

Suppression of Basophil FcεRI Activation by Serum from Active Chronic Idiopathic/Spontaneous Urticaria (CIU/CSU) Subjects

Journal of Investigative Dermatology (2015) **135**, 1454–1456; doi:10.1038/jid.2015.13; published online 5 February 2015

TO THE EDITOR

Chronic idiopathic/spontaneous urticaria (CIU/CSU), which affects ~1% of the United States population, is defined as recurrent hives for >6 weeks (Kaplan and Greaves, 2009). Active CIU/CSU patients uniquely display suppressed basophil FcεRI-mediated histamine release (BHR) (Saini, 2014). This is a feature that improves in disease remission (Kern and Lichtenstein, 1976; Eckman *et al.*, 2008; Oliver *et al.*, 2015). The pathways leading to basophil FcεRI suppression in CIU/CSU are unclear. There is evidence that IgE has a role in urticaria. For example, injection of cold urticaria patients' serum into a healthy subject's skin transfers the sensitivity to the cold stimulus (Gruber *et al.*, 1988). Clinical trials with monoclonal anti-IgE (omalizumab) in subjects with CIU/CSU have shown rapid symptom relief, which implicates IgE in the disease pathway (Gober *et al.*, 2008; Saini *et al.*, 2011; Maurer *et al.*, 2013). Furthermore, the autoimmune theory of CIU/CSU pathogenesis proposes that serum IgG autoantibodies specific to IgE or the IgE receptor directly activate skin mast cells and basophils in a subset of CIU/CSU subjects (Kaplan and Greaves, 2009). In this study, we examined the ability of active CIU/CSU patients' serum to transfer FcεRI-mediated BHR suppression to healthy basophils and further test the contributions of IgE, IgG, and complement in the observed basophil mediator suppression.

Healthy adults ($n=8$, 25% female) had repeated blood collections using

an institutional review board approved protocol. CIU/CSU sera were collected as part of a longitudinal study (Baker *et al.*, 2008). Written informed consent was obtained from all subjects, whether for blood or for sera. Remission sera were collected from CIU/CSU patients with no skin symptoms or medication use for ≥ 2 months (Baker *et al.*, 2008). For CIU/CSU patients using omalizumab, sera collection occurred before and after the patient had been on therapy for 1 to 4 months with marked clinical improvement (Gober *et al.*, 2008). All serum samples were stored at -20°C .

Basophils were purified from venous blood using percoll density gradients (GE Healthcare, Uppsala, Sweden) that were followed by negative selection as described (Vonakis *et al.*, 2007). Purified basophils ($>200,000$ basophils per condition) were cultured overnight (37°C , 18–22 h, 5% CO_2) in Iscove's Modified Dulbecco's Media media (Gibco, New York, NY) $\pm 15\%$ serum v/v (chosen because 15% does not induce BHR; Vasagar *et al.*, 2006). Each culture experiment utilized a single individual's serum (CIU/CSU or autologous). IL-3 ($0.1\text{--}100\text{ ng ml}^{-1}$; R&D Systems, Minneapolis, MN) was added for basophil survival, with no difference observed in results based on the concentration of IL-3. Post-culture analysis of the histamine content of media supernatants and total basophil histamine content revealed no differences between conditions.

After culture, cells were collected, stimulated with polyclonal goat

anti-human IgE ($0.01\text{--}1\text{ }\mu\text{g ml}^{-1}$) or formylmethionine-leucyl-phenylalanine (fMLP-N, 10^{-6} M ; Sigma-Aldrich, Missouri, St Louis, MO) at $>10,000$ basophils per stimulation, and assayed for histamine by fluorimetry (Vonakis *et al.*, 2007). In select experiments, CIU/CSU serum was tested as follows: (i) paired sera from patients before and after remission, (ii) serum depleted of IgE by overnight incubation with omalizumab covalently coupled to Sepharose-CL-4B as previously validated to achieve a 98% IgE depletion rate (Eckman *et al.*, 2008), (iii) serum heat inactivated at 56°C for 30 min to denature IgE and inactivate complement (Ishizaka *et al.*, 1986), and (iv) serum IgG depleted by adsorption with Staphylococcal Protein G Sepharose 4B-CL (Fu *et al.*, 2007; $>93\%$ depletion).

All statistics were performed with Prism 4.0 using the Wilcoxon signed-rank test between the two groups being compared at the same concentration of stimulant. Error bars in the graphs indicate the standard error of the mean (SEM).

Healthy donor basophils cultured in active CIU/CSU subjects' serum exhibited marked suppression of FcεRI-mediated BHR when compared with media (Figure 1a) or media with autologous serum (Supplementary Figure S1a online). In contrast, basophils cultured in media or autologous serum showed no significant difference in subsequent BHR (Supplementary Figure S1b online). Further, fMLP-N-induced BHR did not differ between the media versus CIU/CSU serum or autologous serum versus CIU/CSU serum cultures (data not shown).

In remission, CIU/CSU patients show an increase in their basophil HR profile (Eckman *et al.*, 2008). Therefore, we compared the effect of serum culture

Abbreviations: CIU/CSU, chronic idiopathic/spontaneous urticaria; BHR, basophil histamine release; fMLP-N, formylmethionine-leucyl-phenylalanine

Accepted article preview online 3 February 2015; published online 5 February 2015

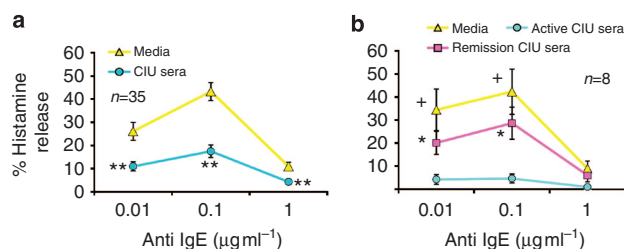


Figure 1. Normal basophils cultured in individual chronic idiopathic/spontaneous urticaria (CIU/CSU) serum show a suppressed basophil histamine release (BHR) profile that reverses in disease remission serum. Purified basophils from healthy donors were cultured overnight in media or CIU/CSU serum and assessed for FcεRI-mediated BHR. (a) CIU/CSU serum-cultured basophils showed significant BHR suppression as compared with parallel media-cultured basophils ($n=35$; $**P<0.0001$ to matched control). (b) Basophils cultured in CIU/CSU patients' serum obtained during active disease showed marked BHR suppression relative to culture in the same subject's serum during remission ($n=8$). No statistical differences were observed between the levels of BHR from cells cultured in serum from CIU/CSU patients in remission or media alone. All media versus active serum statistics were $+P<0.05$, whereas all remission versus active serum statistics were $*P<0.01$.

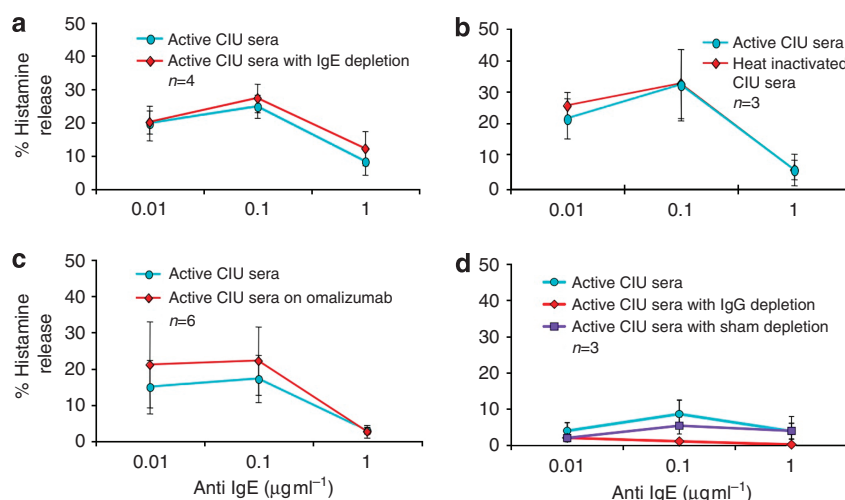


Figure 2. The source of the suppressive factor is not complement, IgE, or IgG autoantibody. FcεRI-mediated basophil histamine release (BHR) suppression does not appear to be affected by (a) serum IgE depletion by immunoabsorption ($n=4$) or (b) heat inactivation of IgE and complement ($n=3$). The three sera used in a contained 33, 212, and 355 ng ml⁻¹ of IgE, and after immunoabsorption, levels were reduced to 2% of original values. (c) Serum from patients pre and post omalizumab therapy ($n=6$) showed no differences in the degree of histamine suppression observed. (d) IgG was depleted from active CIU/CSU serum using protein G Sepharose and evaluated in comparison to the untreated serum for BHR suppression ($n=3$). No statistical differences were seen among the active, IgG-depleted, or sham-depleted sera.

from CIU/CSU patients during active disease and in remission. Basophils cultured in remission serum displayed significantly less BHR suppression compared with active serum culture and were similar to culture in media alone (Figure 1b).

Given that omalizumab therapy rapidly reduces symptoms in CIU/CSU patients (Gober *et al.*, 2008; Saini *et al.*, 2011; Maurer *et al.*, 2013), we explored the role of IgE in conveying BHR

suppression. IgE depletion by immunoabsorption failed to change the degree of BHR suppression obtained with intact serum (Figure 2a). Similarly, the use of heat-inactivated serum, inactivating IgE and complement, also did not alter BHR suppression after culture (Figure 2b). Serum from patients with CIU/CSU disease before and after clinical improvement on omalizumab therapy (Figure 2c) did not differ in their capacity to transfer BHR suppression.

Likewise, basophils cultured in IgG-depleted CIU/CSU serum showed similar suppression of BHR to sham-depleted serum (Figure 2d).

An unclear mechanism suppresses the FcεRI pathway of blood basophils in subjects with active CIU/CSU. We have reported altered expression of inhibitory molecules in the IgE receptor signaling pathway (Vonakis *et al.*, 2007). In remission, basophil FcεRI histamine response increases toward a more normal profile (Eckman *et al.*, 2008). To gain insights into factors responsible for basophil FcεRI suppression, we examined the effects of culture of healthy donor basophils with well-characterized CIU/CSU patients' serum. We consistently observed marked suppression of FcεRI-mediated BHR in cultures using active CIU/CSU patients' serum but found that the serum from patients in remission had less capacity to suppress basophil HR.

Given the putative roles of IgE in CIU/CSU in disease, IgE depletion and heat denaturation of serum from active CIU/CSU patients were examined for its' impact on the suppression of donor BHR. Such approaches failed to alter the ability of these sera to suppress basophil mediator release. Omalizumab therapy led to rapid symptom relief in CIU/CSU subjects with symptom rebound when therapy was discontinued (Maurer *et al.*, 2013). However, culture with CIU/CSU serum in the presence of therapeutic levels of omalizumab also failed to impair the transfer of BHR suppression. These observations suggest that the suppressive factor in the serum of CIU/CSU patients acts through the IgE receptor pathway but is not IgE. Furthermore, IgG depletion of active CIU/CSU serum also failed to alter the transfer of mediator suppression, indicating that IgG antibodies are not directly involved in the phenomenon.

Although the nature of the serum factor responsible for suppressing BHR in CIU/CSU remains unknown, its significance as a potential disease biomarker for CIU/CSU is apparent, but more work is needed. The suppression factor is present in active disease, reduced as the patient experiences natural disease remission, and is independent of IgE and IgG antibodies. Elucidation of the nature

of this factor may provide insights into the pathogenesis of CIU/CSU.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank Becky Vonakis, Don MacGlashan, and John Eckman for their comments, suggestions, and support for this paper. SSS received support as Cosner Scholars in Translational Research from the Johns Hopkins University. This work was supported by the Asthma and Allergic Disease Research Centers grant U19AI070345 from the National Institutes of Health and AAAA Bridge Award.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/jid>

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Loss of Primary Cilia in Melanoma Cells Is Likely Independent of Proliferation and Cell Cycle Progression

Journal of Investigative Dermatology (2015) **135**, 1456–1458; doi:10.1038/jid.2015.22; published online 26 February 2015

TO THE EDITOR

Accurate diagnosis of melanoma is valuable in reducing melanoma mortality and morbidity. A number of candidate biomarkers have been developed for melanoma (Mandala and Massi, 2014); however, none has been accepted as being accurate, easily interpretable, and practical in diagnosing histologically challenging melanocytic lesions. Recently, it was found that primary cilia exist in melanocytes and melanoma cells and that primary cilia were frequently lost in melanomas (Kim *et al.*, 2011; Le Coz *et al.*, 2014), suggesting that the primary cilium may be used as a biomarker for melanoma. However, it remained unclear whether the loss of cilia in melanoma was due to increased proliferation or cell cycle progression. The statistical power of using primary

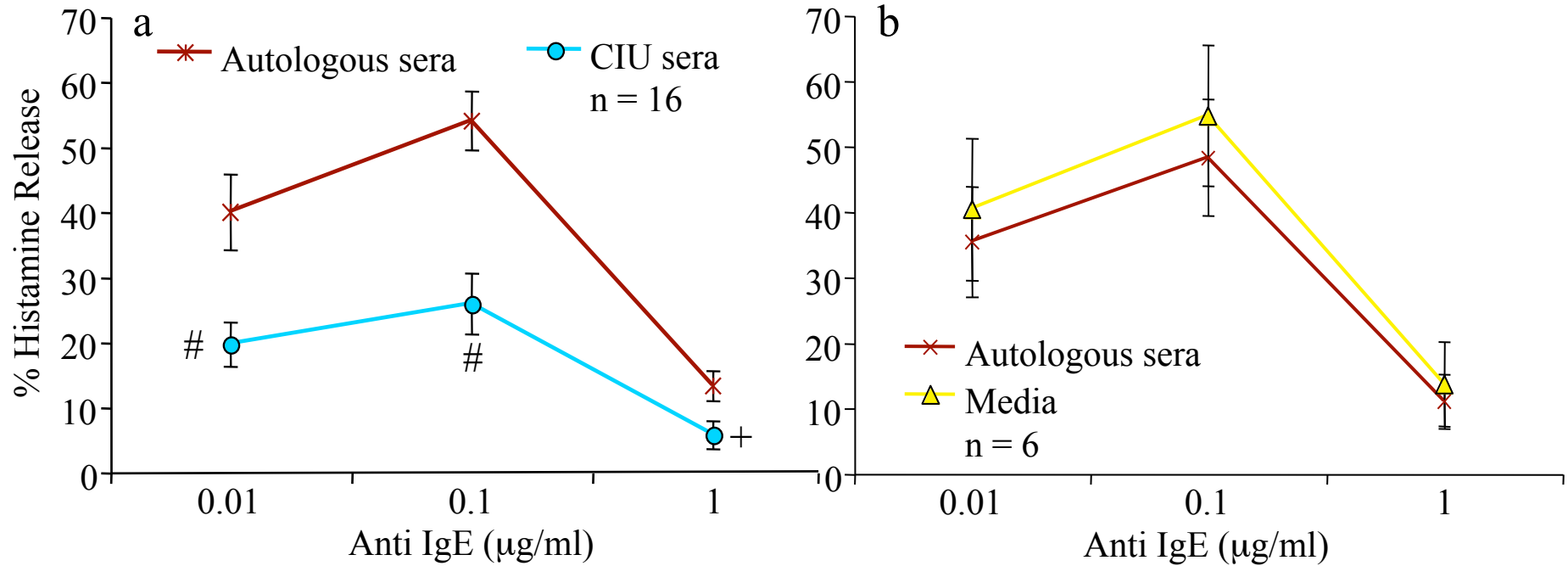
cilium as a biomarker also remained to be established.

Here, we independently evaluated primary cilia by immunofluorescence microscopy on 87 cases of melanocytic nevi and melanomas obtained from the archival collections of the Department of Pathology of Stony Brook Medicine between 2003 and 2011. This study was approved by the IRB of Stony Brook University. ARL13B (Proteintech, Chicago, IL), acetylated α -tubulin (Sigma, St. Louis, MO), and ACIII (Santa Cruz Biotechnology, Dallas, TX) were used to detect primary cilia; gamma-tubulin and chibby (CBY1, (Takamaru *et al.*, 2003)) were used to detect basal body; SOX10 (Santa Cruz Biotechnology) and S100 (BioCare Medical, Concord, CA) were used to detect melanocytes and melanoma cells, as described previously (Dai *et al.*, 2011).

Comparable results were obtained from all antibodies of each category (Supplementary Figure S1 and S2 online). An average of 366 (97–959) cells were evaluated for each specimen.

Remarkable decrease in ciliated cells was found in all types of melanoma when compared with nevi. Specifically, $24.85 \pm 6.33\%$ of melanocytes in nevi were ciliated (Figure 1a). In contrast, percentages of ciliated cells were reduced to 4.36 ± 2.13 , 2.85 ± 2.43 , and $1.16 \pm 0.70\%$ in melanoma *in situ*, primary melanoma, and metastatic melanoma, respectively (Figure 1b–d). The decrease was statistically significant ($P = 2.50 \times 10^{-17}$ by two-way analysis of variance; Figure 1e). In comparison with nevi, all types of melanoma demonstrated a highly significant reduction in ciliated cells ($P < 1 \times 10^{-9}$ by the Tukey test).

Proliferating cells typically show higher rates of cilia formation compared



Supplemental Figure

Normal basophils cultured in CIU serum show a suppressed histamine release profile. Purified blood basophils from healthy donors were cultured overnight in media, autologous serum, or CIU serum and then assessed for Fc ϵ RI mediated histamine release. a) CIU serum cultured basophils showed significant Fc ϵ RI mediated BHR suppression as compared to parallel autologous serum cultured basophils (+p<0.05, #p<0.001 relative to matched control) (n=16). b) Media and autologous serum cultures (n=6) showed similar BHR profiles.