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# IgE and IgE Autoantibodies in Patients with Autoimmune Thyroid Disorders and Their Relatives[1] (40142)

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Autoimmunity has been generally considered to be involved in the pathogenesis of Graves' disease and Hashimoto's thyroiditis. Antithyroid antibodies can be detected in nearly all patients (1); and autoantibodies of IgG, IgA, and IgM classes have been described (2).

The possibility that IgE autoantibodies might be involved in thyroid diseases has not been much investigated although in one immunofluorescent study, IgE was said to be localized in the stroma and follicular basement membrane of thyroid glands from some patients with Graves' disease (3).

We speculated that sera from patients with autoimmune thyroid disorders might contain IgE autoantibodies specifically directed towards thyroid antigens. It was also conceivable that IgE deposition in the thyroid might be due to an IgE antibody to IgG or altered IgG. Deposition of IgE by either mechanism might initiate subsequent immunological or inflammatory responses.

We chose the radioallergosorbent (RAST) test as the best currently available technique for detecting specific IgE antibodies (4). Plasmas from relatives of thyroid disease patients were also tested, since it is known that familial aggregations of cases may occur with Graves' disease and Hashimoto's thyroiditis, and antithyroid antibodies are ofttimes found in their sera (5). In addition, we measured the plasma IgE levels of all subjects.

*Materials and methods. Patients and controls.* We randomly selected 10 plasma samples from each of four subject groups. These were patients with Graves' disease, patients with Hashimoto's thyroiditis, and their immediate relatives. Plasmas were separated from heparinized blood. Eight of the ten Graves' disease patients had antimicrosomal hemagglutinating antibodies, titers ranging from 1:100 to 1:6400. Four out of 10 had antithyroglobulin antibodies, titers ranging from 1:70 to >1:5120. Two out of 10 Hashimoto's disease patients had antithyroglobulin antibodies and four of 10 had antimicrosomal antibodies.

As controls, we used serum and heparinized plasma from 10 healthy hospital workers who did not have autoantibodies to thyroglobulin or thyroid microsomal antigens. We compared heparinized plasma with serum from normal subjects and found identical binding to antigen-coated discs.

*Antigen preparations.* Thyroid extract: Human thyroid tissue obtained at surgery or autopsy was sliced and extracted in 0.14 *M* NaCl at 4° overnight. The extract was stored at −20°C for periods of up to 3 years. The pooled extract was centrifuged at 17,600*g* for 20 min. The supernatant was dialyzed against 0.5 *M* $NaHCO_3$ pH 8.1 for 48 hr and then was concentrated by negative pressure dialysis.

Thyroid homogenate: Previously extracted human thyroid tissue was homogenized in 0.14 *M* NaCl with a Waring blender. The homogenate was centrifuged at 600*g* for 30 min. This supernatant was dialyzed and concentrated in the same manner as for preparing thyroid extract.

*Thyroglobulin.* Thyroglobulin was prepared from the soluble thyroid extract by ammonium sulfate precipitation (6). The thyroglobulin was lyophilized from distilled water and stored at −20°. The lyophilized material was reconstituted with 0.5 *M* $NaHCO_3$ pH 8.4 before coupling with the discs.

*Aggregated IgG.* One percent human IgG (Cohn fraction II) in 0.14 *M* NaCl was heated at 63° for 20 min. After overnight storage at 4° the supernatant was dialyzed and concentrated. Sodium azide, 0.01%, was added to each antigen preparation.

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*Radioallergosorbent technique (RAST).* Antigen was coupled to cyanogen bromide activated filter paper discs at a concentration of 25 mg/ml in 0.5 *M* $NaHCO_3$, pH 8.4 (7). Discs prepared in this way contained insolubilized antigens since the discs were able to remove non-IgE autoantibodies from the sera we studied. Thyroglobulin and thyroid extract-coated discs significantly decreased antithyroglobulin but did not lower antimicrosomal hemagglutinin titers. Homogenate-coated discs partially absorbed both antithyroglobulin and antimicrosomal hemagglutinins from the serum (Table I).

*Specific IgE assay.* Antigen-coupled discs placed in individual polystyrene tubes were preincubated in 50 μl incubation buffer (phosphate buffer 0.1 M pH 7.4, 0.3% human serum albumin, 0.05% Tween 20, 0.02% sodium azide) enriched to a 5% concentration of human serum albumin. Overnight incubation with 25 μl of test serum or plasma followed. After washing with 0.14 *M* NaCl, 100 μl of $^{125}$I-labelled rabbit anti-PSIgE (Fc) was added (8). Following 18 hours incubation, the total radioactivity of each tube and radioactivity bound to the disc were assayed. Duplicate assays were performed on all specimens. The difference in total count rate of duplicate assays was less than 5%. Results were expressed as a percentage of the total counts added to the tube. (Hereafter, the term, % bound radioactivity, is used.) If the count rates of bound radioactivity in duplicate samples differed by more than 20%, the results were rejected.

*Total IgE measurements.* Individual cellulose discs coupled with rabbit anti-PSIgE (Fc) were placed in polystyrene tubes with 50 μl of incubation buffer containing 5% human serum albumin. Subject's serum or plasma, 25 μl, was added and incubated overnight. Bound IgE was detected with $^{125}$I-labelled rabbit anti-PSIgE (Fc). IgE concentrations were obtained from a standard curve using a serum known to contain 3,600 IU of IgE/ml. The technique used was sensitive to 0.5 IU/ml but was most precise between 3 and 600 IU/ml.

*Statistics.* Student's *t* test was applied for the analysis of specific IgE assay results. The Mann-Whitney U test was applied for the analysis of total IgE levels, since serum IgE levels have a multimodal rather than a normal distribution in larger samples (9).

*Results.* Total plasma IgE levels of patients with Hashimoto's thyroiditis were significantly lower than those of a simultaneously assayed control group ($P < 0.025$, Mann-Whitney U test). The same tendency was observed in the plasma from relatives of patients with Hashimoto's thyroiditis and several low values were seen in the patients with Graves' disease. Total circulating IgE levels for all the groups studied are presented in Fig. 1.

Small amounts of $^{125}$I-anti-IgE bound to the discs coupled with each of the antigens. Sera from normal subjects promoted this binding to antigen-coupled discs to a similar degree. Results of these assays for the various thyroidal antigens used, as well as for aggregated IgG, are shown in Table II. Binding with these antigens varied slightly. It is likely that the differences between mean binding to discs coupled with thyroglobulin and with thyroid homogenate related to the antigen used. The reproducibility of the procedures used was found to be excellent in repeated assays employing thyroid extract-coupled discs. All assays using a specific antigen were

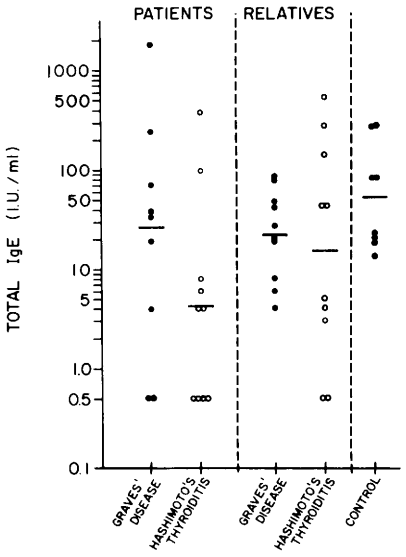


FIG. 1. Total plasma IgE concentration (IU/ml). Each bar represents the geometric mean for the group. Total IgE values less than 0.5 IU/ml were considered to be 0.5 for the calculation of the geometric mean. The Hashimoto's thyroiditis patients' IgE levels were significantly lower than those of controls ($P < 0.025$, Mann-Whitney U test).

performed at the same time, facilitating comparison of control and patient groups. Inspection of the data for individual sera failed to reveal any which differed remarkably from the group as a whole.

To assess the reproducibility and specificity of the procedures used, we selected from each of the patient groups a plasma which promoted relatively great binding of $^{125}$I-anti-IgE and one promoting relatively little binding. This was done for each type of antigen-coated disc. Of the 19 pairs thus reassayed, 18 were found to have the same relationship of greater vs. lesser binding as in the original assay. The same pairs of plasma specimens were then assayed with discs to which no antigen was bound and 10 pairs now showed reversal of the greater-lesser binding relationship. This suggests specificity in the binding of $^{125}$I-anti-IgE to the antigen-coated discs preincubated with serum but not to uncoated discs preincubated with the same sera. Two sera with high total levels of IgE and large amounts of IgE antibody to wheat and rice did not react differently than controls with the antigen-coupled discs used for this study. There was no relationship between total serum IgE levels and $^{125}$I-anti-IgE binding to thyroid extract or thyroglobulin-coated discs.

TABLE I. ABSORPTION OF ANTITHYROGLOBULIN AND ANTIMICROSOMAL HEMAGGLUTINATING ANTIBODIES BY ANTIGEN-COATED DISCS.

| Serum # | Control | Antigen-coated disc: Thyroglobulin | Extract | Homogenate |
|---|---|---|---|---|
| Antithyroglobulin | | | | |
| 13 | 1:20 | ND* | <1:10 | <1:10 |
| 6 | 1:640 | 1:80 | 1:80 | <1:10 |
| 8 | <1:10 | <1:10 | <1:10 | ND |
| Antimicrosomal | | | | |
| 13 | 1:400 | ND | 1:400 | <1:100 |
| 6 | 1:6400 | ND | 1:6400 | 1:600 |
| 8 | 1:400 | 1:400 | 1:400 | ND |

* ND—not done.

*Discussion.* We are unaware of previous reports of IgE levels in autoimmune thyroid disease although other immunoglobulins have been measured. Barkas *et al.* reported increased levels of IgG in sera from groups of Hashimoto's and Graves' disease patients (10). Glynne and Thomson found that mean IgG, IgA and IgM were not different from their normal group except for a group of patients with relapsed Graves' disease whose mean IgG level was elevated to 1930 mg/ml (11). IgE levels bore no relationship to the titers of autoantibodies.

Since our groups were small and we did not obtain information about allergic diseases, some caution is necessary in evaluating the apparent lowering of IgE levels in Hashimoto's thyroiditis. On the other hand, the geometric mean of the total IgE levels of our control group, 50.6 IU/ml, is comparable to that of 41.9 or 42.9 IU/ml obtained previously in this laboratory for nonallergic control groups with a double antibody radioimmunoassay using the same standard serum (12) and also similar to values obtained in larger control populations (9).

None of the patients had significant amounts of IgE antibodies to thyroidal antigens. In addition to the studies described, we have assayed several selected human sera containing large amounts of IgG antithyroglobulin or IgG antithyroid microsomal antibodies. None has promoted any more anti-IgE binding to antigen-coated discs than the plasmas described in this study.

The RAST technique is a sensitive, specific method for demonstrating IgE antibodies. All reagents used in this study were active in other immunologic systems, i.e. the thyroid antigens were active in hemagglutination and

TABLE II. RESULTS OF SPECIFIC IgE AUTOANTIBODY ASSAYS % BINDING OF $^{125}$I-ANTI-IgE, MEAN ± SD.

| | (Number of Subjects) | | | |
|---|---|---|---|---|
| | Thyroid extract | Thyroglobulin | Thyroid homogenate | Aggregated IgG |
| Patients with Graves' disease | 3.9 ± 0.4 (8) | 4.5 ± 0.3 (8) | 7.5 ± 0.6 (9) | 7.2 ± 0.4 (10) |
| Patients with Hashimoto's thyroiditis | 4.1 ± 0.4 (10) | 4.5 ± 0.5 (10) | 7.5 ± 0.4 (9) | 7.4 ± 0.4 (10) |
| Immediate relatives of Graves' disease patients | 3.9 ± 0.2 (10) | 4.3 ± 0.3 (10) | 7.4 ± 0.4 (10) | 7.4 ± 0.4 (10) |
| Immediate relatives of Hashimoto's thyroiditis patients | 3.8 ± 0.3 (9) | 4.9 ± 0.9 (10) | 7.0 ± 0.3 (10) | 7.6 ± 0.5 (10) |
| Controls | 4.0 ± 0.3 (9) | 4.6 ± 0.6 (8) | 7.3 ± 0.5 (10) | 7.5 ± 0.4 (10) |

complement fixation assays and the same batch of $^{125}$I-labelled rabbit anti-IgE worked well in several unrelated RAST assays. Direct evidence that antigens were bound to the discs was provided by the finding that antigen-coupled discs absorbed antithyroglobulin and antimicrosomal hemagglutinating antibodies from the plasma. Also, there were consistent differences in the plasmas in their ability to promote $^{125}$I-anti-IgE binding, even though there was no significant difference between the normal and the diseased groups in this regard. Anti-tissue antibodies can be demonstrated in many normal sera with sensitive techniques, so we have considered the possibility that the low level of binding of $^{125}$I-anti-IgE may reflect the presence of IgE autoantibodies in all the groups, including the normal controls. Another possibility, however, is that this binding represents a variable degree of background or nonspecific binding in the system used.

Our pooled human thyroid glands did not contain tissue from Hashimoto's thyroiditis patients, and Graves' disease thyroid glands made up only a minor fraction of the pool. Therefore, IgE autoantibodies to antigenic products of diseased glands might have been missed. Such antibodies might explain the reported IgE deposition in diseased glands which could then be regarded as a consequence, not a cause, of the disease.

*Summary.* We have investigated the possibility that immunoglobulin E (IgE) antibodies are involved in the pathogenesis of autoimmune thyroid diseases. For this purpose, the radioallergosorbent technique was used to search for IgE autoantibodies to various thyroidal antigens and to aggregated IgG in patients with Hashimoto's thyroiditis, Graves' disease, and their immediate relatives. Thyroidal antigens used were thyroglobulin, thyroid extract and thyroid homogenate. No definite evidence, however, was found for the presence of circulating IgE autoantibodies to these antigens.

Plasma IgE concentration in the same subjects was measured with a cellulose disc radioimmunoassay method. The IgE levels of a group of Hashimoto's thyroiditis patients were found to be significantly lower than those of a control group.

These studies provide little support for hypotheses of IgE participation in the pathogenesis of autoimmune thyroid diseases. The immunopathologic significance, if any, of the lowering of total IgE levels in patients with Hashimoto's disease is presently unknown.

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