Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: http://ees.elsevier.com

The central role of endothelium in hereditary angioedema due to C1 inhibitor deficiency

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ARTICLE INFO

Keywords
Endothelium
Hereditary angioedema
Permeability
Biomarker
C1 inhibitor
Pathophysiology

ABSTRACT

An impairment of the endothelial barrier function underlies a wide spectrum of pathological conditions. Hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE) can be considered the "pathophysiological and clinical paradigm" of Paroxysmal Permeability Diseases (PPDs), conditions characterized by recurrent transient primitively functional alteration of the endothelial sieving properties, not due to inflammatory-ischemic-degenerative injury and completely reversible after the acute flare. It is a rare yet probably still underdiagnosed disease which presents with localized, non-pitting swelling of the skin and submucosal tissues of the upper respiratory and gastrointestinal tracts, without significant wheals or pruritus. The present review addresses the pathophysiology of C1-INH-HAE with a focus on the crucial role of the endothelium during contact and kallikrein/kinin system (CAS and KKS) activation, currently available and emerging biomarkers, methods applied to get new insights into the mechanisms underlying the disease (2D, 3D and in vivo systems), new promising investigation techniques (autonomic nervous system analysis, capillaroscopy, flow-mediated dilation method, non-invasive finger plethysmography). Hints are given to the binding of C1-INH to endothelial cells. Finally, crucial issues as the local vs systemic nature of CAS/KKS activation, the episodic nature of attacks vs constant C1-INH deficiency, pros and cons as well as future perspectives of available methodologies are briefly discussed.

1. Introduction

1.1. The complexity of the vascular endothelium

Nowadays it is recognized that the vascular endothelium is not only a passive physical barrier that separates blood from the surrounding tissues: it is actually an active player, able to continuously and dynamically regulate pivotal cardiovascular functions.

Remarkably, in response to shear stress, changes of the chemical environment, fluctuations in the concentration of vasoactive agents, endothelial cells can process multiple and even contradictory messages, coordinating all the inputs to produce an efficient response. This is the result of the heterogeneity of endothelial cells thanks to which the endothelium may be considered as a complex organ, whose properties exceed those of single cells [1].

Through the release of a variety of mediators (e.g. nitric oxide, prostacyclins, endothelin, superoxide and thromboxane) endothelial cells modulate vasodilation and vasoconstriction, with a net impact on blood flow and blood pressure [2]. The blood clotting cascade, fibrinolysis and platelet activation are regulated by endothelial factors [3], as is also angiogenesis [4].

A fine tuning of endothelial permeability is crucial for complex processes as inflammation and immune response [5]. Significant effort has been done to deepen our knowledge on the processes induced by inflammatory agonists/immune mediators to promote generation of intercellular gaps, leading to leukocyte diapedesis, increased delivery of molecules and circulating substances across the endothelial barrier.

While passage of fluids from the intravascular to the interstitial space occurs via diffusion and filtration, the passage of proteins and macromolecules is regulated by interendothelial junctions (paracellular pathway) [6] or biochemical transporters, fenestrae and vescicular transport systems (transcellular pathway) [7]. Among specialized selective junctional systems (mainly adherens junctions and tight junctions), a pivotal role is played by Vascular endothelial cadherin (VE-cadherin, also known as CD144), whose phosphorylation and internalization (in-

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https://doi.org/10.1016/j.intimp.2020.106304
Received 12 August 2019; Accepted 10 Febraury 2020
Available online xxx
1567-5769/

duced by mediators as histamine and vascular endothelial growth factor) is responsible for alterations of the endothelial sieving properties [8,9].

The subsequent defects in endothelial permeability can lead to edema and increase in interstitial pressure, which in turn can induce compression and altered tissue perfusion.

An impairment of the endothelial barrier function underlies a wide spectrum of pathological conditions. Alterations of the endothelial morphology and function (thickening of the intima, proliferation of smooth muscle cells, formation of the fibrous plaque, changes of the vasa vasorum, loss of endothelium-derived nitric oxide, hyper-adhesiveness of the vascular lining toward platelets) are at the basis of the processes leading to atherosclerosis, which is responsible for coronary, cerebral, peripheral artery and aortic diseases.

Over time, the term "endothelial dysfunction" has been used with reference to the loss of endothelium's ability to regulate vascular resistance. Therefore, this alteration might imply effective chronic structural changes of the endothelial cell barrier (as in pulmonary hypertension and neurodegenerative disorders) or also conditions characterized by a transient alteration of endothelial morphology and function, which is restored almost to normal after the resolution of the acute phase. Among the latter conditions is sepsis, which is characterized by endothelial dysfunction due above all to functional changes (increased leukocyte adhesion and trafficking, altered vasomotor tone, loss of barrier function, shifts in hemostatic balance and programmed cell death), even though endothelial structural changes can also occur (nuclear vacuolization, cytoplasmic swelling, cytoplasmic fragmentation, denudation, and/or detachment).

However, nowadays it is clear that it is possible to identify also a variety of diseases, the so-called Paroxysmal Permeability Diseases (PPDs) [10], which are due to or exacerbated by recurrent alteration of endothelial permeability, with no inflammatory, degenerative, ischemic vascular injury and with complete *restitutio ad integrum* after each paroxysmal alteration. Among these conditions are different forms of primary angioedema (hereditary/acquired angioedema due to C1 inhibitor deficiency, idiopathic histaminergic and non-histamnergic angioedema, hereditary angioedema with normal C1 inhibitor), Idiopathic Systemic Capillary Leak Syndrome and other yet poorly defined forms of periodic edema (e.g. recurrent retroperitoneal edema, Gleich's syndrome) [10].

The clinical condition which serves as "pathophysiological and clinical paradigm" of this nosological entity is undoubtedly hereditary angioedema due to C1-inhibitor deficiency (C1-INH-HAE).

1.2. Hereditary angioedema

1.2.1. Angioedema with and without wheals

Angioedema is a localized self-limiting swelling with variable etiology. It can arise with wheals in the setting of allergic reactions or urticaria (either acute or chronic, spontaneous or inducible) as a result of mast cell degranulation. Angioedema without wheals can still be mediated by histamine, but can also be independent of mast cells and in this event it stands as a separate entity and can be inherited or acquired.

In 2014 a comprehensive classification of "angioedema without wheals" was elaborated, identifying three types of hereditary angioedema (genetic C1-INH deficiency, normal C1-INH with Factor XII mutations and unknown origin) and four types of acquired angioedema (due to C1-INH deficiency, related to ACE inhibitors intake, idiopathic histaminergic and idiopathic non-histaminergic) [11].

C1-INH-HAE is an autosomal dominant disorder due to mutations in one of the two alleles of *SERPING1*, the gene coding for C1-INH, located on chromosome 11q12–q13.1. The gene consists of eight exons distributed over 17 kb, with introns containing repetitive Alu sequences [12]. About 500 different mutations causing C1-INH-HAE have been described so far (http://www.hgmd.cf.ac.uk).

Phenotypic effects of these mutations are quantitative (C1-INH-HAE type I, 85% of cases) or functional (C1-INH-HAE type II, 15% of the cases) deficiencies in C1-INH [13].

C1-INH-HAE has a prevalence which ranges from 1:50,000 to 1:100,000 throughout the world [14]. Acquired C1 inhibitor deficiency: (C1-INH AAE) is about 10 times rarer and commonly associated with lymphoproliferative diseases [15].

Nowadays new mutations underlying the pathogenesis of some more challenging forms of angioedema keep being discovered, as a mutation in the angiopoietin 1 gene [16], in the plasminogen gene [17] and in the kininogen gene [18].

Due to marked heterogeneity of clinical presentations and to lack of straightforward genotype-phenotype correlation, comprehensive knowledge of angioedema phenotypes is crucial for a correct diagnosis and for choosing the appropriate therapeutic approach [19].

Among hereditary angioedema, the best characterized form is due to C1 inhibitor deficiency (C1-INH-HAE), which is the focus of the present review.

1.2.2. The clinical picture of hereditary angioedema due to C1 inhibitor deficiency (C1-INH-HAE)

Clinically, C1-INH-HAE is characterized by localized, non-pitting edema of the skin and submucosal tissues of the upper respiratory and gastrointestinal tracts, without significant wheals or pruritus, due to a temporary increase in vascular permeability. Attacks involving the upper airways can be rapidly fatal leading to asphyxia, while abdominal crises can be so painful that they can mimic surgical emergencies [11,19].

In C1-INH-HAE, angioedema has slow progression, with an increase during the first 12–36 h and complete remission in 2–4 days.

In the first accurate description of angioedema, Quincke observed that "acute circumscribed edema of the skin" (then entitled: "angioneurotic") could occur with and without urticaria. Three centuries earlier, Marcello Donati (*De medica historia mirabili libri*, Mantua) described a boy presenting with wheals (rash) on the face and angioedema of the lips after eating eggs. In 1888 Osler presented the full clinical picture and the familial nature of hereditary angioedema (HAE) [20]. These three seminal descriptions already contain the critical issues that should be considered when dealing with angioedema.

The overall burden of chronically recurrent angioedema is remarkable, due to personal-domestic-social and occupational disability and to exposure to the risk of death (when the respiratory tract is involved leading to asphyxia) [11,13]. Along with the limitations related to acute attacks, patients experience substantial impairment also in between attacks due to the fear for upcoming ones [21].

2. Pathophysiology of C1-INH-HAE

2.1. The contact activation system (CAS) and kallikrein/kinin system (KKS)

After the detailed description of the clinical picture of hereditary angioedema by Osler (1888) [20], it took about 75 years until a group of researchers headed by Dr. Virginia Donaldson (1963) [22] shed light on the central role of C1 inhibitor in the pathophysiology of HAE. This seminal discovery paved the way to the work of many subsequent researchers who gave their contribution to the understanding of the complex pathogenetic pathways underlying this clinical condition, allowing to test and design more and more "targeted" therapies [23].

C1-INH belongs to the superfamily of serine protease inhibitors (SERPIN) that all together constitute 20% of the plasma proteins [24,25]. It is a glycoprotein of 478 amino acid residues encoded by the *SERPING1* gene on chromosome 11 [26]. It regulates several serine proteases of the clotting, fibrinolytic, complement and kinin sys-

tems and is the only known physiologic inhibitor of C1r and C1s [27]. Thus, edema associated with C1-INH deficiency has been previously considered as the result of an uncontrolled activation of the complement system, with release of a kinin from C2 [28]. It is now proved that the swellings characterizing C1-INH-HAE primarily involve activation of the contact system and kinin-forming pathway [29,30]. These pathways move from activated factor XII (FXIIa) and plasma kallikrein (PK), both controlled by C1-INH, with cleavage of high molecular weight kininogen (HK) and final release of the nonapeptide bradykinin (BK) [31]. Bradykinin binds specific B2 receptors (B2Rs), which are G-protein-coupled receptors that activate the nitric oxide (NO) pathway, causes prostacyclin production, uncoupling of endothelial cell junctions, thus enhancing endothelial permeability and eventually causing edema [32,33]. B2Rs are are ubiquitous and constitutively expressed; in inflammatory conditions, the bradykinin B1 receptor (B1R), another G-protein-coupled receptor, may be expressed, further increase of vascular permeability. B1R has no affinity for BK, but better recognizes des-Arg9-BK originating from the C-terminal truncation of BK by carboxypeptidase M or N.

Moreover, nowadays we know that there are 2 major kinin-generating proteases: plasma kallikrein and tissue kallikrein (KLK-1). The latter is considered to have protective action on cardiovascular and renal functions [34]. KLK-1 produces primarily Lys-BK from low molecular weight kininogen, which is equipotent with BK at the human B2 receptor, but KLK-1 has never been associated to HAE. On the other hand, being Lys-des-Arg9-BK the only natural kinin sequence with a subnanomolar affinity for B1R, this suggests that the tissue kallikrein-kinin system and B1R may be strongly integrated [35].

Selective blockade of B2R with icatibant is an effective treatment for angioedema attacks and it improves outcomes [34]. Contrary to B2R, the involvement of B1R in HAE is still elusive.

Remarkably, even though the terms 'kallikrein/kinin system' (KKS) and 'contact activation system'

(CAS) are often erroneously used as synonyms and despite undeniable overlap between the two systems, it is of pivotal importance to underline that the activation of the KKS (with subsequent release of BK) may be independent of activation of CAS, since PK can be activated in both a FXIIa-dependent and a FXIIa-independent manner [36]. Besides FXIIa, other recognized activators of prekallikrein are prolylcarboxypeptidase (PRCP) [37] and heat shock protein 90 (Hsp90) [38].

Most of plasma prekallikrein (about 85%) circulates in complex with HK, through which it is recruited to the surface of endothelial cells. Indeed, through its domains 3 and 5, HK interacts with cytokeratin 1 (CK1), C1q receptor (gC1qR) and urokinase plasminogen activator receptor (uPAR) to form a multiprotein receptor complex on endothelial surface [39,40]. Also FXIIa can directly bind to endothelial cells surface through the same multiprotein assembly, but not necessary to the same binding sites as HK [41].

The precise mechanism/s by which initiation of kallikrein-kinin forming cascade occurs on the endothelial cell surface is still subtle. Activation of plasma occurs upon incubation with human umbilical vein endothelial cells. Zinc-dependent binding of plasma proteins to the above-described multiprotein assembly is a requisite for activation to take place. In vitro data support both factor XII-dependent and factor XII-independent mechanisms; the latter require a cell-derived protease to activate prekallikrein and the presence of HK and zinc ions [42].

Recent research has also allowed to unravel that contact and plasminogen activation system are strictly functionally coupled and that not only FXIIa can act as plasminogen activator, but also plasmin can act as FXII activator [43].

The abnormal hyperactivation of CAS/KKS, with increased production of BK from HK mediated by PK, is currently considered the main trigger of the attacks.

Indeed, the first obvious treatment strategy used was the replacement of the deficient protein with C1 inhibitor concentrate. Afterwards, evidence of the efficacy of icatibant served as further confirmation that the constitutively expressed bradykinin B2 receptor plays a key role in HAE pathogenesis [33].

Recent evidence shows that inflammatory mechanisms (eg, heparin released from allergen-activated mast cells) can initiate formation of bradykinin and its breakdown metabolites (some of which, such as Des-Arg BK, are still active mediators of angioedema) and that regulation of the bradykinin B2 receptors (and possibly also B1 receptors) plays a crucial role in bradykinin-induced vascular hyperpermeability [44,45]. However, as above mentioned, concerning the role of B1 receptors in humans, caution is required when translating results of experimental studies to clinical situations.

Since the lack of C1- INH as well as its reduced activity is constant, as shown by the low levels of the complement component 4 (C4) which is a biochemical alteration found in the large majority of patients [11], the observation that attacks are recurrent and patients are completely asymptomatic during intercritical phases is intriguing and challenging. The direct implication is the presence of inducible cofactors, which can have a role to determine the onset and development of acute attacks (most of which are unpredictable, even if acute infections, traumas and psychological stress are often reported as triggers). The role of cofactors has also been hypothesized to explain the reasons why the disease is characterized by an intrinsic high unpredictability (timing and features of attack recurrences), both among affected family members and in the very same patient throughout different periods of life.

As BK activity is directed on endothelial cells, which in turn are responsible for the leakage of plasma in the interstitial space and for the angioedema formation, understanding the activity and the function of endothelial cells in this setting is of paramount importance.

Current knowledge about endothelial cells in C1-INH-HAE is the aim of this review.

2.2. The endothelium during angioedema attacks

2.2.1. The crucial role of endothelium during contact – kallikrein/kinin system activation

The endothelium has a crucial role in triggering angioedema attacks, through local CAS/KKS system activation. In fact, Factor XII, the leading activator of the contact system, is located on the vessel walls after the exposition of a binding site. The actual receptor is still matter of debate: negative charged molecules have been proposed by Hofman et al. because they promptly activate the enzyme *in vitro* (for example, kaolin or dextran sulfate) [46], while, as above-mentioned, other Authors suggest a role for a protein receptor, namely urokinase plasminogen activator receptor (u-PAR) and cytokeratin-1 (CK-1) complex [47].

Either way, autoactivation of Factor XII activates PK, which in turn proteolyzes HK and releases BK, which exerts its paracrine activity through the B2R exposed on the endothelial cells themselves. Contact system activation has the potential to spread systemically due to the activated soluble form of Factor XII (ß-FXIIa) [46].

The underlying mechanisms, which boost the onset of the process in a certain area rather than in another one and the reasons why the reactions tend to occur locally rather than spreading systemically have not been elucidated yet. However, a local priming of the endothelial cells could be hypothesized, allowing to explain also the reasons why sometimes an attack involves different not adjacent sites at the same time.

The binding of C1-INH to activated endothelial cells has been shown [48–51]. Notwithstanding, the influence of this binding on C1-INH regulatory activity of complement and contact systems remains controversial. Plasma kallikrein has been shown to be relatively

less susceptible to inhibition by C1-INH when bound to endothelial cells [52].

First of all, the C1-INH binding partner on the endothelial surface has not been identified yet and could vary in different situations. It has been proven that pdC1-INH binds with low affinity to P- and E-selectin adhesion molecules expressed on the endothelial cell surface in the early stages of inflammation. Indeed, Kajdácsi et al. demonstrated that endothelial cells are activated during HAE attacks in C1-INH deficient patients and that soluble E-selectin was significantly increased in patients' plasma [53]. In this context, the binding of C1-INH to selectin molecules on the endothelial surface may serve to localize and concentrate C1-INH at these sites, which would result in more efficient local inhibition of activation of the complement and contact systems.

On the other hand, recent studies suggest a previously unrecognized involvement of the ficolins and mannose-associated serine proteases (MASPs) in the pathophysiology of C1-INH-HAE [54–58]. Evaluation of the lectin pathway of complement during attack and symptoms free periods suggested an activation of the ficolin-lectin pathway during attacks [57]. In particular, the serum levels of mannose-associated serine protease 1 (MASP-1) and its complex formation with C1-INH have been linked to the severity of C1-INH-HAE [58].

Additionally, a new mechanism of bradykinin production by MASP-1 was demonstrated, independent from factor XII and kallikrein. Definitely, MASP-1 could contribute to a yet unrecognized effect on bradykinin levels in C1-INH-HAE patients [59].

C1-INH binding to endothelial cells, both via ficolins or lectins, is mediated by the carbohydrate linked to the molecule, especially in its N-terminal domain. The impact of C1-INH glycosylation on C1-INH binding to endothelial cells waits to be addressed.

2.2.2. The search for a biomarker

C1-INH-HAE is characterized by the unpredictable nature of attacks, that sometimes can be elicited by known triggers, but more often affect the patients unexpectedly. Frequency, severity and location of attacks deeply differ from patient to patient and, even in the same patient, they may change throughout the course of life. The severity of the clinical phenotype strongly influences the burden of the disease and patients' quality of life.

Besides the correct diagnosis of C1-INH-HAE, which has clear and worldwide accepted criteria, the identification of disease-specific biomarkers that could reflect disease activity and response to therapy still remains an open question. Some clinical conditions can be confusing also for patients with firm C1-INH-HAE diagnosis. For example, abdominal attacks in C1-INH-HAE patients sometimes mimic acute surgical abdomen (whereas C1-INH-HAE patients can also suffer from abdominal pain of different etiology as in the general population); until now the response to therapy is fundamental to distinguish these clinical entities; an acute attack biomarker could be useful to unravel these complex situations.

Although C1-INH and C4 are crucial diagnostic biomarkers, they cannot be measured in all hospital settings on a routine basis, and never in emergency settings, so results are usually available a few days or weeks later. In 2016 Bork et al. showed that shortened activated partial thromboplastin time (aPTT) may help to diagnose C1-INH-HAE and C1-INH-AAE when other tests are not available, above all in emergency setting [60]. aPTT is a routine and easy screening test for the intrinsic and common pathways of plasmatic blood coagulation. Elevated plasma levels of D-dimer, a marker of fibrin degradation, have been found in C1-INH-HAE patients both in symptom-free period and during acute attacks [61]. aPTT and D-dimer are easily available in hospital settings, and also in emergency, but they are both not specific for angioedema, so probably not useful in the daily routine.

The search of ideal biomarkers is a very difficult task. Kaplan and Maas have outlined in a recently published review the fundamental characteristics of a molecule to be named a biomarker [62]. It is not just a molecule whose quantity or activity correlates with a disease feature, but it also needs to help with patients' management in a practical way. Therefore, it needs to be taken from an easily accessible sample type like blood and have a long half-life in blood. Moreover, the available assays are requested to have a minimum set of properties; they have to be specific and sensitive, robust, rapid, affordable and sustainable from an economic point of view.

Half-life is a fundamental point that has hampered the scientists to use bradykinin as a biomarker; it is an obvious potential one for its pathobiologic link to disease mechanisms, but its half-life is too short and its dosage is technically challenging. In order to circumvent this obstacle, Suffritti et al. have tried and succeeded in finding a surrogate of BK that could be used as biomarker. In 2014 they have shown that cleaved high-molecular-weight kininogen (cHK), produced by the cleavage of BK from HK, correlates with disease states in C1-INH-HAE [63]. cHK plasmatic levels are lower in patients with unfrequent attacks (<12 attacks/year). Its practical utility has been recently proved in a clinical trial, in which the Western blot assay measuring cHK has been used in order to evaluate the pharmacodynamic profile of lanadelumab, a monoclonal antibody inhibitor of kallikrein. This assay has numerous practical limitations that have been possibly overcome by an immune-assay for cHK detection using a monoclonal antibody ELISA method developed in 2017 by Hofman et al [64].

Even though they are far from being used in practical life, there are many proposals for new biomarkers, and the most promising sources appear to be the components of plasma contact system, endothelial cell-related and inflammatory factors. We will focus on endothelium-derived and vasoactive mediators, in line with the topic of this review.

Since BK tends to be increased in plasma of C1-INH-HAE patients, as shown by the evidences exposed of HK consumption even during remission phase [63], a homeostasis-preserving mechanism could be hypothesized, leading to enhanced vasoconstriction and increased release of anti-permeability factors in plasma of C1-INH-HAE patients, in order to counteract BK activity. Surprisingly, as explained into details in the following paragraph, vascular endothelial growth factors (VEGF-A, VEGF-C), Angiopoietin 1 (Angpt1) and 2 (Angpt2), adrenomedullin and phospholipase A2 (PLA_2), all known to drive vasodilation and permeabilization, were found to be increased in intercritical phases [65–68].

VEGF-A and VEGF-C are expressed in a broad variety of tissues, including endothelial cells, in response to tissue hypoxia. Their plasma levels are higher in patients during intercritical phase than in normal controls; moreover, they are significantly higher in patients with more frequent attacks, suggesting a pathogenetic role as predisposing factors [65]. Plasma concentrations of VEGF-A and VEGF-C are not altered during attack compared to remission [69].

Angpt2, a proangiogenic factor involved in endothelial homeostasis, mainly produced by endothelial cells, shows a similar pattern in remission phase, being higher in C1-INH- HAE patients and, among them, in those with more frequent attacks [65].

Angpt2, as well as the vascular endothelial-protein tyrosine phosphatase (VE-PTP), is a negative regulator of the tyrosine kinase receptor Tie2, which is located predominantly on vascular endothelial cells and plays a central role in vascular stability. In fact, Angpt2 counteracts the stabilizing effects of Angpt1: Angpt1 is able to activate Tie2 inducing its autophosphorylation, leading to the activation of downstream signaling pathways that stabilize the endothelium and guarantee cell junction integrity; on the opposite, Angpt2 inactivates Tie2, destabilizing the vasculature and making endothelial cells more responsive to the effects of VEGF and other inflammatory cytokines, thus increasing endothelial permeability [70].

The Angpt2/ Angpt1 ratio was decreased during angioedema attacks; in fact, concentrations of Angpt1 were increased during at-

tacks compared to symptoms-free periods, whereas Angpt2 levels were not altered [69].

The recent identification of a missense mutation in the angiopoietin 1 gene (*ANGPT1*, c.807G > T, p.A119S) in a family with previously "unknown HAE" has highlighted that in these patients a reduction of Angpt1 multimeric forms leads to reduced ability to bind the Tie2 receptor, with the result of increased vascular leakage [16]. This discovery draws possible links between apparently separated types of hereditary angioedema, highlighting once more the complexity of the systems involved and the likely cross-talks between different mediators and signaling pathways.

Blood endothelial cells and vascular permeability can be modulated by the PLA_2 superfamily, including secreted PLA_2 ($sPLA_2$) and platelet activating factor acetylhydrolase (PAF-AH). We have recently described that plasma levels of $sPLA_2$ group 2A (PLA2G2A) and PAF-AH are altered in C1-INH-HAE patients during symptom-free periods compared to healthy donors [68]. Differences between patients with a high vs low frequency of attacks for PAF-AH but not for sPLA2 activity were found. We also demonstrated that both sPLA2 and PAF-AH activity are reduced during attacks if compared with the basal condition [69]. Therefore, they could be potential biomarkers to distinguish the acute attack phase from the remission period.

Differently from the above-mentioned mediators, levels of endothelin-1 and arginine vasopressin, peptides derived respectively from endothelium and hypothalamus, both of which show vasoconstrictor activity, were found to be not different in C1-INH-HAE as compared to the normal control [66]. During an acute HAE attack endothelin-1 and arginine vasopressin increase and could theoretically have a role to counterbalance BK activity [66]; adrenomedullin, broadly expressed peptide, has vasodilatory activity but counteracts the BK-driven vasopermeabilization, and is found elevated as well during attacks [66].

Another mediator that deserves some attention is nitric oxide (NO), a vasoactive molecule, which is physiologically released by the endothelium through the endothelial nitric oxide synthase (eNOS). It is remarkable that eNOS activity is regulated by many proteins, among which Hsp90, thus supporting the hypothesis that eNOS activity might be altered in case Hsp90 is released after endothelial activation. Demirtuk et al. observed that eNOS plasma levels are increased in C1-INH-HAE patients both in remission and during attacks, and eNOS metabolites are elevated during attacks period [71]. This may reflect a sustained hyperpermeability state in C1-INH-HAE patients, and make this pathway a potential source of biomarkers, even if further studies are needed.

Adhesion molecules VE-cadherin, Endocan and vascular cell adhesion molecule 1 (VCAM-1) were showed to be increased in C1-INH-HAE patients, so representing potential biomarkers [72,73].

Finally, both von Willebrand factor antigen (vWf antigen) and collagen binding activity (vWfcb activity), important co-factor involved in coagulation process, have been shown to be increased in C1-INH HAE patients.

Recent observations show that levels of oxidative products of metabolism (ROS) are lower in controls than in C1-INH-HAE and FXII-HAE patients and that ROS are higher in C1-INH-HAE subjects than in FXII subjects [74].

In Table 1 a summary of mediators is shown, with indications about their biological role together with changes observed in C1-INH-HAE patients both during remission and acute attack.

Fig. 1 highlights the crucial role of the endothelium in C1-INH-HAE pathophysiology.

3. In vitro methods to get insights into the mechanisms underlying CL-INH-HAE

Endothelial cell monolayers are a good *in vitro* model to study different aspects of vascular leakage [75]. When forming a monolayer, cultured endothelial cells are connected to each other by cell junctions (such as adherens, tight, and gap junctions) which are essential to ensure vascular integrity and to control permeability. Vasopermeabilizing factors, such as BK, can induce cytoskeleton modification and formation of paracellular gaps that can be studied *in vitro*.

3.1. Two dimensional systems

Traditionally, two experimental approaches are used to assess vascular permeability in endothelial cell culture: diffusion of labeled macromolecules through a cell monolayer grown on a porous filter (transwell permeability assays) and measurements of transendothelial electrical resistance (TEER) by impedance of cell monolayers [75].

Both methods offer some advantages but presents also some limitations concerning sensitivity, time and spatial resolution and feasibility [76].

Impedance measurements allow monitoring of changes in the cell-monolayer in real time and are ideally suited to study time courses; moreover, they provide a high sensitivity. However, this technique, besides being expensive, has a limited spatial resolution due to the size of the electrodes used. Furthermore, permeability is evaluated indirectly and does not allow identification of the real cause for the change in impedance that may be due to changes in cell-cell contacts, but may also be caused by other factors, such as altered ion currents, cell adherence to the microelectrodes or cell membrane permeability.

Transwell assay have the advantage to allow assessment of the size selectivity of intercellular barriers as different size (from 4 to 150 kDa) of labeled macromolecule can be employed as macromolecular tracers. The choice of the proper tracer molecules is critical, molecules foreign to the system under study should be preferred as otherwise cell activation or transcytosis cannot be excluded. Limitation of this approach are its low sensitivity, delayed time between measurements, need for absolute coverage of the transwell membrane by cell monolayer, to avoid unspecific leakage, and dependence of the support used (different material and pore size) that can alter cell adhesion to the substratum.

The main drawback of both methods is that none allows for spatial resolution of local changes in endothelial permeability, an important aspect to study HAE mechanism *in vitro*. Moreover, both assays are unable to evaluate permeability in endothelial monolayer grown on 3D substrates and exposed to shear stress, a key feature of bradykinin-induced leakage in HAE.

More recently, a third method was developed to bypass these limitations. This method is called eXpress Permeability Testing (XPerT) assay and allows both visualization and rapid quantification of trans-monolayer permeability. This assay takes advantage on high affinity interactions of a FITC-conjugated avidin, added in the culture medium, to the biotinylated extracellular matrix (gelatin or collagen) immobilized on the bottom of culture vessels [77].

Very few works are present directly assessing permeability in the C1-INH-HAE context through *in vitro* and in vivo models of vascular leakage.

Bossi et al used a transwell model system to investigate the effect on vascular permeability induced by plasma samples obtained from patients with C1-INH deficiency [44]. They showed that attack phase plasma (APL) induced vascular leakage, whereas remission plasma (RPL) elicited a modest effect. The plasma permeabilizing effect was delayed compared to the rapid effect of bradykinin and was prevented by blocking the gC1q receptor–high-molecular-weight kininogen interaction, as well as by a combination of B2 and B1 receptors antagonists, while was partially inhibited by using an antagonist at a time. They conclude that, additionally to the already recognized B2 receptor, both B1 receptor and gC1q receptor are involved in the vascular leakage induced by plasma from patients affected by hereditary and acquired angioedema due to C1 inhibitor deficiency. Since the role of B1R in C1-

Table 1
Summary of biomarkers discussed in the review, listed in alphabetical order. ↑: increased in comparison with healthy controls; ↓: reduced in comparison with healthy controls; = : unchanged in comparison with healthy controls; ↓↓: reduced during attacks in comparison with remission phase; ↑↑: increased during attacks in comparison with remission phase; * : reduced during acute attacks in comparison with the remission phase, no comparison between acute attack and healthy control available; ADMA: asymmetric dimethylarginine; aPTT: activated partial thromboplastin time; C1-INH: C1-inhibitor; HK: High molecular weight kininogen; E-selectin: endothelial selectin; eNOS: endothelial nitric oxide synthetase MASP: mannose-binding lectin-associated serine protease; MASP-1/C1-INHc: MASP-1/C1-INH complexes; PAF-AH: platelet activating factor acetylhydrolase; ROS: reactive oxygen species; sPLA2: secreted phospholipases A_2; sPLA2G2A: secreted phospholipases A_2 group IIA; unk: unknown; VCAM: Vascular cell adhesion molecule; VE-cadherin: vascular endothelial cadherin; VEGF: vascular endothelial growth factor; vWf: von Willebrand factor; vWfcb: von Willebrand factor collagen binding.

Biomarker	Biological role	C1-INH-HAE patients remission phase	C1-INH-HAE patients acute attack	Correlation with attack frequency	References
ADMA	Nitric oxide synthase inhibitor	↑	unk	unk	[90]
Adrenomedullin	Mediator of vasodilation, endothelial barrier stabilization	=	↑	unk	[66]
Angiopoietin 1	Mediator of endothelial barrier stabilization	↑	↑↑	none	[65,69]
Angiopoietin 2	Mediator of inflammation, angiogenesis	↑	↑	positive	[65,69]
aPTT	In vitro coagulation assessment	↓	unk	unk	[60]
C1-INH	Plasma protease inhibitor	↓	↓↓	none	[63]
C4	Complement component	↓	↓↓	negative	[63]
Cleaved HK	Contact system component	↑	↑↑	positive	[63]
D-dimer	Fibrinolysis product	↑	↑	unk	[61]
Endocan (soluble form)	Intercellular adhesion molecule	↑	unk	unk	[72]
Endothelin 1	Mediator of vasoconstriction	=	↑	unk	[66]
eNOS	Nitric oxide synthase	↑	↑	unk	[71]
eNOS metabolites	Nitric oxide degradation products	=	↑	unk	[71]
E-selectin (soluble form)	Intercellular adhesion molecule	↑	↑	unk	[53]
MASP-1	Complement component	↓	unk	negative	[58,59,78]
MASP-1/C1-INHc	Complement activation product	↓	unk	negative	[58,59,78]
PAF-AH	Platelet activating factor (PAF) inactivator	↑	*	positive	[69]
ROS	Oxidative products of metabolism	↑	unk	unk	[74]
sPLA2 activity	Phospholipide digestion – functional assay	↑	*	none	[68]
sPLA2G2A	Plasma phospholipidase	↑	*	none	[68]
Arginine vasopressin	Mediator of vasoconstriction, renal water reabsorption	=	↑	unk	[66]
VCAM-1 (soluble form)	Intercellular adhesion molecule	↑	unk	unk	[72]
VE-cadherin (soluble form)	Intercellular adhesion molecule	=	↑	unk	[73]
VEGF-A	Mediator of inflammation, angiogenesis	↑	↑	positive	[65,69]
VEGF-C	Mediator of lymphangiogenesis	↑	↑	positive	[65,69]
vWf antigen	Factor VIII stabilization	=	↑	unk	[53]
vWfcb activity	vWf – functional assay	=	↑	unk	[53]

INH-HAE patients is still controversial and multiple pathways have been proposed to generate BK-related peptides from blood, the use of specific activators and/or inhibitory agents would have been precious to highlight the molecular mechanism underlying the reported observations.

The *in vitro* results were confirmed through in vivo experiments in male Wistar Kyoto rats. The permeability of mesenteric vessels to fluorescein isothiocyanate labelled bovine serum albumin (FITC-BSA) after topical administration of either APL or RPL was analyzed by intravital microscopy. APL caused extravasation of FITC-BSA, whereas RPL failed to induce vascular leakage. FITC-BSA leakage was totally abrogated by reperfusion with a mixture of the B1R and B2R antagonists before the addition of APL.

Debreczeni et al. chose alternative *in vitro* models of vascular leakage to investigate the effect of MASP-1 on endothelial permeability. They showed that MASP-1 has potent permeability increasing effects; real-time micro electric sensing revealed that MASP-1 decreases the impedance of HUVEC monolayers and XperT assay demonstrated a MASP-1 dose-dependent increase of endothelial paracellular transport. Moreover, they showed that the molecular mechanisms underlying this phenomenon comprise protease activated receptor-1 mediated intracellular Ca^{2+}-mobilization, Rho-kinase activation dependent myosin light chain (MLC) phosphorylation, cytoskeletal actin rearrangement, and disruption of interendothelial junctions. Finally, using a whole-transcriptome microarray they show that MASP-1 significantly altered the expression of 25 permeability-related genes, among which the most important is the up-regulation of bradykinin receptor B2R. They therefore speculated that MASP-1 may play a role in the pathomechanism of diseases, where edema formation and complement lectin pathway activation are simultaneously present; raising the possibility that MASP-1 may be a promising target of anti-edema drug development [78]. However, further experiments are needed to clarify the exact role of MASP-1 in generating kining physiologically and in HAE.

3.2. Three dimensional systems

Recently, much work has been devoted to design novel microfluidic three dimensional devices, which enable to culture endothelial cells and to study the endothelial barrier function under dynamic physiological flow conditions.

Some of these systems constitute really powerful tools, which allow to tightly control the fluidodynamic – mechanical and chemical microenvironment.

In order to study alterations of endothelial cells' permeability we designed the "microvasculatures-on-a-chip", an *in vitro* model that mimics important features of microvessel networks [79].

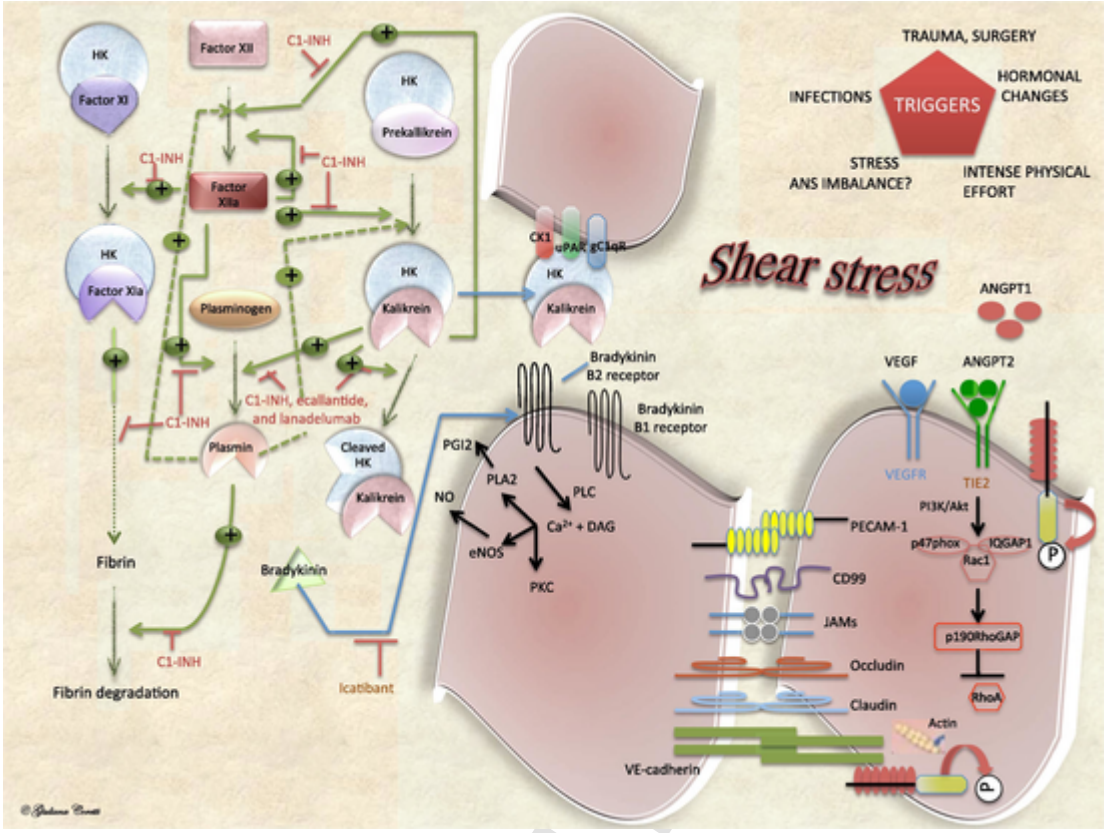

Fig. 1. Representation of the crucial role of the vascular endothelium in the pathogenesis of hereditary angioedema due to C1 inhibitor deficiency (C1-INH-HAE). The endothelium is continuously exposed and responds to hemodynamic stimuli (*shear stress*) and signals from both surrounding tissues and flowing blood, which can have an impact on the mechanisms required for barrier maintenance and stabilization. Under resting conditions, endothelial barrier function is maintained thanks to transmembrane adhesion proteins such as VE-cadherin at adherens junctions and claudin at tight junctions, anchored to the cortical actin cytoskeleton as well as by the glycocalyx surface layer (*not shown*). A variety of factors (including trauma, stress, hormonal changes, intense physical effort) can be triggers of acute C1-INH-HAE attacks. The activation of the contact and kallikrein/kinin systems on the endothelial cell surface moves from activated factor XII (FXIIa) and plasma kallikrein, with cleavage of high molecular weight kininogen (HK) and final release of the nonapeptide bradykinin (BK). BK binds specific G-protein-coupled receptors, the main of which is the B2 receptor, which is a constitutively expressed receptor, generating vasodilator mediators as nitric oxide (NO) and prostacyclin (PGI2), inducing phosphorylation of the interendothelial junction VE-cadherin and its associated catenins resulting in internalization and degradation of VE-cadherin with destabilization of the barrier and contraction of the actomyosin cytoskeleton. This cascade leads to enhanced endothelial permeability, passage of fluids from the intravascular to the extravascular space and subsequent edema formation. VE-cadherin phosphorylation may occur not only in response to a wide spectrum of mediators (including VEGF), but also through inhibition of associated phosphatases (eg the phosphatase VE-PTP). BK can also bind the Bradykinin B1 receptor, which is an inducible receptor expressed during acute flares. Besides BK, other mediators are implicated in C1-INH-HAE angioedema. VEGFs and angiopoietins may induce a state of 'vascular preconditioning' that can predispose to angioedema attacks. Angiopoietin 2 (Angpt2) counteracts the stabilizing effects of Angiopoietin-1 (Angpt1); it inactivates Tie2, destabilizing the vasculature and making endothelial cells more responsive to the effects of VEGF. VE-PTP dephosphorylates Tie2 further limiting Tie2 signaling. Proteolytic activities are shown with green arrows (dashed lines depict putative secondary pathways); steps inhibited by C1 inhibitor and newer treatments (ecallantide, icatibant, and lanadelumab) are indicated with red T-bars. Intracellular pathways are briefly indicated. HK, high molecular weight kininogen; BK, bradykinin; ANGPT1, angiopoietin 1; ANGPT2, angiopoietin 2; C1-INH, C1 esterase inhibitor; Factor XIIa, activated Factor XII; TIE2, tunica interna endothelial cell kinase 2; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; PLC, phospholipase C; DAG, diacylglycerol; Ca^{2+}, calcium; PLA2, phospholipase A2; PGI2, prostacyclin; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; PKC, protein kinase C; PECAM-1, platelet endothelial cell adhesion molecule- 1 (also known as cluster of differentiation 31); CD99, CD99 antigen (mediating transendothelial migration of human monocytes and lymphocyte recruitment); JAMs, junctional adhesion molecules; VE-cadherin, vascular endothelial cadherin; ANS, autonomic nervous system; uPAR, urokinase plasminogen activator receptor; CK 1, cytokeratin 1.

Microchannel networks (composed of four successive branching points and smallest 30 μm square section) have been fabricated using a soft-lithography technique. The inlet of the microchip has been connected to a fluid reservoir via a silicone tubing, and the outlet to a 1 ml syringe installed on a high precision syringe pump (KDS Legato 110) used in withdraw mode at imposed flow rate. Afterwards, the inner surfaces of the channels has been coated with fibronectin, and Human Umbilical Vein Endothelial Cells (HUVECs) were subsequently cultured within the networks, in the presence of a steady flow of culture medium, ensuring a physiologically relevant level of fluid shear stress at the wall. Via this procedure, endothelial cells formed a perfectly confluent monolayer on all the walls of the circuit, displaying a glycocalyx that fully lines the lumen of the microchannels.

After creating a continuous monolayer in the channels, HUVECs' were stained and subsequently exposed to a constant flow of either culture medium (control), plasma from healthy volunteer or bradykinin (Sigma, USA) diluted in endothelial cells culture medium.

Permeability studies were conducted assessing high-affinity interactions between the ligand, fluorescein isothiocyanate-conjugated avidin (finally added to the perfusion solution) and biotinylated fibronectin used as a matrix [77].

Images obtained with Laser Scanning Confocal Fluorescence Microscope revealed that no major difference can be detected in fluorescence intensity histogram peak or width between the control and the 50%-plasma from healthy volunteer, while in the circuit exposed to 25 μM solution of bradykinin the histogram is clearly shifted to larger intensity values compared to that obtained for the control. Therefore, being the microfluidic network able to detect changes of endothelial permeability induced by a permeabilizing mediator as bradykinin, the experimental system seems to be promising to get deeper insights into the mechanisms underlying HAE as well as other even rarer forms of angioedema [10].

Fig. 2 summarizes the above-mentioned 2D and 2D systems used to investigate C1-INH-HAE pathophysiology.

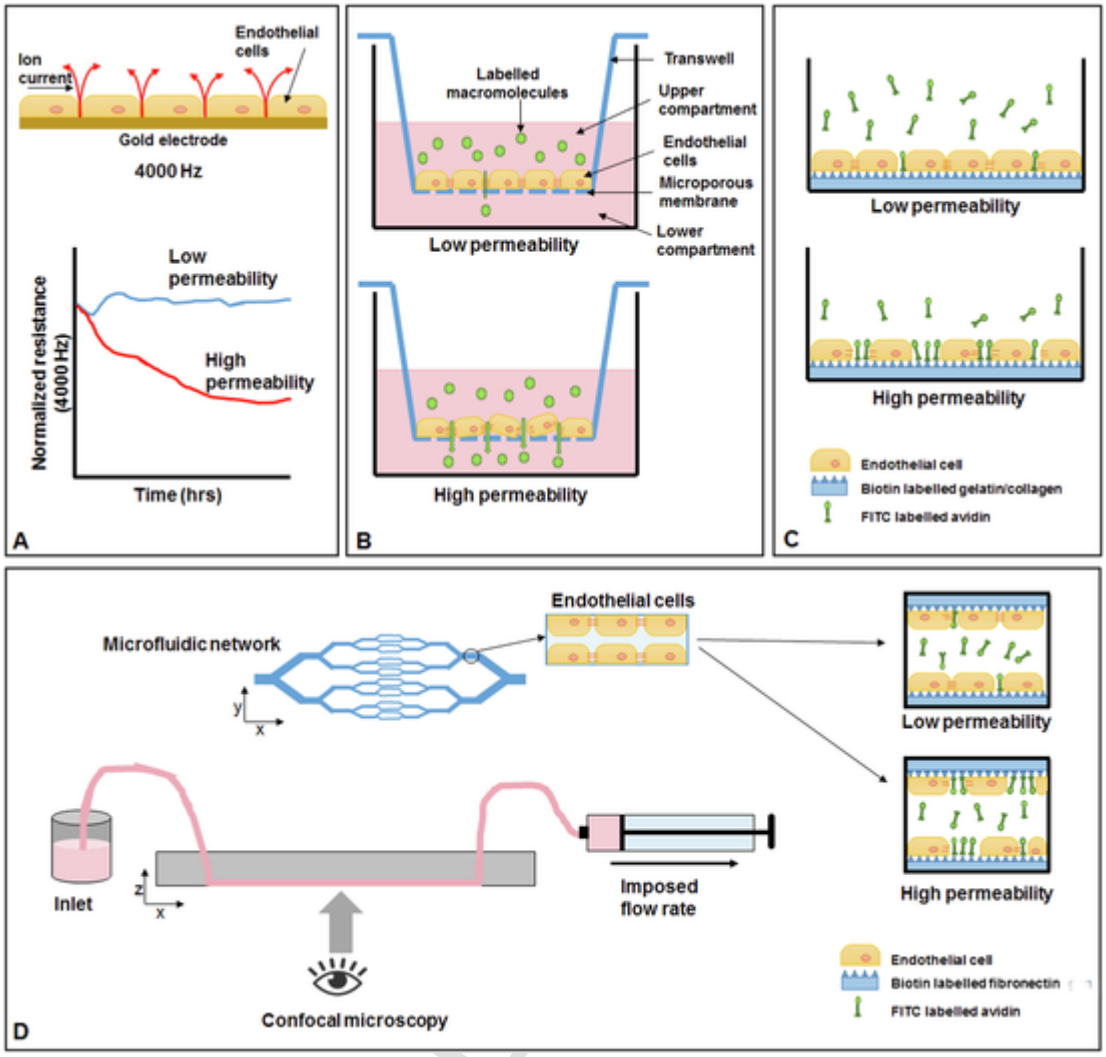

Fig. 2. 2D and 3D methods to assess vascular permeability. (A) Measurements of impedance of endothelial cell monolayers at low AC frequencies (4000 Hz) to evaluate barrier function changes. The resistive portion of the impedance is used as a parameter for endothelial permeability. An increase in resistance indicates augmented permeability. (B) Transwell permeability assay to measure diffusion of labelled macromolecules through a cell monolayer grown on a porous filter. When permeability increases, a major number of labelled molecules passes through the membrane in the lower compartment. (C) The Express permeability testing assay (XPerT) for quantification of trans-monolayer permeability. When cellular changes giving rise to augmented permeability arise, more FITC-conjugated avidin molecules can bind to the biotinylated extracellular matrix resulting in an increase in fluorescence signal. D) Scheme of a microfluidic three-dimensional device ("microvasculature-on-a-chip") to assess endothelial permeability. Cell are grown in a microchannel network and are cultured under an imposed flow rate, subjected to shear stress. Endothelial permeability assessment is based on high-affinity interactions between biotinylated fibronectin used as a matrix for HUVECs and FITC-conjugated avidin added to the perfusion solution, with confocal microscopy used for cell imaging.

4. In vivo methods to investigate the mechanisms underlying C1-INH-HAE

4.1. Studies in animal models

In order to investigate the role of C1-INH in vivo and mimic HAE three different C1-INH knockout mouse models were generated.

In the first model, generated by gene trapping, the *Serping1* gene was targeted into a splice acceptor site in intron 6 (210 bp upstream of exon 7) [32]. *Serping1* gene expression is not totally abolished but results in transcription of a shortened C1-INH variant composed of exons 1–6 fused to construct coded reporter gene (β-geo).

The second one was generated by random mutagenesis, a single base transition (G to A) at position +1 in the splice donor site of intron 2 caused skipping of exon 2 and a subsequent frame-shift resulting in complete abolition of C1-INH protein synthesis [80].

Finally, for the third one CRISPR/Cas9 technology was used to generate a C1-INH deficient mouse in which a 10-bp deletion created a frame-shift causing the introduction of a premature stop codon in exon 3 and the production of a truncated protein consisting of 43 amino acids [81].

In none of the three models C1-INH protein was detected, nevertheless these mice present slightly different phenotypes.

Both the first and third mouse models share a common phenotype; they present increased vascular permeability of skin and internal organs and decreased C1-INH and C4 levels. Moreover, similar to human, reversal of vascular leakage by treatment with human C1-INH, DX88 (a plasma kallikrein inhibitor), or the bradykinin B2R antagonist Hoe140 was demonstrated in the first model. However, neither models developed spontaneous or triggered swelling episodes of skin or organs comparable to HAE patients.

Conversely, the second model shows a phenotype closer to HAE patients. The *Serping1*$^{-/-}$ mice presented normal vascular permeability under non-stimulated conditions, whereas stimuli such as topical application of heparin provoked excessive vascular leakage. Moreover, intradermal injections of heparin and C48/80 (a degranulator of mast

cells and eosinophils) triggered excessive skin edema that exceeded the leakage observed in WT mice.

However, none of the mouse models showed spontaneous episodes of swelling involving the skin or organs as in the human disease.

In general, increased vascular permeability is present in these C1-INH knockout mice models; although no direct studies on endothelium were performed, its role in vascular leakage is suggested. Han and coworkers showed a correlation between peripheral vascular permeability and the activation of bradykinin B2R, which is constitutively expressed on endothelial cells [32]. The treatment with the B2R antagonist Hoe140 protected the C1-INH KO mice from enhanced vascular permeability. Moreover, the double-knockout mice (*Serping1*$^{-/-}$, *Bdkrb2*$^{-/-}$) with deficiency of both C1-INH and B2R, showed a diminished vascular permeability compared to the single C1-INH deficient mice.

Probably a better model to evaluate the role of endothelium in vascular permeability would be a tissue-specific knockout animal in which B2R is deleted only in endothelial cells.

Recently, a tissue-specific transgenic rat overexpressing the bradykinin B2R in endothelial cells (B2Rover) was generated using the vascular endothelial cadherin promoter [82].

Differently from the previous murine models, acute angioedema attacks can be induced in this rat by topical applications of mustard oil. Moreover, although no skin swelling was observed, intestinal swellings was present in some animals indicating spontaneous and sporadic occurrence of abdominal angioedema.

Another approach used to replicate the C1-INH deficiency associated with HAE in mouse models is knockdown by the use of antisense oligonucleotides (ASOs).

Bhattacharjee et al. identified a C1-INH targeted ASO that effectively reduced C1-INH mRNA expression in the liver and C1-INH plasma protein levels [83]. Comparably to *Serping1*$^{-/-}$ mice, C1-INH ASO treated mice did not exhibit spontaneous swelling events seen in HAE patients but they showed increased basal vascular permeability as seen in two of the knockout models.

Besides peripheral vascular permeability, effect of C1-INH depletion on blood–brain barrier (BBB) integrity and brain function was also investigated [84].

Circulating C1-INH was knocked down in wild-type mice using ASO, without affecting its expression in peripheral immune cells or the brain. Long-term depletion of circulating endogenous C1-INH was shown to cause neurovascular dysfunction, neuroinflammation, and behavioral deficits mediated by the activation of the vascular kinin pathway. Although this paper is not directly related to angioedema, the Authors performed investigation on endothelial cells in the brain that could be applied also to investigate the role of endothelium in angioedema. They found significant increases in gene expression of B1R and B2R in the brains of C1-INH ASO-treated mice compared to controls. Moreover, analyzing endothelial cells in the brain they show a decreased expression of CD31, PECAM1 and occludin, a tight-junction protein that degrades with increased blood–brain barrier permeability, in the CA1 region of the hippocampus in C1INH ASO-treated mice.

4.2. Studies in C1-INH-HAE patients

4.2.1. Heart rate variability analysis

When investigating possible triggers or precipitating factors for different kinds of PPDs, it is clear that, while some factors appear to be detectable more easily, as for instance trauma, surgery or menstrual cycle for C1-INH-HAE or intense physical effort and infections - inflammatory conditions for both C1-INH-HAE and diseases as idiopathic systemic capillary leak syndrome, others are often reported by patients but their relationship with recurrences is more complex, as stressful events.

It is known that mental stress, through activation of the sympathoadrenal system, activates both coagulation and fibrinolysis [85] . Given that as above mentioned, the role of fibrinolysis as a general trigger of attacks is emerging [43], the link between stress, hyperfibrinolysis and contact system activation should not be underestimated.

The frequency and severity of angioedema recurrences differ from patient to patient and in the same patient throughout life. C1-INH plasma levels do not satisfactorily account for the variability in clinical phenotypes [19]. In angioedema recurrences, the release of bradykinin occurs locally and is facilitated by trauma and psychological stress [86,87]. While it sounds logical to speculate that endothelial cells injured by trauma could become local activators of the contact system, pathways linking psychological stress to increased endothelial permeability are not obvious [36].

To investigate autonomic nervous system (ANS) modulation in patients affected by hereditary angioedema due to C1 inhibitor deficiency, we performed heart rate variability analysis in C1-INH-HAE patients, at steady state and in a controlled condition of stress induced by the orthostatic challenge (tilt testing) [88]. 23 C1-INH-HAE patients during remission and 24 healthy volunteers were studied.

A three lead-ECG, beat-by-beat plethysmograph arterial blood pressure and respiratory movements were recorded continuously for 10 min in clinostatism (rest, R) and for 10 min in orthostatism using a head-up tilt table with a 75° inclination (tilt, T). Autoregressive spectral analysis allowed us to identify the main oscillatory patterns embedded in the signal: low-frequency (LF, ranging from 0.04 to 0.15 Hz) oscillations, markers of sympathetic modulation; high-frequency (HF, ranging from 0.15 to 0.4 Hz) component, marker of parasympathetic modulation, synchronous with respiration. Blood samples were collected during rest and tilt condition to assess C1-INH antigenic and functional levels, C1q and C4 antigens, cHK and plasma catecholamines.

The results show that mean systolic arterial pressure (SAP) is significantly higher in C1-INH-HAE patients than in controls both at R and during T. Tilt induced a significant increase in SAP and its variability only in controls, but not in the HAE patients' group. LFnu (low-frequency component, marker of sympathetic modulation, expressed in normalized units) increased significantly after orthostatic challenge both in C1-INH-HAE patients and in controls, suggesting a preserved response to the orthostatic challenge. However, only in healthy subjects tilting induced a significant increase of LF/HF ratio, an index of sympathovagal balance. cHK, marker of contact system activation, is increased in C1-INH-HAE patients compared to controls and tilt test induces a significant increase in cHK only in C1-INH-HAE patients. Noradrenaline was higher in patients at R ($p = 0.05$) and increased in both groups after tilt test.

Our preliminary results suggest that autonomic modulation is altered in C1-INH-HAE even during remission periods, with an impairment in the response to a stressful stimulus. The increase of HK cleavage with tilt test suggests a link with contact system activation.

A major finding of our study is that patients with C1-INH-HAE, and no concomitant co-morbidities, have an increased sympathetic modulation at rest, associated with a blunted response to a sympatho-excitatory stimulus such as head-up tilt. Moreover, tilt test in these patients was associated with a significant increase in HK cleavage, confirming a correlation between stress and bradykinin production.

4.2.2. Measurement of impaired endothelial function

In order to investigate the impact of hereditary C1-INH deficiency on atherosclerosis, Demirtürk et al. performed measurement of coronary flow reserve (CFR) in the left anterior descending coronary artery using transthoracic doppler harmonic echocardiography at baseline and following dipyridamole infusion in 26 C1-INH-HAE patients as compared to 30 healthy controls [89]. They demonstrated that the mean CFR value was significantly lower in the HAE patient group than in the control group ($p < 0.001$). Even though no statistically significant differences were observed in the intima-media thickness (IMT) in

the carotid artery between the two study groups, a trend towards higher values in HAE patients was reported. These findings suggest microvascular early endothelial dysfunction, with development of atherosclerotic plaques in C1-INH-HAE subjects. However, it should be noted that most patients were under long term prophylaxis with danazol and this might have affected the results.

In a recent study Firinu et al. report that the atherosclerotic process previously revealed in coronary arteries affects also the peripheral vessels in C1-INH-HAE patients [90]. Given the hypothesis that endothelial dysfunction implies an altered response to a variety of patho-physiological stimuli such as reactive hyperemia, the Authors assessed endothelial function by means of non-invasive finger plethysmography (reactive hyperaemia index: RHI) and levels of asymmetric dimethylarginine (ADMA) -which is a strong inhibitor of nitric oxide synthesis- by high-performance liquid chromatography. Their results show that in C1-INH-HAE patients RHI was lower (2.03 ± 0.46 vs. 2.82 ± 0.34, $p < 0.0001$) and ADMA higher (0.636 ± 7 vs. 585 ± 5 micromol/L, $p < 0.01$) than in controls. There was a statistically significant inverse correlation between RHI and patients' ADMA levels ($r = -0.516$, $p = 0.009$). No statistically significant differences were observed between C1-INH-HAE and FXII-HAE subgroups. Interestingly, these patients were studied during the attack-free period and none of them was under attenuated androgens (a factor which could have shifted the lipid profile to a pro-atherogenic phenotype). No significant correlations were detected between RHI and gender, age at disease onset, disease duration and severity scores (all p = ns).

These results cast new light on the role of bradykinin in ADMA production, through stimulation of B2 receptors, stimulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and production of reactive oxygen species (ROS) or reduction of dimethylarginine dimethylaminohydrolase activity.

However, the topic is still controversial and Nebenfuhrer et al. in a very recent study report no signs of endothelial dysfunction in HAE patients [91]. They performed ultrasound assessment of endothelium-dependent flow-mediated dilation of the brachial artery in 33 C1-INH-HAE patients and in 30 healthy matched controls. No difference was found in endothelial function (reactive hyperemia, RH) between patients (median, 9.0; 25–75%percentile, 6.3–12.9) and controls (median, 7.37; 25–75%percentile, 4.52–9.93). Moreover, no alterations were detected in danazol-treated patients. They could not find significant differences in RH values of patients with low and high attack frequency. Surprisingly, in C1-INH-HAE patients no differences were found in the RH values of smokers and non smokers and the correlation between the RH levels and the LDL-C/HDL-C ratio or FMD and FRS was detected only in the danazol-non treated subgroup.

Therefore, the Authors speculate that bradykinin may have a cardioprotective role, through production of nitric oxide and prostaglandin I2 (prostacyclin), which have vasodilating, anti-thrombotic and inflammation-modulating effects.

However, the Authors themselves admit that the lower age of patients enrolled in the study (as compared to previous studies) might have affected the results.

Further studies are needed to investigate endothelial dysfunction in HAE patients.

4.2.3. Nailfold videocapillaroscopy

Paralleling studies performed in connective tissue diseases, nailfold videocapillaroscopy (NVC) has recently been applied to investigate structural alterations of the microvasculature in C1-INH-HAE patients [92]. Cesoni Marcelli et al. enrolled twenty-eight C1-INH-HAE patients (mean age 35 years [8–73];14 male) in remission, ten patients with ACE-I-AE (mean age 67 years [46–76]; 7 male) and thirty-eight controls. NVC was performed, evaluating the following features: capillary morphology, distribution, density and length; intercapillary distance; apical, internal and external diameter.

Interestingly, the results showed that C1-INH-HAE patients had increased apical, internal and external diameter compared to controls (28 [23–40] vs 22 [16–29] μm median values (interquartile ranges), $p < 0.01$; 23 [20–27] vs 20 [18–22] μm, $p < 0.01$; 81 [70–89] vs65 [55–73] μm, $p < 0.001$, respectively). The capillary density was decreased in patients as compared to controls (4/mm [4–5] vs5/mm [5–6], $p < 0.001$). C1-INH-HAE patients had a higher prevalence of alterations in capillary distribution as compared to controls (irregular vs ordered, $p < 0.01$). Moreover, a positive correlation was found between internal and apical diameter ($p < 0.05$), external and apical ($p < 0.0001$) and external and internal diameter ($p < 0.05$), while capillary density was negatively correlated with apical diameter ($p < 0.05$). No differences were found in capillary morphology and length and intercapillary distance. NVC did not highlight any significant microvascular alteration in ACE-I-AE patients as compared to controls. NVC performed in two C1-INH-HAE patients during acute attacks involving the hands and the abdomen detected no changes compared to the remission phase.

Therefore, microvascular structural alterations found in C1-INH-HAE seem to be a constant feature (not related either to acute/ remission phase or to site of the attack) and they could play a role which is not negligible in angioedema pathogenesis.

5. Discussion

Hereditary angioedema due to C1 inhibitor deficiency is undeniably the paradigm of Paroxysmal Permeability Disorders, conditions characterized by recurrent episodes of increased vascular permeability which has a transient nature and is usually self-limiting, but can be extremely dangerous in certain circumstances, as when the upper airways are involved (with the risk of death due to asphyxia) or when the clinical picture of abdominal acute attacks leads to unnecessary surgery.

Starting from the very first description of the disease in 1888 and from the identification of C1 inhibitor deficiency in 1963, knowledge about this condition has expanded remarkably over the years, leading to the possibility to design more and more drugs and to tailor patients' treatment with an individualized perspective. The present review underlines the crucial role of the endothelium in the pathophysiology of this disease: the endothelium is not just a passive bystander which is the target of action of ever increasing identified possible mediators; the endothelium is rather an active player, whose stability-instability is determined by an ever changing bi-directional interplay with the surrounding environment, characterized by constant exposure to flow and related shear stress and integration in a complex living organism, with all related modulators (as autonomic nervous system modulation of cardiovascular activity).

Awareness of the above-mentioned complexity can help elaborating hypotheses on the most intriguing yet challenging topics related to C1-INH-HAE.

The first one regards the localized nature of acute C1-INH-HAE attacks. A question which is still unanswered is whether swelling involving a specific site should be regarded as the local expression of a systemic derangement or not [93]. With this regard, some evidence exists about local generation of bradykinin, with possible local activation of the kinin-releasing system by rarely identified triggers (physical trauma, oral surgery. etc), as demonstrated by higher BK levels in the venous compartment of the affected limb as compared to those of the unaffected limb [86]. This is consistent with the usual absence of hypotension during HAE attacks.

On the other hand, the localized nature of angioedema attacks despite a systemic activation of the CAS/KKS might be supported by the following considerations: endothelial cells are intrinsically different from site to site, they may gain a local activation status and a sort

of priming (sometimes due to or exacerbated by specific local triggers) because of exposure to specific mediators (as Angpt2 and VEGF), to expression/upregulation of inducible receptors (as bradykinin B1 receptor), to local alterations of the glycocalyx, interendothelial cell junctions and activation of specific intracellular pathways. Interestingly, since bradykinin B2 receptor is rapidly desensitized after interaction with BK, while B1Rs are expressed longer on endothelial cells after induction [94], expression of B1Rs in specific sites has been envisaged as a pivotal mechanism explaining the localized nature of attacks. However, efficacy of icatibant, antagonist of the B2R, in reverting the hyperpermeability state should be taken into consideration and the role of constitutively expressed B2R cannot be underestimated.

The systemic hypothesis is corroborated by the finding of increased values of activation products (as cHK) in plasma samples from C1-INH-HAE patients as well as by attacks involving multiple sites. The presence of prodromal signs and symptoms in some cases also suggests a systemic process [46].

The priming of endothelial cells, chronically exposed to hyperpermeabilizing factors even during the intercritical periods, and the complexity of the mechanisms activated to answer to a high variety of stimuli (and possibly inability to adapt to ever changing conditions) might support the threshold hypothesis, according to which C1-NH-HAE patients do carry a stable deficiency which makes them more prone to develop hyperpermeability and this lower threshold for edema formation is easily overcome episodically when a wide spectrum of possible factors interact one with another. This issue may help explaining the episodic nature of acute attacks despite a constant underlying deficit as well as the reasons why the disease shows an unpredictable pattern of attacks recurrence, both among affected family members and in the very same patient in different periods of life.

The seek for biomarkers is ongoing, but caution is warranted, given that, as some Authors have underlined, for most of the proposed biomarkers a causality correlation has not been proven so far [95].

In vitro studies have helped acquiring many of the currently shared notions about the pathogenesis of angioedema. However, it should be underlined that cautious selection of endothelial cells used in *in vitro* essays is of pivotal importance: the phenotype of endothelial cells differs substantially from one vascular bed to another one. Since angioedema episodes occur preferentially in the skin, in some papers, endothelial cells were also isolated from human adult dermal microvasculature (ADMECs) rather than from the umbilical vein (HUVECs). Alternatively, cells can be obtained from commercial suppliers that offer arterial, venous or microvascular endothelial cells from different organs. To avoid an influence of individual variability, pooling cells from four-five donors is recommended. With division/passage number primary endothelial cells tend to differentiate and loose some functions, including the ability to induce oxidative stress etc. Therefore, endothelial cell lines are not recommended since classical physiology could be missing.

Endothelial cells in culture downregulate a number of genes that are expressed in situ, since in most cell culture systems shear stress, which is able to up regulate several genes, is missing. Three dimensional models with endothelial cells continuously exposed to physiological flow conditions are undeniably promising tools to overcome these limitations. Preliminary results with exposure to bradykinin are promising and open the way to many possible further investigations, last but not least the possibility to test drugs and their delivery.

New techniques as HRV analysis, nailfold capillaroscopy, non-invasive finger plethysmography and reactive-hyperemia-based methods may help to shed more light on the mechanisms underlying angioedema, hopefully giving new insights that may help also to better individualize therapy not only in C1-INH-HAE but also in other types of angioedema.

Finally, we also speculate that C1-INH-HAE may serve as useful model to understand endothelial behaviour in a wide spectrum of clinical conditions.

Acknowledgments

We would like to heartily thank Prof Marco Cicardi, who was a great Mentor to all of us, in science as well as in life.

We thank Prof. C. Misbah, Dr. Lionel Bureau, Dr. D. Tsvirkun for the design of the microvasculature on a chip model and for the fruitful contribution which led to the development of the protocol to test endothelial permeability in the microfluidic device.

We thank Dr. Giuliana Marzia Coretti for her more than precious help with artwork representing the crucial role of endothelium in the pathogenesis of angioedema due to C1 inhibitor deficiency.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2020.106304.

References

[1] J.G. McCarron, C. Wilson, H.R. Heathcote, X. Zhang, C. Buckley, M.D. Lee, Heterogeneity and emergent behaviour in the vascular endothelium, Curr. Opin. Pharmacol. 45 (2019) 23–32.
[2] A. Sandoo, J.J. van Zanten, G.S. Metsios, D. Carroll, G.D. Kitas, The endothelium and its role in regulating vascular tone, Open Cardiovasc. Med. J. 4 (2010) 302–312.
[3] V.W. van Hinsbergh, Endothelium–role in regulation of coagulation and inflammation, Semin Immunopathol 34 (2012) 93–106.
[4] G.L. Semenza, Vascular responses to hypoxia and ischemia, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 648–652.
[5] J.S. Pober, W.C. Sessa, Evolving functions of endothelial cells in inflammation, Nat. Rev. Immunol. 7 (2007) 803–815.
[6] E. Dejana, E. Tournier-Lasserve, B.M. Weinstein, The control of vascular integrity by endothelial cell junctions: molecular basis and pathological implications, Dev. Cell 16 (2009) 209–221.
[7] A.M. Dvorak, S. Kohn, E.S. Morgan, P. Fox, J.A. Nagy, H.F. Dvorak, The vesiculo-vacuolar organelle (VVO): a distinct endothelial cell structure that provides a transcellular pathway for macromolecular extravasation, J. Leukoc. Biol. 59 (1996) 100–115.
[8] M.Y. Radeva, J. Waschke, Mind the gap: mechanisms regulating the endothelial barrier, Acta Physiol. Oxf (2018) 222.
[9] F. Orsenigo, C. Giampietro, A. Ferrari, M. Corada, A. Galaup, S. Sigismund, G. Ristagno, L. Maddaluno, G.Y. Koh, D. Franco, V. Kurtcuoglu, D. Poulikakos, P. Baluk, D. McDonald, M. Grazia Lampugnani, E. Dejana, Phosphorylation of VE-cadherin is modulated by haemodynamic forces and contributes to the regulation of vascular permeability in vivo, Nat. Commun. 3 (2012) 1208.
[10] M.A. Wu, D. Tsvirkun, L. Bureau, I. Boccon-Gibod, M. Inglebert, A. Duperray, L. Bouillet, C. Misbah, M. Cicardi, Paroxysmal permeability disorders: development of a microfluidic device to assess endothelial barrier function, Front Med. (Lausanne) 6 (2019) 89.
[11] M. Cicardi, W. Aberer, A. Banerji, M. Bas, J.A. Bernstein, K. Bork, T. Caballero, H. Farkas, A. Grumach, A.P. Kaplan, M.A. Riedl, M. Triggiani, A. Zanichelli, B. Zuraw, E.A.A.C.I. Hutpo, Classification, diagnosis, and approach to treatment for angioedema: consensus report from the Hereditary Angioedema International Working Group, Allergy 69 (2014) 602–616.
[12] P.E. Carter, C. Duponchel, M. Tosi, J.E. Fothergill, Complete nucleotide sequence of the gene for human C1 inhibitor with an unusually high density of Alu elements, Eur. J. Biochem. 197 (1991) 301–308.
[13] H. Longhurst, M. Cicardi, Hereditary angio-oedema, Lancet 379 (2012) 474–481.
[14] E. Aygoren-Pursun, M. Magerl, A. Maetzel, M. Maurer, Epidemiology of Bradykinin-mediated angioedema: a systematic investigation of epidemiological studies, Orphanet J. Rare Dis. 13 (2018) 73.
[15] M.A. Wu, R. Castelli, The Janus faces of acquired angioedema: C1-inhibitor deficiency, lymphoproliferation and autoimmunity, Clin. Chem. Lab. Med. 54 (2016) 207–214.
[16] V. Bafunno, D. Firinu, M. D'Apolito, G. Cordisco, S. Loffredo, A. Leccese, M. Bova, M.P. Barca, R. Santacroce, M. Cicardi, S. Del Giacco, M. Margaglione, Mutation of the angiopoietin-1 gene (ANGPT1) associates with a new type of hereditary angioedema, J. Allergy Clin. Immunol. 141 (3) (2018) 1009–1017.
[17] K. Bork, K. Wulff, L. Steinmuller-Magin, I. Braenne, P. Staubach-Renz, G. Witzke, J. Hardt, Hereditary angioedema with a mutation in the plasminogen gene, Allergy 73 (2) (2018) 442–450.
[18] K. Bork, K. Wulff, H. Rossmann, L. Steinmuller-Magin, I. Braenne, G. Witzke, J. Hardt, Hereditary angioedema cosegregating with a novel kininogen 1 gene mutation changing the N-terminal cleavage site of bradykinin, Allergy 74 (12) (2019) 2479–2481.
[19] M.A. Wu, F. Perego, A. Zanichelli, M. Cicardi, Angioedema phenotypes: disease expression and classification, Clin. Rev. Allergy Immunol. 51 (2016) 162–169.
[20] W. Osler, Landmark publication from The American Journal of the Medical Sciences: Hereditary angio-neurotic oedema. 1888, Am. J. Med. Sci. 339 (2010) 175–178.

[21] T. Caballero, E. Aygoren-Pursun, A. Bygum, K. Beusterien, E. Hautamaki, Z. Sisic, S. Wait, H.B. Boysen, The humanistic burden of hereditary angioedema: results from the Burden of Illness Study in Europe, Allergy Asthma Proc. 35 (2014) 47–53.
[22] V.H. Donaldson, R.R. Evans, A biochemical abnormality in herediatry angioneurotic edema: absence of serum inhibitor of C' 1-esterase, Am. J. Med. 35 (1963) 37–44.
[23] A. Reshef, M. Kidon, I. Leibovich, The story of angioedema: from quincke to bradykinin, Clin. Rev. Allergy Immunol. 51 (2016) 121–139.
[24] S.C. Bock, K. Skriver, E. Nielsen, H.C. Thogersen, B. Wiman, V.H. Donaldson, R.L. Eddy, J. Marrinan, E. Radziejewska, R. Huber, et al., Human C1 inhibitor: primary structure, cDNA cloning, and chromosomal localization, Biochemistry 25 (1986) 4292–4301.
[25] P.E. Carter, B. Dunbar, J.E. Fothergill, Genomic and cDNA cloning of the human C1 inhibitor. Intron-exon junctions and comparison with other serpins, Eur. J. Biochem. 173 (1988) 163–169.
[26] A.E. Davis 3rd, A.S. Whitehead, R.A. Harrison, A. Dauphinais, G.A. Bruns, M. Cicardi, F.S. Rosen, Human inhibitor of the first component of complement, C1: characterization of cDNA clones and localization of the gene to chromosome 11, Proc. Natl. Acad Sci. U.S.A. 83 (1986) 3161–3165.
[27] A.E. Davis 3rd, P. Mejia, F. Lu, Biological activities of C1 inhibitor, Mol. Immunol. 45 (2008) 4057–4063.
[28] V.H. Donaldson, O.D. Ratnoff, W. Dias Da Silva, F.S. Rosen, Permeability-increasing activity in hereditary angioneurotic edema plasma. II. Mechanism of formation and partial characterization, J. Clin. Invest. 48 (1969) 642–653.
[29] T. Fields, B. Ghebrehiwet, A.P. Kaplan, Kinin formation in hereditary angioedema plasma: evidence against kinin derivation from C2 and in support of "spontaneous" formation of bradykinin, J. Allergy Clin. Immunol. 72 (1983) 54–60.
[30] J. Nussberger, M. Cugno, C. Amstutz, M. Cicardi, A. Pellacani, A. Agostoni, Plasma bradykinin in angio-oedema, Lancet 351 (1998) 1693–1697.
[31] S. de Maat, C. Tersteeg, E. Herczenik, C. Maas, Tracking down contact activation - from coagulation in vitro to inflammation in vivo, Int. J. Lab. Hematol. 36 (2014) 374–381.
[32] E.D. Han, R.C. MacFarlane, A.N. Mulligan, J. Scafidi, A.E. Davis 3rd, Increased vascular permeability in C1 inhibitor-deficient mice mediated by the bradykinin type 2 receptor, J. Clin. Invest. 109 (2002) 1057–1063.
[33] K. Bork, J. Frank, B. Grundt, P. Schlattmann, J. Nussberger, W. Kreuz, Treatment of acute edema attacks in hereditary angioedema with a bradykinin receptor-2 antagonist (Icatibant), J. Allergy Clin. Immunol. 119 (2007) 1497–1503.
[34] F. Alhenc-Gelas, N. Bouby, J.P. Girolami, Kallikrein/K1, kinins, and ACE/kininase II in homeostasis and in disease insight from human and experimental genetic studies, therapeutic implication, Front. Med. (Lausanne) 6 (2019) 136.
[35] L.M. Leeb-Lundberg, F. Marceau, W. Muller-Esterl, D.J. Pettibone, B.L. Zuraw, International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences, Pharmacol. Rev. 57 (2005) 27–77.
[36] A.H. Schmaier, The contact activation and kallikrein/kinin systems: pathophysiologic and physiologic activities, J. Thromb. Haemost. 14 (2016) 28–39.
[37] Z. Shariat-Madar, F. Mahdi, A.H. Schmaier, Recombinant prolylcarboxypeptidase activates plasma prekallikrein, Blood 103 (2004) 4554–4561.
[38] K. Joseph, B.G. Tholanikunnel, A. Bygum, B. Ghebrehiwet, A.P. Kaplan, Factor XII-independent activation of the bradykinin-forming cascade: Implications for the pathogenesis of hereditary angioedema types I and II, J. Allergy Clin. Immunol. 132 (2013) 470–475.
[39] A.H. Schmaier, The plasma kallikrein-kinin system counterbalances the renin-angiotensin system, J. Clin. Invest. 109 (2002) 1007–1009.
[40] Z. Shariat-Madar, F. Mahdi, A.H. Schmaier, Assembly and activation of the plasma kallikrein/kinin system: a new interpretation, Int. Immunopharmacol. 2 (2002) 1841–1849.
[41] F. Mahdi, Z.S. Madar, C.D. Figueroa, A.H. Schmaier, Factor XII interacts with the multiprotein assembly of urokinase plasminogen activator receptor, gC1qR, and cytokeratin 1 on endothelial cell membranes, Blood 99 (2002) 3585–3596.
[42] K. Joseph, B. Ghebrehiwet, A.P. Kaplan, Activation of the kinin-forming cascade on the surface of endothelial cells, Biol. Chem. 382 (2001) 71–75.
[43] C. Maas, Plasminflammation-an emerging pathway to bradykinin production, Front. Immunol. 10 (2019) 2046.
[44] F. Bossi, F. Fischetti, D. Regoli, P. Durigutto, B. Frossi, F. Gobeil Jr., B. Ghebrehiwet, E.I. Peerschke, M. Cicardi, F. Tedesco, Novel pathogenic mechanism and therapeutic approaches to angioedema associated with C1 inhibitor deficiency, J. Allergy Clin. Immunol. 124 (1303–1310) (2009) e1304.
[45] M. Magerl, M. Bader, A. Gompel, K. Joseph, A.P. Kaplan, G. Kojda, T. Renne, M. Wirth, M. Maurer, M.K. Church, Bradykinin in health and disease: proceedings of the bradykinin symposium 2012, Berlin 23–24 August 2012, Inflamm. Res. 63 (2014) 173–178.
[46] Z.L. Hofman, A. Relan, S. Zeerleder, C. Drouet, B. Zuraw, C.E. Hack, Angioedema attacks in patients with hereditary angioedema: local manifestations of a systemic activation process, J. Allergy Clin. Immunol. 138 (2016) 359–366.
[47] F. Bossi, E.I. Peerschke, B. Ghebrehiwet, F. Tedesco, Cross-talk between the complement and the kinin system in vascular permeability, Immunol. Lett. 140 (2011) 7–13.
[48] L. Bergamaschini, S. Gatti, L. Caccamo, P. Prato, L. Latham, P. Trezza, M. Maggioni, G. Gobbo, L.R. Fassati, C1 inhibitor potentiates the protective effect of organ preservation solution on endothelial cells during cold storage, Transplant Proc. 33 (2001) 939–941.
[49] S. Cai, A.E. Davis 3rd, Complement regulatory protein C1 inhibitor binds to selectins and interferes with endothelial-leukocyte adhesion, J. Immunol. 171 (2003) 4786–4791.
[50] R. Gesuete, C. Storini, A. Fantin, M. Stravalaci, E.R. Zanier, F. Orsini, H. Vietsch, M.L. Mannesse, B. Ziere, M. Gobbi, M.G. De Simoni, Recombinant C1 inhibitor in brain ischemic injury, Ann. Neurol. 66 (2009) 332–342.
[51] S. Caccia, R. Castelli, D. Maiocchi, L. Bergamaschini, M. Cugno, Interaction of C1 inhibitor with thrombin on the endothelial surface, Blood Coagul. Fibrinol. 22 (2011) 571–575.
[52] S. Ravindran, T.E. Grys, R.A. Welch, M. Schapira, P.A. Patston, Inhibition of plasma kallikrein by C1-inhibitor: role of endothelial cells and the amino-terminal domain of C1-inhibitor, Thromb. Haemost. 92 (2004) 1277–1283.
[53] E. Kajdacsi, P.K. Jani, D. Csuka, L.A. Varga, Z. Prohaszka, H. Farkas, L. Cervenak, Endothelial cell activation during edematous attacks of hereditary angioedema types I and II, J. Allergy Clin. Immunol. 133 (2014) 1686–1691.
[54] M. Cedzynski, K. Madalinski, H. Gregorek, A.S. Swierzko, E. Nowicka, K. Obtulowicz, K. Dzierzanowska-Fangrat, U. Wojda, D. Rabczenko, M. Kawakami, Possible disease-modifying factors: the mannan-binding lectin pathway and infections in hereditary angioedema of children and adults, Arch. Immunol. Ther. Exp. (Warsz) 56 (2008) 69–75.
[55] L. Varga, G. Szeplaki, J. Laki, A. Kocsis, K. Kristof, P. Gal, Z. Bajtay, J. Wieslander, M.R. Daha, P. Garred, H.O. Madsen, G. Fust, H. Farkas, Depressed activation of the lectin pathway of complement in hereditary angioedema, Clin. Exp. Immunol. 153 (2008) 68–74.
[56] D. Csuka, L. Munthe-Fog, M.O. Skjoedt, A. Kocsis, Z. Zotter, P. Gal, L. Varga, H. Farkas, G. Fust, P. Garred, The role of ficolins and MASPs in hereditary angioedema due to C1-inhibitor deficiency, Mol. Immunol. 54 (2013) 271–277.
[57] D. Csuka, L. Munthe-Fog, E. Hein, Z. Zotter, Z. Prohaszka, H. Farkas, L. Varga, P. Garred, Activation of the ficolin-lectin pathway during attacks of hereditary angioedema, J. Allergy Clin. Immunol. 134 (1388–1393) (2014) e1381.
[58] C.B. Hansen, D. Csuka, L. Munthe-Fog, L. Varga, H. Farkas, K.M. Hansen, C. Koch, K. Skjodt, P. Garred, M.O. Skjoedt, The levels of the lectin pathway serine protease MASP-1 and its complex formation with C1 inhibitor are linked to the severity of hereditary angioedema, J. Immunol. 195 (2015) 3596–3604.
[59] J. Dobo, B. Major, K.A. Kekesi, I. Szabo, M. Megyeri, K. Hajela, G. Juhasz, P. Zavodszky, P. Gal, Cleavage of kininogen and subsequent bradykinin release by the complement component: mannose-binding lectin-associated serine protease (MASP)-1, PLoS One 6 (2011) e20036.
[60] K. Bork, G. Witzke, Shortened activated partial thromboplastin time may help in diagnosing hereditary and acquired angioedema, Int. Arch. Allergy Immunol. 170 (2016) 101–107.
[61] M. Cugno, A. Zanichelli, A.G. Bellatorre, S. Griffini, M. Cicardi, Plasma biomarkers of acute attacks in patients with angioedema due to C1-inhibitor deficiency, Allergy 64 (2009) 254–257.
[62] A.P. Kaplan, C. Maas, The search for biomarkers in hereditary angioedema, Front. Med. (Lausanne) 4 (2017) 206.
[63] C. Suffritti, A. Zanichelli, L. Maggioni, E. Bonanni, M. Cugno, M. Cicardi, High-molecular-weight kininogen cleavage correlates with disease states in the bradykinin-mediated angioedema due to hereditary C1-inhibitor deficiency, Clin. Exp. Allergy 44 (2014) 1503–1514.
[64] Z.L.M. Hofman, S. de Maat, C. Suffritti, A. Zanichelli, C. van Doorn, S.A.E. Sebastian, N. Veszeli, D. Csuka, T. Renne, G. Pasterkamp, M. Cicardi, H. Farkas, C.E. Hack, C. Maas, Cleaved kininogen as a biomarker for bradykinin release in hereditary angioedema, J. Allergy Clin. Immunol. 140 (6) (2017) 1700–1703.e8.
[65] S. Loffredo, M. Bova, C. Suffritti, F. Borriello, A. Zanichelli, A. Petraroli, G. Varricchi, M. Triggiani, M. Cicardi, G. Marone, Elevated plasma levels of vascular permeability factors in C1 inhibitor-deficient hereditary angioedema, Allergy 71 (2016) 989–996.
[66] E. Kajdacsi, P.K. Jani, D. Csuka, L. Varga, Z. Prohaszka, H. Farkas, L. Cervenak, Novel vasoregulatory aspects of hereditary angioedema: the role of arginine vasopressin, adrenomedullin and endothelin-1, J. Clin. Immunol. 36 (2016) 160–170.
[67] G. Castellano, C. Divella, F. Sallustio, V. Montinaro, C. Curci, A. Zanichelli, E. Bonanni, C. Suffritti, S. Caccia, F. Bossi, A. Gallone, F.P. Schena, L. Gesualdo, M. Cicardi, A transcriptomics study of hereditary angioedema attacks, J. Allergy Clin. Immunol. 142 (2018) 883–891.
[68] S. Loffredo, A.L. Ferrara, M. Bova, F. Borriello, C. Suffritti, N. Veszeli, A. Petraroli, M.R. Galdiero, G. Varricchi, F. Granata, A. Zanichelli, H. Farkas, M. Cicardi, G. Lambeau, G. Marone, Secreted phospholipases A2 in hereditary angioedema with C1-inhibitor deficiency, Front. Immunol. 9 (2018) 1721.
[69] A.L. Ferrara, M. Bova, A. Petraroli, N. Veszeli, M.R. Galdiero, M. Braile, G. Marone, L. Cristinziano, S. Marcella, L. Modestino, H. Farkas, S. Loffredo, Hereditary angioedema attack: what happens to vasoactive mediators?, Int. Immunopharmacol. 78 (2019) 106079.
[70] S.M. Parikh, Angiopoietins and Tie2 in vascular inflammation, Curr. Opin. Hematol. 24 (2017) 432–438.
[71] M. Demirturk, A. Gelincik, S. Cinar, M. Kilercik, E. Onay-Ucar, B. Colakoglu, N. Arda, S. Buyukozturk, G. Deniz, Increased eNOS levels in hereditary angioedema, Int. Immunopharmacol. 20 (2014) 264–268.
[72] M. Demirturk, T.S. Akpinar, M. Kose, A. Gelincik, B. Colakoglu, S. Buyukozturk, Endocan: a novel marker of endothelial dysfunction in C1-inhibitor-deficient hereditary angioedema, Int. Arch. Allergy Immunol. 174 (2017) 104–107.
[73] L. Bouillet, I. Vilgrain, VE-cadherin, a potential marker for endothelial cell activation during hereditary angioedema attacks, J. Allergy Clin. Immunol. 134 (2014) 241.
[74] S.R. Del Giacco, D. Firinu, P.L. Minciullo, M.P. Barca, P.E. Manconi, G. Tartarisco, M. Cristani, A. Saija, S. Gangemi, Oxidative stress markers in patients with hereditary angioedema, Arch. Med. Sci. 15 (2019) 92–98.
[75] A. Ludwig, A. Sommer, S. Uhlig, Assessment of endothelial permeability and leukocyte transmigration in human endothelial cell monolayers, Methods Mol. Biol. 763 (2011) 319–332.
[76] I. Bischoff, M.C. Hornburger, B.A. Mayer, A. Beyerle, J. Wegener, R. Furst, Pitfalls in assessing microvascular endothelial barrier function: impedance-based devices versus the classic macromolecular tracer assay, Sci. Rep. 6 (2016) 23671.
[77] O. Dubrovskyi, A.A. Birukova, K.G. Birukov, Measurement of local permeability at subcellular level in cell models of agonist- and ventilator-induced lung injury, Lab. Invest. 93 (2013) 254–263.

[78] M.L. Debreczeni, Z. Nemeth, E. Kajdacsi, E. Schwaner, V. Mako, A. Masszi, Z. Doleschall, J. Rigo, F.R. Walter, M.A. Deli, G. Pal, J. Dobo, P. Gal, L. Cervenak, MASP-1 increases endothelial permeability, Front. Immunol. 10 (2019) 991.
[79] D. Tsvirkun, A. Grichine, A. Duperray, C. Misbah, L. Bureau, Microvasculature on a chip: study of the Endothelial Surface Layer and the flow structure of Red Blood Cells, Sci. Rep. 7 (2017) 45036.
[80] C. Oschatz, C. Maas, B. Lecher, T. Jansen, J. Bjorkqvist, T. Tradler, R. Sedlmeier, P. Burfeind, S. Cichon, S. Hammerschmidt, W. Muller-Esterl, W.A. Wuillemin, G. Nilsson, T. Renne, Mast cells increase vascular permeability by heparin-initiated bradykinin formation in vivo, Immunity 34 (2011) 258–268.
[81] T. Qiu, M.J. Chiuchiolo, A.S. Whaley, A.R. Russo, D. Sondhi, S.M. Kaminsky, R.G. Crystal, O.E. Pagovich, Gene therapy for C1 esterase inhibitor deficiency in a Murine Model of Hereditary angioedema, Allergy 74 (2019) 1081–1089.
[82] C.L. Veronez, S. Maghsodi, M. Todiras, E. Popova, A.F. Rodrigues, F. Qadri, J.B. Pesquero, M. Bader, Endothelial B2-receptor overexpression as an alternative animal model for hereditary angioedema, Allergy 74 (10) (2019) 1998–2002.
[83] G. Bhattacharjee, A.S. Revenko, J.R. Crosby, C. May, D. Gao, C. Zhao, B.P. Monia, A.R. MacLeod, Inhibition of vascular permeability by antisense-mediated inhibition of plasma kallikrein and coagulation factor 12, Nucleic Acid Ther. 23 (2013) 175–187.
[84] D. Farfara, E. Feierman, A. Richards, A.S. Revenko, R.A. MacLeod, E.H. Norris, S. Strickland, Knockdown of circulating C1 inhibitor induces neurovascular impairment, glial cell activation, neuroinflammation, and behavioral deficits, Glia 67 (2019) 1359–1373.
[85] R. von Kanel, Acute mental stress and hemostasis: When physiology becomes vascular harm, Thromb. Res. 135 (Suppl 1) (2015) S52–S55.
[86] J. Nussberger, M. Cugno, M. Cicardi, A. Agostoni, Local bradykinin generation in hereditary angioedema, J. Allergy Clin. Immunol. 104 (1999) 1321–1322.
[87] Z. Zotter, D. Csuka, E. Szabo, I. Czaller, Z. Nebenfuhrer, G. Temesszentandrasi, G. Fust, L. Varga, H. Farkas, The influence of trigger factors on hereditary angioedema due to C1-inhibitor deficiency, Orphanet J Rare Dis 9 (2014) 44.
[88] M.A. Wu, F. Casella, F. Perego, C. Suffritti, N. Afifi Afifi, E. Tobaldini, A. Zanichelli, C. Cogliati, N. Montano, M. Cicardi, Hereditary angioedema: assessing the hypothesis for underlying autonomic dysfunction, PLoS ONE 12 (2017) e0187110.
[89] M. Demirturk, N. Polat, G. Guz, A. Gurdal, I. Altun, A. Gelincik, B. Toz, H. Oflaz, B. Colakoglu, M. Dal, S. Buyukozturk, There is an increased risk of atherosclerosis in hereditary angioedema, Int. Immunopharmacol. 12 (2012) 212–216.
[90] D. Firinu, P.P. Bassareo, A.M. Zedda, M.P. Barca, A. Crisafulli, G. Mercuro, S. Del Giacco, Impaired endothelial function in hereditary angioedema during the symptom-free period, Front. Physiol. 9 (2018) 523.
[91] Z. Nebenfuhrer, E. Szabo, E. Kajdacsi, K.V. Kohalmi, I. Karadi, A. Zsary, H. Farkas, L. Cervenak, Flow-mediated vasodilation assay indicates no endothelial dysfunction in hereditary angioedema patients with C1-inhibitor deficiency, Ann. Allergy Asthma Immunol. 122 (2019) 86–92.
[92] A. Cesoni Marcelli, M. Bova, A. Petraroli, S. Loffredo, A. Ferrara, L. Carucci, I. Mormile, G. Spadaro, A. Genovese, Nailfold videocapillaroscopic findings in bradykinin-mediated angioedema, Allergy 74 (Suppl. 106) (2019) 217, doi:10.1111/all.13959.
[93] S. De Maat, Z.L.M. Hofman, C. Maas, Hereditary angioedema: the plasma contact system out of control, J. Thromb. Haemost. 16 (2018) 1674–1685.
[94] J. Enquist, C. Skroder, J.L. Whistler, L.M. Leeb-Lundberg, Kinins promote B2 receptor endocytosis and delay constitutive B1 receptor endocytosis, Mol. Pharmacol. 71 (2007) 494–507.
[95] S. De Maat, Z.L.M. Hofman, C. Maas, Hereditary angioedema: the plasma contact system out of control: reply, J. Thromb. Haemost. 16 (2018) 2349–2351.