibody activities was around May–July. Compared to this, the peak was around October, that is 7 months after the attacks of rhinitis, for both anti-thyroid-peroxidase and antithyroglobulin antibodies, though the pattern was slightly different in individual patients. IgE antibodies, including that against cedar-pollen-specific IgE, bind to mast cells and basophils. Anti-TSH-receptor antibodies, which usually belong to IgG, bind to TSH receptors in the thyroid gland. On the other hand, antithyroglobulin and anti-thyroid-peroxidase antibodies have no such binding site, and thus consumption is different from the above 2 antibodies. These differences may be relevant to the differences of the patterns of antibody increase.

Even in group B, 2 patients (cases 6 and 8) showed an increase in anti-thyroid-peroxidase antibody in spring. In case 6, this antibody was increased before any attack of allergic rhinitis. An increase in antithyroglobulin antibodies was also observed in case 8 in spring, although it occurred before allergic rhinitis. They did not have concurrent respiratory disease in spring, and we do not know the reason why these 2 cases showed an antibody increase.

It is important to elucidate the mechanism of antibody increase. Allergic rhinitis is an inflammatory disease localized in the nose and eyes. If the involved lymph

nodes draining the thyroid gland and mucosal respiratory tract are, at least in part, the same, some Th2-derived cytokines produced during allergen recognition can indirectly drive and amplify the ongoing autoantibody production by local B cells. IL-4, a Th2-associated cytokine, stimulates the production of IgG1, IgG3 and IgG4, as well as IgE [22]. Another Th2 cytokine, IL-13, stimulates IgE and IgG4 antibody production [23]. In this study, the increased antithyroid autoantibodies did not belong to the IgE fraction. It may be worthwhile to examine the IgG subclasses of these autoantibodies. Activation of a local Th2 immune reaction may stimulate systemic immunity toward Th2 predominance, but the measurement of circulating cytokines failed to prove this possibility.

In conclusion, local allergic reactions to environmental antigens in the nose and eyes may stimulate an immune reaction to Th2 predominance in the involved lymph node and may activate autoantibody production in the thyroid gland.

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