Independent Evaluation of a Commercial Test for “Autoimmune” Urticaria in Normal and Chronic Urticaria Subjects


TO THE EDITOR

Although the pathogenesis of the majority of chronic idiopathic urticaria (CIU) patients is unknown, approximately 40% of patients are proposed to have “functional” IgG autoantibodies against either the high-affinity IgE receptor (FcεRIa) or IgE as measured by the basophil histamine-releasing activity (HRA) assay (Ferrer and Kaplan, 2007). This assay involves incubating basophils from a healthy donor with a CIU patient’s serum and measuring histamine release. A “positive” result is often judged in relation to basophil HRA levels obtained with serum from a non-CIU population. Some investigators have proposed that the presence of “functional” autoantibodies offers a pathogenic explanation for patients’ symptoms or provides a rationale for treatment with immunomodulatory therapy such as cyclosporine (Sabroe and Greaves, 2006). However, the HRA assay, which is considered the “gold standard” (Ferrer and Kaplan, 2007) for “functional” autoantibodies, has limitations. Its performance depends on the unique characteristics of the healthy basophil donors (Eckman et al., 2008). In addition, interlaboratory reproducibility of this test has not been possible to assess because of a lack of universally available standardized reagents.

An alternative line of investigation has shown that basophils from active CIU subjects manifest a suppressed IgE receptor-mediated histamine degranulation (Ferrer and Kaplan, 2007). We have stratified CIU subjects into responder (CIU R) and nonresponder (CIU NR) functional phenotypes based on the profile of ex vivo activation of their basophils by an optimal concentration (0.1 μgml⁻¹) of polyclonal anti-IgE (Vonakis et al., 2007). Subjects with <10% histamine release with anti-IgE stimulation were classified as CIU NR, whereas subjects with histamine release ≥10% were classified as CIU R. This basophil classification remains consistent over the course of active disease (Eckman et al., 2008). Interestingly, increased basophil IgE-receptor-mediated histamine release occurs in both groups as subjects enter disease remission (Eckman et al., 2008). Using a previously identified immunoenzymometric assay (IEMA), we observed a similar prevalence and concentration of IEMA-detected autoantibodies in the CIU R, CIU NR, and non-CIU subject groups (Eckman et al., 2008). We have previously reported that positive HRA presence occurs at a similar frequency in CIU R, CIU NR, and nonatopic, healthy (normal) subjects (Vonakis et al., 2007). The positive HRA results we obtained in the non-CIU subjects have been challenged (Kaplan and Joseph, 2007).

The purpose of this study was to assess HRA activity in sera from CIU and non-CIU subjects by the CU Index test by IBT Laboratories (Lenexa, KS). Further, we examined the relationship of HRA activity to previously determined CIU basophil classification and the presence of IEMA-detected autoantibodies.

Following consent, whole-blood was collected for basophil and serology studies from subjects with a physician-determined diagnosis of CIU (n = 21) or non-CIU controls (n = 22; ages 18–65, 12 female, 10 male, 9 atopic, 13 nonatopic by history). None of the non-CIU subjects had any known autoimmune disease. Protocols were approved by the Johns Hopkins Institutional Review Board and the Western Institutional Review Board and were in adherence to the Declaration of Helsinki Principles (Eckman et al., 2008). Basophil functional phenotyping studies were performed as described...
CU Index values were compared to subject classification (see Figure 1). Positive CU Index values were seen in both CIU and non-CIU subjects, and therefore, positive HRA presence is not exclusively observed in CIU subjects. The CU Index values were higher in the CIU group (median 17.6; \( n = 21 \)) as compared to the non-CIU group (median 6.2; \( n = 22 \), \( P = 0.03 \), Mann-Whitney test). Moreover, CIU subjects (57%) were significantly more likely to have a positive HRA test than non-CIU subjects (23%; \( P = 0.03 \), Fisher’s exact test). In CIU subjects, the presence of IEMA-detected IgG anti-FcεRIz and/or anti-IgE (see Figure 1a; closed symbols) was not associated with a positive CU Index (\( P = 0.33 \); Fisher’s exact test). Among CIU basophil phenotype subsets, CIU-unclassified subjects (median 17.6; \( n = 7 \)) had significantly higher CU Index values than the CIU R group (median 6.2; \( n = 6 \); \( P = 0.01 \), Mann-Whitney test). The CIU NR subjects’ CU Index values were not significantly different from the levels in the other CIU groups. Between the non-CIU populations; nonatopic, non-CIU subjects (\( n = 13 \)) were more likely to have a positive test than atopic, non-CIU subjects (\( n = 9 \); \( P = 0.05 \), Fisher’s exact test).

The significantly higher frequency and magnitude of CU Index values in the CIU group as compared to non-CIU group support the general hypothesis that serological factors in CIU subjects affect donor basophils differently than non-CIU subjects. However, these data raise questions about the clinical utility of HRA testing in the diagnosis and management of “autoimmune” CIU. The existence of strongly positive HRA levels in nonatopic, non-CIU patients is similar to what we observed in our previous studies (Vonakis et al., 2007), and the reasons for this are not currently understood. In addition, there was no relationship between IEMA-detected autoantibodies and a positive CU Index. This lack of diagnostic specificity of the CU Index, and thus HRA, raises concerns about its general usefulness in providing clarity on the disease’s pathogenesis to CIU patients. It may be argued that this lack of specificity may be due to the improper selection of the basophil donor for the HRA assay. It is not clear from the literature how many non-CIU subjects should be screened for low histamine release in choosing the correct basophil donor. However, we assumed that the basophil donor was optimized for this commercial assay. Because of the lack of consensus on the laboratory protocols for HRA, and thus the definition of “chronic autoimmune urticaria”, it cannot be said definitively that the IBT assay used in this report makes the distinction between autoimmune and idiopathic urticaria. In addition, the IBT assay that did not establish serological factors in CIU patients affect donor basophils differently than non-CIU subjects. However, these data raise questions about the clinical utility of HRA testing in the diagnosis and management of “autoimmune” CIU. The existence of strongly positive HRA levels in nonatopic, non-CIU patients is similar to what we observed in our previous studies (Vonakis et al., 2007), and the reasons for this are not currently understood. In addition, there was no relationship between IEMA-detected autoantibodies and a positive CU Index. This lack of diagnostic specificity of the CU Index, and thus HRA, raises concerns about its general usefulness in providing clarity on the disease’s pathogenesis to CIU patients. It may be argued that this lack of specificity may be due to the improper selection of the basophil donor for the HRA assay. It is not clear from the literature how many non-CIU subjects should be screened for low histamine release in choosing the correct basophil donor. However, we assumed that the basophil donor was optimized for this commercial assay. Because of the lack of consensus on the laboratory protocols for HRA, and thus the definition of “chronic autoimmune urticaria”, it cannot be said definitively that the IBT assay used in this report makes the distinction between autoimmune and idiopathic urticaria. In addition, the IBT assay that did not establish serological factors in CIU patients affect donor basophils differently than non-CIU subjects. However, these data raise questions about the clinical utility of HRA testing in the diagnosis and management of “autoimmune” CIU. The existence of strongly positive HRA levels in nonatopic, non-CIU patients is similar to what we observed in our previous studies (Vonakis et al., 2007), and the reasons for this are not currently understood. In addition, there was no relationship between IEMA-detected autoantibodies and a positive CU Index. This lack of diagnostic specificity of the CU Index, and thus HRA, raises concerns about its general usefulness in providing clarity on the disease’s pathogenesis to CIU patients. It may be argued that this lack of specificity may be due to the improper selection of the basophil donor for the HRA assay. It is not clear from the literature how many non-CIU subjects should be screened for low histamine release in choosing the correct basophil donor. However, we assumed that the basophil donor was optimized for this commercial assay. Because of the lack of consensus on the laboratory protocols for HRA, and thus the definition of “chronic autoimmune urticaria”, it cannot be said definitively that the IBT assay used in this report makes the distinction between autoimmune and idiopathic urticaria. In addition, the IBT assay that did not establish a positive result was because of the IgG fraction of serum. The method for defining a positive value is different...
from many other descriptions of the HRA assay. A lack of specificity also has been seen with the autologous serum skin test, which is another measure of “functional” autoantibodies in CIU. The autologous serum skin test is positive in up to 37% of non-CIU subjects (Guttman-Yassky et al., 2007).

On a limited basis, HRA-positive CIU subjects have been reported to have more severe disease (Sabroe et al., 2002) and to be more likely to have thyroid autoantibodies (Kikuchi et al., 2003) than HRA-negative subjects. However, skin biopsies show similar pathology in HRA-positive versus HRA-negative subjects with CIU (Sabroe et al., 1999; Ying et al., 2002). More importantly, in making decisions about starting immunomodulatory medication such as cyclosporine in CIU patients, HRA measurement is not clearly needed, because the presence of “functional” autoantibodies is not correlated to clinical response (Morgan and Khan, 2008).

At the present time, available assays for autoimmunity are flawed and do not consistently assist clinicians in their understanding of CIU’s pathogenesis. What is truly needed is a reproducible assay to advance the specific definition of autoimmune urticaria.

**CONFLICT OF INTEREST**
The authors state no conflict of interest.

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**TO THE EDITOR**
Basal cell carcinoma (BCC) is the most common cancer in humans. Very often patients are prone to develop multiple lesions at different body sites (Rama-chandran et al., 1999, 2001; and cited therein; Heitzer et al., 2007). Recently, several studies have been performed to determine the clonal origin of BCC in patients with multiple lesions (Walsh et al., 1998; Saldanha et al., 2002; Shulman et al., 2006). For instance, Shulman et al. (2006) performed loss of heterozygosity (LOH) analysis and X-chromosome inactivation studies to determine the clonal origin of multiple BCCs from patients exhibiting lesions at different anatomical locations. They reported that in all BCCs displaying LOH, the same allele was lost in multiple tumors from a given patient. Furthermore, an identical X-chromosome inactivation pattern was noted in certain tumors. Hence, they suggested that the majority of BCCs in an individual might originate from one single tumor cell clone, independent of the anatomical site and time of lesion occurrence. However, van Steensel and Frank (2006) questioned the theory of monoclonality of multiple BCCs. They stated that it is important to sequence the PTCH gene to prove monoclonality of multiple BCCs because PTCH mutations are a hallmark of BCCs. If identical PTCH mutations are present in distinct BCCs at different anatomical locations from individual patients with multiple lesions, it would indicate monoclonality. If not, then it would suggest that each tumor arises independently from a distinct initiated (progenitor) cell.