

Activation of blood coagulation in chronic urticaria: pathophysiological and clinical implications

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Abstract Chronic urticaria (CU) is a skin disorder characterized by the recurrent eruption of short-lived wheals accompanied by redness and itching for at least 6 weeks. The wheals can be associated with angioedema. CU is considered an autoimmune disease in about 50% of cases with the presence of circulating histamine releasing auto-antibodies mainly directed against the high affinity IgE receptor FcεRI on mast cells and basophils or against IgE. In several CU cases regarded as idiopathic; the actual pathophysiological mechanisms are still unknown. Some patients with CU do not respond to antihistamines and require the use of systemic steroids or cyclosporin, which are, however, not always effective. In CU, several investigators have demonstrated the activation of coagulation that is due to the involvement of eosinophils and a tissue factor pathway with generation of thrombin potentially contributing to an increased vascular permeability. CU

patients often present with elevation of coagulation and fibrinolysis markers, such as prothrombin fragment F1+2 and D-dimer, which correlate with the disease severity. Preliminary data indicate that anticoagulant treatment with heparin and warfarin may be effective in reducing the symptoms of this disorder. Taken together, all these findings provide the rationale for proposing clinical trials on the use of anticoagulant drugs as adjuvant treatment in CU patients.

Keywords Chronic urticaria · Coagulation · Eosinophil · Tissue factor · Autoimmunity

Introduction

Chronic urticaria (CU) is a skin disorder characterized by the recurrent eruption of short-lived wheals accompanied by redness and itching for at least 6 weeks [1]. The wheals are skin lesions characterized by localized edema of the upper dermis. They are usually pale in the center, with a red surrounding flare when they erupt and become pink as they mature (Fig. 1a). The wheals can be associated with deeper swelling of the dermis and subcutaneous and sub-mucosal tissues, which is called angioedema (Fig. 1b). Wheals and angioedema often coexist in urticaria, but may occur alone [2].

CU is a rather frequent disease with an estimated prevalence of 0.5–1% [3, 4] and a female predominance (female/male ratio: 2/1) [4]. CU has a heavy impact on the quality of life; in fact, health status scores in patients with CU are comparable to those reported from patients with coronary artery disease, particularly in terms of work performance, sleep disruption, emotional reactions and social interactions [5].

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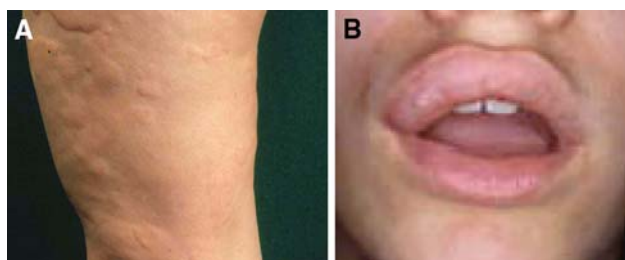


Fig. 1 **a** Multiple pink wheals on a leg in chronic urticaria. **b** Angioedema involving the upper lip in a chronic urticaria patient

Classification of chronic urticaria

The most useful classification of CU is that based on the clinical aspects (Table 1), and distinguishes urticarias provoked by physical stimuli (physical urticaria), skin contact with biologic or chemical agents (contact urticaria), or small vessel vasculitis (urticarial vasculitis) from all other presentations, which are grouped under the umbrella term: “ordinary” CU. The present review focuses on this last subset of CU. The timing of onset and duration of wheals may be helpful to classify the form of CU. The wheals of ordinary CU usually fade after 2–24 h. Physical urticarias usually appear within 10 min after the trigger stimulus (cold, heat, pressure, physical exercise, aquagenic or sun exposure) and clear within 1 h. An exception is represented by the so-called “delayed” pressure urticaria that characteristically arises several hours after sustained pressure, and lasts at least a day. Contact urticaria arises within 10–30 min following exposure to the contactant, and resolves over about 2 h. Finally, the wheals of urticarial vasculitis usually last several days; moreover, they tend to burn rather than itch [2, 3].

Pathogenesis of chronic urticaria

Several factors have been advocated as causes of the disease, including emotional disorders [6, 7], food allergy,

intolerance to food additives [8] and chronic infections like *Helicobacter pylori* [9]. More recently, experimental and clinical findings have supported the autoimmune origin in several cases of CU [3]. CU is thought to have an autoimmune basis in approximately 45% of patients. In a variable proportion (30–60%) of patients with active disease, the intradermal injection of autologous serum (autologous serum skin test, ASST) causes a wheal-and-flare reaction, and the serum from some CU patients is able to induce histamine release from cultured basophils of healthy subjects. Both phenomena have been ascribed to circulating IgG specific for the high affinity IgE receptor FcεRI present on mast cells and basophils or for IgE [10–12]. The IgG antibodies activate the classical complement cascade [13, 14], and purification of IgG subclasses demonstrate histamine-releasing activity predominantly within subclasses 1 and 3 [15]. Other autoantibodies may also contribute to CU. A recent large in vitro study identifies autoantibodies to CD23 (the low-affinity IgE receptor) in 65% of patients with CU [16]. This autoantibody is able to cause mast cell degranulation in vitro indirectly through the release of the major basic protein from eosinophils. The quite frequent association of CU with antithyroid antibodies [17–19], markers of autoimmunity [20], further supports the autoimmune nature of the disease.

The wheals and itching that characterize CU are considered due to activation of dermal mast cells that secrete preformed mediators, including histamine (the main cause of pruritus), proteases, interleukin-1 and tumor necrosis factor-α [3]. Although a large number of naturally occurring immunological and nonimmunological agents are known to be capable of activating dermal mast cells, only anti-IgE and anti-FcεRI autoantibodies and complement C5a are of established importance in CU [3]. The most important advances in the knowledge of CU pathophysiology that have marked the last two decades are summarized in Fig. 2.

Open questions

Although autoimmunity explains some aspects of CU pathogenesis, several points remain unclear. It is generally accepted that autoantibodies to IgE or to the high-affinity IgE receptor, FcεRI, which are commonly regarded as the most relevant pathogenic factors in this disease, can be detected in sera of only 25–50% of patients with CU [11, 12, 21–23]. Another important problem is the discrepancy between skin response to autologous serum and the histamine release from cultured basophils. The ASST elicits a wheal-and-flare reaction in about 50% of CU patients [1, 24], and sera from only half of those who score positive on ASST are positive for in vitro histamine release assay

Table 1 Clinical classification of chronic urticaria

Ordinary urticaria (the most common form, generally without a defined triggering stimulus)
Physical urticaria (triggered by different physical stimuli)
Aquagenic urticaria
Cholinergic urticaria (heat, physical exercise)
Cold urticaria
Delayed pressure urticaria
Solar urticaria
Contact urticaria (skin contact with biologic or chemical agents)
Urticarial vasculitis (small vessel vasculitis)

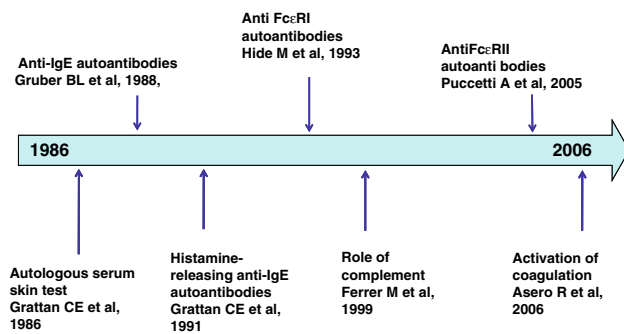


Fig. 2 Chronology of the key events in the investigation of chronic urticaria pathophysiology in the last two decades. The observation that injection of autologous serum causes a wheal and flare reaction in about 50% of chronic urticaria patients by Grattan et al. [10] in 1986 paved the way for the concept of chronic urticaria as an autoimmune/autoreactive disease. In the subsequent years, histamine releasing autoantibodies directed against IgE [11, 22] and against the high affinity IgE receptor [12] have been identified in about 30% of chronic urticaria patients. The role of complement in the amplification of histamine release induced by autoantibodies is demonstrated by Kaplan's group [13]. In 2005, Puccetti et al. [16] report autoantibodies directed against the low affinity IgE receptor in about 65% of chronic urticaria patients. These autoantibodies are shown to induce eosinophil activation with release of the major basic protein. Finally, in 2006 our group [29] demonstrates the activation of the coagulation cascade, supporting its involvement in chronic urticaria pathophysiology. Coagulation activation stemmed from eosinophil expression of tissue factor has been confirmed by us as well as by other groups [35–37, 40–42]

(HRA) [24]. Moreover, Fagiolo et al. [25] find that sera from CU patients containing anti-FcεRI autoantibodies retain the ability to induce a wheal-and-flare reaction upon intradermal injection of autologous serum even after depletion of IgG. These findings suggest the possible involvement of other factors both in the autoreactive state detected by ASST and in the pathogenesis of CU.

Involvement of blood coagulation in chronic urticaria

Pathophysiological aspects

Recently, some evidence of the possible involvement of the coagulation cascade in the pathogenesis of CU has emerged. We observed that positive autologous skin test rises up from 50 to 80% if autologous-citrated plasma instead of autologous serum is injected [26], and that CU patients show elevated plasma levels of prothrombin fragment F1+2, a polypeptide of about 34 kD that is released into the circulation during the activation of prothrombin to thrombin by activated factor X (FXa) [26]. Interestingly, F1+2 plasma levels were found to be related to urticaria severity at the time plasma was collected [26]. The abovementioned findings may suggest that CU is

associated with the generation of thrombin, and that the severity of the disease is paralleled by the amount of thrombin generated. Thrombin is a serine protease involved in hemostasis as well as in vessel wound healing, revascularization, and tissue remodeling [27]. It is of note for the pathophysiology of CU that thrombin is able to cause a relevant increase in vascular permeability [28, 29] and to activate mast cells, triggering their degranulation [30, 31]. In subsequent studies we find that CU patients show an activation of the tissue factor (TF) pathway of coagulation cascade [32], and that in patients with severe disease such activation can be so pronounced as to produce an elevation of plasma levels of D-dimer, the last being regarded as a sign of fibrinolysis [33]. The increase of F1+2 and D-dimer has been reported also in angioedema due to C1-inhibitor deficiency [34, 35] in which are observed the activation of not only of the TF pathway, but also the contact system [34]. Both F1+2 and D-dimer plasma levels prove to be extremely high during an acute phase of severe CU but are completely normal after remission [33]. In another study [32], immunohistochemical experiments show TF expression in skin specimens from CU patients. Recently, we demonstrated that TF is expressed by eosinophils present in the inflammatory infiltrate of CU skin lesions [36]. The nature of the TF expressing cell is revealed by performing double-staining studies that show co-localization of TF and eosinophil cationic protein, a classic cell marker of the eosinophil. These data highlight the importance of eosinophils in CU as a source of TF, in keeping with studies showing that eosinophils store TF and rapidly transfer it to the cell membrane during activation [37]. The strong expression of TF in CU lesional skin may be due to eosinophil activation, even if patients with CU virtually never show peripheral eosinophilia, probably because TF specifically facilitates the early transendothelial migration of the eosinophils [37]. The activation of the TF pathway of coagulation results in the generation of thrombin that, in experimental models, has been shown to induce edema through an increase in vascular permeability [28, 29] by a direct effect on endothelial cells [38] and indirectly by a thrombin-related release of inflammatory mediators. Most effects of thrombin are probably histamine mediated (and hence mast cell mediated), as they have been reported to be reduced by anti-histamines and mast cell granule depletion in animal models [29]. Furthermore, thrombin triggers mast cell degranulation [31] and may activate protease activated receptor-1 on mast cells [30] in animal models. The activation of blood coagulation in patients with CU has been recently confirmed by other independent researchers [39–41], and thus can be added to the mechanisms involved in CU (Fig. 3). In particular, Wang et al. [39] find increased plasma levels of FVIIa (marker of the activation of the TF pathway), thrombin-antithrombin complex

(marker of thrombin generation) and D-dimer (marker of fibrin degradation), which are correlated to each other in CU patients. Thrombin–antithrombin complex and D-dimer plasma levels are also found increased in CU by Fujii et al. [40]. Khalaf et al. [41] confirm high levels of F1+2 during active CU and a significant decrease during remission.

Clinical implications

CU patients show a marked increase in plasma markers of thrombin generation and fibrinolysis during severe exacerbations of the disease [33]. The activation of coagulation and fibrinolysis decreases till complete normalization during remission of CU [33, 40], and, as far as we know, is not associated with an increased risk of thrombosis. Whether this phenomenon (activation of coagulation/fibrinolysis) is relevant to the disease pathophysiology, or simply acts as an amplification system is still to be defined; however, the fact that it parallels the activity of CU may provide the rationale for the evaluation of anticoagulant and antifibrinolytic therapy in patients with CU. Heparin is an anticoagulant that potentiates the effect of antithrombin, a plasma protein that binds irreversibly to some serine protease enzymes (mainly factor Xa and thrombin) and blocks their

activity. Scattered reports indicate that heparin can be effective in the treatment of CU [42, 43]. Meyer-De Schmid and Neuman [42] observe a persistent remission in four CU patients (complete in 3 and partial in 1) treated with intravenous unfractionated heparin at dosage of 50–100 mg twice a day corresponding to 5,000–10,000 U. Chua and Gibbs [43] observe a complete remission in a CU patient refractory to antihistamine and immunosuppressive treatments during administration of subcutaneous unfractionated heparin (5,000 U twice a day) with a relapse when heparin was discontinued. Moreover, oral anticoagulant drugs improve clinical symptoms in patients with CU unresponsive to antihistamines in a small but double-blind, placebo-controlled trial in which six of eight CU patients responded to warfarin [44]. In a recent uncontrolled study, Mahesh et al. [45] obtain remission with warfarin at a therapeutic dose in four out of five CU patients (complete in 2 and partial in 2). The antifibrinolytic agent tranexamic acid, which is very effective in angioedema patients [46], has been proposed also in the treatment of CU with a good clinical response in two CU patients [47], but its effectiveness was not confirmed in a double-blind study [48]. Taken together, all these findings provide the rationale for proposing clinical trials on the use of anticoagulant drugs as adjuvant treatment in CU patients.

Conflict of interest statement The authors declare that they have no conflict of interest related to the publication of this manuscript.

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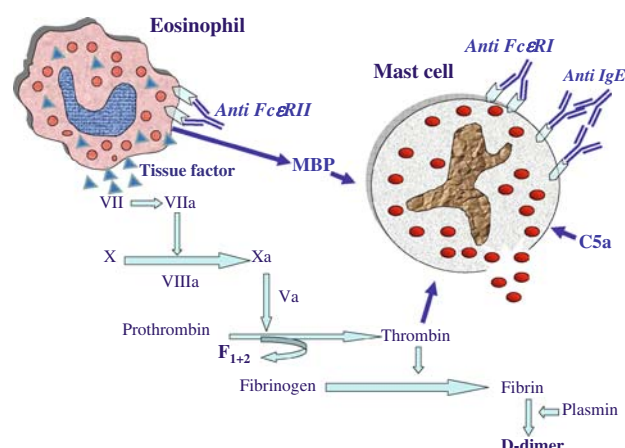


Fig. 3 Mechanisms of eosinophil and mast cell activation in chronic urticaria (CU). Mast cells release histamine and other inflammatory mediators after stimulation by autoantibodies directed against the high-affinity IgE receptor (FcεRI) and IgE, complement anaphylatoxin C5a, eosinophil-derived major basic protein (MBP) and possibly other molecules. Eosinophils are activated by autoantibodies directed against the low-affinity IgE receptor (FcεRII) and potentially by other factors, release MBP and express tissue factor which in turn activates the coagulation cascade (factors VII, X, V and prothrombin) leading to thrombin generation. Experimental data in animal models indicate that thrombin also induces mast cell degranulation. Thrombin generation is demonstrated in CU patients by the increased plasma levels of the fragment F1+2 released from prothrombin after its activation. Finally, fibrin degradation is documented by elevated plasma levels of the fibrin fragment D-dimer in CU patients with active disease

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