Crosstalk of immune cells in chronic spontaneous urticaria

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Abstract

Chronic spontaneous urticaria (CSU) is defined as the recurrent episodes of spontaneous wheals and/or angioedema for more than 6 weeks, and at least twice a week. The core link in the pathogenesis of CSU is the activation of mast cells and other immune cells caused by various reasons. These activated immune cells release a series of inflammatory active mediators such as histamine, arachidonic acid metabolites, chemokines, etc., resulting in clinical features such as wheals and/or edema. However, the specific mechanisms leading to the activation of various immune cells have not been fully elucidated. Previous evidence has shown that about 50% of CSU patients have potential autoimmune reactions. Here we review the functional implication of immune cells in CSU focusing on the crosstalk between them and discuss whether their crosstalk promotes the occurrence and development of the disease.

KEYWORDS: crosstalk, immune cells, mast cells, pathogenesis, urticaria

1. Introduction

Overtime, the prevalence of chronic urticaria has increased globally.[1] The updated EAACI/GA2LEN/EDF/WAO guideline for chronic urticaria now clearly divided into chronic inducible urticarias (CIndU) and chronic spontaneous urticaria (CSU), previously termed chronic idiopathic urticaria (CIU).[2] Urticaria is manifested

by rapid wheals and/or angioedema, often accompanied by itching and/or burning. Wheals usually have a fleeting nature, with the skin returning to its normal appearance, usually within 30 minutes to 24 hours. Besides, patients generally have milder systemic symptoms and may be accompanied by other autoimmune diseases.[2] Here we will review and summarize the immune-related parts of the pathogenesis of CSU, focusing on the crosstalk between immune cells involved in this disease. The understanding of these mechanisms will enable us to diagnose and manage CSU patients better, contributing to research and develop innovative and efficient treatments.

2. Autoimmune pathogenesis of chronic spontaneous urticaria

About 35%-45% of CSU patients can detect IgG autoantibodies, and 5%-10% can detect IgE autoantibodies.[3] Schmetzer et al[4] found that more than 200 kinds of IgE autoantigens in CSU patients, and proposed that IL-24 is a common, specific and functional autoantigen of IgE antibody in CSU patients. IgE antibodies against thyroid peroxidase (TPO) was also found in a large group of CSU patients, which could activate mast cells obviously. [5,6] There have been a couple of studies suggesting subjects with CSU have autoimmune basis, for example, intradermal injection of serum from subjects with CSU into autologous skin can cause wheals at the injection site, which is the autologous serum skin test (ASST).[7,8] Subsequent studies have shown that IgG antibodies against IgE in patients or its high affinity receptor (FcεRI) can cause mast cells degranulation and basophil activation to release a series of inflammatory mediators, finally leading to wheals or angioedema. [9,10]（**Fig.1**）The Histamine release test and Basophil activation test（BAT）also support this view.[11,12]

In patients with CSU, infiltrating inflammatory cells are mainly located in the dermis and deep dermis, and there is almost no difference between patients with and without anti-FcεRI and/or anti-IgE autoantibodies.[7] Evidence shows that eosinophils, neutrophils, basophils, and macrophages in lesions are significantly higher than healthy subjects.[8,13,14] However, whether skin mast cells in CSU are increased or not remains controversial. Some studies reported an increase[13,15] while other study reported that mast cells decreased slightly compared with the control group.[16]

3. The crosstalk of immune cells in CSU

CSU is an allergic disease mediated by mast cells and other immune cells, such as basophils, T lymphocytes and eosinophils, neutrophils, B lymphocytes, macrophages and monocytes, both in number and activity, have more or less changed. The changes in the release of inflammatory mediators after activation of each type of immune cells will also affect other cells, including histamine, prostaglandin D2 (PGD2), C5a, thrombin, tissue factor (TF), major basic protein (MBP), eosinophil cationic protein (ECP), cytokines (interleukin, chemokine, interferon and tumor necrosis factor), etc., leading to a late-phase-like perivascular infiltration around small

venules and wheal formation. Above immune cells involved in CSU and the main mechanism of activation are summarized in Table 1.

Since mast cells are considered to play the most important role in the pathogenesis of urticaria, both the crosstalk between mast cells and other immune cells（**Fig.2**）and the interaction between B lymphocytes and other immune cells will be discussed in detail.

3.1 Mast cells and basophils：

Both mast cells and basophil cells release large amounts of histamine after activation. One of the histamine receptors, H4R, was expressed in both human mast cells and basophils.[17] The stimulation of mast cells by histamine through H4R may have three functions. First, it increases the signal transduction and chemotaxis of mast cells, thereby promoting their accumulation at the site of an allergic response. Second, it upregulates the expression of FcεRI on mast cells, leading to allergen-induced activation.[18] Third, it causes intracellular calcium mobilization.[19] Finally, H4R-mediated mast cell activation leads to the expression of several pro-inflammatory cytokines and chemokines, such as IL-4,IL-5,IL-6, tumor necrosis factor α (TNF-α), tumor growth factor-β1 (TGF-β1), RANTES, IL-8, macrophage inflammatory protein 1α (MIP-1α), and monocyte Chemoattractant Protein 1 (MCP-1).[20] Histamine also induce the chemotaxis migration of basophils through H4R.[21]

Activated mast cells (mainly the tcMC subtype) can release PGD2, [22] Which could be activated and chemotactic by the receptor chemoattractant receptor homologous molecule 2 (CRTH2) expressed by basophil.[23] However, CRTH2 expression was inhibited in CSU patients.[24] This is because PGD2 binds to CRTH2, resulting in receptor internalization. The cellular effects of CRTH2-activated include shape change, chemotactic activity, upregulation of CD11b, Th2 cell production of Th2 cytokines, eosinophil release of ECP, and allergen-induced enhancement of basophil histamine release, suggesting that PGD2 enhances antigen-mediated histamine release from basophils.[25]

Patients with CSU have elevated levels of IL-3 in peripheral circulation, which can be released by activated mast cells. IL-3 is essential for basophil development and survival. In addition, it activates and enhances basophil responses to other stimuli and can up-regulate the expression of FcεRI in primary human basophils.[26]

Serum levels of IL-4 were reduced in CSU patients.[27-29] However, some studies have suggested that the plasma IL-4 level in CSU patients is increased, which is positively correlated with the total IgE level.[30] Since total IgE serum levels are often elevated (up to 50%) in CSU patients, they can be used as a marker of CSU.[31] Therefore, the role of IL-4 in CSU is also worthy of attention. What is not disputed is that IL-4 is significantly expressed in the diseased skin.[14,16,32] IL-4 is mainly derived from T cells, basophils and mast cells. A robust IL-4 production of basophils in response to IgE plus antigens.[33] It has been described as an effective

regulator of human mast cell phenotype, growth, and differentiation.[34,35] Some reports have shown that IL-4 can synergistically act with IgE to up-regulate the expression of FcεRI on mast cells surface.[36] Thienemann et al [37] elegantly described that in mature cutaneous mast cells, IL-4 treatment significantly increased the survival rate of cutaneous mast cells, although no effect of IL-4 on the expression of c-KIT or FcεRI-α was observed. However, IL-4 reduces the ability of mast cells to adhere to extracellular matrix.[38] Thus, basophils can influence mast cells in the inflammatory site by producing IL-4.

The results clearly showed that serum MRGPRX2 levels were significantly higher in patients with severe CSU.[39] MRGPRX2, a receptor on mast cells, basophils, and eosinophils associated with IgE-independent degranulation, has been reported to be highly expressed on cutaneous mast cells in patients with severe CSU. The involvement of MRGPRX2 is associated with pro-inflammatory basophilic and eosinophilic effects, such as calcium mobilization, increased survival, and cytokine release. Mast cells produce IL-3 and IL-5 that enhance the expression of MRGPRX2, which may lead to a pro-inflammatory vicious cycle.[40,41]

Plasma levels of IL-31 and IL-33 in CSU patients were significantly higher than those in healthy controls.[42] IL-31 is strongly expressed in the serum and skin of patients with CSU and is released from isolated basophils after anti-IgE, IL-3, or fMLP stimulation. IL-31 also induces the release and chemotaxis of IL-4 and IL-13.[43] The IL-33 in serum is mainly from activated CD4+ T cells, and IL-33 is also released by skin mast cells and macrophages.[44] IL-33 acts through its receptor ST2, which is highly expressed in Th2 cells, mast cells, basophils, eosinophils, and innate lymphocytes (ILC2s) that produce type 2 cytokines.[45] IL-33 plays an important role in the activation of mast cells, and it induces the synthesis and secretion of IL-31 from LAD2 mast cells. The induction effect is enhanced in the presence of IgE or IgG antibody, and IL-4 can also enhance this effect. IL-33 itself does not induce mast cells degranulation, but IL-33 can enhance allergy stimulation to mast cells and basophils, promoting the maturation of mast cells and can be released by mast cells activated by allergic stimulation.[46]IL-33 pretreatment not only increased threshing and the number of mast cells producing chemokines, but also increased the magnitude of single mast cell threshing and chemokine production. This finding also supports that view.[47] We demonstrated a cellular crosstalk mechanism，by this mechanism, the activated mast cells crosstalk with the IL-33 receptor-carrying basophils, prompting these basophils to adopt a unique response signal rich in neutrophil-related molecules. [45]

3.2 Mast cells and eosinophils：

Mast cells and eosinophils are the key effector cells of CSU. Some studies suggest that there is a physical contact between mast cells and eosinophils in the late and chronic stages of allergic inflammation. Transmission electron microscopy (TEM)

showed that mast cells and eosinophils adhere to each other during co-culture in vitro. In other words, mast cells and eosinophils showed signs of physical contact and mutual activation during co-culture. These results indicate that mast cells and eosinophils can form an effector unit during allergic diseases.[48] **(Fig.3)** This MC-Eos interplay can improve the survival rate of eosinophils in vitro. There is a complex network of paracrine and membrane interactions between mast cells and eosinophils.[49] It was found that the physical contact between mast cells and eosinophils was mediated by CD48 (on mast cells)-2B4 (on eosinophils).[49] Eosinophils enhance the release of basal mast cells mediators with CD48-2B4 and jointly stimulate IgE activated mast cells. Eosinophils also lower the IgE response threshold of mast cells by delivering costimulatory signals integrated into IgE mediated pathways. However, mast cells induced eosinophil activation does not require CD48-2B4 exposure, resting and IgE stimulated mast cells leads to eosinophils migration and activation via paracrine. The increase of TNF-a release was also observed in long-term co-culture. Eosinophils also showed enhanced expression of intercellular adhesion molecule-1 (ICAM-1), which was dependent on direct contact with mast cells. TNF-a released during long-term co-culture of MC-Eos also increased ICAM-1 in eosinophils. ICAM-1 signal was associated with prolonged survival of eosinophils and enhanced MC-Eos adhesion. Leukotrienes produced by activated mast cells/eosinophils may also be involved in cellular interactions, as they both express leukotriene receptors.[50] The binding of mast cell DNAM-1（CD226）to eosinophil Nectin-2(CD112) has also been implicated in eosinophil-augmented degranulation of mast cells, with important possible consequences on chronic allergic processes and other diseases on which these two cells are associated.[51,52] CD226 synergizes with FcεRI on mast cells, and its engagement augments degranulation through a pathway involving Fyn, linker of activation of T-cells, phospholipase Cγ2, and CD18. This costimulatory response might be a critical component in allergic inflammation such as rhinitis, asthma and CSU closely related to autoimmunity. Thus, it is crucial to demonstrate the MC–Eos interplay in skin lesions of CSU patients, blocking this interface may have a critical value in future therapy of CSU.

In addition to physical contact, mast cells and eosinophils can interact with each other through inflammatory mediators and related receptors. Eosinophils show a large number of activated receptors. They express activated receptors of various chemokines (i. e. CCR3, CXCR3, CXCR4, CCR5, CCR6, etc.), interleukin (i. e. IL-3R, IL-4R, IL-5R, IL-13R, ST2, etc.), amines (i. e. Histamine receptors), phosphoryl-associated molecular pattern molecules (i. e. Toll-like receptors) and complement system (i. e. C3A, C5A, etc.) on their surface, as well as inhibitory receptors like CD300a and Siglecs.[22]

Mast cells recruit eosinophils to the diseased skin by releasing eotaxin, an effective agonist of CCR3.[53,54] Mast cells release a large amount of histamine after activation, and one of the histamine receptors, H4R, is expressed on eosinophils.[17] Histamine

enhances the expression of eosinophil adhesion molecules through H4R, resulting in increased eosinophil migration.[55] PGD2 released by mast cells can also induce chemotaxis of eosinophils,[23] promoting the activation of eosinophils and release of ECP.[25] Tryptase, produced by mast cells, stimulates the activation of eosinophils to produce IL-6 and IL-8 by cleavage of protease activated receptor 2 (PAR-2).[56]

The tMC mainly produces IL-5 and IL-6.[57] Hong et al[58] showed that the levels of histamine, LTC4, TNF-α, TGF-β, IL-4, IL-5 and IL-6 in patients with CSU were significantly higher than those in healthy controls. In humans, the effects of IL-5 are limited to basophils and eosinophils. The expression of IL-5Rα on basophils was three times lower than that on mature eosinophils. IL-5 is an important cytokine for priming and survival of mature eosinophils, and for proliferation and maturation of their progenitors. It is speculated that IL-5 is involved in the development and maintenance of the innate inflammatory process in the spontaneous wheals.[8,59]

Selective expression of Siglec-8 in human eosinophils and mast cells has been demonstrated. In eosinophils, the involvement of Siglec-8 leads to apoptosis,[60] IL-33 (produced by mast cells) triggers Siglec-8-mediated eosinophil apoptosis through β2 integrins.[61] In mast cells, however, Siglec-8 cross-linking resulted in severe inhibition of IgE receptor-induced histamine and PGD2 release without apoptosis.[62-64]

The activity of tissue transglutaminase 2 (TG2) in the serum of patients with CSU was significantly higher than that of healthy controls. Experiments showed that TG2 came from mast cells, which may induce the production of cytokines in the way of autocrine and paracrine, leading to the activation of T cells, eosinophils and other inflammatory cells, thus leading to the pathogenesis of CSU.[58]

Plasma matrix metalloproteinase-9 (MMP-9) levels in CSU are increased because TNF-α release leads to the upregulation of these two genes in mast cells, and MMP-9 levels are correlated with disease severity.[65-67] Mast cells, eosinophils or activated T cells may be potential sources of MMP-9 that may promote the migration of eosinophils and lymphocytes (especially CD4+T cells) to the skin.[68]

Eosinophils can affect mast cells in the following ways. The extrinsic coagulation cascade in CSU is activated by eosinophil derived tissue factor,[69,70] triggering the production of thrombin and C5a. Thrombin acts on PARs (PAR1 and PAR2) to mediate mast cell adhesion and degranulation [71]. It also causes increased endothelial cell permeability, resulting in the formation of cutaneous wheals and angioedema.[72] However, another study has shown that activated exogenous coagulation factors do not activate human skin mast cells and basophils by themselves, but by producing complement C5a acting on C5a receptor (C5aR).[73]

The activated eosinophils release MBP, ECP, eosinophil peroxidase (EPO) and other inflammatory mediators,[74] MBP, ECP and EPO induce histamine release from mast cells and basophils through MRGPRX2, [40,75] MRGPRX2 has been shown

to be up-regulated in mast cells in the skin of patients with severe chronic urticaria. In addition, MBP activates human mast cells through integrin-b1 (expressed on the surface of mast cells).[76] Besides, eosinophils derived stem cell factor may recruits and activates mast cells.[77]

Translation control tumor protein (TCTP), also known as histamine releasing factor, has the ability to activate mast cells. The expression of dimer TCTP was significantly increased in CSU patients. After stimulation with dimer TCTP, the activation of basophils and mast cells were significantly increased. The study found that the level of dimer TCTP was significantly positively correlated with the level of ECP, indicating that eosinophils may indirectly participate in the activation of basophils and mast cells through this mechanism.[78]

3.3 Mast cells and T cells:

Several reports have shown that there is a complex interaction between mast cells and activated T lymphocytes at the site of inflammation **(Fig.3)**. They can make physical contact (heterotypic adhesion) through adhesion molecules, which can be activated to release inflammation related mediators (histamine, TNF-α, MMP-9, IL-4, TNF-α, IL-6, metallopeptidase inhibitor 1, etc.) These cytokines and proteases regulate extracellular matrix degradation during T cell mediated inflammation, and are also essential for leukocyte exudation and recruitment to affected parts.[79-81] It is proved that this activation pathway can also lead to the expression and release of IL-8, which is an effective chemokine to induce neutrophil migration.[82] These studies suggest that activated T cells may play a role in the pathogenic activation of mast cells. Mast cells express costimulatory molecules CD80 (B7-1), CD86 (B7-2) and adhesion molecule CD54 (ICAM-1), all of which are involved in T cell activation.[83,84] The interaction between mast cells and T cells is at least partially mediated by the adhesion molecule ICAM-1 and its ligand leukocyte function related antigen-1 (LFA-1), because the addition of antibodies against these two molecules inhibits adherent-induced degranulation of mast cells.[85] The heterotypic adhesion suggests that mast cells have a broad ability to directly mediate the activation of T cells, suggesting that human mast cells may be involved in inducing adaptive immune responses by recruiting and activating T cells in allergic reactions or autoimmune diseases. But the limited evidence for this effect comes from the use of in vitro co-culture systems,[80,85,86] Because of the heterogeneity of mast cells from different races and different tissues, the development of models to evaluate these effects in vivo will be a great progress in mast cell and T cell biology. Especially in patients with CSU, mast cells and T cells are abundant in the lesion area, but whether there is heterotypic adhesion between mast cells

and T cells needs to be determined by immunofluorescence or electron microscopy.

It has been found that mast cells can also be activated by microvesicles released by T cells, allowing them to respond to the inflammatory site without contact with the T cells **(Fig.3)**. Activated T cells released microvesicles carrying similar Mast cells activators. Thus, by releasing microvesicles, T cells deliver activated surface molecules in a way that does not require physical contact between cells, and encourage mast cells to release inflammatory mediators such as histamine and IL-8.[87] Further analysis showed that T-cell-derived microvesicles, rather than FcεRI crosslinking, induced IL-24 gene transcription and protein production in mast cells.[88] Shefler et al [89] describes elegantly that T-cell derived microvesicle, as an intercellular vector of functional miR-4443, may regulate PTPRJ gene expression heteromorphically in mast cells, thereby regulating ERK-phosphorylation and IL-8 release in mast cells. Mast cell-microvesicle interaction enables activated T cells to promote the remote-contact-mediated activation of mast cells, said mast cells are not located in close physical proximity to the inflammatory site, which provides a new mechanism for chronic inflammatory skin disease, but its role in CSU needs to be confirmed.

In addition to the interaction between physical contact and mast cell-microvesicle, mast cells and T cells can also interact with each other through inflammatory mediators and related receptors. One of the histamine receptors, H4R, is also expressed on T cells.[17] H4R is involved in the pathogenesis of allergy and inflammation by activating Th2 cells and Th17 cells.[90]Enhancement of Th2 cytokine secretion (such as IL-5, IL-4, IL-10, and IL-13) and inhibition of Th1 cytokine production (IFN-γ, IL-12, and IL-2) are mediated by histamine. Thus, histamine regulates the efficient balance between Th1 and Th2 cells by aiding the transfer to Th2.[91] In addition, T cells enhance mast cell proliferation, maturation, and reactivity by secreting IL-6 after FcεRI aggregation.[92,93] T-cell-derived IL-4 can also induce chemotaxis of mast cells.[94]

3.4 Mast cells and neutrophils:

Mast cells influence neutrophils in the following ways: mast cells initiate the early stage of neutrophilic recruitment by releasing the chemical inducer CXCL1/CXCL2. Upon reaching the stimulated tissue, neutrophils further penetrate in a macrophage-dependent manner (macrophages also synthesize CXCL1/CXCL2 neutrophils chemokines).[95] granulocyte-macrophage colony stimulating factor (GM-CSF) derived from mast cells activated by IgE cross-linking significantly prolonged the survival of neutrophils.[96] IL-1β expression was found to be elevated in both diseased and non-diseased skin of CSU patients, and mast cells secreted IL-1β and induced neutrophil migration and vascular leakage.[93] The heterotypic adhesion of mast cells to T cells leads to the expression and release of IL-

8, which is an effective chemokine that induces neutrophil migration, thereby promoting neutrophil aggregation in the diseased skin.[82]

3.5 Mast cells and monocytes:

The expression of chemokines CCL2 and CXCL8 in monocytes of CSU patients was upregulated, reflecting the high responsiveness of monocytes. CXCL8/IL-8 is chemotactic to neutrophils, lymphocytes, and monocytes, activates a variety of cells, including monocytes, macrophages, lymphocytes, eosinophils, and basophils, and is associated with chronic inflammatory diseases. CCL2 causes the activation of mast cells, mainly basophils.[97] After being activated, monocytes release MCP-1, an effective histamine-releasing factor of mast cells and basophil, which can cause the activation of mast cells and basophil to release histamine and other inflammatory mediators.[98] In addition to chemokines, monocytes also influence mast cells by releasing TF. Mononuclear TF expression was enhanced in CSU patients compared with healthy donors. It may be induced by agonists of TLR 1, 2, 4, and 5, triggering exogenous coagulation pathways and increased vascular permeability in a histamine-independent manner, indirectly triggering the activation of mast cells and basophils, leading to the formation of wheal and angioedema.[99]

3.6 Mast cells and macrophages:

One of the histamine receptors, H4R, is also expressed on macrophages.[17] In the local microenvironment dominated by Th2, histamine exists in high concentration, and C3aR is down-regulated on human M2 macrophages. This is induced by IL-4 and histamine via H4R. Reduced C3aR expression through H4R in response to IL-4 or histamine may have an anti-inflammatory effect by reducing sensitivity to C3a-induced downstream signals, thereby helping to regulate local inflammatory responses in the skin. This mechanism may be related to the pathogenesis of CSU.[100]

After FcεRI aggregation, macrophages secrete IL-6 to enhance mast cell proliferation, maturation, and reactivity.[92,93] The significant increase of IFN-λ1 levels in epidermis and peripheral blood of CSU patients suggested that IFN-λ1 May play an important role in the pathogenesis of CSU. In CSU blood CD8+ T cells express more of IFN-λ1, and in the skin, mast cells, eosinophils, B cells, neutrophils and macrophages may be sources of IFN-λ1. In mice, IFN-λ1 can induce mast cells and eosinophils, the accumulation of inflammatory cells, and increases the number of these cells in a dose-dependent manner.[101]

3.7 Crosstalk between B lymphocytes and other immune cells

Autoantigens (such as TPO) in patients with CSU can induce B cells to produce IgE / IgG antibodies. IgE / IgG binds to FcεRI-α of mast cells / basophils and other target cells in FC segment. When the same antigen is contacted again, the antigen binds to two or more IgE that have been bound to the target cells FcεRI is cross-linked, which leads to a series of activation reactions and release a large number of inflammatory mediators.[5] In addition to mast cells and basophils, IgE receptors were also expressed on the surface of eosinophils. It was found that there was anti-CD23 antibody in a subgroup of CSU. The anti-CD23 antibody was activated by binding to FcεRII on the surface of eosinophils infiltrating the sk in of patients. [102]

And beyond that, T cells are able to influence B cells by secreting cytokines. In CSU, the level of intracellular IL-10 (produced by Th1 and Th2 cells) is increased, and IL-10 can activate B cells to produce autoantibodies.[103] The low level of IL-21 was observed in CSU, which was negatively correlated with total IgE, suggesting that IL-21 may be involved in the immunopathogenesis of CSU.[30] One of the functions of IL-21 is to induce apoptosis of antigen-specific B cells.[104] Therefore, the decrease of IL-21 alleviates the inhibition of B cell proliferation, which may lead to the increase of B cells with the progress of CSU. In addition, IL-21 seems to be a key cytokine for maintaining low IgE levels under physiological and pathological conditions. The lack of IL-21 significantly enhanced the IgE homoswitch and antigen driven clonal expansion of IgE+ cells, which triggered the increase of IgE and led to the occurrence of diseases.[105]

Basophil derived IL-4 and IL-6 also act on B cells or plasma cells to enhance their survival and proliferation and promote humoral immunity.[106]

Above cytokines described in CSU subjects are summarized in Table 2.

4. Conclusion and future directions

Since approximately 45-50% of CSU patients have autoantibodies, there is no doubt that further research is needed to target the activation of immune cells by autoantibody pathways. In addition to the intervention of autoantibodies, the mechanisms of crosstalk among various immune cells include physical contact activation and other pathways. This includes the heterotypic adhesion between mast cells and T cells and an effector unit formed by mast cells and neutrophils. The physical contact between cells promotes mutual activation and release of a large number of inflammatory factors to a certain extent. Beyond that, activated T cells can also promote the release of histamine, IL-8 and other inflammatory mediators by mast cells through the function of microvesicles, which provides a new mechanism for the pathogenesis of chronic inflammatory skin diseases, but it remains to be verified in CSU. Inflammatory mediators such as histamine, PGD2, C5a, thrombin, TF, MBP-1, ECP, and cytokines (i. e. interleukin,

chemokine, interferon and tumor necrosis factor) play an important role in regulating the activation or inhibition of immune cells through the communication network between these cells, and further affect the incidence and mitigation of CSU.

On account of mast cells and basophils play a major role in the pathogenesis of the CSU, the current research mostly focuses on their single functions, while the significances of T cells, neutrophils and eosinophils in this disease is still not be unified definitely, and about whether the various types of immune cells have physical interactions remains to be seen. Along with the increase of the disease rates over the years, some patients may suffer more than one episode of CSU during their lifetime. Considering the recurrences, disabling symptoms, and significant impact on quality of life, further studies are vitally needed to advance the understanding of pathogenic factors that trigger skin symptoms even systemic symptoms in CSU, especially the specific role played by immune cells, and to assist in the selection of therapeutics, properly and effectively.

TABLES

Table1. Immune cells involved in CSU

Cell type	The tissue level	Cell activity	Mechanism of activation	Main inflammatory mediators in CSU	Ref
Mast cells	Increased / Decreased	Activation and degranulation	Autoimmunity (autoantibodies against IgE or FcεRI-α); dysregulation of the signaling pathways (increased SYK and decreased SHIP); Activation of extrinsic coagulation pathway (increased level of thrombin, D-dimer, FVIIa, F1+2, complement C5a and TF); physical contact with activated T cells and eosinophils, etc.	Histamine, LTC4, PGD2, tryptase, IL-1β, IL-4, IL-5, IL-8, IL-9, IL-13, IL-31, IL-33, GM-CSF, MMP-9, CXCL1/2, TNF-α	[5,13,15,16,32,48-50,57,69,79-81,85,107-116]
Basophils	Increased	Activation	Autoimmunity (autoantibodies against IgE or FcεRI-α); dysregulation of the signaling pathways (increased SYK and decreased SHIP); MCP-1, MBP, IL-3, IL-33, etc.	Histamine, protease, IL-4, IL-6, IL-31, IL-33, TNF-α, CXCL1	[13,77,98,102,106,117-121]
Eosinophils	Increased	Activation	Autoimmunity (autoantibodies against CD23 and FCεRII); physical contact with mast cells; IL-5, TNF-α, etc.	MBP, ECP, EPO, TF, VEGF, PAF, MMP-9, IL-6, IL-8, IL-9	[13,32,48-50,102]
T cells	Decreased Th1/Th17 /Treg cells; Increased Th2/ Th9 cells (the serum level)	Imbalance of Th1 / Th2 cytokines	Imbalance of humoral immunity; physical contact with mast cells; histamine, IL-5, IL-6, IL-18, PGD2, TG2, MMP-9, etc.	IL-9, IL-10, IL-13, IL-17, IL-23, IL-25, IL-33, TSLP, and TNF-α	[32,44,79-81,85,103,122-140]
B cells	Increased (the serum level)	Activation	Autoimmunity	IgE, IgG, IgM, IgA	[3,9,102,141-143]

Macrophages	Increased	Activation	Histamine, IFN-λ1	CXCL1/CXCL2, IL-6, IL-18, IL-33, IFN-λ1	[13,32,92,95,101,144-147]
Monocytes	/	Activation	IL-4, IL-8, CXCL8	IL-18, CCL2, MCP-1, CXCL8, TF	[97,99]
Neutrophils	Increased	Activation	Histamine, IL-1 β , IL-8, IL-18, CXCL1/2, CXCL8, GM-CSF	MPO	[8,13,32,95,148,149]

Table2. Crosstalk between immune cells involved in CSU

Cytokine	Receptors	The serum level	Cell sources	Cell targets	Major functions	Ref
IL-1β	IL-1 type 2 receptor	Increased	Mast cells	Neutrophils	Induction of neutrophils migration and vascular leakage	[93,148]
IL-2	IL-2R	Decreased	CD4+and CD8+ activated T cells	CD4+ and CD8+ T cells, B cells	Proliferation of effector T and B cells; development of Treg cells; growth factor for B cells and stimulus for antibody synthesis	[29,124]
IL-3	IL-3 receptor α+β c (CD131)	Increased	T cells, macrophages, Mast cells, NK cells, eosinophils	Basophils, eosinophils	Activation of basophils and eosinophils; upregulation the expression of FcεRI in basophils and improvement of cell viability	[26,106]
IL-4	IL-4R type I, IL-4R type II	Decreased/ Increased	TH2 cells, Basophils, Mast cells	T cells, B cells, Mast cells, monocytes	Activation of basophils and T cells; enhancement of humoral immunity; recruitment of eosinophils; Induction of monocytes and TH2 differentiation; survival factor for B and T cells	[27,28,30,33-38,94,127]
IL-5	IL-5R	Increased	Mast cells, TH2 cells, activated eosinophils	Eosinophils, basophils	Increment of eosinophils chemotactic activity and adhesion capacity	[8,57,59,150,151]
IL-6	IL-6R (sIL-6R) gp130	Increased	T cells, basophils, mast cells, macrophages	B cells, mast cells	B-cell differentiation and production of IgG, IgM, and IgA; Enhancement of mast cell proliferation, maturation, and reactivity	[57,92,93,106]
IL-8	CXCR1 and CXCR2	Increased	Mast cells, eosinophils	Neutrophils, NK cells, T cells, basophils, and eosinophils	Chemoattractant for neutrophils, NK cells, T cells, basophils, and eosinophils	[8,82,87,97]

IL-9	IL-9R	Increased	T cells, mast cells, eosinophils	B, T, and mast cells	T cell and mast cell growth factor; inhibition of TH1- cytokines; proliferation of CD8+ T cells and mast cells	[130-136]
IL-10	IL-10R1/IL-10R2 complex	Increased	T cells, B cells	T cells, B cells	Inhibition of the function of TH1 and TC1; activation of B cells and induction of auto-antibodies by B cells	[103,131,137-140]
IL-13	IL-13R1a1 and IL-13R1a2	Increased	T, NKT, and mast cells; basophils and eosinophils	B cells, mast cells, eosinophils	Activation of eosinophils and mast cells; recruitment and survival of eosinophils	[127,128]
IL-17	IL-17R	Increased	TH17 cells	Monocytes, macrophages, B and T cells	Induction of proinflammatory cytokines, chemokines, and metalloproteases; recruitment and activation of neutrophils	[126]
IL-18	IL-18R	Increased	Macrophages	T cells, NK cells, macrophages	Induction of IFN-γ in the presence of IL-12; enhancement of NK cell cytotoxicity, promoting TH1 or TH2 cell responses depending on cytokine milieu	[144-147]
IL-21	IL-21R	Decreased	T cells	CD4+T cells, CD8+T cells, B cells, DCs, macrophages	Induction of antigen-specific B-cell apoptosis; inhibition of B-cell proliferation	[30,104,105,152,153]
IL-23	IL-23R	Increased	Macrophages	T cells (TH17 cells), NK cells, eosinophils, monocytes, macrophages	A supporting role in the continued stimulation and survival of Th17 cells; induction of the secretion of IL-17 by non-T cells	[126]

IL-24	L-20R1/IL-20R2 and IL-22R1/ IL-20R2	/	T cells, monocytes, B cells	/	An autoantigen in chronic spontaneous urticaria	[4]
IL-25	IL-17RA and IL-17RB	Increased	T cells, mast cells, eosinophils, basophils	TH2 memory cells, basophils, NKT cells, macrophages	Induction of TH2 responses and inhibition of both TH1 and TH17 responses; induction of IgE, IgG1, IL-4, IL-5, IL-9, IL-13 production	[29,44,124]
IL-31	IL-31RA/OSMRβ	Increased	T cells, mast cells, basophils	Eosinophils, mast cells, basophils	Induction of IL-6, IL-8, CXCL1, CXCL8, CCL2, and CCL8 production in eosinophils	[42,43,46]
IL-33	ST2	Increased	TH2 cells, macrophages, mast cells, eosinophils, basophils	Basophils, mast cells, eosinophils, DCs, macrophages, NK cells, NKT cells, T lymphocytes, B lymphocytes	Enhanced integrin expression in basophils and eosinophils; induction of the synthesis and secretion of IL-31 by mast cells; enhancement on allergic stimulation of mast cells and basophils; promotion of mast cells maturation	[42,43,45-47,61,145]
IL-35	IL-12Rβ2/gp130; IL-12Rβ2/IL-12Rβ2; gp130/gp130	Decreased	Treg cells, monocytes	NK cells and activated T cells	Reduction of effector T-cell proliferation; Increase of IL-10 production and Treg proliferation	[154-156]
TNF-α	TNFR1 (p55/60, CD120a) and TNFR2 (p75/80, CD120b)	Increased	T cells, mast cells, basophils	Eosinophils	Activation of eosinophils; Increase the expression of eosinophils ICAM-1	[29,44,58,124,126]
IFN-γ	IFNGR1/IFNGR2	Decreased	T cells, mast cells, macrophages	Eosinophils, lymphocytes, mast cells, macrophages, and neutrophils	Aggregation of eosinophils, lymphocytes, mast cells, macrophages, and neutrophils	[27-29,44,124]

IFN-λ1	IFNLR1 and IL-10R2	Increased	T cells, macrophages, mast cells	Eosinophils, lymphocytes, mast cells, macrophages, and neutrophils	Induction of mast cells, lymphocytes, neutrophils and macrophages accumulation; regulation of T h1/Th2 responses	[101]
TGF-β	TβR-I and TβR-II	Decreased	Eosinophils, macrophages, Treg cells	T cells, NK cells, monocytes, macrophages, neutrophils, and eosinophils	Reduction of mast cells expression of FcεRI; regulation of the differentiation of several TH cell subsets and induction of Treg cells; immune tolerance	[58]

FIGURE LEGENDS

Figure1. The activation of mast cells and basophils in patients with chronic spontaneous urticaria mediated by autoantibodies. Mast cells and basophils are activated by IgE antibodies against its high affinity receptor (FcεRI) or IgG antibodies against IgE/FcεRI, and release several mediators [i. e. histamine, tryptase, Leukotriene C4 (LTC4), prostaglandin D2 (PGD2), etc.] that concur to produce the marked vasodilation that stands at the basis of both wheal-and flare reaction and angioedema.

Figure2. The interactions between main effector cells involved in chronic spontaneous urticaria. PGD2, prostaglandin D2; MBP, major basic protein; MCP-1, monocyte chemotactic and stimulating factor; CCL, C-C motif chemokine ligand; CXCL, C-X-C motif chemokine ligand; SCF, stem cell factor; ECP, eosinophil cationic protein; EPO, eosinophil peroxidase; TG2, tissue transglutaminase2; GM-CSF, granulocyte-macrophage colony stimulating factor; PAF, platelet activating factor; MMP-9, matrix metalloproteinase-9; IFN-λ1, interferon-λ1.

Figure3. Physical contact between mast cells and T cells/eosinophils. Mast cells and activated T cells in the inflammation site can perform physical contact (heterotypic adhesion) mediated by adhesion molecules (i. e. ICAM-1 [on mast cells], LFA-1 [on T cells]), thereby being activated to release inflammation-related mediators (histamine, TNF-α, MMP-9, interleukin, metallopeptidase inhibitor 1, etc.). Heterotypic adhesion also shows that mast cells have a broad ability to directly mediate T cell activation. In addition, mast cells are activated by microvesicles released by T cells that carry activating factors, responding to the site of inflammation without contact with T cells. Mast cells and eosinophils. Mast cells and eosinophils have been observed in the late and chronic stages of allergic inflammation to regulate each other's functions by forming an effect unit.CD48 (on mast cells) and CD244 (on eosinophils), DNAM-1 (on mast cells) and Nectin-2 (on eosinophils) have been reported to mediate this effect.

Figure1

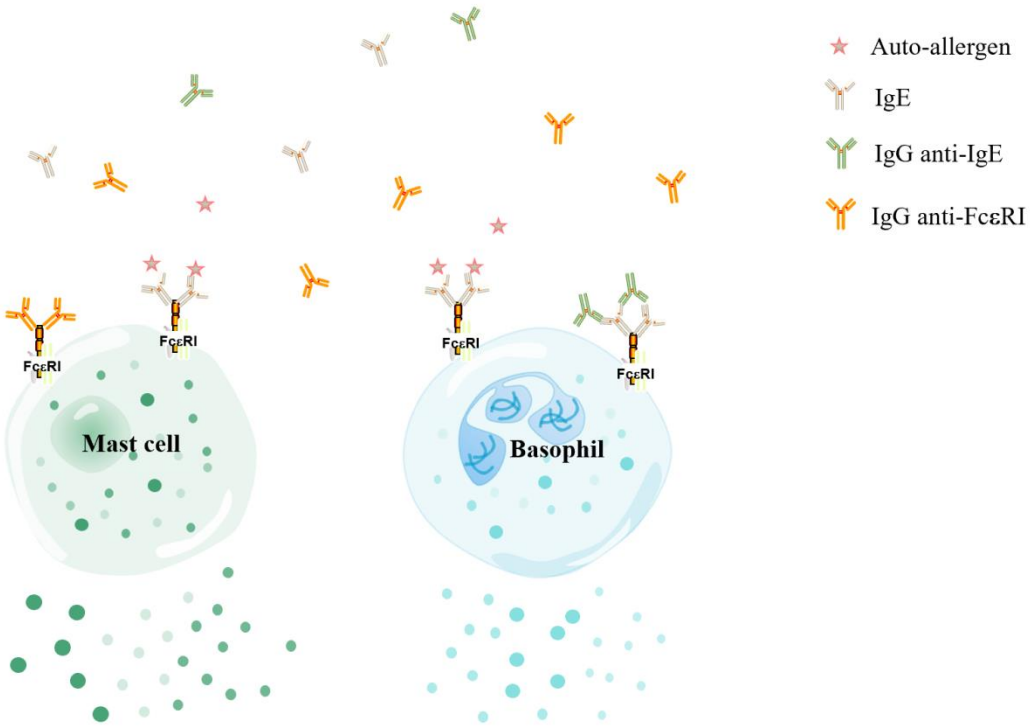


Figure2

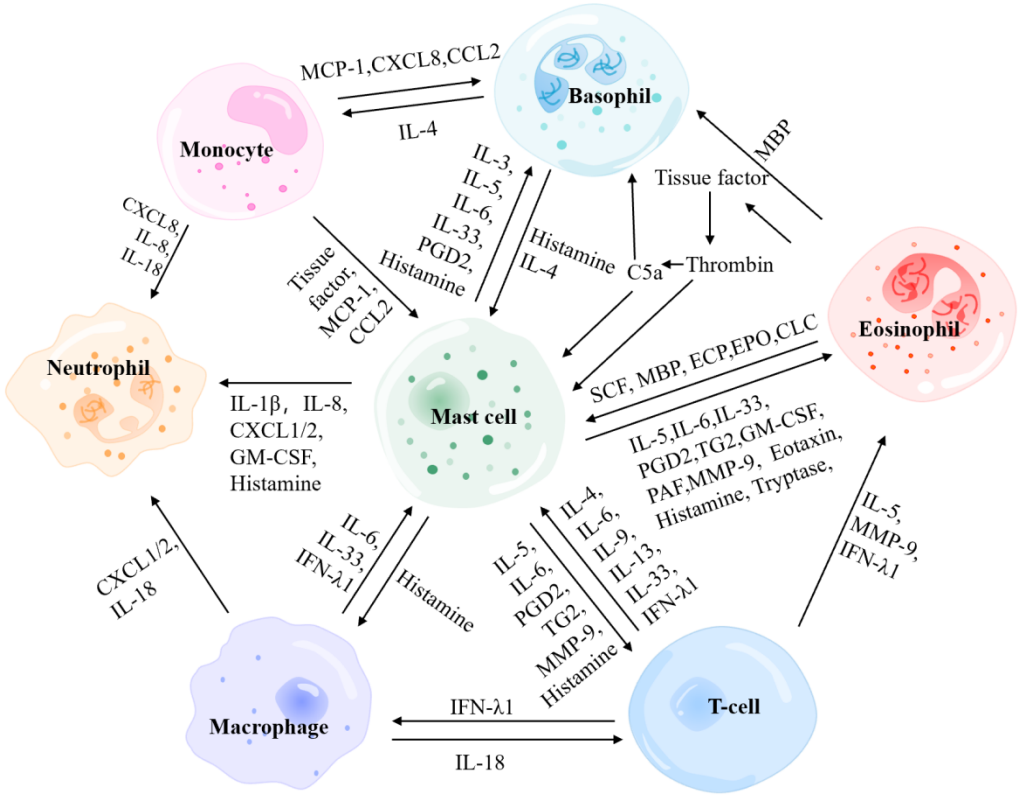
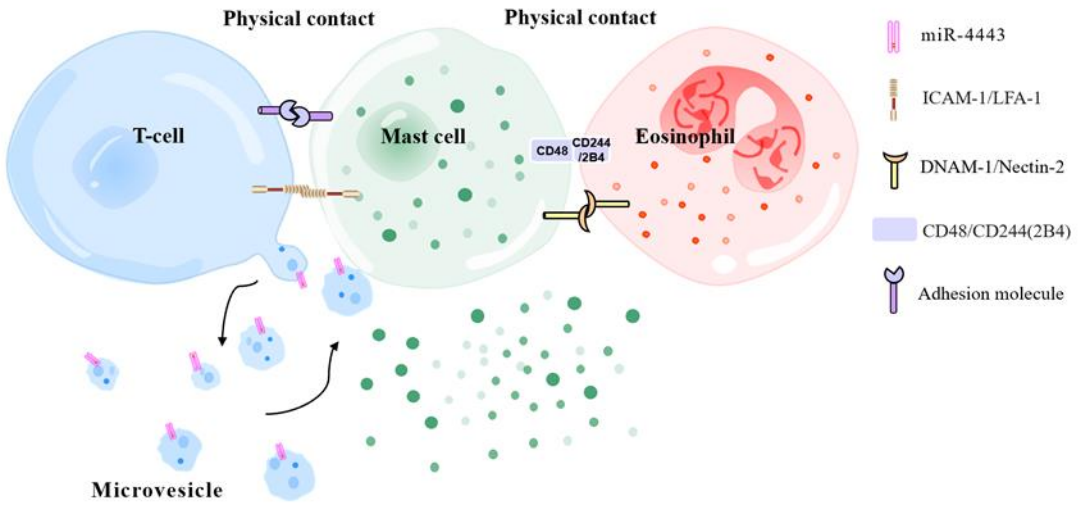


Figure3



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