

# The role of eosinophils in chronic spontaneous urticaria

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Chronic spontaneous urticaria (CSU) is considered to be primarily a mast cell–driven disease. However, recent evidence suggests that eosinophils may also have an axial role in symptomatology. Histologic studies have demonstrated the presence of both eosinophils and eosinophil granules, indicative of activation, in CSU lesions. Although many allergic and inflammatory conditions are associated with a peripheral blood eosinophilia, the converse appears to be the case in CSU, with a peripheral blood eosinopenia being observed in many patients. Possible mechanisms include the depletion of blood eosinophils by recruitment into the skin during active disease and immunologic destruction in the blood. We also address in some detail the interactions between eosinophils and mast cells, particularly the cytokine cross-talk of these cells and mediator release possibly leading to clinical symptoms. Also, activation by eosinophil proteins of the coagulation pathway leads to the generation of thrombin and increased mast cell degranulation. Finally, treatments aimed at reducing eosinophil accumulation and activation, such as the anti-IL-5 antibodies mepolizumab, reslizumab, and benralizumab, have been reported to reduce CSU symptoms. Clearly, a new picture of an important role of

eosinophils in the pathogenesis of CSU is emerging. (J Allergy Clin Immunol 2020;■■■■:■■■■-■■■■.)

**Key words:** Urticaria chronic spontaneous urticaria, mast cell, eosinophil

Eosinophils and mast cells, both cells of the innate immune system, have bidirectional communication pathways and important synergistic immunologic functions.<sup>1</sup> In parasitic infections, the mast cell and the eosinophil act in tandem to eliminate the parasite. The initial response is IgE-dependent activation of mast cells to induce vasodilatation, itching, and mucus secretion in mucous membranes. In addition, mast cell cytokines stimulate a T<sub>H</sub>2 inflammation,<sup>2</sup> resulting in the recruitment of eosinophils.<sup>3,4</sup> The role of the eosinophil, which is essentially a secondary effector cell, is to kill parasitic worms, particularly in larval stages, by secretion of their cytotoxic proteins, major basic protein (MBP), eosinophil cationic protein (ECP), and the less toxic eosinophil-derived neurotoxin (EDN).<sup>4</sup>

Allergy (of the immediate type) can essentially be considered an inappropriate parasite-killing response whereby the immune system recognizes common allergens, such as grass and tree pollens, house dust mites, and peanuts as though they are parasites.<sup>5</sup> Again, IgE-dependent mast cell activation is the primary stimulus. In allergic rhinitis, for example, mast cell histamine causes vasodilatation, rhinorrhea, itching, and sneezing. The release of mast cell cytokines stimulates a T<sub>H</sub>2 response with the influx of eosinophils whose cytotoxic proteins cause sustained allergic inflammation and exacerbate nasal congestion.<sup>6,7</sup>

Now let us consider chronic spontaneous urticaria (CSU), a mast cell–driven skin disease characterized by the recurrence of transient wheals, angioedema, or both for more than 6 weeks.<sup>8</sup> Mast cell degranulation induced via the high-affinity IgE receptor (FcεRI) is considered one of the most frequent initiating factors in CSU.<sup>9</sup> This is evidenced by the effectiveness of omalizumab (anti-IgE) in reducing the symptoms of the disease.<sup>10</sup> In CSU, FcεRI-dependent mast cell degranulation may occur in either of 2 ways, as shown in Fig 1. The first is type 1 autoallergy, in which a patient's IgE is directed to autoallergens, such as thyroid peroxidase<sup>11</sup> and IL-24.<sup>12,13</sup> The second is type 2 autoimmunity, in which IgG-autoantibodies to IgE or FcεRI initiate mast cell activation.<sup>14,15</sup> Mast cell cytokines could initiate a local late-phase response that would be expected to result in eosinophil infiltration and activation, as in the response to parasites and allergy.

## THE BIOLOGY AND FUNCTION OF EOSINOPHILS

Eosinophils are circulating innate immune cells representing 1% to 3 % of the white blood cells. They originate from

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P. Kolkhir was supported by a GA<sup>2</sup>LEN fellowship. This project benefitted from the support of the GA<sup>2</sup>LEN network of Urticaria Centers of Reference and Excellence ([www.ga2len-ucare.com](http://www.ga2len-ucare.com)) and the Russian Academic Excellence Project 5-100.

Disclosure of potential conflict of interest: S. Altrichter is or was recently a speaker and/or advisor for and/or has received research funding from Allakos, AstraZeneca, Moxie, and Sanofi. P. Kolkhir is or was recently a speaker for Novartis and Roche. M. Maurer is or was recently a speaker and/or advisor for and/or has received research funding from Allakos, Aralez, AstraZeneca, FAES, Genentech, Menarini, Novartis, Leo Pharma, Lilly, Moxie, MSD, Roche, Sanofi, UCB, and Uriach. M. Church has been a speaker or consultant for Almirall, FAES Pharma, Menarini, Moxie, MSD, Novartis, UCB Pharma, Sanofi-Aventis, and Uriach. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication November 14, 2019; revised February 26, 2020; accepted for publication March 2, 2020.

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0091-6749/\$36.00

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<https://doi.org/10.1016/j.jaci.2020.03.005>

**Abbreviations used**

|         |                                                |
|---------|------------------------------------------------|
| CCL11:  | Eotaxin-1                                      |
| CCL24:  | Eotaxin-2                                      |
| CLC:    | Charcot-Leyden crystal protein                 |
| CSU:    | Chronic spontaneous urticaria                  |
| ECP:    | Eosinophil cationic protein                    |
| EDN:    | Eosinophil-derived neurotoxin                  |
| EPO:    | Eosinophil peroxidase                          |
| MBP:    | Major basic protein                            |
| MCP3:   | Monocyte chemotactic protein-3                 |
| PAF:    | Platelet-activating factor                     |
| PAR:    | Protease-activated receptor                    |
| Siglec: | Sialic acid-binding immunoglobulin-like lectin |
| VEGF:   | Vascular endothelial growth factor             |

multipotent hematopoietic stem cells in the bone marrow.<sup>16</sup> When eosinophils are terminally differentiated, the presence of IL-5 is crucial for entering the blood stream,<sup>17,18</sup> where they persist in the circulation for 8 to 12 hours without proliferation. Chemokines, especially eotaxin-1 (CCL11), eotaxin-2 (CCL24), monocyte chemotactic protein-3 (MCP3), and RANTES, which bind to the  $\beta$ -chemokine receptor CCR3 on eosinophils, have been shown to be responsible for eosinophil recruitment to sites of allergic inflammation in the airways,<sup>19,20</sup> skin, and other tissues. Once migrated to tissues, eosinophils are able to survive for additional 8 to 12 days in the absence of stimulation.<sup>21</sup>

Besides contributing to (immune) homeostasis, eosinophils are commonly known for their role as effector cells in allergies (especially in asthma and allergic rhinitis), fueling chronic inflammation and tissue destruction. Together with mast cells, they represent the key effector cells in allergic inflammation, thus being termed the *allergic effector unit*.<sup>22</sup> Furthermore, they contribute to autoimmune disorders (eg, bullous pemphigoid, inflammatory bowel disease, eosinophilic granulomatosis with polyangiitis, eosinophilic myocarditis, neuromyelitis optica, primary biliary cirrhosis),<sup>23</sup> cardiovascular diseases, and cancer.<sup>24</sup> In patients with CSU, eosinophils are abundant along with mast cells at sites of skin lesions, and they are also present in nonlesional skin.<sup>25</sup>

Here, we review the evidence that supports a contribution of eosinophils to the pathogenesis of CSU, their role as a biomarker, and their value as a target of treatment of patients with CSU.

## WHAT IS THE EVIDENCE FOR EOSINOPHIL INVOLVEMENT IN CSU?

The presence of eosinophil infiltration in urticarial skin lesions of patients with CSU was demonstrated as early as in the 1980s by Grattan et al in patients with CSU who were injected with autologous sera.<sup>26</sup> Subsequent studies have confirmed eosinophil infiltration in CSU lesions,<sup>27</sup> together with lymphocytes,<sup>28</sup> and small numbers of other cells, such as neutrophils and basophils.<sup>25,28-32</sup> Interestingly, eosinophils and microvascular changes persist in uninvolved skin, which together with increased mast cell numbers, suggests that nonlesional skin is primed for further whealing.<sup>25</sup> Furthermore, reports of mononuclear cell and eosinophil perivascular infiltration into the areas of wheals have also been reported in some forms of chronic inducible urticaria (eg, delayed pressure urticaria and solar urticaria).<sup>30,33-38</sup>

Immunocytochemical studies using eosinophil granular proteins, particularly MBP, have explored eosinophil degranulation in chronic urticaria. In an initial study, Peters et al<sup>39</sup> demonstrated the occurrence of eosinophil degranulation in lesional biopsy specimens from 12 of 28 patients with chronic urticaria. Subsequent studies have reported large numbers of extracellular eosinophil granules in the vicinity of disrupted eosinophils in long-duration wheals (defined as lasting >24 hours) compared with in short-duration wheals.<sup>27,40</sup>

## HOW ARE EOSINOPHILS RECRUITED INTO THE SKIN?

Recruitment of eosinophils to sites of allergic inflammation in the skin and other tissues is driven by IL-5, which is essential for the expansion and mobilization of eosinophils from the bone marrow,<sup>41</sup> as well as by chemotactic chemokines, especially eotaxin-1 and eotaxin-2, MCP3, and RANTES. These chemokines bind to the  $\beta$ -chemokine receptor CCR3 on eosinophils, which drives the eosinophil recruitment to sites of allergic inflammation in the airways.<sup>19,20</sup> There are essentially 2 critical steps involved in eosinophil recruitment, beginning with eosinophil adhesion to endothelial cells with the aid of the adhesion molecules  $\beta$ 1 integrin and  $\beta$ 2 integrin, as well as selectins expressed<sup>42</sup> on the surface of endothelial cells. After the process of adhesion is complete, migration of eosinophils into tissues takes place under the influence of the aforementioned chemotactic factors<sup>43</sup> (Fig 1).

Recruitment of eosinophils to sites of allergic inflammation usually involves IL-5–producing T<sub>H</sub>2 cells,<sup>44</sup> as well as tissue-resident type 2 innate lymphoid cells constitutively expressing IL-5 and thereby supporting their homeostasis under steady-state conditions.<sup>45</sup> As these cells have not as yet been described as being pathophysiologically involved in CSU, their role in eosinophil recruitment in CSU remains elusive. Other cells that are capable of eosinophil recruitment are dermal fibroblasts, which have been identified as an important source of eotaxin on stimulation with TNF- $\alpha$  and IL-4.<sup>46</sup> In addition, basophils are involved in eosinophil recruitment, as shown in a model of IgE-dependent skin inflammation in which IgE-activated basophils produced IL-4 on interaction with endothelial cells. In turn, IL-4 promotes the upregulation of vascular cell adhesion molecule 1 on the vascular endothelium, which is necessary for eosinophil entry.<sup>47</sup> Interestingly, dupilumab, a mAb against IL-4 and IL-13 receptors, was effective in CSU in an early study.<sup>48</sup> Additionally, IgE-activated basophils expressing Fc $\epsilon$ RI have been demonstrated to play an active role in eosinophil recruitment through the secretion of IL-4/IL-13, which leads to STAT6-dependent expression of CCL24 from endothelial cells and extravasation of eosinophils into the tissues.<sup>49</sup>

Mast cells are a source of various mediators. They have been shown to release IL-5 and eotaxins and could thereby directly mobilize and recruit eosinophils into the skin.<sup>50</sup> Furthermore, they can stimulate dermal fibroblasts via TNF- $\alpha$ <sup>51</sup> or activate and recruit basophils via IL-3,<sup>52-54</sup> and they could thereby also be indirectly involved in eosinophil recruitment. In CSU, these mechanisms have not been proved as of yet, but they seem feasible.

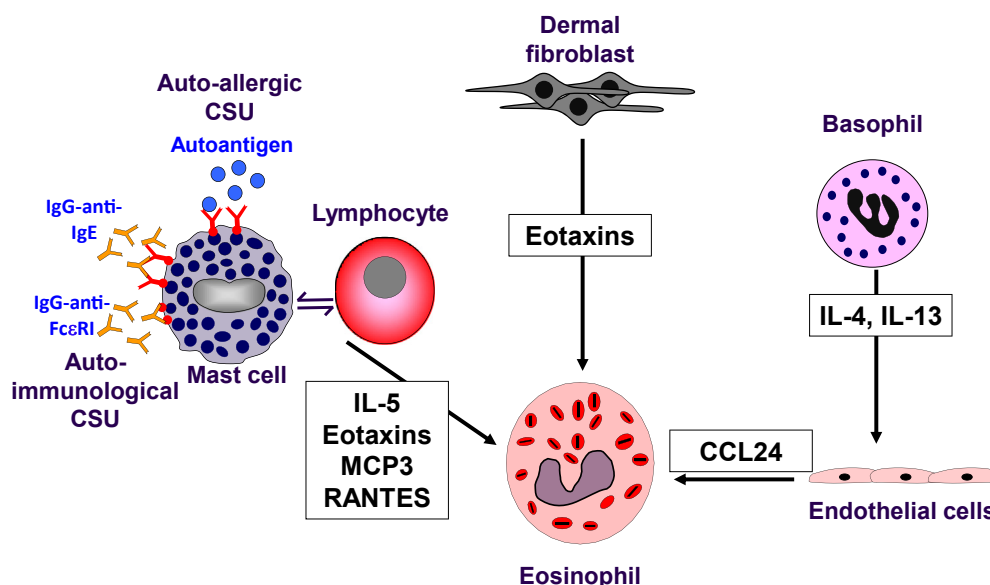


**FIG 1.** Mechanisms of eosinophil recruitment into the skin in CSU. CCL, C-C motif chemokine ligand; MCP, mast cell protease.

### ARE HIGHER SKIN NUMBERS OF EOSINOPHILS LINKED TO HIGHER EOSINOPHIL COUNTS IN THE BLOOD OF PATIENTS WITH CSU?

In many diseases such as allergic, infectious, inflammatory, and neoplastic disorders, tissue eosinophilia had been associated with peripheral blood eosinophilia ( $\geq 450$ –550 eosinophils per  $\mu\text{L}$ ).<sup>55</sup> In CSU, however, the converse appears to be the case. Eosinopenia (ie, low absolute eosinophil counts in the blood) was first described in an individual patient with CSU in 1981.<sup>56</sup> In the ensuing years, eosinopenia has been reported in further individual case reports of patients presenting with urticaria.<sup>50,57,58</sup> Recently, we identified eosinopenia to be present in about 10% of patients with CSU ( $n = 129$  in a cohort of 1259 patients with CSU).<sup>59</sup> For the definition of eosinopenia/low blood eosinophil counts, we used a cutoff of less than  $0.05 \times 10^9/\text{L}$  (50 eosinophils per  $\mu\text{L}$ ) because it was frequently used in the literature.<sup>60,61</sup> The rate of eosinopenia was considerably higher in comparison with that in the general population, in which when the same cutoff of 50 eosinophils per  $\mu\text{L}$  was used, eosinopenia was found to be present in about 5% of the general population in the United Kingdom (44,112 of 775,231 individuals).<sup>61</sup> In our population, 3% of patients with CSU had a complete absence of eosinophils in the blood and 81% patients with eosinopenia also had basopenia,<sup>59</sup> which is a well-known clinical feature of high disease activity and serum autoreactivity.<sup>62</sup>

### HOW DOES EOSINOPENIA DEVELOP IN CSU?

Currently, the mechanisms leading to eosinopenia in CSU are unknown. There are, however, several hypothetical mechanisms that have been described.

First, blood eosinophils may be depleted from the blood by being recruited to the skin during active disease. This is supported by findings of significantly higher numbers of eosinophils and basophils in lesional than in nonlesional skin of patients with CSU and significantly more eosinophils in

nonlesional skin from patients with CSU than in nonlesional skin from healthy control subjects.<sup>25,27,30,31,63</sup> Fujisawa et al also observed eosinophil infiltration in urticarial lesions in 7 of 9 severely affected patients with CSU but not in the skin from normal subjects.<sup>64</sup>

Second, eosinophils may be immunologically destroyed in the blood of patients with CSU. This hypothesis is supported by the fact that some patients with CSU can have low eosinophil levels in both blood and skin. For example, in a 71-year-old woman with chronic urticaria and vitiligo, a lack of eosinophils, basophils, and IgE was seen in her blood, bone-marrow, and skin.<sup>58</sup> This phenomenon may be explained by autoimmune antieosinophil and/or antibasophil serum activity. In another patient with CSU, complete eosinopenia and basopenia were detected along with IgE and IgG, anti-IgA antibodies, circulating immune complexes, and IgA deficiency.<sup>57</sup> Interestingly, a complement-dependent IgG-related activity was observed in the serum of a patient with urticaria and complete eosinopenia. This patient's serum cytotoxicity degranulated human eosinophils *in vitro* without affecting neutrophils. How common this is is currently unclear, and further studies are needed to confirm this observation and characterize the prevalence and relevance of eosinophil-activating serum factors in CSU. In *in vivo* experiments, the same serum was able to suppress eosinophil counts in monkeys for up to 12 hours.<sup>56</sup> In another study, all eosinophils and basophils of patients with atopic dermatitis and healthy subjects were degranulated and then destroyed after their blood was mixed with the plasma from a patient with eosinopenia, basopenia, alopecia, hemolytic anemia, IgA deficiency, and a very low total IgE level.<sup>65</sup>

Third, it is known that immunosuppressive therapy (eg, corticosteroids and cyclosporine) can lead to eosinophil depletion.<sup>66</sup> In some cases of CSU, a complete blood count may have been performed during or shortly after such treatment with immunosuppressive drugs, resulting in an iatrogenically induced low cell number.

Finally, eosinopenia may be associated not with CSU but with comorbid diseases described in CSU. For example, IgA deficiency, which has been described in some patients with CSU, has also been associated with eosinopenia.<sup>57,67</sup>

## MAST CELL-EOSINOPHIL INTERACTIONS

Eosinophils are able to establish physical contact with mast cells via its surface receptors sialic acid-binding immunoglobulin-like lectin 7 and sialic acid-binding immunoglobulin-like lectin 8 (Siglec-7 and Siglec-8),<sup>68,69</sup> CD300a, CD48, CD244/2B4, or Nectin2/CD112.<sup>70</sup> Additionally, eosinophils express a variety of surface receptors to bind secreted mediators (ie, protease-activated receptor 2 [PAR2], ST2, chemokine receptor homologous molecule expressed on T<sub>H</sub> 2 [CRTH2], TNF- $\alpha$ R, IL-5R, GM-CSF-R, IL-4R, CCRR3, histamine receptors [H1-H4], and sphingosine-1-phosphate [S1P] receptors).<sup>70</sup> Thus, various mediators released by mast cells or through an interaction of mast cells with other cells (eg, eotaxin, IL-5, GM-CSF, histamine, PGD<sub>2</sub>, and platelet-activating factor [PAF]) can lead to eosinophil recruitment into the skin.<sup>50</sup> Consequently, activation of infiltrating eosinophils by, for example, IL-5, GM-CSF, or PAF may provoke the persistent activation of mast cells by secreted eosinophil granule proteins such as major basic protein-1 (MBP1) and major basic protein-2 (MBP2), eosinophil peroxidase (EPO), the ribonuclease A superfamily members ECP and eosinophil-derived neurotoxin, and the acidic Charcot-Leyden crystal protein (CLC [or galectin-10]). Because of their biochemical properties, these hydrophobic and cationic proteins are not only highly toxic to parasites and bacteria, but they can also activate neutrophils and platelets, and they stimulate histamine release from basophils and mast cells.<sup>64,71,72</sup> In addition, eosinophil granule proteins are able to persist in the skin for up to several weeks. In Hartley guinea pigs, skin-injected ECP, EDN, and EPO persisted for 1 to 2 weeks, whereas MBP was still detectable after 6 weeks.<sup>73</sup> This could maintain the persistent state of inflammation in long-duration wheals (the late-phase reaction). For example, activation of the low-affinity IgE receptor Fc $\epsilon$ RII/CD23 on eosinophils by IgG-anti-Fc $\epsilon$ RII/CD23 leads to secretion of MBP by eosinophils, with consequent histamine release from basophils.<sup>74</sup> Alternatively, activation of the coagulation cascade by tissue factor produced by eosinophils may lead to generation of thrombin.<sup>75</sup> Thrombin can induce mast cell degranulation<sup>76</sup> via action on PARs and may activate C5, generating C5a.<sup>77</sup> C5a may be responsible for the augmented histamine release after crosslinking of IgE receptor by IgG-anti-Fc $\epsilon$ RI (autoimmunity type IIb). Specific IgE against tissue factor,<sup>78</sup> IL-24,<sup>12</sup> and other autoallergens can mediate the release of mediators by peripheral basophils and/or mast cells (autoimmunity type I). Finally, MBP and EPO can induce degranulation of human skin mast cells through nonimmunologic activation of MAS-related G protein-coupled receptor X2 (MRGPRX2).<sup>64</sup>

## EOSINOPHIL FUNCTION AND CHRONIC URTICARIA

Cellular activation of eosinophils occurs during inflammatory responses and is mainly mediated by GM-CSF, IL-5, IL-3, and IL-13, leading to degranulation and consequent mediator production. Extracellular release of cytotoxic cationic proteins following degranulation may contribute to the pathogenesis of urticaria by activation of skin mast cells.<sup>42</sup> Additionally, it has been observed

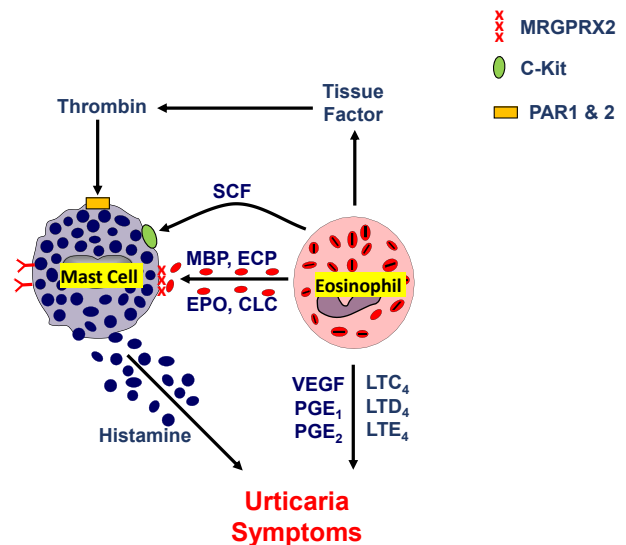


**FIG 2.** Schematic model of the interaction of eosinophils with mast cells in CSU. LTC<sub>4</sub>, Leukotriene C<sub>4</sub>; LTD<sub>4</sub>, leukotriene D<sub>4</sub>; LTE<sub>4</sub>, leukotriene E<sub>4</sub>; MRGPRX2, MAS-related G protein-coupled receptor X2; PGE<sub>1</sub>, prostaglandin E<sub>1</sub>; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; SCF, stem cell factor.

that patients with CSU have increased levels of vascular endothelial growth factor (VEGF), a very potent regulator of angiogenesis as well as a major mediator in vascular permeability. VEGF was found to colocalize with ECP, supporting eosinophils being the main cellular source of VEGF in chronic urticaria lesional skin.<sup>79</sup>

Eosinophils may enhance chronic urticaria in 3 ways (Fig 2). First, eosinophil-derived stem cell factor promotes the recruitment and local maturation of mast cells in the tissues.<sup>24,80,81</sup> Second, eosinophil proteins, such as MBP, ECP, and EPO, can provoke mast cell degranulation.<sup>64,79,82-86</sup> Third, activated eosinophils also express tissue factor, the main initiator of the coagulation cascade leading to thrombin formation.<sup>42,79,87,88</sup> Thrombin in turn, as demonstrated in experimental models, may directly activate mast cells by interaction with the PARs PAR1 and PAR2.<sup>89</sup> Not only may eosinophil infiltration contribute to tissue edema of lesional skin in CSU,<sup>25,27,30-32</sup> but the increased persistence of eosinophils in nonlesional skin and the deposition of their granule proteins, together with increased mast cells, could prime the skin for further whealing.<sup>25</sup>

## WHAT IS THE CLINICAL RELEVANCE OF EOSINOPENIA IN CSU?

The clinical relevance of eosinopenia in CSU is, as yet, unclear. However, correlation of eosinopenia with clinical parameters in patients with CSU has revealed that eosinopenia is linked to higher disease activity and a poor response to treatment.<sup>59</sup> Furthermore, overall lower blood eosinophil numbers were identified in patients with evidence of type IIb autoimmune CSU (eg, patients with CSU with a positive autologous serum skin test result, a positive basophil histamine release assay result, or high levels of IgG-anti-thyroid peroxidase). Most importantly, eosinopenia in CSU was significantly associated with a failure to respond to the guideline-recommended treatment with both second-generation antihistamines and omalizumab. Here, the combination of eosinopenia and basopenia better predicted

nonresponse to second-generation antihistamines than did eosinopenia alone (odds ratio of 9.5 vs 4.8).

## EOSINOPHIL-TARGETED TREATMENT CAN BENEFIT PATIENTS WITH CHRONIC URTICARIA INCLUDING CSU

Mast cell-targeted therapies, such as H<sub>1</sub>-antihistamines and anti-IgE (omalizumab), in the treatment of CSU are now well established.<sup>8,10,90,91</sup> But can eosinophil-targeted treatments be of benefit? The first indication that they could was seen in our clinic in Berlin in 2017 when a woman with severe asthma was prescribed mepolizumab, a humanized mAb directed against IL-5.<sup>92</sup> In addition to her asthma, this patient had longstanding and recalcitrant CSU. From the day after initiation of treatment with mepolizumab, the patient noted a dramatic and sustained improvement of her urticarial symptoms.<sup>93</sup> By 4 weeks, her urticaria was well controlled, and by 8 weeks she was symptom-free. Following discontinuation of mepolizumab, the signs and symptoms of CSU recurred, indicating that mepolizumab controlled symptoms rather than curing the condition. Later the same year, another woman with CSU, cold urticaria, and severe nonallergic refractory eosinophilic asthma was treated with an anti-IL-5 antibody. This time, the antibody was reslizumab.<sup>94</sup> Immediately following reslizumab injection her symptoms of severe CSU and cold urticaria improved; she was completely symptom-free and remained so for the duration of reslizumab treatment. The third patient was an 80-year-old man with severe uncontrolled eosinophilic asthma for which treatment was initiated with subcutaneous benralizumab, an anti-IL-5 receptor antibody.<sup>95</sup> He also had symptomatic dermatographism, which was controlled only with oral prednisolone. His dermatographism was markedly improved after the first injection of benralizumab; the results of provocation tests for dermatographism were negative by 3 months.<sup>96</sup>

Although the patients in the aforementioned studies had concomitant asthma with systemic eosinophilia, the question of whether anti-IL-5 therapy would be of benefit in all patients with urticaria arises. To examine this issue, benralizumab and mepolizumab are currently under evaluation in CSU (in clinical trials with the identifiers NCT03183024 and NCT03494881, respectively).

Another eosinophil-targeted drug, antolimumab, a humanized mAb to Siglec-8, is being evaluated in a phase IIa, open-label pilot study (NCT03436797) in patients with CSU, cholinergic urticaria, and symptomatic dermatographism. Antolimumab inhibits mast cell activity and depletes eosinophils by targeting Siglec-8, an inhibitory receptor and member of the CD33-related family that is expressed on mast cells, eosinophils, and basophils.<sup>97</sup>

## UNANSWERED QUESTIONS, STUDIES THAT SHOULD BE DONE, CONCLUSIONS

Taken together, the data that we have presented are strong circumstantial evidence that eosinophils contribute to the pathogenesis of CSU, at least in a subgroup of patients.

Eosinopenia is seen in a subpopulation of patients with CSU, and its rates are likely to be higher than in the general population. In CSU, eosinopenia is linked to high disease activity, type IIb autoimmunity, and poor response to treatment.

Many questions remain to be answered. These include, but are not limited to How are eosinophils activated in CSU? How are eosinophils recruited to sites of whealing? Are eosinophils also recruited to sites of angioedema? Do eosinophils contribute to mast cell activation? and Does the involvement of eosinophils make a difference to the course of the disease, the clinical features, and the response to treatment?

Further studies are needed to address these and other questions, which may help to advance our understanding of CSU and improve its treatment.

We thank Beate Schinzel for help with searching the literature and preparing this article.

## REFERENCES

- Robida PA, Puzzovio PG, Pahima H, Levi-Schaffer F, Bochner BS. Human eosinophils and mast cells: birds of a feather flock together. *Immunol Rev* 2018;282:151-67.
- Hepworth MR, Danilowicz-Luebert E, Rausch S, Metz M, Klotz C, Maurer M, et al. Mast cells orchestrate type 2 immunity to helminths through regulation of tissue-derived cytokines. *Proc Natl Acad Sci U S A* 2012;109:6644-9.
- Huang L, Appleton JA. Eosinophils in helminth infection: defenders and dupes. *Trends Parasitol* 2016;32:798-807.
- Ramirez GA, Yacoub MR, Ripa M, Mannina D, Cariddi A, Saporiti N, et al. Eosinophils from physiology to disease: a comprehensive review. *Biomed Res Int* 2018;2018:9095275.
- Fitzsimmons CM, Falcone FH, Dunne DW. Helminth Allergens, Parasite-specific IgE, and its protective role in human immunity. *Front Immunol* 2014;5:61.
- Naclerio RM, Bachert C, Baraniuk JN. Pathophysiology of nasal congestion. *Int J Gen Med* 2010;3:47-57.
- Pawankar R, Mori S, Ozu C, Kimura S. Overview on the pathomechanisms of allergic rhinitis. *Asia Pac Allergy* 2011;1:157-67.
- Zuberbier T, Aberer W, Asero R, Abdul Latiff AH, Baker D, Ballmer-Weber B, et al. The EAACI/GA(2)LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria. *Allergy* 2018;73:1393-414.
- Kolkhir P, Church MK, Weller K, Metz M, Schmetzer O, Maurer M. Autoimmune chronic spontaneous urticaria: what we know and what we do not know. *J Allergy Clin Immunol* 2017;139:1772-81.e1.
- Chang TW, Chen C, Lin CJ, Metz M, Church MK, Maurer M. The potential pharmacologic mechanisms of omalizumab in patients with chronic spontaneous urticaria. *J Allergy Clin Immunol* 2015;135:337-42.
- Altrichter S, Peter HJ, Pisarevskaja D, Metz M, Martus P, Maurer M. IgE mediated autoallergy against thyroid peroxidase—a novel pathomechanism of chronic spontaneous urticaria? *PLoS One* 2011;6:e14794.
- Schmetzer O, Lakin E, Topal FA, Preusse P, Freier D, Church MK, et al. IL-24 is a common and specific autoantigen of IgE in chronic spontaneous urticaria. *J Allergy Clin Immunol* 2017; In Press.
- Lakin E, Church MK, Maurer M, Schmetzer O. On the lipophilic nature of autoreactive IgE in chronic spontaneous urticaria. *Theranostics* 2019;9:829-36.
- Schoepke N, Asero R, Ellrich A, Ferrer M, Gimenez-Arnau A, Grattan CEH, et al. Biomarkers and clinical characteristics of autoimmune chronic spontaneous urticaria (aiCSU): results of the PURIST study. *Allergy* 2019;74:2427-36.
- Grattan CE, Francis DM, Hide M, Greaves MW. Detection of circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. *Clin Exp Allergy* 1991;21:695-704.
- Uhm TG, Kim BS, Chung IY. Eosinophil development, regulation of eosinophil-specific genes, and role of eosinophils in the pathogenesis of asthma. *Allergy Asthma Immunol Res* 2012;4:68-79.
- Collins PD, Marleau S, Griffiths-Johnson DA, Jose PJ, Williams TJ. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J Exp Med* 1995;182:1169-74.
- Kopf M, Brombacher F, Hodgkin PD, Ramsay AJ, Milbourne EA, Dai WJ, et al. IL-5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity* 1996;4:15-24.
- Humbles AA, Lu B, Friend DS, Okinaka S, Lora J, Al-Garawi A, et al. The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness. *Proc Natl Acad Sci U S A* 2002;99:1479-84.

20. Dahinden CA, Geiser T, Brunner T, von Tscharnher V, Caput D, Ferrara P, et al. Monocyte chemotactic protein 3 is a most effective basophil- and eosinophil-activating chemokine. *J Exp Med* 1994;179:751-6.
21. Rothenberg ME, Owen WF Jr, Silberstein DS, Soberman RJ, Austen KF, Stevens RL. Eosinophils cocultured with endothelial cells have increased survival and functional properties. *Science* 1987;237:645-7.
22. Minai-Fleminger Y, Levi-Schaffer F. Mast cells and eosinophils: the two key effector cells in allergic inflammation. *Inflamm Res* 2009;58:631-8.
23. Diny NL, Rose NR, Cihakova D. Eosinophils in autoimmune diseases. *Front Immunol* 2017;8:484.
24. Galdiero MR, Varricchi G, Seaf M, Marone G, Levi-Schaffer F, Marone G. Bidirectional mast cell-eosinophil interactions in inflammatory disorders and cancer. *Front Med (Lausanne)* 2017;4:103.
25. Kay AB, Ying S, Ardelean E, Mlynek A, Kita H, Clark P, et al. Elevations in vascular markers and eosinophils in chronic spontaneous urticarial wheals with low-level persistence in uninvolved skin. *Br J Dermatol* 2014;171:505-11.
26. Grattan CE, Wallington TB, Warin RP, Kennedy CT, Bradfield JW. A serological mediator in chronic idiopathic urticaria—a clinical, immunological and histological evaluation. *Br J Dermatol* 1986;114:583-90.
27. Toyoda M, Maruyama T, Morohashi M, Bhawan J. Free eosinophil granules in urticaria: a correlation with the duration of wheals. *Am J Dermatol* 1996;18:49-57.
28. Elias J, Boss E, Kaplan AP. Studies of the cellular infiltrate of chronic idiopathic urticaria: prominence of T-lymphocytes, monocytes, and mast cells. *J Allergy Clin Immunol* 1986;78:914-8.
29. Saini SS. Chronic spontaneous urticaria: etiology and pathogenesis. *Immunol Allergy Clin North Am* 2014;34:33-52.
30. Ying S, Kikuchi Y, Meng Q, Kay AB, Kaplan AP. TH1/TH2 cytokines and inflammatory cells in skin biopsy specimens from patients with chronic idiopathic urticaria: comparison with the allergen-induced late-phase cutaneous reaction. *J Allergy Clin Immunol* 2002;109:694-700.
31. Caproni M, Volpi W, Macchia D, Giomi B, Manfredi M, Campi P, et al. Infiltrating cells and related cytokines in lesional skin of patients with chronic idiopathic urticaria and positive autologous serum skin test. *Exp Dermatol* 2003;12:621-8.
32. Martins CF, Morais KL, Figueroa P, Dias NF, Valente NS, Maruta CW, et al. Histopathological and clinical evaluation of chronic spontaneous urticaria patients with neutrophilic and non-neutrophilic cutaneous infiltrate. *Allergol Int* 2018;67:114-8.
33. Haas N, Motel K, Czarnetzki BM. Comparative immunoreactivity of the eosinophil constituents MBP and ECP in different types of urticaria. *Arch Dermatol Res* 1995;287:180-5.
34. Leiferman KM, Norris PG, Murphy GM, Hawk JL, Winkelmann RK. Evidence for eosinophil degranulation with deposition of granule major basic protein in solar urticaria. *J Am Acad Dermatol* 1989;21:75-80.
35. Murphy GF, Austen KF, Fonferko E, Sheffer AL. Morphologically distinctive forms of cutaneous mast cell degranulation induced by cold and mechanical stimuli: an ultrastructural study. *J Allergy Clin Immunol* 1987;80:603-11.
36. Irwin RB, Lieberman P, Friedman MM, Kaliner M, Kaplan R, Bale G, et al. Mediator release in local heat urticaria: protection with combined H1 and H2 antagonists. *J Allergy Clin Immunol* 1985;76:35-9.
37. Mayou SC, Kobza Black A, Eady RA, Greaves MW. Cholinergic dermatographism. *Br J Dermatol* 1986;115:371-7.
38. Mekori YA, Dobozi BS, Schocket AL, Kohler PF, Clark RA. Delayed pressure urticaria histologically resembles cutaneous late-phase reactions. *Arch Dermatol* 1988;124:230-5.
39. Peters MS, Schroeter AL, Kephart GM, Gleich GJ. Localization of eosinophil granule major basic protein in chronic urticaria. *J Invest Dermatol* 1983;81:39-43.
40. Koh YI, Choi IS, Lee SH, Lee JB, Park CH, Hong SN. Localized heat urticaria associated with mast cell and eosinophil degranulation. *J Allergy Clin Immunol* 2002;109:714-5.
41. Blanchard C, Rothenberg ME. Biology of the eosinophil. *Adv Immunol* 2009;101:81-121.
42. Cugno M, Marzano AV, Tedeschi A, Fanoni D, Venegoni L, Asero R. Expression of tissue factor by eosinophils in patients with chronic urticaria. *Int Arch Allergy Immunol* 2009;148:170-4.
43. Staumont-Salle D, Dombrowicz D, Capron M, Delaporte E. Eosinophils and urticaria. *Clin Rev Allergy Immunol* 2006;30:13-8.
44. Islam SA, Chang DS, Colvin RA, Byrne MH, McCully ML, Moser B, et al. Mouse CCL8, a CCR8 agonist, promotes atopic dermatitis by recruiting IL-5+ T(H)2 cells. *Nat Immunol* 2011;12:167-77.
45. Nussbaum JC, Van Dyken SJ, von Moltke J, Cheng LE, Mohapatra A, Molofsky AB, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* 2013;502:245-8.
46. Miyamasu M, Nakajima T, Misaki Y, Izumi S, Tsuno N, Kasahara T, et al. Dermal fibroblasts represent a potent major source of human eotaxin: in vitro production and cytokine-mediated regulation. *Cytokine* 1999;11:751-8.
47. Cheng LE, Sullivan BM, Retana LE, Allen CD, Liang HE, Locksley RM. IgE-activated basophils regulate eosinophil tissue entry by modulating endothelial function. *J Exp Med* 2015;212:513-24.
48. Lee JK, Simpson RS. Dupilumab as a novel therapy for difficult to treat chronic spontaneous urticaria. *J Allergy Clin Immunol Pract* 2019;7:1659-61.e1.
49. JU Eberle, Radtke D, Nimmerjahn F, Voehringer D. Eosinophils mediate basophil-dependent allergic skin inflammation in mice. *J Invest Dermatol* 2019;139:1957-65.e2.
50. Asero R, Cugno M, Tedeschi A. Eosinophils in chronic urticaria: supporting or leading actors? *World Allergy Organ J* 2009;2:213-7.
51. Okayama Y, Ono Y, Nakazawa T, Church MK, Mori M. Human skin mast cells produce TNF-alpha by substance P. *Int Arch Allergy Immunol* 1998;117(Suppl 1):48-51.
52. Ishizuka T, Okayama Y, Kobayashi H, Mori M. Interleukin-3 production by mast cells from human lung. *Inflammation* 1999;23:25-35.
53. Kim S, Prout M, Ramshaw H, Lopez AF, LeGros G, Min B. Cutting edge: basophils are transiently recruited into the draining lymph nodes during helminth infection via IL-3, but infection-induced Th2 immunity can develop without basophil lymph node recruitment or IL-3. *J Immunol* 2010;184:1143-7.
54. Zellweger F, Buschor P, Hobi G, Brigger D, Dahinden CA, Villiger PM, et al. IL-3 but not monomeric IgE regulates Fc epsilon R1 levels and cell survival in primary human basophils. *Cell Death Dis* 2018;9:510.
55. Kovalszki A, Weller PF. Eosinophilia. *Prim Care* 2016;43:607-17.
56. Franklin W, Goetzl EJ. Total absence of eosinophils in a patient with an allergic disorder. *Ann Intern Med* 1981;94:352-3.
57. Freeman GL. Syndromes associated with eosinopenia. *Allergy* 1998;53:331-3.
58. Juhl L, Venge P. Total absence of eosinophils in a patient with chronic urticaria and vitiligo. *Eur J Haematol* 1988;40:368-70.
59. Kolkhir P, Church MK, Altrichter S, Stahl Skov P, Hawro T, Frischbutter S, et al. Eosinopenia, in chronic spontaneous urticaria, is associated with high disease activity, autoimmunity and poor response to treatment. *J Allergy Clin Immunol Pract* 2019; In Press.
60. Karakostas S, Kalemaki D, Tzagarakis E, Lydakis C. Pitfalls in studies of eosinopenia and neutrophil-to-lymphocyte count ratio. *Infect Dis (Lond)* 2018;50:163-74.
61. Shah AD, Denaxas S, Nicholas O, Hingorani AD, Hemingway H. Low eosinophil and low lymphocyte counts and the incidence of 12 cardiovascular diseases: a CALIBER cohort study. *Open Heart* 2016;3:e000477.
62. Folci M, Heffler E, Canonica GW, Furlan R, Brunetta E. Cutting edge: biomarkers for chronic spontaneous urticaria. *J Immunol Res* 2018;2018:5615109.
63. Spry CJ, Tai PC, Barkans J. Tissue localization of human eosinophil cationic proteins in allergic diseases. *Int Arch Allergy Appl Immunol* 1985;77:252-4.
64. Fujisawa D, Kashiwakura J-i, Kita H, Kikukawa Y, Fujitani Y, Sasaki-Sakamoto T, et al. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J Allergy Clin Immunol* 2014;134:622-33.e9.
65. Juhl L, Michaelsson G. A new syndrome characterised by absence of eosinophils and basophils. *Lancet* 1977;1:1233-5.
66. Altman LC, Hill JS, Hairfield WM, Mullarkey MF. Effects of corticosteroids on eosinophil chemotaxis and adherence. *J Clin Invest* 1981;67:28-36.
67. Gleich GJ, Klion AD, Lee JJ, Weller PF. The consequences of not having eosinophils. *Allergy* 2013;68:829-35.
68. Munitz A, Bachelet I, Eliashar R, Moretta A, Moretta L, Levi-Schaffer F. The inhibitory receptor IRp60 (CD300a) suppresses the effects of IL-5, GM-CSF, and eotaxin on human peripheral blood eosinophils. *Blood* 2006;107:1996-2003.
69. Floyd H, Ni J, Cornish AL, Zeng Z, Liu D, Carter KC, et al. Siglec-8. A novel eosinophil-specific member of the immunoglobulin superfamily. *J Biol Chem* 2000;275:861-6.
70. Gangwar RS, Landolina N, Arpinati L, Levi-Schaffer F. Mast cell and eosinophil surface receptors as targets for anti-allergic therapy. *Pharmacol Ther* 2017;170:37-63.
71. Gleich GJ, Adolphson CR, Leiferman KM. The biology of the eosinophilic leukocyte. *Annu Rev Med* 1993;44:85-101.
72. Acharya KR, Ackerman SJ. Eosinophil granule proteins: form and function. *J Biol Chem* 2014;289:17406-15.
73. Davis MD, Plager DA, George TJ, Weiss EA, Gleich GJ, Leiferman KM. Interactions of eosinophil granule proteins with skin: limits of detection, persistence, and vasopermeabilization. *J Allergy Clin Immunol* 2003;112:988-94.
74. Puccetti A, Bason C, Simeoni S, Millo E, Tinazzi E, Beri R, et al. In chronic idiopathic urticaria autoantibodies against Fc epsilon R1/CD23 induce histamine release via eosinophil activation. *Clin Exp Allergy* 2005;35:1599-607.

75. Sakurai Y, Morioke S, Takeda T, Takahagi S, Hide M, Shima M. Increased thrombin generation potential in patients with chronic spontaneous urticaria. *Allergol Int* 2015;64:96-8.
76. Razin E, Marx G. Thrombin-induced degranulation of cultured bone marrow-derived mast cells. *J Immunol* 1984;133:3282-5.
77. Krisinger MJ, Goebeler V, Lu Z, Meixner SC, Myles T, Prydzial EL, et al. Thrombin generates previously unidentified C5 products that support the terminal complement activation pathway. *Blood* 2012;120:1717-25.
78. Cugno M, Asero R, Ferrucci S, Lorini M, Carbonelli V, Tedeschi A, et al. Elevated IgE to tissue factor and thyroglobulin are abated by omalizumab in chronic spontaneous urticaria. *Allergy* 2018;73:2408-11.
79. Tedeschi A, Asero R, Marzano AV, Lorini M, Fanoni D, Berti E, et al. Plasma levels and skin-eosinophil-expression of vascular endothelial growth factor in patients with chronic urticaria. *Allergy* 2009;64:1616-22.
80. Hartman M, Piliponsky AM, Temkin V, Levi-Schaffer F. Human peripheral blood eosinophils express stem cell factor. *Blood* 2001;97:1086-91.
81. Oliveira SH, Lukacs NW. Stem cell factor: a hemopoietic cytokine with important targets in asthma. *Curr Drug Targets Inflamm Allergy* 2003;2:313-8.
82. Grattan CE, Winkelmann RK, Leiferman KM. Eosinophil major basic protein in autologous serum and saline skin tests in chronic idiopathic urticaria. *Br J Dermatol* 1997;136:141-2.
83. Kay AB, Clark P, Maurer M, Ying S. Elevations in T-helper-2-initiating cytokines (interleukin-33, interleukin-25 and thymic stromal lymphopoietin) in lesional skin from chronic spontaneous ('idiopathic') urticaria. *Br J Dermatol* 2015;172:1294-302.
84. Juhlin L, Pihl-Lundin I. Effects of antihistamines on cutaneous reactions and influx of eosinophils after local injection of PAF, kallikrein, compound 48/80 and histamine in patients with chronic urticaria and healthy subjects. *Acta Derm Venereol* 1992;72:197-200.
85. Neves JS, Radke AL, Weller PF. Cysteinyl leukotrienes acting via granule membrane-expressed receptors elicit secretion from within cell-free human eosinophil granules. *J Allergy Clin Immunol* 2010;125:477-82.
86. Oliver ET, Chichester K, Devine K, Sterba PM, Wegner C, Vonakis BM, et al. Effects of an oral CRTh2 antagonist (AZD1981) on eosinophil activity and symptoms in chronic spontaneous urticaria. *Int Arch Allergy Immunol* 2019;179:21-30.
87. Kay AB, Ying S, Ardelean E, Mlynec A, Kita H, Clark P, et al. Calcitonin gene-related peptide and vascular endothelial growth factor are expressed in lesional but not uninvolved skin in chronic spontaneous urticaria. *Clin Exp Allergy* 2014;44:1053-60.
88. Criado PR, Antinori LC, Maruta CW, Reis VM. Evaluation of D-dimer serum levels among patients with chronic urticaria, psoriasis and urticarial vasculitis. *An Bras Dermatol* 2013;88:355-60.
89. Zhang H, Yang H, Ma W, He S. Induction of IL-13 production and upregulated expression of protease activated receptor-1 by RANTES in a mast cell line. *Cytokine* 2011;53:231-8.
90. Magerl M, Staubach P, Altrichter S, Ardelean E, Krause K, Metz M, et al. Effective treatment of therapy-resistant chronic spontaneous urticaria with omalizumab. *J Allergy Clin Immunol* 2010;126:665-6.
91. Maurer M, Church MK, Goncalo M, Sussman G, Sanchez-Borges M. Management and treatment of chronic urticaria (CU). *J Eur Acad Dermatol Venereol* 2015;29(Suppl 3):16-32.
92. Walsh GM. Mepolizumab-based therapy in asthma: an update. *Curr Opin Allergy Clin Immunol* 2015;15:392-6.
93. Magerl M, Terhorst D, Metz M, Altrichter S, Zuberbier T, Maurer M, et al. Benefit of mepolizumab treatment in a patient with chronic spontaneous urticaria. *J Dtsch Dermatol Ges* 2018;16:477-8.
94. Deeks ED, Brusselle G. Reslizumab in eosinophilic asthma: a review. *Drugs* 2017;77:777-84.
95. FitzGerald JM, Bleecker ER, Nair P, Korn S, Ohta K, Lommatzsch M, et al. Benralizumab, an anti-interleukin-5 receptor alpha monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2016;388:2128-41.
96. Bergmann KC, Altrichter S, Maurer M. Benefit of benralizumab treatment in a patient with chronic symptomatic dermatographism. *J Eur Acad Dermatol Venereol* 2019.
97. Kiwamoto T, Kawasaki N, Paulson JC, Bochner BS. Siglec-8 as a drugable target to treat eosinophil and mast cell-associated conditions. *Pharmacol Ther* 2012;135:327-36.