

## Laboratory Evaluation of Autoimmune Chronic Urticaria

Chronic urticaria is a common skin disorder affecting up to 1% of the general population. Studies have demonstrated that 30–50% of patients with chronic urticaria have an autoimmune etiology. The basophil histamine release test has been considered the “gold standard” to diagnose autoimmune chronic urticaria. Known as a “functional” test, it measures histamine released by donor basophils in response to the patient’s serum. In order to provide accurate and reproducible results, laboratories performing the test must have experience with the assay, including appropriate validation and control measures. The continued investigation into the pathogenesis of chronic urticaria should lead to additional tests to help physicians evaluate and understand the immunologic factors responsible for causing this challenging, chronic disease.

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### Background

Chronic urticaria (CU) is characterized by recurrent, transitory, pruritic, erythematous wheals present for at least six weeks.<sup>1,2</sup> In many cases, the urticaria is idiopathic; however multiple investigators have demonstrated an autoimmune etiology in 30 – 50% of patients.<sup>3-8</sup> The identification of an autoimmune cause can help rule out the need to continue searching for dietary or other etiologies, and for those with severe disease, can inform therapeutic decisions, such as when to use immunomodulatory agents.

The earliest direct evidence for establishing autoimmune chronic urticaria comes from studies in which patient serum was injected into the same patient’s uninvolved skin, which is monitored for a wheal and flare response. This *in vivo* procedure, known as the autologous serum skin test (ASST), was first used in the 1940s to diagnose autoimmune chronic urticaria.<sup>9</sup> It is used today, though it is time consuming, requires an on-site laboratory to prepare the serum, and demands experience with immediate-allergy skin testing to obtain accurate, reproducible results. To provide a more convenient, reliable method of diagnosing autoimmune CU, *in vitro* testing measuring basophil histamine release was developed.

### Basophil Histamine Release Testing

The basophil histamine release test has evolved over the past 20 years to be considered by many as the “benchmark diagnostic test”<sup>2</sup> to identify autoimmunity as the cause of a patient’s CU. In this assay, patient sera is mixed with donor basophils, allowing autoantibodies present in the patient’s sera to bind to the donor cells, activate them and trigger histamine release. The level of histamine released is then quantified using an immunoassay, with high levels suggesting autoimmune chronic urticaria.<sup>10</sup> Recently, other assays using flow cytometry to measure up-regulated markers such as CD203c, have also been described, though these are not as well studied as the histamine release assay.

The use of donor basophils in this assay has posed problems for many academic and research laboratories. Due to the inherent variability of donor basophils, it is necessary to develop sophisticated testing and validation methodologies to ensure consistent results. As part of our development of the CU Index™ test in 2006, IBT focused on

### Chronic Urticaria and Thyroid Autoantibodies

Since Leznoff and colleagues first proposed the “syndrome” of autoimmune thyroid disease and chronic urticaria in 1989,<sup>21</sup> investigators have found that patients with autoimmune CU are more likely to have thyroid autoantibodies.<sup>22</sup> Though most patients are euthyroid, some are hyper- or hypothyroid. The strong association between CU and thyroid autoantibodies has prompted many to include measurement of plasma thyroid stimulating hormone and thyroid autoantibodies in the workup of CU patients.

The relationship between these two autoimmune disorders does not appear causal; rather certain individuals seem to be more prone to development of autoantibodies.

normalization between donor cell populations; our protocol includes rigorous screening to ensure that donor basophils do not produce high basal levels of histamine in response to healthy non-CU serum. Figure 1 illustrates, for five different donors, the low positive rate (less than 6.5%) in response to normal, non-CU serum.

For additional monitoring, IBT includes multiple non-CU, positive and negative controls in each CU Index test run, helping to ensure that the donor basophils remain consistent. As a result of our strict screening process and rigorous quality controls, IBT provides CU analysis that is reliable and consistent, allowing physicians and patients to have confidence in our results.

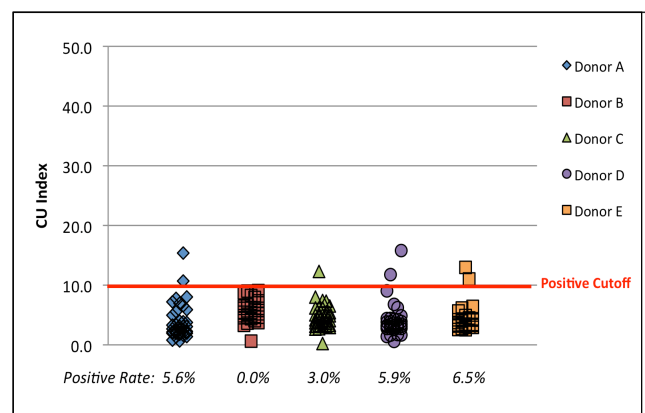


Figure 1: The CU Index test was performed with serum from non-CU individuals (n = 31 to 36). The positive rate (CU Index > 10) ranged from 0% to 6.5%, with no result >18.

## Autoantibodies and Chronic Urticaria

Research into the pathogenesis of chronic urticaria has led to the discovery of autoantibodies with multiple specificities that are now understood to be causative for CU.<sup>11-16</sup> These autoantibodies are:

- IgG anti-IgE, accounting for 5-10% of cases<sup>6</sup>
- IgG with specificity for the  $\alpha$ -chain of the high affinity IgE receptor (Fc $\epsilon$ R1 $\alpha$ ), responsible for 35-40% of cases<sup>6</sup>
- The low affinity IgE receptor Fc $\epsilon$ R11 / CD23, accounting for 5-10% of cases

In order to identify autoantibodies that may be responsible for CU, IBT has recently developed and validated a binding assay to detect autoantibodies against IgE, the Anti-IgE Test. In our validation work, we found that significantly more patients with autoimmune CU (positive CU Index) had elevated anti-IgE (20.6% versus 3.3% for normal health controls and 4.9% for idiopathic CU.<sup>17</sup> The use of the Anti-IgE assay can provide physicians more information about the disease process in their patients.

### Anti-Fc $\epsilon$ R1 $\alpha$ Binding Assays

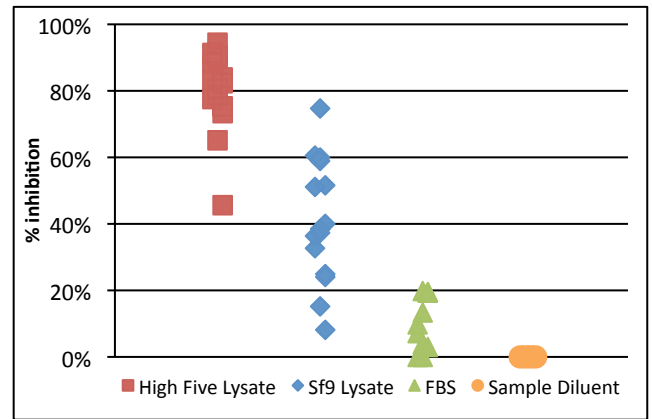
Studies of binding assays developed to detect antibodies against Fc $\epsilon$ R1 $\alpha$  have demonstrated mixed results, with some providing clear distinction between CU and controls<sup>18</sup> and others finding no significant difference in antibody levels between healthy individuals and CU patients.<sup>19</sup>

As part of our research into this area and our effort to develop a reliable assay to detect anti-Fc $\epsilon$ R1 $\alpha$ , we have discovered that the preparation of Fc $\epsilon$ R1 $\alpha$  (Heska) used may be the cause of some of these conflicting results. Using this protein preparation in a binding assay, we found that we could also detect antibodies against the Fc $\epsilon$ R1 $\alpha$  in both CU and non-CU populations. Investigating further, we discovered that the recombinant Fc $\epsilon$ R1 $\alpha$  protein is typically produced using High Five<sup>TM</sup> insect cells. These cells attach a glycosylation motif to the protein, causing it to be immunogenic in a large percent of the population.<sup>20</sup> This glycosylation motif has been shown to cause false positive reactions in other diagnostic tests.<sup>20</sup>

In an internal study, (Figure 2) we found that binding to Fc $\epsilon$ R1 $\alpha$  is inhibited by a lysate from the High Five cell line in a dose-dependent fashion. By contrast, binding was not inhibited by FBS or by a sample diluent. Using a different insect cell lysate (Sf9), binding was also inhibited, though to a lesser degree.

As a result of our research, we concluded that:

- The source of Fc $\epsilon$ R1 $\alpha$  protein can be a strong determinant of assay specificity.
- Use of Fc $\epsilon$ R1 $\alpha$  produced in High Five cells can result in false positives, and may invalidate results obtained with this protein.



**Figure 2:** Inhibition of binding to Fc $\epsilon$ R1 $\alpha$ . Neither Sf9 lysate nor Fetal Bovine Serum (FBS) contain the glycosylation motif believed to make High Five Lysate immunogenic.

In order to address the issues of potential impurities in the recombinant protein and of the glycosylation produced by High Five cells, IBT has developed a novel assay that uses an alternative preparation of Fc $\epsilon$ R1 $\alpha$ . This assay is in the final stages of development and validation, and we expect that it will detect Fc $\epsilon$ R1 $\alpha$  autoantibodies with high sensitivity and specificity.

Autoantibodies to the low affinity IgE receptor, Fc $\epsilon$ R11, are also thought to be responsible for a small percentage of autoimmune CU. Research into these autoantibodies is evolving, though there is not yet a test available to detect them in chronic urticaria.

## Conclusions

It is now recognized that autoimmune chronic urticaria is a disease that is distinct from “idiopathic” CU. With recent diagnostic advances, clinicians now have tools, such as the CU Index<sup>TM</sup> test, that can definitively diagnose the disease, ending the time consuming search for other causes and providing information that can help guide the choice of therapies. With the addition of immunoassays to detect the presence of autoantibodies that can cause CU, such as Anti-IgE, and Anti-Fc $\epsilon$ R1 $\alpha$ , clinicians will be able to detect more patients with autoimmune CU, and will be able to pinpoint the precise immune cause.

### **IBT Provides a Full Menu of Testing for Chronic Urticaria**

- CU Index<sup>TM</sup> panel (Test code 403005)  
This panel contains the following assays:
  - CU Index<sup>TM</sup> test (Test #2103)
  - Anti-Thyroglobulin IgG (Test #2005)
  - Anti-Thyroid Peroxidase IgG (Test #322)
  - Thyroid Stimulating Hormone (Test #2004)
- Anti-IgE (Test #2105)
- Anti-Fc $\epsilon$ R1 $\alpha$  - Launching Summer 2009

## References

1. Greaves M. Chronic urticaria. *J Allergy Clin Immunol.* 2000;105(4):664-672.
2. Greaves MW, Tan KT. Chronic urticaria: recent advances. *Clin Rev Allergy Immunol.* 2007;33:134-143.
3. Tong LJ, Balakrishnan G, Kochan JP, et al. Assessment of autoimmunity in patients with chronic urticaria. *J Allergy Clin Immunol.* 1997;99(4):461-465.
4. 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.* 1998; 101:672-676.
5. Puccetti A, Bason C, et al. In chronic idiopathic urticaria autoantibodies against FcεRII/CD23 induce histamine release via eosinophil activation. *Clin Exp Allergy.* 2005;35:1599-1607.
6. Soundararajan S, Kikuchi Y, Joseph K, et al. Functional assessment of pathogenic IgG subclasses in chronic autoimmune urticaria. *J Allergy Clin Immunol.* 2005;115:815-21.
7. Platzer MH, Grattan CEH, Poulsen LK, and 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.* 2005; 60:1152-1156.
8. Schocket AL. Chronic urticaria: pathophysiology and etiology, or the what and why. *Allergy Asthma Proc.* 2006;27:90-95.
9. Malmros H. Auto serum test (AST). *Nordisk Med* 1946;29:150-1.
10. Altrich ML, Halsey JF, and Altman L. Comparison of the in vivo autologous skin test with in vitro diagnostic tests for diagnosis of chronic autoimmune urticaria. *Allergy Asthma Proc.* 2009;30:28.34.
11. Cirillo R, Triggiani M, Siri L et. al. Cyclosporin A rapidly inhibits mediator release from human basophils presumably by interacting with cyclophilin. *J Immunology.* 1990;144:3891-3897.
12. Marone G, Spadaro G, Palumbo C, et al. The anti-IgE/anti-FcεRIα antibody network in allergic and autoimmune diseases. *Clinical and Experimental Allergy.* 1999;29:17-27.
13. 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.* 1993; 328:1599-604.
14. Sabroe RA, Greaves MW. Chronic idiopathic urticaria with functional autoantibodies: 12 years on. *Br. J Dermatol.* 2006;154:813-819.
15. Kaplan AP. Chronic urticaria: pathogenesis and treatment. *J Allergy Clin Imm.* 2004;114:465-74.
16. 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.* 2000; 106:567-72.
17. Altrich ML, Bohn J, and Halsey JF. Presence of IgG Anti-IgE Autoantibodies in Chronic Urticaria and Atopic Dermatitis. *J Allergy Clin Immunol.* 2009;123:S107.
18. Fiebiger E, Hammerschmid F, Stingle G, and Maurer D. Anti FcεRIα Autoantibodies in Autoimmune-mediated Disorders. *J Clin Invest.* 1998;101:243-251.
19. Eckman JA, Hamilton RG, Gober LM, Sterba PM, and Saini SS. Basophil Phenotypes in Chronic Idiopathic Urticaria in Relation to Disease Activity and Autoantibodies. *J Invest Derm.* 2008; 128:1956-1963.
20. Hancock K, Someet Narang S, Pattabhi S, et. al. False positive reactivity of recombinant, diagnostic, glycoproteins produced in High Five™ insect cells: Effect of glycosylation. *J Immunological Methods.* 2008;330:130-136.
21. Leznoff A, Sussman GL. Syndrome of idiopathic chronic urticaria and angioedema with thyroid autoimmunity: a study of 90 patients. *J Allergy Clin Immunol.* 1989;84:66-71.
22. Zauli D, Grassi A, Ballardini G, et al. Thyroid autoimmunity in chronic idiopathic urticaria: implications for therapy. *Am J Clin Dermatol.* 2002;3(8):525-8.



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