Immunological signature of chronic spontaneous urticaria (Review)

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Abstract. Chronic urticaria (CU) is a condition characterized by intensely pruritic, edematous, erythematous papules lasting for more than 6 weeks. Over half of the cases have concomitant swelling of deeper tissues, known as angioedema. The socio-economic burden of the disease is significant. Unfortunately, patients with severe CU, refractory to conventional treatment, have limited and expensive therapeutic options. The pathogenesis of CU is not yet completely understood. Therefore, elucidating the pathophysiological mechanisms involved would potentially identify new therapeutic targets. It has been accepted in recent years that mast cells and their activation, followed by excessive degranulation represent the key pathophysiological events in chronic spontaneous urticaria (CSU). The triggering events and the complexity of the effector mechanisms, however, remain intensely debated topics with conflicting studies. One pathogenetic mechanism incriminated in chronic spontaneous urticaria is the response mediated by the high-affinity receptor for IgE (FcεRI) expressed on mast cells. Increasing recognition of chronic spontaneous urticaria as an autoimmune disease linked to the cytokine-chemokine network imbalance resulting from alteration of innate immune response is another pathogenetic explanation. It is likely that these different pathological mechanisms are more interconnected, both acting synergistically, rather than separately, to produce the clinical expression of CU. The discovery and understanding of pathogenic mechanisms represent the premise for the development of safe and effective immunomodulators and targeted biological treatment for severe, refractory CU.

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Contents

1. Introduction

According to the 2018 European Academy of Allergology and Clinical Immunology (EAACI) guideline, urticaria is a disease characterized by the development of an urticarial rash (also called hives/wheals), angioedema, or wheals plus angioedema (1). By definition, chronic spontaneous urticaria (CSU) is a diagnosis of exclusion after ruling out other conditions defined by the appearance of the wheals, angioedema or both. Some of these conditions are: Autoinflammatory syndromes, anaphylaxis, vasculitis (bradykinin-mediated) or bradykinin-mediated angioedema, including hereditary angioedema (defined by angioedema unaccompanied by urticaria) (1). Urticaria is classified based on the duration of the symptoms into acute (≤6 weeks) or chronic (>6 weeks). Furthermore, in relation to precipitating factors, urticaria is subclassified into spontaneous (does not involve any identifiable specific trigger) or inducible (has a known trigger) (1). The present review examined chronic urticaria (CU), specifically CSU.

The effect of CSU on patients, their family and friends, their jobs, the health system and society is substantial. The use of reported patient measurements, such as urticaria activity score (UAS), Angioedema Activity Score (AAS), Quality of Life Questionnaire (CU-Q2oL), AE-QoL Questionnaire and Urticaria Control Test (UCT) in clinical trials has helped to better define the multi-faced effect of CSU (2-6). Numerous studies based on these and data obtained from these assessments have helped highlight the impact and reveal that CSU influences the lives of patients both functionally, objectively and subjectively (7-9). In 1997 O'Donnell *et al* revealed that the health scores of patients with CSU were comparable to those reported in patients with coronary artery disease (10).

The costs for treating this disease are also extremely high, both for patients and society (11-13).

In recent years, a research team coordinated by Maurer revealed that the essential pathophysiological element in CSU is the mast cell (14). From what is known at present, a brief overview could be summarized by the release of histamine and other mediators, such as platelet activating factor (PAF) and cytokines by activated mast cells leading to sensory nerve stimulation, vasodilation and plasma extravasation, as well as the recruitment of other immune cells to the urticaria site. Moreover, the signals for activating mast cells leading to hives are incomplete and poorly understood and appear complicated, intricate and heterogeneous (14).

From a histological point of view, edema appears in the upper and middle dermis, due to dilation and increase of postcapillary venules and lymphatic vessel permeability in the upper dermis, leading to extravasation of fluids in the tissues. Regarding angioedema, the same changes occur, only that these changes are located initially in the deep dermis ultimately extending to the subcutaneous tissue. In the lesional skin affected by the hives, there is almost always a perivascular inflammatory infiltrate, composed of neutrophils with or without eosinophils, basophils, macrophages and T cells and an increase in the number of endothelial cells, adhesion molecules, neuropeptides and growth factors. In urticaria, there is no necrosis of the vascular wall, this being a pathognomonic sign of vasculitis (15-19). Even in the healthy skin of patients with CU, certain changes occur, such as eosinophilic infiltration, increased expression of adhesion molecules and of some cytokines (20,21). All the aforementioned highlight how complex the pathogenic mechanism of urticaria is and how much it exceeds what is known to date, proving that it is markedly more than the activation, degranulation of mast cells and basophils with the release of vasoactive mediators (22-24). Due to the fact that numerous of these molecular findings are also observed in other autoinflammatory diseases, they cannot be used as biomarkers or be identified as typical changes in CSU. Additional research could lead to an improved understanding and identification of more specific pathogenic pathways.

2. Methods

For this review the Google Scholar browser was used, with the following search terms: 'Mast cells' and/or 'chronic spontaneous urticaria pathogenesis'. The selection period of articles was 2010-2018 and any type of article was selected, being sorted according to relevance. The results produced over 3,000 articles, and among them those referring mainly to the biological treatment known and already used in clinical practice were eliminated, while those focusing on pathogenesis were retained. Following an overview of each of the titles of these articles, the 200 most relevant to our topic were selected. Older publications presented in the selected articles were also reviewed as well and introduced in our study if deemed relevant. Finally, 101 articles helped us in compiling the data presented in this review.

3. Evidence for autoimmune etiology

The first indication that urticaria could have an autoimmune basis, with an intrinsic immune imbalance, excluding extrinsic factors as the cause, comes from the so-called 'autologous serum skin test', in which by intradermal injection of the serum of the CU patient, an erythematous papule is produced at the injection site (25-27).

Previous studies have revealed that IgG antibodies against the IgE or high affinity receptor (FcεRI) of the patient lead to the activation, degranulation of mast cells and the appearance of urticarial lesions, but this does not fully explain the complexity of pathogenesis (14,28-30). Strengthening the theory, it is known that urticarial lesions are induced by vasoactive mediators, the main representative remaining the histamine released by mast cells. Hide *et al* identified IgG autoantibodies against the α subunit of high-affinity IgE receptors in the plasma of patients with CSU (30). These autoantibodies cross-link with IgE receptors, being responsible in some cases for mast cell activation and induction of specific lesions in CSU and other mast cell-mediated autoimmune diseases (30-33). The same theory is supported by studies revealing that patients with CSU have high serum levels of IgE autoantibodies, such as IgE-anti-TPO and IgE-anti-dDNA (31,34,35). The concept of 'overlapping autoimmune diseases' is also presented, suggesting that the concomitant presence of autoimmune diseases is occasionally observed along with CSU (36).

In addition, more than 200 IgE-type autoantigens were found in patients with CSU that were not in the blood of controls. IgE can react with autoantigens identified in the plasma of patients with CSU and other autoimmune diseases (including systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis). This phenomenon stimulates the activation of mast cells in the CSU, which degranulates rapidly under the action of intrinsic factors (autoreactive IgG against IgE and FcεRI, autoreactive IgE against its own antigens) (37). Previous evidence has emphasized the importance of IgE-anti-IL-24, which was revealed in all patients with CSU, suggesting and supporting that IL-24 may be a dermal autoantigen specific to this condition (14,38,39). Therefore, a possible autoimmune mechanism of CSU is the development of autoantibodies for the high affinity IgE receptor, FcεRI, which is found on the surface of mast cells and to a lesser extent on basophils, and is responsible for the activation and degranulation of these cells, followed by the release of vasoactive mediators. Activation of the high affinity IgE receptor, FcεRI, apparently represents an important step in the occurrence of urticarial lesions (35,36,40,41). This receptor is composed of 4 subunits: α, β and two γ, the last two being susceptible to the pathological activity of mast cells when they have an inadequate regulation (42).

A population study of more than 12,000 subjects in Israel revealed an incidence of autoimmune diseases (Sjögren's syndrome, rheumatoid arthritis, celiac disease, type 1 diabetes and systemic lupus erythematosus) in women with CSU, significantly higher than in women without CSU (43). The prevalence of antinuclear antibodies, anti-thyroperoxidase (anti-TPO), anti-thyroglobulin, rheumatoid factor, anti-transglutaminase IgA and anti-cardiolipin have also been revealed to be increased among individuals with CSU and associated autoimmune diseases. Therefore, a common pathophysiological mechanism between CSU and other autoimmune diseases can be considered, due to the increased prevalence of autoantibodies and the existence of a chronic inflammatory

process expressed by a persistently increased mean platelet volume (MPV) (43).

Another important aspect to mention is the association between CSU and autoimmune thyroid diseases. An increased prevalence of Hashimoto's thyroiditis and Basedow-Graves' disease has been revealed in patients with CSU, with increased values of anti-thyroid IgG antibodies (31). Numerous patients diagnosed with urticaria have increased values of anti-thyroglobulin (anti-TG) or antimicrosomal antibodies/anti-TPO (44). Significantly elevated anti-TPO Ac IgE values have also been revealed in patients with CSU, thus supporting the theory of autoallergic mast cell activation that contributes to the pathophysiology of CSU. IgE has been also credited with an important role in autoimmunity, thus complementing its well-known role in defending against helminth infections and exogenous allergens. Concurrently, it has been revealed that these anti-TPO antibodies have the ability to induce degranulation of basophils *in vitro* in the presence of TPO antigens and thus play an important role in the pathogenesis of CSU, which is another argument in favor of the autoimmune etiology of this disease (45-47). Autoallergy thus refers to a type I hypersensitivity reaction, mediated by IgE against its own antigens, which can stimulate the degranulation of mast cells, type I autoimmunity (14,25). It is important to understand the formation of IgE autoantibodies and their contribution to the pathogenesis of CSU compared with IgG autoantibodies. A summary of the autoantibodies identified in CSU are presented in Table I.

In conclusion, it should be generally accepted that autoimmunity (type I, described by IgE antibodies against autoantigens or type 2b, characterized by IgG autoantibodies to IgE or their FcεRI receptor) is considered to be the most common cause of urticaria, with the mast cells rich in high affinity receptors, as the main cellular elements (14,39).

Table I. Autoantibodies identified in chronic spontaneous urticaria.

Autoantibodies	Refs.
IgG antibodies to αFcεRI receptor	(36,37,40-42)
IgG antibodies to IgE	(30)
IgG antibodies anti-TG	(31,44)
IgE and IgG antibodies anti-TPO	(21,22,31,44)
ANA, RF	(30,35,42,43)
ACA-IgG, IgM or IgA	(43)
Anti-transglutaminase IgA antibodies	(43)

Anti-TG, anti-thyroglobulin; anti-TPO, anti-thyroperoxidase; ANA, antinuclear antibodies; RF, rheumatoid factor; ACA, anti-cardiolipin antibodies.

4. Mast cells

Mast cells are round, mononuclear cells up to 25 microns in diameter, with a round or oval-shaped unicellular nucleus, eccentrically positioned and containing numerous cytoplasmic granules. They can be identified by special stains (Giemsa and toluidine blue), which poorly identify granulated immature or degranulated mast cells or by more sensitive techniques, such as tryptase immunohistochemistry. Moreover, mast cells can be marked with monoclonal antibodies that recognize membrane receptor targets of these cells (kit receptor, the high-affinity receptor for IgE) and proteases from intracytoplasmic granules (tryptase, chymase and carboxypeptidase) (48-51).

Mast cells are defined according to the content of their intracytoplasmic granules. The majority of tryptase-rich mast cells are predominantly located in mucous membranes (T-mast cells), while those containing tryptase, carboxypeptidase and chymase predominate in the connective tissue of the skin (TC-mast cells) (52-55). With regard to the mast cells involved in the pathogenesis of CSU, particularly TC-mast cells are referred to, which contain both tryptase and chymase (connective tissue mast cells).

Mast cells in patients with urticaria, similar to those in healthy individuals, are located in the superficial and deep dermis, preferentially in the perivascular and periaxonal spaces (56,57). The largest number of mast cells is identified in the superficial dermis of skin lesions, and few in the deep reticular dermis (58,59). It has been revealed in several studies that the distribution of mast cells in the dermis with urticarial lesions is different from that in healthy dermis, but it has remained ambiguous and unknown whether the number of these cells changes, increasing in urticaria (14,56,57). A previous study regarding CSU revealed that there was an increase in the proportion of dermal mast cells from 11 to 14% (60). This study was not unique, others on the same topic also identified an increased number of mast cells in both injured and intact skin in patients with urticaria compared with the skin of healthy subjects (20,61). Studies supporting this increase indicate that the changes revealed, appear only after at least 10 weeks of illness, thus only in CU (20,60,61). It is surmised that there remains uncertainty as to whether or not the number of mast cells in the dermis of patients with CU changes, because contrary to the studies mentioned in favor of this theory, there are numerous others that demonstrate the opposite, that in fact there are no increases in the number of these cells in the dermis of the CU patients compared with the dermis of the control subjects (56,57,62).

Scanning and transmission electron microscopy has been used since the 1970s to demonstrate mast cell degranulation (63). It was later used to identify mast cell degranulation in the affected skin of individuals with hives as well. Numerous histological studies have revealed mononuclear cells and perivascular eosinophilic infiltration in areas with urticaria wheals (64-67). Thus, mast cell degranulation, an immediate process followed by the observation of persistent eosinophilic infiltrate, suggests that there is also a delayed allergic inflammation in the pathogenesis of CSU, reinforcing the theory that the mechanisms are combined and heterogeneous (20). Furthermore, several cytokines have been identified in CSU, supporting the existence of a chronic inflammatory response, most of them having mast cell origin.

5. Cytokines in CSU

Mast cells release proinflammatory factors including cytokines. IL-31 is involved in the onset of pruritus, the release of

IL-4 and IL-13, chemotactism for basophils, proliferation of the epithelial basal layer and inhibition of filaggrin synthesis. IL-4 and IL-13 direct T-lymphocyte differentiation to T helper 2 and B lymphocytes toward IgE synthesis (68-74). The study of these cytokines has led to new therapeutic approaches, such as the use of nemolizumab (humanized monoclonal antibody against IL-31 receptor A). It has been observed that the itching cannot be completely prevented by antihistamines, which is why the role of other substances implicated in the mechanism of its occurrence have been considered. CSU patients have exhibited higher plasma concentration of IL-31, contributing significantly to the onset of itching. Serum levels of IL-31, a member of the IL-6 cytokine family, are higher in CSU than in control patients, but the values are lower than in patients with atopic dermatitis. Although secreted primarily by Th2 lymphocytes, IL-31 is also released by mast cells and basophils. They produce and release IL-31 through an IgE-dependent mechanism. IgE binding to FcεRI activates mast cells and basophils that release IL-31. Once released, IL-31 has an autocrine effect on basophils by binding to IL-31 receptors (IL-31RA) and oncostatin M (OSM) receptors (which have a role in increasing the binding affinity of IL-31 to IL-31RA) leading to release of Th2-type cytokines: IL-4 and IL-13 (73-76). Nemolizumab is an antibody targeted against the IL-31RA receptor, preventing the binding of IL-31 and consequently the development of the cascade of reactions implicated. The use of this monoclonal antibody in clinical trials has reduced pruritus, demonstrating the major role of IL-31 in this process (77).

IL-33 induces a strong Th2 response and acts as an alarm for the immune system when the tissue is injured (78). A previous study revealed increases of IL-33 in the plasma of patients with CSU (75). IL-33 is expressed locally in inflamed skin, both in keratinocytes and in mast cells, but not in basophils, a situation highlighted in animal models. IL-33 has two actions that may be essential in urticaria: First, it activates itch-mediating sensory neurons, and second, it can modulate aspects of mast cell function, including adhesion, maturation, degranulation, and the production of a variety of Th2 cytokines (17,20,79-81). With this last role, IL-33 contributes to the induction of Th2-type inflammation observed in CU.

IL-25 is a member of the IL-17 cytokine family, the so-called autoimmune cytokine family. It is a potent inflammatory promoter of the Th2 type, found in epithelial cells, mast cells and eosinophils involved in hives. The fact that mast cells could produce IL-25 after IgE-dependent activation suggests a possible pathway of innate immunity that can stimulate Th2 response in CU (82-84). The question of whether it can be considered as an important autoantigen involved in the pathological mechanism deserves to be addressed in future scientific research.

Thymus stromal lymphopoietin (TSLP) is another member of the IL-17 cytokine family, which has also been revealed to be increased in urticarial lesions (75). While the cellular origin of TSLP has not been described in this pathology, an increased number of TSLP-positive mast cells have been observed in the bronchial tissue of patients with asthma (85-87). The primary role of TSLP in the skin is related to the development of Th2 cells by recruitment and activation of dendritic cells. TSLP does not induce degranulation of the mast cells *per se* but stimulates them in the presence of IL-1 and TNF and induces the production and release of other proinflammatory cytokines/chemokines including IL-5, IL-13, IL-6, GM-CSF, CXCL8 and CCL1 (88).

Table II. Treatment in chronic spontaneous urticaria[a].

Step 1: Second-generation H1-antihistamines
If symptom control is not achieved after 2-4 weeks of use or earlier if symptoms are very severe, proceed to step 2
Step 2: Increase second-generation H1-antihistamine dose (up to 4x)
If symptom control is not achieved after 2-4 weeks of use or earlier if symptoms are very severe, proceed to step 3
Step 3: Omalizumab (add-on to second-generation H1-antihistamines)
If symptom control is not achieved after 6 months of use or earlier if symptoms are very severe, proceed to step 4
Step 4: Cyclosporin A (add-on to second-generation H1-antihistamines)

[a]Adapted from ref 1.

6. Treatment in CSU

At present, the therapeutic protocol provided by The EAACI/GA²LEN/EDF/WAO guideline of urticaria can be summarized as revealed in Table II (1).

As it can be observed, although numerous therapies have been tried for refractory CSU treated with four times the dose of second-generation antihistamines, H1, the only ones approved by scientific consensus are omalizumab and cyclosporine A.

Omalizumab is a humanized G1K immunoglobulin monoclonal antibody produced by recombinant DNA technology which binds selectively to human IgE. It was originally approved in the US in 2003 for the treatment of moderate to severe persistent allergic bronchial asthma, and then in the European Union (EU) in 2005. In 2014, omalizumab was also approved in patients with CU, both in the US and in the EU, being the first drug indicated in patients who remain symptomatic despite maximal anti-H1 antihistamine treatment. Accumulated evidence has revealed that IgE, by binding to FcεRI on mast cells, can upregulate the receptors by promoting their proliferation and survival, thereby maintaining an increased number available (89,90). IgE coupling with FcεRI may also decrease the release threshold of mast cells and increase their sensitivity to different stimuli, either by FcεRI or by other receptors, resulting in an overactive degranulation process. Furthermore, the IgE-FcεRI binding potentiates the ability of mast cells to store and synthesize inflammatory mediators and cytokines *de novo*. Omalizumab administration, by depleting IgEs, attenuates their multiple effects, such as managing mast cell activities, thereby reducing the ability of these cells to manifest inflammatory mechanisms in patients with CSU (91). There has even been a decrease in serum IL-31 levels observed after treatment with omalizumab (91), which

leads us to consider this interleukin as a potential biomarker in the diagnosis and monitoring of urticaria treatment, providing hope for the future.

The effectiveness of cyclosporine A in combination with a second-generation H1 antihistamine has been demonstrated in clinical trials on CSU, but this drug cannot be recommended as a standard treatment due to its numerous side effects (92-97). Cyclosporine A is only recommended for patients with severe refractory disease at any dose of antihistamine and omalizumab combination. Compared with long-term use of glucocorticosteroids, cyclosporine A has a more favorable risk/benefit ratio.

7. Conclusions

As it could be perceived in the present review, the immunological signature of urticaria is complicated, with numerous potential players and possible research points. Certainly, many aspects have remained unidentified and require future studies, but exploring each known part of the immunopathological mechanism may pave the way for possible therapeutic targets.

Every question can lead to an answer, thus it is definitely worth asking! Some of our queries after this introduction to the mysteries of CSU would be: i) How important is the role of the mast cell in the pathogenesis of this disease? Does this cell change its location, number and predisposition to degranulation depending on the clinical severity of urticaria? ii) What are the optimal biomarkers for both the diagnosis and monitoring of different treatments in CSU? Can IL-31 be considered a serological response biomarker to omalizumab? iii) The study of the IL-31 target in CSU may represent a new therapeutic approach, which merits further research. Can nemolizumab be used in the treatment of CSU? iv) Evaluation of various cytokine involvement (IL-31, IL-33, IL-25 and TSLP) in the pathophysiological mechanism of urticaria. Does their serum value change in the blood of patients in accordance with the evolution of the disease? Which of these cytokines deserves to be the most studied in this entity? Can IL-25 be considered as an autoantigen? v) Evaluation of IgE, FcεRI, and IgG-anti-IgE and IgG-anti-FcεRI antibodies in CU to provide more evidence of autoimmune etiology. vi) Omalizumab resistance mechanism; when does it become inefficient? vii) Identifying a correlation between the genetic polymorphism of an interleukin with mast cell origin and the predisposition for the development of CSU is also an area which requires study and exploration.

Collectively, this review shed light on new therapeutic targets and identified potential new defense mechanisms against CSU, which is a great burden to sufferers.

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CTD and GAF conceived the present review. CTD developed the theory and performed the data collection. IAM and DMD supervised the findings of this work. CTD, GAF, IP and CP contributed to writing the manuscript and revising it critically for important intellectual content. Data authentication is not applicable. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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