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Skin autoimmunity and blood coagulation

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Abstract

Evidence exists that the immune and coagulation systems are simultaneously activated in some systemic autoimmune disorders. Although proinflammatory mediators induce tissue factor (TF) expression, the main initiator of blood coagulation, activated proteases of coagulation may act on protease-activated receptors (PAR) triggering inflammation. Such a cross-talk amplifies and maintains the activation of both systems. This review focuses on the involvement of immune and coagulation system in two skin disorders as chronic urticaria (CU), autoimmune in about 45% of cases, and bullous pemphigoid (BP), the prototype of autoimmune blistering diseases. Several investigators demonstrated the activation of coagulation in CU through the involvement of eosinophils, of TF pathway with thrombin generation and increased vascular permeability. Preliminary data indicate that anticoagulant treatment with heparin and warfarin may be effective in reducing the symptoms of this disorder. The activation of coagulation seems to display local and systemic implications in BP. Eosinophils' recruitment and thrombin generation locally contribute to the bulla formation and tissue damage. The systemic activation of coagulation may explain the increased thrombotic risk observed in these patients. Taken together, these data provide the rationale for proposing clinical trials on the anticoagulant treatment in both CU and BP patients.

Keywords: *Tissue factor, coagulation, eosinophils, urticaria, bullous pemphigoid*

Introduction

The simultaneous activation of the immune system and blood coagulation after injury is a phylogenetically ancient, adaptive response that can be traced back to the early stages of evolution [1]. The two systems are strictly linked to provide defence against infections and tissue damages, however, sometimes their activation may proceed uncontrolled leading to the development of thrombosis and organ injury, as during sepsis [2].

Inflammation is the link between the immune and the coagulation system. Proinflammatory cytokines such as interleukin 1 (IL-1), IL-6 and tumor necrosis factor alpha (TNF-α) induce the expression of tissue

factor (TF) the main initiator of blood coagulation and downregulate the natural anticoagulants such as antithrombin, protein C and TF pathway inhibitor [3]. TF activates factor VII and the complex of TF with activated factor VII (TF-FVIIa) catalyzes the conversion of factor X–Xa, which forms the prothrombinase complex with factor Va, prothrombin, and calcium, thereby generating thrombin which leads to the formation of fibrin clot from fibrinogen. At the cellular level, the coagulant mediators (FVIIa, FXa, and FIIa) in turn act on protease-activated receptors (PAR) inducing the expression of proinflammatory cytokines [2,4]. This cross-talk between inflammation and coagulation creates a self-refuelling

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loop which amplifies and sustains the activation of both systems [2].

The dysregulation of the immune response may affect the coagulation system as observed in several systemic immune-mediated diseases including systemic lupus erythematosus [5], rheumatoid arthritis [6], and inflammatory bowel diseases [7] with a consequent increased risk for thrombosis. Recent studies suggested the involvement of blood coagulation also in some autoimmune skin disorders whose aspects will be analyzed in the present review. In particular, we will focus on two skin disorders as chronic urticaria, which is considered autoimmune in about 45% of cases and bullous pemphigoid (BP), which is the prototype of autoimmune blistering disease.

Chronic urticaria

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 deeper swelling of the dermis and subcutaneous and submucosal tissues which is called angioedema. Wheals and angioedema often coexist in urticaria but may occur alone [8].

The estimated prevalence of CU is 0.5–1% with women being affected twice as frequently as men [8]. CU is one of the diseases with the greatest impact on quality of life of all cutaneous disorders. 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 [9].

Although a variety of causative factors have been advocated over the years including emotional disorders, food allergy, intolerance to food additives and chronic infections like *Helicobacter pylori*, only recently evidence emerged that some CU patients are suffering from an autoimmune disease [8]. 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 the IgE themselves [10,11]. The IgG antibodies activate the classical complement cascade, and purification of IgG subclasses demonstrated histamine-releasing activity predominantly within subclasses 1 and 3 [12].

The detection of anti FcεRI autoantibodies is difficult due to the interference of the natural IgE,

immune complexes and complement [13], making this investigation currently limited to the research setting. It has been suggested that other autoantibodies may also contribute to CU. A recent large *in vitro* study identified autoantibodies to CD23 (the low-affinity IgE receptor) in 65% of patients with CU [14]. This autoantibody was able to cause mast cell degranulation *in vitro* indirectly through the release of the major basic protein (MBP) from eosinophils. The quite frequent association of CU with antithyroid antibodies, further supports the autoimmune nature of the disease [15].

Another possible mechanism of mast cell activation in CU involves activated T lymphocytes. In fact, it has been demonstrated that activated T lymphocytes induce degranulation and cytokine production by human mast cells following cell-to-cell contact [16]. All these mechanisms are summarized in Figure 1.

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, IL-1, and tumour necrosis factor-α [8]. 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

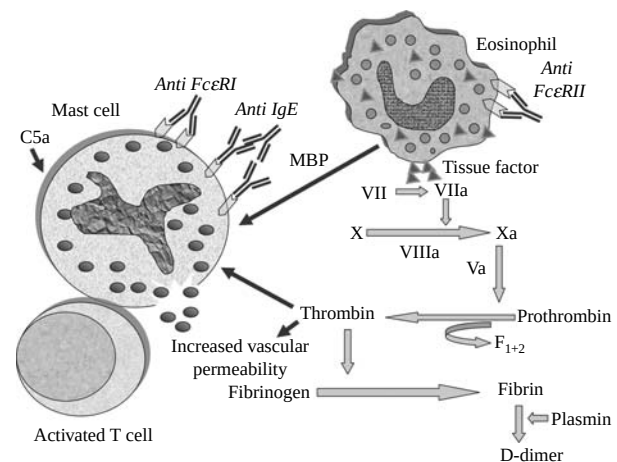


Figure 1. Mechanisms of eosinophil and mast cell activation in CU. Mast cells release histamine and other inflammatory mediators after stimulation by autoantibodies directed against the high-affinity IgE receptor (FcεRI) and IgE, complement anaphylatoxin C5a, eosinophil-derived MBP and possibly other molecules. Eosinophils are activated by autoantibodies directed against the low-affinity IgE receptor (FcεRII), by activated T lymphocytes and potentially by other factors. Upon their activation, eosinophils release MBP and express TF which in turn activates the coagulation cascade (factors VII, X, VIII, V, and prothrombin) leading to thrombin generation. Experimental data in animal models indicate that also thrombin induces mast cell degranulation as well as increased vascular permeability. 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.

autoantibodies and complement C5a are of established importance in CU [8].

Although autoimmunity explains some aspects of CU pathogenesis, several points still remain unclear. It is generally accepted that autoantibodies to IgE or to the high-affinity IgE receptor (FcεRI) can be detected in sera of only 25–50% of patients with CU [11,17]. 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, and sera from only half of those who score positive on ASST are positive for *in vitro* histamine release assay (HRA). These findings suggest the possible involvement of other factors both in the autoreactive state detected by ASST and in the pathogenesis of CU.

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, and that CU patients show elevated plasma levels of prothrombin fragment F1+2, suggesting thrombin generation [18]. In subsequent studies, we found that CU patients show an activation of TF pathway of coagulation cascade [18], and that in patients with a severe disease such activation can be so pronounced to produce an elevation of plasma levels of D-dimer [19]. Immunohistochemical experiments showed TF expression by eosinophils present in the inflammatory infiltrate of CU skin lesions [20]; an example is reported in Figure 2, panel A. 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 [21].

The strong expression of TF in CU lesional skin may be due to eosinophil activation, even if patients with CU virtually never display peripheral eosinophilia, probably because TF specifically facilitates the early transendothelial migration of the eosinophils into the affected tissues [21]. The activation of the TF pathway of coagulation results in the generation of thrombin which, in experimental models, has been shown to induce oedema through an increase in

vascular permeability directly by acting on endothelial cells and indirectly by a thrombin-related release of inflammatory mediators [22,23]. 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 [22]. Furthermore, thrombin triggers mast cell degranulation and may activate protease activated receptor-1 on mast cells [24] in animal models. The activation of the blood coagulation in patients with CU has been recently confirmed by other independent researchers [25–27] and thus can be added to the mechanisms involved in CU (Figure 1).

CU patients show marked increase of plasmatic markers of thrombin generation and fibrinolysis during severe exacerbations of the disease [19]. The activation of coagulation and fibrinolysis decreases till complete normalization during remission [19,26]. Whether this phenomenon (activation of coagulation/fibrinolysis) plays a pivotal role in the disease pathophysiology or acts simply as an amplification system is still to be defined. However, the fact that such an activation parallels the activity of CU may provide the rationale for the evaluation of anticoagulant and antifibrinolytic therapy in patients with CU.

Bullous pemphigoid

BP is an autoimmune skin disease that is characterized by the formation of blisters on normal or erythematous skin as a result of the interaction of autoantibodies with two hemidesmosomal antigens (BP180 and BP230), followed by complement activation and leukocyte infiltration [28]. A variety of activated inflammatory cells, particularly autoreactive T cells and eosinophils, participate in blister formation by producing and releasing a number of cytokines and soluble factors involved in amplifying and maintaining tissue damage [29,30]. Eosinophils are found scattered throughout the upper dermal infiltrate or aggregated at the edge of the dermal-epidermal separation. They are present in the blister space, and their involvement is also demonstrated by the high concentrations of eosinophil cationic protein and MBP in blister fluid and BP sera [31].

Patients with BP display an increased expression and release of various cytokines and soluble factors involved in chemotaxis and eosinophil activation, including IL-5, IL-16, and eotaxin [32], thus confirming the importance of eosinophils in its pathogenesis. Moreover, eosinophil production and release of matrix metalloproteinases (MMPs), elastase and gelatinase may further contribute to tissue damage in BP [33]. In particular, it has been shown that the 92 Kd gelatinase produced by eosinophils at the site of blister formation cleaves the extracellular domain of the 180 Kd recombinant BP antigen, thus

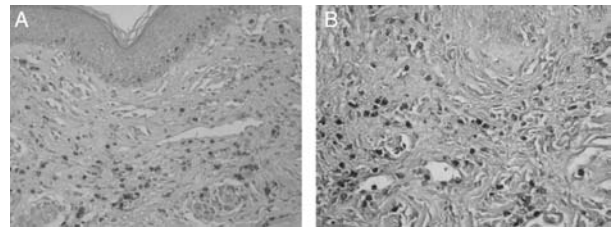


Figure 2. Immunohistochemical studies of lesional skin from CU (panel A) and BP (panel B) (staining for TF; × 400). Both panels show TF expression, which is more intense in BP.

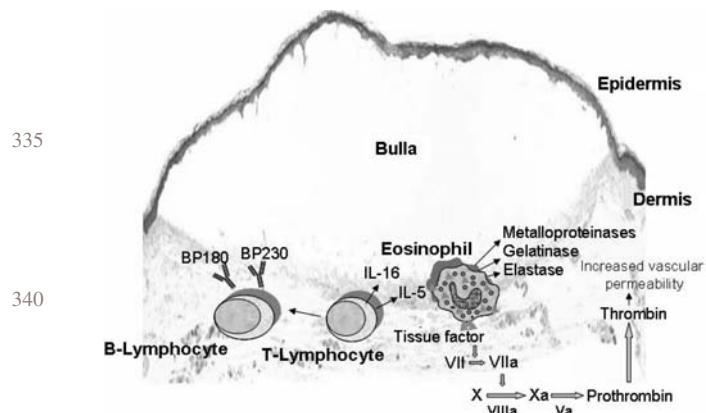


Figure 3. Simplified scheme illustrating the possible involvement of blood coagulation in the pathophysiology of BP. Bulla formation is the result of a dermo-epidermal detachment due to the interaction of autoantibodies with two hemidesmosomal antigens (BP180 and BP230), followed by complement activation and leukocyte infiltration. Autoreactive T lymphocytes cooperate with B lymphocytes in the autoantibody production; in addition, they release cytokines, most notably IL-5 and IL-16, and other soluble factors responsible for the recruitment and activation of eosinophils. In lesional skin, eosinophils produce and release MMPs, elastase and gelatinase which may further contribute to tissue damage. Moreover, eosinophils strongly express TF, which is the main initiator of the coagulation cascade (factors VII, X, VIII, V, and prothrombin) leading to generation of thrombin. This last may increase the permeability of blood vessels, amplifying the inflammatory process.

potentially contributing to blister formation [33]. Eosinophils strongly express TF in BP lesional skin (Figure 2, panel B), as we have recently demonstrated by immunohistochemistry [34]. This is in line with recent findings indicating that eosinophils are a major intravascular location for TF storage and exposure and that TF specifically facilitates the early transendothelial migration of the eosinophils [21].

The role of TF in initiating extrinsic blood coagulation leads to the intriguing hypothesis that the TF-mediated coagulation pathway may be involved in BP (Figure 3). Such an hypothesis is theoretically supported by the fact that both the plasma and blister fluid of our BP patients displayed significantly higher levels of D-dimer and F1+2 than normal plasmas [34]. The observed activation of the extrinsic coagulation pathway generates thrombin, an enzyme which increases permeability of blood vessels [35]. The presence of thrombin may play a role in the pathogenesis of BP by increasing vascular permeability (Figure 3) and favoring the transendothelial migration of inflammatory cells and their accumulation in the skin.

The activation of coagulation, besides the physiopathological effects in the skin microenvironment, may have systemic clinical consequences as a hypercoagulable state leading to thrombotic complications. Although the actual thrombotic risk in BP patients should be further assessed by properly

designed studies, a retrospective analysis revealed the occurrence of various thrombotic complications in 50% of the 130 BP patients seen at our Institute over the last 4 years [34]. In particular, in our series of BP patients, we observed an incidence of venous thrombosis of 8% per year [34], which is clearly higher than in the age-matched general population (2.8–4.1% per year) [36].

In contrast to BP, pemphigus vulgaris (PV), the other important autoimmune blistering disorder, is not characterized by activation of blood coagulation. In fact, PV patients do not display increased markers of thrombin generation and fibrin degradation in their plasma and lack TF immunoreactivity in their lesional skin [37]. Histologically, BP is characterized by a dense dermal inflammatory infiltrate mainly composed by lymphocytes and eosinophils. In contrast, the dermal infiltrate is mild and displays only few eosinophils in PV, the major variant of pemphigus. Clinically, in BP, the blisters are “hot,” due to the presence of an erythematous-edematous halo, while in PV, they are “cold,” usually arising on normal skin. The different intensity of inflammation and the different degree of eosinophil involvement may explain why coagulation is markedly activated in BP but not in PV. The clinical observation that thrombotic risk is increased in BP but not in PV is in line with the aforementioned physiopathological findings.

Clinical and therapeutic implications

The activation of coagulation in autoimmune skin disorders may have two main consequences: on one hand, a local pathogenic role in inducing the skin lesions and on the other hand a systemic role in increasing the thrombotic risk. Concerning the pathophysiology of the skin lesions, thrombin increases the endothelial vascular permeability, thus contributing to the inflammatory processes of both CU and BP.

Scattered reports indicate that heparin, an anticoagulant which potentiates the effect of antithrombin, can be effective in treating CU [38,39]. Moreover, warfarin, an oral anti-vitamin K drug, induced a good response in patients with CU unresponsive to antihistamines in a small but double-blind, placebo-controlled study [40]. We have preliminary data indicating that CU patients with elevated D-dimer often present a more severe disease with reduced response to antihistamines and may benefit of a short-time treatment with low-molecular-weight heparin. To our knowledge, no data are available on the use of anticoagulant drugs in the treatment of BP and this issue should be a matter of further investigation.

Concerning the systemic implications of the coagulation involvement, several data indicate an increased thrombotic risk in BP [34,41]. In contrast, no data are available concerning the incidence of

thrombotic complications in patients with CU and further studies are needed. The retrospective evaluation of our patient series with BP has shown an increased incidence of venous thrombosis. Although most BP patients are elderly, the incidence of venous thrombosis in this kind of patients appears to be significantly increased as compared to the age-matched population of otherwise healthy subjects. BP is the prototype of autoimmune blistering disorder mediated by autoantibodies but in which an important physiopathological role is played by eosinophils. The intense skin inflammatory infiltrate is in fact characterized by the presence of eosinophils together with autoreactive CD4+T lymphocytes and a few other inflammatory cells.

Based on our findings, eosinophils play an important role in the activation of coagulation, an issue which is supported by the scattered reports of thrombotic complications in patients with other eosinophil-mediated diseases, namely the Churg Strauss syndrome and the idiopathic hypereosinophilic syndrome. The increased incidence of venous thrombosis in BP patients raises the question of the indication of anticoagulant treatment in combination with the conventional immunosuppressive therapy.

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