

Indirect Immunofluorescence for the Detection of Autoimmune Urticaria

Journal of Cutaneous Medicine and Surgery
2016, Vol. 20(2) 113–117
© The Author(s) 2015
Reprints and permissions:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1023475415610745
jcms.sagepub.com



Bahar Bahrani¹, Natasha T. Gattey¹, and Peter R. Hull¹

Abstract

Background: An autoimmune basis is believed to be responsible for about half of chronic spontaneous urticaria (CSU) cases. The autologous serum skin test is used as a possible indicator, but there is currently no test that directly indicates an autoimmune etiology. In this study, an indirect immunofluorescence was used to identify patients with autoantibodies directed at mast cells.

Methods: Two substrates were used including paraffin embedded sections of skin biopsies from an infant with bullous mastocytosis and cord blood–derived mast cells (CBMC). Sera from 76 patients with CSU were incubated with substrates and conjugated with human IgG.

Results: Using the bullous mastocytosis preparations, positive indirect immunofluorescence was found in 46% (n = 76), while the CBMC substrate was positive in 39% (n = 70).

Conclusion: The IgG autoantibodies directed at mast cells could be detected in about half the patients with CSU. Indirect immunofluorescence should be considered as an indicator of the autoimmune form of CSU.

Keywords

dermatology, immunology, chronic urticaria, autoimmune, indirect immunofluorescence, mast cells, mastocytosis, chronic spontaneous urticaria, autologous serum skin test, IVIG

Introduction

Chronic spontaneous urticaria (CSU) is defined as the presence of continuous or recurrent, pruritic, transient (lasting less than 24 hours) erythematous wheals, which persist for a minimum of 6 weeks with or without angioedema.¹ Excluded are all causes of physical urticaria and those with a known cause.¹ Chronic spontaneous urticaria is equivalent to the previous designation of chronic idiopathic urticaria. It is generally assumed that about half of all cases of CSU have an autoimmune basis.^{2,3} The autologous serum skin test (ASST) is used clinically as a surrogate indicator of the autoimmune form of CSU. The ASST is not a direct measure of autoimmunity because it is an intradermal response to factors present in the serum that can act on cutaneous mast cells, other cells, or the microvasculature of the skin. It is better defined as a test for determining autoreactivity instead of autoimmunity.

The basophil histamine release assay (BHRA) is another test widely used in the diagnosis of autoimmune form of CSU. It indicates the presence of factor(s) in the patient serum, not necessarily autoantibodies, that cause histamine release from the basophils. Konstantinou et al⁴ have proposed the following for the diagnosis of CSU: positive BHRA to establish functionality in vitro, positive ASST to establish autoreactivity in vivo, and positive immunoassay (ELISA or Western blot) detecting IgG autoantibodies against

Fc ϵ RI α and/or anti-IgE to establish antibody specificity.⁴ There have been no published studies using this combination of diagnostic tools.

Direct and indirect immunofluorescence tests are routinely performed for a range of autoimmune skin disorders, but we could find no published studies using either direct or indirect immunofluorescence in evaluating autoimmune urticaria.

The aim of this study was to develop an indirect immunofluorescence method to detect anti–mast cell IgG antibodies in the sera of patients with CSU.

Materials and Methods

Subjects

Seventy-six CSU patients that were seen in the Division of Dermatology are included in the study. The ratio of female to

¹Division of Dermatology, Department of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

Corresponding Author:

Peter R Hull, Division of Clinical Dermatology and Cutaneous Sciences, Department of Medicine, Dalhousie University, Dickson Building, Halifax, NS B3H 1V7, Canada.
Email: peter.hull@dal.ca

male was 63:13. The age and duration of urticaria is given for 71 of these patients. The age range was from 12 to 84, with a mean age of 45. The duration of CSU was between 0.4 and 55 years, with a mean of 9.1 years.

Autologous Serum Skin Test

All antihistamines were discontinued 3 days prior to the testing. None of the patients tested were on immunosuppressive treatment or receiving intravenous immunoglobulin (IVIG). Fifteen ml of blood was drawn into plain sterile glass tubes and allowed to clot. The tubes were centrifuged at 500 g for 15 minutes and serum collected. Using a 30 gauge needle, 5 test sites 3 cm apart were injected with 0.1 ml of the following: normal saline (control), full strength serum, and 25%, 50%, and 75% dilutions with normal saline. Observations were made at 30 minutes. The test was considered to be positive if a >2 mm wheal larger than the saline site was noted. Uniformly this was greatest at the undiluted injection site, and in all cases there was a graduated response with dilution. In patients with associated dermatographism, care was taken to inject slowly to avoid a pressure effect.

The ASST was performed in all patients and was positive in 34 (45%).

Substrate

Two sources of mast cells were used. These included cord blood–derived mast cells (CBMC), generously donated from Dr Marshall's Lab in Dalhousie University, Halifax, Nova Scotia, Canada, as well as paraffin embedded, skin biopsy sections, derived from a patient with bullous mastocytosis.

Indirect immunofluorescence was performed on slides coated with CBMC slides in the same manner, with the exception of a deparaffinization step.

Multiple dilutions of sera were tested including 1:1 to 1:50. A 1:25 dilution was chosen as it resulted in acceptable background fluorescence.

This study was approved by the Biomedical Ethics Board of the University of Saskatchewan. For more on materials and methods, please see supplementary information in online journal.

Results

Mastocytosis Historical Pathology Slides

Indirect immunofluorescence using mastocytosis historical pathology slides (Figures 1 and 2) as a substrate was performed with 76 CSU patient sera (see Table 1 for results). Positive indirect immunofluorescence was observed in 35 (46%) cases and was positive in 17 (50%) of patients who had a positive ASST score ($n = 34$). In patients treated with IVIG, 61% ($n = 21$) had positive immunofluorescence. Eighty percent of patients with a

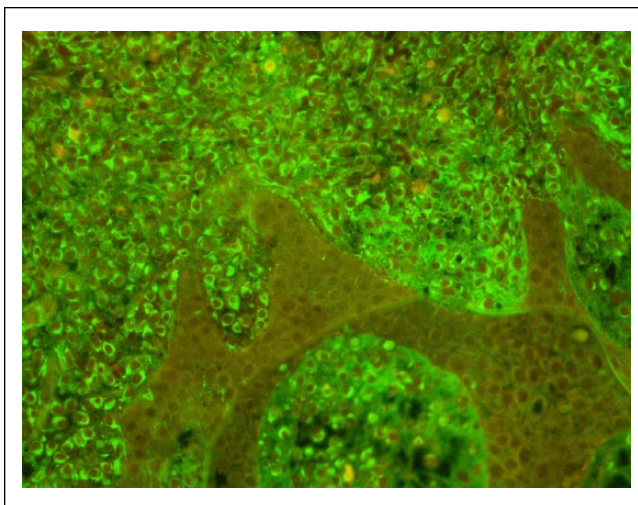


Figure 1. Positive indirect immunofluorescence using mastocytosis slides as a substrate.

clinical history of an associated autoimmune disease had positive indirect immunofluorescence.

CBMC Cytospin Slides

Indirect immunofluorescence with CBMC cytopsin slides as a substrate was performed with 76 CSU patient sera (Figure 3 and 4). The indirect immunofluorescence score for 4 slides were deemed to be indeterminate, and 2 were judged to have excessive background fluorescence. Positive indirect immunofluorescence was observed in 27 (39%, $n = 70$). There was a positive immunofluorescence in 16 (52%) of patients who had a positive ASST score ($n = 31$).

Cohen's kappa statistics was performed to examine the agreement between the results of the indirect immunofluorescence of mastocytosis historical pathology and the CBMC. The kappa coefficient was calculated to be 0.54, with a moderate agreement between the 2 substrates. Control sera from 2 unaffected subjects were negative using both substrates.

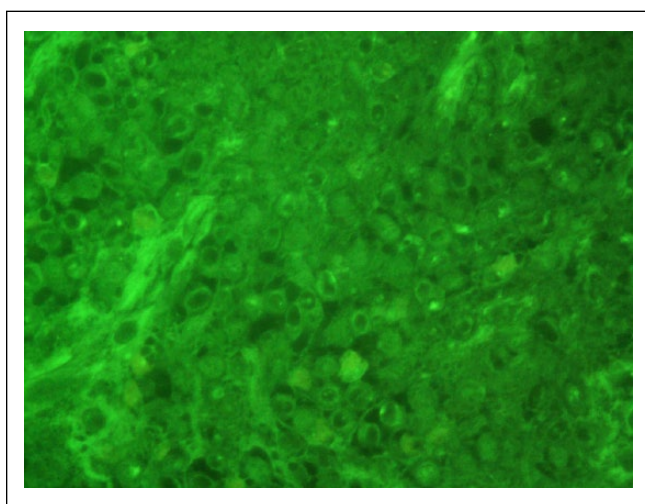
Discussion

An autoimmune etiology is thought to account for 30% to 50% of CSU cases.^{2,3} Leznoff et al⁵ was the first to suggest that there is an autoimmune basis for CSU based on the association between CSU and thyroid autoimmune disease, as 22 (12%) of the 182 patients he examined had anti-thyroid antibodies. Anti-IgE antibodies in sera of CSU patients were shown by Gruber et al⁶ to be involved in the pathogenesis of CSU. Grattan et al⁷ then revealed the presence of anti-IgE antibodies with histamine-releasing properties using a BHRA. By 1993, functional antibodies against the IgE receptor (Fc ϵ RI) were reported using antibody neutralization assays.⁸ Functional IgG autoantibodies are present

Table 1. Results.

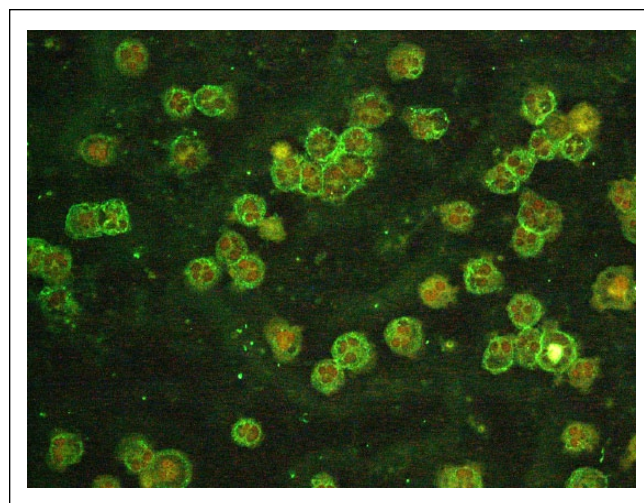
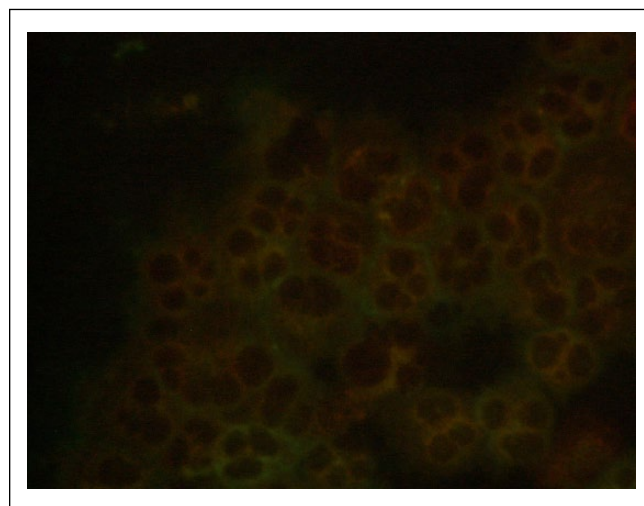
Substrate	No IVIG, %	IVIG, %
Mastocytosis slides		
AB+ and ASST+	16.36	61.9
AB+ and ASST–	30.91	0
AB– and ASST–	43.64	38.1
AB– and ASST+	9.09	0
CBMC		
AB+ and ASST+	18.37	65.00
AB+ and ASST–	26.53	35.00
AB– and ASST–	46.94	0
AB– and ASST+	8.16	0

Abbreviations: AB, antibody; ASST, autologous serum skin test; CBMC, cord blood–derived mast cells; IVIG, intravenous immunoglobulin.

**Figure 2.** Negative indirect immunofluorescence using mastocytosis slides as a substrate.

with affinity to the α -chain of Fc ϵ RI in 35% to 40% and to IgE in 5% to 10% of cases.^{6–8} Sabroe et al⁹ classified 78 CSU patients into 5 subtypes: 26% with autoantibodies against anti-Fc ϵ RI α with histamine releasing properties, 15% with autoantibodies against anti-Fc ϵ RI α without histamine releasing properties, 9% autoantibodies with anti-IgE properties, 9% histamine releasing factors specific for mast cells, and 41% with no identifiable factor.⁹

The ASST, an in vivo test involving injecting patient serum into the dermis of the skin, was first described in 1986 by Grattan et al.¹⁰ It is reported to be positive in 35% to 50% of patients.^{2,3,11} A positive ASST validates the presence of circulating factor(s) in the serum that are responsible for CSU. However, a positive ASST has also been found in allergic rhinitis and in healthy controls.¹² ASST is better defined as a test for determining autoreactivity instead of autoimmunity. Studies have suggested that positive ASST patients have higher disease activity, increased systemic symptoms, and require higher amounts of antihistamines.^{13–15} However,

**Figure 3.** Positive immunofluorescence using cord blood–derived mast cells as the substrate.**Figure 4.** Negative immunofluorescence using cord blood–derived mast cells as the substrate.

other studies have found no correlation with disease severity and quality of life between ASST positive and negative patients.^{16,17}

The BHRA and basophil activation test have been used in the diagnosis of autoimmune form of CSU. The BHRA measures the amount of histamine released from activated basophils, while the basophil activation test relies on the expression of CD63 or CD203c basophil surface activation markers.^{18–20} These tests, on their own, indicate the presence of serum factors responsible for the activation and degranulation of basophils. Other weaknesses of the test include variability among basophil donors and limitations in access to such assays, as they are not routinely available.

Sabroe et al²¹ examined ASST sensitivity and specificity of CSU patients using BHRA as the gold standard. They

calculated the sensitivity to be 65% and 71% and specificity of 81% and 78% for 2 patient populations.²¹

If, instead, the indirect immunofluorescence is used as a measure of an autoimmune urticaria, the ASST has a sensitivity of 34.62% (95% CI, 17.25%-55.66%) and a specificity of 82.76% (95% CI, 64.21%-94.09%). The ASST has low sensitivity but a reasonably high specificity in the prediction of mast cells avid IgG antibodies. This suggests that mast cell binding antibodies may occur in patients that are ASST negative.

Functionality

If the ASST is used instead as an indicator of functional autoantibodies capable of causing mast cell degranulation and the production of a wheal, then the indirect immunofluorescence method, using the mastocytosis slide substrate, suggests that in about half the cases these autoantibodies were functional. Intravenous immunoglobulin was the treatment of choice in patients showing a positive ASST and having severe, incapacitating urticaria, not responsive to high dose antihistamines. In this group, the percentage of patients with both a positive ASST and positive indirect immunofluorescence (mast cell substrate) was 62%. The presence of antibodies against Fc ϵ RI α in IVIG was reported by Horn et al.²¹ IVIG may be interfering with the indirect immunofluorescence scores by contributing to the percentage of nonfunctional anti-mast cell autoantibodies.

In the less severe patients not receiving IVIG (n = 21) and with a positive ASST, the indirect immunofluorescence was positive in only 16%.

Conclusion

As in other autoimmune skin diseases, we have demonstrated that it is possible to detect anti-mast cell IgG antibodies by indirect immunofluorescence. However, we have not established the target on the mast cell. We presume in most cases it is the high affinity IgE receptor. In addition, although we have suggested the use of the ASST as an indicator of functionality of the detected autoantibodies, future studies need to combine indirect immunofluorescence with other methods such as the basophil activation test to confirm this. Future studies should also be conducted prospectively to avoid the possible confounding influence of treatment.

Acknowledgments

We acknowledge the University of Saskatchewan, College of Medicine for funding this project. Sincere thanks to Dr Jean Marshall's lab in Dalhousie University for cord blood-derived mast cells cytospin slides.

Authors' Note

Presented at the 2014 Canadian Dermatology Association, Toronto, Ontario.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Dean's Project funding, College of Medicine, University of Saskatchewan.

References

1. Maurer M, Bindslev-Jensen C, Gimenez-Arnau A, et al. Chronic idiopathic urticaria (CIU) is no longer idiopathic: time for an update. *Br J Dermatol*. 2013;168:455-456.
2. Ferrer M, Kinet J, Kaplan A. Comparative studies of functional and binding assays for IgG anti-Fc ϵ RI α (-subunit) in chronic urticaria. *J Allergy Clin Immunol*. 1998;101:672-676.
3. Tong L, Balakrishnan G, Kochan J, et al. Assessment of autoimmunity in patients with chronic urticaria. *J Clin Immunol*. 1997;99:461-465.
4. Konstantinou GN, Asero R, Ferrer M, et al. EAACI taskforce position paper: evidence for autoimmune urticaria and proposal for defining diagnostic criteria. *Allergy*. 2013;68:27-36.
5. Leznoff A, Josse RG, Denburg J, et al. Association of chronic urticaria and angioedema with thyroid autoimmunity. *Arch Dermatol*. 1983;119:636-640.
6. Gruber BL, Baeza ML, Marchese MJ, et al. Prevalence and functional role of anti-IgE autoantibodies in urticarial syndromes. *J Invest Dermatol*. 1988;90:213-217.
7. Gratten C, Francis DM, Hide M, et al. Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy*. 1991;21:695-704.
8. Hide M, Francis DM, Gratten CE, et al. Autoantibodies against the high-affinity IgE receptor as a cause of histamine release in chronic urticaria. *N Engl J Med*. 1993;328:1599-1604.
9. Sabroe RA, Fiebiger E, Francis DM, et al. Classification of anti-Fc ϵ RI and anti-IgE autoantibodies in chronic idiopathic urticaria and correlation with disease severity. *J Allergy Clin Immunol*. 2002;110:492-499.
10. Gratten C, Wallington T, Warin R, et al. A serological mediator in chronic idiopathic urticaria—a clinical, immunological and histological evaluation. *Br J Dermatol*. 1986;114:583-590.
11. Fiebiger E, Maurer D, Holub H, et al. Serum IgG autoantibodies directed against the alpha chain of Fc epsilon RI—a selective marker and pathogenetic factor for a distinct subset of chronic urticaria patients? *J Clin Invest*. 1995;96:2606-2612.
12. Guttman-Yassky E, Bergman R, Maor C, et al. The autologous serum skin test in a cohort of chronic idiopathic urticaria patients compared to respiratory allergy patients and healthy individuals. *J Eur Acad Dermatol Venereol*. 2007;21:35-39.
13. Sabroe RA, Seed PT, Francis DM, et al. Chronic idiopathic urticaria: comparison of the clinical features of patients with and without anti-Fc ϵ RI or anti-IgE autoantibodies. *J Am Acad Dermatol*. 1999;40:443-450.
14. Caproni M, Volpi W, Giomi B, et al. Chronic idiopathic and chronic autoimmune urticaria: clinical and immunopathological features of 68 subjects. *Acta Derm Venereol*. 2004;84:288-290.
15. Staubach P, Onnen K, Vonend A, et al. Autologous whole blood injections to patients with chronic urticaria and a

- positive autologous serum skin test: a placebo-controlled trial. *Dermatology*. 2006;212:150-159.
16. Kulthanan K, Jiamton S, Gorvanich T, et al. Autologous serum skin test in chronic idiopathic urticaria: prevalence, correlation and clinical implications. *Asian Pac J Allergy Immunol*. 2006;24:201-206.
 17. Nettis E, Dambra P, D'Oronzio L, et al. Reactivity to autologous serum skin test and clinical features in chronic idiopathic urticaria. *Clin Exp Dermatol*. 2002;27:29-31.
 18. Yasnowsky KM, Dreskin SC, Efaw B, et al. Chronic urticaria sera increase basophil CD203c expression. *J Allergy Clin Immunol*. 2006;117:1430-1434.
 19. 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*. 2000;105:552-560.
 20. De Swerdt A, Van Den Keybus C, Kasran A, et al. Detection of basophil-activating IgG autoantibodies in chronic idiopathic urticaria by induction of CD 63. *J Allergy Clin Immunol*. 2005;116:662-667.
 21. Sabroe RA, Grattan CE, Francis DM, et al. The autologous serum skin test- a screening test for autoantibodies in chronic idiopathic urticaria. *Br J Dermatol*. 1999;140:446-452.

English Language Editing

JCMS is an English language journal and submissions must be presented in a style and grammar consistent with the highest standards of English publications. Although work described in a manuscript may be relevant and appropriate for consideration in our journal, if the manuscript suffers from insufficient English editing, it will be returned to the authors with the suggestion to first seek assistance either from a colleague whose first language is English or from a professional language service. SAGE Publications also offers resources in this area; please visit <http://languageservices.sagepub.com/en/> for more information.

Copyright of Journal of Cutaneous Medicine & Surgery is the property of Decker Publishing and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.