



Chronic urticaria: A disease at a crossroad between autoimmunity and coagulation

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Abstract

Chronic urticaria (CU), defined as recurrence of wheals with or without angioedema for more than 6 weeks, is a quite common disease that may severely worsen the quality of life. Studies carried out during the last 2 decades have demonstrated an autoimmune pathogenesis mediated by functionally active autoantibodies to the high affinity IgE receptor (FcεRI) or to IgE which are able to induce histamine release from basophils and mast cells. However, such mechanism can be detected in less than 50% of patients only. The present article reviews recent findings showing an additional pathogenic mechanisms in CU patients: activation of the coagulation cascade resulting in thrombin production. Thrombin is a serine protease which may play a key role in urticaria, being able to induce edema through an increase in vascular permeability, mast cell activation and degranulation, and to induce the production of the anaphylotoxin C5a. Such mechanism seems to be active in the majority of CU patients, however their relationship with anti-FcεRI or anti-IgE autoantibodies is still matter of research.

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Keywords: Chronic urticaria; Autoimmunity; Coagulation cascade; Histamine; Thrombin

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1. Introduction

Chronic urticaria (CU), defined as the recurrent occurrence of short-lived wheals with or without angioedema for more than 6 weeks, is a rather common disorder. It has been estimated that 0.1% of the general population is suffering from this disease [1], but the real prevalence probably exceeds this figure. CU may appear at any age although it occurs more frequently in adults, affects more women than men [2,3], and is characterized by a familial pattern [4]. (Fig. 1).

2. Chronic urticaria as an autoimmune disease: a short history

The pathogenesis of CU has long remained a mystery. This disease has been ascribed to anxiety, underlying

infectious diseases, or intolerance to foods or to food additives for years. As a consequence, patients have been prescribed long-lasting restriction diets, serial stools examinations, repeated *Helicobacter pylori* eradication treatments, and/or dental radiograms followed by surgical eradication of apical granulomas; in most cases with little or no benefit and discomfort. Finally (and fortunately) substantial advances in our understanding of the pathogenesis of this disease have been recorded during the last 2 decades. This new history has been started in 1986 with the observations by Grattan and co-workers that the intradermal injection of autologous serum caused a wheal-and-flare reaction in a significant proportion of CU patients [5]. Such a finding suggested the possible presence of circulating histamine-releasing factors as a possible pathogenic factor. Actually, a couple of years later Gruber et al.

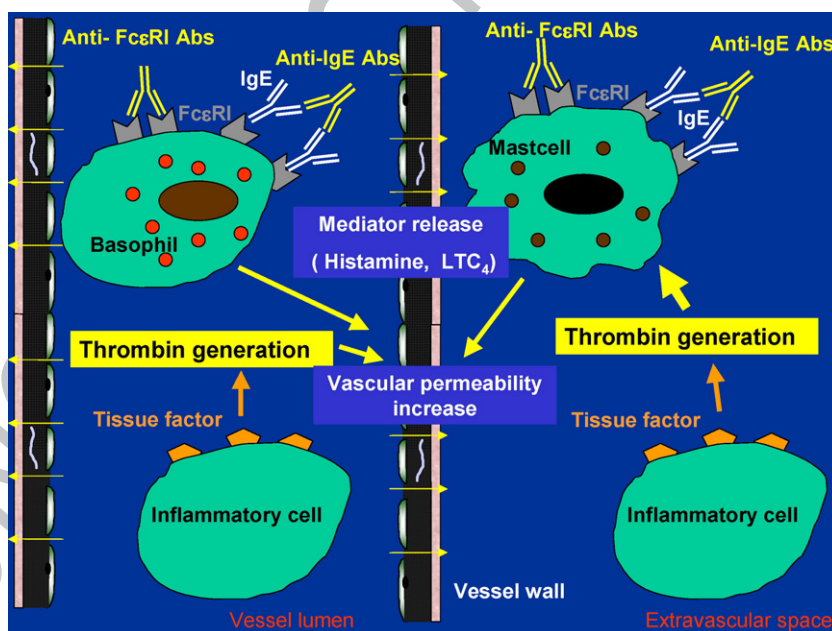


Fig. 1. Summary of the events possibly involved in the pathogenesis of chronic urticaria. Functional autoantibodies to the high affinity IgE receptor, FcεRI, or to IgE induce histamine release intravascularly from basophils (left side of the picture) and extravascularly from mast cells (right side of the picture). Contemporarily (or alternatively) the expression of tissue factor by hitherto not yet identified inflammatory cells triggers the activation of the extrinsic pathway of coagulation. Generated thrombin causes vasodilation and increases vascular permeability, and induces direct mast cell degranulation.

detected nonfunctional (i.e. not able to induce basophil degranulation in-vitro) IgG anti-IgE autoantibodies in 3/6 and 5/9 patients with CU and cold urticaria, respectively [6]. Unfortunately, however, the same type of nonfunctional autoantibodies were subsequently detected also in sera from many atopic patients and normal subjects as well. Finally, in 1991 Grattan et al found for the first time functional anti-IgE autoantibodies [7], and two years later the same group concluded that an IgG autoantibody specific for the α subunit of the high affinity IgE receptor (Fc ϵ RI) was the main mediator of whealing in CU. Such autoantibody could be detectable in about 25% of CU patients [8]. This finding was confirmed by independent groups [9,10], and subsequent studies pointed out that functional anti-IgE antibodies can be detected in about 5% of CU patients only [1].

3. Pathogenic relevance of autoantibodies in cu

Both anti-Fc ϵ RI and anti-IgE antibodies are able to cause direct cross linking of adjacent Fc ϵ RI receptors, thus inducing mast-cell and basophils activation and release of histamine and of other pro-inflammatory mediators. Further, the binding of autoantibodies to receptors activates the complement cascade which results in the generation of anaphylotoxins. Kaplan and co-workers recently demonstrated that in patients with CU, in-vitro histamine release from mast cells is complement-dependent [11] and that the anaphylotoxin C5a enhances histamine release induced by Fc ϵ RI autoantibodies [12].

4. Histopathology and inflammatory aspects

CU shows significant inflammatory signs. A perivascular infiltrate of lymphocytes (mainly CD4+), monocytes, neutrophils, and eosinophils recalling the late-phase antigen-induced reaction, characterizes the sites of whealing [13,14]. Activated CD4+ T cells have been found in CU patients suggesting that these cells are involved in the disease pathogenesis [15]. Such cells might reside in close proximity to mast cells inducing mast cell degranulation, as indicated by in vitro experimental findings [16]. Furthermore, normal basophils stimulated with sera from CU patients generate *de-novo* leukotriene C₄ (LT C₄) [17], an arachidonic acid derivative that is able to induce wheal-and-flare reaction upon intradermal injection in humans.

5. In-vivo and in vitro diagnostic tests

The autologous serum skin test (ASST) has become a popular and readily available means to detect auto-

reactivity in patients with CU in-vivo. In this test 0.05 ml of fresh, sterile serum are injected intradermally into the volar side of the forearm; an equivalent amount of sterile saline is injected at least 3 cm apart as negative control. Readings are taken at 20 min; the occurrence of a wheal-and-flare reaction at the site of serum injection in the absence of any reaction with saline is considered as a positive result. Clearly, the test is not informative in patients characterized by marked dermatographism.

In-vitro histamine release assays (HRA) can be carried out as well. In these assays basophils or mast cells from normal donors are stimulated with sera from CU patients and released histamine is measured in the supernatant by fluorometric methods or immunoassays. Unfortunately these in-vitro tests are time consuming, can be carried out only in selected centers, and show high inter-assay variability as histamine releasability of cells from different donors may change to a large extent.

6. Inconsistencies and problems

Although, at a first glance, the autoimmune origin of CU might seem to explain the pathogenesis of this disorder satisfactorily, several aspects remain unclear.

The first evident problem is the discrepancy between ASST and HRA. The ASST elicits a wheal-and-flare reaction in about 50% of CU patients [1,18], and sera from only a proportion (about 50%) of those who score positive on ASST are able to induce histamine release in-vitro from cultured basophils from one normal donor [18]. A better concordance between in-vivo and in-vitro test can be achieved if sera from patients who score strongly positive on ASST (i.e. a selected subset of patients with CU) are tested on both basophils and mast cells from different donors [19]. However, it should be pointed out that about 50% of CU patients score negative on ASST and a significant proportion of those who score positive is unable to induce any histamine release in-vitro. Interestingly, the reverse condition (i.e. positive HRA in patients negative on ASST) seldom occurs. In a previous study we observed that histamine-releasing activity in-vitro is associated with serum fraction >100 kDa, and hence presumably with functionally active IgG autoantibodies [18]. These observations do suggest that although the presence of circulating autoantibodies is always associated with ASST autoreactivity, skin reactivity can also occur in the absence of circulating autoantibodies.

The second observation rising problems on the interpretation of ASST/HRA was made some years ago by Fagiolo and co-workers [20]. They found that sera from CU patients containing Fc ϵ RI autoantibodies

retained their ability to induce a wheal-and-flare reaction upon intradermal injection of autologous serum even after depletion of IgG. This finding puts the role played by autoantibodies into question and clearly suggests the possible involvement of other factors both in the auto-reactive state detected by ASST and in the pathogenesis of CU. In effect, anti-FcεRI autoantibodies are detected in a variable proportion of patients with CU but it is generally accepted that such proportion is well below 50% [1,10,20–24]. Although it has been claimed that most CU patients will eventually turn out to an overt autoimmune disease, at least 50% of the cases has to be considered idiopathic up to now.

7. Novel findings

One novel observation was that heparin exerted an inhibitory effect both on ASST [18,24] and HRA [18]. In view of its immediacy the in-vivo effect on ASST was suggested to represent a direct effect at a cellular level such as the inhibiting binding activity towards soluble histamine releasing factors [24]. Moreover, importantly, heparin exerted its inhibitory effect also in ASST-positive patients whose sera were not able to induce any histamine release in-vitro, whereas it did not inhibit the wheal-and-flare reaction induced by a SPT (skin prick test) with histamine or with a specific allergen extract in allergic subjects [18]. These observations suggested that histamine is not the target of heparin, at least in-vivo. In-vitro, heparin is known to inhibit basophil histamine release induced by different agents such as anti-IgE, FMLP (formyl-methionyl-leucyl-phenylalanine), and interleukin-3 (IL-3), all of which interact with distinct receptors and activate basophils via various pathways. We were able to show that the in-vitro inhibitory effects of heparin were dose-dependent [18]. Besides the well known anticoagulant activities, heparin interacts with a number of plasma proteins and mast cell surface components, thus its true pharmacological effect is still matter of research [25].

Another recent interesting observation is the discrepancy between skin testing with autologous serum (ASST) and autologous plasma (APST) anticoagulated with Na citrate. Whereas the former, as previously discussed, scores positive in about 50% of cases, the latter does in about 90% of patients. Since autoantibodies are equally present in serum and plasma, this finding pointed to a possible role of clotting factors in the wheal-and flare reactions induced by autologous plasma. In effect, our recent studies showed significantly elevated plasma levels of prothrombin fragment F_{1+2} in CU patients. F_{1+2} is a polypeptide of about 34 kDa that is

released into the circulation during the activation of prothrombin to thrombin by factor Xa [26]. This observation suggests that thrombin generation does take place in CU and also explains the efficacy of drugs active on the coagulation system reported in some patients. In fact, both heparin (which highly increases the antithrombin activity in plasma) and oral anticoagulant therapy (which reduces thrombin generation in vivo [27]) have been shown to be effective in the treatment of CU [28,29]. In experimental models, thrombin was able to induce edema through an increase in vascular permeability [30,31] which can be obtained by a direct effect of thrombin on endothelial cells [32] and indirectly by a thrombin related release of inflammatory mediators, an effect that is reduced by anti-histamines and totally abolished if mast cells granules are depleted [31]. Moreover, thrombin triggers mast cell degranulation [33] and may activate protease activated receptor-1 on mast cells [34]. Finally, thrombin is able to generate C5a in the absence of C3, thus bypassing the whole first part of the complement cascade [35].

In view of this observation we investigated the coagulation system both forward and backwards in patients with CU [36]. We confirmed that mean F_{1+2} levels are significantly higher in patients than in controls and showed that patients showing high F_{1+2} levels display a more severe disease. Mean D-dimer levels were also higher in patients than in normal controls and, again, elevated D-dimer levels were associated with a more severe disease activity. Finally, factor VIIa levels were higher in patients than in controls as well and were statistically correlated with fragment F_{1+2} levels. By contrast, factor XIIa levels were similar in patients and controls. These findings suggest an activation of the extrinsic pathway of the coagulation cascade. Immunohistochemical analysis of skin biopsies showed tissue factor (TF) expression by upper dermal inflammatory cells in CU patients (n. 10) but not in normal controls (n. 10). We concluded that chronic urticaria is characterized by an activation of the extrinsic pathway of the coagulation with the involvement of inflammatory cells, leading to the formation of thrombin which might be responsible for generating C5a and increasing vascular permeability. On the other hand, the excess in fibrin formation could be counter-balanced by an enhanced fibrinolysis, as suggested by the increase in D-dimer levels.

8. Conclusions

Although histamine release mediated by autoantibodies probably represents the main pathogenic mechanism

in patients with chronic urticaria displaying a more severe disease [37], such pathogenic mechanism seems to be effective in a minority of patients [38]. Other mechanisms, namely the activation of the extrinsic coagulation pathway with thrombin generation might play a role in the majority of patients. Whether the two mechanisms co-exist acting synergistically or are somehow linked or sequential remains to be established.

Take-home messages

- Autoimmune pathogenic mechanisms have been suggested to mediate chronic urticaria.
- Autoantibodies to FcεRI or to IgE are found in about 30% of chronic urticaria patients.
- An activation of the extrinsic pathway of the coagulation cascade resulting in thrombin generation seems to occur in the majority of patients with CU.
- Thrombin is able to induce mast cell degranulation and C5a production.
- Thrombin generation can be detected in the majority of CU patients.

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