

Evaluation of Chronic Urticaria in Patients with Hashimoto Thyroiditis

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The coincidence of Hashimoto thyroiditis (HT) and chronic idiopathic urticaria (CIU) is a commonly observed phenomenon in western New York. Previous literature suggested that there may be a direct relationship between them. We undertook these studies to determine whether humoral or cell-mediated mechanisms might link HT and CIU.

Skin biopsies from patients with CIU, with or without HT, were indistinguishable by light microscopy. No immune complex deposition was observed, although only the skin from patients with CIU and HT contained perivascular fibrin deposits. Similarly, immunohistochemical studies evaluating cellular expression of CD3, CD4, CD8, CD20, and CD68 failed to differentiate between CIU with or without HT. Analysis of V β restriction in thyroid tissue of patients with HT and the skin of patients with CIU and HT by *in situ* polymerase chain reaction failed to reveal any oligoclonal T-lymphocyte subpopulations. In contrast, only patients with CIU and HT had anti-Fc ϵ RI antibodies in their sera that could induce degranulation of normal basophils. Some sera from patients with CIU and HT caused degranulation of normal basophils in the absence of anti-Fc ϵ RI. The factor causing basophil degranulation in these sera was not determined. Patients with CIU and HT failed to improve clinically with thyroid replacement therapy. All CIU patients were equally well managed with symptomatic therapies.

In conclusion, HT likely represents a marker of other autoimmunity, rather than being a direct causative agent in CIU. Management of CIU, with or without HT and with or

without anti-Fc ϵ RI antibodies, should be the same. Future studies will have to examine whether cell-mediated responses participate in CIU, especially in association with HT.

KEY WORDS: urticaria; autoimmunity; Hashimoto thyroiditis.

INTRODUCTION

The pathogenesis of chronic urticaria remains poorly understood. Despite extensive clinical investigations, no cause is identified in the majority of patients (1). Treatment is largely symptomatic. However, in the past decade, new concepts have emerged regarding its pathogenesis, at least in a subgroup of patients.

Since most patients with chronic urticaria are not atopic, Mathews (2) proposed that autoimmunity may be a contributing factor. An increasing body of literature has accumulated to support this hypothesis (3–5). This includes several observations: (a) an allergic etiology cannot be found in most patients (1); (b) injecting autologous serum under the skin of patients with urticaria leads to localized urticaria (6, 7); (c) CD4⁺ lymphocytes are found in the infiltrate in the dermis of chronic urticaria patients, similar to other organ-specific autoimmune diseases (8, 9); and (d) autoantibodies reacting with IgE and the high-affinity receptor for IgE—Fc ϵ RI—have been detected in the sera of patients with chronic urticaria (10–12). Recently, increased CD40L expression on activated T cells and increased bcl-2 expression in activated B and T lymphocytes were found in severe chronic urticaria patients (13). Similar genetic dysregulation has been observed in patients with autoimmune diseases such as systemic lupus erythematosus (14, 15).

The association between chronic idiopathic urticaria (CIU) and thyroid autoimmunity (TA), was first described by Leznoff *et al* (16) in 1983. They reported an increased incidence of Hashimoto's thyroiditis (HT)

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among patients with chronic urticaria. In a larger study, Leznoff and Sussman (17) found 14.4% incidence of thyroid autoimmunity among 624 patients with chronic urticaria. Several other investigators have reported a similar association (18–20). The basis for such an association, if any, is not well understood. Some investigators used thyroid hormones in the therapy of CIU with variable results (21, 22).

We undertook this study because of the large number of patients with chronic urticaria and/or HT that were referred to our service. We sought to determine whether there were differences in the clinical features of patients with chronic urticaria, whether or not they had HT. We sought to examine several hypotheses for how these two entities might be related: (a) immune complexes produced in the course of HT thyroiditis are trapped in the skin and cause urticaria; (b) inflammatory cells activated in the thyroid are directed toward a cross-reactive antigen existing in the skin; (c) there is no direct relationship between the inflammation in the thyroid and the skin, but autoimmunity directed toward Fc ϵ RI coexists with autoimmunity directed toward the thyroid gland in susceptible patients; and (d) there is no relationship between chronic urticaria and HT, but they are both common disorders that are bound to coexist in a small percentage of people.

METHODS

Patient Population and Control

Medical charts of 610 patients who were referred to local allergists for the evaluation and management of chronic urticaria were reviewed. Chronic urticaria was defined as the daily or almost daily recurrent itchy skin eruption for over 6 weeks. Sixty of these patients also met the criteria for HT, which was defined as a titer of anti-thyroglobulin antibodies (TG) >2 IU/ml and/ or anti-thyroid peroxidase (TPO) antibodies at a titer >2 IU/ml. Fourteen of the patients with HT were receiving thyroid replacement for treatment of hypothyroidism. Of the patients with both CIU and HT, 27 patients agreed to participate in the studies (group 1). Measurement of thyroid-stimulating hormone (TSH) was done in all patients. Five patients with CIU and negative thyroid antibodies were enrolled as group 2. Patients were followed for an average of 12 months. Patients were defined as responders to therapy if their urticaria either disappeared or decreased in the frequency of occurrence and skin distribution by greater than 50%. Nine healthy laboratory personnel with no history of chronic urticaria

served as controls (group 3). Thyroid tissues from eight other female patients with HT that had been removed because of a fear of malignancy were obtained from the Department of Pathology at Roswell Park Cancer Institute (RPCI).

Patients with urticarial vasculitis, physical urticaria, dermatographism, and systemic lupus erythematosus (SLE) were excluded. None of the study patients were taking corticosteroids or other immunosuppressive drugs at the time of enrollment.

Informed consents were obtained from all the patients. The institutional review board of Kaleida Health and the SUNY at Buffalo School of Medicine and Biomedical Sciences approved the study protocol.

Methods

Sera were obtained from all patients and stored at -80°C . Two 3-mm skin biopsies were taken from each patient. One was fixed in 10% formalin and then embedded in paraffin. The other was used for immunofluorescence studies. Four-micron sections were cut from each biopsy and either stained with hematoxylin and eosin for histopathological examination, or used for immunohistochemical or *in situ* polymerase chain reaction (PCR) studies.

Thyroid tissues obtained from RPCI were investigated for the same microscopic features, immunohistochemical studies, and *in situ* PCR evaluations as the skin biopsies. None of the chronic urticaria patients in this study had a medical indication for a thyroid biopsy or thyroidectomy, so there was no opportunity to simultaneously evaluate the skin and thyroid of the same patient.

Immunohistochemistry

Immunohistochemical staining was performed using the Ventana basic diaminobenzidine (DAB) detection system, according to the manufacturer's instructions. Briefly, sections were deparaffinized and digested with pepsin. The inhibitor solution was added to remove the endogenous peroxidase activity for 4 min at 37°C . Incubation with the primary antibody was performed for 30 min at room temperature. Murine monoclonal anti-human CD20 antibody (B lymphocyte), murine monoclonal anti-human CD68 antibody (macrophage), rabbit polyclonal anti-human CD3 antibody (T lymphocyte), murine monoclonal anti-human CD4 ("helper" T lymphocyte), and anti-human CD8 ("killer" T lymphocyte) antibodies (Dako, Cambridge, England) were used. The slides were then incubated with biotin-conjugated goat anti-murine antibodies or goat-anti-rabbit antibodies, for

8 min at 37°C. Next, the slides were incubated for 8 min with avidin horseradish peroxidase. The specific antibody, secondary antibody, and avidin enzyme complex were then visualized using DAB. Copper enhancement was performed and the slides counterstained with hematoxylin. Positive immunostaining resulted in brown labeling of positive cells. All skin and thyroid tissues were evaluated for expression of CD3, CD4, CD8, CD20, and CD68.

Immunofluorescence

Tissues were sectioned and air dried thoroughly. The fluorescein-conjugated goat anti-human IgG, IgA, IgM, C3, fibrin (Dake, Cambridge, England) were diluted 1:1000 with PBS and centrifuged to remove precipitated debris. The slides were washed gently in PBS for 15 min, then placed in a moist chamber. Diluted antibody conjugates were placed on the tissue and incubated at room temperature for 30 min. Slides were then washed in PBS for 15 min. Johnson's media was placed on each cover slip and the slides visualized by fluorescence microscopy.

In Situ PCR

Three sections from each tissue sample, 4 μ m in thickness, were placed on glass microscopic slide. They were deparaffinized with xylene and alcohol, and digested with pepsin (2 mg/ml) (Roche, IN). The optimal digestion conditions were determined using the hot start maneuver as described by Nuovo (23). Tissue digestion was terminated by placing the slide in Tris solution (PH 7.5) and then ethanol. The slides were then left to air dry. The tissue samples and negative controls were then incubated overnight with RNAase-free, DNAase I solution (Roche, IN) in a humidity chamber at 37°C. Positive control sections received no DNAase I. The slides were then placed in Tris solution, followed by ethanol.

In situ PCR solution was prepared according to the manufacturer's instructions (Perkin-Elmer) using DNA polymerase, RNAase inhibitor, gene amplification buffer, digoxigenin-labeled dNTP, and 2% BSA and specific primers for various T-cell receptor families. Specific V β primers and C β primers for the T-cell receptor families V beta 2, 3, 6, 7, 8, 20 were synthesized according to previously published studies (24). Using the assembly tool, *in situ* PCR solution was placed on the tissue samples using Amplicover disks (Perkin Elmer). *In situ* PCR was completed by placing the slides in a gene-amp thermocycler that used 20 cycles of 60° F for 1 min and 94°F for 30 sec. Detection of the amplified

signal was done using anti-digoxigenin-AP antibodies (Roche, Germany) and NBT/BCIP (Roche), according to the manufacturer's instructions. Tissue signal was considered acceptable if good cell morphology was maintained, the positive control signal was present in >50% of cells, and the negative control did not show any detectable signal. The entire tissue section was then screened for the presence of a signal with the designated primers.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis Immunoblotting

Purified recombinant human high affinity IgE receptor, alpha chain-HSA fusion protein(130 kDa), produced in CHO cells was obtained from Novartis. This protein was electrophoresed at 750 ng/lane in 10–12% Tris-glycine gels (Invitrogen, Netherlands) and then blotted overnight onto nitrocellulose membranes, as previously described (25). The membranes then were blocked with 5% powdered milk in PBS for 1 hr at 20° C. Membranes then were incubated with patients' sera at various dilutions and with positive and negative sera at a dilution of 1:200 for 30 min at 20° C. (The negative and positive control sera were kindly provided by Allen Kaplan, University of South Carolina.) After washing three times with TBS for 15 min each at 20° C, membranes were incubated with anti-human IgG (H+L)-AP conjugate (Promega, Madison, WI) at a dilution of 1:7500 for 1 hr at 20° C. After washing three times with TBS, antibody binding was visualized by incubating the membrane with NBT/BCIP (Sigma, St. Louis, MO), according to the manufacturer's instructions.

Basophil Histamine Release Assay

The basophil histamine release assay was performed according to previously published protocols (26). In short, basophil-enriched leukocytes were obtained from normal donors using 6% dextran sedimentation. The cells were washed and resuspended in assay buffer containing 10 mM HEPES, 137 mM NaCl, 2.7 mM KCL, 0.4 M NaH₂PO₄, 0.03% human albumin, 5 mM glucose, 2 mM calcium, and 1 mM magnesium, with pH 7.4. Leukocyte suspensions were incubated in a 37° C water bath for 5 min and then aliquoted into polypropylene tubes at a final reaction volume of 200 μ l per tube. Challenge stimuli were sera from individual patients with urticaria, normal subjects, and known positive and negative control sera. Calcium ionophore A23187 was used as a positive control to demonstrate histamine release. Basophils were incubated with the challenge

Table I. Patient Characteristics

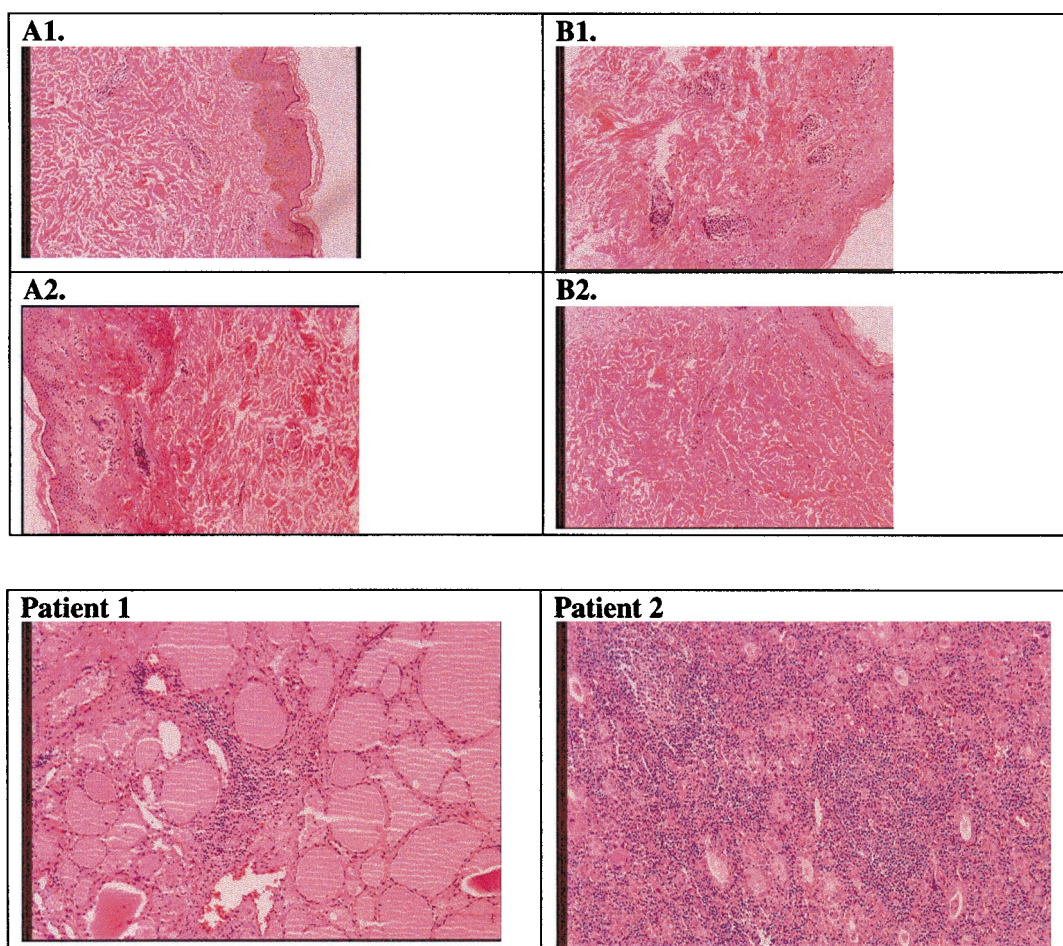
Group	1	2	3
Clinical presentation ^a	HT, CIU (N = 27)	CIU (N = 5)	Control (N = 9)
Sex (F/M)	25/2	5/0	4/5
Age range (mean)	12–60 (37)	14–39 (27)	18–48 (32)
Atopy	5	0	1
IgE level	5	0	ND
Duration of urticaria (years)	1–20 (4)	1–15 (5)	0
Elevated thyroid antibodies	27	0	N/D
History of hypothyroidism	14	0	0
Other diseases	4	0	0
Medical therapy			
Antihistamines	27	5	0
Thyroid hormones	17	0	0

^aHT Hashimoto's thyroiditis; CIU, chronic idiopathic urticaria; ND, not done.

stimuli for 40 min at 37° C. The cells were centrifuged at $500 \times g$ for 15 min and the supernatant removed for the histamine assay. The cell pellet was resuspended in assay buffer and boiled to release residual cellular histamine. The cell particles were again centrifuged at $500 \times g$ and the supernatant removed for the histamine assay. Hista-

mine released was assessed by enzyme-linked immunosorbent assay according to the manufacturer's instructions (Immuno Biological laboratories, Hamburg, Germany).

The percent histamine release in individual samples was expressed as the ratio of histamine released in the



first incubation to the histamine released in the first incubation plus histamine released by boiling.

RESULTS

Patient Characteristics

The characteristics of the patients used in this study are shown in Table I. Thirty-two patients who met the criteria for CIU were enrolled. Twenty-nine were females. Age ranged from 12 to 60 years with a mean of 37 years in group 1, 14 to 39 years with a mean of 27 in group and 18 to 48 years with a mean of 38 years in group 3. Only five patients from group 1 were atopic by

history and skin testing, none in group 2, and one normal control. The duration of urticaria ranged from 1 to 20 years. Five patients had elevated IgE levels. One patient in group 1 was diagnosed with Graves' disease and treated with radioactive iodine ablation before being treated with thyroid hormone for secondary hypothyroidism. There with no change in the course of her urticaria. Overall, 17 patients received thyroid hormone replacement, 14 for overt hypothyroidism and 3 as a trial in the therapy of their HT-related urticaria (22). In almost all the patients, the diagnosis of HT preceded the appearance of urticarial lesions. Three patients had other diseases (diabetes mellitus in one, multiple sclerosis in one, congestive heart failure in one). All of group 1 and

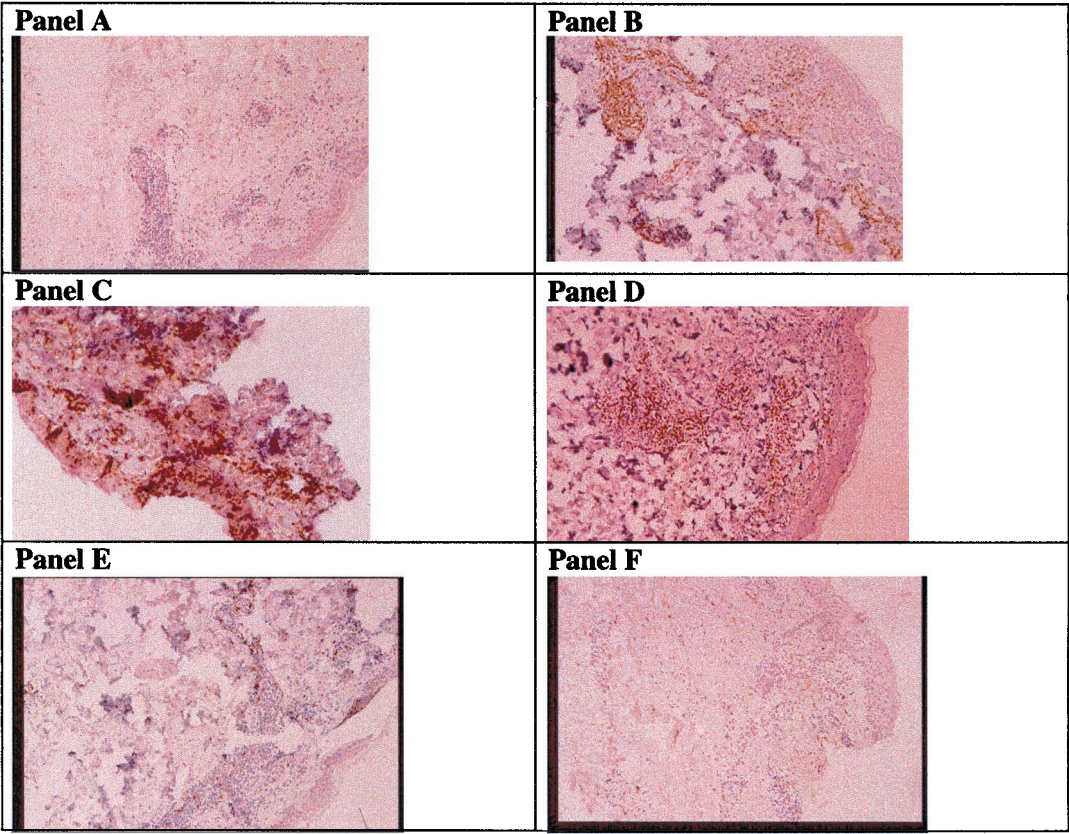


Fig. 1. (opposite, top) Histopathologic findings of the skin of patients with urticaria, either without (A1 and A2) or with (B1 and B2) Hashimoto thyroiditis. Sections of skin were stained with hematoxylin and eosin and examined by light microscopy at 20× magnification.

Fig. 2. (opposite, middle) Histopathologic findings of the thyroid gland of two patients with Hashimoto thyroiditis. Sections of thyroid gland were stained with hematoxylin and eosin and examined by light microscopy at 20× magnification.

Fig. 3. (opposite, bottom) Representative series of immunohistochemical stains on the skin of a patient with chronic urticaria and Hashimoto thyroiditis. (Panel A) Negative control; (Panel B) anti-CD3; (Panel C) anti-CD4; (Panel D) anti-CD8; (Panel E) anti-CD20; (Panel F) anti-CD68.

group 2 patients were receiving some type of antihistamine in their therapy.

Clinical Observations

Most patients continued to have relapses of urticaria with no change in the course of their disease after treatment with thyroid hormone. Three patients in group 1 reported reduction in the number of their urticarial lesions upon the institution of thyroid hormone therapy. All these patients had evidence of hypothyroidism. None of the three euthyroid patients, who were given a trial of thyroid hormone therapy, demonstrated any improvement in their disease. Thyroid hormone withdrawal was instituted in four patients, with no change in their urticaria. There was no significant difference between patients in groups 1 and 2 with regards to the number of their lesions or the frequency of their relapses. There was no significant difference in the severity of urticaria or the response to therapy between patients with or without anti-FcεRI antibodies.

Histopathologic Features

SKIN. The classic histopathologic appearance of urticaria was observed in the skin of all the patients in groups 1 and 2. This includes dermal edema, dilated blood vessels, and a perivascular infiltrate, which consisted mainly of lymphocytes, eosinophils, macrophages, and mast cells. None of these findings were identified in the normal control. Interestingly, the skin of patients with urticaria that did not have recognizable skin lesions at the time of biopsy also showed the same histopathologic findings as the skin of patients with overt urticaria. No vasculitis was seen in any of the skin biopsies. There was no difference in the histopathologic appearance of the skin of patients in groups 1 and 2 (Fig. 1). There was no difference in the histopathologic appearance of the skin of patients with or without anti-FcεRI antibodies (data not shown).

THYROID. Thyroid tissue from patients with Hashimoto thyroiditis revealed a prominent cellular infiltrate that consisted mainly of lymphocytes. Lymphoid follicles were present in almost all of the tissues, associated with varying degrees of follicular destruction and oxyphilic epithelial metaplasia. Fibrosis was observed in four of eight glands studied (Fig. 2).

Immunohistochemistry

SKIN. A summary of the immunohistochemical findings in the skin are shown in Table II. A representative

Table II. Summary of Cutaneous Immunohistochemical Findings^a

Patient	Group #	CD3	CD4	CD8	CD20	CD68
LH 30-1	1	++	++	++	—	++
LG 30-2	1	++	—	+	—	+
RC 30-3	1	++	++	++	—	++
LB 30-4	1	++	+	++	—	++
DM 30-5	1	++	+++	++	—	++
CE 30-6	1	++++	++++	++	++	++
MS 30-7	1	++	++	+	—	++
KL 30-8	1	++	+++	++	—	++
MD 30-9	1	++	+++	++	—	++
LB 30-10	2	+	+++	++	—	++
JA 30-11	3	—	—	—	—	—
JS 30-12	1	+	++	+	—	++
LM 30-13	1	++	+	++	—	++
RW 30-14	2	++	+++	++	—	++
JZ 30-15	1	++	+++	++	—	++
AE 30-16	1	++	+++	++	—	++
PH 30-19	1	++	+++	++	—	++
SH 30-21	2	++	+++	++	—	++
VS 30-61	1	++	+++	++	—	++
MB 30-68	1	++	++	+	—	++
CL 30-69	1	+++	+++	++	—	++
RV 30-70	1	+++	+++	++	—	++

^a++++ Prominent, +++ moderate, ++ few, + rare, — absent. Immunohistochemical staining was done as described in materials and methods. Grading of the intensity of staining was performed by a pathologist (MZ). See Fig. 3 for a representative sample.

skin biopsy is shown in Fig. 3, demonstrating the anti-CD3, CD4, CD8, CD20, and CD68 staining.

Almost all patients biopsied had a perivascular infiltrate that consisted of CD4+ and CD8+ T lymphocytes. There was a predominance of CD4+ over CD8+ lymphocytes in all the skin biopsies. Less frequently, macrophages, eosinophils, and mast cells also were seen. None of the skin biopsies contained B lymphocytes, except for that of one patient who had particularly severe and chronic urticaria (CE). There was no significant difference in the nature of the cutaneous cellular infiltrate between patients in groups 1 or 2, or between patients who did or did not produce anti-FcεRI antibodies.

THYROID. A summary of the immunohistochemical findings in the thyroid are shown in Table III. A representative thyroid study is shown in Fig. 4, demonstrating the anti-CD3, CD4, CD8, CD20, and CD68 staining.

There was a predominance of CD3+, CD4+, and CD20+ lymphocytes in the tissues examined. The lymphoid follicles consisted mainly of CD20+ cells. Of interest, CD4 staining was found in the thyroidal epithelial cells. Cells that were both CD3+ and CD4+ cells were found primarily in the perifollicular regions.

Immunofluorescence

There was subtle fibrin deposition in the dermal blood vessels of patients from group 1, but not groups 2 or 3.

Table III. Summary of Thyroid Immunohistochemical Findings^a

Patient	CD3	CD4	CD8	CD20	CD68
TH 99-7372	++++	+++	*	++	++
TH 97-343	++	++++	+++	++	++
TH 99-6875	++++	++	++	+++	+++
TH 99-836	+++	+++	*++	*++	++
TH 99-9200	++++	++++	*++	++++	+++
TH 95-7204	+++	++	*+++	+++	++++
TH 96-3215	++	+	+	++++	+++
					++++
TH 96-4607	++	—	—	+++	

^a++++ Prominent, +++ moderate, ++ few, + rare, — absent, * thyroid epithelial staining. Immunohistochemical staining was done as described in materials and methods. Grading of the intensity of staining was performed by a pathologist (MZ). See Fig. 4 for a representative sample.

No IgG, IgM, IgA, or C3 deposits were detected in any of the specimens examined.

In Situ PCR

The findings are summarized in Table IV. Representative skin and thyroid tissue *in situ* PCR studies for V β 6 and V β 8 are shown in Figs. 5 and 6, respectively.

Among the 22 patients tested, analysis of the TCR V β restriction of the lymphocytic infiltrate of the skin biopsies demonstrated TCR V β 2 expression in three patients, one showed TCR V β 8 expression, and two patients showed TCR V β 6 expression. All patients were from group 1, except the patient with V β 8 expression who belonged to group 2. The rest of the skin biopsies showed no significant expression of the V β families analyzed. In no case did a majority of infiltrating cells express a TCR utilizing a single V β family.

In contrast to the skin, three of the thyroid tissue showed V β 8 expression but no expression of the other five V β families that were analyzed. In no case did a majority of infiltrating cells express a TCR utilizing a single V β family.

SDS-PAGE Immunoblotting

The findings are summarized in Table V. A representative Western blot assay is shown in Fig. 7.

Anti-Fc ϵ RI antibodies were detected at a titer of 1:200 or greater in the sera of eight patients with CIU (25%). Two normal control sera had similar antibodies at similar dilutions. Twenty sera from patients with SLE or various systemic vasculitides were also tested. Of those, only one patient with SLE and no history of urticaria had anti-Fc ϵ RI antibodies.

Basophil Histamine Release Assay

The findings are summarized in Table VI. Sera of 15 out of 32 patients with CIU (47%) released 10% or greater histamine from the basophils of a nonatopic donor. All of those patients except three had HT. Histamine release ranged from 12 to 64%. Calcium ionophore released 35% histamine. Five of eight sera (62%) from patients with CIU and demonstrated to have anti-Fc ϵ RI antibodies by the Western blot assay caused significant histamine release in this assay. Four of these positive sera were from patients with HT. Of note, the sera from the two normal controls and the one SLE patient, which contained anti-Fc ϵ RI antibodies (Table V), did not cause significant histamine release. In addition, basophil histamine release was induced by the sera of some patients with CIU that did not have anti-Fc ϵ RI antibodies; 80% of these sera were from patients with HT.

DISCUSSION

We have demonstrated in this study that the histopathologic and immunohistochemical analyses of urticaria from patients with or without HT are largely indistinguishable. Subtle differences were noted in the immunofluorescent studies. While none of the biopsies demonstrated immune complex deposition, only the skin biopsies from HT patients showed fibrin deposition. No oligoclonal populations of lymphocytes could be demonstrated in the skin of urticaria patients with or without HT or in the thyroid glands of patients with HT. Sera from patients with CIU and HT were more likely to induce histamine release from the basophils of normal donors than sera from patients with CIU alone. In only some cases did the ability to induce basophil histamine release correlate with the presence of antibodies to Fc ϵ RI. Antibodies to Fc ϵ RI in the sera of normal donors and SLE patients without urticaria did not induce basophil histamine release.

The histopathologic, immunohistochemical, and *in situ* PCR studies argue against a direct relationship between HT and chronic urticaria. There is no evidence for immune complexes in the skin. There is no evidence that cellular immunity initiated in the thyroid leads to pathological changes in the skin, although these studies are unable to rule out the possibility that a small population of cells does function in this way. Investigators examining thyroid tissues in HT, including the work in these studies, have had difficulty in demonstrating restriction in the lymphocyte subpopulations residing in the thyroid gland and have argued that multiple different epitopes are recognized simultaneously in HT (27–29).

We did find small aggregates of V β 8- or V β 6-restricted T lymphocytes in the skin of some patients with CIU and HT and cannot rule out the possibility that these cells came from the thyroid gland. Aggregates of V β 8-restricted T lymphocytes were found in several of the thyroid glands from patients with Hashimoto thyroiditis. In the absence of local B lymphocytes or antibodies, these T lymphocytes would have to induce mast cell degranulation by releasing chemokines or tachykinins to cause urticaria (30–32).

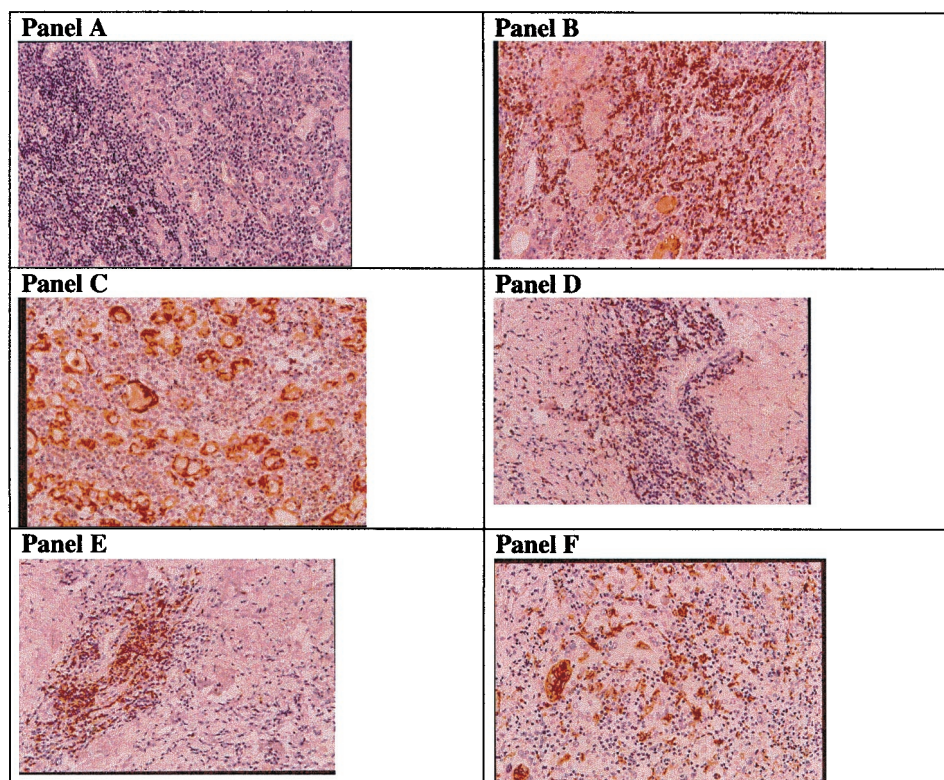
Furthermore, the clinical data argue against a direct relationship between HT and CIU. In our study, the incidence of TA in patients with CIU was 10%. This is similar to the incidence of TA in other studies evaluating patients with CIU (17, 19) and in the normal population. Anti-thyroid antibodies have been estimated to be present in 9–18% of the normal adult female population (33–35). The use of thyroid hormone did not appear to influence the course of the urticaria in any of the patients with HT, whether or not they had hypothyroidism. This is in contrast to one study, in which thyroid hormone replacement caused resolution of chronic urticaria in euthyroid patients (22). Other investigators have noted similar clinical responses to thyroid replacement as in this study (17).

Table IV. Summary of in Situ PCR Findings in Skin and Thyroid Tissue^a

Patient	VB2	VB3	VB6	VB7	VB8	VB20
SH 30-21	—	—	—	—	+	—
MB 30-68	+	—	—	—	—	—
CL 30-69	+	—	—	—	—	—
DM 30-5	+	—	+	—	—	—
JS 30-12	—	—	+	—	—	—
TH 95-7204	—	—	—	—	+	—
TH 99-7372	—	—	—	—	+	—
TH 99-0836	—	—	—	—	+	—

^aThe designated V β families were evaluated in the skin and thyroid tissues of the listed patients. A + sign designates a V β family for which several aggregates of positive lymphocytes were identified (see Fig. 5 and 6).

The next issue regarding the relationship between CIU and HT is whether patients with CIU and HT have a more generalized autoimmune phenotype that includes antibodies to Fc ϵ RI. Our data suggest that patients with CIU and HT are more likely than CIU patients, normal donors, or patients with other autoimmune diseases to have anti-Fc ϵ RI antibodies that induce histamine release from normal donors. However, not all patients with HT had these antibodies, some patients with CIU without HT had these antibodies, and sera of some HT patients



without these antibodies induced significant basophil histamine release. We found anti-FcεRI antibodies in 25% of CIU patients, of whom 80% had HT; 57% of the anti-FcεRI antibodies from CIU and HT patients induced basophil histamine release. One study reported an incidence of 37% for CIU patients with anti-FcεRI antibodies (36) and another 50% (37). The former studied noted anti-FcεRI antibodies only in CIU patients, not in normal controls or patients with atopic dermatitis. The latter study noted that only 9% of the CIU sera but also 7% of normal sera induced histamine release from normal

basophils. A recent study identified anti-FcεRI antibodies that could induce basophil histamine release among patients with CIU and atopy, and normal controls. Many of these antibodies were found to recognize tetanus toxoid (38). Finally, a recent evaluation of patients with multiple etiologies for acute and chronic urticaria identified antibodies to FcεRI in the sera of patients with chronic urticaria from a wide variety of different causes, including drug allergy, food allergy, *Helicobacter pylori* infection, and various autoimmune diseases (39). Thus, antibodies to FcεRI likely participate in chronic urticaria

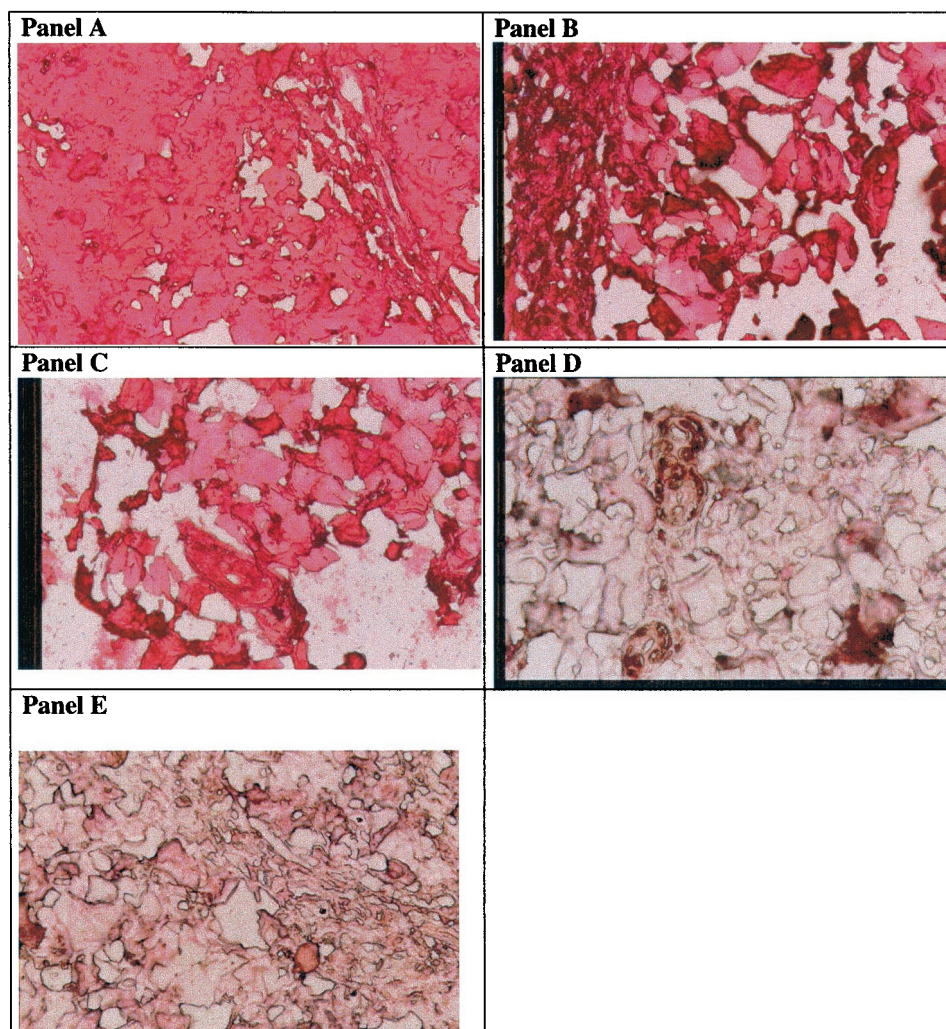


Fig. 4. (opposite, top) Representative series of immunohistochemical stains on the thyroid of a patient with Hashimoto thyroiditis. (Panel A) Negative control; (Panel B) anti-CD3; (Panel C) anti-CD4; (Panel D) anti-CD8; (Panel E) anti-CD20; (Panel F) anti-CD68.

Fig. 5. (opposite, bottom) Representative *in situ* PCR analysis of skin for Vβ 8 and Vβ6 expression in lymphocytes. (Panel A) Negative control; (Panel B) Vβ8 positive control; (Panel C) Vβ8; (Panel D) Vβ6 positive control; (Panel E) Vβ6.

Table V. Summary Of Sera Positive by Western Blot Analysis for Anti-FcεRI Antibodies^a

Patient	Group No.	Serum dilution
LH 30-1	1	1:200
RC 30-3	1	>1:400
KL 30-8	1	1:200
JS 30-12	1	1:400
JZ 30-15	1	1:200
SH 30-21	2	>1:400
BS 30-22	3	1:400
AK 30-25	3	1:200
K.B 30-29	*	1:200
ES 30-59	1	1:400
NM 30-64	1	1:200

*Patient with SLE.

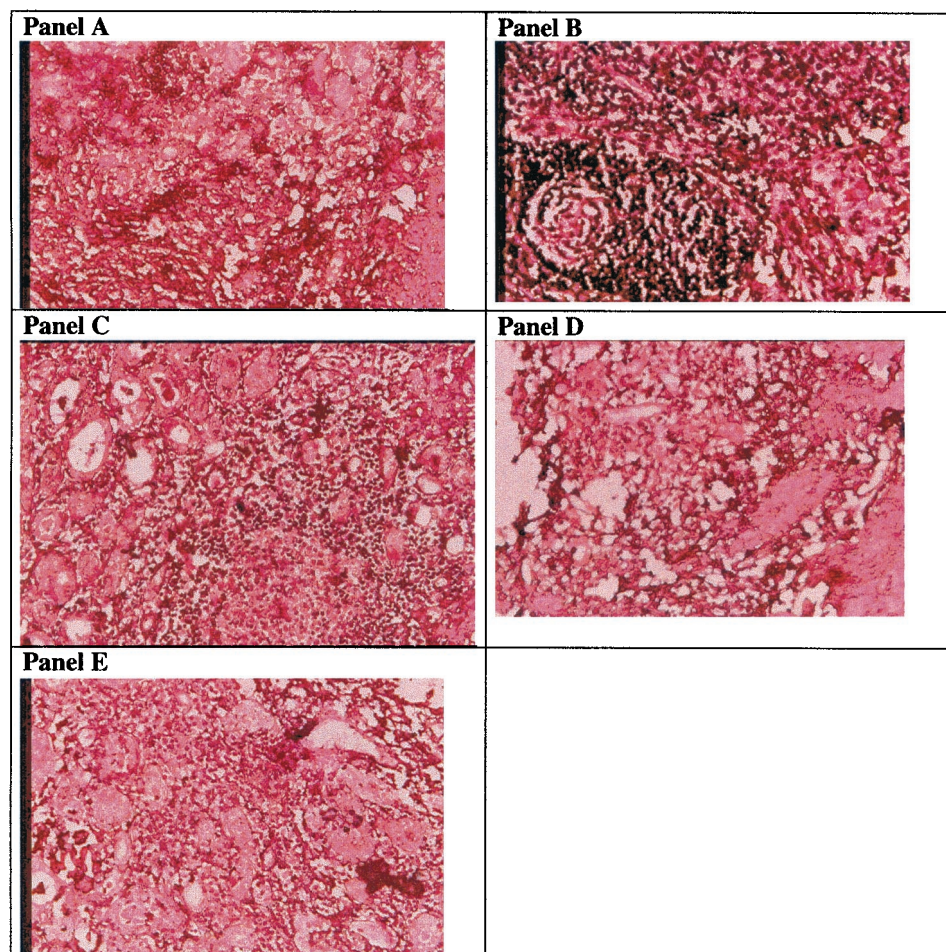
^aWestern blot assays were performed as described in Materials and Methods. A representative anti-FcεRI Western blot is shown in Fig. 7.

Table VI. Summary Of Positive Basophil Histamine Release Assays^a

Patient	Group	Anti-FcεRI antibody	Percent histamine release
LH 30-1	1	+	25
RC 30-3	1	+	18
JS 30-12	1	+	32
ES 30-59	1	+	12
SH 30-21	2	+	36
CL 30-69	1	—	40
MD 30-9	1	—	15
LB 30-10	2	—	22
DM 30-5	1	—	36
AE 30-16	1	—	64
VS 30-61	1	—	15
SR 30-18	1	—	28
JF 30-71	2	—	34
MS 30-7	1	—	42
PH 30-19	1	—	26

^aPositive sera released 48% histamine, negative sera < 5% histamine. The basophil histamine release assay was performed as described in Materials and Methods. The percent histamine release in the amount of histamine released from the reaction vial after the addition of serum divided by the total histamine extracted from the cells.

in a subgroup of patients, but exactly what that subgroup represents is currently unclear. In our study, pathological anti-FcεRI antibodies were associated with HT.



In conclusion, this study suggests that CIU and HT are not directly linked, but HT may be a marker for a subgroup of patients who also have autoimmune responses that may contribute to the presence of urticaria. These autoimmune responses include humoral mechanisms, namely anti-FcεRI antibodies, which were documented in this and other studies. Cell-mediated autoimmune responses resulting in release of chemokines, tachykinins, and/or other histamine-releasing factors, need to be evaluated in future studies. The clinical course of CIU does not appear to be significantly different whether or not it involves HT and/or anti-FcεRI antibodies. Treatment for these various conditions should be identical.

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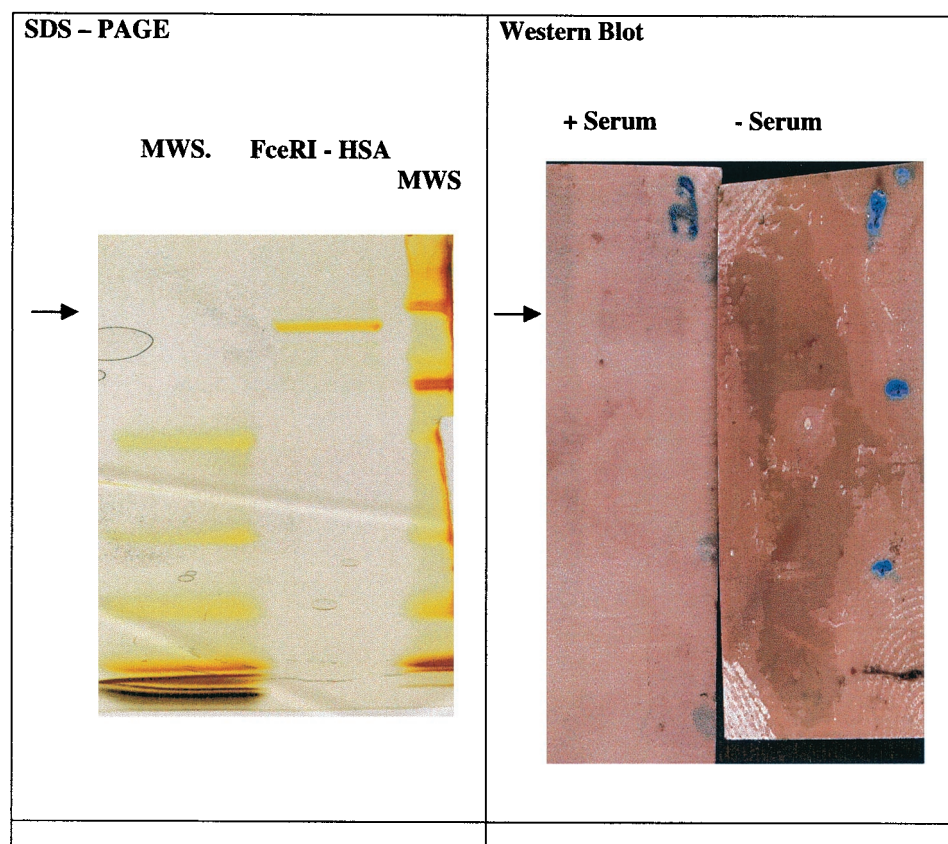


Fig. 6. (opposite, top) Representative *in situ* PCR analysis of thyroid tissue for Vβ8 and Vβ6 expression in lymphocytes. (Panel A) Negative control; (Panel B) Vβ8 positive control; (Panel C) Vβ8; (Panel D) Vβ6 positive control; (Panel E) Vβ6.

Fig. 7. (opposite, bottom) Western blot assay for anti-FcεRI antibodies. (Panel A) The silver stain of the 10-12% SDS-PAGE gel of recombinant FcεRI-HS A protein (Lane 1 = molecular weight markers; Lane 2 = 130-kDa recombinant FcεRI HS A). (Panel B) The Western blots of the FcεRI protein developed with sera from patients with (left panel) or without (right panel) antibodies to FcεRI.

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