

Role of the B1 Bradykinin Receptor and gC1qR/p33 in Angioedema

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KEYWORDS

• Angioedema • Vascular leakage • gC1qR/p33 • BK 1 receptor • BK 2 receptor

KEY POINTS

- The features and signs seen in angioedema (AE) lesions are typical of the inflammatory process, but increased vascular permeability predominates, leading to dermal and subcutaneous edema.
- gC1qR/p33 may represent a natural surface receptor that modulates or triggers the coagulation/kinin cascade, causing the generation of bradykinin (BK) and related kinins.
- Both B1 and B2 BK receptors are involved in AE episodes.
- Experimental data suggest that the blockade of both B1 and B2 receptors, or gC1q/p33, may provide novel therapeutic targets to induce symptom relief.

CLINICAL FEATURES OF ANGIOEDEMA EPISODES

Patients affected by angioedema (AE) are subject to asymmetric, nonerythematous, nonpruritic, localized, transient, episodic swelling of deeper layers of the skin or submucosal tissues of the skin, oropharyngolaryngeal tissue, and/or gastrointestinal wall (**Fig. 1**).¹ When the swelling occurs in the intestine, it causes severe abdominal pain, reminiscent of appendicitis, and intestinal obstructions. When the colon is affected, severe watery diarrhea may occur. Swelling in the larynx is the most dangerous symptom, because the affected individual may suffocate. The features and signs seen in AE lesions are typical of the inflammatory process but increased vascular permeability predominates, leading to massive dermal and subcutaneous edema, becoming

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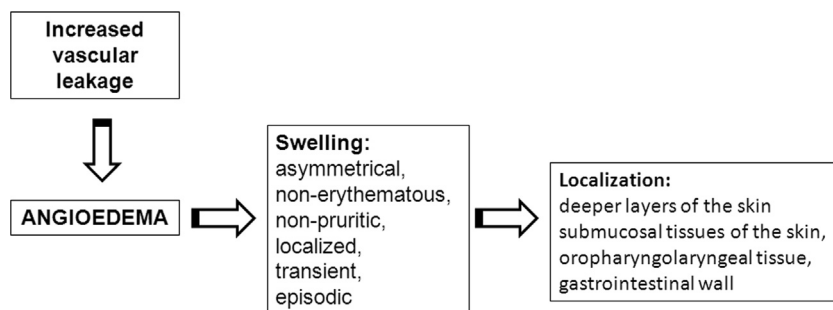


Fig. 1. Clinical signs of angioedema episodes.

particularly life threatening if it occurs in the upper respiratory tract. AE affects at least 20% of the population and several forms recur chronically, causing significant personal, domestic, social, and occupational disability.

Different forms of AE can be distinguished based on the presence or absence of chronic recurrence or significant urticaria. Acute AE is frequently a direct consequence of exposure to a specific substance (usually a food, chemical, or drug) eliciting an allergic/pseudoallergic reaction, which releases histamine. Chronic recurrent AE, occurring with prominent urticarial flare, is typically histamine mediated. More mysterious is the condition of chronically recurrent AE without urticaria. Such episodes may be triggered by trauma, menstrual periods, excessive exercise, or exposure to extremes of temperature or mental stress, but most events remain apparently spontaneous.^{2,3} This situation is frequently seen in cases of inherited C1 inhibitor (C1-INH) deficiency and in AE related to angiotensin-converting enzyme (ACE) inhibitor treatment. AE associated with C1-INH deficiency is an inherited (hereditary angioneurotic edema [HAE]) or acquired (acquired angioedema) clinical condition that affects approximately 1:50,000 subjects in the general population.^{4,5} The inherited deficiency is caused by mutations in the C1-INH gene, whereas the acquired form is the result of protein hypercatabolism, frequently caused by autoantibodies to C1-INH, and is associated with lymphoproliferative disorders and, in a few instances, with solid neoplasia, autoimmune, and infectious diseases.^{6–8} Although the pathogenesis of the swelling associated with HAE was originally thought to be mediated by complement and, more specifically, by C2-derived kinin, evidence now exists that the swelling involves primarily the kinin-forming pathway.^{3,9}

In spite of a lifelong stable C1-INH deficiency or continuous ACE inhibitor treatment, patients with these conditions are only seldom symptomatic, with huge variation in frequency and severity of symptoms. The mechanism(s) responsible for such variability is unexplained and, despite the progress that has been made in recent years, the molecular mechanisms underlying AE, particularly in the chronic forms, are still largely unknown. To unravel the pathogenesis of AE, the molecular players should be identified, including the factors that act to localize the event at a specific site, the mediator(s) that induce permeability on the vascular structures, and the enzymatic systems promoting the release of such mediator(s). The increase of vascular permeability may be induced by different factors. Histamine is the best characterized mediator that causes the vascular response. However, several systems may be activated during AE attacks, and the contact system, the factor XII (FXII)-dependent fibrinolytic cascade, and the complement system seem to be the most important factors involved in the disease.¹⁰

Available evidence suggests that the nonapeptide bradykinin (BK), generated at the endothelial cell surface, may be largely responsible for the vascular permeability seen in most AE, which does not respond to antihistamine. This possibility is supported by the presence of high levels of BK in the plasma of patients during acute attacks but not when in remission. Patients with AE that is responsive to antihistamines do not have increased levels of BK even during acute attacks. These data suggest the existence of 2 distinct groups of AE: histaminergic and nonhistaminergic. Histaminergic AE responds to antihistamines, whereas nonhistaminergic AE is more difficult to treat. In these cases the treatment of AE attacks involves drugs that function as BK B2 receptor antagonists (icatibant/HOE-140) or restore the control of BK formation through the inhibition of kallikrein (Dx88 and plasma-derived C1-INH). The result of the treatment is rapid relief, but complete resolution of symptoms is rarely obtained in less than 12 hours.¹¹

ROLE OF GC1QR IN BK RELEASE ON EC

Because simultaneous and unregulated activation of both the complement (C) and kallikrein-kinin systems on the endothelial surface is largely responsible for the BK-mediated vascular permeability leading to edema, identification and characterization of the molecule or molecules that facilitate the assembly and activation of the kinin-generating pathway have been the focus of intense research over the past 20 years.

The ECs are activated by the late C components from C5 to C9 that form the terminal C complex (TCC). The formation of this complex is initiated with the release of C5b from cleaved C5, followed by progressive binding of the remaining late C components from C6 to C9. This complex may cause cytolysis through a pore formation following insertion into the plasma membrane of target cells,¹² or as a sublytic complex that fails to cause cell lysis despite its binding to the phospholipids bilayer of the cell membrane. The TCC can also assemble in the fluid phase, where it accumulates as a cytolytically inactive complex because of the short half-life of its cell-binding ability. The complex binds the soluble C regulators S protein and clusterin and circulates in plasma or is present in the extravascular fluid as SC5b-9.¹³ We have shown that this complex, despite its inability to cause cytolysis, promotes vascular leakage.¹³ This effect was documented *in vitro* using a Transwell system to evaluate the leakage of fluorescein-labeled bovine serum albumen (BSA) or the *intravital* microscopy to follow the extravasation of circulating FITC-BSA across mesenteric microvessels.¹³ The permeabilizing activity of SC5b-9 was partially inhibited by the B2R antagonist (HOE-140), or the selective platelet activating factor (PAF) receptor antagonist (CV3988) and was completely neutralized by the mixture of the two antagonists, suggesting that the effect of SC5b-9 is mediated by the formation of BK and the release of PAF.

Our observation was further supported by the finding of high levels of SC5b-9 in pleural fluids of patients affected by Dengue virus infections.¹⁴ In these patients, who die mainly from vascular leakage and shock, the nonstructural viral protein 1 (NS1) activates C, resulting in the release of anaphylotoxins and SC5b-9. The plasma levels of NS1 and SC5b-9 correlated with the disease severity, suggesting that the anaphylotoxins and the terminal complex may contribute to the pathogenesis of the vascular permeability. A significant activation of the terminal pathway of the C sequence was also observed during the attack phase of hereditary AE, as documented by the increased levels of SC5b-9 measured in patients during the attack phase.¹⁵ These data, together with the *in vitro* and *in vivo* observations indicating a clear permeabilizing activity of SC5b-9,¹³ suggest the existence of a crosstalk between the C and the kinin systems in induction of vascular leakage during the acute episodes of AE.

To date, 3 endothelial cell-binding sites for high-molecular-weight kininogen (HK) and FXII have been described. These sites include the 33-kDa cell surface receptor for the first component of C C1q (gC1qR/p33),^{16–18} cytokeratin 1,^{19,20} and the urokinase plasminogen activator receptor (uPAR) (Fig. 2).²¹ Although the 3 proteins bind HK and/or FXII in a zinc-dependent manner and are postulated to form a multiprotein receptor complex with each contributing to the recruitment and assembly of the kinin-generating pathway proteins,²² gC1qR/p33, which binds specifically to domain 5 in the light chain of HK,^{18,23} may play a central role by virtue of its high affinity for HK. Although HK binds to isolated gC1qR/p33 with a dissociation constant (K_d) of 0.8 ± 0.7 nM (on-rate constant $[k_{on}] = 12.3 \pm 5.0 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$; off-rate constant $[k_{off}] = 0.8 \pm 0.5 \times 10^{-4} \text{ M}^{-1}\text{s}^{-1}$),²⁴ the binding of HK to gC1qR expressed on endothelial cell surface is 9 ± 2 nM. This binding is completely inhibited by mAb 74.5.2, which recognizes the C-terminal half of gC1qR/p33 corresponding with residues 204 to 218.^{17,25} In contrast, domain 5 of HK, located at the N-terminus of the light chain, is rich in histidine and arginine residues and contains the site for interaction with gC1qR/p33. A 20 amino acid peptide corresponding with domain 5 and termed HKH20 has been synthesized and shown to inhibit the interaction of HK with intact endothelial cells.^{18,23}

gC1qR/p33, uPAR, and CK-1, which are present on the EC surface, are able to bind the molecular complex formed by prekallikrein (PK), HK, and FXII (see Fig. 2).^{26,27} Following this interaction, a serine protease (prolylcarboxypeptidase [PRCP]), expressed on the cell membrane, cleaves PK.²⁸ The activated kallikrein modifies FXII, leading to its activation, which in turn can increase the kallikrein formation on ECs. The main substrate of kallikrein is HK, which releases the vasoactive peptide BK.²⁶ These data suggest that gC1qR/p33 may represent a natural surface receptor that modulates or triggers the coagulation/kinin cascade, causing the generation of the potent proinflammatory peptide BK and related kinins (Lys-BK, des-Arg⁹-BK, Lys-des-Arg⁹-BK).

BK can also be generated through another pathway that starts intracellularly. First, conversion of prekallikrein to tissue kallikrein takes place inside the cell and the kallikrein generated in this manner is then secreted into the pericellular milieu. Second, the secreted kallikrein digests low-molecular-weight kininogen (LK) to generate the decapeptide, lysyl-BK (Lys⁰-BK) or kallidin.^{29,30} Third, kallidin is converted to the nonapeptide BK by the zinc-dependent enzyme called aminopeptidase.³¹ BK produced by either pathway is then degraded in a sequential manner to lower-molecular-weight peptides by 3 kininases including carboxypeptidase N, ACE, and aminopeptidase P.^{31–33} The major plasma enzyme is carboxypeptidase N, which removes the C-terminal arginine from BK to yield an octapeptide, des-arg⁹-BK (Fig. 3).^{32,33} The

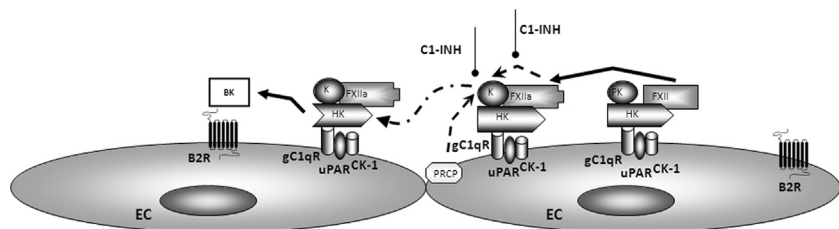


Fig. 2. Role of gC1qR/p33 in angioedema episodes. The molecules factor XII (FXII), prekallikrein (PK), and HK interact with the endothelial cell membrane through a binding site formed by gC1qR/p33, urokinase plasminogen activator receptor (uPAR), and cytokeratin 1 (CK-1). The consequent activation of kallikrein (K) by FXIIa and prolylcarboxypeptidase (PRCP) leads to the generation of BK that can stimulate the B2R.

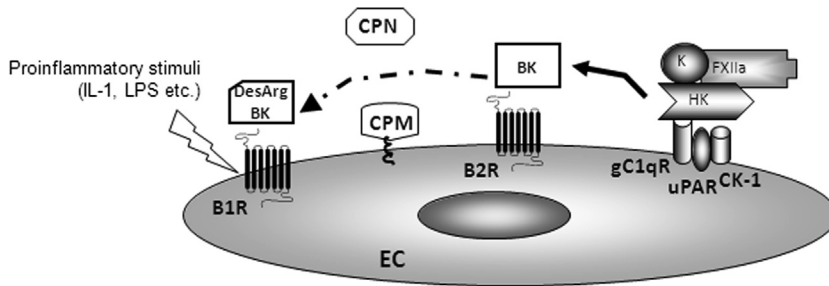


Fig. 3. Role of bradykinin receptors in angioedema episodes. BK released from the activation of the kinin system along the endothelial cell surface can interact with B2 receptors or can be cleaved by carboxypeptidase M (CPM), on the cell membrane, or CPN, in soluble phase, forming des-Arg9-BK, which is the agonist for B1R, membrane expression of which is induced by proinflammatory molecules.

second kinase in plasma is kininase II, which is identical to ACE and is found mainly along the pulmonary vascular endothelial cell surface. This kinase removes the dipeptide phe-arg from the C-terminus of BK to yield a heptapeptide, and a second cleavage removes ser-pro to leave a pentapeptide.³⁴

The release of BK may be controlled by C1-INH, which belongs to a family of serine protease inhibitors called serpins, which together constitute 20% of all plasma proteins.¹ This serpin is a glycoprotein of 478 amino acid residues encoded by a single-copy gene on chromosome 11 and synthesized by the liver, fibroblasts, monocytes, macrophages, ECs, and other cell types.³⁵ C1-INH is the sole known inhibitor of C1r and C1s proteases of the first C component (C1), and moreover inactivates the complex formed by mannose binding protein and mannose-associated serine proteases,³⁶ and inhibits the alternative pathway.³⁷ In addition, C1-INH is able to control the serine proteases of the clotting and the kinin systems, including FXIIa and factor XIa, kallikrein, plasmin, and tissue plasminogen activator, which are activated by injury to blood vessels and by some bacterial toxins.³⁵ C1-INH deficiency leads to the onset of AE. Nevertheless, the possibility that activation of both the complement and the kinin-forming systems may contribute to the edema has not been completely excluded. Activated plasma kallikrein, with the contribution of FXII, initiates the kinin cascade (see Fig. 2), cleaving HK to generate BK. Nussberger and colleagues³⁸ documented that levels of BK were increased in the plasma of patients with AE. FXIIa is also capable of activating C1 and plasmin, leading to the cleavage of C2 into a kininlike fragment (C2 kinin).³⁹ BK, and possibly this fragment, can cause enhanced postcapillary venule permeability, presumably mediated by B1R and B2R localized on the endothelial cell membrane.⁴⁰ This mechanism is responsible for the edema and movement of fluid from the vascular space into the tissues.

We recently used both in vitro and in vivo permeability assays to analyze the ability of plasma collected during the attack phase plasma (APL) from C1-INH-deficient patients to cause endothelial leakage.⁴¹ Using human adult dermal microvascular ECs and ECs isolated from the human umbilical vein, we were able to show that APL induced a delayed FITC-BSA leakage (30 minutes) as opposed to the rapid effect of BK (5 minutes), whereas remission plasma (RPL) elicited a modest effect compared with the control plasma. These data were also confirmed by in vivo experiments using intravascular microscopy. APL, RPL, and BK were administered via topical application on rat mesenteric microvessels. APL induced a significant increase in vascular leakage, whereas RPL had no effect. Incubation of cultured endothelial cells with a

monoclonal antibody against the gC1qR/p33 abrogated the permeabilizing effect of APL observed in the *in vitro* model.

ROLE OF BK RECEPTORS

BK is a powerful endothelium-dependent peptide that, in addition to its well-known function in vasodilation, enhancement of vascular permeability, and smooth muscle constriction, has also been implicated in various pathophysiologic processes including hypotension, tumor angiogenesis and metastasis, and pain.^{30,42} More importantly, BK is thought to play a critical role in the onset of the rare disease HAE. BK action and signaling are mediated by two 7-transmembrane domain-containing G-protein-coupled receptors named B1 receptor (B1R) and B2R. Although the B2R is ubiquitously and constitutively expressed in healthy tissues, B1R is induced and synthesized *de novo* as a consequence of tissue injury or inflammation (see **Fig. 3**).^{40,43} Furthermore, although the B2R displays high affinity for BK and Lys-BK peptide agonists, the B1 receptor displays highest affinity to des-Arg⁹-BK and des-Arg⁹-kallidin. Following initial stimulation of B2 receptors, the action of BK is further enhanced by secondary production of vasodilators such as nitric oxide (NO), and prostacyclins, which exacerbate the inflammatory process.^{40,42} An undesired side effect of ACE inhibitors, a class of drugs used to treat patients with hypertension and congestive heart failure, is the inhibition of BK degradation. This in turn increases plasma levels of BK, thereby causing hypotension and vascular permeability.^{40,43}

The release of BK and related peptides and their interaction with B1R or B2R often lead to pathologic conditions usually characterized by increased vascular permeability. Increased retinal vascular permeability can be caused by loss of retinal endothelial tight junctions. This disorder is mediated by the carbonic anhydrase-I-induced alkalization of the corpus vitreum, which promotes the enzymatic activity of kallikrein and the generation of activated FXII. This activation promotes the formation of kinins, which are eventually responsible for increase of vascular leakage.⁴⁴ The same permeabilizing effect of kinins released after stimulation by carbonic anhydrase-I is presumably responsible for intracerebral hemorrhage and edema.⁴⁴ Experimental evidence underlines that the role of B2R is more important than that of B1R in the blood-brain disruption and brain edema in ischemic stroke.⁴⁵ However, the relevant contribution of B1R has been shown in other pathologic conditions such as endotoxic shock⁴⁶ and maintenance of intra-articular edema in arthritis.⁴⁷ B1R and B2R are also involved in the vascular leakage seen in tumors and infections. In particular, antagonists of B2R are able to suppress vascular permeability in solid tumors and reduce accumulation of ascitic fluid.⁴⁸ Bacterial surfaces can directly activate HK leading to the release of BK, which in turn can be transformed by bacterial proteases to B1 agonists and in this way contribute to bacterial dissemination.⁴⁸

The involvement of B2R in the onset of HAE symptoms is supported by the ameliorative effect obtained treating the patients with HOE-140 (icatibant), a selective antagonist of B2R.⁴⁹ However, despite the immediate relief obtained with this drug, complete resolution of symptoms requires several hours, raising the hypothesis that other molecules and receptors are involved in the maintenance of AE.

Using both *in vitro* and *in vivo* models, we have shown that HOE-140 induced a partial reduction of APL-induced BSA leakage, as did either of the two B1R antagonists (R715 and R954), whereas combined treatment with B1R and B2R antagonists completely inhibited the leakage. Because B2R is constitutively expressed but B1R expression is induced by proinflammatory stimuli such as IL-1 β , we treated ECs first with IL-1 β and then with APL from patients with inherited or acquired AE. That

treatment induced a further increase of vascular leakage. To further evaluate the role of B1R, brefeldin A, which is a protein trafficking inhibitor, was used, and that treatment was able to reduce the permeabilizing effect of the APL.⁴¹ These data suggest that the interaction between the HK-prekallikren-FXII molecular complex and gC1qR/p33, which leads to the formation of BK and des-Arg9-BK, and B1R expression represent critical steps in the development of AE and that blockade of both B1R and B2R receptors, or gC1q/p33, may provide novel therapeutic tools to better control the symptoms of the acute attack in patients with AE.

SUMMARY

From experimental evidence reported in this article, it is evident that both the kinin and the complement systems are involved in pathologic conditions characterized by increased vascular permeability. These two systems closely cooperate and their interplay between the complement and the kinin systems is established at different levels: the vascular leakage induced by SC5b-9 is mediated by PAF and BK; the essential role played by C1-INH in controlling both the activation of the complement cascade and many of the serine proteases involved in the clotting and kinin formation processes; the presence of gC1qR/p33 onto the EC surface, where it functions as a receptor for the molecular complex HK-PK-FXII leading to the release of BK. All these events indicate that there is a crosstalk between the complement and the kinin systems, and all the activation products coming from both these pathways have a common target: the endothelium. During the AE attacks, the deficiency of C1-INH results in the activation of the serine proteases, leading to the release of BK through the interaction between HK-PK-FXII and gC1qR/p33. Enzymes expressed on the EC membrane can metabolize BK producing the agonist of the B1R, which can then be upregulated by proinflammatory stimuli such as IL-1 β . These data suggest that the blockade of both B1R and B2R, or gC1q/p33, may provide novel therapeutic targets to induce symptom relief.

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