Clinical Commentary Review

The Pathogenesis of Chronic Spontaneous Urticaria: The Role of Infiltrating Cells

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No funding was received for this work.

Conflicts of interest: A. M. Giménez-Arnau received research grants supported by Uriach Pharma, Novartis, grants from Instituto Carlos III- FEDER; is an advisor for Uriach Pharma, Genentech, Novartis, FAES, GSK, Sanofi–Regeneron, Amgen, and Thermo Fisher Scientific; and performs educational activities in Uriach Pharma, Novartis, Genentech, Menarini, LEO-PHARMA, GSK, MSD, Almirall, and Sanofi. L. DeMontjoye is a medical investigator/advisor and educational activities for Novartis. Y. Yanase received honorarium from Novartis. M. Hide received research grant from GlaxoSmithKline, Kaken Pharmaceutical, Mitsubishi-Novartis, Sanofi, Taiho Pharma, and Mitsubishi-Tanabe; and received honorarium from Kaken-Pharmaceutical, Kyowa Hakko Kirin, Mitsubishi-Tanabe, MSD, Novartis, Sanofi, Taiho Pharma, Teikoku Seiyaku, and Uriach. A. P. Kaplan is a consultant for Novartis, Genentech, BioCryst, Allakos, Celldex, and Pharvaris; is a lecturer (teaching program) in Pharming and Novartis; and does adjudications of allergic reactions in drug trails (blinded) for Novartis, Genentech, Abb-RISA, and Sanofi-Aventis. None of these pose a conflict of interest regarding this manuscript. The rest of the authors declare that they have no relevant conflicts of interest.

process. Although the role of the cellular infiltrate has not previously been addressed, each constituent can contribute to the overall pathogenesis. It is of interest that CSU responds to corticosteroid, yet, short-term steroids do not affect autoimmunity or degranulation of mast cells, and act on margination of cells along the endothelium and chemotaxis to enter the surrounding dermis. In this review, we address each cell's contribution to the overall inflammatory response, as it is currently understood, with a view toward development of therapeutic options that impede the function of critical cells and/or their secretory products.

Key words: Lymphocyte; Eosinophil; Basophil; Endothelial cell; Neuroinflammation

Abbreviations used: AAb, Autoantibody; ASST, Autologous serum skin test; CGRP, Calcitonin gene-related protein; CIU, Chronic idiopathic urticaria; CSU, Chronic spontaneous urticaria; FIIa, Factor IIa; FXa, Factor Xa; HUVEC, Human umbilical vein endothelial cell; ICAM, Intercellular adhesion molecule; IFN-γ, Interferon gamma; IL, Interleukin; LCN2, Lipocalin 2; LPR, Late-phase reaction; LPS, Lipopolysaccharide; MBP, Major basic protein; MC, Mast cell; MCP, Monocyte chemotactic protein; NK, Neurokinin; PAF, Platelet-activating factor; PAR, Protease-activated receptor; PBMC, Peripheral blood mononuclear cell; PECAM, Platelet endothelial cell adhesion molecule; RANTES, Regulated upon activation normal T-cell expressed and secreted; SCF, Stem cell factor; SHIP, Src-homology-2 containing 5 inositol phosphatase; TF, Tissue factor; TGF-β, Transforming growth factor beta; Th, T helper cell; TNF, Tumor necrosis factor; Treg, Regulatory T cell; VCAM, Vascular cell adhesion molecule; VEGF, Vascular endothelial growth factor

The pathogenesis of chronic spontaneous urticaria (CSU) typically focuses on mechanisms by which cutaneous mast cells (MCs) may be activated to initiate the process. So far there are multiple possibilities; however, all have in common the presumption that the disorder is fundamentally autoimmune and therefore is not driven by exposure to any exogenous agent. The autoimmune process is viewed as serologic but leading to a perivascular infiltration (cellular) about small venules in the skin. IgG antibody may react with the IgE receptor (30%-45%) or IgE itself (5%-10%), activate the MC, fix complement via the classical pathway, and achieve further MC activation by the interaction of C5a with the C5a receptor. Alternatively, IgE antibody may react with a host of autoantigens (eg, interleukin [IL]-24) although one associated with the MC surface is not yet defined. Here too activation can occur that is more closely related to a typical allergic reaction, that is, autoallergy.

These mechanisms have received much attention, and therapies directed to this enhanced MC function are routinely used and are effective. These would include omalizumab and cyclosporine.

There is however another facet to this disease that is largely unexplained yet may be no less pathogenically relevant, namely, the particular cellular infiltrate containing a mixture of T lymphocytes (a mixture of T helper [Th]1 and Th2 subtypes with Th2 being the more prominent), monocytes, eosinophils, basophils, variable numbers of neutrophils, and lymphoid subtypes not yet well characterized. Migration of all of these cells from the blood to the tissue requires a chemotactic response for each and interaction with activated endothelial cells, the latter being a key participant in the process. One piece of evidence that this process is of particular importance is the response to corticosteroid, at least acutely. Steroids appear to have no effect on any of the steps in the autoimmune activation of MCs but do eliminate the chemotactic response that produces the cellular infiltrate. Thus, targeting the infiltrate can work as an effective therapy; however, steroids are the only one that does so and are too toxic for sustained use, and cyclosporine might downregulate this cellular response but acts on MCs and basophils as well.

In this review, we will focus attention on each of the major constituents of the cellular infiltrate, assess the potential contribution of each (including endothelial cells) to the urticarial process, and in addition, will address any role for the innervation of the skin, particularly sensory neurons, to complete our assessment of non-MC contributors to CSU.

Lymphocytes and Involved Cytokines in CSU Pathogenesis

T-cell involvement in wheal pathogenesis

A wheal is the consequence of “actors” involved at different times along its dynamic evolution. The pathogenic relevance of lymphocytes *per se* has not been commonly addressed. In this section, we review current knowledge of its role in the genesis of the wheal.

Cytokine expression has been studied in patients with CSU, in plasma, serum, peripheral blood mononuclear cells (PBMCs), and skin. Naïve T cells can differentiate into Th1, Th2, Th17, Th9, Th22, and regulatory T (Treg) cells depending on the environment, cell interactions, and cytokines. The role of autoimmunity is prominent, with disorders produced by Th1, Th17, and Th22 cells. Because key effector cells in CSU are MCs and basophils, the cytokines acting on them will be explored: either cytokines implicated in cell maturation, or in the activation of MCs and/or basophils. Finally, because IgE is implicated in CSU, and more specifically, IgE autoantibodies (AAbs), cytokines inducing class switching in B cells will also be reviewed.

The primary function of Th1, Th2, and Th17 cells is host defense. Yet Th1 cells and related cytokines can function as proinflammatory effectors in autoimmune disorders.[1] Th2 and Th9 cells are mainly implicated in allergic

diseases, such as asthma and allergic contact dermatitis,[2-4] and some Th2/Th9 cytokines are able to induce the switch of human B cells to produce IgE or to promote MC, basophil, and eosinophil activation. A role for Th17 and Th22 cells in inflammatory and autoimmune disease is well known.[5] IL-17 cells promote recruitment of monocytes and neutrophils and lead to chronic neutrophilic inflammation. Th22 cells can also play either a protective or a detrimental role in autoimmune diseases, depending on the disorder. Finally, Tregs, which produce transforming growth factor beta (TGF-β) and IL-10, are another subset of effector T cells that modulate the immune response to maintain tolerance against self-antigens and thus prevent development of autoimmune diseases.[6]

Th17 cytokines

According to the hypothesis of an autoimmune pathogenesis, Th17 would seem the most likely T helper cytokine profile implicated. Maintenance, survival, and activation of Th17 cells are dependent on IL-23.[7] Th17 cells produce many cytokines, such as IL-17, IL-6, IL-8, IL-22, IL-23, and tumor necrosis factor-α (TNF-α). Among these cytokines, IL-6 is the most commonly studied in CSU and higher IL-6 levels were found both in blood and in skin biopsies of patients.

IL-6 is produced mainly by innate immune cells (macrophages, dendritic cells, and MCs), B cells, and to a lesser extent by Th17 cells and eosinophils.[8] IL-6 is implicated in chronic inflammatory or autoimmune diseases.[9] It promotes Th17 cell lineage and maturation of B cells and plasmablasts and inhibits the induction of Tregs.[9] IL-6 interacts with hepatocytes and induces proteins involved in the acute phase of inflammation such as CRP and fibrinogen. IL-6 is able to increase MC proliferation and promote a more reactive phenotype of MC and induce its chemotaxis.[10-12]

IL-6 seems clearly elevated in the sera or plasma of patients with CSU,[13-24] although not always confirmed.[25-27] Moreover, IL-6 levels correlate with disease activity[13,17-21,23] and has been proposed as a biomarker.[28-30] However, no correlations were seen with autologous serum skin test (ASST) results[14,17-19,26] (Table I).

Table I Summary of reports studying circulating cytokines in plasma or serum of patients with CSU compared with HCs

Cytokine	Patients with CSU vs HCs			Correlation with clinical activity/severity of CSU			Correlation with ASST		
	Increase	Decrease	No difference	Positive	Negative	No correlation	Increased in ASST+	Increased in ASST−	No correlation with ASST
IL-6	13-24		25-27	13,17-21,25		14,27			14,17-19,26
TNF-α	13,14,16,17,21,31,32			7,13,14		21,32	7		14
TSLP		33	25						
IFN-γ	14,15,34		35	14					14
IL-31	36		25			25,26,37			
IL-10	16,21		14,33,38			14,21	14		
TGF-β	14,31,39		38	39		14			14
IL-17	36,39,40		14,41	7,36,39,40		14	7,14		
IL-23	14			7,14			7		14
IL-13	14					14			14
IL-4	35,42	33	14,31			14			14,43
IL-2	13			14					14
IL-5			14,35	14					14
IL-12p70	14,16					14			14
IL-21	14	33		14					14
TNF-β	14			14					14
IL-1β	16		14,31			14			14

IL-22			14			14	14		
IL-28A			14			14			14
IL-35		44							
IL-9	45		25,31			31,45			
IL-15		46				46			
IL-8	47			47					47
IL-18	48		25,26			25,48			26,48
IL-33	36		25,31,39	36		25,39			
IFN-λ1	33								
IL-17A	36,39,40		31	36,39,40	14,38		36,39,40		
IL-17F			14			14			14
GM-CSF			14			14	14		
MIP3a			14			14			14
IL-24	50			50					

Comparison was made for cytokine plasma/serum level between patients with CSU and HCs. For patients with CSU, correlation of cytokine plasma/serum level with disease activity/severity, with ASST response, was investigated. Only statistically significant data with a *P* value of <.05 were included. If the difference between the 2 groups analyzed was ≥.05, it was reported in "no difference."

ASST, Autologous serum skin test; *CSU*, chronic spontaneous urticaria; *GM-CSF*, granulocyte-macrophage colony–stimulating factor; *HC*, healthy control; *IFN*, interferon; *IL*, interleukin; *MIP3a*, XXX; *TGF*, transforming growth factor; *TNF*, tumor necrosis factor.

Recently, mRNA microarray analyses showed an upregulation of IL-6 expression in spontaneous urticarial wheals compared with unaffected skin.[51,52] To date, no trials have been performed to assess the effectiveness of anti–IL-6 therapy in CSU, for example, with tocilizumab that can also effectively reduce immunoglobulins.[53-55] In systemic mastocytosis, a JAK1/JAK2 inhibitor, ruxolitinib (which inhibited the production of IL-6 among other cytokines), showed a convincing decrease in MC-related symptoms.[41,56,57]

IL-17 mediates adverse effects in some autoimmune diseases.[58] Neutrophils are found in urticarial wheals, and IL-17 could contribute to this neutrophil infiltrate. Unfortunately, in CSU, serum levels of IL-17 are not consistent[36,39,40] with positive or negative[14,38] correlation with disease activity. However, in this instance, a positive correlation with ASST was shown.[7,14] Recently, increased Th17 and IL-17 expression in both CD4+ T cells and MCs, residing in close proximity to each other, was described in the skin of patients with severe CSU.[59] In an open study, all the patients (n = 8) treated with the anti–IL-17A antibody, secukinumab, showed significant improvement in CSU disease activity.[60]

The upregulation of IL-beta (IL-1β) mRNA expression in skin samples of urticarial wheals deserves to be investigated further. IL-1β is a multifunctional cytokine, primarily of the innate immune system, and MCs are a source of IL-1β. Dysfunction of IL-1β pathways is implicated in autoinflammatory disorders.[61] In CSU, data regarding IL-1β are few[31] and contradictory results were found concerning IL-1β serum levels (Table I). IL-1β mRNA expression was observed in skin biopsies of positive ASST tests but not in patients with negative ASST and was also upregulated in the spontaneous wheals and PBMCs from patients with CSU. IL-1β induces neutrophil recruitment and extravasation, promotes expansion of Th1 and Th17 cells, and induces a downregulation of Tregs.[62]

IL-22 belongs to the IL-10 cytokine family and is expressed in several chronic inflammatory or autoimmune diseases[63,64] including psoriasis.[65,66] No difference between IL-22 serum levels in patients with CSU versus normal controls was found, whereas patients with CSU with a positive ASST tended toward higher IL-22 serum levels.[14]

IL-23 induces polarization, maintenance, survival, and activation of Th17 cells and secretion of IL-17 by non–T cells.[7,67] Even if data are scarce, it seems interesting to further explore the role of IL-23 in CSU (Tables I and II).[7,14,70] Interestingly, IL-23 serum levels were found to be higher in patients with CSU,[7,14] supernatants of their PBMCs,[7,70] and in those with a positive ASST compared with a negative ASST.[7]

Table II Summary of reports studying cytokine expression in (cultured) PBMCs of patients with CSU (including mRNA expression and protein expression in supernatant) compared with HCs

Cytokine	Patients with CSU vs HCs			Correlation with ASST		
	Increase	Decrease	No difference	Increased in ASST+	Increased in ASST−	No correlation with ASST
IL-6	68			68		
TNF-α	43			43		
IFN-γ	35	69	16,43,70			43,70
IL-10	43,70	16	16,71			43,70
TGF-β			70			70
IL-17			16			
IL-23	7,70			7		70
IL-4	35,70	69,71	16,43	70		14,43
IL-2	16	71	43			43
IL-17A			70			70
IL-24	50					

Comparison was made for cytokine expression between patients with CSU and HCs. For patients with CSU, correlation of cytokine with ASST response was investigated. Only statistically significant data with a *P* value of <.05 were included. If the difference between the 2 groups analyzed was ≥.05, it was reported in "no difference."

ASST , Autologous serum skin test; *CSU* , chronic spontaneous urticaria; *HC* , healthy control; *IFN* , interferon; *IL* , interleukin; *PBMC* , peripheral blood mononuclear cell; *TGF* , transforming growth factor; *TNF* , tumor necrosis factor.

TNF-α is quickly released on activation because it is stored in granules of MCs and induces neutrophil recruitment.[72] Elevated TNF-α serum levels[13,14,16,21,32] often correlate with disease activity[7,13,14] although ASST correlation was variable[7,14] (Table I). Conflicting results were found concerning levels of TNF-α in supernatants of cultured PBMCs[16,43] (Table II). TNF-α has been shown to be elevated in spontaneous wheals and uninvolved skin of patients with CSU versus skin of normal controls. [73] A few case reports and small controlled studies showed the efficacy of TNF- α inhibitors in CSU.[33,74,75]

Th1 cytokines

Th1 cells mainly produce interferon gamma (IFN-γ). In CSU, data on detection of IFN-γ in serum are contradictory and thus unconvincing (Table I).[14,15,34,35] Levels of IFN-γ in supernatants of stimulated PBMCs are shown in Table II.[16,35,43,69,70] IFN-γ expression was found to be elevated in spontaneous wheals versus control skin,[76] with fewer numbers of cells expressing IFN-γ as compared with cells expressing IL-4 or IL-5. Inconsistent results were also found when the nonlesional skin of patients with CSU was compared with spontaneous wheals, or control skin.[77]

Th2 cytokines

Th2 cells produce many cytokines, mainly IL-4, IL-5, and IL-13, but also IL-9, IL-24, IL-31, and IL-33. Th2 cells are mainly implicated in allergic diseases, stimulating IgE production and MC, basophil, and eosinophil activation.

IL-4 is produced by Th2 cells and also by basophils and MCs.[42,78] IL-4 contributes to IgE synthesis induced by activated CD4+ or CD8+ T cells.[79] IL-4 also upregulates the expression of high-affinity IgE receptors (FcεRI).[80] Circulating IL-4, as well as IL-4 produced by PBMCs, does not seem elevated in the majority of patients with CSU and here too results are conflicting (Tables I and II).[33,35,42,69-71] Furthermore, no correlation with disease activity or ASST could be made.[14,43] Nevertheless, IL-4 was elevated in skin biopsies of spontaneous wheals compared with control skin.[76] Like IL-4, IL-13 induces class switch of B cells to IgE,[42,81] but IL-13 serum levels in patients with CSU do not correlate with disease activity.[14] Recently, the efficacy of dupilumab (monoclonal antibody blocking IL-4 and IL-13) was reported in 6 patients with CSU.[82] A phase 2a multicenter (NCT03749135) and a phase III study, CUPID, are ongoing in CSU.

IL-5 primes the eosinophil responses to chemokines and is important for their development and survival. IL-5 produced by MCs has autocrine effects by promoting MC proliferation in the presence of stem cell factor (SCF) and

increasing cytokine production.[37] IL-5 serum levels in patients with CSU are conflicting[14,35] (Table I); however, IL-5 expression is increased in wheals compared with nonlesional skin and control skin[76] (Table III).

Table III Summary of reports studying cytokines in wheals of patients with CSU

	vs HC skin	vs uninvolved skin (CSU)
IL-4	↑ [76]	ND[76]
TNF-α	↑ [73]	↑ [73]
IL-5	↑ [76]	↑ [76]
IFN-γ	↑ [76]	ND[76]
IL-17	↑ [59]	
IL-3	↑ [73]	↑ [73]
IL-13	ND[76]	ND[76]
IL-33	↑ [76,77]	↑ [76,77]
IL-25 IL-24 I	↑ [76]	↑ [76]
	↑ [50]	

Only statistically significant data with a *P* value of <.05 were included. If the difference between the 2 groups analyzed was ≥.05, it was reported in "no difference."

CSU, Chronic spontaneous urticaria; *HC*, healthy control; *IFN*, interferon; *IL*, interleukin; *TNF*, tumor necrosis factor.

IL-24 belongs to the IL-10 cytokine family involved in autoimmune diseases and skin inflammation.[49,63,65,83,84] The IL-24 gene and protein expression are increased in PBMCs of a subset of patients with CSU. It is detected in spontaneous urticarial wheals, but not those induced by autologous serum injection (ASST).[50] Interestingly, Schmetzer et al[85] detected IgE against IL-24 in the sera of patients with CSU and correlated this directly with CSU activity. They also showed that, *in vitro*, exogenous IL-24 induced histamine release from human MCs sensitized by IgE against IL-24 from patients with CSU, but not with IgE from normal controls.[85]

IL-31 is produced by Th2 cells and MCs. IL-31 induces basophil recruitment and secretion of IL-4 and IL-13. IL-31 also stimulates eosinophils to produced proinflammatory cytokines such as IL-6, and chemokines,[86] and it is a mediator of pruritus apart from that seen with histamine. Table I shows IL-31 to be higher in CSU.[36] IL-31 can be modulated by omalizumab treatment.[87]

IL-33 is a member of the IL-1 superfamily.[88] IL-33 induces CD4+ T cells, MCs, eosinophils, and basophils to produce type 2 cytokines. IL-33 could be produced by MCs and causes their activation as well as their maturation. IL-33 therefore regulates IgE-dependent inflammation. Existing data with regard to IL-33 blood levels in CSU are variable[25,31,36,48,49] (Table I). However, Kay et al[77] found higher IL-33+, IL-25+, and TSLP+ cells in spontaneous wheals than in uninvolved skin and normal controls.

Th9 cytokines

IL-9 is produced by Th9 cells, eosinophils, neutrophils, and MCs. IL-9 is a stimulus for recruitment of MCs.[89] Experimental models in mice suggest that IL-9 and IL-10 contribute to CSU development via activation of the JAK/STAT signaling pathway.[90] One study found elevated IL-9 levels in patients with CSU[45] (Table I).

Regulatory cytokines

Tregs produce mainly IL-10 and TGF-β. In CSU, circulating Tregs (CD4+CD25+FOXP3+) seem reduced and/or defective compared with normals.[91] The reduced frequency of Tregs is consistent with an autoimmune hypothesis for CSU.[92-94] IL-10 also has inhibitory effects on eosinophil survival. Yet in CSU, IL-10 expression is not clearly reduced. Data concerning the IL-10 expression in patients with CSU versus controls or mRNA levels in PBMCs have no consistent pattern[14,16,21,33,38,43,70,71] (Tables I and II).

TGF-β1 in skin could act as an anti-inflammatory cytokine by reducing the expression of FcεRI on the MCs. In studies investigating the role of TGF-β1 in CSU, findings are interesting but not sufficient to evaluate its role in CSU pathogenesis[14,31,38,39,70] (Table I).

To conclude, new studies are in progress regarding the role of T cells and cytokines in the wheal pathogenesis. Recently, IL-6 and IL-1β mRNA expression was increased in skin biopsies of positive ASST versus negative ASST in patients with CSU. IL-6 and IL-1β mRNA expression was upregulated in spontaneous wheals and PBMCs from patients with CSU.[68] Cytokines such as TNF-α, IL-4, or IL-5 are involved in wheal pathogenesis. Data concerning others cytokines, for instance, IL-8,[47] IL-13, IL-15,[46] IL-21,[46] IL-22, IL-23, IL-24,[50] and IL-35,[44] are insufficient to allow conclusions, but they deserve to be investigated. This knowledge may lead to the development of future biotherapies.

Basophils

The basophil is thought to have an important role in the pathogenesis of CSU given its similarities to the MC as a major source of histamine and expression of the high-affinity receptor for IgE. Developmentally, however, they differ substantially. Basophils, as a granulocyte, shares a common precursor with the eosinophil[95] whose final stages of differentiation are dependent on IL-3 and IL-5, respectively, whereas the precursor of the MC is derived from fetal tissue (connective tissue MCs including those of skin) and within the bone marrow (inducible mucosal MCs) as separate lineages,[96] more closely related to the monocyte. Their development is dependent on the interaction of SCF with c-kit as well as TGF-β. Final differentiation of all MC subtypes occurs within the tissue to which they migrate, so that cutaneous MCs differ in many respects from MCs elsewhere.

Basophils account for approximately 1% of circulatory leucocytes and are not normally found in tissues unless they migrate there, a process dependent on cytokines and chemokines (chemotactic cytokines). Here there is considerable overlap with those factors causing eosinophil migration. Among those are VLA expression for binding to endothelial cell vascular cell adhesion molecule (VCAM)-1[97] and responsiveness to chemokines monocyte chemotactic protein (MCP)-1, MCP-3, MCP-4, regulated upon activation normal T-cell expressed and secreted (RANTES), the eotaxins, the prostaglandin D2 receptor CRTH-2, and the anaphylatoxin C5a.[98-100]

Basophil number and functional properties appear to be altered in patients with CSU. Within the circulation, basophil numbers are depleted in at least 50% of patients[101,102] and may relate to severity of disease.[103] Furthermore, when one checks basophil function employing a stimulant that acts thru the IgE receptor (rabbit anti-IgE receptor is often used), the basophils are hyporesponsive[104] as if they had been desensitized. Activation thru some other receptor is normal, for example, histamine release due to C5a interaction with the C5a receptor. The most likely explanation for the basopenia is migration of basophils from the circulation to the skin[101] where the basophils are likely active because chemotactic factors are typically secretagogues. Degranulated basophils within the circulation may also be missed.[105] The hyporesponsiveness of circulating basophils appears due to increased levels of intracellular phosphatases (particularly src-homology-2 containing 5 inositol phosphatase [SHIP]-1 and SHIP-2) that remove phosphate residues from key signal transduction molecules that are required for normal responsiveness.[106] Decreased basophil CRTH2 receptors for PGD_2 likely reflect internalization due to PGD_2 exposure[107] consistent with increased expression of other markers associated with basophil activation.[108] The cause of the increased phosphatases is unknown, but its importance is highlighted by the changes observed during symptom reversal. Whether improvement is spontaneous, that is, remission, or induced by therapy such as use of omalizumab, the basopenia reverses and the basophils become more responsive to anti-IgE.[102,109] Although these changes are coincidental with improvement, it is perhaps paradoxical that basophil histamine release (using circulating basophils) increases at a time that urticarial lesions in the skin quiet down. The functional basophil phenotypes (normal or hyporesponsive) remain stable in symptomatic subjects and are unrelated to the presence or absence of AAbs.[110] Such basophils appear to have increased surface expression of FcεRIα, which correlates with elevated IgE levels, and upregulation of responsiveness to various agonists if exposed to IL-3[111,112] as reported previously for basophils in general[113] and did correlate with a positive ASST[111] typically reflective of the presence of IgG AAb to FcεRIα.

Basophil donors from normal subjects have typically served as an indicator of the presence of antibodies to the high-affinity IgE receptor[114] that activate basophils as well as MCs.[114,115] The incidence of such antibodies varies within the literature from 25% to 45% in patients with CSU and an additional 5% to 10% in those who have IgG anti-IgE. The antireceptor antibody–FcεRIα interaction activates the classical complement pathway[116] to liberate C5a,[117] which substantially augments histamine release when compared with the purified AAb. Most patients have IgG_1 and/or IgG_3 subclasses of antireceptor antibody as expected.[118] The second most common IgG subclass is IgG_2, which when isolated did not cause histamine release although they were often positive by immunoblot versus the α chain of the IgE receptor emphasizing the possibility of false-positive binding results using ELISA methodology where the incidence of such antibodies is reported as 55% to 65%.[110] Of particular note regarding specificity is the increased incidence of such antibodies in a variety of autoimmune diseases[119] all of which were negative for histamine release, the sole exception being positives for histamine release seen in patients with SLE in a later study,[120] yet below the incidence seen in CSU. Basophils can also be employed to verify the presence of IgE antibodies to putative autoallergens (autoallergy) by passively sensitizing normal basophils with patients' IgE[85] and adding antigen or adding the putative autoantigen to patients' basophils known to have the requisite IgE antibody present. A cell surface autoantigen or skin autoantigen that can stimulate MCs or basophils is not yet identified.

Eosinophils in CSU

Evidence for eosinophil involvement in CSU

Eosinophils are multifunctional leukocytes involved in the host defense against infections as well as in repair of injured tissue; however, eosinophils also play critical roles in the pathophysiology of allergic and inflammatory diseases.[76] Although many allergic and inflammatory conditions are associated with a peripheral blood eosinophilia, circulating eosinophil numbers are only rarely increased in patients with CSU,[121] even if these cells are always present in the perivascular cellular infiltrate that characterizes the disease, particularly in lesional skin.[121,122] Recent studies show that the recruitment of eosinophils in the skin may lead to blood eosinopenia[122] by a mechanism that

resembles very much the one leading to the much more frequent basopenia. The recruitment of eosinophils in the skin is mediated by a number of cytokines including IL-5, eotaxin 1, eotaxin 2, MCP-3, chemokine C-C motif ligands 4, and RANTES, released by several cell types, including Th2, type 2 innate lymphoid cells-2, MCs, dermal fibroblasts, endothelial cells, and basophil granulocytes.[123] Once migrated into the skin, eosinophils appear all but an innocent bystander, and the more they are studied the clearer it appears that they play an active role within the inflammatory network that characterizes chronic urticaria. In fact, there is little doubt that in CSU, skin eosinophils are activated. The eosinophil-derived major basic protein (MBP) can be measured in urticarial lesions even when eosinophils cannot be detected.[124] Interestingly, this seems the case mainly in patients lacking IgG AAbs to the high-affinity IgE receptor (ie, those patients who do not have a type IIb autoimmune urticaria after the Gell-Coombs classification),[121] which we now know have in several cases an IgE-mediated autoimmune disease (ie, a type I autoimmune [autoallergic] urticaria). Interestingly, MBP has been detected in ASST site biopsies, along with eosinophil infiltration.[125] Notably, the MBP is able to induce MC activation and degranulation,[126] thus representing one possible mechanism for histamine release. The activation of eosinophils appears to occur via different mechanisms in CSU. One is an autoimmune mechanism mediated by circulating IgG AAbs directed against the low-affinity IgE receptor expressed on the surface of these cells. These AAbs have been detected in approximately 65% of patients with chronic urticaria,[127] and their presence was confirmed recently.[128]

Eosinophil interactions with other cells in CSU

In addition, eosinophils may be recruited and activated by several mediators and cytokines released by activated MCs. These include IL-5, TNF-α, platelet-activating factor (PAF), and eotaxin.[129,130] The MC/eosinophil cross-talk sustaining a reciprocal recruitment and activation is also shown by the secretion of SCF, a growth and maturation factor for MCs, released by activated eosinophils.

Although other cell types such as endothelial cells[131] and peripheral monocytes[132] may also show this feature, eosinophils are able to store tissue factor (TF) and to transfer it rapidly to the cell membrane on activation.[133] The potentially relevant role played by these cells in the pathogenesis of CSU has been further highlighted by the observation that patients with a severe disease are characterized by an intense activation of the extrinsic pathway of the coagulation cascade.[134-136] Immunohistochemical experiments using double staining showed that in CSU skin, TF colocalizes with eosinophil cationic protein, a classic cell marker of eosinophils.[137] Notably, several activated coagulation factors (eg, thrombin, and FVIIa/factor Xa FXa/TF complex) are able to stimulate histamine release from MCs via the activation of protease-activated receptor (PAR)-1 and PAR-2 receptors, respectively.[138,139] Activated coagulation factors can induce MC and basophil degranulation by activating fraction 5 of complement.[140] Thrombin-induced inflammation is probably limited by the MCs themselves via the secretion of protease 4 in a sort of feedback mechanism.[141]

Finally, a significant increase in vascular endothelial growth factor (VEGF) has been detected in patients with CSU.[142] VEGF is the most potent regulator of angiogenesis presently known and one major mediator of vascular permeability; furthermore, it exerts a vasodilator effect through an increase of nitric oxide production by endothelial cells.[143] VEGF was found to stimulate the activation of the extrinsic coagulation pathway, including modulation of TF expression[144] and intercellular gap formation on vascular endothelial cells, an effect that was synergistically increased in the presence of TNF-α and lipopolysaccharide (LPS).[145] In patients with CSU, the main source of VEGF is eosinophils.[142]

Clinical relevance

The activation of eosinophils in urticaria lesional skin and the subsequent initiation of the coagulation cascade may have clinical effects not only in eliciting wheal eruption, but also at the systemic level. However, patients with CSU are not reported to have an increased risk for thrombotic events probably due to the fact that the activation of coagulation occurs mostly locally (ie, extravascularly[145]), where thrombin may contribute to increasing vascular permeability and inducing MC degranulation. These findings have provided the rationale of both controlled studies and case series supporting the effectiveness of both oral anticoagulants and heparin in some patients with refractory CSU.[146-148]

Another potential clinical implication of eosinophil involvement in CSU may be related to the presence of AAbs of the IgG class to the low-affinity IgE receptor that is expressed on eosinophils[127,128] and AAbs of both IgG and IgE classes to TF in a proportion of patients with CSU[128] that may influence the clinical response to the anti-IgE therapy.

Finally, interestingly, mepolizumab (anti–IL-5) and benralizumab (anti–IL-5 receptor), 2 monoclonal Abs leading to the abolition of eosinophil activity by similar mechanisms, were found to be highly effective in a proportion of patients with severe, unremitting urticaria. With benralizumab, 5 of 12 patients were asymptomatic and 2 others markedly improved, similar to early studies of omalizumab.[149]

In conclusion, there is ample evidence supporting the notion that eosinophils are leading actors in the inflammatory processes that underlie CSU.

The Role of Neutrophils in CSU

The evidence for PMN involvement in CSU

The presence of neutrophil infiltration in urticarial skin lesions of patients with CSU has been demonstrated by many studies. Sánchez and Benmamán[150] reported 3 histopathological patterns in 36 patients with CU:

neutrophilic, lymphocytic, and mixed type, but there was no specific association with the clinical presentation. Sugita et al[151] reported an apparent infiltration of neutrophils in 94% of biopsied wheals from 35 patients with chronic idiopathic urticaria (CIU). However, in only 5 patients were neutrophils, the predominant cell, seen. In cases where eosinophil infiltration predominates, neutrophils are still the dominant leucocyte in blood and there was no eosinophilia. Martins et al[152] reported 2 histological groups: (1) predominance of neutrophils or eosinophils (42.4%) and (2) predominance of lymphocytes (57.6%) in 93 patients with CSU. There was no significant correlation between histological group and laboratory tests.

For autologous serum-induced wheals in chronic autoimmune urticaria, Grattan et al[153] performed serial biopsies of ASST wheals. Perivascular neutrophils and eosinophils were seen as early as 30 minutes, becoming more intense and diffuse over 2 hours. T lymphocyte numbers were increased by 2 hours. By 48 hours, the neutrophils were clearing, but eosinophils and lymphocytes persisted. The inflammation resembled the late phase of IgE-mediated immediate hypersensitivity reactions. Caproni et al[154] examined 28 patients with CU with positive ASST at different evolutive stages, measuring expression of CD3, CD4, CD8, tryptase, eosinophil cationic protein, myeloperoxidase, basophil granular protein, IL-4, IL-5, IL-8, CCR3 and CXCR3, intercellular adhesion molecule (ICAM)-1, VCAM, and ELAM. ASST-induced wheals involve a prevalent role of lymphocytes, with strong neutrophil infiltration and activity and involvement of CXCR3 and CCR3 chemokines. For spontaneous wheals, Sabroe et al[123] studied biopsy specimens from wheals present for less than 4 hours and greater than 12 hours in 22 patients with CIU. Neutrophil and eosinophil accumulation occurred early in the evolution of a wheal, but eosinophil activation might occur later or be more persistent in patients without AAbs against FcεRI and/or IgE. Another study of 37 patients with CU who were positive or negative for anti-IgE receptor antibodies had neutrophil predominance in 28% and 47%, respectively.[155]

Neutrophils accumulate in allergic late-phase reactions (LPRs)

A recent study suggested the role of neutrophils as antigen-presenting cells in LPRs.[156] After a cutaneous allergen challenge, there were significant increases in the numbers of intradermal CD3+, CD4+, CD8+, and CD25+ T cells, eosinophils, neutrophils, basophils, and macrophages in patients with CSU compared with nonatopic control subjects.[76] The profile of inflammatory infiltrate of CSU was similar to that of allergen-induced skin LPR[76] with a mixed T-cell subclass distribution of Th1 and Th2 cells as was also reported in biopsies of ASST reactions.[154] Later, another study reported increased expression of Th2-initiating cytokines (IL-33, IL-25, and thymic stromal lymphopoietin) in the lesional skin of patients with CSU.[77]

How are PMN recruited in the skin?

The major mediators involved in the recruitment of neutrophils to sites of active inflammation included bacterial components, C3a and C5a, TNF-α, IL-17, CXCL8/IL-8, IL-36, IL-1, lipocalin-2 (LCN2), leukotriene B4, PAF, plasma kallikrein, matrix metalloproteinases, and myeloperoxidase inhibitors.[157] Lesional skin in CSU had significantly more CD31+ endothelial cells, CD31+ blood vessels, neutrophils, and eosinophils than uninvolved skin, suggesting a link between inflammatory cells and vascular leakage.[158] Basophils and macrophages and CD3+ T cells were also increased but less marked. This may explain how in CSU, as with the LPR, MC activation leads to the release of IL-8 and eotaxin, chemotactic factors for neutrophils and eosinophils, respectively.[158-161] Another study reported that lesional skin in CSU contained significantly more calcitonin gene-related protein (CGRP+) and VEGF+ cells than nonlesional skin.[162] CGRP was expressed mainly by neutrophils and eosinophils. Increased expression of CGRP and VEGF was detected only in lesional skin that indicated that they might have a role in wheeling and tissue edema.

The levels of neutrophil activation and related cytokines were studied in 88 patients with ASA-intolerant CU. The serum levels of myeloperoxidase and IL-18 were higher than normal controls.[163] Another study reported mean levels of serum LCN2, TNF-α, IL-6, and IL-10 that were significantly higher in 191 patients with CU than in controls.[164] LCN2 levels decreased significantly in refractory CU compared with patients who responded to antihistamines. Increased serum LCN2 in patients with CU was associated with a decrease in urticarial activity scores.

What is the clinical relevance?

Elevation in the absolute neutrophil count and neutrophil/lymphocyte ratio was reported to be associated with a poor prognosis in childhood CSU, and the neutrophil/lymphocyte count can be used as a remission marker.[165] A study of 74 patients with CSU receiving omalizumab 300 mg every 4 weeks for at least 1 year resulted in a substantially increased peripheral blood basophil count and reduced peripheral blood neutrophil especially in the first 3 months.[166] The authors proposed that the alterations in peripheral blood cell ratios might contribute to or reflect the therapeutic effect of omalizumab in CSU. In another retrospective study of 74 patients with CSU receiving omalizumab 300 mg every 4 weeks, the neutrophil count, the neutrophil/lymphocyte ratio, and the neutrophil/monocyte ratio showed a statistically significant reduction after 12 weeks of omalizumab treatment.[167]

Taken together, the data suggest that neutrophils might contribute to the pathogenesis of CSU, at least in a subgroup of patients; however, mechanistic details are not yet known.

The role of vascular endothelial cells in CSU

Plasma leakage from skin microvessels in the dermis is crucial to produce edema and the flare reaction, clinically recognized as wheals of urticaria. The critical role of histamine released from skin MCs and/or basophils for vascular permeability in the pathogenesis of CSU has been proven by the effectiveness of H1 antihistamines. Besides histamine, other mediators, such as VEGF, thrombin, bradykinin, and/or PAF, may induce plasma leakage through

gap formation of vascular endothelial cells via specific receptors.[168] Moreover, an increase of leukocytes, such as eosinophils, neutrophils, and basophils, is observed in the lesional skin of CSU.[137,138,169]

To date, several reports suggest that the expression levels of cell adhesion molecules, such as P-selectin, E-selectin, ICAM, VCAM, and platelet endothelial cell adhesion molecule (PECAM, CD31), are upregulated in the lesional skin of patients with CSU.[158,170,171] Histamine and thrombin rapidly induce surface expression of P-selectin stored in endothelial cells within few minutes.[182] Moreover, inflammatory cytokines, such as TNF-α and IL-1β, contribute to the late phase (4-24 hours) expression of P-selectin, E-selectin, ICAM, and VCAM on the surface of endothelial cells,[172,173] Because P-selectins and E-selectins are required for the rolling process, and ICAM, VCAM, and PECAM are required for the firm adhesion process of leukocytes and/or for passing through the blood vessel wall, it is feasible that upregulation of cell adhesion molecules expressed on the vascular endothelial cells contributes to the infiltration of leukocytes into dermis in the pathogenesis of CSU. However, detailed roles of adhesion molecules expressed on endothelial cells other than the role in inflammatory cell infiltration as a consequence of MC activation remained unknown.

As described in the sections above, a number of studies have suggested the involvement of the blood coagulation/fibrinolysis system, especially of the TF-triggered extrinsic coagulation pathway, in the pathogenesis of CSU. In patients with CSU, plasma levels of prothrombin fragment 1+2 and D-dimer are higher than those in normal controls and correlate with disease severities.[136,174] Sakurai et al[175] revealed the elevation of extrinsic blood coagulation potential in the patients with CSU. Plasminogen activator and urokinase released from vascular endothelial cells may contribute to the activation of the fibrinolysis process that produces D-dimer.[176] Moreover, *in vitro* studies focused on the role of vascular endothelial cells have unveiled a role of vascular endothelial cells in the cascade of the blood coagulation pathway triggered by histamine and inflammation in the pathogenesis of CSU.[131,177] Both human umbilical vein endothelial cells (HUVECs) and human dermal microvascular endothelial cells express a large amount of TF on the surface of cells in response to the combination of 2 categories of TF inducers: one including histamine and VEGF, and the other including LPS, TNF-α, IL-33, and IL-1β.[131,144] Moreover, TF expressed on HUVECs induces activation of the extrinsic coagulation pathways and produces activated coagulation factors, such as FXa and factor IIa (FIIa). FXa and FIIa then induce gap formation of vascular endothelial cells via PAR-1 and leakage of plasma with AAbs against IgE or FcεRI, and autoantigens for specific IgE on MCs in the skin, resulting in the activation of the MCs and edema formation.[131,177] Thus, TF on endothelial cells may be a good target to prevent TF-extrinsic coagulation pathway-plasma leakage axis in the treatment of severe and refractory CSU. However, patients with CSU do not usually show apparent thrombosis, presumably due to homeostatic activation of fibrinolysis. The actual expression of TF on vascular endothelial cells in the lesion of CSU remained to be confirmed in the patients.

Neuroinflammation and the Afferent Nervous System

A relationship between hives that persist and the “nervous system” has been assumed once physicians/researchers began to consider the possible cause of CSU. Although few believe that an underlying psychogenic condition (eg, chronic anxiety) is the cause, most are of the opinion that stress can exacerbate hives, and all agree that urticaria is certainly a cause of stress. For those who see no effect of stress on any objective manifestation of urticaria, the interpretation is that tolerance for the discomfort, including itch, is lessened by stress, leading to complaints of “worsening.”

Anatomically, efferent nerve cells are in proximity to MCs,[178] and we know that histamine stimulation of sensory nerves (type C unmyelinated neurons) can initiate impulses migrating through the dorsal root ganglion to the spinal cord that then branch or interconnect in multiple ways. Some have connections (prespinal cord) that meet adjacent nerves that conduct back to the skin (antidromic conduction), which leads to the release of neuropeptides, particularly substance P. This causes “reflex” vasodilatation of arterioles and is responsible for the axon reflex, the third element of the “triple response of Lewis.” The axon reflex causes redness far beyond histamine diffusability. Substance P also has the ability to further activate cutaneous MCs if sufficiently high levels are reached,[179] and blood levels are indeed elevated in CSU[180] and produce a positive wheal and flare on skin testing that is augmented in CSU.[181]

Besides the aforementioned branch points, synapses within the spinal cord then project to the brain and lead to the perception of itch. For years, it was assumed that fibers mediating pruritus and pain were identical, and that the differing extent of stimulation might be responsible for different sensations. We have recently discovered that there are separate nerve fibers mediating itch that travel along with fibers mediating pain within the lateral spinothalamic tract.[182]

Neuropeptides released from nerve endings that could contribute to the pathogenesis of CSU are many, and further work is needed to delineate specific contributions of each assuming that many are relevant. These include substance P, neurokinin A, and CGRP.

Substance P and neurokinin A are derived by alternative splicing from the same preprotachykinin A gene and interact with the same neurokinin (NK)-1 receptor. Inhibitors of the NK-1 receptor are being developed, which is most likely relevant to histamine-dependent pruritis.[181] There is also a receptor on skin MCs, termed MRGPRX2, that is responsive to substance P as well as other neuropeptides.[183]

Because the pruritis of CSU is as responsive to therapy as are the urticarial lesions,[184] the mediators downstream are ultimately dependent on MC activation. In this respect, the circumstance may differ from other causes of pruritis that are chronic (eg, kidney disease, liver disease, lymphoma, or prurigo nodularis) and that may include atopic dermatitis in which normal appearing (but often dry) skin can be pruritic and antihistamine resistance is the norm. A role for other receptors, such as opioid and cannabinoid receptors, PAR receptors or receptors for cytokines such as IL-31, IL-4, and IL-13 appears likely .[185]

Concluding Comments

The presence or absence of any significant cellular infiltrate within the dermis differentiates many types of chronic urticaria. Most inducible urticarias lack such an infiltrate perhaps because the stimulus is fleeting, even if repetitive. These would include cold urticaria, symptomatic dermatographism, cholinergic urticaria, solar urticaria, local heat urticaria, aquagenic urticaria, and vibratory urticaria/angioedema. On the other hand, CSU has a prominent cellular infiltrate[186] with features similar to the allergic LPR.[153] Perhaps a sustained stimulus leads to this result. The one inducible urticaria that has a significant cellular infiltrate is delayed pressure urticaria.[187] Although the lesion appears hours after the stimulus, the initiating stimulus is typically sustained (walking or being seated a long time) or repetitive (hammering) relative to the other inducible urticaria where stimuli ranging from a few seconds to a few minutes suffice.

The cellular infiltration seen in patients with CSU involves all the cellular elements of blood many of which have been considered in this review (Figures 1 and 2). We have not discussed monocytes, in particular, although they are plentiful when cells within skin biopsies are counted,[187] and have enhanced TF expression.[132] By definition, MCP 1-4 may be responsible for their accumulation along with the other cell types. Lymphocyte subtypes that have not been considered include NK cells and NKT cells. We have not considered B lymphocytes because they are not seen within the urticarial lesions although they certainly have a systemic role in the production of the AAbs described above.

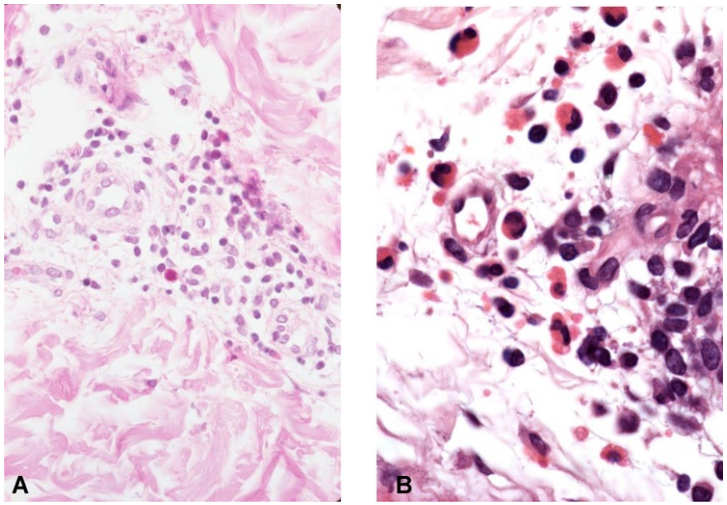

Figure 1 Skin biopsies representative of chronic spontaneous urticaria with a non-necrotizing perivascular infiltration of cells. **A,** Predominance of T lymphocytes and monocytes. **B,** Mononuclear cells with prominence of eosinophils.

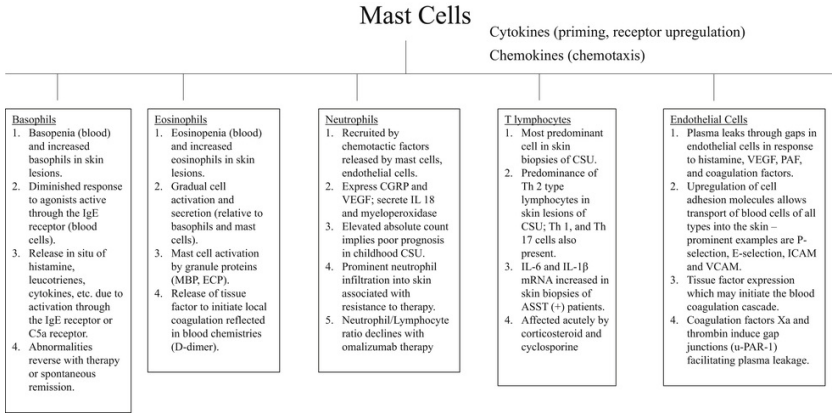

Figure 2 Functions of basophils, eosinophils, neutrophils, T lymphocytes, and endothelial cell in chronic spontaneous urticaria. *ASST*, Autologous serum skin test; *CGRP*, calcitonin gene-related protein; *CSU*, chronic spontaneous urticaria; *ECP*, XXX; *ICAM*,

intercellular cell adhesion molecule; *MBP*, major basic protein; *PAF*, platelet-activating factor; *VCAM*, vascular cell adhesion molecule; *VEGF*, vascular endothelial growth factor.

Although methods such as immunohistochemistry[76] can certainly shed light on the function of cells within the perivenular infiltrate, their contribution *in vivo* can perhaps best be inferred from clinical studies of the efficacy of drugs that target particular cells or their products. Rituximab is reported to be effective (case reports)[188] presumably by preventing AAb synthesis although it is not generally recommended. Corticosteroids inhibit the function of T lymphocytes and eosinophils, and prevent egress of virtually all cell types from the blood stream into tissues, including skin. The latter effect certainly includes T lymphocytes, basophils, and eosinophils, and possibly monocytes. Neutrophil egress and function, as is seen in asthma, may be more resistant to steroid effects.

For the future, we envision studies of drugs with increasing specificity to determine the effect on the clinical course of CSU. We can target IL-17 (secukinumab), Th2-dependent cytokines, IL-4 and IL-13 (dupilumab), Th2- and eosinophil-dependent cytokines, IL-5 and its receptor (mepolizumab, reslizumab, and benralizumab), complement C5a receptor (avdoralimab), and MC and eosinophil cell surface Siglec-8 (lirentelimab) to name some being studied at present. This has been recently reviewed.[189]

Uncited Reference and Figure

172. Figure 2.

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