absent circulating basophils identified previously (Grattan et al., 1997). It is therefore of interest that the mean level of anti-FcεRIα detected by immunoenzymatic assay was substantially and significantly higher than other chronic urticaria patients with enough circulating basophils to assess responsiveness to anti-IgE. This would support the possibility that the level of autoantibody may be a factor in determining functionality. Testing chronic urticaria basophil responsiveness to a monoclonal anti-FcεRIα in addition to anti-IgE would seem appropriate in the context of defining basophil phenotypes in a disease that is believed to be caused by both. Debate about the importance of autoantibodies in urticaria pathogenesis will no doubt continue but it is important not to be side-lined from the autoimmune hypothesis on the strength of work that only shows part of a much wider portfolio of clinical and laboratory evidence supporting the concept.

Clive E.H. Grattan¹ and Elena Borzova¹
¹Department of Dermatology, Norfolk & Norwich University Hospital, Norwich, UK E-mail: clive.grattan@nnuh.nhs.uk

REFERENCES


Response to Grattan et al.


TO THE EDITOR

We appreciate the comments by Dr Grattan and Dr Brozova to our article (Eckman et al., 2008) concerning blood basophil phenotypes and their relationship to autoantibodies in patients with chronic idiopathic urticaria (CIU). However, we would like to respond to their comments.

Grattan stated, “Unfortunately the histamine releasing activity of sera was not assessed by Eckman et al…” We have three main concerns with histamine-releasing activity (HRA). First, the reliance on a normal basophil donor is a major concern given the literature’s demonstration of significant differences in HRA results depending on who is chosen as the basophil donor (Grattan et al., 1999; Kikuchi and Kaplan, 2001). Second, the reagents and methods are not standardized, and they differ with regard to the basophil isolation method, the ratio of CIU sera to donor basophils, the use of IL-3 as a basophil enhancer, and the interpretation and variability (poor replication) of a positive results (Vonakis and Saini, 2005). Furthermore, verification of HRA’s specificity for autoantibodies by preincubating a CIU patients sera with soluble FcεRIα and observing a reduction in HRA are sparse and shown for only selected datasets. HRA’s specificity is shown by preincubating the IgG fraction of CIU patients sera with soluble FcεRIα and then showing a subsequent reduction in HRA in comparison to a sham control. Hid et al. only show this inhibition in 4/17 patients, Kikuchi and Kaplan only show inhibition in 7/111 patients, and Fiebiger et al. in 9/50 patients (complete sera; IgG fraction not isolated; Hide et al., 1993; Fiebiger et al., 1998; Kikuchi and Kaplan, 2001). The inhibition profiles in these studies are significantly different. In addition, Fiebiger showed 9 HRA-negative/
imunoenzymetric assay-positive subjects had enhancement (rather than inhibition) in HRA when sera were preincubated with soluble FcRRI (Fiebiger et al., 1998). Therefore, this test’s ability to reproducibly and specifically detect functional autoantibodies (versus another serological factor) is limited in our opinion. In addition, if “local tissue concentrations of autoantibody rather than circulating plasma concentrations may determine whether sufficient receptors can be cross-linked or not to initiate degranulation”, then serum HRA is flawed in that it reflects serum concentrations of a factor. Therefore, we focused on a reproducible immunoenzymetric assay to detect autoantibodies.

Grattan questions our use of the term “idiopathic” urticaria. The term “autoimmune” remains dependent on the use of the highly variable HRA assay as described above. Until tests to more accurately define autoimmunity are developed, we feel compelled to use the term “idiopathic”.

Grattan stated, “The key to understanding the etiology of urticaria is what stimulates degranulation of the cutaneous mast cells, rather than circulating basophils...” We do not argue that the cutaneous mast cell is central to the pathology in CIU disease. However, a role for blood basophils in CIU is supported by a number of findings, some of which are noted by Grattan in previous publications. Blood basopenia in active CIU has been described for decades (Grattan, 2001). Blood basophilia is inversely correlated with urticarial disease activity (Grattan et al., 2003). In CIU lesional sites, intradermal basophils are also increased compared to healthy, nonatopic control patients (Ying et al., 2002). In CIU, short-term use of corticosteroids is effective in controlling flares of CIU (Sabroe and Greaves, 2006), yet it does not affect skin mast cell histamine release (Schwiebert et al., 1996). In addition, basophil numbers increase after treatment with oral corticosteroids whereas they decrease in non-CIU subjects; this finding suggests an impairment of basophil recruitment to the skin (Grattan et al., 2003). In summary, as noted by Grattan and in his words, these findings support the idea that basophils “may contribute directly to the pathogenesis of urticarial wheals in “idiopathic” urticaria” (Grattan, 2001).

Our approach to understanding the role that a particular “factor” plays in the etiology of CIU is to evaluate differences in the presence, level or structure of the “factor” or its target during active disease as compared to disease remission. In active CIU disease, suppressed IgE receptor-mediated histamine degranulation by blood basophils has been observed by several investigators (Greaves et al., 1974; Kern and Lichtenstein, 1976; Sabroe et al., 1998; Luquin et al., 2005; Vonakis et al., 2007). In our current study, we found a significant increase in CIU patients basophils’ IgE receptor-mediated degranulation mediated by a polyclonal anti-IgE in 6 out of 6 patients in CIU disease remission (Figure 4a, Eckman et al., 2008), and since publication, we have observed another CIU patient enter remission with a similar dramatic rise in basophil IgE receptor-mediated degranulation (0-56%). Likewise, Kern and Lichtenstein (1976) showed similar changes in 5 out of 5 patients. Grattan suggests that testing basophil responsiveness to a monoclonal anti-FcRRI in addition to anti-IgE should be performed; however, previous studies have shown that in CIU patients, basophil receptor-mediated degranulation to anti-IgE is similar to anti-FcRRI (Sabroe et al., 1998). As only ~40% of patients with CIU are thought to have “functional” serum autoantibodies (IgG anti-FcRRI and/or anti-IgE; Ferrer and Kaplan, 2007), it is highly unlikely that all 12 patients (6 from Eckman et al., 1 additional from our ongoing experience, and 5 from Kern and Lichtenstein) with suppressed basophil degranulation possessed functional autoantibodies, yet all 12 patients showed increases in their basophil histamine release profile during disease remission. This observation may not only indicate a disease related suppressive serological factor beyond autoantibodies, but also suggests that basophil function is tied to CIU disease severity and is independent of autoantibody status or presence. We would like to briefly reply to several other criticisms of our paper. Grattan suggested that “complement”, “subclass of IgG”, and “conditional autoimmunity” are “additional factors” that may make the autoantibodies functional. Although a role for complement dependence for serum HRA is provided by in vitro studies, it has yet to be firmly established in vivo (Fiebiger et al., 1998; Kikuchi and Kaplan, 2002). Fiebiger et al. (1998) examined the IgG subclasses of detected IgG anti-FcRII. The IgG1 and/or IgG3 subclasses were the more prevalent subclasses of IgG anti-FcRII detected in the serum of CIU subjects (91% of IgG anti-FcRII) as compared to subjects with other skin diseases (44%; Fiebiger et al., 1998). However, the relationship is far from perfect and is likely only a partial explanation for the presence of “non-functioning” autoantibodies in non-CIU subjects. As mentioned, the accurate quantification of the subclass distribution of IgG autoantibodies is an arduous task because of the lack of subclass-specific standards for the antibodies of interest and different binding constants for the different subclass detection antibodies (Hamilton, 1987). Conditional autoimmunity is a theory that states the functionality of autoantibodies depends on the receptor occupancy by IgE under the control of local environmental influences (Miescher et al., 2001). This theory lacks direct experimental evidence in CIU.

In summary, we have shown for the first time that basophil phenotypes among CIU patients (CIU R and CIU NR) appear independent of immunoenzymetric assay measured IgG anti-FcRRI and IgG anti-IgE antibodies. In addition, these phenotypes are stable during active disease. Finally, similar to the finding of Kern and Lichtenstein, we found an enhancement in IgE-receptor mediated degranulation in disease remission. Collectively, the data support a direct role of basophils in contributing to CIU’s pathogenesis. Greater insight into CIU may be gained from future studies examining basophils/mast cells or serological factors as they relate to active disease and again in remission. At the present time, available assays for autoimmunity are flawed and do not
AM Nelson et al.
Temporal Changes in Gene Expression

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 declare no conflict of interest.

John A. Eckman1, Robert G. Hamilton1 and Sarbjit S. Saini1

1Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
E-mail: jeckman1@jhmi.edu

REFERENCES

Ferrer M, Kaplan AP (2007) Chronic urticaria: what is new, where are we headed. Allergol Immunopathol (Madri) 35:57-61

Luquin E, Kaplan AP, Ferrer M (2005) Increased responsiveness of basophils of patients with chronic urticaria to sera but hypo-responsiveness to other stimuli. Clin Exp Allergy 35:456-60

Isotretinoin Temporally Regulates Distinct Sets of Genes in Patient Skin


TO THE EDITOR

The goal of this study is to gain insight into putative pathways by which 13-cis retinoic acid (13-cisRA) improves acne by comparing the temporal changes in gene expression in the skin of acne patients. Gene array analysis and immunohistochemistry were performed on skin biopsies from patients at baseline and after 8 weeks of isotretinoin therapy and compared to data obtained at 1 week (Nelson et al., 2008).

As 13-cisRA drastically decreases sebaceous gland size after 16 weeks (Goldstein et al., 1982); we chose an 8-week time point to examine changes in skin histology and gene expression. All protocols were approved by the Institutional Review Board of The Pennsylvania State University College of Medicine and were conducted according to the principles outlined in the Declaration of Helsinki. Eight patients who were prescribed isotretinoin for their severe acne enrolled in the study after giving informed consent and 5-mm punch biopsies of uninvolved skin were taken from their upper backs at baseline and 8 weeks of treatment. Demographic data are presented in Figure 1a. After 8 weeks, sebaceous gland size was reduced by 76% (4.17-fold) compared to baseline (P=0.009) (Figure 1b and d). At 1 week, glands were decreased by approximately 49% (not significant; Figure 1c and d).

Gene array expression analysis was performed on biopsies at baseline and after 8 weeks of therapy. Using a false discovery rate of 0.05 corresponding to