



Crosstalk between the renin–angiotensin, complement and kallikrein–kinin systems in inflammation

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Abstract | During severe inflammatory and infectious diseases, various mediators modulate the equilibrium of vascular tone, inflammation, coagulation and thrombosis. This Review describes the interactive roles of the renin–angiotensin system, the complement system, and the closely linked kallikrein–kinin and contact systems in cell biological functions such as vascular tone and leakage, inflammation, chemotaxis, thrombosis and cell proliferation. Specific attention is given to the role of these systems in systemic inflammation in the vasculature and tissues during hereditary angioedema, cardiovascular and renal glomerular disease, vasculitides and COVID-19. Moreover, we discuss the therapeutic implications of these complex interactions, given that modulation of one system may affect the other systems, with beneficial or deleterious consequences.

Angiotensin

A group of peptide hormones that are effectors of the renin–angiotensin system and are involved in control of vascular tone, blood pressure, fluid and salt balance, cell proliferation, fibrosis and inflammation.

The renin–angiotensin system (RAS), the complement system and the kallikrein–kinin system (KKS) each consists of a large number of distinct plasma and membrane-bound proteins and receptors that can become activated through proteolytic cascades. Notably, all three systems are excessively activated during inflammation, have potent pro-inflammatory and prothrombotic effects and increase vascular permeability, leading to oedema. The RAS has a major role in the control of vascular tone, blood pressure, fluid and salt balance, cell proliferation, fibrosis and inflammation¹. The complement system, which can be activated via the classical, alternative and lectin pathways, forms an integral part of innate immunity by eliciting local inflammation and clearance mechanisms, including opsonization, chemotaxis, phagocytosis, anaphylaxis, cytolysis and autophagy, and by interacting with pathways of adaptive immunity in response to pathogens, altered and apoptotic cells, immune complexes or polymeric forms of immunoglobulin as well as pentraxins². The KKS releases vasoactive kinins, such as bradykinin, which induce vasodilation, vascular leakage and pain. The KKS can be induced both in plasma, where it is referred to as the ‘plasma KKS’, and in tissue, where it is known as the ‘tissue KKS’. These systems not only differ in location; they also have different enzymes and release distinct kinins. The plasma KKS is tightly linked with the contact system, which is activated by factor XII (FXII; also called ‘Hageman factor’). In addition to kinin release, FXII also initiates the

intrinsic pathway of coagulation³, leading to thrombus formation. Here we refer to the plasma KKS and the contact system as the ‘plasma KKS/contact system’.

In this Review, we describe the RAS, the complement system and the plasma KKS/contact system and their role in inflammation, with a particular focus on the mechanisms of crosstalk between the three systems. We elaborate on the role of these interactions in inflammatory disorders such as hereditary angioedema (HAE), cardiac injury, glomerulonephritis, vasculitis and severe COVID-19, and discuss the therapeutic implications of the interactions between the three systems.

The RAS

The RAS is composed of proteins, peptides, enzymes and receptors, and the main physiological functions of the RAS are determined by the balance of two counter-regulatory pathways, the canonical and non-canonical RAS pathways (FIG. 1; Supplementary Table 1). These allow the regulation of blood pressure, fluid and salt homeostasis, vasoconstriction or vasodilation, vascular permeability, oxidative stress, cell death or proliferation, inflammatory modulation (both pro-inflammatory and anti-inflammatory effects), angiogenesis, fibrosis and coagulation/impaired fibrinolysis⁴. Whereas the immediate effects of the RAS are related to maintaining blood flow and adequate blood pressure as well as electrolyte balance, prolonged stimulation contributes to the development of hypertension and

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<https://doi.org/10.1038/s41577-021-00634-8>

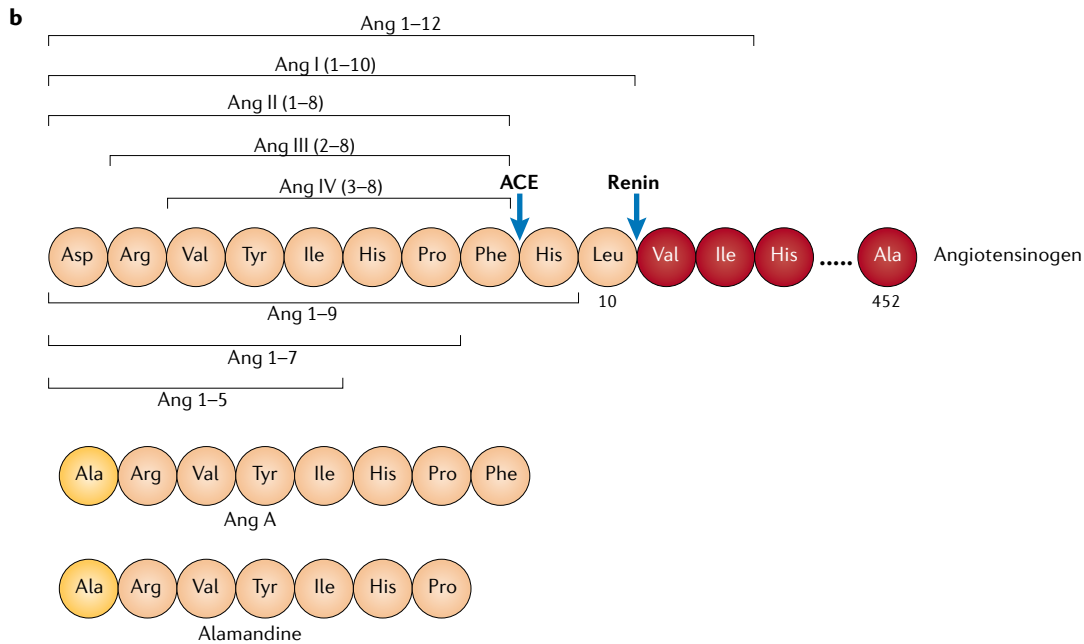
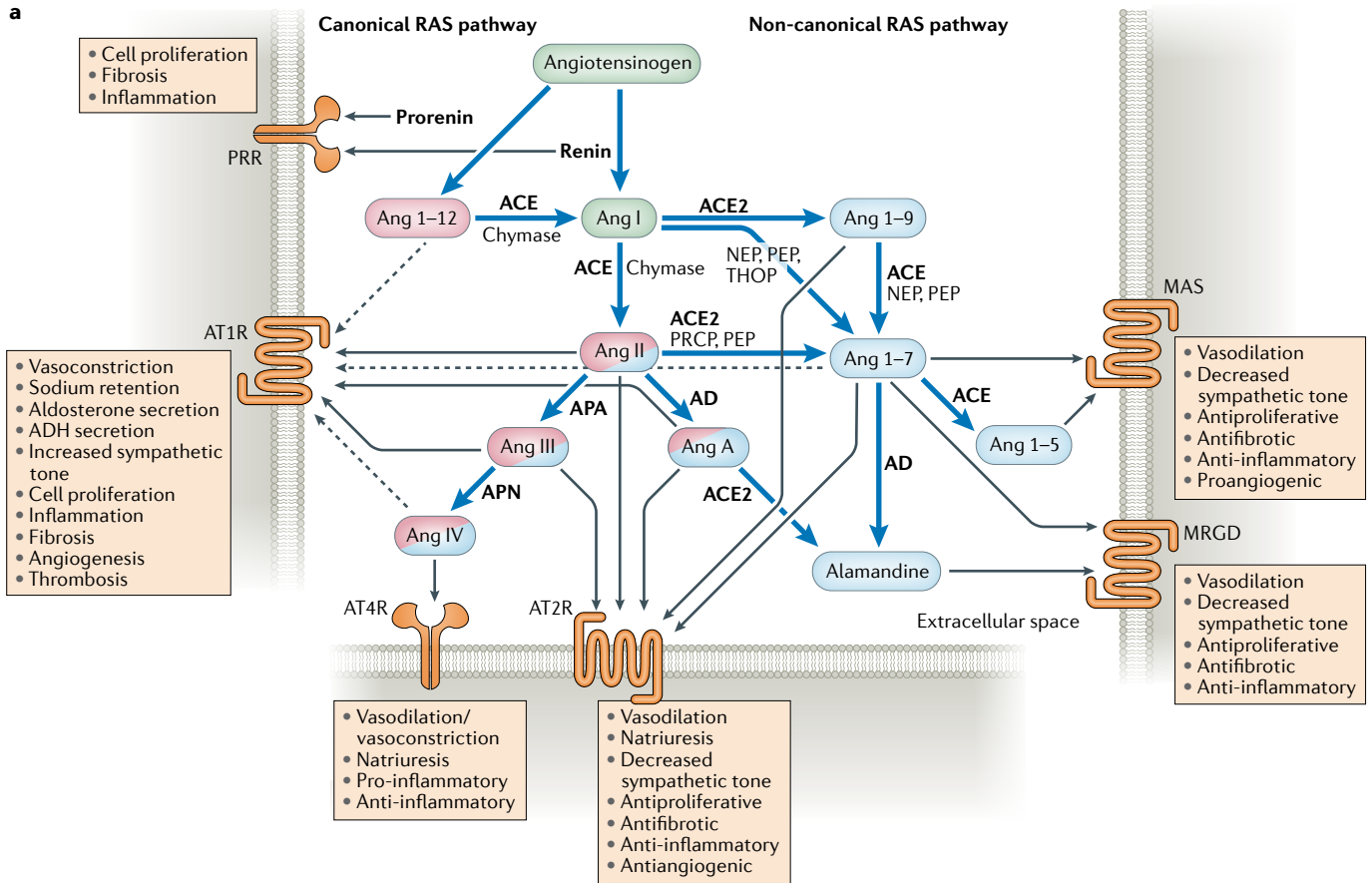
Kinin

A group of peptides that are effectors of the kallikrein-kinin system and induce vasodilation, vascular leakage and pain, cardinal signs of inflammation.

cardiovascular and renal diseases as well as inflammatory diseases and inflammatory complications in infectious diseases, but may also yield protective countereffects as described later.

A central component of the RAS is angiotensin-converting enzyme 2 (ACE2), which directs the RAS catalytic process towards the non-canonical pathway (FIG. 1). ACE2 is an integral membrane protein and, as such, is also

the cellular receptor for the spike proteins of severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2 (REFS^{5,6}). However, its soluble form also acts as an enzyme and can bind the viral spike protein⁷. ACE2 shares 42% homology with the enzyme ACE⁸ and is present in/on various types of cells in the heart, arteries (endothelial and smooth muscle cells), intestine, kidneys, lungs and brain⁹. ACE2 decreases angiotensin II (Ang II)



Pentraxins

A family of innate pattern recognition proteins, of which C-reactive protein (CRP), serum amyloid P and pentraxin 3 (PTX3) activate the classical pathway of the complement system.

Intrinsic pathway of coagulation

A cascade of serial activation of the coagulation factors. Factor XII (FXII; in activated form FXIIa), FXI and FIX generate a complex between FVIIIa and FIXa that activates FX. FXa with FVa form the prothrombinase complex that cleaves prothrombin into thrombin.

Membrane attack complex

An assembly of complement proteins C5b, C6, C7 and C8 and a number of C9 molecules forming a cytolytic pore in the cell membrane.

levels by directly generating Ang 1–7 from Ang II (FIG. 1) and by degrading Ang I, the precursor of Ang II, to Ang 1–9. Ang 1–7 exerts inhibitory effects on inflammation, fibrosis and cell proliferation via its receptor MAS. *Ace2*-knockout mice develop enhanced pulmonary vascular permeability and oedema in a lung injury model^{10,11}, an effect that might be due to excess cleavage of Ang I to Ang II by ACE and lack of Ang II degradation (FIG. 1). This mechanism has been suggested to occur in patients with COVID-19, where binding of SARS-CoV-2 to ACE2 downregulates ACE2 on cells¹². ACE2 can be cleaved from the cell membrane by the enzyme ADAM17. Ang II can promote ADAM17 activity and ACE2 shedding¹³, which leads to reduced ACE2 tissue expression and elevated circulating levels of ACE2, with pathological effects in conditions such as cardiac failure and hypertension¹⁴.

The main mechanism of RAS inhibition occurs through a negative-feedback loop mediated via Ang II on renin. This feedback does not appear to be direct, as the receptors for Ang II, the Ang II type 1 receptors (AT1Rs) on renin-producing cells, are not involved¹⁵. Instead, changes in blood pressure and sodium retention induced by angiotensins regulate renin levels¹⁶. Blockade of the canonical RAS pathway (for example, by use of an ACE inhibitor) increases renin levels¹⁷ and

promotes the catalytic breakdown of Ang II through the non-canonical pathway, as well as Ang 1–7 stimulation of MAS¹⁸, leading to vasodilation, decreased vascular tone and natriuresis (FIG. 1).

An interesting aspect of receptor specificity in the RAS is biased agonism⁴, whereby the position of the ligand determines the nature of the intracellular signalling cascades. For example, both Ang II and Ang 1–7 can bind to AT1R, but they induce different intracellular responses¹⁹. Moreover, Ang 1–7 blocks the effects of Ang II on the same receptor¹⁹. Biased agonists may accelerate receptor internalization, and this phenomenon could potentially be exploited therapeutically. Furthermore, certain G-protein-coupled receptors (GPCRs) have been found to form heterodimers (for example, AT1R and AT2R²⁰ as well as AT2R and MAS²¹), adding additional complexity to the signal interaction.

The complement system

When stimulated by pathogens, apoptotic cells, immune complexes or immunoglobulin polymers, the complement system elicits local inflammation and clearance mechanisms, including opsonization, chemotaxis, phagocytosis, anaphylaxis, cytolysis and autophagy. Moreover, components of the complement system interact with pathways and cells of both innate and adaptive immunity². The complement system may be activated on cell membranes, in the fluid phase and even intracellularly²². The three pathways of activation (the classical, lectin and alternative pathways, depicted in FIG. 2) respond to different stimuli but all ultimately generate C3 and C5 convertases and the membrane attack complex. Cleavage products of complement proteins are biologically active and can induce anaphylaxis (C3a and C5a), chemotaxis and phagocytosis (C3a and C5a) and opsonization (C3b, iC3b and C3dg). This robust defence system has multiple inhibitors, both soluble and expressed on cell surfaces, to prevent excessive activation (Supplementary Table 1). For a more comprehensive overview of the complement system, the reader is referred to REF.² Here we focus on the components of the complement system that are important for inflammation and coagulation or thrombosis and are known to interact with the RAS or the plasma KKS/contact system (TABLE 1).

The classical pathway of complement activation is initiated when the complement component C1q binds to the Fc domains of IgM or IgG. Together with two molecules of the serine protease C1r and two molecules of the serine protease C1s, C1q forms a complex called 'C1q2s2', and within this complex, C1s possesses the enzymatic activity that cleaves C2 and C4. C1q itself is composed of 18 polypeptides and has a hexameric structure, with heterotrimeric polypeptide chains forming a total of six subunits. Each heterotrimer has a collagen-like domain at the amino terminus (N terminus) and a globular head domain termed 'gC1q' at the carboxy terminus (C terminus). The gC1q domain can bind both to its receptor and to immunoglobulins. The multifunctional gC1q receptor (gC1qR)²³ is expressed by endothelial cells and platelets²⁴ and also binds thrombin, vitronectin²⁵, fibrinogen, high-molecular-weight

Fig. 1 | Overview of the renin–angiotensin system. a | The renin–angiotensin system (RAS) is initiated by renin, which is secreted by the juxtaglomerular cells of the afferent renal arterioles in response to decreased perfusion, in response to low sodium levels, upon β_1 -adrenergic receptor stimulation or upon stimulation by nitric oxide (NO) or prostaglandin^{259,260}. The renin precursor prorenin and renin both bind to prorenin receptor (PRR)²⁶¹. In the circulation, renin cleaves angiotensinogen into angiotensin I (Ang I). Prorenin can also cleave angiotensinogen into Ang I, to a certain extent, when bound to PRR. Ang I can also be generated through the cleavage of Ang 1–12 by angiotensin-converting enzyme (ACE)²⁴⁰ or by chymase in cardiac tissue²⁶². The ensuing proteolytic cascade can be divided into the canonical and non-canonical pathways. The canonical pathway is initiated when ACE, or chymase in the heart, cleaves Ang I into Ang II. Binding of Ang II or its cleavage products Ang III or Ang A to the G-protein-coupled receptor Ang II receptor type 1 (AT1R) leads to vasoconstriction, an increase in blood pressure and other effects^{4,221}. The non-canonical pathway is initiated by Ang I cleavage by ACE2 to Ang 1–9, which is further cleaved into Ang 1–7 (mainly by ACE). In the non-canonical pathway, binding of Ang II, Ang III, Ang 1–9, Ang 1–7 and Ang A to AT2R counteracts effects mediated by AT1R. Ang IV (generated from Ang III when cleaved by aminopeptidase N (APN)) is involved in both the canonical pathway and the non-canonical pathway and binds to angiotensin 4 receptor (AT4R), also known as insulin-regulated aminopeptidase (IRAP). Binding of Ang IV to AT4R has antiapoptotic and both pro-inflammatory and anti-inflammatory effects, as it regulates responses by competitive inhibition of the enzyme/receptor^{218,263,264}. Ang 1–7 binds to the receptors MAS and MAS-related G-protein-coupled receptor (MRGD), and alamandine, which is generated from Ang A or Ang 1–7, also binds to MRGD. Both Ang 1–7 and alamandine have antihypertensive, anti-inflammatory and antifibrotic effects²⁶⁵. The non-canonical and canonical RAS pathways can counterbalance each other^{218,263–265}. For example, Ang II binds to both AT2R (non-canonical pathway) and AT1R (canonical pathway) but exerts a more robust effect on AT1R^{99,266}. AT2R can, however, be upregulated during pathological conditions⁹⁹. Effectors are depicted in circles and the enzymes catalysing their cleavage are listed next to blue arrows. Effectors that predominantly participate in the canonical pathway are coloured pink and those that predominantly participate in the non-canonical pathway are coloured blue. Effectors that participate in both pathways are coloured in both pink and blue, with the predominant colour indicating the more prominent pathway. Black arrows indicate which molecule binds to which receptor, with dashed lines depicting weak receptor binding. **b** | Amino acid sequences of angiotensin peptides. The cleavage sites of ACE and renin are indicated by blue arrows. AD, aspartate decarboxylase; ADH, antidiuretic hormone; APA, aminopeptidase A; NEP, neprilysin; PEP, prolylendopeptidase; PRCP, prolylcarboxypeptidase; THOP, thimet oligopeptidase.

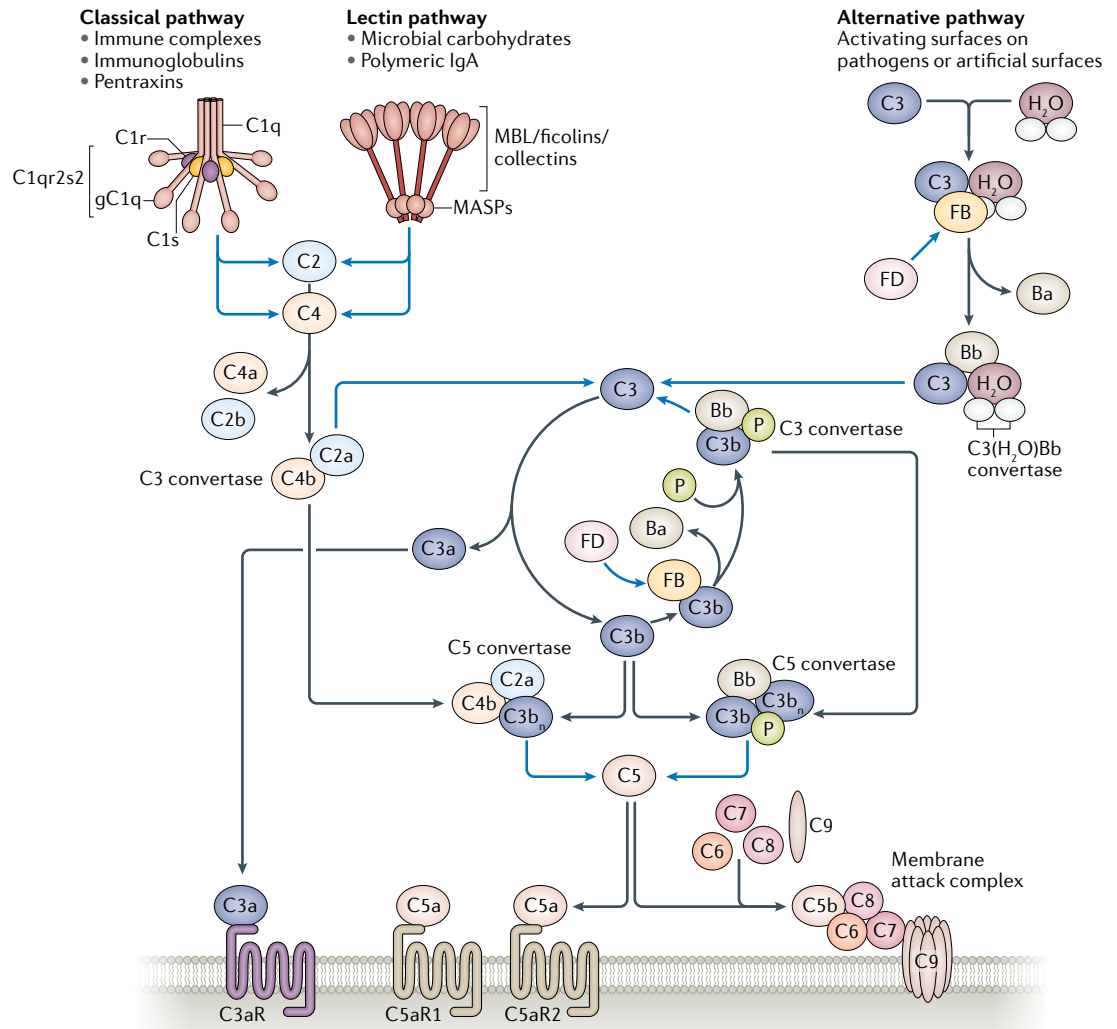


Fig. 2 | Overview of the complement system. The complement system is a network that can be divided into the classical, lectin and alternative pathways. The classical pathway is activated when C1s within the C1q₂s₂ complex cleaves C2 into C2a and C2b and cleaves C4 into C4a and C4b. Likewise, in the lectin pathway, mannose-binding lectin (MBL) serine protease 1 (MASP1) and MASP2 can also cleave C2 and C4. The classical and lectin pathways converge at the level of C2 and C4 cleavage, where a complex between C2a and C4b forms the C3 convertase. The alternative pathway exhibits a constant low-grade activation on biological surfaces whereby hydrolysed C3, C3(H₂O), binds to factor B (FB), which is cleaved by factor D (FD) into the Bb and Ba fragments. The Bb fragment contributes to the formation of the initial convertase C3(H₂O)Bb. This convertase cleaves C3 into C3a and C3b. C3b forms a complex with FB, which is again cleaved by FD into Bb, to form C3bBb, the C3 convertase. This convertase is stabilized by properdin (P). The formation of C3 convertases (C4b2a in the classical and lectin pathways and C3bBb in the alternative pathway) is amplified by the C3b-generation amplification loop (centre). By addition of extra C3bs to the C3 convertases, these are transformed into C5 convertases. C5 convertases cleave C5 into C5a and C5b, which promotes the assembly of the pore-forming unit C5b-9 within the cell membrane; this process is called the ‘terminal lytic complement pathway’. Binding of C3a to the receptor C3aR and of C5a to the C5a receptors C5aR1 and C5aR2 induces potent inflammatory responses. Blue lines depict enzymatic activity. Black lines depict binding or conversion into cleavage products. gC1q, globular head of C1q.

kininogen and FXII, in addition to C1q²⁶. Of note, high-molecular-weight kininogen and FXII are components of the KKS. gC1qR can initiate the classical pathway of complement activation, even in the absence of immunoglobulins²⁷, and can also activate the plasma KKS/contact system (FIGS 3, 4a).

The lectin pathway of complement activation is initiated by mannan-binding lectin serine proteases (MASPs), which also cleave complement components C2 and C4 (REF. 28). MASP1 and MASP2 can also cleave

prothrombin into thrombin^{29,30}, and MASP1 activates thrombin-activatable fibrinolysis inhibitor (TAFI), suggesting that activation of the lectin pathway may promote thrombosis by stabilizing fibrin and prolonging fibrinolysis²⁹. MASP1 also contributes to thrombus formation *in vivo*³¹. MASPs are inhibited by C1 inhibitor; however, HAE, which is associated with C1 inhibitor deficiency, is not associated with excessive clotting³². MASP1 was also reported to cleave high-molecular-weight kininogen *in vitro*, thereby releasing bradykinin, whereas

MASP2 was shown to cleave high-molecular-weight kininogen but bradykinin is not released³³. Furthermore, MASP1 was recently found to upregulate the kinin B2 receptor³⁴ described later.

The main inhibitor of both the classical pathway and the lectin pathway of the complement system is C1 inhibitor³⁵, which directly inhibits the enzymatic activity of C1r/C1s in the classical pathway and MASP1/MASP2 in the lectin pathway. C1 inhibitor can also inhibit components of the KKS, such as activated FXII (FXIIa), FXI and plasma kallikrein, as well as plasmin, thereby affecting the fibrinolytic system³⁵. Active plasma kallikrein is inhibited by both C1 inhibitor and the large glycoprotein α_2 -macroglobulin³⁶. In the absence of sufficient levels of C1 inhibitor, FXII will autoactivate to form FXIIa and convert plasma prekallikrein (also called ‘Fletcher factor’) to kallikrein and FXI to FXIa³⁷ and also allow uninhibited activation of the classical and lectin pathways of complement, ultimately leading to increased vascular leakage and inflammation.

The KKS/contact system

The plasma KKS and the tissue KKS are independent systems that both release kinins as effector peptides from kininogens through cleavage by two different kallikreins. The plasma KKS is tightly interlinked with the contact system, which is integral to the intrinsic

pathway of coagulation and thrombus formation. Both involve plasma prekallikrein and FXII; the latter is activated upon contact with charged natural or artificial surfaces^{3,38}, followed by rapid reciprocal activation of plasma prekallikrein into plasma kallikrein and FXII into FXIIa (FIG. 3). Plasma kallikrein releases bradykinin from high-molecular-weight kininogen, and FXIIa initiates blood coagulation by activating FXI. FXIIa also activates plasma kallikrein. The tissue KKS is initiated by the serine protease tissue kallikrein, which is activated, probably intracellularly, by an unknown protease, liberating kallidin from low-molecular-weight kininogen. Although there are many tissue kallikreins, only one is involved in kinin generation^{39,40} (Supplementary Table 1).

The only two precursor proteins for kinins in humans, high-molecular-weight kininogen and low-molecular-weight kininogen, are synthesized mainly in the liver (Supplementary Table 1). The mRNAs encoding these proteins are generated by alternative splicing. In the blood, high-molecular-weight kininogen circulates in complex with plasma prekallikrein or FXI of the intrinsic coagulation system. Plasma prekallikrein is inactive in this complex, but when the complex binds to the surface of endothelial cells, plasma prekallikrein can be activated by prolylcarboxypeptidase (PRCP) or heat shock protein 90 (HSP90) to plasma kallikrein^{41,42} (FIG. 3). More important, however, is its activation by FXII,

Table 1 | Vascular and inflammatory effects of the RAS, the complement system and the KKS

Component	Vasotonus	Vascular permeability	Neutrophil chemotaxis	Inflammation	Thrombosis	Fibrinolysis
RAS						
Ang II	↑ ^a , ↓ ^b (REF. ⁴)	↑ ^a (REF. ⁵⁹)	↑ ²¹⁴	↑ ^a , ↓ ^b (REF. ⁴)	↑ ²¹⁵	↓ ^c (REF. ²¹⁶)
Ang III	↑ ^a (REF. ²¹⁷), ↓ ^b (REF. ⁴)	–	–	↑ ^a (REF. ²¹⁷), ↓ ^b (REF. ⁴)	–	–
Ang IV	↓ ⁴ , ↑ ^d	–	–	↑, ↓ ^d (REFS ^{218,219})	↑ ²¹⁵	–
Ang 1–9	↓ ²²⁰	–	–	↓ ²²⁰	–	↓ ²¹⁶
Ang 1–7	↓ ⁹²	–	↓ ⁹²	↓ ⁹²	↓ ¹²⁵	–
Ang A	↑ ^a , ↓ ^{b,e} (REF. ²²¹)	–	–	↑ ^a , ↓ ^{b,e} (REF. ²²¹)	–	–
Alamandine	↓ ²²²	–	–	↓ ¹⁰³	–	–
Complement system						
C3a	↑, ↓ ^f (REFS ^{223,224})	↑ ²²⁴	↑ ⁷¹	↑ ⁹ (REF. ²²⁵)	↑ ^{151,226}	–
C4a	–	↑ ²²⁷	–	– ^h	–	–
C5a	↑, ↓ ^f (REFS ^{223,224})	↑ ^{72,74,224}	↑ ⁷⁵	↑ ^{70,74}	↑ ^{70,73}	–
C5b-9	–	↑ ⁷⁹	–	↑ ²²⁸	↑ ^{137,149}	–
KKS						
Bradykinin	↓ ³	↑ ^{3,229}	↑ ¹³⁴	↑ ²³⁰	↓ ^{3,38}	↑ ²³¹
DesArg ⁹ -bradykinin	↓ ⁵⁴	↑ ⁶⁷	↑ ^{134,232}	↑ ^{230,233}	↓ ²³⁴ ↑ ²³⁵	0 ²³⁶
Kallidin	↓ ⁵⁴	↑ ⁶⁷	↑ ¹³⁴	↑ ²³⁰	0 (REF. ²³⁷) or ↓ ²³⁴	↑ ²³⁴
DesArg ¹⁰ -kallidin	↓ ⁵⁴	↑ ⁶⁷	↑ ¹³⁴	↑ ²³⁰	↓ ²³⁴ , ↑ ²³⁵	0 (REF. ²³⁶)

Ang, angiotensin; KKS, kallikrein–kinin system; RAS, renin–angiotensin system; ↑, effect increased; ↓, effect decreased; 0, no effect; –, data not available. ^aSignalling via Ang II type 1 receptor (AT1R); for Ang II this is the main effect (FIG. 1). ^bSignalling via AT2R (FIG. 1). ^cAng II inhibits fibrinolysis by releasing plasminogen activator inhibitor 1. ^dAng IV has a weaker signal via AT1R than angiotensin 4 receptor (AT4R) (FIG. 1). ^eAng A has a weak vasopressor and inflammatory effect via AT1R. ^fThe vasodilatory effect is secondary to anaphylatoxic effects. ^gThe C3a receptor has also been shown to have anti-inflammatory properties²³⁸. ^hThe inflammatory potential of C4a signalling, if any, is currently unclear²³⁹.

which can also bind to endothelial cells via three different factors: heparan sulfate proteoglycans⁴³, urokinase plasminogen activator receptor (uPAR)⁴⁴ and gC1qR⁴⁵. gC1qR and uPAR are each complexed with cytokeratin 1 (REF.⁴⁶). FXII autoactivates to form FXIIa when it encounters negatively charged surfaces. These can be artificial material such as glass or plastic, or natural factors such as the subendothelial matrix of vessels after injury, or DNA, polyphosphates and heparin released from activated neutrophils, platelets and mast cells, respectively^{47,48}. In addition to activating FXI to FXIa and thereby the intrinsic coagulation system, FXIIa also cleaves plasma prekallikrein into plasma kallikrein as mentioned earlier. This initiates a feedforward loop that activates more FXII (FIG. 3). Since plasma kallikrein releases the nonapeptide bradykinin from high-molecular-weight kininogen, its activation leads to a rapid burst in local bradykinin production, with marked effects on the surrounding tissue. Bradykinin mediates inflammation by inducing vasodilation and fluid extravasation, resulting in redness (rubor), heat (calor) and swelling (tumour), three of the four cardinal signs of inflammation. The fourth sign, pain (dolour), is mediated by kinin receptors on nociceptive nerve endings⁴⁹. In addition, bradykinin induces the release of nitric oxide (NO), prostacyclin and tissue plasminogen activator, and has thrombin-inhibitory effects^{3,38,50} (TABLE 1).

In addition to kallikrein, other proteases, which do not belong to the KKS, have been described as releasing kinin peptides from high-molecular-weight kininogen. These peptides can activate the kinin receptors

B1R and B2R⁵¹ (BOX 1). One example of such a peptide is PR3-kinin, which has two additional amino acids at both ends of the bradykinin sequence and is released by neutrophil-derived proteinase 3 (REF.⁵²).

Kinins have a very short half-life (less than 1 minute) in tissues and blood as they are quickly metabolized by various proteases^{39,53}. Removal of the N-terminal lysine by aminopeptidases (such as aminopeptidase N) transforms kallidin to bradykinin. However, since both kallidin and bradykinin activate B2R, this does not alter signalling⁵⁴. Carboxypeptidase M, on cell surfaces, and carboxypeptidase N (CPN; also called 'kininase I'), in plasma, release the C-terminal arginine from bradykinin and kallidin, generating desArg⁹-bradykinin (DABK) and desArg¹⁰-kallidin (DAKD), respectively, which are both B1R agonists (BOX 1). Various peptidases degrade kinins further. Neprilysin and ACE (also called kininase II) are two prominent peptidases, and both liberate dipeptides from the C terminus of kallidin and bradykinin, thereby abolishing their biological activity³⁹ (TABLE 2). ACE inhibition therefore increases bradykinin levels⁵⁵.

Interactions in inflammation

The interactions between the RAS, the complement system and the KKS/contact system (FIG. 4a; TABLE 2) play an important role in maintaining vasotonus and blood pressure, vasodilation and initiating thrombosis, as well as the removal of microorganisms, or their components, and dying cells. These interactions, when excessive, can lead to disease-related pathology due to increased

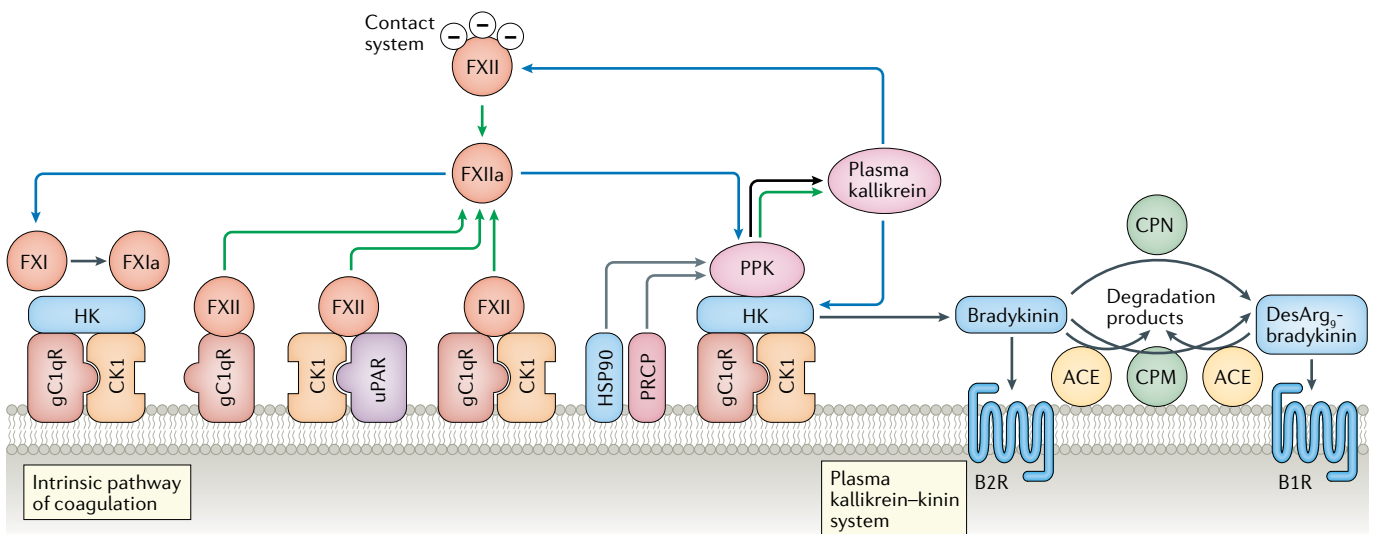


Fig. 3 | Overview of the plasma kallikrein–kinin and contact systems. The plasma kallikrein–kinin and contact systems and their activation on endothelial cells. The zymogen factor XII (FXII) binds to urokinase plasminogen activator receptor (uPAR) in complex with cytokeratin 1 (CK1) or the globular head of the C1q receptor (gC1qR) alone or in complex with CK1 (REF.³⁷) and is autoactivated, or is activated by plasma kallikrein, into the enzymatically active FXIIa. Alternatively, in the contact system, FXII is activated to FXIIa when it encounters negatively charged surfaces. Plasma prekallikrein (PPK) is cleaved into plasma kallikrein, its active form, by soluble or bound FXIIa²⁶⁷. On the surface of endothelial cells, PPK, when bound to high-molecular-weight kininogen (HK), can also be activated to plasma kallikrein by heat shock protein 90 (HSP90) or by prolylcarboxypeptidase (PRCP) (HSP90

and PRCP act as cofactors rather than enzymes). Thus, prekallikrein can be converted to plasma kallikrein both by FXIIa and by autoactivation in the presence of cofactors. Plasma kallikrein cleaves HK to release the nonapeptide bradykinin, and FXIIa activates FXI by cleaving it to form FXIa, thereby initiating the intrinsic coagulation cascade³. Bradykinin exerts inflammatory effects via the B2 receptor (B2R). Bradykinin is degraded to desArg⁹-bradykinin by carboxypeptidase M (CPM) on the cell surface or by carboxypeptidase N (CPN) in the fluid phase. DesArg⁹-bradykinin binds preferentially to the B1 receptor (B1R). Both kinins are further degraded by angiotensin-converting enzyme (ACE). Green arrows show that autoactivation of a protein into its activated form can occur, blue arrows indicate enzymatic activity, black arrows point to cleavage products and grey arrows indicate cofactors.

Chagas disease

A tropical infection caused by the intracellular protozoan *Trypanosoma cruzi*.

vasopermeability, vasodilation, leukocyte chemotaxis, inflammation, thrombosis or fibrinolysis (TABLE 1).

Vascular tone, inflammation and permeability. Kinin activation in the vasculature induces inflammation, neutrophil chemotaxis, vasodilation and generally an increase in vasopermeability (TABLE 1). The KKS regulates vascular tone but has different effects depending on the affected cell type. In endothelial cells, kinins increase intracellular Ca^{2+} levels, leading to vasodilation via the activation of endothelial NO synthase, the production of NO and the generation of prostacyclin⁵⁶. In smooth muscle cells, NO activates guanylate cyclase and thereby increases the levels of the nucleotide cGMP, and prostacyclin upregulates the nucleotide cAMP, both inducing smooth muscle relaxation. Moreover, kinins induce vasodilation via the release of endothelial-derived hyperpolarizing factor⁵⁷. By contrast, the increase in intracellular Ca^{2+} levels induced by kinins can also lead to the constriction of smooth muscle cells via Ca^{2+} /calmodulin-induced myosin light chain phosphorylation. Therefore, kinins can both contract or dilate vessels depending on the strength of signalling of kinin receptors, their level of expression on endothelial and smooth muscle cells and the amount of peptides that reach these cell types. In most arteries, kinins cause endothelium-dependent relaxation, resulting in a decrease in blood pressure when bradykinin is injected intravenously. However, kinins can also induce endothelium-dependent contractions in veins⁵⁸.

Ang II increases vasopermeability and vasotonus via AT1R⁵⁹ but may also decrease vasotonus via AT2R (FIG. 4b; TABLE 1). Ang II induces the expression of vascular endothelial growth factor (VEGF) in human vascular smooth muscle cells⁶⁰. Ang 1–7 and bradykinin induce a synergistic vasodilatory effect that is abolished by either of their respective antagonists⁶¹ and involves AT2R signalling⁶², which stimulates bradykinin production⁶³. This vasodilatory effect is reduced in kininogen-deficient rats, demonstrating the interaction between the KKS and the RAS⁶⁴. The combined effect of RAS signalling and bradykinin release affects natriuresis and blood pressure. In renal proximal tubules, AT2R activation by its agonist, compound 21, induces natriuresis and lowers blood pressure. In Ang II-mediated hypertension associated with stimulation of AT1R via the canonical pathway of the RAS, AT2R counter-regulates blood pressure elevation by increasing renal production of bradykinin and promoting renal NO release⁶⁵. The natriuretic effect involves the bradykinin–NO–cGMP pathway; this can be blocked by the small-molecule B2R antagonist icatibant⁶⁶.

Kinins mediate a drastic increase in vascular permeability (FIG. 4b). The increase in cytosolic levels of Ca^{2+} results in a shortening of contractile proteins within the endothelial cells, the destabilization of adherens junctions, the formation of fenestrations in the walls of microvascular tissue and the extravasation of plasma constituents and leukocytes⁵⁷. Similar effects are mediated by the complement system components C3 and C5 (TABLE 1). C3 and C5 cleavage generates the anaphylatoxins C3a (77 amino acids) and C5a (74 amino

acids, with a C-terminal receptor-binding octapeptide), respectively. These bind to GPCRs on the endothelium and trigger intracellular activation pathways such as mitogen-activated protein kinase ERK1/2 signalling^{68,69}, cytokine release^{68,70} and recruitment of leukocytes⁷¹, thereby inducing inflammation. C5a induces vascular permeability (FIG. 4b) through endothelial cell retraction⁷², tissue factor expression, and basophil and mast cell degranulation^{73,74}. C5a binds to two GPCRs, C5aR1 (also known as CD88) and C5aR2 (also known as C5L2 or GPR77). Binding to C5aR1 induces the aforementioned pro-inflammatory response, whereas signalling through C5aR2, which also binds desArg-C5a, has an opposing effect on C5a–C5aR1 activity⁶⁹ but also plays a role in vascular inflammation. C5aR2 was recently shown to transport tissue-derived C5a into the vascular lumen and thereby induce neutrophil recruitment and adhesion⁷⁵. Overall, excess complement activation on the vascular endothelium promotes inflammation by inducing leukocyte recruitment and extravasation. The enhanced vascular permeability can lead to oedema. These mechanisms are crucial in the development of sepsis⁷⁶.

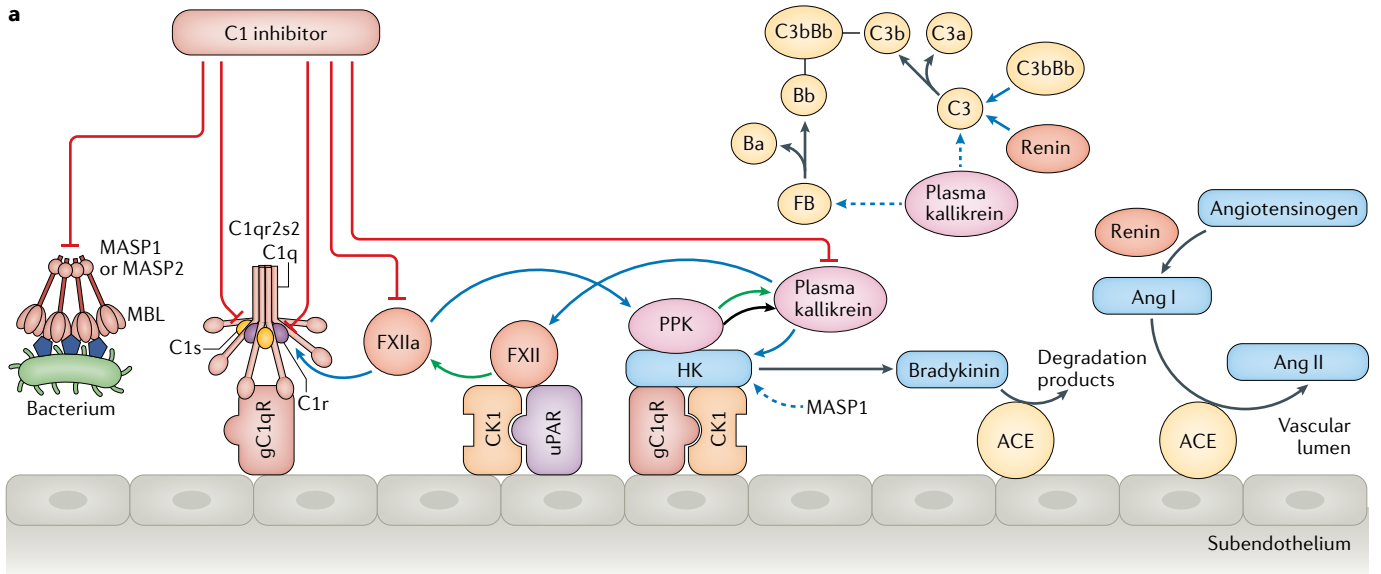
In a hamster model of Chagas disease, plasma leakage occurs in the cheek pouch. Targeting C5aR1 and B2R reduced vascular leakage⁷⁷, suggesting that both C5a and bradykinin contribute to vascular permeability. Patients with severe capillary leakage after bone marrow transplantation improved in response to treatment with C1 inhibitor and exhibited reduced plasma levels of C5a, suggesting it may contribute to capillary leakage⁷⁸. C5b-9 can also increase vascular permeability and leakage⁷⁹ as demonstrated in human umbilical vein endothelial cells and rat mesenteric endothelium. C5b-9 induced the accumulation of kinins in the culture medium, suggesting that kinins mediated the response, and indeed a B2R antagonist or kallikrein inhibitor partially blocked C5b-9-induced vascular leakage⁷⁹. This indicates an interaction between the terminal complement pathway and KKS activation in mediating vascular permeability.

Hereditary angioedema. Dysfunctional or deficient C1 inhibitor or dysfunctional FXII can result in angioedema. HAE is associated with mutations in the C1 inhibitor gene. In type 1 HAE, the mutation leads to lower levels of C1 inhibitor, whereas in type 2 HAE, the levels of C1 inhibitor may be normal, but the protein is dysfunctional. Both types are autosomal dominant. In patients with normal C1 inhibitor function, mutations have been found in genes encoding FXII, plasminogen, angiopoietin 1, myoferlin and kininogen^{80,81}. Acquired C1 inhibitor dysfunction or deficiency is due to the presence of circulating autoantibodies³⁷.

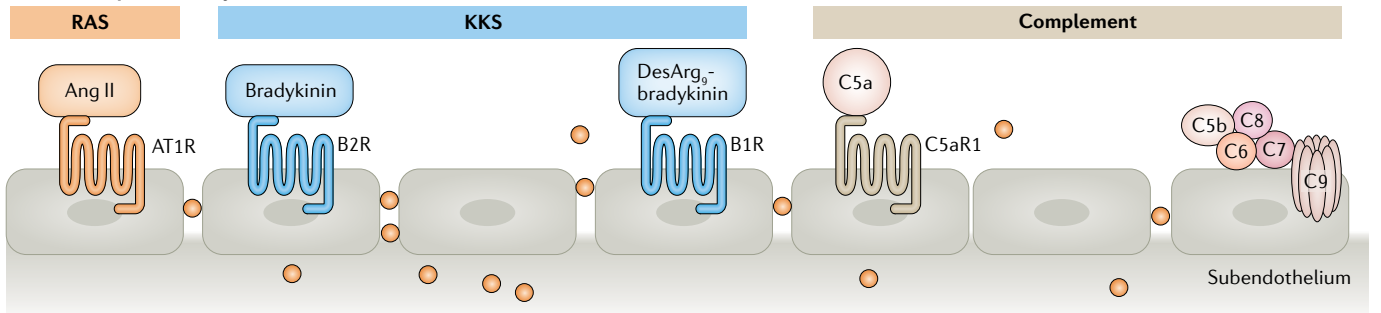
HAE is characterized by the activation of the plasma KKS/contact system due to C1 inhibitor dysfunction, leading to the release of bradykinin⁸². Bradykinin then binds to B2R, which increases vascular permeability and thereby causes local extravasation of plasma and tissue swelling⁸³. The swelling may be local or generalized and can be life-threatening when airways are affected. Patients with C1 inhibitor dysfunction have high levels of circulating bradykinin⁸⁴. In addition to the plasma KKS/contact system, the classical pathway

of the complement system is activated in these patients, who generally have low levels of C4 and high levels of soluble C5b-9 (REF.³²). However, complement activation is not the primary mediator of the swelling⁸⁵. The fibrinolytic pathway is also activated and increased levels

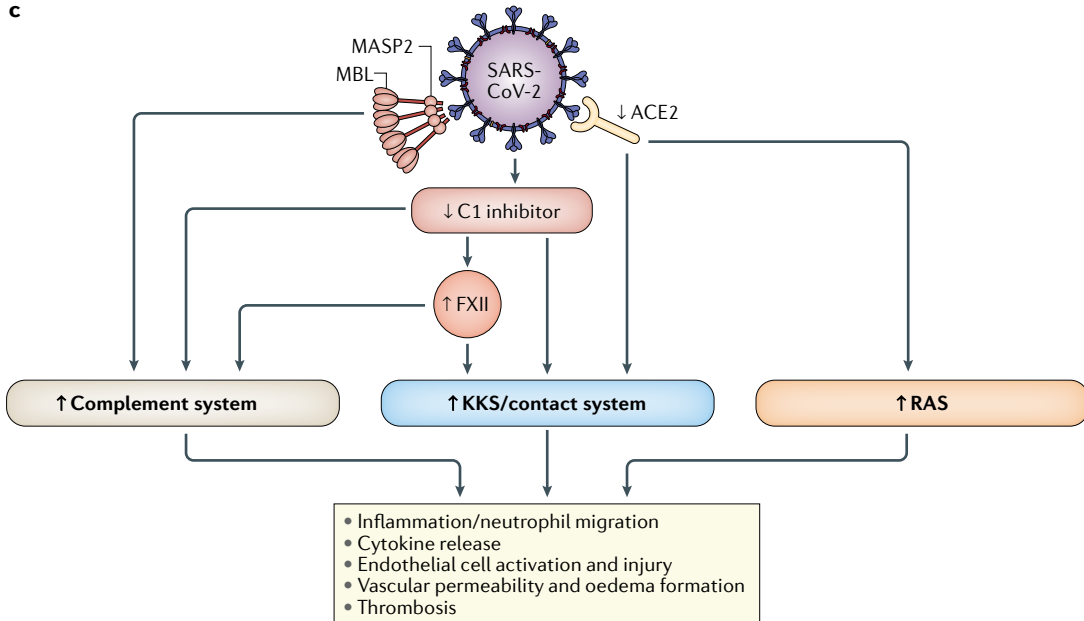
of plasmin- α 2-antiplasmin complexes are observed³². Overall, C1 inhibitor dysfunction leads to excess KKS activation and bradykinin-mediated oedema as well as complement activation with release of the anaphylatoxins C3a and C5a³⁷.



b Vascular permeability



c



HAE can be treated with purified C1 inhibitor, with the B2R antagonist icatibant or with kallikrein inhibitors such as the small molecule ecallantide⁸⁶, the monoclonal antibody ranadelumab⁸⁷ or the oral inhibitor berotralstat⁸⁸.

Cardiovascular injury and protection. The RAS, the complement system and the KKS interact in the induction of cardiovascular injury and hypertension as well as in cardioprotection. The pro-inflammatory and profibrotic cellular effects of the canonical RAS pathway can contribute to the development of vascular inflammation, resulting in hypertension, atherosclerosis, cardiovascular disease and renal disease, whereas the non-canonical RAS pathway generally has a protective role. Ang II plays a central role in the inflammatory response by signalling via AT1R and exerting a pro-inflammatory effect on endothelial cells, vascular smooth-muscle cells and leukocytes⁸⁹. This includes promoting the release of chemokines and cytokines, the expression of cellular adhesion molecules, the induction of dendritic cell

migration and endothelial cell proliferation, as well as promoting monocyte adhesion to endothelial cells^{90,91}.

The activation of the non-canonical pathway of the RAS leads to the generation of anti-inflammatory peptides with cardioprotective effects, which can reduce the size of myocardial infarcts, improve cardiac function, decrease arrhythmias, prevent the development of hypertrophy and modulate the release of pro-inflammatory cytokines⁹². Ang 1–9 was shown to reduce cardiac inflammation in a rat model⁹³. Additionally, Ang 1–7 can inhibit ACE and induce vascular relaxation⁹⁴. Ang 1–9 and Ang 1–7 (REF.⁹⁵) can potentiate the effects of bradykinin on B2R, and all three peptides are degraded by ACE⁹⁶ (TABLE 2). The RAS peptides do not engage the B2R itself but increase the release of arachidonic acid and NO that is induced by bradykinin⁹⁷. Importantly, neprilysin activity and bradykinin levels are elevated in infarcted heart tissue⁹⁸.

The activation of the bradykinin–NO–cGMP pathway through AT2R has a cardioprotective effect⁹⁹. In a porcine model of myocardial ischaemia, an AT1R blocker reduced myocardial infarct size, and this cardioprotective effect was abrogated by an AT2R antagonist or by the B2R antagonist icatibant¹⁰⁰. Similar findings were demonstrated in B2R-deficient mice, where the cardioprotective effect of ACE inhibition or AT1R blockade was diminished relative to that in wild-type mice¹⁰¹, suggesting that B2R has an important cardioprotective role. In *Agtr2*^{-/-} mice (*Agtr2* encodes AT2R), AT1R expression is upregulated¹⁰² and an imbalance between AT1R and AT2R expression favours enhanced inflammatory responses.

The effectors in the non-canonical RAS pathway, including the angiotensin family member alamandine¹⁰³, have anti-inflammatory and antiproliferative effects¹⁰⁴. Several therapeutics for heart failure, left ventricular hypertrophy, hypertension, proteinuria, renal fibrosis and inflammation^{99,105} target the canonical RAS pathway, thereby diverting peptide ligands to the non-canonical pathway and providing agonists for the stimulation of AT2R and MAS (BOX 2, Supplementary Box 1). These therapeutics include renin and ACE inhibitors, AT1R antagonists and neprilysin inhibitors in combination with AT1R blockers, termed ‘ARN inhibitors’¹⁰⁶. Furthermore, a number of investigational therapeutics are currently in preclinical and clinical development; these include aminopeptidase A inhibitors, MAS agonists, NPA7 (a peptide combining particulate guanylyl cyclase A activation with MAS agonism), the AT2R agonist compound 21, recombinant ACE2 (REF.¹⁰⁷), Ang 1–9 (REF.⁹³), angiotensin 4 receptor (AT4R) inhibitors and angiotensinogen antisense oligonucleotides^{104,108}.

During cardiovascular inflammation and RAS activation, the complement system also becomes activated. For example, mice infused with Ang II exhibit an increase in plasma levels of C3a and C5a and have perivascular deposition of C3b in cardiac tissue. C5aR-deficient mice as well as mice treated with anti-C5 or anti-C5aR antibodies were shown to be protected from cardiac inflammation induced by Ang II (REF.¹⁰⁹). Moreover, C3aR-deficient and C5aR-deficient mice had lower blood pressure than wild-type mice and were protected from renal and

Fig. 4 | Interactions between the renin–angiotensin, complement and kallikrein–kinin systems on the vascular wall. **a** | Joint pathways of the renin–angiotensin system (RAS), complement system and kallikrein–kinin system (KKS) on the endothelium. Certain components of the complement system and the KKS bind to the same surface receptor, the globular head of C1q receptor (gC1qR). It can bind C1q or, in complex with cytokeratin 1 (CK1), it binds high-molecular-weight kininogen (HK) and plasma prekallikrein (PPK). C1 inhibitor is a common inhibitor of plasma kallikrein, activated factor XII (FXIIa), mannose-binding lectin (MBL) serine proteases (MASPs), C1s and C1r. Plasma kallikrein cleaves HK, releasing bradykinin. In vitro it was also shown to cleave complement factor B (FB) and C3; however, this has not been confirmed in vivo. Renin cleaves both angiotensinogen into angiotensin I (Ang I) and C3 into C3a and C3b allowing formation of more C3bBb. Angiotensin-converting enzyme (ACE) degrades bradykinin to metabolites and also converts Ang I to Ang II. Red lines depict inhibition, green arrows indicate that autoactivation of a protein can occur, blue arrows indicate enzymatic activity (dashed if shown only in vitro) and black arrows point to cleavage products. **b** | Mechanisms of vascular permeability and leakage induced by the RAS, the KKS and the complement system. Vascular permeability via the RAS is induced by Ang II signalling via Ang II type 1 receptor (AT1R). In the KKS, vasopermeability is associated with bradykinin and desArg⁹-bradykinin signalling via the receptors B2R and B1R, respectively, and in the complement system with C5a signalling via the C5a receptor (C5aR1) and deposition of C5b-9 within the cell membrane. **c** | Mechanisms of endothelial cell injury, thrombosis, vasopermeability/oedema and inflammation induced by the RAS, the KKS/contact system and the complement system during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. After binding to pulmonary epithelial cells via surface-expressed ACE2, SARS-CoV-2 undergoes endocytosis, thereby reducing the amount of ACE2 on cell surfaces. This creates an imbalance in the RAS with increased activation of the canonical pathway. ACE2 inactivates desArg⁹-bradykinin¹⁷⁰. Decreased levels of ACE2 therefore increase desArg⁹-bradykinin levels and signalling via B1R in the KKS, leading to inflammation¹⁷¹. SARS-CoV-2 interacts with C1 inhibitor¹⁸⁴, the level of which was found to be reduced in bronchoalveolar lavage fluid from patients with COVID-19 (REF.¹⁷⁶). Downregulation of C1 inhibitor will allow activation of the classical and lectin pathways of the complement system, and the nucleocapsid proteins of SARS-CoV and SARS-CoV-2 were shown to bind to MASP2 and initiate the lectin pathway of the complement system¹⁹⁵. The KKS/contact system is also activated because C1 inhibitor inhibits activated factor XII (FXIIa). Furthermore, activated FXII cleaves C1r²⁵⁴, activating the classical pathway of the complement system. Endothelial cells become injured through excess activation of the three pathways. Endothelial cell activation leads to increased vascular permeability (as shown in part **b**), resulting in pulmonary oedema. Ang II, C3a and C5a as well as the kinin effectors bradykinin and desArg⁹-bradykinin promote neutrophil recruitment and inflammation. Neutrophil proteases can further activate the KKS by cleavage of HK. As listed in TABLE 1, effectors of the KKS and FXII, effectors of the RAS canonical pathway and of the complement system induce thrombosis. uPAR, urokinase plasminogen activator receptor.

Box 1 | The kinin B1 and B2 receptors

The G-protein-coupled receptors B1R and B2R are the only known receptors for kinins^{54,268}. Since B1R is expressed only at very low levels under normal conditions, most physiological effects of kinins are dependent on the presence of B2R. B2R is constitutively expressed on the cell surface, but after stimulation by its ligands, kallidin or bradykinin, it undergoes internalization and sequestration by endocytosis²⁶⁸. In addition to its role in the kallikrein-kinin system, bradykinin signalling via B2R induces renin expression²⁶⁹. Moreover, angiotensin II type 2 receptor (AT2R) and B2R can form heterodimers²⁷⁰, leading to an increase in ligand-induced nitric oxide release²⁷¹. Similarly, B2R and MAS²⁷² and B2R and AT1R²⁷³ can form heterodimers.

B1R is less responsive to bradykinin and kallidin than to their carboxypeptidase metabolites, desArg⁹-bradykinin (DABK) and desArg¹⁰-kallidin (DAKD)^{54,274}. In humans, unlike in mice, DAKD is several orders of magnitude more active than DABK, and even kallidin has considerable affinity for B1R^{54,275}. B1R is quickly upregulated after tissue injury or inflammatory insults²⁶⁸.

Inflammatory mediators such as IL-1 β and TNF can induce the expression of B1R by inducing the activation of transcription factors such as NF- κ B, AP-1 and CREB, which bind to the B1R promoter²⁷⁶. In contrast to most other G-protein-coupled receptors, B1R is not internalized and desensitized following ligand binding, and therefore exerts prolonged signal transduction²⁶⁸. B1R signalling can promote chronic inflammation since cytokines released from infiltrating leukocytes in affected tissues can induce B1R expression. Kinin-induced activation of B1R on endothelial cells and neutrophils plays an important role in neutrophil accumulation and activation in inflammatory conditions^{133,134,233,277}.

vascular damage caused by Ang II infusion¹¹⁰. This was found to be partly due to the presence of C3aR and C5aR on regulatory T cells¹¹⁰, but the precise mechanisms by which protection occurs remain to be elucidated. Thus, the blockade of complement receptors might attenuate Ang II-mediated target organ damage.

Complement-associated glomerular inflammation.

Activation of the alternative pathway of the complement system can be initiated by renin, an enzyme secreted solely in the kidney. In *in vitro* experiments, renin was shown to cleave both angiotensinogen and C3. Renin cleavage of C3 into C3a and C3b¹¹¹ enables C3b to bind to factor B, which can then be cleaved into Ba and Bb by factor D¹¹². This results in the formation of the C3bBb complex, which is the C3 convertase (FIG. 4a). Such renin-mediated C3 cleavage also occurs in the presence of serum¹¹¹. Renin levels are higher in the kidney than in the circulation¹¹³. In patients with the complement-mediated kidney disease C3 glomerulopathy, the direct renin inhibitor aliskiren decreased complement activation systemically (as measured by higher levels of circulating C3 as well as decreased C3a and C5a levels) and in renal tissue (as evidenced by reduced C3 and C5b-9 deposition in glomeruli and reduced glomerular basement membrane thickness). Furthermore, patients treated with aliskiren exhibited stabilized or improved renal function and reduced proteinuria over a long follow-up period (BOX 2). The effects of aliskiren could not be evaluated in a mouse model as murine renin does not cleave C3 (REF.¹¹¹). However, a study using aliskiren in double transgenic rats that express human renin and angiotensinogen genes showed that aliskiren reduced both C3 deposition and C5b-9 deposition in renal sections¹¹⁴.

Renin-mediated C3 cleavage is sufficient to trigger activation of the complement system via the alternative pathway (FIG. 4a), but further activation of this pathway and amplification would, under physiological

conditions, be inhibited by functional complement regulators in the fluid phase and on cell membranes. Furthermore, as renin has more than one substrate, it is plausible that the presence of angiotensinogen may limit the degree of C3 cleavage. In complement-mediated renal diseases such as C3 glomerulopathy, complement regulators have been found to be dysfunctional due to mutations, or the C3 convertase may be stabilized by the circulating autoantibody C3 nephritic factor¹¹⁵. This will allow uninhibited complement activation once renin-mediated C3 cleavage is triggered.

Thromboinflammation. Thromboinflammation is a condition that arises from an interaction between thrombosis and inflammation involving the endothelium, leukocytes and platelets as well as the complement system, the KKS/contact system, the coagulation system and the fibrinolytic system^{116,117}. Thromboinflammation is a serious complication of severe infection, deep vein thrombosis, stroke and other inflammatory disorders^{116,118}. FXIIa can promote inflammation by activating plasma kallikrein and inducing the KKS/contact system. The zymogen FXII is also involved in neutrophil migration as demonstrated in a mouse model of wound healing¹¹⁹ and, via intracellular AKT2 signalling, in neutrophil extracellular trap formation¹²⁰. Furthermore, FXII can autoactivate to form FXIIa on DNA in neutrophil extracellular traps¹²¹. FXIIa is also involved in thrombosis by its role in activating the intrinsic pathway of coagulation (FIG. 3). Accordingly, mice that lack FXII are protected from collagen-adrenaline-induced or polyphosphate-induced thromboembolism and lethality^{48,122,123}. Both FXII-deficient and plasma prekallikrein-deficient mice show reduced thrombosis after endothelial injury^{123,124}.

Plasma prekallikrein-deficient mice exhibit lower levels of bradykinin, as less is generated from high-molecular-weight kininogen, as well as lower expression of B2R. These mice overexpress MAS to compensate for the reduced levels of B2R. The interaction of Ang 1-7 with MAS increases plasma levels of prostacyclin¹²⁵, thereby increasing the expression of the transcription factors SIRT1 and KLF4 and reducing the expression of tissue factor, which lowers the risk of thrombosis¹²³. B2R-deficient mice also show partial protection in models of endothelial injury-induced thrombosis due to AT2R and MAS overexpression¹²⁵.

Even animals that lack high-molecular-weight kininogen are partially protected from thrombosis induced by endothelial cell injury^{124,126}. This implies that for the initial induction of thrombus formation at subendothelial surfaces, complexes of high-molecular-weight kininogen with plasma prekallikrein or with FXI are important^{127,128}.

In mouse models of stroke and other brain injuries, B1R deficiency or inhibition of B1R was shown to be beneficial^{129,130}. Plasma kallikrein and FXIIa have thus become targets for antithrombotic treatment strategies without the risk of bleeding episodes¹²¹. Accordingly, the reduction or inhibition of plasma kallikrein or FXIIa by small molecules, peptides, antibodies, small interfering RNAs or antisense oligonucleotides has been shown to be protective in animal models of thrombosis and is

currently being tested in clinical trials^{131,132}. In addition to their antithrombotic action, such drugs would also have anti-inflammatory effects by inhibiting kinin generation. This may be particularly important in diseases

characterized by thromboinflammation, a combination of neutrophil migration and activation that includes the release of neutrophil extracellular traps containing DNA, followed by platelet activation. Neutrophil migration can

Table 2 | Enzymatic interactions between the RAS, the complement system and the KKS

Enzyme	RAS		Complement system		KKS	
	Forms	Cleaves	Forms	Cleaves	Forms	Cleaves
Renin	Ang I (REF. ⁴)	–	C3b, C3a ¹¹¹	–	–	–
ACE	Ang I (REF. ²⁴⁰)	Ang I (REF. ⁴)	–	–	–	BK ^{39,53}
	Ang II (REF. ⁴)	Ang 1–9 (REF. ⁹²)	–	–	–	KD ⁵³
	Ang 1–7 (REF. ⁹²)	Ang 1–7 (REF. ⁹²)	–	–	–	DABK ⁵³
ACE2	Ang 1–9 (REF. ¹)	Ang I (REF. ¹)	–	–	–	DAKD ⁵³
	Ang 1–7 (REF. ¹)	Ang II (REF. ¹)	–	–	–	DABK ^{170,171}
	Alamandine ¹	Ang A ¹	–	–	–	DAKD ¹⁷⁰
Chymase	Ang II (REF. ¹)	Ang I (REF. ⁴)	–	–	–	BK ²⁴¹
		Ang 1–12 (REF. ²⁴²)	–	–	–	KD ²⁴¹
NEP	Ang 1–7 (REF. ²⁴³)	Ang I (REF. ¹⁸⁶)	–	–	–	BK, KD ²⁴⁴
		Ang 1–9 (REF. ⁹²)	–	–	–	DABK ²³⁰
THOP	Ang 1–7 (REF. ⁹²)	Ang I (REF. ⁹²)	–	–	–	DAKD ²⁴⁵
		Ang II (REF. ²⁴⁷)	–	–	–	BK ²⁴⁶
PRCP ^a	Ang 1–7 (REF. ²⁴⁷)	Ang II (REF. ²⁴⁷)	–	–	–	DABK ²⁴⁸
KLK	Ang II (REFS ^{249,250})	–	C3a ²⁵¹	–	BK	HK ⁵³
		–	–	–	KD ⁵³	–
PKa ^b	–	–	C3b, C3a ^{252,253}	–	BK ³	HK ³
FXIIa ^c	–	–	–	C1r ²⁵⁴	–	–
APN	Ang IV (REF. ⁹²)	Ang III (REF. ⁹²)	–	–	BK ⁵³	KD
		–	–	–	–	DAKD ⁵³
APP	–	–	–	–	–	BK
		–	–	–	–	DABK ¹⁰⁴
APA	Ang III (REF. ¹⁰⁴)	Ang II (REF. ¹⁰⁴)	–	–	–	–
PEP	Ang 1–7 (REF. ⁹²)	Ang I (REF. ⁹²)	–	–	–	–
		Ang II	–	–	–	–
		Ang 1–9 (REF. ⁹²)	–	–	–	–
AD	Ang A ⁹²	Ang II (REF. ⁹²)	–	–	–	–
	Alamandine ⁹²	Ang 1–7 (REF. ⁹²)	–	–	–	–
CPM ^d	–	–	–	–	DABK	BK ²⁵⁵
		–	–	–	DAKD ⁵³	KD ⁵³
CPN ^d	–	–	–	C3a	DABK	BK ²⁵⁶
		–	–	C5a ²⁵⁷	DAKD ⁵³	KD ⁵³
TAFIa ^d	–	–	–	C3a ^{256,258}	DABK ²⁵⁸	BK ^{256,258}
		–	–	–	C5a ²⁵⁸	–
MASP1	–	–	–	–	BK ³³	HK ³³

AD, aspartate decarboxylase; Ang, angiotensin; ACE, angiotensin-converting enzyme; APA, aminopeptidase A; APN, aminopeptidase N; APP, aminopeptidase P; BK, bradykinin; CPM, carboxypeptidase M; CPN, carboxypeptidase N; DABK, desArg⁹-bradykinin; DAKD, desArg¹⁰-kallidin; FXIIa, activated factor XII; HK, high-molecular-weight kininogen; KD, kallidin; KKS, kallikrein-kinin system; KLK, tissue kallikrein; MASP1, mannan-binding lectin serine protease 1; NEP, neprilysin; PEP, prolylendopeptidase; PKa, plasma kallikrein; PRCP, prolylcarboxypeptidase; RAS, renin-angiotensin system; TAFIa, activated thrombin-activatable fibrinolysis inhibitor (also known as carboxypeptidase U or carboxypeptidase B2); THOP, thimet oligopeptidase. ^aPRCP activates plasma prekallikrein to plasma kallikrein and also degrades Ang II to Ang 1–7. ^bIn addition to cleaving high-molecular-weight kininogen, releasing bradykinin domain 4, plasma kallikrein also cleaves C3 into C3b, and possibly even factor B into Bb²⁵³, thereby activating the alternative pathway of the complement system to form the C3 convertase. ^cFXIIa converts plasma prekallikrein to plasma kallikrein and thereby releases bradykinin in the fluid phase³⁷, while it can also initiate complement activation by cleavage of C1r. ^dC3a and C5a are degraded by CPN and by TAFIa. TAFIa and CPN remove the carboxy-terminal arginine to form the desArg derivative of C3a and C5a. Both TAFIa and CPN cleave the Arg residue off bradykinin while generating the B1 receptor agonist desArg⁹-bradykinin. Similarly, CPM can digest carboxy-terminal Arg residues of kinins.

Box 2 | Pharmacological interactions between the RAS, the complement system and the KKS

A number of pharmacological antagonists, inhibitors or agonists, listed in Supplementary Box 1, have been developed to modulate the renin–angiotensin system (RAS), the complement system and the plasma kallikrein–kinin system (KKS)/contact system. Due to the multiple interactive effectors and signalling pathways, as discussed in the main text and summarized in TABLE 2, an agonist or antagonist affecting components of one pathway may also affect other pathways.

ACE, neprilysin inhibitors and AT1R blockers

Angiotensin-converting enzyme (ACE) inhibitors, which are among the most prescribed drugs in cardiovascular diseases, prevent bradykinin degradation and sensitize the receptor B2R, with cardioprotective and renoprotective effects^{278–280}. By increasing the levels of bradykinin, ACE inhibitors can cause effects similar to those observed in individuals with C1 inhibitor deficiency leading to angioedema²⁸¹. Angiotensin II type 1 receptor (AT1R) blockers can also increase bradykinin levels⁹⁸. Combined inhibitors of neprilysin and ACE, the two kinin-degrading enzymes, such as omapatrilat, were removed from the market because they caused an unacceptably high incidence of angioedema²⁸². The more recently approved ARN LCZ696 (Entresto), a combination of the neprilysin inhibitor sacubitril and the AT1R blocker valsartan, appears to be safer in this respect²⁸¹.

ACE inhibitors and AT1R blockers are widely used in patients with chronic renal diseases to reduce proteinuria and improve renal outcome²⁸³. In diseases with vascular inflammation, such as vasculitis, the KKS is activated and contributes to pathogenesis^{158,160}. Thus, treatment with ACE inhibitors, which increase bradykinin levels, could potentially have adverse effects. This raises the question whether these substances are suitable for patients with inflammatory kidney diseases that are driven by kinin activity. However, the renoprotective effects may outweigh the undesirable inflammatory consequences, and these aspects remain to be elucidated. B1R antagonists are not yet commercially available (Supplementary Box 1) but may play a role in the treatment of renal inflammation and could possibly be used instead of AT1R antagonists in patients who do not tolerate them well.

C1 inhibitors

C1 inhibitors block the classical and lectin pathways of the complement system as well as the plasma KKS/contact system and are used for the treatment of patients with hereditary angioedema. In vitro studies showed that C1 inhibitor reduced the release of B1R-positive microvesicles from endothelial cells that potentiate neutrophil migration¹³³, and it could therefore be explored as a treatment for neutrophil-mediated inflammation.

Renin inhibitors

ACE inhibition or AT1R blockade increases renin levels¹⁷ by eliminating angiotensin II-mediated feedback on renin. RAS blockade in the absence of renin inhibition could therefore potentially be detrimental in complement-mediated diseases by increasing renin levels because renin can cleave and activate C3 (REFS^{111,284}). This is currently being addressed in a crossover clinical trial comparing the renin inhibitor aliskiren with the ACE inhibitor enalapril in patients with C3 glomerulopathy²⁸⁵.

be enhanced by kinins^{133,134}, and platelet activation by extracellular DNA is FXII dependent¹³⁵. Thus, FXIIa or plasma kallikrein inhibition can reduce both neutrophil migration and platelet activation and thereby potentially ameliorate thromboinflammation^{118,120,136}.

The complement system also plays an important role in thromboinflammation¹¹⁷. Complement activation on the endothelium leads to cellular activation and induces the expression of adhesion molecules, the release of cytokines and chemokines, a decrease in NO production, an increase in shedding of complement-coated endothelial microvesicles¹³⁷ and the assembly of C5b-9, ultimately leading to cell lysis¹³⁸. Tissue factor activity on the endothelium will lead to a procoagulant phenotype and is dependent on the generation of C5b-9 (REF.¹³⁹). Moreover, C5b-9 deposition on the endothelium promotes the assembly of the prothrombinase complex and thrombin generation¹⁴⁰. Thrombin can cleave both C3 and C5 and perpetuate complement activation^{141,142}.

In addition, complement activation on the endothelium triggers the release of von Willebrand factor (VWF)¹⁴³ onto the vessel wall, which then promotes platelet adhesion, and VWF multimers form strings on the endothelium that serve as a platform for the deposition of more C3 via the alternative complement pathway¹⁴⁴. Accordingly, high plasma levels of C3 have been associated with a risk of venous thromboembolism¹⁴⁵.

The complement components C3 and C4 can bind to platelets¹⁴⁶, C3b can bind to P-selectin and propagate complement activation¹⁴⁷ and C5b-9 assembly can activate platelets¹⁴⁸ and promote thrombin-mediated platelet aggregation¹⁴⁹. C5b-9 deposition on the platelet membrane induces the release of platelet-derived microvesicles and results in a prothrombotic and procoagulant platelet surface¹³⁷. Platelet microvesicles promote coagulation as they express both phosphatidylserine and tissue factor¹⁵⁰. C3a and C5a are generated in the process of complement activation on platelets²⁴, and C3a will further promote platelet activation¹⁵¹. C5a induces the release of thromboxane 4, an eicosanoid that is a potent platelet aggregator, from neutrophils⁷⁰. Both C5a and C5b-9 contribute to platelet and neutrophil activation during extracorporeal circulation¹⁵². In turn, platelet activation in itself can induce complement activation on the cell surface both via the classical pathway and via the alternative pathway^{24,147}. Importantly, uninhibited complement activation on the endothelium¹⁵³ or on platelets¹⁵⁴ will promote thrombosis and microangiopathy.

Vasculitis. Vasculitides are disorders characterized by severe inflammation of the vasculature with multi-organ damage affecting the kidneys, intestines, respiratory tract, skin, joints and other organs. Tissue injury involves endothelial cell damage and neutrophil-mediated inflammation¹⁵⁵. The KKS and the complement system can both be activated during vasculitis. In patients with anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and other vasculitides, plasma kallikrein activity and levels of kinins are increased, whereas plasma high-molecular-weight kininogen is degraded^{52,156–158}. Neutrophil-derived proteases such as proteinase 3 can generate vasoactive kinin peptides that bind to the B1R⁵². Blocking B1R ameliorates vascular inflammation in animal models of glomerulonephritis and vasculitis^{159,160}. B1R was detected on leukocyte-derived and endothelial cell-derived microvesicles in the circulation of patients with vasculitis^{133,161}. The microvesicles were capable of transferring the receptor to recipient cells to induce KKS activation and, importantly, B1R on the vesicles served as a neutrophil chemoattractant, which could, thereby, promote inflammation. C1 inhibitor reduced the release of B1R-positive endothelial microvesicles in vitro¹³³.

In patients with AAV, complement activation occurs primarily via the alternative pathway¹⁶². Circulating complement-coated endothelial microvesicles were detected during the acute phase of vasculitis¹⁶³. Blocking B1R and B2R inhibited complement activation on microvesicles in an in vitro perfusion model. In a mouse model of nephrotoxic serum-induced glomerulonephritis, which mimics vasculitis, a B1R

Extracorporeal circulation
Temporary circulation and oxygenation of blood through an instrument that takes over bodily functions. For example, a cardiopulmonary bypass is a form of extracorporeal circulation.

Microangiopathy
A pathological lesion affecting small vessels, primarily capillaries, characterized by endothelial cell injury, detachment of endothelial cells, accumulation of amorphous material in the subendothelial space, platelet aggregation, vascular luminal narrowing and partial or complete vessel occlusion.

Endotheliitis

Generalized inflammation and damage to the endothelium with leukocyte infiltration into the perivascular space.

antagonist reduced glomerular complement deposition, a finding that could have potential therapeutic implications because complement activation on the endothelium is an important feature of vasculitides¹⁶³. Sera from patients with AAV have also been shown to induce the shedding of tissue factor-positive microvesicles from neutrophils in vitro. Similarly, in vitro experiments showed that C5a-primed neutrophils treated with ANCA release tissue factor-positive microvesicles, an effect that is abrogated by treatment with a C5aR antagonist¹⁶⁴. This suggests that complement activation contributes to excess coagulability in AAV. In patients with vasculitis, the small-molecule C5aR antagonist avacopan was shown to sustain remission¹⁶⁵.

SARS-CoV-2 infection and inflammation. Severe SARS-CoV-2 infection is associated with multi-organ failure, primarily affecting the lungs, heart, liver and kidneys. Patients present with histopathological lesions indicative of endotheliitis, diffuse thrombotic microangiopathy and vasculitis^{166,167}. The RAS has been of particular interest in this context as ACE2, in addition to its carboxypeptidase function in the non-canonical pathway of the RAS, is also the receptor for SARS-CoV-2 and the related virus SARS-CoV¹⁶⁸. ACE2 is expressed on lung alveolar epithelial cells, as well as in many other organs⁹. Upon binding of the virus spike protein to ACE2, the virus–receptor complex is taken up by endocytosis. This leads to a downregulation of ACE2 on cell surfaces¹¹ (FIG. 4c), where ACE2 should have a protective effect¹⁰, and a reduction in the generation of Ang 1–7. This results in unhindered activation of the canonical RAS pathway, without counterbalance by the non-canonical RAS pathway, with profound deleterious pro-inflammatory and prothrombotic effects in the lungs, heart and kidneys¹⁶⁹.

ACE2 can inactivate DABK by cleaving off its C-terminal amino acid¹⁷⁰. Therefore, decreased levels of ACE2 will result in increased DABK activity (FIG. 4c) and promote signalling via B1R and contribute to lung inflammation¹⁷¹. Since the availability of ACE2 is reduced in the lungs of patients with COVID-19, DABK may be stabilized and contribute to the pathogenesis of the disease. A case report of a patient who was successfully treated with recombinant ACE2, leading to markedly reduced Ang II and increased Ang 1–7 and Ang 1–9 levels, has been published¹⁷².

Conflicting data have been reported with regard to Ang II levels in patients with COVID-19. One study showed elevated levels of Ang II, correlating with the degree of lung injury¹⁷³, a second study showed that Ang II levels were higher in severely ill patients¹⁷⁴, whereas a third study did not detect altered levels of ACE2 and Ang II in serum¹⁷⁵. These data may, however, not fully reflect cellular and tissue levels as gene expression profiles in cells from bronchoalveolar lavage fluid from patients with COVID-19 showed upregulation of ACE2, angiotensinogen, renin, AT1R and AT2R and downregulated ACE¹⁷⁶. Upregulation of ACE2 seemingly conflicts with data showing that ACE2 expression is decreased on the surface of cultured cell lines during SARS-CoV uptake¹¹. However, ACE2 is physiologically upregulated

in conditions in which the effects of the canonical RAS pathway and Ang II are counterbalanced by the non-canonical RAS pathway, such as in hypertension, cardiomyopathy¹⁷⁷, heart failure and chronic kidney disease¹⁷⁸, and ACE2 upregulation may increase the risk of severe COVID-19 (REF.¹⁷⁷). Moreover, ACE2 levels are elevated in the plasma of severely ill patients with COVID-19 (REF.¹⁷⁹). Downregulation of ACE will theoretically lead to increased levels of bradykinin. Gene expression of high-molecular-weight kininogen, kallikreins and B1R and B2R was upregulated in cells from bronchoalveolar lavage fluid¹⁷⁶. Activated products of the KKS might also play a pivotal role in the inflammatory processes in COVID-19 (REFS^{180,181}). Severely ill patients exhibited reduced plasma levels of high-molecular-weight kininogen, prekallikrein and factor XII (REF.¹⁸²), which could potentially lead to oedematous lung injury¹⁸³. The *SERPING1* gene, which encodes C1 inhibitor, was downregulated in bronchoalveolar lavage fluid from these patients¹⁷⁶, allowing KKS/contact system activation, and SARS-CoV proteins have been shown to interact with C1 inhibitor¹⁸⁴. The combination of a decrease in ACE level and increased levels of AT1R, AT2R, B1R and B2R creates an imbalance that results in increased vascular permeability, bradykinin-driven inflammation and RAS/KKS-mediated thrombosis (FIG. 4c). KKS-mediated thromboinflammation may also be involved in this process given that increased platelet/neutrophil aggregates and augmented FXII activity have been observed in patients with severe COVID-19, which may cause embolisms, particularly in the lung¹⁸⁵.

ACE inhibitors do not affect ACE2 activity¹⁸⁶. Studies of animal models and patients treated with ACE inhibitors and Ang II receptor blockers (ARBs) have produced conflicting results with regard to the effect of these drugs on ACE2 expression, although studies have not explicitly addressed its expression on lung epithelium¹⁸⁷. ACE inhibitors and ARBs may have a protective effect with regard to the pro-inflammatory and profibrotic effects induced by the canonical RAS pathway. Indeed, in mice injected with the SARS-CoV spike protein, RAS blockade attenuated lung injury¹¹. Although the effects of ACE inhibitors and ARBs in COVID-19 are still being evaluated, there is a general consensus that these drugs should not be withdrawn in patients being treated for cardiovascular disease, diabetic nephropathy or hypertension^{187–189} and that they do not increase the risk of hospitalization¹⁹⁰ or death¹⁹¹. ACE inhibitors and ARBs were shown to reduce the severity of COVID-19 in patients with hypertension¹⁹².

The complement system plays a prominent role in the immune response to viral infections during acute lung injury^{193,194}, where it contributes to systemic inflammation, coagulopathy, thrombosis and multi-organ dysfunction. The nucleocapsid proteins of SARS-CoV and SARS-CoV-2 bind to MASP2, thereby activating the lectin pathway of the complement system¹⁹⁵, and the viral spike proteins activate the alternative pathway of complement¹⁹⁶. In vitro experiments showed that SARS-CoV-2-infected cells upregulate the transcription of genes encoding C3, factor B, C1s and C1r, and the increased expression of C3 and factor B was confirmed

Cytokine storm

A systemic inflammatory disorder triggered by pathogens, genetic or autoimmune diseases or iatrogenic treatments leading to hyperactivation of immune cells, such as T cells, macrophages and dendritic cells, and extremely high levels of circulating pro-inflammatory cytokines resulting in multi-organ failure and a high risk of death.

in bronchoalveolar lavage fluid from patients with COVID-19 (REF.¹⁹⁷). Proteomic profiles of sera from patients with severe COVID-19 revealed elevated levels of complement proteins, including C5, C6, factor B and CPN¹⁹⁸, as well as C5a or soluble C5b-9 (REFS^{199–202}). The levels of C3a and soluble C5b-9 were higher in patients with thromboembolic events²⁰³. Deposits of C3, C4, mannose-binding lectin (MBL), MASP2 and C5b-9 were demonstrated in the lungs, and deposits of C3 and MASP2 were demonstrated in the kidneys, of patients with severe COVID-19 (REFS^{204,205}). The severe inflammatory response to SARS-CoV-2 may be attributed, in part, to elevated levels of C5a, which acts as a neutrophil chemoattractant that can also contribute to inducing a cytokine storm and increase vascular permeability^{166,193}. C1 inhibitor has been proposed to interact with SARS-CoV-2 proteins¹⁸⁴, causing a physiological C1 inhibitor deficiency. Such a scenario would promote unbridled activation of the plasma KKS/contact system and complement system (FIG. 4c), which, together with activation of tissue factor and the extrinsic coagulation pathway, would contribute to extensive thromboembolism.

Treatment with C1 inhibitor, narsoplimal (a MASP2 inhibitor), C3 inhibitor (AMY-101) or eculizumab (anti-C5) was effective in uncontrolled studies and in case reports of patients with COVID-19 (REFS^{200,201,206–210}). A small phase II trial of the C5a-targeted monoclonal antibody IFX-1 (vilobelimab) produced promising results²¹¹. Likewise, in case-control studies, blockade of B2R with icatibant was shown to be effective in a limited number of patients^{212,213}, and use of the plasma kallikrein

inhibitor lanadelumab or FXIIa-inhibiting drugs (Supplementary Box 1) have been proposed as potential therapies^{180,181}. The timing of intervention is likely to be crucial, as inhibition of a fulminant inflammatory process once it has occurred may be less effective.

Conclusion

Severe inflammation is a complex condition involving multiple pathways and mediators. Blocking the RAS, the complement system or the plasma KKS/contact system individually may not be sufficient in hyperinflammatory disorders such as sepsis or COVID-19. The RAS, the complement system and the plasma KKS/contact system interact and contribute to the development of shock, severe cytokine storm and capillary leakage. The three pathways may either potentiate or counterbalance each other. The large number of interactions between components of the three systems lead us to suggest that the three systems actually constitute subdivisions of a joint protein cascade. Systemic and tissue inflammation can be effectively treated by blockade of the canonical RAS pathway or potentiation of the non-canonical RAS pathway, inhibition of complement activation via its three pathways and/or inhibition of the KKS at the level of enzymatic activation or receptor signalling. Understanding the intricate interactions between the three systems is of crucial importance for the treatment of inflammatory and infectious diseases as pharmacological interference with one system may have beneficial or deleterious effects in the other systems.

Published online 10 November 2021

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Acknowledgements

Z.B. is supported by the Crafoord Foundation, the Swedish Kidney Foundation and the Maggie Stephens Foundation. M.B. is supported by the German Research Foundation (SFB1365). D.K. is supported by the Swedish Research Council (2017-01920), the Knut and Alice Wallenberg Foundation (Wallenberg Clinical Scholar 2015.0320), the Skåne Centre of Excellence in Health, the IngaBritt and Arne Lundberg's Research Foundation and the Olle Engkvist Byggmästare Foundation.

Author contributions

All authors researched data for the article. Z.B., M.B. and D.K. contributed substantially to discussion of the content. All authors wrote the article and reviewed the manuscript before submission.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Immunology thanks Johannes Stegbauer, Allen P. Kaplan, Berhane Gherbehiet and Claudia Kemper for their contribution to the peer review of this work.

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Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information

The online version contains supplementary material available at <https://doi.org/10.1038/s41577-021-00634-8>.

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