

Comparison of the *in vivo* autologous skin test with *in vitro* diagnostic tests for diagnosis of chronic autoimmune urticaria

Michelle L. Altrich, Ph.D.,* John F. Halsey, Ph.D.,* and Leonard C. Altman, M.D.#

ABSTRACT

Previous studies indicate that 30–50% of chronic urticaria patients have an autoimmune etiology. Clinical diagnosis of autoimmune urticaria is supported with the autologous serum skin test. The purpose of this study was to compare two laboratory tests for measurement of IgG autoantibodies to IgE or IgE receptors and compare the results with the autologous serum and plasma skin tests. We performed skin tests and two functional *in vitro* tests, basophil histamine release, and CD63 up-regulation to detect autoantibodies relevant to autoimmune urticaria. Both sera and citrated plasma were evaluated in the autologous skin test and histamine release assay. Thyroid autoantibodies were also measured. Basophils were incubated with patient plasma, sera, buffer, or anti-IgE. The cells were analyzed for CD63 expression and the supernatants were recovered for histamine analysis. There was high correlation between CD63 up-regulation and histamine release assays, but histamine release was more sensitive. There was a high concordance between sera and citrated plasma for the skin test. Sera from chronic urticaria patients produced higher mean histamine release (23%) compared with citrated plasma (12%). Thirty-one percent of patients positive in the histamine release assay were also positive for thyroid autoantibodies. This compares with 12% who were negative in the histamine release assay. These data show that *in vitro* basophil histamine release can be used to measure antibodies to FcεRI, FcεRII/CD23, or IgE and identify patients with autoimmune urticaria.

(Allergy Asthma Proc 30:28–34, 2009; doi: 10.2500/aap.2009.30.3185)

Key words: Anti-FcεR, autoantibodies, autoimmune, autologous skin test, basophil, CD63, chronic urticaria, histamine release test, *in vivo* test, thyroid autoantibodies

Chronic urticaria is a common skin disorder affecting 0.1–1% of the general population. It is characterized by recurrent, transitory, pruritic erythematous wheals present for at least 6 weeks.^{1,2} Although chronic urticaria is not life-threatening, it can adversely affect a patient's quality of life. One report relates the stress from chronic urticaria as equivalent to that of a patient waiting for bypass surgery and indicates that chronic urticaria affects the quality of life in multiple areas including work, social life, home relationships, and hobbies.^{3,4} Although chronic urticaria primarily affects adults, recent reports document that it is also prevalent in children.^{5,6} Although the cause of urticaria is often unclear; multiple investigators have indicated

an autoimmune etiology in 30–50% of chronic urticaria patients.^{7–13} Recent evidence has also suggested that the basophils in patients with chronic urticaria may be dysregulated.^{14–18}

The first reports of autoimmune chronic urticaria were indirect. In the 1960s there were reports that antibody–antigen interactions could cause basophil/mast cell degranulation.¹⁹ Moreover, an association between chronic urticaria and autoimmune thyroid disease has been recognized since the early 1980s.^{20,21} Specifically, it has been shown that a higher percentage of patients with chronic urticaria show autoantibodies against either thyroid peroxidase (TPO) or thyroglobulin (Tg)²² than a normal healthy population. There is also evidence that HLA DRB4 class II alleles may predispose individuals to autoimmune chronic urticaria.²³

The earliest direct evidence for autoimmune chronic urticaria comes from studies that showed that the injection of patient serum into the dermis of the skin caused a wheal and flare response. This *in vivo* test, the autologous serum skin test (ASST), was first used in the 1940s to diagnose autoimmune chronic urticaria.²⁴ A later report found that this response was only detected if the disease was active.^{25,26} The active factor in the serum was shown to be heat stable at 56°C. Subsequent work identified the serum factor as an IgG autoantibody, which, in most cases, was directed against

From *IBT Laboratories, Lenexa, Kansas, and #Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington and Northwest Asthma and Allergy Center, Seattle Washington

Presented in part (poster presentations) at the 2007 meeting of the Western Society of Allergy, Asthma, and Immunology, Wailea, Hawaii, January 2007, the 2007 meeting of the American Academy of Allergy, Asthma, and Immunology, San Diego, California, February 2007 and the 2008 Combined Meeting on Allergy, Asthma, and Immunology, Puerto Vallarta, Mexico, January 2008

Funding: IBT Laboratories

All study participants provided informed consent

Address correspondence and reprint requests to Michelle Altrich, Ph.D., IBT Laboratories, 11274 Renner Boulevard, Lenexa, KS 66219

E-mail address: michellea@ibtllabs.com

Copyright © 2009, OceanSide Publications, Inc., U.S.A.

the IgE receptor on cutaneous mast cells and less frequently to IgE itself.²⁷⁻³¹ A recent report by Asero *et al.* suggested that the autologous plasma skin test (APST) was a more sensitive assay than the ASST for detecting autoantibodies³² in chronic urticaria. This report has not been confirmed by others.

Detailed analysis has shown that the antireceptor autoantibodies bind to either the $\alpha 2$ -subunit of the IgE receptor and compete with IgE for binding or to the $\alpha 1$ -subunit, in which case they are noncompetitive. Other studies have suggested that only IgG1 and IgG3 subclasses are responsible for autoimmune chronic urticaria and that complement activation is involved. IgG1 and IgG3 are efficient in activating the complement cascade leading to C5a generation and this peptide is a potent histamine releaser from mast cells.³³

Performance of the ASST is time-consuming and requires an on-site laboratory to prepare the serum and experience performing immediate allergy skin testing. There is also a risk of injecting the wrong serum into a patient. Therefore, the availability of a commercial *in vitro* diagnostic test would facilitate the diagnosis of autoimmune chronic urticaria. *In vitro* assays for the diagnosis of autoimmune chronic urticaria have been used for several years but heretofore have only been available in research facilities. One obstacle that has slowed the development of routine clinical tests for autoimmune chronic urticaria is the lack of a gold standard test to establish positive and negative predictive values. Some consider the ASST to be that standard; and others feel the basophil histamine test should be considered the gold standard.

These prior research studies used both donor mast cells and basophils to look for autoantibodies in patient sera.³⁴ In addition, basophils from both atopic and nonatopic donors have been used and some reports indicate that basophils from atopic donors are more sensitive.³⁵ Initial experiments used histamine release to measure the degree of basophil activation, hence, the presence of autoantibodies. More recently, up-regulation of basophil surface activation markers CD63 or CD203c has been used.^{8,36-39}

In the current study we compared two *in vivo* and two *in vitro* diagnostic tests for autoimmune chronic urticaria. The *in vivo* autologous skin test compared both serum and citrated plasma, and the *in vitro* tests compared histamine release with CD63 up-regulation. We also evaluated thyroid function and antithyroid autoantibody levels in the same group of chronic urticaria patients.

We found a high degree of concordance with serum and plasma in the autologous skin test, but a higher percentage of histamine release with serum. *In vitro*, histamine release and CD63 were both useful in the diagnosis of autoimmune chronic urticaria, but histamine release was more sensitive. Similar to other in-

vestigations, we found that chronic urticaria patients had a higher incidence of thyroid autoantibodies. This study indicates that serum and plasma are comparable for *in vivo* skin testing but serum is better than plasma for performing *in vitro* assays. Furthermore, *in vitro* basophil histamine release is a useful diagnostic test for confirming the diagnosis of autoimmune chronic urticaria.

METHODS

Patients and Controls

One hundred one patients from an allergy practice who fit established criteria for chronic urticaria and 11 healthy controls were selected for study. Patients had no identifiable trigger in any case. Patients had autologous skin testing performed and serum and plasma samples were taken for *in vitro* analysis. Controls were not skin tested but provided specimens for use *in vitro*. All study participants provided informed consent.

Skin Testing

To perform the ASST and APST, 50 μ L of autologous serum (ASST) or plasma (APST) was injected intradermally into the patient's forearm. Equal volumes of histamine (0.10 mg/mL; Hollister-Stier Laboratories, Spokane, WA) and saline served as the positive and negative controls. Immediately after injection the sizes of the wheal and flare in millimeters at each site were recorded. Measurements were made again after 15 minutes, and in some individuals readings were also taken at 30 minutes. In preliminary studies skin tests were read at 15, 30, and 60 minutes. All positive reactions were observed within 30 minutes and longer observation did not alter the results. A positive result was defined as an increase in the wheal reaction size of >3 mm with no change or reduction in size of the saline control.

In Vitro Autoimmune Chronic Urticaria Testing

CD63 Up-Regulation. Sodium heparinized whole blood collected from a single nonatopic donor was used as a source of basophils throughout the study. Basophils were enriched by an initial slow $200 \times g$ centrifugation; the plasma and buffy coat were removed and centrifuged at $500 \times g$. After the second centrifugation, the plasma was discarded. The enriched basophil preparation was diluted in stimulation buffer containing IL-3 (FLOW CAST kit; ALPCO, Salem, NH) and incubated with either patient sera, a buffer control, or anti-Fc ϵ RI α (FLOW CAST kit) as a positive control for 30 minutes at 37°C. Cells were stained with anti-IgE fluorescein isothiocyanate and anti-CD63 phycoerythrin (FLOW CAST kit) and analyzed on the FC500 (Beckman Coulter, Fullerton, CA).

Basal level of CD63 expression was determined with the negative control.

Histamine Release. Enriched basophils prepared as described previously were diluted in stimulation buffer containing IL-3 and incubated with patient or control sera or plasma, buffer control, or anti-IgE (2 μ g/mL; Beckman Coulter) as a positive control for 30 minutes in a 37°C water bath. The cells were then centrifuged and the supernatants were recovered. Two aliquots of cells were lysed by three rounds of freeze and thaw for determination of total histamine content. Using a quantitative enzyme immunoassay (Beckman Coulter), the histamine released into the supernatant was measured and compared to the total histamine in the basophils to generate a percent histamine release.

Thyroid Autoantibody Measurement

Levels of serum antibodies against TPO and Tg were measured on the Immulite 2000 (Siemens, Los Angeles, CA). Normal reference values were based on manufacturer's recommendation. Normal values are <35 IU/mL for anti-TPO and <40 IU/mL for anti-Tg. Thyroid stimulating hormone (TSH) levels in serum were measured using the Rapid TSH kit (Siemens) also on the Immulite 2000. For this method the normal reference range is between 0.4 and 4.0 mIU/mL.

Statistics

Regression analysis was used to compare the CD63 and histamine assays. Technologists who performed the *in vitro* assays were blinded to the skin test results.

RESULTS

Comparison between Histamine Release and CD63 Up-Regulation

A single donor was used for the studies illustrated in this article. The donor went through a qualification process before his basophils were selected for *in vitro* assays. Initially, donor basophils were incubated with a panel of control (nonchronic urticaria) sera and the positive control, anti-IgE. To qualify the donor's basophils for testing, the cells had to show low percent histamine release and CD63 expression when incubated with control sera and high percent histamine release and CD63 expression in response to anti-IgE.

A positive result in each test was based on the mean plus two standard deviations of a control population. For the histamine release assay the mean plus two standard deviations was 12.9% and 16.4% for CD63. Validation of the histamine release assay showed an intra-assay variability of 8% and an inter-assay variability of 5.3%. Sera from ASST⁺ chronic urticaria (CU) patients ($n = 24$) produced a mean histamine release of 28.4% compared with 9.5% with sera from ASST⁻ CU

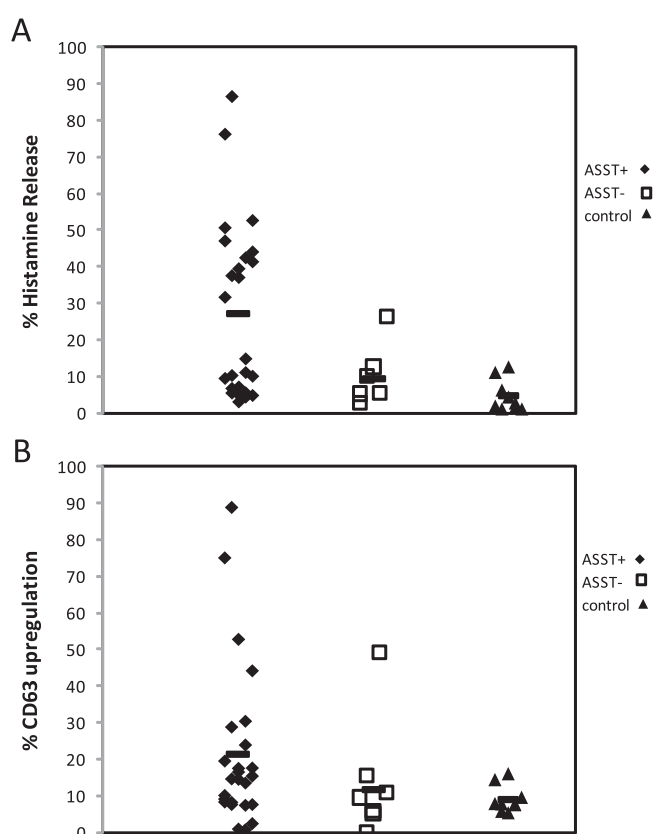


Figure 1. Autologous serum skin test–positive (ASST⁺) chronic urticaria patients show higher reactivity in basophil histamine release and CD63 up-regulation assays. Using donor basophils from a single atopic donor, sera from chronic urticaria patients (ASST⁺ and ASST⁻) and normal controls were evaluated in the (A) histamine release assay and the (B) CD63 up-regulation assay (B).

patients ($n = 7$) and 4.8% with control sera ($n = 11$; Fig. 1 A). Similarly, ASST⁺ CU patient sera caused 21.4% of donor basophils to up-regulate CD63, compared with 11.9% for ASST⁻ CU sera and 9.0% for control sera (Fig. 1 B). In this study, 55% of ASST⁺ CU and 14% of ASST⁻ CU patients were positive in the histamine release assay; while 44% of ASST⁺ CU and 14% of ASST⁻ CU patients were positive in the CD63 assay. Although the correlation coefficient between the results of the two assays was low ($r^2 = 0.54$; Fig. 2), the concordance (positive versus negative) was higher (75%; Table 1). The histamine release assay showed higher sensitivity.

Comparison between ASST and APST

A recent report suggested that performing the autologous skin test with citrated plasma rather than serum resulted in a higher percentage of positive results.³² We evaluated 65 chronic urticaria patients with both the ASST and the APST. We found a very high 87.7% (57/65) correlation between the two methods of skin

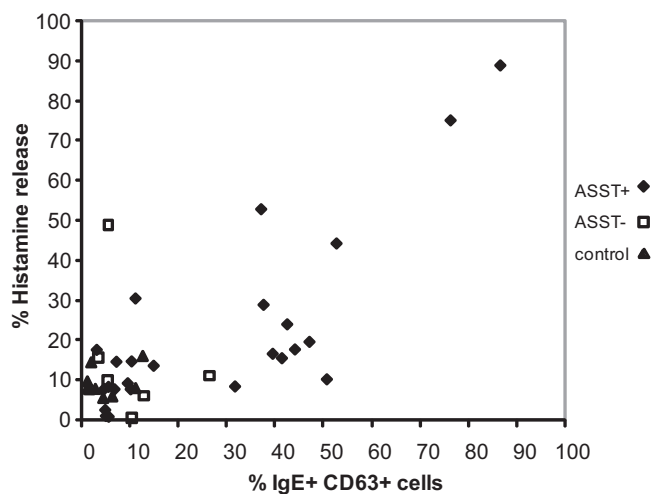


Figure 2. Comparison between histamine release and CD63 up-regulation as in vitro diagnostic tests for autoimmune chronic urticaria. Donor basophils from a single atopic donor were incubated with sera from chronic urticaria patients autologous serum skin test (ASST⁺ and ASST⁻) and normal controls and evaluated in the histamine release assay and the CD63 up-regulation assay.

Table 1 Comparison between two *in vitro* assays

	CD63 Up-Regulation	
	Positive	Negative
Histamine release		
Positive	28%	16%
Negative	9%	47%

Table 2 Comparison between the ASST and APST

	ASST	
	Positive	Negative
APST		
Positive	31	7
Negative	1	26

n = 65.

APST = autologous plasma skin test; ASST = autologous serum skin test.

testing (Table 2). Thirty-one or 47.7% of patients were positive on both the ASST and the APST; 26 or 40% of patients were negative on both tests. Only 8 patients (12.3%) were positive on one test and negative on the other. This suggests that either serum or citrated plasma can be used in the autologous skin test.

Comparison between Serum and Plasma in the Histamine Release Assay

Using basophils from a single nonatopic donor, we extended our comparison between serum and citrated

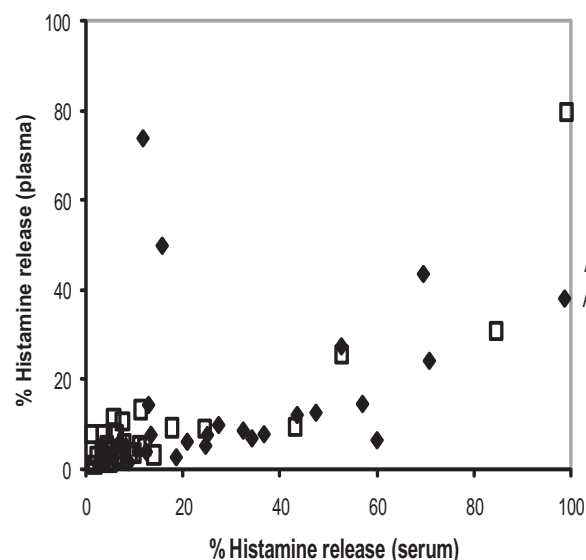


Figure 3. Sera from chronic urticaria patients' causes higher levels of basophil histamine release compared with plasma. Using donor basophils from a single atopic donor, serum and plasma from chronic urticaria patients were used as stimulants in the histamine release assay ($p < 0.01$).

plasma to the histamine release test. Serum induced a mean histamine release of 22.7%, and citrated plasma induced a mean histamine release of only 11.5% ($p < 0.01$). This lower mean percentage of histamine release with citrated plasma also resulted in a lower percentage of samples that were scored positive; 41.5% of serum samples resulted in a positive result compared with only 20% with citrated plasma. Figure 3 is a graphic representation of these data. This indicates that functional autoantibodies can be detected with both serum and citrated plasma; however, serum is the better specimen and the only specimen accepted for clinical testing.

Correlation between Autoimmune Thyroid Disease and Chronic Urticaria

Previous studies have found a higher occurrence of autoimmune thyroid disease in chronic urticaria patients compared with healthy controls.²² In this study we measured the levels of anti-Tg, anti-TPO, and TSH in a subset of chronic urticaria patients. A greater percentage of patients that tested positive in the histamine release assay had thyroid autoantibodies (Table 3). The presence of these autoantibodies did not result in abnormal TSH levels in most patients. This indicates that patients remained euthyroid despite the presence of circulating thyroid autoantibodies. The same correlation held true when patients were grouped based on reactivity in the ASST (Table 3).

Table 3 Thyroid status in HR⁺ and HR⁻ and ASST⁺ and ASST⁻ chronic urticaria patients

	HR ⁺ (n = 24)	HR ⁻ (n = 40)	ASST ⁺ (n = 27)	ASST ⁻ (n = 29)
Anti-TPO	33.3%	10.0%	25.9%	13.8%
Anti-Tg	20.8%	0.0%	18.5%	0.0%
Abnormal TSH	4.2%*	5%	7.4%*	3.4%

*One patient had low TSH levels.

ASST = autologous serum skin test; HR = histamine release; Tg = thyroglobulin; TPO = thyroid peroxidase; TSH = thyroid stimulating hormone.

DISCUSSION

Chronic urticaria is a difficult disease in which to establish the etiology. A major advance in recent years has been the recognition that 30–50% of chronic urticaria patients have an underlying autoimmune disease causing the disorder.^{7–13} However, diagnosis of the autoimmune form has been limited to specialty clinics with in-house laboratories able to perform the autologous skin test and university research groups that are capable of performing *in vitro* testing to identify relevant autoantibodies. This has left a deficit in routine clinical practice. Here, we describe the standardization of the *in vitro* histamine release test for use in routine diagnostic testing. Also, we compared the use of serum and plasma for performing *in vivo* and *in vitro* tests and verified previous reports that document elevated thyroid autoantibodies in patients with chronic urticaria.

Our results extend the findings of other groups indicating that *in vitro* functional tests can be used in the diagnosis of autoimmune chronic urticaria.^{11,12} Our results agree with others in the literature and an imperfect correlation between *in vitro* functional tests and the results of the ASST test. The reason for these differences is unclear. One explanation may be that the skin test measures the response of mast cells, whereas the *in vitro* assays use basophils.⁹ It is interesting to note that patients with chronic urticaria have involvement of skin mast cells but have less involvement of other tissue mast cells such as those in the lung and gastrointestinal tract.

Both histamine release and CD63 up-regulation are useful assays to measure the activation of donor basophils. However, because of the availability of a standardized histamine ELISA, the ease of batching samples and a better concordance of histamine release and ASST results rather than CD63 up-regulation and ASST results, we have standardized the histamine ELISA for commercial use. In this study we also performed further analysis comparing citrated plasma and serum as specimens for use in the assay. Our data indicate that both specimens can induce histamine release from donor basophils; however, serum results in a higher percentage of positive results. The reason for this difference is not clear.

When comparing serum and citrated plasma in the *in vivo* autologous skin test we found a high 87.7% concordance between the matched sets of specimens. This is in contrast to a recent report from Asero *et al.* suggesting that the use of citrated plasma was more sensitive in detecting autoantibodies against IgE and/or IgE receptors.³² We have no clear explanation why there is a discrepancy between the Asero study and ours; however, our finding agrees with most of the published literature that used serum to perform this test. The presence of the citrate in the plasma may increase the threshold necessary for histamine release in the donor basophils. It appears that either serum or citrated plasma can be used in the skin test to diagnose autoimmune chronic urticaria.

It may also be useful to evaluate all patients with chronic urticaria for thyroid function and thyroid-specific autoantibodies. Previous studies²² have shown a higher frequency of thyroid-specific autoantibodies in chronic urticaria patients when compared with the general population and our data show that a higher percentage of patients with autoimmune chronic urticaria, confirmed either with a skin or *in vitro* test, have thyroid antibodies than chronic urticaria patients with negative skin or histamine release tests. This does not suggest a cause and effect relationship between autoimmune chronic urticaria and autoimmune thyroid disease. It more likely suggests that individuals that are predisposed to one autoimmune disease are at risk of other types of autoimmune pathology. Although most patients with thyroid autoantibodies are euthyroid, there are isolated case reports of chronic urticaria patients improving with thyroxine treatment.³⁷ Furthermore, the detection of thyroid autoantibodies may precede the development of hypothyroidism and this test can serve as a marker to be followed serially over time.

Diagnosis of autoimmune chronic urticaria is important in clinical practice. First, it gives the patient peace of mind by understanding the etiology of their disease. Second, it should reduce the use of allergy skin tests to foods or aeroallergens that are often incorrectly identified as causal factors. In addition, by providing a mechanistic explanation it should eliminate unproven treatments such as rotation diets, food elimination tri-

als, and preservative and dye-free diets. For the same reason, the test result may prevent patients from searching for treatments from alternative practitioners. Patients with chronic urticaria may require immunomodulatory therapy such as cyclosporine, methotrexate, or hydroxychloroquine.^{41–46} These medications can be used if patients fail to respond to front-line treatment with non- or low-sedating antihistamines such as cetirizine, levocetirizine,^{47,48} fexofenadine,⁴⁹ loratadine,⁵⁰ desloratadine,⁵¹ or sedating antihistamine such as hydroxine or doxapim even at higher than recommended doses.⁵² Cyclosporine has been studied extensively with chronic urticaria patients in combination with antihistamines. A double-blind study with ASST⁺ patients found significant benefit with the use of 4 mg/kg of daily cyclosporine.⁴¹ Cyclosporine has also been shown to be effective in ASST⁺ patients at doses of 2.5–5 mg/kg.^{42,43}

Methotrexate^{44,45} and hydroxychloroquine⁴⁶ have also been shown to be effective in limited studies. In clinical practice, weekly pulse therapy with methotrexate has been beneficial in producing control and/or complete remission in ~70% of patients.⁴⁵ The drug is given weekly starting at 7.5 mg the 1st week and 15 mg each week thereafter. Once control is complete, antihistamines should be gradually reduced and stopped, and if the patient is symptom free without antihistamines, methotrexate can be reduced by 2.5 mg weekly. Hydroxychloroquine has also been used with benefit, but controlled studies are lacking. The usual adult dose is 200 mg twice daily.

Many physicians now recognize autoimmune urticaria as a distinct subgroup of chronic urticaria patients and exclude them from the “idiopathic” category. Practicing allergists report that the determination of an autoimmune etiology is clinically helpful because, as mentioned, it reduces the need for extensive testing in search of an exogenous cause and, ultimately, may reduce unnecessary laboratory testing. In addition, immunomodulatory drugs may be initiated sooner in chronic urticaria patients who are known to have an autoimmune process.^{53,54} For these reasons, a widely available *in vitro* test to identify the autoantibodies to FcεRI, IgE, or FcεRII/CD23 should be very useful to all physicians treating patients with chronic urticaria.

REFERENCES

- Greaves M. Chronic urticaria. *J Allergy Clin Immunol* 105:664–672, 2000.
- Greaves MW, and Tan KT. Chronic urticaria: Recent advances. *Clin Rev Allergy Immunol* 33:134–143, 2007.
- O'Donnell BF, Lawlor F, Simpson J, et al. The impact of chronic urticaria on the quality of life. *Br J Dermatol* 136:197–201, 1997.
- Weldon DR. Quality of life in patients with urticaria. *Allergy Asthma Proc* 27:96–99, 2006.
- Brunetti L, Francavilla R, Miniello VL, et al. High prevalence of autoimmune urticaria in children with chronic urticaria. *J Allergy Clin Immunol* 114:922–927, 2004.
- Du Toit G, Prescott R, Lawrence P, et al. Autoantibodies to the high-affinity IgE receptor in children with chronic urticaria. *Ann Allergy Asthma Immunol* 96:341–344, 2006.
- Tong LJ, Balakrishnan G, Kochan JP, et al. Assessment of autoimmunity in patients with chronic urticaria. *J Allergy Clin Immunol* 99:461–465, 1997.
- Ferrer M, Kinet JP, and Kaplan AP. Comparative studies of functional and binding assays for IgG anti-FcεRIα (α-subunit) in chronic urticaria. *J Allergy Clin Immunol* 101:672–676, 1998.
- Frieri M, and Madden J. Chronic steroid resistant urticaria. *Ann Allergy* 70:1, 1993.
- Puccetti A, Bason C, Simeoni S, et al. In chronic idiopathic urticaria autoantibodies against FcεRII/CD23 induce histamine release via eosinophil activation. *Clin Exp Allergy* 35:1599–1607, 2005.
- Soundararajan S, Kikuchi Y, Joseph K, et al. Functional assessment of pathogenic IgG subclasses in chronic autoimmune urticaria. *J Allergy Clin Immunol* 115:815–821, 2005.
- Platzer MH, Grattan CEH, Poulsen LK, et al. Validation of basophil histamine release against the autologous serum skin test and outcome of serum-induced basophil histamine release studies in a large population of chronic urticaria patients. *Allergy* 60:1152–1156, 2005.
- Schocket AL. Chronic urticaria: Pathophysiology and etiology, or the what and why. *Allergy Asthma Proc* 27:90–95, 2006.
- Vonakis BM, Vasagar K, Gibbons SP Jr, et al. Basophil FcεRI histamine release parallels expression of SH2-containing inositol phosphatases in chronic idiopathic urticaria. *J Allergy Clin Immunol* 119:441–448, 2007.
- Eckman JA, Hamilton RG, Gober LM, et al. Basophil phenotypes in chronic idiopathic urticaria in relation to disease activity and autoantibodies. *J Invest Dermatol* 128:1956–1963, 2008. (Advance online publication.)
- Brodell LA, and Beck LA. Differential diagnosis of chronic urticaria. *Ann Allergy Asthma Immunol* 100:181–188, 2008.
- Brodell LA, Beck LA, and Saini SS. Pathophysiology of chronic urticaria. *Ann Allergy Asthma Immunol* 100:291–297, 2008.
- Baker R, Vasagar K, Ohameje N, et al. Basophil histamine release activity and disease severity in chronic idiopathic urticaria. *Ann Allergy Asthma Immunol* 100:244–249, 2008.
- Rorsman H, Slatkin MW, Harber LC, et al. The basophil leukocyte in urticarial hypersensitivity to physical agents. *J Invest Dermatol* 39:493–499, 1962.
- Leznoff A, Josse RG, Denburg J, et al. Association of chronic urticaria and angioedema with thyroid autoimmunity. *Arch Dermatol* 119:636–640, 1983.
- Martinez S, Vanté I, Pacheco C, et al. Resolution of chronic urticaria and autoimmune markers following surgery in Hashimoto's thyroiditis. Presented at the 2007 annual meeting of the American College of Allergy, Asthma, and Immunology. Arlington Heights, IL: ACAAI. Abstract P261.
- Zauli D, Grassi A, Ballardini G, et al. Thyroid autoimmunity in chronic idiopathic urticaria: implications for therapy. *Am J Clin Dermatol* 3:525–528, 2002.
- O'Donnell BF, O'Neill CM, Francis DM, et al. Human leucocyte antigen class II associations in chronic idiopathic urticaria. *Br J Dermatol* 140:853–858, 1999.
- Malmros H. Auto serum test (AST). *Nordisk Med* 29:150–151, 1946.
- Grattan CEH, Wallington TB, Warin RP, et al. A serological mediator in chronic idiopathic urticaria—a clinical, immunological and histological evaluation. *Br J Dermatol* 114:583–590, 1986.
- Frieri M. Complement related diseases. *Allergy Asthma Proc* 23:319–324, 2002.

27. Marone G, Spadaro G, Palumbo C, et al. The anti-IgE/anti-FcεRIα antibody network in allergic and autoimmune diseases. *Clin Exp Allergy* 29:17–27, 1999.
28. Hide M, Francis DM, Grattan CEH, et al. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med* 328:1599–1604, 1993.
29. Sabroe RA, and Greaves MW. Chronic idiopathic urticaria with functional autoantibodies: 12 years on. *Br J Dermatol* 154:813–819, 2006.
30. Kaplan AP. Chronic urticaria: Pathogenesis and treatment. *J Allergy Clin Immunol* 114:465–474, 2004.
31. Fagiolo U, Kricek F, Ruf C, et al. Effects of complement inactivation and IgG depletion on skin reactivity to autologous serum in chronic idiopathic urticaria. *J Allergy Clin Immunol* 106:567–572, 2000.
32. Asero R, Tedeschi A, Riboldi P, et al. Plasma of patients with chronic urticaria shows signs of thrombin generation, and its intradermal injection causes wheal-and-flare reactions much more frequently than autologous serum. *J Allergy Clin Immunol* 117:1113–1117, 2006.
33. Fiebiger E, Hammerschmid F, Stingl G, et al. Anti-FcεRIα autoantibodies in autoimmune-mediated disorders. *J Clin Invest* 101:243–251, 1998.
34. Asero R, Lorini M, Chong SU, et al. Assessment of histamine-releasing activity of sera from patients with chronic urticaria showing positive autologous skin test on human basophils and mast cells. *Clin Exp Allergy* 34:1111–1114, 2004.
35. Gyimesi E, Sipka S, Danko K, et al. Basophil CD63 expression assay on highly sensitized atopic donor leucocytes—A useful method in chronic urticaria. *Br J Dermatol* 151:388–396, 2004.
36. Yasnowsky KM, Dreskin SC, Efaw B, et al. Chronic urticaria sera increased basophil CD203c expression. *J Allergy Clin Immunol* 117:1430–1434, 2006.
37. Wedi B, Novacovic V, Koerner M, et al. Chronic urticaria serum induces histamine release, leukotriene production, and basophil CD63 surface expression—Inhibitory effects of anti-inflammatory drugs. *J Allergy Clin Immunol* 105:552–560, 2000.
38. De Swerd A, Van Den Keybus C, Kasran A, et al. Detection of basophil-activating IgG autoantibodies in chronic urticaria by induction of CD63. *J Allergy Clin Immunol* 116:662–667, 2005.
39. Altrich ML, Halsey JF, and Altman LC. Evaluation of functional assay for the diagnosis of autoimmune urticaria. *J Allergy Clin Immunol* 119:S199, 2007 (Abs).
40. Bangash SA, and Bahna SL. Resolution of Chronic urticaria and angioedema with Thyroxine. *Allergy and Asthma Proc*, 26:415–417, 2005.
41. Grattan CEH, O'Donnell BF, Francis DM, et al. Randomized double blind study of cyclosporin in chronic idiopathic urticaria. *Br J Dermatol* 143:365–372, 2000.
42. Barlow RJ, Kobza Black A, and Greaves MW. Treatment of severe, chronic urticaria with cyclosporine A. *Eur J Dermatol* 3:273–275, 1993.
43. Toubi E, Blant A, Kessel A, et al. Low-dose cyclosporin A in the treatment of severe chronic idiopathic urticaria. *Allergy* 52:312–316, 1997.
44. Gach JE, Sabroe RA, Greaves MW, et al. Methotrexate-responsive chronic idiopathic urticaria: A report of two cases. *Br J Dermatol* 145:340–343, 2001.
45. Altman L. Autoimmune urticaria. *Pediatr Asthma Allergy Immunol* 20:196–200, 2007.
46. Reeves GEM, Boyle MJ, Bonfield J, et al. Impact of hydroxy-chloroquine therapy on chronic urticaria: Chronic autoimmune urticaria study and evaluation. *Intern Med J* 34:4:182–186, 2004.
47. Garg G, and Thami GP. Comparative efficacy of cetirizine and levocetirizine in chronic idiopathic urticaria. *J Dermatolog Treat* 18:23–24, 2007.
48. Dubuske LM. Levocetirizine: The latest treatment option for allergic rhinitis and chronic idiopathic urticaria. *Allergy Asthma Proc* 28:724–734, 2007.
49. Spector SL, Shikar R, Harding G, et al. The effect of fexofenadine hydrochloride on productivity and quality of life in patients with chronic idiopathic urticaria. *Cutis*. 79:157–162, 2007.
50. Pons-Guiraud A, Nekam K, Lahovsky J, et al. Emedastine difumarate versus loratadine in chronic idiopathic urticaria: A randomized, double-blind, controlled European multicentre clinical trial. *Eur J Dermatol* 16:649–654, 2006.
51. DuBuske L. Desloratadine for chronic idiopathic urticaria: A review of clinical efficacy. *Am J Clin Dermatol* 8:271–283, 2007.
52. Kaplan AP. Chronic urticaria: Pathogenesis and treatment. *J Allergy Clin Immunol* 114:465–474, 2007.
53. Sands MF, Blume JW, and Schwartz SA. Successful treatment of 3 patients with recurrent idiopathic angioedema with omalizumab. *J Allergy Clin Immunol* 120:979–981, 2007.
54. Spector SL, and Tan RA. Effect of omalizumab on patients with chronic urticaria. *Ann Allergy Asthma Immunol* 99:190–193, 2007. □

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.