



Invited Review Article

Urticaria and basophils

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ABSTRACT

Chronic spontaneous urticaria (CSU) is a common skin disease without an etiology in the vast majority of cases. The similarity of symptoms and pathology to allergen-induced skin reactions supports that skin mast cell IgE receptor activation is also involved in CSU. Accumulating evidence also supports a role for blood basophils in disease expression. Blood basopenia is noted in active CSU disease with the recruitment of blood basophils to skin lesion sites. Blood basophils further display altered IgE receptor mediated degranulation patterns in two phenotypes that improve in remission. In active CSU subjects, changes in IgE receptor signaling molecule expression levels accompany the altered degranulation function in blood basophils. The success of therapies targeting IgE in CSU patients have also shown that altered blood basophil phenotypes and enumeration have potential use as a disease biomarker.

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Basophils in urticaria - introduction

Urticaria or hives is a common skin manifestation of an allergic response such as observed in anaphylaxis to drugs or foods. Urticarial eruptions can occur with or without angioedema and are classified by the time of symptom duration and inciting factor. Recurrent hive episodes for less than six weeks are classified as acute urticaria and can occur in 20% of the population.¹ In chronic urticaria (CU), symptoms are persistent on most days for over six weeks, lack an obvious external trigger in 80% of cases, and termed chronic spontaneous urticaria (CSU).¹ The remaining 20% of CU cases are related to specific stimuli (cold, heat, pressure) or physical conditions (elevated body temperature) and are termed chronic inducible urticaria (CIndU).

The underlying pathogenesis for CSU has mainly focused on the activation of skin mast cells via multiple possible mechanisms. The consensus view is that mast cells are activated and release mediators such as histamine that provoke symptoms of itch, lead to vasodilation and recruitment of other immune cells to the skin lesion site. Evidence points to a role for IgE and IgE receptor (FcεRI) activation in CSU disease given the success of a monoclonal IgG anti-IgE (omalizumab) in moderating or eliminating skin symptoms.² Thus, there is interest in better defining the contribution of IgE bearing cells such as mast cells and basophils in this disorder. A role for the blood basophil is forwarded given the capacity of these leukocytes to migrate into skin tissues and their ability to release

inflammatory mediators that could contribute to the disease symptoms. This review will examine the recent evidence for the role for basophils in CSU. Unique features of blood basophils in active CSU patients are basopenia, basophil infiltration of skin wheals and altered IgE based degranulation pathways.³

Basopenia in CSU-association with disease measures

Evidence has been accumulating for the role of basophils in CSU pathogenesis.^{3,4} Basophils were first identified by Paul Erlich in 1879. Blood basophils constitute less than 1% of blood leukocytes and share features with mast cells including a common progenitor, cytoplasmic granules containing histamine and expression of surface IgE receptors.⁵ Basophils can produce histamine, leukotriene C4, and cytokines such as IL-4, IL-13, and IL-31 in response to IgE receptor activation.^{5,6} They express IL-3 receptors (CD123) on their surface and depend on this cytokine for differentiation and activation. They can also degranulate to non-IgE dependent pathways such as C5a, fMLP and IL-31. The recruitment of basophils to allergen challenged sites such as the nose, skin and lung is well-documented as part of the late phase of allergen challenge responses.⁷ The exact contributions of basophils to disease is being explored given better tools to isolate roles for basophils.

For over 6 decades, it has been noted that basopenia occurs in persons with chronic urticaria. Rorsman *et al.* did not find reduced bone marrow production of basophils in CSU patients with basopenia and postulated that intravascular destruction was the cause of basopenia.⁸ A reduction in numbers of blood basophils was later found to be correlated with increased symptoms of itch and hives

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and observed in a subset of CSU patients with antihistamine resistance.^{9,10} A longitudinal study of CSU patients with repeated basophil enumeration and clinical symptom scoring has revealed that an interval increase in circulating basophil numbers was correlated with reductions in the reported current itch and hive symptom scores.¹¹ During the course of CSU disease, we have observed rising blood basophil counts with natural remission.^{11,12} Collectively these findings suggest that basopenia is the result of basophil recruitment to the skin and that basophils contribute to skin symptoms. In CSU patients with extreme basopenia, the possibility still exists for intravascular destruction via the presence of functional autoantibodies¹³ (see below).

Blood basophil enumeration has also been examined in the setting of CSU therapies. On a larger scale, blood samples from antihistamine refractory CSU patients enrolled in omalizumab Phase III studies were examined at baseline and after twelve weeks of therapy. An omalizumab dose-dependent improvement in clinical symptoms was observed that was mirrored by a similar patterns of increase in blood basophil presence.¹⁴ Smaller studies have confirmed that among blood leukocytes, only basophil percentages increase in CSU patients on omalizumab therapy.¹⁵ In a single case report,¹⁶ rapid clinical improvement with omalizumab therapy was related to rising basophil counts while reductions in basophil counts and a return of clinical symptoms were noted after terminating omalizumab therapy. Recently, the use of antihistamine treatment with symptom improvement has also been noted to increase basophil numbers.¹⁷ These therapeutic studies support that improvement in skin symptoms is associated with reduced basophil migration to the skin and rising circulating basophil numbers, offering a potential biomeasure for clinical improvement.

Detection of basophils in skin tissue of CSU

The development of basophil specific antibodies to proteins such as basogranulin (BB1) has allowed validation of the idea that basophils traffick to active skin lesion sites.^{18,19} Earlier work showed that biopsies of urticarial skin lesions resemble the infiltrate found in allergen challenge late phase sites, with a predominance of lymphocytes, eosinophils, and basophils.²⁰ A study of skin biopsies across multiple skin diseases demonstrated the highest basophil numbers were detected in pruritic skin diseases such as CSU and prurigo along with evidence of an activated status of blood basophils as measured by increased expression of CD203c.²¹ Although lesional sites had the greatest numbers of infiltrating basophils, even non-lesional sites had evidence of elevated basophils as compared to healthy skin suggesting general recruitment signals in CSU skin.^{22,23}

Basophil recruitment pathways to the skin

Proposed pathways for basophil recruitment to the skin are not fully identified but include the PGD2 pathway via the CRTh2 receptor which is expressed on basophils and other cell types recruited to lesions such as eosinophils and Th2 cells.²⁴ It is notable that both blood basophils and eosinophils have reduced levels of CRTh2 expression relative to healthy controls suggesting ligand mediated downregulation.²⁴ It is proposed that PGD2 production by skin mast cells is enhanced in local skin tissues, but systemic levels of prostaglandin metabolites measured in urine have not been found to be significantly different between patients and controls.²⁵ Another potential basophil recruitment pathway involves CCR3 given evidence for elevations in serum eotaxin²⁶ and reduced CCR3 expression by both basophils and eosinophils.²⁷

A clinical study of an oral CRTh2 antagonist in CSU patients revealed reversal of depressed CRTh2 levels on blood basophils, but

not on eosinophils. In contrast, in CSU patients given the CRTh2 antagonist circulating numbers of eosinophils increased from baseline, but not basophils, suggesting differences in active recruitment pathways for these two cell types to the skin.²⁸

The degree of basophil migration may be influenced by the level of cellular activation, often measured on basophils by flow cytometry for CD63 or CD 203c (Table 1).²⁹ A modest increase in the expression of CD63, a degranulation marker, was noted on basophils of CSU patients but not CD203c as compared to healthy controls.³⁰ Those with detectable serum autoantibodies did not have elevated CD203c or CD63 measures on their blood basophils. In a recent study from Japan, no differences were observed in either CD 63 or CD203c on CSU subjects' basophils relative to controls, but the investigators did confirm basopenia.³¹

A recent murine model directly examined the issue that the onset of acute skin inflammation was associated with directed blood basophil skin migration.¹⁷ In the initial hours after application of an antigen to the skin, a rapid reduction in blood basophil numbers was noted with the arrival of GFP tagged basophils to skin sites. At later timepoints such as 24–48 h post allergen application, peripheral blood basophil numbers were noted to normalize due to bone marrow compensation occurring after the initial skin event. This model suggests that dynamic shifts between skin migration of basophils, circulating numbers and bone marrow production are the factors that led to basopenia or normalization of blood basophil numbers. It remains unclear how current therapeutic interventions such as omalizumab can alter basophil migration to skin, but perhaps may impact the activation status of circulating basophils that limits their transit into active sites. It is also unknown if basophils can re-enter the circulation after migration to the skin.

Basophil functional phenotypes – association with disease measures

Since the 1970's it has been recognized that basophils from CSU patients have a reduced degree of histamine release after IgE receptor activation.^{32,33}

Table 1
Features associated with basophil activation markers.

Marker	Description	Stimuli
CD63	Lysosomal associated membrane protein, on the surface of cytoplasmic histamine granules	IgE receptor mediated activation leads to histamine granule fusion and exposure of CD63 to cell surface, correlated with histamine degranulation
CD203c	Ectonucleotide pyrophosphatase, present on surface of resting cells	Epitope is exposed with IgE receptor activation and also exposure of basophils to IL-3

Table 2
CSU basophil functional phenotypes.

Subset	
CSU-Responder (CSU-R)	Histamine release to an optimal dose of cross-linking anti IgE that is >10% of total histamine content
CSU-Non-responder (CSU-NR)	Histamine release to an optimal dose of cross-linking anti IgE that is ≤ 10% of total histamine content
CSU- Non-responder Basopenics	Histamine release to an optimal dose of cross-linking anti IgE that is ≤ 10% of total histamine content and significant reduction of blood basophil numbers (less than 8000/ml)

Nearly twenty years ago, two distinct basophil functional phenotypes were defined based on the magnitude of observed IgE receptor triggered histamine release (Table 2). The first degranulation profile was termed CSU-Responder (CSU-R) based on > 10% release of total cellular histamine content whereas the other was called CSU-Non-responder (CSU-NR) based on <10% release of total histamine content.³⁴ The abnormal responsiveness appears to be due to elevated expression of intracellular phosphatases (SHIP-2) in CSU-NR, which regulate IgE signaling pathways essential for histamine release, while other pathways for degranulation are unaffected (FMLP, MCP-1).^{34,35} A more recent study used flow cytometry to measure CD63 induction on CSU basophils (also called the basophil activation test) as a surrogate measure for histamine release after IgE receptor triggering. They confirmed the presence of the previously defined two degranulation profile patterns of CSU R, CSU NR, and offered a third subset called CSU NR basopenic.³⁶ Another recent study confirmed the altered IgE pathway histamine release subsets and found that non-IgE stimulation with C5a led to degranulation in all groups.³¹ Of note, testing of ClnDU basophils did not reveal evidence of altered IgE pathways to degranulation supporting this is a unique feature of basophils of CSU subjects.³⁷

Longitudinal examination of active CSU patients has shown these functional phenotypes are stable during the course of CSU disease.¹² In remission, basophil IgE receptor abnormalities are noted to improve and points again towards basophils as a participant in disease.^{11,12,33} Paradoxically, the blood basophils of patients who recover from CSU release more histamine when challenged through the IgE receptor than they did during active disease. It is also notable that the serum from active CSU subjects, but not serum taken during CSU remission, can transfer the suppressed IgE receptor phenotypes to healthy donor basophils after overnight culture.³⁸ The serum factor responsible for this suppression appears to be independent of IgG, IgE or complement.

The relationship between disease measures and these CSU disease associated basophil functional phenotypes has also been investigated.³⁹ Subjects with a CSU-R phenotype had evidence for longer disease duration as compared to basopenics and CSU-NR subjects. Measure of severe disease, such as the use of a steroid courses in the previous year was more often reported by basopenic subjects and least often by CSU-R. Basopenic subjects also reported higher scores for itch and hives. Furthermore, quality of life measures such as Skindex were found to be correlated to shifts in basophil functional phenotype with improved Skindex scores related to increased basophil *in vitro* IgE receptor mediated histamine measures.¹¹

In a mechanistic clinical study involving omalizumab therapy of CSU subjects and impact on IgE bearing cell types, baseline status of CSU-R as compared to CSU-NR and basopenia was found to be predictive of a more complete and rapid response to omalizumab treatment.⁴⁰ Besides functional phenotype, low baseline IgE and lower basophil FcεRI display were also noted to be associated with poorer response to omalizumab.

Impact of autoantibodies on basophil features

One of the oldest theories of disease pathogenesis is that a subset of CSU patients possess IgG autoantibodies that target either IgE or IgE receptors (FcεRI), and drive the activation of mast cells and basophils.⁴¹ This autoimmune concept of disease was derived from the recognition that other autoimmune disorders such as thyroid disease were more prevalent in patients with CSU^{42,43} and has recently been reviewed.⁴⁴ In the 1990s, IgG autoantibodies to IgE or IgE receptors were proposed to be present in 30–40% of individuals.⁴¹ Assays to detect such autoantibodies included autologous serum skin testing, basophil histamine release assays or plate based ELISAs.

It was clear that individual assay results often did not overlap, leading to much debate about defining assays for autoimmunity.⁴⁵ More rigorous criteria to detect such functional autoantibodies has lowered estimates to 7–8% of CSU subjects.^{45,46} This IgG autoantibody theory has been termed Type IIb autoimmunity and its clinical significance has been the topic of other reviews and will not be covered in depth here.^{3,47–50} It is worth noting that several bio-measures have been considered as associated with Type IIb autoimmunity in CSU including lower basophil counts and low IgE.⁵⁰

A newer concept in the autoimmune theories of pathogenesis is that IgE autoantibodies (Type I) are present to self-antigens such as thyroid peroxidase, IL-24, and are proposed to drive CSU disease via exposure to autoallergens in local skin sites.⁵⁰ Of significance, recent studies have found that both types I and II autoantibodies can co-occur in the same CSU patient, such as both IgE and IgG anti-FcεRI.^{51,52} Biomeasures such as basopenia and low IgE can also appear in Type I autoimmunity.⁵² Others have also noted both IgA and IgM anti-FcεRI leading to overlap in autoantibody isotypes and mixing of types I and II in the same individual.⁵³ Given the evolving picture of overlap in types I and II autoimmunity, previously described as distinct entities, defining autoimmune CSU and its disease implications will require further effort to clarify.

An older study by Grattan *et al.* used 2 methods to quantify basophils (flow cytometry and manual counts with granule stains) in CSU patients and relate these to the presence of autoantibody presence. They identified subjects with IgG autoantibodies using a functional assay that measures the ability of CSU serum to cause donor basophil histamine release. They observed that subjects with evidence of serum evoked histamine releasing activity (HRA) had the greatest degree of basopenia.⁵⁴ This again raises the question whether basopenic CSU subjects have basophils that are more “activated” or adherent to endothelial cells, or display greater migration to the skin, or have a shortened lifespan due to easier destruction based on the activity of circulating autoantibodies.

In regards to basophil functional phenotypes, the prevalence of IgG autoantibodies to either IgE or to FcεRI measured by immunoenzymatic assays (IEMA) did not differ among the 2 identified basophil functional phenotypes (CSU-R or CSU NR), but it was noted that a greater prevalence of autoantibodies was seen in subjects with extreme basopenia.¹² This is similar to the finding of the Grattan study but used a separate assay method for autoantibodies. In this IEMA IgG autoantibody study, a subset of CSU subjects entered remission and displayed a marked increase in basophil degranulation via IgE pathway induced histamine release. However, there was no major shift noted in IEMA measured IgG autoantibody titers in active or remission serum.

In a recent mechanistic study using omalizumab in CSU, several individuals with extreme basopenia (<8000/ml) were enrolled and noted to have functional IgG autoantibodies¹³ using more rigorous assays (reproducible serum evoked histamine release with multiple basophil donors, serum evoked response that was subject to inhibition by BTK inhibitors, and HRA residing in purified IgG serum fractions). This more recent study again demonstrated the association of basopenia with the presence of IgG functional autoantibodies and a resistance to complete therapeutic response to omalizumab.

Basophils and therapeutic response prediction - insights from clinical studies

There are several lines of evidence that suggest extreme basopenia (defined as less than 5–8000 basophils per cubic ml of blood) have more severe disease features, are less responsive to antihistamines, less responsive to omalizumab, and more consistently found to have autoantibodies (see Table 3). Rijavec *et al.* found

Table 3
Features associated with extreme basopenia.

Characteristic	Reference
Increased IgG autoantibodies	Grattan 1997, ⁵⁴ Eckman 2008, ¹² MacGlashan 2021 ¹³
Higher symptom scores	Johal 2021, ⁴⁰ Huang 2020, ³⁹ Grattan 2003 ⁹
Antihistamine resistance	Kolkhir 2020, ⁵⁶ Magen 2011 ¹⁰
Lower serum IgE/lower basophil FcεRI	MacGlashan 2020, ¹³ Fok JS 2021, ⁵⁷ Deza 2017 ⁵⁸
Poorer response to omalizumab	Johal 2021, ⁴⁰ Rijavec 2021 ⁵⁵

basophils less than 1700 cells/ml were a negative predictor for response to omalizumab.⁵⁵ Overlap of low basophil numbers, low IgE and low receptor density have been noted.¹³

It has also been noted that the lack of serum based CD203c activation with CSU patient serum predicts for better response to omalizumab, whereas the presence of an active CIU index may favor responsiveness to cyclosporine.

Early work with omalizumab identified that the impact of omalizumab on blood basophils and mast cells varied in timing in allergic subjects.⁵⁹ The kinetics of cellular phenotypic shift due to reductions in circulating free IgE resulting in reduced FcεRI density and allergen driven responses in mast cells and basophils has been assessed in allergic subjects with airway and food allergy.^{59–61} The examination of blood samples from subjects undergoing omalizumab therapy (dosed based on weight and baseline IgE) for allergic asthma revealed that blood basophils rapidly reduced surface display of IgE and IgE receptors (typically 1 week, >90%).⁵⁹ This also led to a rapid reduction in allergen mediated responses by blood basophils.^{62,63} In contrast, corresponding skin biopsies after 1 week of therapy of these same subjects demonstrated stable skin mast cell densities of IgE receptors and stable allergen mediated skin reactions. The timing for change of mast cell IgE responses to allergen was found to require several weeks (70 days in Beck study⁵⁹) and six to eight weeks in other later studies.⁶⁴ These timed observations of cellular changes have been previously summarized.⁶⁵ The timing of change may be related to differences in the lifespan of the two cell types, which is much longer for skin mast cells.

These early kinetic observations for skin mast cell changes were confirmed in skin biopsies of CSU patients undergoing omalizumab therapy⁶⁶ along with the more rapid change in blood basophils.⁶⁷ The longer time required to alter skin mast skin allergen responses holds relevance for current theories of Type I IgE autoantibodies driving disease symptoms in CSU. If IgE directed against self-antigens was a principal driver of disease symptoms, then omalizumab would be expected to take 6–8 weeks to alter skin mast cell autoIgE driven responses, such as observed in multiple past allergen trials monitoring allergen skin test responses.^{60,61,68} However, a study using an identical omalizumab dosing strategy for allergic subjects (weight and baseline IgE based) in CSU patients selected for autoIgE expression (IgE anti-TPO)⁶⁹ showed that the timing of skin symptom improvement to be much earlier (1–2 weeks) than predicted by past studies to change allergen specific IgE responses of skin mast cells. In fact, the timing for clinical symptom response using a fixed omalizumab dosing strategy is similar to that found in autoIgE -TPO dosed trial,² suggesting another explanation may be responsible for the clinical benefits observed.

The ability to predict clinical responses to IgE lowering therapy has been an active area of work and suggest negative predictors include low basophil counts,^{40,55} low serum IgE^{57,70,71} values and associated low levels of basophil IgE receptors,^{58,67} presence of IgG

functional autoantibodies to FcεRI,¹³ and the basophil non-responder functional phenotype.⁴⁰

An additional insight of the impact of omalizumab on basophil IgE receptor functions is related to changes in basophil sensitivity. We have recently examined the impact of omalizumab therapy on blood basophil IgE receptor functions. Shifts in the balance between receptor numbers and Syk, a key regulator of the response has shown an increase in cellular sensitivity.⁷² We have noted that regardless of the baseline CSU phenotype (responder or non-responder), a net rise in basophil IgE receptor activation response occurs, most markedly in those donors with a non-responder phenotype.¹³ It has also been seen with allergen mediated responses to cat.⁷²

The ratios of open to occupied receptors are altered under IgE binding therapies such as omalizumab. The typical shift permits a transient period of increased open receptors (and potentially an opportunity for IgG anti FcεRI antibodies to bind with greater ease and potentially promote increase skin symptoms).

Conclusion

A role for basophils in human skin disease such as CSU has been gaining interest over the last several years. The recognition that basophils migrate into urticaria sites is well established as is the wide range of inflammatory mediators they can produce via IgE receptor activation. The altered IgE degranulation profiles of basophils in active disease and improvement in remission also supports the involvement of basophils in disease along with linkage of distinct phenotypes with disease measures. The relationship of basopenia with disease severity and interval improvement in disease symptoms with rising blood basophil numbers further support their contribution to CSU itch and hive symptoms. Likewise, the degree of symptom improvements by drug interventions such as omalizumab offer further insights into the participation of this blood leukocyte in skin disease.

Conflict of interest

The author declares that there are research interests with NIH, Novartis, Sanofi, Regeneron, Amgen, and other interest includes consultant to Allakos, Granular Therapeutics, Novartis, Aquestive, Regeneron, Escient, Innate, Celltrion, Sanofi.

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