Cell Calcium 77 (2019) 39–48

Contents lists available at ScienceDirect

Cell Calcium

journal homepage: www.elsevier.com/locate/ceca

# Store-operated calcium entry in thrombosis and thrombo-inflammation

Elmina Mammadova-Bach[a], Magdolna Nagy[b], Johan W.M. Heemskerk[b], Bernhard Nieswandt[a,*], Attila Braun[a,*]

[a] Institute of Experimental Biomedicine, University Hospital and Rudolf Virchow Centre, University of Würzburg, Würzburg, Germany
[b] Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, the Netherlands

ARTICLE INFO

*Keywords:*
SOCE
Platelets
Immune cells
Arterial thrombosis
Ischemic stroke
Thrombo-inflammation

ABSTRACT

Cytosolic free calcium ($Ca^{2+}$) is a second messenger regulating a wide variety of functions in blood cells, including adhesion, activation, proliferation and migration. Store-operated $Ca^{2+}$ entry (SOCE), triggered by depletion of $Ca^{2+}$ from the endoplasmic reticulum, provides a main mechanism of regulated $Ca^{2+}$ influx in blood cells. SOCE is mediated and regulated by isoforms of the ion channel proteins ORAI and TRP, and the transmembrane $Ca^{2+}$ sensors stromal interaction molecules (STIMs), respectively. This report provides an overview of the (patho)physiological importance of SOCE in blood cells implicated in thrombosis and thrombo-inflammation, *i.e.* platelets and immune cells. We also discuss the physiological consequences of dysregulated SOCE in platelets and immune cells and the potential of SOCE inhibition as a therapeutic option to prevent or treat arterial thrombosis as well as thrombo-inflammatory disease states such as ischemic stroke.

## 1. Introduction

Cytosolic free $Ca^{2+}$ ions act as an ubiquitous second messenger that regulates many important functions in platelets and immune cells, including cytoskeletal reorganisation, cell adhesion, migration, proliferation and apoptosis [1]. Physiological agonists can increase the cytosolic level of $Ca^{2+}$ by inducing $Ca^{2+}$ release from various intracellular stores as well as by $Ca^{2+}$ entry from the extracellular milieu into the cytosol. In essentially all blood cells, $Ca^{2+}$ entry is regulated by voltage-independent channels, located in the plasma membrane. Both in the anucleated platelets and in nucleated immune cells, several organelles have been described as $Ca^{2+}$ stores, including the dense tubular system or sarco/endoplasmic reticulum, lysosome-like acidic organelles and mitochondria [1–4]. In blood cells, cation channels can be regulated by activation of receptors or by depletion of intracellular $Ca^{2+}$ stores, which then triggers the process of store-operated $Ca^{2+}$ entry (SOCE), [4–6]. Because of its electrophysiological properties, $Ca^{2+}$ release activated $Ca^{2+}$ current ($I_{CRAC}$) has been identified as the current facilitating SOCE [5].

The molecular identity of the $Ca^{2+}$ sensor in the stores responsible for channel opening remained elusive until 2005, when the stromal interaction molecules (STIMs) were identified as reticular membrane located $Ca^{2+}$ sensors [7–9]. Shortly afterwards, three isoforms of transmembrane channel protein ORAI (ORAI1-3) were identified as the main $Ca^{2+}$ channels interacting with STIM proteins [10–15]. In platelets and immune cells, transient receptor potential (TRP) channels and ORAI isoforms may form functional units, which are coupled to STIM1 and inositol 1,4,5-trisphosphate ($IP_3$) receptor channels, thereby enhancing the $Ca^{2+}$ entry [4,16–20]. For platelets, a role of SOCE has been elucidated in thrombotic processes [19,20], while activation of SOCE in immune cells is considered to have a major role in the regulation of inflammation [18]. In the present review, we connected these findings to better understand how the molecular components of SOCE contribute to the whole spectrum of thrombotic and thrombo-inflammatory diseases.

## 2. Calcium homeostasis in platelets

### 2.1. Calcium store release

Platelets are anucleated cell fragments, released by megakaryocytes (MKs) into the bone marrow sinusoids, circulating in the blood and safeguarding vascular integrity. Upon vessel wall injury, platelets are recruited to exposed subendothelial extracellular matrix (ECM) proteins and become activated [21]. The activation process includes fast rearrangement of the platelet cytoskeleton, leading to shape change. Upon platelet activation, intracellular alpha (α)- and dense (δ) granules containing secondary mediators, such as fibrinogen (FGN), von

* Corresponding authors at: Institute of Experimental Biomedicine, University Hospital and Rudolf Virchow Centre, University of Würzburg, Josef-Schneider-Str 2, 97080 Würzburg, Germany.
*E-mail addresses:* bernhard.nieswandt@virchow.uni-wuerzburg.de (B. Nieswandt), attila.braun@virchow.uni-wuerzburg.de (A. Braun).

https://doi.org/10.1016/j.ceca.2018.11.005
Received 7 September 2018; Received in revised form 31 October 2018; Accepted 14 November 2018
Available online 23 November 2018
0143-4160/ 

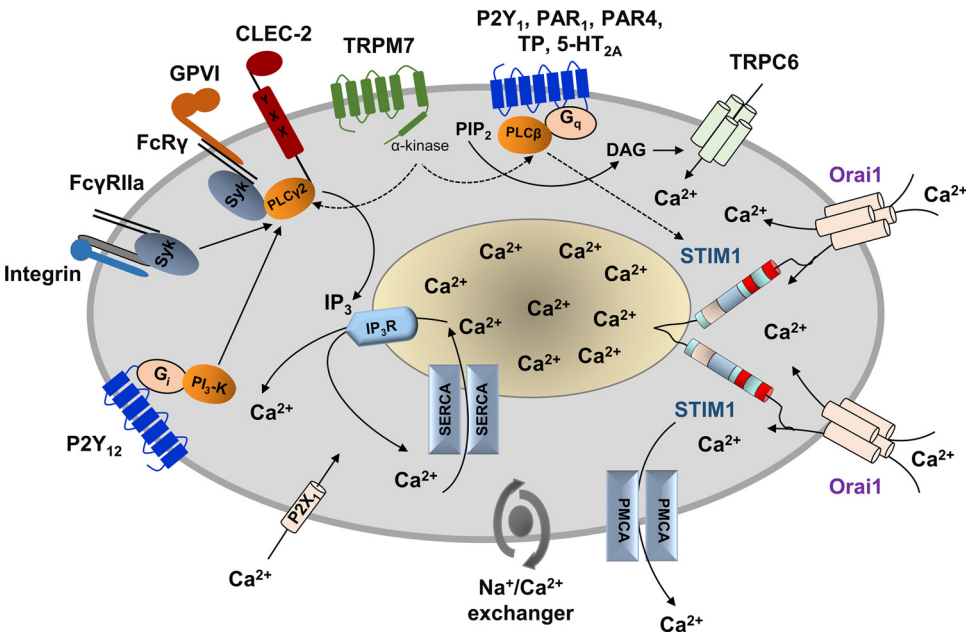


**Fig. 1.** Calcium store release in platelets. After receptor activation, phospholipase C (PLC) isoforms hydrolysephosphatidilinositol-4,5-bisphosphate ($PIP_2$) to inositol-1,4,5-trisphosphate $IP_3$ and diacylglycerol (DAG). IP3 releases $Ca^{2+}$ from the intracellular stores, in turn STIM1 opens Orai1 channels in the plasma membrane, a process known as store-operated calcium entry (SOCE), whereas DAG mediates non-SOCE through transient receptor potential channel 6 (TRPC6). Direct receptor-operated calcium (ROC) channel, $P2X_1$, and a $Na^+/Ca^{2+}$ exchanger (NCX) contribute to the elevation of intracellular $Ca^{2+}$ $[Ca^{2+}]i$. Sarco/endoplasmic reticulum $Ca^{2+}$ ATPases (SERCA) and plasma membrane $Ca^{2+}$ ATPases (PMCA) pump $Ca^{2+}$ back into the stores or through the plasma membrane to outside of the cell, respectively. $IP_3R$: IP3-receptor; GPVI: glycoprotein VI; FcRγ: Fc receptor γ chain; FcγRIIa: Fc γ receptor IIa; CLEC-2: C-type lectin-like receptor 2; PI3-K: phosphatidylinositol 3-kinase; Syk: spleen tyrosine kinase; $P2Y_1$ and $P2Y_{12}$: purinergic G protein-coupled receptor 1 and 12; PAR1 and 4: protease-activated receptor 1 and 4; TP: Thromboxane receptor; 5-$HT_{2A}$: serotonin 2A receptor; Trpm7: Transient receptor potential melastanin 7.

Willebrand Factor (vWF) and adenosine di-, tri-phosphate (ADP/ATP), serotonin are released, along with the production of short-lived thromboxane A2 (TxA2) and hydroxyeicosatetraenoic acid (HETE). These autocrine agents along with locally produced thrombin reinforce the activation process, and cause further recruitment of circulating platelets to the injury site, thereby leading to the formation of thrombi, which can seal the wounded vessel [21]. If occurring in diseased vessels, such as in atherosclerotic arteries subjected to plaque rupturing, the same reactions can lead to acute vessel occlusion, causing life-threatening disease states such as thromboembolism, myocardial infarction or ischemic stroke [22–25].

Signalling-dependent control of $Ca^{2+}$ homeostasis plays a crucial role in the regulation of platelet shape change and adhesion, and also in α- and δ- granule secretion and thrombus growth. The majority of platelet-activating receptors act through stimulation of phospholipase C (PLC) isoforms, which catalyse the hydrolysis of phosphatidyl 4,5-bisphosphate ($PIP_2$) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate ($IP_3$) [26–28], (Fig. 1). These second messengers control the processes of receptor- and store-operated $Ca^{2+}$ entry (ROCE and SOCE). Whilst $IP_3$ induces release of $Ca^{2+}$ through $IP_3$ receptors located in the endoplasmic reticulum, DAG regulates $Ca^{2+}$ entry through protein kinase C and activation of DAG-sensitive $Ca^{2+}$ channels [26–28].

Human and mouse platelets express several PLC isoforms, *i.e.* PLCβ2/3 and PLCγ2. The β-type isoforms are regulated by G-protein coupled receptors (GPCR), including the PAR receptors for thrombin, the purinergic receptors for ADP, and the thromboxane A2 receptor (TP) for TxA2, through coupling to the α-subunit of the G-protein, Gq [29]. The isoform PLCγ2 becomes activated *via* the receptors glycoprotein (GP)VI, C-type lectin-like receptor-2 (CLEC-2), integrins α2β1 and αIIbβ3, and to a limited extent *via* the GPIb-V-IX complex, and in human platelets *via* Fc gamma receptor IIA (FcγRIIA), [21].

In platelets collagen-dependent activation of GPVI causes tyrosine phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) located in the cytoplasmic tail of Fc receptor gamma (FcR-γ) chain. Recruitment and activation of Src family kinases is then followed by activation of spleen tyrosine kinase (Syk), phosphorylation of the adaptor molecule linker of activated T cells (LAT), and formation of the LAT signalosome. The latter recruits PLCγ2 from the cytoplasm to the plasma membrane, where it becomes active [30,31]. Activation of the various PLC isoforms thus converges in a common pathway of $IP_3$-mediated $Ca^{2+}$ store depletion.

### 2.2. Calcium stores in platelets

In human platelets, at least three separate $Ca^{2+}$ stores have been reported: the dense tubular system (equivalent to the sarco/endoplasmic reticulum), lysosome-like acidic organelles and mitochondria. The existence of distinct $Ca^{2+}$ stores has been proposed based on the different affinities to $IP_3$ [4], on the expression of two different sarco/endoplasmic reticulum $Ca^{2+}$ ATPase SERCA isoforms [4,32–34] and on their sensitivity to the thapsigargin (TG). The SERCA2b isoform displaying high sensitivity to TG is expressed on dense tubular system [35]. The SERCA3 isoform is expressed in lysosome-like acidic organelles [36,37] and less sensitive to TG, but high sensitivity was found to 2,5-di-(tert-butyl)-1,4-benzohydroquinone (TBHQ), [33]. Nevertheless, there is a consensus that the major intracellular $Ca^{2+}$ pool in platelets is located in the dense tubular system and the release of $Ca^{2+}$ from this TG-sensitive $Ca^{2+}$ stores is considered to be a key modulator of platelet SOCE [4].

### 2.3. Store-operated calcium channels in platelets

It has become clear that SOC channels are major regulators of extracellular $Ca^{2+}$ entry in platelets, similarly as in other electrically non-excitable cells [4,6]. In this section, we discuss the molecular composition of the platelet SOC channels.

#### 2.3.1. ORAI1

The ORAI proteins encompass a family of plasma membrane-resident channels with four transmembrane domains and intracellularly located C and N termini [10,11]. In mammalians, the family includes three isoforms: ORAI1, 2 and 3. ORAI1 is considered to be the main pore-forming unit of SOC channels with the capability to form multimers that make up a highly selective $Ca^{2+}$ channel [12,13,38]. Studies on genetically engineered mice lacking functional ORAI1 have established its importance in SOCE in many cell types [18]. In mouse and human platelets, all three isoforms of the ORAI channel family have been detected, with ORAI1 being the predominant isoform in both

species [39]. Messenger ribonucleic acids (mRNAs) of ORAI1 are abundantly expressed in human and mouse platelets, primary murine MKs, and megakaryocytic cell lines, at levels far above those of ORAI2, ORAI3, TRPC1 or other TRP isoforms [39,40]. In line with this, platelets isolated from *Orai1*$^{-/-}$ bone-marrow chimeric mice showed a strongly decreased SOCE [39].

Patients with severe combined immunodeficiency have been identified with an ORAI1$^{R93W}$ missense mutation, resulting in the substitution of a highly conserved arginine residue in the first transmembrane domain of ORAI1 [11]. Heterozygous expression of the ORAI1$^{R93W}$ mutation appeared to have a dominant negative effect on SOCE, probably inducing abnormal folding of the channel complex, which inhibits channel opening [11,41]. Platelets from ORAI1$^{R93W}$ mice, similarly to platelets from bone marrow chimeric *Orai1*$^{-/-}$ mice, displayed a strongly reduced SOCE. Yet, specific differences were reported between ORAI1$^{R93W}$ [41] and *Orai1*$^{-/-}$ platelets (39, 42).

Whereas *Orai1*$^{-/-}$ platelets showed a selective defect in GPVI signalling [39,42], ORAI1$^{R93W}$ platelets had a more global defect in integrin activation in response to GPVI as well as GPCR agonists [41]. Such a difference could possibly be explained by increasing expression of other ORAI isoforms (ORAI2 and ORAI3) in *Orai1*$^{-/-}$ platelets, thus partially compensating for the loss of ORAI1 [39]. Another explanation for this may be that the R93W mutation not only inactivates the ORAI1 channel, but also modifies the function of the ORAI1 signalosome *e.g.* by triggering a negative feedback signal from the channel to a GPCR. In both ORAI1 mutant platelets, residual $Ca^{2+}$ entry was detected after TG stimulation, suggesting that other $Ca^{2+}$ channels can play an additional role in this process.

#### 2.3.2. TRPC1

The TRP proteins belong to one of the largest ion channel superfamilies and grouped into seven related families, *i.e.* TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), TRPA (ankyrin) and TRPN (no mechanoreceptor potential C (NOMPC)-like); the latter is found only in invertebrates and fish [43]. Some members of the canonical TRP subfamily (TRPC1-7) can be activated by SOCE [44–46] and appear to be associated to ORAI isoforms [4,6]. Platelets express two isoforms, *i.e.* TRPC1 and TRPC6 [44,47,48]. Especially the latter is highly expressed in the platelet plasma membrane and mediates non selective-cation influx following binding of DAG [48,49].

Before the identification of the ORAI proteins, TRPC1 was considered as a major SOC channel in platelets. A conformational coupling model was proposed, stipulating that TRPC1 mediates SOCE following its interaction with type II $IP_3$ receptors [50,51]. Later studies however suggested that TRPC1 is located in the intracellular membranes of human platelets [48], supporting a different role for the channel rather than activation of SOCE in the plasma membrane. Moreover, TRPC1-deficient mice displayed intact SOCE activity, unaltered $Ca^{2+}$ homeostasis, and unchanged platelet activation responses both *in vitro* and *in vivo* experimental settings [52]. In addition, studies on human platelets indicated that inhibitory anti-TRPC1 antibodies used in previous studies to demonstrate the function of the channel [50,53,54], had no specific effect on SOCE and failed to bind to the protein [52]. Altogether, these results made it clear that SOCE in platelets involves other $Ca^{2+}$ channels, such as ORAI1 than TRPC1 [10,11]. It has been proposed that TRP channels, including TRPC1 can form heteromeric ion channels together with ORAI channels [6,55,56]. Recently, Liao et al., showed existence of functional interactions between Orai1 and TRPC under the influence of calcium sensor STIM1.

### 2.4. Calcium sensors in platelets

#### 2.4.1. STIM1

The first description of a role for STIM1 in platelet SOCE came from studies using a mouse strain named *Saxcoburggotski* (*Stim1*$^{Sax}$), which carry a gain of function mutation in the EF hand domain of STIM1. The selective amino acid substitution of the EF hand domain of STIM1 showed that this mutation changes the conformation of the sensor to be constitutively active, and hence to mimic $Ca^{2+}$ store depletion without $IP_3$-mediated action [7,9,57]. Strikingly, the homozygous *Stim1*$^{Sax}$ mutation leads to embryonic lethality due to severe haemorrhages in different organs, whereas heterozygous mice are viable but developed severe thrombocytopenia [57]. Heterozygous mutant mice display splenomegaly, caused by rapid expansion of MKs in the splenic red pulp. Interestingly, the *Stim1*$^{Sax/+}$ MKs showed normal proplatelet formation *in vitro*, suggesting enhanced clearance of the mutant platelets by the reticuloendothelial system in the spleen rather than defective platelet production to be responsible for the low platelet count [57]. It was confirmed that *Stim1*$^{Sax/+}$ platelets had increased basal levels of cytosolic $Ca^{2+}$, due to a leaky $Ca^{2+}$ store, and showed abnormally activated $Ca^{2+}$ channels, which together led to enhanced phosphatidylserine (PS) exposure and αIIbβ3 integrin activation in the absence of agonist stimulation [57]. In the presence of TG, *Stim1*$^{Sax/+}$ platelets showed a faster store depletion, which was accompanied by impaired SOCE. Whereas *Stim1*$^{Sax/+}$ platelets were unresponsive to agonists of (hem)ITAM-linked receptors such as collagen-related peptide or rhodocytin, the $Ca^{2+}$ responses to GPCR agonists (ADP or thrombin) were nearly normal, indicating that the accelerated $Ca^{2+}$ store depletion functionally compensated for the lack of SOCE in integrin αIIbβ3 activation and platelet degranulation after GPCR stimulation [57].

Genetic deletion of STIM1 in mice allowed analysis of the role of this $Ca^{2+}$-regulating protein in more detail. Due to perinatal lethality of the constitutive knock-out, bone marrow chimeras needed to be generated, resulting in animals with a deficiency of STIM1 in blood cells, including platelets [58–60]. Measurements of cytosolic $Ca^{2+}$ levels showed that *Stim1*$^{-/-}$ platelets still displayed a small residual SOCE in response to TG, but a severely impaired $Ca^{2+}$ increase was observed with all major platelet agonists. The TG-sensitive $Ca^{2+}$ store release was reduced, thus pointing to an essential role of STIM1 in the regulation of $Ca^{2+}$ store refilling [42,58]. A pronounced defect was observed upon activation of (hem)ITAM receptors, while the platelets displayed only minor defects in integrin αIIbβ3 activation or in granule release in response to GPCR agonists [58].

Although both gain (*Stim1*$^{Sax}$) and loss (*Stim1*$^{-/-}$) of function mutations of STIM1 led to impaired (hem)ITAM receptor signalling in platelets, the underlying molecular mechanisms remain unclear. Since the $Ca^{2+}$ store content in both mouse strains was abnormal [57,58], a hypothesis is that ionic stress in platelets can induce a negative feedback mechanism to (hem)ITAM receptors, which then negatively regulates GPVI- and CLEC-2-induced activation cascades. Alternatively, a dysregulated $Ca^{2+}$ store release and SOCE in both STIM1-mutant platelets no longer amplifies GPVI and CLEC-2 receptor activation through a defective $Ca^{2+}$-dependent phosphorylation or dephosphorylation of the LAT signalosome.

It has been shown that also platelets from serotonin transporter knock-out (*5-Htt*$^{-/-}$) and granule release defective (*Unc13d*$^{-/-}$) mice have a strongly reduced SOCE [61], indicating an essential role of platelet-released secondary agonists to amplify the channel activity of ORAI1. In contrast to *Stim1*$^{-/-}$ platelets, *Orai1*$^{-/-}$ platelets display a normal $Ca^{2+}$ store content and $Ca^{2+}$ store release after TG or agonist stimulation, thus explaining the consistently higher cytoplasmic $Ca^{2+}$ rises in these cells compared to *Stim1*$^{-/-}$ platelets. Studies analysing platelets from *Orai1*$^{-/-}$ bone marrow chimeras revealed a less severe phenotype than in *Stim1*$^{-/-}$ bone marrow chimeras [39,58], *i.e.* milder defects in GPVI-induced integrin activation and platelet degranulation and thrombus formation under flow *in vitro*.

In addition, recently we showed the existence of functional interplay between STIM1 and Transient receptor potential melastanin 7 (TRPM7) channel, which contains a cytosolic domain of serine/threonine α-kinase [62]. Global deletion of TRPM7 kinase activity in mice (*TRPM7*$^{R/R}$) resulted in inhibited (hem)ITAM-PLCγ2 and PAR-

PLCβ3–mediated intracellular $Ca^{2+}$ mobilisation [62]. Moreover, activation of STIM1 function with TG-mediated $Ca^{2+}$ store depletion, bypassing receptor-mediated PLC activation, resulted in strongly reduced SOCE in *TRPM7*$^{R/R}$ platelets, suggesting the involvement of TRPM7 kinase activity in the regulation of STIM1-induced SOCE [62]. Of note, ORAI1 and STIM1 functions are regulated by multiple phosphorylation of serine residues [63,64]. Future studies are needed to investigate in more details, whether TRPM7 kinase may phosphorylate serine/threonine residues in the protein complex of SOCE.

### 2.5. Receptor-operated calcium entry

Besides SOCE, also other $Ca^{2+}$ influx mechanisms exist in platelets. These include a ROCE pathway, induced by DAG-mediated TRPC6 activation, and a pathway of $Ca^{2+}$ influx through the ATP-gated purinergic receptor channel $P2X_1$.

#### 2.5.1. TRPC6

TRPC6, which is abundantly expressed in both mouse and human MKs and platelets, has been proposed as a non-SOC channel [48]. This was based on the findings that TRPC6 activation is strongly dependent on phospholipase C (PLC) and D (PLD)-mediated DAG production, but independent of $Ca^{2+}$ release from intracellular stores. In addition, TRPC6 is a substrate for cAMP protein kinase and phosphorylation does not affect $Ca^{2+}$ entry through the channel [48]. TRPC6 contains two sites for cyclic nucleotide kinase action at RRQT and KKLS [48], however it is unknown whether both sites are phosphorylated and their role on TRPC6 function. More recently, Nishida et al., showed that inhibition of cyclic guanosine 3',5'-monophosphate (cGMP)-selective phosphodiesterase 5 induces phosphorylation of TRPC6 proteins at Thr69 [65], leading to inhibition of TRPC6-mediated $Ca^{2+}$ signalling. The authors proposed that cGMP-dependent protein kinase (PKG)-mediated inhibition of TRPC6 channel activity is required for the pathological cardiac hypertrophy [65]. In addition, PKC also can inhibit TRPC6 activity by phosphorylation of $Ser^{448}$ [66]. Both PKC and TRPC6 are activated by GPCR signalling, suggesting that PKC may act as negative feedback mechanism on TRPC6 activity, thereby to weaken $Ca^{2+}$ entry [66].

In TRPC6-deficient mouse platelets, TG-induced SOCE was not altered, suggesting that it does not act as a SOC channel or other $Ca^{2+}$ channels compensate for its function. Although TRPC6-mediated $Ca^{2+}$ influx has been described in many studies, contradictory results exist regarding the importance of TRPC6 in platelet physiology. In platelets from *Trpc6*$^{-/-}$ mice or in human platelets in the presence of non-selective TRPC6 blockers, lower aggregation responses were observed [67]. In contrast, using other *Trpc6*$^{-/-}$ knock-out mouse model, no obvious changes in $Ca^{2+}$ homeostasis or platelet function could be observed, although $Ca^{2+}$ entry induced by the DAG analogue 1-oleoyl-2-acetyl-sn-glycerol was virtually abolished [68]. The latter study also indicated that TRPC6 in mouse platelets provides a major route of DAG-mediated $Ca^{2+}$ entry [68]. Other authors however suggested that TRPC6 is not only involved in ROCE, but can also contribute to SOCE in platelets [49].

To further investigate the relations between SOCE and ROCE, mice lacking both ORAI1 and TRPC6 were generated [69]. *Orai1*$^{-/-}$*Trpc6*$^{-/-}$ platelets displayed a further reduction in TG-induced SOCE, when compared to *Orai1*$^{-/-}$ platelets, indicating the existence of a crosstalk between the TRPC6 and ORAI1 channels [69]. However, the crosstalk was independent of biochemical interactions between ORAI1 and TRPC6 in the SOCE complex, since ORAI1-mediated SOCE amplified DAG production through the activation of PLC and PLD isoforms, thereby increasing the channel activity of TRPC6 [69]. Furthermore, the platelets from *Orai1*$^{-/-}$*Trpc6*$^{-/-}$ mice displayed a severe $Ca^{2+}$ deficit in resting state and upon activation – *i.e.* reduced $Ca^{2+}$ store content and $Ca^{2+}$ levels in the cytosol [69].

#### 2.5.2. $P2X_1$

The $P2X_1$ purinoceptor acts as an ATP-gated ion channel with relatively high $Ca^{2+}$ permeability in the platelet plasma membrane [70,71]. Activation of the $P2X_1$ channel causes a rapid $Ca^{2+}$ influx, dense granule centralisation, shape change and a low level of platelet aggregation [70,72]. Moreover, $P2X_1$ activation enhances platelet responses to thrombin and collagen [70,72]. Typically, the activation of the $P2X_1$ channel is transient due to its fast desensitization by released ADP. Therefore, to study the $P2X_1$ channel *in vitro*, a high concentration of apyrase is required to scavenge ADP [71].

Studies on $P2X_1$ knock-out mice (*P2X1*$^{-/-}$) provided most definitive evidence for an important role of this channel in platelet function [73]. Bleeding times in *P2X1*$^{-/-}$ mice remained unchanged as compared to wild-type mice, indicating normal haemostasis [73]. Under flow conditions, at high shear, however, platelets from *P2X1*$^{-/-}$ mice displayed reduced thrombus formation [73]. Moreover, *P2X1*$^{-/-}$mice were resistant to systemic thromboembolism, induced by the injection of a mixture of collagen and adrenaline, and in a model of arterial thrombosis induced by laser injury [73]. In contrast, overexpression of human $P2X_1$ in transgenic mice resulted in a prothrombotic phenotype [74]. Therefore, the $P2X_1$ channel has been proposed as an attractive target for the development of antithrombotic drugs [71,75,76]. One such drug, NF449, which is an analogue of suramin, a sulfated naphthylamine, was reported to display good selectivity for P2X protein family members and to inhibit thrombus formation *in vivo* [77]. Studies with NF449 demonstrated a dose-dependent inhibition of systemic thromboembolism in mice, which was accompanied by reduced platelet consumption [77]. Interestingly, *Orai1*$^{-/-}$ mice were protected also for systemic thromboembolism triggered by intravenous injection of collagen and epinephrine thrombogenic mixture [39].

$P2X_1$ channel activity also induces calmodulin-dependent myosin light chain kinase activity [78], which modulates cytoskeletal rearrangements and movement of intracellular granules, thereby triggering platelet shape change. Further studies are necessary to investigate whether ORAI1-mediated SOCE also regulates the channel activity of $P2X_1$ and downstream responses.

## 3. Platelet SOCE in arterial thrombosis and haemostasis

Considering that $Ca^{2+}$ signalling is a crucial pathway in platelet activation, regulatory proteins involved in this process could be targets for antithrombotic therapy. In various genetic mouse models, attenuation of intracellular $Ca^{2+}$ signals, in particular SOCE, was found to affect arterial thrombus growth and/or stability. The decreased $Ca^{2+}$ entry in response to (hem)ITAM-linked receptor agonists observed in *Stim1*$^{-/-}$ and *Orai1*$^{-/-}$ platelets resulted in impaired whole-blood thrombus formation *ex vivo*. At high shear rates, resembling arterial flow conditions, the overall volume of thrombi formed by *Stim1*$^{-/-}$ and *Orai1*$^{-/-}$ platelets on collagen were reduced by 70% and 46%, respectively, compared to wild-type [39,58]. In standard aggregometry (*i.e.* stirred suspensions), integrin activation, granule release and aggregation of *Stim1*$^{-/-}$ and *Orai1*$^{-/-}$ platelets were defective in the presence of lower concentrations of GPVI agonists, but not affected in response to GPCR agonists [39,79]. In line with *ex vivo* flow adhesion assays, *Stim1*$^{-/-}$ and *Orai1*$^{-/-}$ chimeric mice were found to be protected from arterial thrombosis in different *in vivo* models, albeit to a different extent. The most pronounced protection of both mouse strains was observed in a mechanical injury model of the abdominal aorta, where thrombus formation is triggered predominantly by collagens exposed at the site of endothelial damage [39,58]. Interestingly, in a chemically induced injury model using topical application of ferric chloride ($FeCl_3$) on mesenteric arterioles, *Stim1*$^{-/-}$ chimeric mice displayed impaired thrombus formation and vessel occlusion, whilst this process was not affected in *Orai1*$^{-/-}$ chimeric mice. This could be explained by the fact that GPCR-induced $Ca^{2+}$ responses are lower in *Stim1*$^{-/-}$ platelets than in *Orai1*$^{-/-}$ platelets [39,58]. Accordingly,

*Orai1*$^{-/-}$/*Trpc6*$^{-/-}$ chimeric mice have also normal thrombus formation in the $FeCl_3$-injured mesenteric arteries highlighting the importance of efficient $Ca^{2+}$ store depletion and contribution of other $Ca^{2+}$ channels to $FeCl_3$-induced thrombus formation, probably enhancing $P2X_1$ or other TRP channel activity [69].

Thrombus formation at high shear rates was also impaired in conditional knock-out mice with a megakaryocyte/platelet specific deletion of Stim1 gene (Stim1$^{f/f\ PF4-Cre}$), [80], however to less extent than in *Stim1*$^{-/-}$ chimeric mice [58] in which SOCE of immune cells in addition to platelets SOCE was also defective. However, platelets from both mouse strain displayed a decreased PS exposure. *In vivo* thrombus stability of Stim1$^{f/f\ PF4-Cre}$ mice were correlated with the delayed formation of fibrin at sites of vascular injury [80], suggesting that the procoagulant role of STIM1-mediated SOCE in platelets plays an important role in thrombus stability and growth.

*Stim1*$^{Sax/+}$ mice have been also studied in several models of arterial thrombosis. These mice showed decreased vessel occlusion in the mechanically injured abdominal aorta, but not of $FeCl_3$-injured mesenteric arterioles [57]. Of note, *Stim1*$^{Sax/+}$ mice exhibit marked thrombocytopenia which likely contributes to the decreased vessel occlusion in the aorta [57].

So far, studies in platelets isolated from *Stim2*$^{-/-}$ mice showed no abnormalities and $Ca^{2+}$ responses to common platelet agonists were unaltered compared to wild-type controls [42]. The unchanged $Ca^{2+}$ store release and SOCE in STIM2 deficient platelets point to substantial redundancy with the physiological functions of STIM1. Although STIM2 is highly expressed in the MK lineage, platelets from STIM2 knock-out mice did not show defects in platelet production or thrombus formation [42].

In humans, rare loss of function mutations in the *ORAI1* and *STIM1* genes have been reported, leading to altered platelet SOCE [81]. These mutations profoundly affect TG-induced SOCE [82] and seem to be associated with mild thrombocytopenia, but not with a bleeding phenotype. Interestingly, both loss- and gain-of-function mutations in ORAI1 and STIM1 are accompanied by a lower platelet count, for reasons that are not yet clear. A recent report investigated the effect of several mutations in ORAI1 and STIM1 on whole-blood thrombus formation on collagen and non-collagen surface *ex vivo* [82]. It appeared that both loss-of-function (ORAI1$^{R91W}$) and gain-of function (ORAI1$^{G98S}$) variants of ORAI1 lead to decreased thrombus formation on collagen microspots, and in particular to a decreased procoagulant activity indicated by reduced PS exposure. Interestingly, these mutations also decreased platelet aggregation and procoagulant activity on vWF/rhodocytin and vWF/FGN microspots. On the other hand, a heterozygous STIM1$^{R429C}$ mutation, in the STIM1-Orai-activating region did not affect thrombus formation [82]. It was proposed that depending on the activated signalling pathway, the platelet SOCE defect and the mild thrombocytopenia in different ways might alter the thrombus-forming process [82].

The roles of murine STIM1 and ORAI1 have also been studied in haemostasis. Tail bleeding times were normal in *Stim1*$^{-/-}$ or *Orai1*$^{-/-}$ bone marrow chimeric mice. Similarly to patients, loss of STIM1 or ORAI1 has not been associated with a bleeding diathesis [39,58]. In contrast, the gain-of function mutation of STIM1 in *Stim1*$^{Sax/+}$ mice results in reduced platelet count, dysregulated platelet function and consequently prolonged bleeding [57]. A similar phenotype was observed in patients with the Stormorken [83,84] and York syndromes [85] carrying gain of function mutations in the *STIM1* gene.

## 4. Platelet SOCE in ischemic stroke

Platelets play a critical role in the progression of ischemic stroke [23]. In mice, the transient middle cerebral artery occlusion (tMCAO) model is widely used to study mechanisms underlying post-ischemic infarct progression in the brain [23,86,87], which is also seen in stroke patients where brain infarcts frequently grow despite recanalization of a previously occluded cerebral artery. Platelets are known to contribute to this reperfusion injury but the underlying mechanisms are only poorly understood. Interestingly, in both *Stim1*$^{-/-}$ and *Orai1*$^{-/-}$ bone-marrow chimeras infarct volumes at 24 h after induction of tMCAO were markedly reduced, when compared to wild-type mice [39,58]. No intracranial haemorrhages were observed, suggesting a net protective effect on stroke with blood cells lacking STIM1 or ORAI1. Importantly, the reduced infarct sizes were accompanied by significantly improved neurological and motoric functions of the chimeric animals in comparison to the control mice [39,58].

When primary neurons isolated from ORAI1 or STIM1 deficient mice were subjected to ischemia *in vitro*, a similar degree of SOCE was observed as wild-type cells [39,58], whereas the neurons from *Stim2*$^{-/-}$ mice showed a reduced $Ca^{2+}$ store content and SOCE [88]. These results highlight a regulatory role of STIM2 in neuronal SOCE, relevant for the development of experimentally induced ischemic stroke. Taken together, this points to SOCE as a potential target to suppress ischemic insults, *i.e.* by blocking $Ca^{2+}$-induced platelet activation and reducing neuronal cell death. In line with this suggestion, in the tMCAO model, wild-type mice pre-treated with the SOCE blocker 2-aminoethoxydiphenyl borate (2 APB) were protected from cerebral infarct growth, in a similar manner as seen with *Orai1*$^{-/-}$ bone marrow chimeric mice [79]. However, the molecular composition of the SOC complex in ischemic neurons has not been elucidated, leaving the possibility that other ORAI and TRP isoforms regulate SOCE in this cell type.

## 5. Platelets and immune cells in thrombo-inflammation

The current evidence suggests that at ischemic vascular lesions, the rapid intravascular activation of platelets and endothelial cells increases thrombotic events (Fig. 2). Collagen–vWF–GPIb platelet activation axis and factor XIIa (FXIIa)-mediated intrinsic coagulation pathways are important players in these events [23,87,89]. At initial steps, interactions of platelets subendothelial ECM components lead to platelet adhesion, activation, thereby enhancing thrombin generation and fibrin formation, which lead to thrombus formation. This process also involves interactions of platelet GPVI with its receptors, thereby enhancing thrombus growth [23,90,91]. Recruitment and activation of immune cells from peripheral blood can trigger fibrin formation, which also contribute to thrombotic events [23,89,92]. In endothelial and immune cells, the increased expression of leukocyte adhesion molecules, such as P-selectin, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and platelet-endothelial cell adhesion molecule (PECAM) further stimulate the transmigration of immune cells [92,93]. At later time points of the stroke event, the ruptured blood-brain barrier exposes ischemic brain tissue to inflammatory molecules, cytokines, proteases, reactive oxygen species (ROS) derived from platelets and different immune cell subsets [89,94,95], thereby leading to neuronal damages (Fig. 2).

Emerging insights indicate an important link between thrombotic and inflammatory pathways in ischemic stroke. Accordingly, stroke can be redefined as a thrombo-inflammatory disorder [23,87,92,96]. In this section, we review the role of SOCE in platelets and immune cells in the context of thrombo-inflammation, referring to studies from mice and patients lacking functional STIM or ORAI proteins.

### 5.1. Role of SOCE in different immune cells

#### 5.1.1. Neutrophils

Neutrophils are first responders of the immune system, which are rapidly recruited into tissues in response to inflammation or infection. Neutrophils recognise antibody-opsonized pathogens and immune complexes containing antigens and antibodies *via* the Fcγ receptors on their cell surface. This leads to immune complexes in the phagosome and phagocytosis of pathogens, with both processes relying on the production of ROS. Besides the Fcγ receptors, also GPCR and integrins

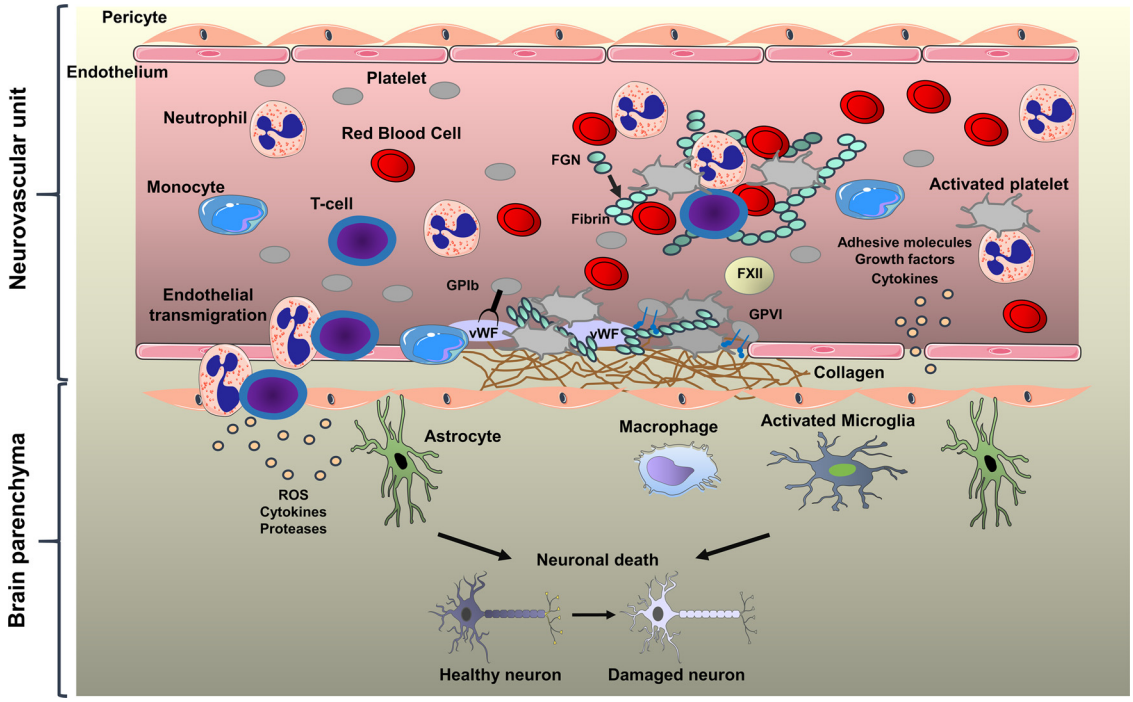


**Fig. 2.** Mechanisms of thrombo-inflammation in brain ischemic stroke.
Upon ischemic vascular injury, blood platelets adhere to exposed von Willebrand factor (vWF) through platelet Glycoprotein Ib alpha (GPIbα), thereby enhancing thrombin generation and fibrin formation, which lead to thrombus formation. This process also involves interaction of GPVI with fibrillar collagen and fibrin, leading to the platelet adhesion, activation and subsequently thrombus formation and growth. In addition, activation of intrinsic coagulation pathway and fibrin generation lead to the recruitment of circulating platelets and immune cells, and subsequently to the release of inflammatory cytokines, oedema, activation of microglia and finally to neuronal death. During ischemic insults, activated neutrophils and T cells enhance the expression of adhesion molecules and release of growth factors and cytokines in endothelium and immune cells. Crosstalk between neutrophils, platelets and T cells lead to thrombus and fibrin formation, thrombin activation and induction of extrinsic tissue factor pathway. Immune cells may also contribute to ischemic stroke by migrating and infiltrating in brain parenchyma, thereby releasing ROS, inflammatory cytokines and proteases, which also lead to neuronal damage.

can regulate $Ca^{2+}$ influx in neutrophils, where SOCE is the main mechanism of $Ca^{2+}$ entry [16]. Early studies showed that in the neutrophil-like HL-60 cells, small interfering RNA (siRNA)-mediated knock-down of STIM1 impairs $Ca^{2+}$ entry in response to formyl peptide or TG, resulting in a decreased production of ROS [97]. Using mice lacking STIM1, STIM2 or both in the myeloid and neutrophil-specific lineage, Clemens and collaborators showed that STIM1 and STIM2 cooperatively regulate neutrophil SOCE. ROS production, neutrophil degranulation and phagocytosis are minimally impaired in the absence of STIM2, suggesting that STIM1 is the major $Ca^{2+}$ sensor required for neutrophil functions [98]. Interestingly, STIM2-deficient, but not STIM1-deficient, neutrophils display a marked defect in cytokine production and protected in a model of systemic inflammatory response syndrome [98].

Patients carrying a missense ORAI1 variant has no alteration in leukocyte cell counts, suggesting that SOCE is not required for granulocyte development [16]. However, receptor-induced $Ca^{2+}$ influx was virtually abolished in activated neutrophils from immuno-deficient patients with strongly altered ORAI1 functions, indicating that the major route of SOCE triggered by ORAI1 isoform in these cells [99–101]. On other hand, in neutrophil like HL-60 cells, siRNA-mediated knock-down of ORAI1 decreased SOCE and production of ROS in response to stimulation through Fcγ receptors or GPCR by no more than 20–50%. [102–104], suggesting the presence of an alternative mechanism. Similar results were obtained using *Orai1*$^{+/-}$ murine neutrophils [102].

Neutrophil recruitment to inflammatory sites involves chemokine release and $\beta_2$-integrin activation, with both events being co-regulated by the cytosolic $Ca^{2+}$ level [105–107]. Inhibition of SOCE in human neutrophils by genetic deletion of ORAI1 delayed integrin $\beta_2$-dependent adhesion and integrin clustering, suggesting a role of SOCE in neutrophil attachment to the endothelium [102,108]. In contrast to ORAI1, in murine neutrophils STIM1 appeared to be dispensable for cellular migration, indicating a complex sensing mechanism in this cell type [109].

Neutrophils contribute to the disruption of blood brain barrier through the release of proteases and ROS, thereby inducing cerebral oedema and neurotoxicity [94]. It has been also proposed that neutrophil and platelet interaction aggravates ischemic brain damage and causes thrombo-inflammatory injury. This interaction is considered to be important for the extravasation [110]. Recently, it has been shown that the migration of neutrophils into the brain is dependent on the platelet GPIb-V-IX complex [111]. Neutrophil migration also involves TRPM7-channel activity by facilitating $Ca^{2+}$ oscillations. It has been suggested that interplay between TRPM7 and glycoprotein CD147 regulates neutrophil chemotaxis, adhesion and migration in rheumatoid arthritis conditions [112]. Recently, using bone marrow chimeric mice, we showed that the kinase activity of TRPM7, which regulates SOCE, is involved in ischemic stroke [62]. After tMCAO, the protective effects observed in different groups of bone marrow chimeric *Trpm7*$^{R/R}$ mice was not only due to the lack of TRPM7 kinase activity in platelets and immune cells [62], but also suggest the involvement of cells located in the brain vessels and neuronal tissue. However, the detailed role of TRPM7 kinase-mediated SOCE in neutrophils and other immune cells need to be further investigated in ischemic conditions.

Together, these data suggest that SOCE mediates many functions of neutrophils, which may occur in thrombo-inflammatory diseases. Whether SOCE components only in neutrophils or platelets or in bi-directional way could mediate these processes is simulating several questions, which need to be addressed in the future.

#### 5.1.2. Macrophages

Macrophages are phagocytes derived from activated monocytes with central roles in both innate and adaptive immunity [113]. Macrophages play an important role in phagocytosis of cell debris and pathogens, and the production of cytokines such as of interleukin (IL)-1β, IL-6 and IL-12, which in turn activate other immune cells. The recognition of antibody-opsonized pathogens by macrophages is mediated through Fc gamma (Fcγ) receptors [113].

Initial studies demonstrated that STIM1 and SOCE are essential for Fcγ receptor activation and auto-immune inflammation [114]. Upon cross-linking of Fcγ receptors, *Stim1*$^{-/-}$ macrophages displayed markedly reduced phagocytosis of opsonized blood, while the production of inflammatory cytokines, macrophage inflammatory protein-2 (MIP-2) and tumor necrosis factor alpha (TNFα) was only moderately impaired [114]. This work further indicated that STIM1 was indispensable for the development of monocytes and their differentiation into macrophages [114]. Deficiency in STIM1 also resulted in resistance to experimental IgG-dependent immune thrombocytopenia, anaphylaxis, and acute pneumonitis [114]. Later evidence indicated that also STIM2 contributes to Fcγ receptor activation, suggesting a partial functional redundancy between the STIM isoforms in this regulation of inflammation [115].

Recently, using *Stim1*$^{-/-}$ and *Stim2*$^{-/-}$ bone marrow chimeras, it was demonstrated that either isoform is dispensable for key effector functions of macrophages and dendritic cells, such as Fcγ receptor-dependent and -independent phagocytosis, phagolysome fusion, cytokine production and NLR family, pyrin domain containing 3 (NLRP3) inflammasome activation [116]. Altogether, these data indicate that depending of the inflammatory context, SOCE can regulate proinflammatory pathways in macrophages, but not other effector functions [116].

TRMP7 channel is also mediator of macrophage functions. TRPM7-deficient macrophages displayed decreased Toll-like receptor-4 (TLR-4) internalisation and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) activation in following Lipopolysaccharide (LPS) challenge [117]. Interestingly, these defects were due to diminished TRPM7-mediated $Ca^{2+}$ influx [117]. In addition, in LPS-induced peritonitis model, *Trpm7*$^{fl/fl\text{-}LysM\text{-}Cre}$ mice had decreased serum cytokine levels after LPS treatment, preventing pathological inflammation [117]. However, whether these functions in macrophages involve TRPM7-mediated SOCE remain still elusive.

After stroke, ischemic brain tissues are abundant in macrophages derived from either resident microglia or infiltrating monocytes [118]. Interestingly, it has been shown that expression profile of genes involved in $Ca^{2+}$ signalling enriched in migrating monocytes during ischemic stroke [119]. Whether SOCE activity may be involved in the regulation of macrophage function in stroke, future studies needed to establish this process using a wide range of thrombo-inflammatory conditions.

#### 5.1.3. T cells

SOCE regulates many important functions of T cells, including proliferation, differentiation and cytokine production. Activation of the T cell receptor signalosome triggers intracellular $Ca^{2+}$ store depletion and subsequent SOCE, and activates a $Ca^{2+}$-dependent calcineurin-nuclear factor of activated T cells (NFAT) pathway. T cells isolated from SOCE-defective patients fail to proliferate in response to receptor stimulation, and accordingly, display impaired NFAT signalling thereby blocking downstream cytokine production [120,121]. In line with these data, murine T cells lacking STIM1 or STIM2 produced less cytokines and displayed reduced nuclear translocation of the transcription factor NFAT [59].

Homozygous ORAI1$^{R93W}$ mice die after birth, but foetal liver chimeras are viable and T cells develop normally, despite defective SOCE. However, the effector functions of T cells were markedly impaired in these mice, including the production of IL-2, IL-4, IL-17, interferon (IFN)-γ and TNFα, [99], indicating a crucial role of ORAI1-mediated SOCE in the regulation of the inflammatory response. In Type 2 T helper (Th2) cells from STIM1 or ORAI1-deficient mice, the activity of $CD4^+$ T helper cells is also dependent on SOCE, as apparent from the decreased production of IL-4 and IL-10 [99]. This effect was linked to impaired functions of Type 1 T helper (Th1) and T helper 17 (Th17) cells, and a shortage of IFN-γ and IL-17 A production [99,122]. *Stim1*$^{-/-}$ mice were found to be more resistant to induction of experimental autoimmune encephalomyelitis than *Stim2*$^{-/-}$ mice, which is consistent with only partial involvement of STIM2 in cytokine production and T cell function [122,123].

Using conditional knock-out mouse models, it was recently shown that genetic deletion of *Orai1* reduces SOCE in mouse T cells, whereas deletion of *Orai2* increases SOCE. This may suggest that ORAI2 forms heteromeric channels with ORAI1, in which ORAI2 attenuates the function of ORAI1 and limits SOCE [124]. Interestingly, deletion of *Orai2* enhanced SOCE in naïve T cells but not in effector T cells. Combined deletion of *Orai1* and *Orai2* completely abolished SOCE, suggesting that both isoforms mediate SOCE in these cells. Moreover, *Orai1*$^{-/-}$*Orai2*$^{-/-}$ double knock-out mice displayed severe defects in T cell functions *in vitro,* and impaired T cell-mediated immune responses *in vivo,* such as antibody production after viral infection, colitis or graft-versus-host disease [124].

Recent studies also highlighted the role of TRPM7 in human T cell proliferation. Pharmacological inhibition of TRPM7 in human T cells leads to growth arrest and reduces cell proliferation [125]. In addition, TRPM7 was described to be involved in the migration of human T cells [126]. Consistently, Romagnani et al., showed that TRPM7 kinase activity is essential for T-cell colonisation and alloreactivity in the gut [127]. More recently, it has been shown that the deletion of TRPM kinase activity in mice results in enlarged spleens, reduced T-cell proliferation and decreased SOCE [128]. In future, it could be important to investigate whether the observed functions of TRPM7-kinase mediated SOCE in mouse cells could be applied to humans.

*Rag1*$^{-/-}$ mice lacking both mature T and B cells are profoundly protected from experimentally induced focal cerebral ischemia [129]. Adoptive transfer of T cells, but not of B cells, could fully restore the susceptibility to stroke progression. Importantly, platelet activation pathways were unaltered in *Rag1*$^{-/-}$ mice [129], suggesting that the observed effect was not linked to altered thrombus formation. However, adoptive transfer of T cells into platelet-depleted *Rag1*$^{-/-}$ mice did not result in increased infarct sizes [129], pointing to a crosstalk between platelets and T cells in the progression of ischemic stroke.

Regulatory T ($T_{reg}$) cells are a subset of T lymphocytes that regulate immune homeostasis by maintaining immunological self-tolerance. The development of such cells is dependent on the forkhead box P3 (Foxp3) transcription factor [130]. Kleinschnitz et al., showed that $Foxp3^+$ $T_{reg}$ cells can interact with cerebral endothelial cells and platelets, thereby promoting ischemic neurodegeneration after the ischemic insult [131]. In mice, inactivation of the *Stim1/Stim2* genes significantly diminished the population of $Foxp3^+$ $T_{reg}$ cells in the thymus and lymphoid organs [59]. However, the $T_{reg}$ cells developed normally in *Stim1*$^{-/-}$ or *Orai1*$^{-/-}$ mice, which may point to a role of STIM2 backing up the SOCE activity for $T_{reg}$ cell development [99,132]. On other hand, SOCE was shown to regulate $T_{reg}$ suppressive functions. Thus, the few $CD4^+CD25^+$ Foxp3 $T_{reg}$ cells deficient in both STIM1 and STIM2 were unable to suppress the proliferation of wild-type $CD4^+$ T cells [101]. $T_{reg}$ cells isolated from STIM1- or ORAI1-deficient animals displayed only a moderate reduction in this suppressive activity [99,132]. Altogether, these data point on potential crosstalk between STIM1 and STIM2 in the regulation of $T_{reg}$ cell function.

Although T cells are important players of thrombo-inflammatory diseases, the role of SOCE-mediated T-cell functions in such conditions is not established. Moreover, T cell-endothelial cell-platelet interactions may influence thrombo-inflammatory process, it appears interesting whether this cellular crosstalk may be regulated by SOCE.

## 6. Therapeutic potential of SOCE inhibitors for thrombo-inflammatory diseases

While a range of synthetic pharmacological compounds are known to inhibit the channels responsible for SOCE, several of these can also affect the activities of other, non-SOC channels. One of the most widely studied drugs is 2 APB. This compound was discovered as a non-competitive lipophilic antagonist of the reticular $IP_3$ receptors, inhibiting $Ca^{2+}$ store depletion, but in addition it attenuates SOCE and the TRPC3 channel in the plasma membrane [133,134]. Subsequently, it appeared that 2 APB can also decrease the activities of TRPM6 and TRPM7 channels in various cell types [135]. Interestingly, at high concentrations, 2 APB inhibits ORAI1 and ORAI2, but stimulates ORAI3 [136]. Recently, Wei et al., showed that 2 APB has also inhibitory effects on functional coupling between STIM1 and ORAI1 [137]. Treatment with 2 APB reduced platelet responses and partially protected mice from cerebral infarct progression in the tMCAO model [79], but the target of the compound responsible for these effects has not been established. 2 APB may also influence many functions in immune cells. In mast cells, 2 APB inhibited cell degranulation and release of inflammatory cytokines, such as TNFα and IL-4 [138]. In addition, in bone marrow-derived macrophages and dendritic cells, 2 APB strongly impaired many effector functions, including phagocytosis, inflammasome activation and priming of T-cells [116]. In contrast, macrophages and dendritic cells from mice with conditional deletion of *Stim1* and *Stim2* genes displayed no major functional defects [116], suggesting that in these cells 2 APB-induced effects occur in SOCE-independent manner.

Another well-studied SOCE blocker is the synthetic estrogen, diethylstilbestrol (DES), which was discovered as an agonist of estrogen receptors [139]. Initially, DES was used as a therapeutic agent for women, for whom estrogen replacement therapy was indicated. DES was also shown to upregulate IFNγ production in splenic lymphocytes [140]. Later, DES was found to inhibit SOCE in platelets and vascular smooth muscle cells [141].

3,5-Bis(trifluoromethyl) pyrazole derivative (BTP2) inhibits SOCE in platelets and macrophages. In primary macrophages, BTP2-inhibited SOCE occurred through the suppression of Fcγ receptor-mediated signalling. Treatment with BTP2 strongly attenuated skin and lung inflammation in mice [142]. The compound was found to suppress anti-CD3 antibody-induced $Ca^{2+}$ mobilisation in T cells, thereby affecting cytokine production and cell proliferation [143–145], but the underlying molecular mechanisms are still unknown. Besides ORAI, BTP2 may also suppress the channel activity of TRPC3 and TRPC5 [146], although it may activate TRPM4 channels [147]. These diverse actions of BTP2 on cation homeostasis suggest that immune-modulatory effects will depend on the channel composites of the investigated cell types.

A pyrazole derivative, GSK-7975 A, has been described to inhibit ORAI-mediated SOCE, likely by blocking the pore-forming unit of the channel [148,149]. In human embryonic kidney 293 (HEK293) cells expressing the $ORAI^{E106D}$ variant, modifying the structure of the channel pore, it appeared that the inhibitory potency of GSK-7975 A on SOCE was significantly reduced [149]. Interestingly, in HEK293 cells, GSK-7975 A was also found to inhibit TRPV6 channels [148,149]. In addition, van Kruchten et al., showed that GSK-7975 A, like a novel compound Synta66, inhibited platelet-dependent coagulation and thrombus formation as potently as 2 APB [79].

Altogether these studies indicate that SOCE inhibitors influence many functions of platelets and immune cells, which may contribute to thrombo-inflammation. Future studies are needed to investigate the mechanisms induced by SOCE inhibitors in more details.

## 7. Conclusions

In this review we summarised important aspects of the complex roles of SOCE in platelets and immune cells. In platelets, SOCE is mainly regulated by STIM1 and ORAI1, whereas other isoforms have less prominent roles in these cells in thrombosis and haemostasis. Studies using knock-out mice indicated that in platelets inhibition of ORAI1-mediated SOCE may present an attractive strategy to prevent arterial thrombosis and ischemic stroke. In immune cells, inhibition of SOCE protected mice from various inflammatory diseases. A better understanding of fine-tuning of SOCE in thrombosis and thrombo-inflammation is required to address a large panel of therapeutic strategies for selective SOC channel inhibition in both platelets and immune cells.

## Disclosure

The authors report no conflicts of interest

## Acknowledgements

E.M.B., B.N. and A.B. are supported by the Deutsche Forschungsgemeinschaft (SFB/TR 240). M.N. and J.W.M.H. acknowledge support from the Cardiovascular Center (HVC), Maastricht University Medical Center$^{+}$, and the 5$^{th}$ Meuse-Rhine Interregional Programme Polyvalve.

## References

[1] J.W. Putney, Origins of the concept of store-operated calcium entry, Front. Biosci. (Schol Ed.) 3 (2011) 980–984.
[2] P.B. Stathopulos, M. Ikura, Store operated calcium entry: from concept to structural mechanisms, Cell Calcium 63 (2017) 3–7.
[3] D.E. Clapham, Calcium signaling, Cell 131 (6) (2007) 1047–1058.
[4] D. Varga-Szabo, A. Braun, B. Nieswandt, Calcium signaling in platelets, J. Thromb. Haemost. 7 (7) (2009) 1057–1066.
[5] M. Muik, R. Schindl, M. Fahrner, C. Romanin, Ca(2+) release-activated Ca(2+) (CRAC) current, structure, and function, Cell. Mol. Life Sci. 69 (24) (2012) 4163–4176.
[6] A.B. Parekh, J.W. Putney Jr, Store-operated calcium channels, Physiol. Rev. 85 (2) (2005) 757–810.
[7] J. Liou, et al., STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx, Curr. Biol. 15 (13) (2005) 1235–1241.
[8] J. Roos, et al., STIM1, an essential and conserved component of store-operated Ca2+ channel function, J. Cell Biol. 169 (3) (2005) 435–445.
[9] S.L. Zhang, et al., STIM1 is a Ca2+ sensor that activates CRAC channels and migrates from the Ca2+ store to the plasma membrane, Nature 437 (7060) (2005) 902–905.
[10] M. Vig, et al., CRACM1 is a plasma membrane protein essential for store-operated Ca2+ entry, Science 312 (5777) (2006) 1220–1223.
[11] S. Feske, et al., A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function, Nature 441 (7090) (2006) 179–185.
[12] M. Prakriya, et al., Orai1 is an essential pore subunit of the CRAC channel, Nature 443 (7108) (2006) 230–233.
[13] A.V. Yeromin, et al., Molecular identification of the CRAC channel by altered ion selectivity in a mutant of Orai, Nature 443 (7108) (2006) 226–229.
[14] M. Vig, et al., Defective mast cell effector functions in mice lacking the CRACM1 pore subunit of store-operated calcium release-activated calcium channels, Nat. Immunol. 9 (1) (2008) 89–96.
[15] Y. Gwack, et al., Hair loss and defective T- and B-cell function in mice lacking ORAI1, Mol. Cell. Biol. 28 (17) (2008) 5209–5222.
[16] P.J. Shaw, S. Feske, Physiological and pathophysiological functions of SOCE in the immune system, Front. Biosci. (Elite Ed.) 4 (2012) 2253–2268.
[17] S. Bertin, E. Raz, Transient receptor potential (TRP) channels in T cells, Semin. Immunopathol. 38 (3) (2016) 309–319.
[18] S. Feske, ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca2+ entry in the immune system and beyond, Immunol. Rev. 231 (1) (2009) 189–209.
[19] A. Braun, T. Vogtle, D. Varga-Szabo, B. Nieswandt, STIM and Orai in hemostasis and thrombosis, Front. Biosci. (Landmark Ed.) 16 (2011) 2144–2160.
[20] D. Varga-Szabo, A. Braun, B. Nieswandt, STIM and Orai in platelet function, Cell Calcium 50 (3) (2011) 270–278.
[21] H.H. Versteeg, J.W. Heemskerk, M. Levi, P.H. Reitsma, New fundamentals in hemostasis, Physiol. Rev. 93 (1) (2013) 327–358.
[22] B. Nieswandt, I. Pleines, M. Bender, Platelet adhesion and activation mechanisms in arterial thrombosis and ischaemic stroke, J. Thromb. Haemost. 9 (Suppl 1) (2011) 92–104.
[23] B. Nieswandt, C. Kleinschnitz, G. Stoll, Ischaemic stroke: a thrombo-inflammatory disease? J. Physiol. 589 (17) (2011) 4115–4123.
[24] S.P. Jackson, Arterial thrombosis–insidious, unpredictable and deadly, Nat. Med. 17 (11) (2011) 1423–1436.
[25] T. Vogtle, D. Cherpokova, M. Bender, B. Nieswandt, Targeting platelet receptors in thrombotic and thrombo-inflammatory disorders, Hamostaseologie 35 (3) (2015) 235–243.
[26] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics,

homeostasis and remodelling, Nat. Rev. Mol. Cell Biol. 4 (7) (2003) 517–529.
[27] J.W. Heemskerk, N.J. Mattheij, J.M. Cosemans, Platelet-based coagulation: different populations, different functions, J. Thromb. Haemost. 11 (1) (2013) 2–16.
[28] L. Lian, et al., The relative role of PLCbeta and PI3Kgamma in platelet activation, Blood 106 (1) (2005) 110–117.
[29] F. Swieringa, M.J. Kuijpers, J.W. Heemskerk, P.E. van der Meijden, Targeting platelet receptor function in thrombus formation: the risk of bleeding, Blood Rev. 28 (1) (2014) 9–21.
[30] S.P. Watson, J.M. Auger, O.J. McCarty, A.C. Pearce, GPVI and integrin alphaIIb beta3 signaling in platelets, J. Thromb. Haemost. 3 (8) (2005) 1752–1762.
[31] B. Nieswandt, S.P. Watson, Platelet-collagen interaction: is GPVI the central receptor? Blood 102 (2) (2003) 449–461.
[32] B. Brune, V. Ullrich, Different calcium pools in human platelets and their role in thromboxane A2 formation, J. Biol. Chem. 266 (29) (1991) 19232–19237.
[33] L. Cavallini, M. Coassin, A. Alexandre, Two classes of agonist-sensitive Ca2+ stores in platelets, as identified by their differential sensitivity to 2,5-di-(tert-butyl)-1,4-benzohydroquinone and thapsigargin, Biochem. J. 310 (Pt 2) (1995) 449–452.
[34] B. Papp, et al., Demonstration of two forms of calcium pumps by thapsigargin inhibition and radioimmunoblotting in platelet membrane vesicles, J. Biol. Chem. 266 (22) (1991) 14593–14596.
[35] J. Enouf, et al., Human platelets express the SERCA2-b isoform of Ca(2+)-transport ATPase, Biochem. J. 286 (Pt 1) (1992) 135–140.
[36] F. Wuytack, et al., A sarco/endoplasmic reticulum Ca(2+)-ATPase 3-type Ca2+ pump is expressed in platelets, in lymphoid cells, and in mast cells, J. Biol. Chem. 269 (2) (1994) 1410–1416.
[37] R. Bobe, et al., The rat platelet 97-kDa Ca2+ATPase isoform is the sarcoendoplasmic reticulum Ca2+ATPase 3 protein, J. Biol. Chem. 269 (2) (1994) 1417–1424.
[38] M. Vig, et al., CRACM1 multimers form the ion-selective pore of the CRAC channel, Curr. Biol. 16 (20) (2006) 2073–2079.
[39] A. Braun, et al., Orai1 (CRACM1) is the platelet SOC channel and essential for pathological thrombus formation, Blood 113 (9) (2009) 2056–2063.
[40] G. Tolhurst, et al., Expression profiling and electrophysiological studies suggest a major role for Orai1 in the store-operated Ca2+ influx pathway of platelets and megakaryocytes, Platelets 19 (4) (2008) 308–313.
[41] W. Bergmeier, et al., R93W mutation in Orai1 causes impaired calcium influx in platelets, Blood 113 (3) (2009) 675–678.
[42] K. Gilio, et al., Roles of platelet STIM1 and Orai1 in glycoprotein VI- and thrombin-dependent procoagulant activity and thrombus formation, J. Biol. Chem. 285 (31) (2010) 23629–23638.
[43] B. Nilius, G. Owsianik, The transient receptor potential family of ion channels, Genome Biol. 12 (3) (2011) 218.
[44] R.N. Carter, et al., Molecular and electrophysiological characterization of transient receptor potential ion channels in the primary murine megakaryocyte, J. Physiol. 576 (Pt 1) (2006) 151–162.
[45] E. den Dekker, et al., Expression of transient receptor potential mRNA isoforms and Ca(2+) influx in differentiating human stem cells and platelets, Biochim. Biophys. Acta 1539 (3) (2001) 243–255.
[46] K.S. Authi, TRP channels in platelet function, Handb. Exp. Pharmacol. 179 (2007) 425–443.
[47] S.L. Brownlow, S.O. Sage, Transient receptor potential protein subunit assembly and membrane distribution in human platelets, Thromb. Haemost. 94 (4) (2005) 839–845.
[48] S.R. Hassock, M.X. Zhu, C. Trost, V. Flockerzi, K.S. Authi, Expression and role of TRPC proteins in human platelets: evidence that TRPC6 forms the store-independent calcium entry channel, Blood 100 (8) (2002) 2801–2811.
[49] I. Jardin, P.C. Redondo, G.M. Salido, J.A. Rosado, Phosphatidylinositol 4,5-bisphosphate enhances store-operated calcium entry through hTRPC6 channel in human platelets, Biochim. Biophys. Acta 1783 (1) (2008) 84–97.
[50] J.A. Rosado, S.O. Sage, Coupling between inositol 1,4,5-trisphosphate receptors and human transient receptor potential channel 1 when intracellular Ca2+ stores are depleted, Biochem. J. 350 (Pt 3) (2000) 631–635.
[51] J.A. Rosado, S. Jenner, S.O. Sage, A role for the actin cytoskeleton in the initiation and maintenance of store-mediated calcium entry in human platelets. Evidence for conformational coupling, J. Biol. Chem. 275 (11) (2000) 7527–7533.
[52] D. Varga-Szabo, et al., Store-operated Ca(2+) entry in platelets occurs independently of transient receptor potential (TRP) C1, Pflugers Arch. 457 (2) (2008) 377–387.
[53] J.A. Rosado, S.O. Sage, Activation of store-mediated calcium entry by secretion-like coupling between the inositol 1,4,5-trisphosphate receptor type II and human transient receptor potential (hTrp1) channels in human platelets, Biochem. J. 356 (Pt 1) (2001) 191–198.
[54] H.L. Ong, et al., Specific detection of the endogenous transient receptor potential (TRP)-1 protein in liver and airway smooth muscle cells using immunoprecipitation and Western-blot analysis, Biochem. J. 364 (Pt 3) (2002) 641–648.
[55] B. Minke, TRP channels and Ca2+ signaling, Cell Calcium 40 (3) (2006) 261–275.
[56] E. Carafoli, L. Santella, D. Branca, M. Brini, Generation, control, and processing of cellular calcium signals, Crit. Rev. Biochem. Mol. Biol. 36 (2) (2001) 107–260.
[57] J. Grosse, et al., An EF hand mutation in Stim1 causes premature platelet activation and bleeding in mice, J. Clin. Invest. 117 (11) (2007) 3540–3550.
[58] D. Varga-Szabo, et al., The calcium sensor STIM1 is an essential mediator of arterial thrombosis and ischemic brain infarction, J. Exp. Med. 205 (7) (2008) 1583–1591.
[59] M. Oh-Hora, et al., Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance, Nat. Immunol. 9 (4) (2008) 432–443.
[60] Y. Baba, et al., Essential function for the calcium sensor STIM1 in mast cell activation and anaphylactic responses, Nat. Immunol. 9 (1) (2008) 81–88.
[61] K. Wolf, et al., Partially defective store operated calcium entry and hem(ITAM) signaling in platelets of serotonin transporter deficient mice, PLoS One 11 (1) (2016) e0147664.
[62] S.K. Gotru, et al., TRPM7 kinase controls calcium responses in arterial thrombosis and stroke in mice, Arterioscler. Thromb. Vasc. Biol. 38 (2) (2018) 344–352.
[63] T. Kawasaki, T. Ueyama, I. Lange, S. Feske, N. Saito, Protein kinase C-induced phosphorylation of Orai1 regulates the intracellular Ca2+ level via the store-operated Ca2+ channel, J. Biol. Chem. 285 (33) (2010) 25720–25730.
[64] E. Pozo-Guisado, F.J. Martin-Romero, The regulation of STIM1 by phosphorylation, Commun. Integr. Biol. 6 (6) (2013) e26283.
[65] M. Nishida, et al., Phosphorylation of TRPC6 channels at Thr69 is required for anti-hypertrophic effects of phosphodiesterase 5 inhibition, J. Biol. Chem. 285 (17) (2010) 13244–13253.
[66] S.M. Bousquet, M. Monet, G. Boulay, Protein kinase C-dependent phosphorylation of transient receptor potential canonical 6 (TRPC6) on serine 448 causes channel inhibition, J. Biol. Chem. 285 (52) (2010) 40534–40543.
[67] E.V. Paez Espinosa, J.P. Murad, H.J. Ting, F.T. Khasawneh, Mouse transient receptor potential channel 6: role in hemostasis and thrombogenesis, Biochem. Biophys. Res. Commun. 417 (2) (2012) 853–856.
[68] G. Ramanathan, et al., Defective diacylglycerol-induced Ca2+ entry but normal agonist-induced activation responses in TRPC6-deficient mouse platelets, J. Thromb. Haemost. 10 (3) (2012) 419–429.
[69] W. Chen, et al., Orai1-induced store-operated Ca(2+) entry enhances phospholipase activity and modulates canonical transient receptor potential channel 6 function in murine platelets, J. Thromb. Haemost. 12 (4) (2014) 528–539.
[70] C. Gachet, Regulation of platelet functions by P2 receptors, Annu. Rev. Pharmacol. Toxicol. 46 (2006) 277–300.
[71] C. Gachet, P2 receptors, platelet function and pharmacological implications, Thromb. Haemost. 99 (3) (2008) 466–472.
[72] M.P. Mahaut-Smith, K.A. Taylor, R.J. Evans, Calcium signalling through ligand-gated ion channels such as P2X1 receptors in the platelet and other non-excitable cells, Adv. Exp. Med. Biol. 898 (2016) 305–329.
[73] B. Hechler, et al., A role of the fast ATP-gated P2X1 cation channel in thrombosis of small arteries in vivo, J. Exp. Med. 198 (4) (2003) 661–667.
[74] C. Oury, et al., Overexpression of the platelet P2X1 ion channel in transgenic mice generates a novel prothrombotic phenotype, Blood 101 (10) (2003) 3969–3976.
[75] B. Hechler, C. Gachet, Purinergic receptors in thrombosis and inflammation, Arterioscler. Thromb. Vasc. Biol. 35 (11) (2015) 2307–2315.
[76] C. Oury, C. Lecut, A. Hego, O. Wera, C. Delierneux, Purinergic control of inflammation and thrombosis: role of P2X1 receptors, Comput. Struct. Biotechnol. J. 13 (2015) 106–110.
[77] B. Hechler, et al., Inhibition of platelet functions and thrombosis through selective or nonselective inhibition of the platelet P2 receptors with increasing doses of NF449 [4,4',4'',4'''-(carbonylbis(imino-5,1,3-benzenetriylbis-(carbonylimino))) tetrakis -benzene-1,3-disulfonic acid octasodium salt], J. Pharmacol. Exp. Ther. 314 (1) (2005) 232–243.
[78] E. Toth-Zsamboki, et al., P2X1-mediated ERK2 activation amplifies the collagen-induced platelet secretion by enhancing myosin light chain kinase activation, J. Biol. Chem. 278 (47) (2003) 46661–46667.
[79] R. van Kruchten, et al., Antithrombotic potential of blockers of store-operated calcium channels in platelets, Arterioscler. Thromb. Vasc. Biol. 32 (7) (2012) 1717–1723.
[80] F. Ahmad, et al., Relative contributions of stromal interaction molecule 1 and CalDAG-GEFI to calcium-dependent platelet activation and thrombosis, J. Thromb. Haemost. 9 (10) (2011) 2077–2086.
[81] R.S. Lacruz, S. Feske, Diseases caused by mutations in ORAI1 and STIM1, Ann. N. Y. Acad. Sci. 1356 (2015) 45–79.
[82] M. Nagy, et al., Variable impairment of platelet functions in patients with severe, genetically linked immune deficiencies, Haematologica 103 (3) (2018) 540–549.
[83] D. Misceo, et al., A dominant STIM1 mutation causes Stormorken syndrome, Hum. Mutat. 35 (5) (2014) 556–564.
[84] G. Morin, et al., Gain-of-function mutation in STIM1 (P.R304W) is associated with stormorken syndrome, Hum. Mutat. 35 (10) (2014) 1221–1232.
[85] T. Markello, et al., York platelet syndrome is a CRAC channelopathy due to gain-of-function mutations in STIM1, Mol. Genet. Metab. 114 (3) (2015) 474–482.
[86] W.M. Clark, N.S. Lessov, M.P. Dixon, F. Eckenstein, Monofilament intraluminal middle cerebral artery occlusion in the mouse, Neurol. Res. 19 (6) (1997) 641–648.
[87] P. Kraft, B. Nieswandt, G. Stoll, C. Kleinschnitz, Acute ischemic stroke. New approaches to antithrombotic treatment, Nervenarzt 83 (4) (2012) 435–449.
[88] A. Berna-Erro, et al., STIM2 regulates capacitive Ca2+ entry in neurons and plays a key role in hypoxic neuronal cell death, Sci. Signal. 2 (93) (2009) ra67.
[89] S.F. De Meyer, et al., Thromboinflammation in stroke brain damage, Stroke 47 (4) (2016) 1165–1172.
[90] I. Induruwa, S.M. Jung, E.A. Warburton, Beyond antiplatelets: the role of glycoprotein VI in ischemic stroke, Int. J. Stroke 11 (6) (2016) 618–625.
[91] S. Dutting, M. Bender, B. Nieswandt, Platelet GPVI: a target for antithrombotic therapy?!, Trends Pharmacol. Sci. 33 (11) (2012) 583–590.
[92] G. Stoll, C. Kleinschnitz, B. Nieswandt, Combating innate inflammation: a new paradigm for acute treatment of stroke? Ann. N. Y. Acad. Sci. 1207 (2010) 149–154.
[93] C.J. Frijns, L.J. Kappelle, Inflammatory cell adhesion molecules in ischemic cerebrovascular disease, Stroke 33 (8) (2002) 2115–2122.

[94] G.C. Jickling, et al., Targeting neutrophils in ischemic stroke: translational insights from experimental studies, J. Cereb. Blood Flow Metab. 35 (6) (2015) 888–901.
[95] D.M. Hermann, C. Kleinschnitz, M. Gunzer, Implications of polymorphonuclear neutrophils for ischemic stroke and intracerebral hemorrhage: predictive value, pathophysiological consequences and utility as therapeutic target, J. Neuroimmunol. 321 (2018) 138–143.
[96] M. Mezger, et al., Platelets and vascular inflammation of the brain, Hamostaseologie 35 (3) (2015) 244–251.
[97] S. Brechard, S. Plancon, C. Melchior, E.J. Tschirhart, STIM1 but not STIM2 is an essential regulator of Ca2+ influx-mediated NADPH oxidase activity in neutrophil-like HL-60 cells, Biochem. Pharmacol. 78 (5) (2009) 504–513.
[98] R.A. Clemens, J. Chong, D. Grimes, Y. Hu, C.A. Lowell, STIM1 and STIM2 cooperatively regulate mouse neutrophil store-operated calcium entry and cytokine production, Blood 130 (13) (2017) 1565–1577.
[99] C.A. McCarl, et al., Store-operated Ca2+ entry through ORAI1 is critical for T cell-mediated autoimmunity and allograft rejection, J. Immunol. 185 (10) (2010) 5845–5858.
[100] F. Le Deist, et al., A primary T-cell immunodeficiency associated with defective transmembrane calcium influx, Blood 85 (4) (1995) 1053–1062.
[101] C.A. McCarl, et al., ORAI1 deficiency and lack of store-operated Ca2+ entry cause immunodeficiency, myopathy, and ectodermal dysplasia, J. Allergy Clin. Immunol. 124 (6) (2009) 1311–1318 e1317.
[102] U.Y. Schaff, et al., Orai1 regulates intracellular calcium, arrest, and shape polarization during neutrophil recruitment in shear flow, Blood 115 (3) (2010) 657–666.
[103] N. Steinckwich, V. Schenten, C. Melchior, S. Brechard, E.J. Tschirhart, An essential role of STIM1, Orai1, and S100A8-A9 proteins for Ca2+ signaling and FcgammaR-mediated phagosomal oxidative activity, J. Immunol. 186 (4) (2011) 2182–2191.
[104] S. Brechard, C. Melchior, S. Plancon, V. Schenten, E.J. Tschirhart, Store-operated Ca2+ channels formed by TRPC1, TRPC6 and Orai1 and non-store-operated channels formed by TRPC3 are involved in the regulation of NADPH oxidase in HL-60 granulocytes, Cell Calcium 44 (5) (2008) 492–506.
[105] W. Schorr, D. Swandulla, H.U. Zeilhofer, Mechanisms of IL-8-induced Ca2+ signaling in human neutrophil granulocytes, Eur. J. Immunol. 29 (3) (1999) 897–904.
[106] D.B. Graham, et al., Neutrophil-mediated oxidative burst and host defense are controlled by a Vav-PLCgamma2 signaling axis in mice, J. Clin. Invest. 117 (11) (2007) 3445–3452.
[107] C. Hellberg, L. Molony, L. Zheng, T. Andersson, Ca2+ signalling mechanisms of the beta 2 integrin on neutrophils: involvement of phospholipase C gamma 2 and Ins(1,4,5)P3, Biochem. J. 317 (Pt 2) (1996) 403–409.
[108] N. Dixit, et al., Leukocyte function antigen-1, kindlin-3, and calcium flux orchestrate neutrophil recruitment during inflammation, J. Immunol. 189 (12) (2012) 5954–5964.
[109] H. Zhang, et al., STIM1 calcium sensor is required for activation of the phagocyte oxidase during inflammation and host defense, Blood 123 (14) (2014) 2238–2249.
[110] M. Koupenova, L. Clancy, H.A. Corkrey, J.E. Freedman, Circulating platelets as mediators of immunity, inflammation, and thrombosis, Circ. Res. 122 (2) (2018) 337–351.
[111] J.A. Giles, et al., Neutrophil infiltration to the brain is platelet-dependent, and is reversed by blockade of platelet GPIbalpha, Immunology 154 (2) (2018) 322–328.
[112] C.H. Wang, et al., CD147 up-regulates calcium-induced chemotaxis, adhesion ability and invasiveness of human neutrophils via a TRPM-7-mediated mechanism, Rheumatology (Oxford) 53 (12) (2014) 2288–2296.
[113] P.J. Murray, T.A. Wynn, Protective and pathogenic functions of macrophage subsets, Nat. Rev. Immunol. 11 (11) (2011) 723–737.
[114] A. Braun, et al., STIM1 is essential for Fcgamma receptor activation and autoimmune inflammation, Blood 113 (5) (2009) 1097–1104.
[115] G. Sogkas, et al., Cooperative and alternate functions for STIM1 and STIM2 in macrophage activation and in the context of inflammation, Immun. Inflamm. Dis. 3 (3) (2015) 154–170.
[116] M. Vaeth, et al., Ca2+ signaling but not store-operated Ca2+ entry is required for the function of macrophages and dendritic cells, J. Immunol. 195 (3) (2015) 1202–1217.
[117] M.S. Schappe, et al., Chanzyme TRPM7 mediates the Ca(2+) influx essential for lipopolysaccharide-induced toll-like receptor 4 endocytosis and macrophage activation, Immunity 48 (1) (2018) 59–74 e55.
[118] S. Fumagalli, C. Perego, F. Pischiutta, E.R. Zanier, M.G. De Simoni, The ischemic environment drives microglia and macrophage function, Front. Neurol. 6 (2015) 81.
[119] G. Kronenberg, et al., Distinguishing features of microglia- and monocyte-derived macrophages after stroke, Acta Neuropathol. 135 (4) (2018) 551–568.
[120] S. Feske, R. Draeger, H.H. Peter, K. Eichmann, A. Rao, The duration of nuclear residence of NFAT determines the pattern of cytokine expression in human SCID T cells, J. Immunol. 165 (1) (2000) 297–305.
[121] S. Feske, J. Giltnane, R. Dolmetsch, L.M. Staudt, A. Rao, Gene regulation mediated by calcium signals in T lymphocytes, Nat. Immunol. 2 (4) (2001) 316–324.
[122] J. Ma, C.A. McCarl, S. Khalil, K. Luthy, S. Feske, T-cell-specific deletion of STIM1 and STIM2 protects mice from EAE by impairing the effector functions of Th1 and Th17 cells, Eur. J. Immunol. 40 (11) (2010) 3028–3042.
[123] M.K. Schuhmann, et al., Stromal interaction molecules 1 and 2 are key regulators of autoreactive T cell activation in murine autoimmune central nervous system inflammation, J. Immunol. 184 (3) (2010) 1536–1542.
[124] M. Vaeth, et al., ORAI2 modulates store-operated calcium entry and T cell-mediated immunity, Nat. Commun. 8 (2017) 14714.
[125] S. Zierler, et al., Waixenicin A inhibits cell proliferation through magnesium-dependent block of transient receptor potential melastatin 7 (TRPM7) channels, J. Biol. Chem. 286 (45) (2011) 39328–39335.
[126] Z. Kuras, Y.H. Yun, A.A. Chimote, L. Neumeier, L. Conforti, KCa3.1 and TRPM7 channels at the uropod regulate migration of activated human T cells, PLoS One 7 (8) (2012) e43859.
[127] A. Romagnani, et al., TRPM7 kinase activity is essential for T cell colonization and alloreactivity in the gut, Nat. Commun. 8 (1) (2017) 1917.
[128] P. Beesetty, et al., Inactivation of TRPM7 kinase in mice results in enlarged spleens, reduced T-cell proliferation and diminished store-operated calcium entry, Sci. Rep. 8 (1) (2018) 3023.
[129] C. Kleinschnitz, et al., Early detrimental T-cell effects in experimental cerebral ischemia are neither related to adaptive immunity nor thrombus formation, Blood 115 (18) (2010) 3835–3842.
[130] S. Sakaguchi, M. Miyara, C.M. Costantino, D.A. Hafler, FOXP3+ regulatory T cells in the human immune system, Nat. Rev. Immunol. 10 (7) (2010) 490–500.
[131] C. Kleinschnitz, et al., Regulatory T cells are strong promoters of acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature, Blood 121 (4) (2013) 679–691.
[132] N. Beyersdorf, et al., STIM1-independent T cell development and effector function in vivo, J. Immunol. 182 (6) (2009) 3390–3397.
[133] H.T. Ma, et al., Requirement of the inositol trisphosphate receptor for activation of store-operated Ca2+ channels, Science 287 (5458) (2000) 1647–1651.
[134] K.T. Ma, B.C. Guan, Y.Q. Yang, A.L. Nuttall, Z.G. Jiang, 2-Aminoethoxydiphenyl borate blocks electrical coupling and inhibits voltage-gated K+ channels in guinea pig arteriole cells, Am. J. Physiol. Heart Circ. Physiol. 300 (1) (2011) H335–346.
[135] M. Li, J. Jiang, L. Yue, Functional characterization of homo- and heteromeric channel kinases TRPM6 and TRPM7, J. Gen. Physiol. 127 (5) (2006) 525–537.
[136] A. Lis, et al., CRACM1, CRACM2, and CRACM3 are store-operated Ca2+ channels with distinct functional properties, Curr. Biol. 17 (9) (2007) 794–800.
[137] M. Wei, et al., Molecular mechanisms underlying inhibition of STIM1-Orai1-mediated Ca(2+) entry induced by 2-aminoethoxydiphenyl borate, Pflugers Arch. 468 (11-12) (2016) 2061–2074.
[138] N.M. Ng, S.P. Jiang, W. Zhang, 2-Aminoethoxydiphenyl borate reduces degranulation and release of cytokines in a rat mast cell line, Eur. Rev. Med. Pharmacol. Sci. 16 (8) (2012) 1017–1021.
[139] K.S. Korach, M. Metzler, J.A. McLachlan, Estrogenic activity in vivo and in vitro of some diethylstilbestrol metabolites and analogs, Proc Natl Acad Sci U S A 75 (1) (1978) 468–471.
[140] E. Karpuzoglu-Sahin, B.D. Hissong, S. Ansar Ahmed, Interferon-gamma levels are upregulated by 17-beta-estradiol and diethylstilbestrol, J. Reprod. Immunol. 52 (1-2) (2001) 113–127.
[141] S.I. Zakharov, et al., Diethylstilbestrol is a potent inhibitor of store-operated channels and capacitative Ca(2+) influx, Mol. Pharmacol. 66 (3) (2004) 702–707.
[142] G. Sogkas, E. Rau, F. Atschekzei, S.N. Syed, R.E. Schmidt, The pyrazole derivative BTP2 attenuates IgG immune complex-induced inflammation, Inflammation 41 (1) (2018) 42–49.
[143] K. Ohga, R. Takezawa, Y. Arakida, Y. Shimizu, J. Ishikawa, Characterization of YM-58483/BTP2, a novel store-operated Ca2+ entry blocker, on T cell-mediated immune responses in vivo, Int. Immunopharmacol. 8 (13-14) (2008) 1787–1792.
[144] T. Yoshino, et al., YM-58483, a selective CRAC channel inhibitor, prevents antigen-induced airway eosinophilia and late phase asthmatic responses via Th2 cytokine inhibition in animal models, Eur. J. Pharmacol. 560 (2-3) (2007) 225–233.
[145] E. Jara, et al., Delphinidin activates NFAT and induces IL-2 production through SOCE in T cells, Cell Biochem. Biophys. 68 (3) (2014) 497–509.
[146] L.P. He, T. Hewavitharana, J. Soboloff, M.A. Spassova, D.L. Gill, A functional link between store-operated and TRPC channels revealed by the 3,5-bis(trifluoromethyl)pyrazole derivative, BTP2, J. Biol. Chem. 280 (12) (2005) 10997–11006.
[147] R. Takezawa, et al., A pyrazole derivative potently inhibits lymphocyte Ca2+ influx and cytokine production by facilitating transient receptor potential melastatin 4 channel activity, Mol. Pharmacol. 69 (4) (2006) 1413–1420.
[148] G. Owsianik, D. D'Hoedt, T. Voets, B. Nilius, Structure-function relationship of the TRP channel superfamily, Rev. Physiol. Biochem. Pharmacol. 156 (2006) 61–90.
[149] I. Derler, et al., The action of selective CRAC channel blockers is affected by the Orai pore geometry, Cell Calcium 53 (2) (2013) 139–151.