A critical appraisal of the role of intracellular Ca^{2+} -signaling pathways in Kawasaki disease

Jeroen Bijmens, Ludwig Missiaen, Geert Bultynck, Jan B. Parys*

KU Leuven, Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine, B-3000 Leuven, Belgium

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ABSTRACT

Kawasaki disease is a multi-systemic vasculitis that generally occurs in children and that can lead to coronary artery lesions. Recent studies showed that Kawasaki disease has an important genetic component. In this review, we discuss the single-nucleotide polymorphisms in the genes encoding proteins with a role in intracellular Ca^{2+} signaling: inositol 1,4,5-trisphosphate 3-kinase C, caspase-3, the store-operated Ca^{2+} -entry channel ORAI1, the type-3 inositol 1,4,5-trisphosphate receptor, the $\text{Na}^+/\text{Ca}^{2+}$ exchanger 1, and phospholipase CB4 and CB1. An increase of the free cytosolic Ca^{2+} concentration is proposed to be a major factor in susceptibility to Kawasaki disease and disease outcome, but only for polymorphisms in the genes encoding the inositol 1,4,5-trisphosphate 3-kinase C and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger 1, the free cytosolic Ca^{2+} concentration was actually measured and shown to be increased. Excessive cytosolic Ca^{2+} signaling can result in hyperactive calcineurin in T cells with an overstimulated nuclear factor of activated T cells pathway, in hypersecretion of interleukin-18 and tumor necrosis factor- α by monocytes/macrophages, in increased urotensin-2 signaling, and in an overactivation of vascular endothelial cells.

1. Introduction

Kawasaki disease (KD) was first described by the pediatrician Tomisaku Kawasaki in Japanese in 1967 and in English in 1974 [1,2]. KD occurs worldwide, but is especially frequent in Southeast Asia. The prevalence of KD in children younger than 5 years is the highest in Japan, followed by Korea and Taiwan. The incidence in Japan is 265/100,000 [3] and more than 1 in every 100 Japanese children will develop KD [4]. The incidence is 10–20 times higher than in Western countries. The incidence rate and the number of patients with KD are increasing steadily.

KD is an acute multi-systemic self-limited vasculitis of the small- and medium-sized arteries that predominantly occurs in infants and children under 5 years old. The disease is characterized in its acute phase by persistent high fever for at least 5 days and a multi-system inflammation with a polymorphous red skin rash, bilateral bulbar conjunctival congestion without discharge, diffuse oropharyngeal mucosal

inflammation with a strawberry tongue and bright red fissured lips, bright red swollen hands and feet followed by desquamation beginning at the tips of the fingers and toes, and swollen cervical lymph nodes, which are characteristically located on one side [2,5]. The diagnosis is made according to clinical data, as no specific diagnostic laboratory tests are available [6]. KD then develops into a localized inflammation focused primarily at the coronary arteries, resulting in coronary artery dilatation, aneurysms, fistulas and stenosis, which in turn can lead to sudden death [5,7,8]. KD has replaced acute rheumatic fever as the leading cause of acquired pediatric heart disease in developed countries [4,9].

Many decades have passed since the discovery of this complex disease, but its exact etiology and pathophysiology remain unclear. The most recent hypothesis suggests that an environmental agent or a pathogenic microorganism triggers an abnormal innate and adaptive immune response in genetically susceptible individuals [8,10]. Although the seasonal pattern, the geographical localization and the symptomatology resembling common childhood infections suggest an infectious

Abbreviations: $[\text{Ca}^{2+}]_{\text{cyt}}$, free cytosolic Ca^{2+} concentration; CRAC, Ca^{2+} -release activated Ca^{2+} entry; EBV, Epstein-Barr virus; ER, endoplasmic reticulum; IP_3 , inositol 1,4,5-trisphosphate; IP_4 , inositol 1,3,4,5-tetrakisphosphate; IP_3R , IP_3 receptor; KD, Kawasaki disease; NCX, $\text{Na}^+/\text{Ca}^{2+}$ exchanger; NFAT, nuclear factor of activated T cells; NF- κB , nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, nucleotide-binding domain, leucine-rich-repeat-containing family, pyrin domain-containing 3; PMCA, plasma-membrane Ca^{2+} -ATPase; SERCA, sarco/endoplasmic-reticulum Ca^{2+} -ATPase; SNP, single-nucleotide polymorphism; SOCE, store-operated Ca^{2+} entry; STIM, stromal interaction molecule; TCR, T-cell receptor.

* Corresponding author at: KU Leuven Campus Gasthuisberg, O&N I, Herestraat 49 – Bus 802, 3000 Leuven, Belgium.

Email address: jan.parys@kuleuven.be (J.B. Parys)

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etiology, no causative infectious organism has been isolated so far from affected children [11,12]. KD depends on a heterogeneous interaction between various immune cells [13–15]. Macrophages and activated dendritic cells are present in close contact to T cells in the coronary arteritis lesions [16–18].

A single infusion of a high dose of intravenous immunoglobulins over a 12-hour period, combined with high doses of aspirin, is the standard treatment of KD [19–22]. The immunoglobulins decrease the rate of aneurysm formation from 25% to less than 5% [23,24]. Resistance to this therapy, defined as recrudescence of fever more than 36 h after intravenous immunoglobulin completion [25], continues to be a problem worldwide, and these patients need adjunctive therapies to reduce inflammation.

Abnormal cytosolic Ca^{2+} signaling plays a role in the development of multiple human diseases, including KD [26]. With the development of genome-wide association studies and linkage studies [27,28], glimpses of the genetic component of KD have been exposed. Some genetic aberrances occurring in KD patients are located in genes linked with Ca^{2+} signaling. In this review, we will provide a critical assessment of the role of Ca^{2+} signaling in the pathophysiology of KD. We will describe the relevant Ca^{2+} -signaling pathways in healthy cells, with a focus on T cells. We will then discuss the genetic susceptibility to KD involving Ca^{2+} -signaling pathways. We will end with some models of how dysfunctional Ca^{2+} -signaling pathways might cause KD and its complications.

2. Intracellular Ca^{2+} signaling in T cells

We will primarily discuss intracellular Ca^{2+} signaling in T cells, given the important role of this cell type in the pathogenesis of KD [16–18] and will mainly focus on those molecules of the Ca^{2+} -signaling toolkit that are thought to be important in the process.

Antigen binding to the T-cell receptor or ligation of chemokine receptors activates respectively phospholipase $\text{C}\gamma 1$ and phospholipase $\text{C}\beta$ [29]. These phospholipases hydrolyze phosphatidylinositol 4,5-bisphosphate in the plasma membrane to diacylglycerol and inositol 1,4,5-trisphosphate (IP_3). IP_3 diffuses in the cytosol and binds to and activates an IP_3 receptor (IP_3R). The IP_3Rs are Ca^{2+} -permeable ion channels localized predominantly in the endoplasmic-reticulum (ER) membrane and are encoded by a family of three genes (*ITPR1*, *ITPR2* and *ITPR3*). All three isoforms have been detected in T cells [30]. Their relative expression varies among T-cell subtypes and various physiological and pathological conditions. Binding of IP_3 to its receptor triggers the release of Ca^{2+} from the ER Ca^{2+} stores. This process only results in a short-lasting rise of the free cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{cyt}}$). A more long-lasting rise of the $[\text{Ca}^{2+}]_{\text{cyt}}$ requires the activation of a sustained influx of extracellular Ca^{2+} through activation of ‘capacitative’ or store-operated Ca^{2+} entry (SOCE). In T cells, the Ca^{2+} -release activated Ca^{2+} (CRAC) channels in the plasma membrane are the main SOCE channels. They can be formed by the 4-transmembrane domain-containing proteins ORAI1, ORAI2 and ORAI3, although quantitatively the latter two contribute much less to SOCE than the former. In T cells ORAI1 and ORAI2 are expressed at much higher levels than ORAI3 [31]. The depletion of the ER Ca^{2+} stores is the stimulus for the opening of the CRAC channels and the influx of extracellular Ca^{2+} . The reduction in the ER $[\text{Ca}^{2+}]$ is sensed by two transmembrane proteins located in the ER membrane, stromal interaction molecule (STIM) 1 and STIM2. Dissociation of Ca^{2+} from the luminal EF-hand domains of STIM1 and STIM2 results in conformational changes that enable them to bind to and open the ORAI channels in the plasma membrane [32,33]. Although both STIM1 and STIM2 promote SOCE into T cells [34], STIM2 activates Ca^{2+} influx upon smaller decreases in ER $[\text{Ca}^{2+}]$ and was proposed to be involved in a housekeeping feedback regula-

tion to keep the basal $[\text{Ca}^{2+}]_{\text{cyt}}$ and the ER $[\text{Ca}^{2+}]$ within tight limits [35].

Two enzymatic pathways terminate IP_3 signaling. One involves hydrolysis of IP_3 by IP_3 5-phosphatase, which yields the inactive compound inositol 1,4-bisphosphate. Another enzymatic pathway involves IP_3 3-kinase which phosphorylates IP_3 to form inositol 1,3,4,5-tetrakisphosphate (IP_4), a compound that is unable to trigger intracellular Ca^{2+} mobilization. There are 3 isoforms of the latter enzyme encoded by 3 genes: *ITPKA*, *ITPKB* and *ITPKC* [36]. The C isoform was inducible in peripheral blood mononuclear cells and in leukemic cell lines by a phorbol ester and a Ca^{2+} ionophore [23]. The $[\text{Ca}^{2+}]_{\text{cyt}}$ returns to baseline due to both Ca^{2+} reuptake into the ER via the sarco/endoplasmic-reticulum Ca^{2+} -ATPase (SERCA) Ca^{2+} pumps and Ca^{2+} extrusion across the plasma membrane via the plasma-membrane Ca^{2+} -ATPase (PMCA) Ca^{2+} pumps [37]. The PMCA family comprises four members, two of which are expressed in T cells: PMCA1 and PMCA4 [38]. The $\text{Na}^+/\text{Ca}^{2+}$ exchangers, of which 3 isoforms have been cloned in mammals (NCX1, NCX2 and NCX3) [39], seem to be unimportant for removing cytosolic Ca^{2+} from T cells [40,41].

3. Susceptibility genes to KD

A recent systemic review and meta-analysis revealed that 62 genes may be correlated with the susceptibility to KD, and that 47 genes may be associated with the incidence of coronary artery lesions [42]. Several functional single-nucleotide polymorphisms (SNPs) were discovered in genes that encode proteins linked to Ca^{2+} signaling (Table 1). These genes are discussed below.

3.1. *ITPKC*

The *ITPKC* gene encodes IP_3 3-kinase C. This enzyme terminates intracellular Ca^{2+} signaling by converting the Ca^{2+} -mobilizing second messenger IP_3 – IP_4 .

The *ITPKC* gene is the first and probably the most important candidate gene predisposing to KD. Onouchi et al. [27] performed a genome-wide linkage study for KD and identified region 19q13 as a region where microsatellite alleles were shared more commonly than expected. They subsequently reported that the functional SNP rs28493229 in the *ITPKC* gene in that region was associated with an increased KD susceptibility and with subsequent development of coronary artery lesions [23]. SNP rs28493229 consistently associates with KD susceptibility and with coronary artery lesions across diverse ethnic/racial populations [42–47]. Some studies found no association between SNP rs28493229 and KD [48–52] but this could be attributed to the matching of the control group, to the lower frequency of the risk-

Table 1
Susceptibility genes to KD having a link with intracellular Ca^{2+} signaling.

Candidate gene	Encoded protein	Function
<i>ITPKC</i>	IP_3 3-kinase C	Converts IP_3 into IP_4
<i>CASP3</i>	Caspase-3	Plays a central role in apoptosis Cleaves various proteins including NFAT1 and the type-1 IP_3R
<i>ORAI1</i>	SOCE channel ORAI1	Sets up influx of extracellular Ca^{2+} in conditions of a decreased $[\text{Ca}^{2+}]$ in the ER
<i>ITPR3</i>	Type-3 IP_3R	Releases Ca^{2+} from the ER
<i>SLC8A1</i>	$\text{Na}^+/\text{Ca}^{2+}$ exchanger 1	Sets up influx of extracellular Ca^{2+} or removal of intracellular Ca^{2+} depending on the Na^+ and Ca^{2+} gradients
<i>PLCB1/PLCB4</i>	Phospholipase $\text{C}\beta 1/4$	Catalyzes the formation of IP_3 and diacylglycerol

conferring C allele in some populations, or to an insufficient sample size. Also other SNPs in the *ITPKC* gene have been reported. SNP rs2290692 was associated with susceptibility to KD and with coronary artery lesion formation in Han Chinese children [50] but not in Korean children [47]. SNP rs7251246 was associated with coronary artery lesion formation and therefore with the severity of KD [53].

3.2. *CASP3*

The *CASP3* gene encodes caspase-3, which is an effector cysteine-dependent protease playing a central role in apoptosis targeting many cellular proteins including the nuclear factor of activated T cells 1 (NFAT1) [54] and the type-1 IP₃R [55]. Caspase-3 also plays a role in non-apoptotic cellular activities in T cells [56,57].

Onouchi et al. [58] performed a positional candidate gene study of the 4q34–q35 region in which they earlier found a positive linkage [27]. They identified a set of multiple variants in the *CASP3* gene that conferred susceptibility to KD. SNPs rs2720378, rs72689236 and rs113420705 were associated with KD susceptibility [42,52,58–60]. SNP rs113420705 was also associated with coronary artery lesion formation [60].

3.3. *Orai1*

The *Orai1* gene encodes the SOCE channel Orai1 in the plasma membrane. Ca²⁺-release activated Ca²⁺ entry through the SOCE channel is essential for T-cell activation and is linked with immune deficiency syndromes [61].

No association between SNPs found in *Orai1* or in the genes for its ER Ca²⁺ sensors *STIM1* and *STIM2* and KD susceptibility were found in Taiwanese children [62]. Onouchi et al. [63] found a common non-synonymous SNP in exon 2 of the *Orai1* gene (rs3741596) and a rare 6-base-pair in-frame insertion variant (rs141919534) associated with KD susceptibility in a Japanese population.

3.4. *ITPR3*

The *ITPR3* gene encodes the type-3 IP₃R, a Ca²⁺-release channel located in the ER membrane that raises the [Ca²⁺]_{cyt}. This isoform plays an important role in apoptosis [64], as it preferentially transmits Ca²⁺ signals into the mitochondria [65,66]. SNPs in the *ITPR3* gene are associated with autoimmune diseases like systemic lupus erythematosus, rheumatoid arthritis and Graves' disease, possibly by their effect on the apoptotic pathway in auto-reactive T cells [67].

SNP rs2229634 in the *ITPR3* gene was associated with the development of coronary artery aneurysms, and with elevated serum levels of C-reactive protein [68]. However, there was no link between this SNP and an elevated risk of KD. The authors acknowledged that this SNP could just be a tag SNP, as it is located about 500kb centromeric to the human leukocyte antigen class II genes *DQ* and *DR* in a region that contains genes encoding molecules that either confer risk factors or protection in many infections or autoimmune diseases. Nonetheless, they advocated the idea that the type-3 IP₃R was involved in the pathogenesis of KD because of its role in apoptosis and in the pathogenesis of various autoimmune diseases.

3.5. *SLC8A1*

The *SLC8A1* gene encodes the Na⁺/Ca²⁺ exchanger 1, which depending on the respective electrochemical gradients for Na⁺ and Ca²⁺ can mediate either the influx of extracellular Ca²⁺ or the extrusion of Ca²⁺ across the plasma membrane. This transporter was expressed in myofibroblasts and inflammatory cells in coronary arteries and in cardiomyocytes of deceased KD patients [28].

SNPs rs10490051, rs12989852 and rs13017968 in the *SLC8A1* gene were associated with a higher KD susceptibility [28]. SNP rs13017968 also influenced the risk of aneurysm formation and dilatation [28].

3.6. *PLCB4/PLCB1*

The *PLCB4* and *PLCB1* genes encode respectively phospholipase C β 4 and C β 1, which hydrolyze phosphatidylinositol 4,5-bisphosphate in the plasma membrane to diacylglycerol and IP₃.

SNP rs6140791 between the *PLCB4* and *PLCB1* genes was associated with the risk of coronary artery lesions [69].

3.7. Genetic synergism

The many genes involved in KD [42] probably interact with one another to increase the risk for the disease and for its complications [4]. Patients with the combined rs28493229 polymorphism in the *ITPKC* gene encoding IP₃ 3-kinase C and the rs113420705 polymorphism in the *CASP3* gene encoding caspase-3 had a higher risk of resistance to intravenous immunoglobulins, while a polymorphism in only one of the two genes was not associated with the response to the treatment [20,21,58,60,70]. Although rs1561876 in the *STIM1* gene was not associated with KD risk, gene-gene interactions with *CASP3* rs2720378 and *IL-2* rs2069762 encoding interleukin-2 resulted in a stronger combined effect of the SNPs and a synergistic risk to develop KD [52]. Reactivation of a Bacillus Calmette-Guérin vaccine scar in children older than 24 months requires a synergism between polymorphisms in the *ITPKC* gene encoding IP₃ 3-kinase C and in the *SLC11A1* gene encoding an iron-transporting protein [47].

4. Role of abnormal Ca²⁺ signaling in the pathogenesis of KD

4.1. Overactivation of the Ca²⁺/NFAT pathway in T cells

Ca²⁺ signals in T cells activate various transcription factors. We focus on the NFAT pathway, which is the best studied Ca²⁺-signaling pathway [29]. NFAT in its inactivated state is phosphorylated on multiple sites and kept stable in the cytosol. In the presence of oscillatory Ca²⁺ signals with the appropriate frequency [71–73], Ca²⁺-calmodulin activates calcineurin, a Ca²⁺-calmodulin-dependent serine/threonine protein phosphatase. Calcineurin dephosphorylates the three Ca²⁺-sensitive forms of NFAT present in the cytosol of T cells: NFAT1, NFAT2 and NFAT4 [74]. Dephosphorylated NFAT enters the cell nucleus, where it drives the transcription of genes important for T-cell activation such as those coding for various proliferation regulators and cytokines (e.g. interleukin-2). The conversion of IP₃ to IP₄ terminates the Ca²⁺ signal and thereby acts as a negative regulator of the Ca²⁺/NFAT pathway. Upon rephosphorylation in the nucleus, NFAT is transported back to the cytosol.

Caspase-3 is activated in apoptotic T cells by a variety of pathways including death-receptor driven caspase-8 cleavage and mitochondrial driven caspase-9 cleavage. But caspase-3 is also activated and up-regulated following T-cell stimulation [57,75]. NFAT1 is a key transcription factor for the *CASP3* gene [76]. Caspase-3 cleaves hundreds of proteins including NFAT1 and therefore acts as a negative regulator of the Ca²⁺/NFAT pathway [54]. Caspase-induced NFAT1 cleavage may downregulate T cell-receptor signaling and induce the T-cell anergy [77] described in KD patients [78]. Caspase-3 also cleaves the type-1 IP₃R in apoptotic T cells [55], but the role of this effect is unclear, especially since only a small fraction of IP₃Rs is fragmented and since fragmentation occurs very late during apoptosis [79,80]. Site-specific cleavage of the type-1 IP₃R by caspase-3, depending on the experimental condition, inhibited [55] or enhanced channel activity [80,81]. Recent studies revealed that the N- and C-terminus of the type-1-IP₃R

fragments produced by caspase-3-mediated cleavage remained associated within a tetrameric channel complex that was able to flux Ca^{2+} in response to extracellular agonists [82,83]. Nevertheless, the channels made up by such fragments displayed distinct functional features and set up unique spatiotemporal Ca^{2+} -signaling profiles, potentially relevant for cell-death and survival processes [80].

SNPs rs28493229 and rs7251246 are located in intron 1 of the *ITPKC* gene encoding IP_3 3-kinase C. SNP rs28493229 resulted in a substitution of a G by a C nucleotide at nine nucleotides from the 5' splice site [23]. The mutant C allele of this polymorphism resulted in less efficient mRNA splicing and in 30% less mature *ITPKC* mRNA as compared to the protective G allele. This should lower the expression level of IP_3 3-kinase C, one of the enzymes that degrades IP_3 . The mechanism by which SNP rs7251246 affects gene expression is unclear, but splicing could be involved [53]. Animal experiments revealed that the reduced expression of IP_3 3-kinase C is associated with KD. A single intraperitoneal injection of *Lactobacillus casei* cell-wall extract in mice creates a murine model of KD that reproducibly induces coronary arteritis that is histologically similar to the coronary arteritis observed in KD in humans [18,84,85]. Injection of the extract induced coronary arteritis in 100% of *Itpkc*-deficient mice and only in 38% of wild-type mice [86]. The severity score of the arteritis was also 8-times higher in *Itpkc*-deficient mice than in wild-type mice.

Onouchi et al. [23] proposed a model for explaining how the SNPs in the *ITPKC* gene encoding IP_3 3-kinase C would lead to KD. The reduced expression of IP_3 3-kinase C phosphorylates the Ca^{2+} -mobilizing IP_3 during T-cell activation more slowly to the inactive IP_4 , leading transiently to a higher IP_3 concentration, more release of Ca^{2+} from the ER, and a more pronounced $[\text{Ca}^{2+}]_{\text{cyt}}$ rise (Fig. 1). This would result in an overstimulation of NFAT, an enhanced T-cell activation, an increased production of interleukin-2, an increased proliferation of pro-inflammatory T cells and an intensified T cell-related immune response. The association of SNP rs28493229 with erythema and induration of a Bacillus Calmette-Guérin vaccine scar in the acute phase of KD is compatible with an immune hyperreactivity [43]. To the best of our knowledge, no studies are available that linked the polymorphisms in the *ITPKC* gene with measurements of the $[\text{Ca}^{2+}]_{\text{cyt}}$ in T cells of KD patients.

The above model can explain why thimerosal, an organomercury compound added as preservative to several pediatric vaccines, can lead to KD. Thimerosal, a thiol-oxidizing agent, is a well-known sensitizer of the IP_3R [87,88], specifically activating the type-1 IP_3R by oxidizing

cysteine residues [89–91]. Thimerosal may induce autoimmunity by sensitizing IP_3Rs resulting in an increased release of Ca^{2+} [4,92]. This mechanism could explain why only children with genetic sensitivity develop KD, while vaccination programs with vaccines containing thimerosal were universal. Thimerosal may therefore be an environmental factor needed to develop KD.

The importance of the Ca^{2+} /NFAT pathway was confirmed by the demonstration of SNPs in the *CASP3* gene. SNP rs113420705 (formerly rs72689236) resulted in a substitution of a G by an A nucleotide in the 5'-untranslated region of the gene. The DNA sequence surrounding this SNP showed an enhancer activity, with NFAT being a transactivator for this site responsible for the upregulation of *CASP3* transcription after T cell-receptor ligation [58]. The mutant A allele of polymorphism rs113420705 was characterized by a weakened binding of NFAT to the DNA sequence surrounding the SNP and resulted in a decreased expression of caspase-3 at the mRNA level in immune effector cells as compared to the protective G allele. Another SNP (rs2720378) may also affect caspase-3 expression, especially since this SNP may affect transcription-factor-binding site activity [52]. A decreased expression of caspase-3 could play a role in the pathogenesis of KD via two ways (Fig. 1). Firstly, the diminished cleavage of NFAT1, one of the substrates of caspase-3 in T cells, would lead to an overactivation of the Ca^{2+} /NFAT pathway, an overproduction of downstream molecules and a hyperactivation of the immune system [58,70]. This view however contrasts with a recent report showing that cleavage of the type-1 IP_3R by caspase-3 induced more oscillatory Ca^{2+} signals and more translocation of NFAT to the nucleus [80]. Secondly, since caspase-3 plays an important role during the execution phase of T-cell apoptosis, the decreased expression level of caspase-3 may result in less T-cell apoptosis and therefore facilitate a sustained activation of immune cells and a progression of KD inflammation [42]. A delayed apoptosis has indeed been described in peripheral blood lymphocytes and neutrophils from KD patients [93,94].

The effect of polymorphism rs3741596 in the *ORAI1* gene encoding a SOCE channel involves a change of a serine to a glycine in the second extracellular loop of this Ca^{2+} -entry channel [63]. In contrast to the importance of the first extracellular loop in Ca^{2+} selectivity, the precise role of the second extracellular loop is not known, and therefore the effect of this alteration is unknown. The insertion variant rs141919534 on the other hand leads to an elongation of the channel protein by 2 amino acids. The modified tertiary structure of the *ORAI1* channel could raise the affinity between *ORAI1* and *STIM1* [63]. This could result in more influx of extracellular Ca^{2+} , with a resulting overstimulation of the Ca^{2+} /NFAT pathway and T-cell overactivation. Future measurements of the $[\text{Ca}^{2+}]_{\text{cyt}}$ should clarify the role of *ORAI1* gene polymorphisms.

Although SNP rs1561876 in the *STIM1* gene was not associated with KD risk, gene-gene interactions with the *CASP3* and the *IL-2* genes encoding respectively caspase-3 and interleukin-2 resulted in a stronger combined effect of this SNP and a synergistic risk to develop KD [52]. SNP rs1561876 in the *STIM1* gene was predicted to affect the binding of transcription factors and microRNAs, implying that the role of *STIM1* should be further investigated.

The exact role of SNP rs2229634 in the *ITPR3* gene encoding the type-3 IP_3R must also be further investigated [68]. This isoform plays a crucial role in apoptosis [64,65], and its expression was selectively increased during T-cell apoptosis [64]. The detected SNP in exon 20 of the *ITPR3* gene resulted in the replacement of one base by another one without affecting the resulting amino-acid sequence. At least in species with large populations, even such synonymous mutations in exons may have constrained effects and affect expression levels of genes through their potential influence on splicing and/or mRNA stability [95]. It has however not yet been clarified whether this SNP influences the expression level of the type-3 IP_3R [68].

Fig. 1. Role of T cells in the pathogenesis of KD. Activation of the T-cell receptor results in an IP_3 -dependent $[\text{Ca}^{2+}]_{\text{cyt}}$ rise. Ca^{2+} activates the calcineurin/NFAT pathway, leading to T-cell activation. Caspase-3 is activated/upregulated by various pathways, including Ca^{2+} /calcineurin/NFAT1-dependent upregulation of caspase-3-expression levels. Caspase-3 induces apoptosis but also acts as a negative regulator of NFAT1. ↑: Increased in KD; ↓: decreased in KD. Abbreviations: IP_3 , inositol 1,4,5-trisphosphate; NFAT, nuclear factor of activated T cells; TCR, T-cell receptor.

The discovery that functional polymorphisms within the *ITPKC* gene encoding IP₃ 3-kinase C are associated with both susceptibility to KD and with the risk for coronary artery lesions, and the finding that activated T cells play an important role in KD, led to the use of cyclosporine A in children refractory to immunoglobulins [96–98]. This calcineurin inhibitor potently suppresses the activity of T cells by negatively regulating the Ca²⁺/NFAT pathway, where it inhibits NFAT dephosphorylation and thus its translocation to the nucleus. Cyclosporine A led to a rapid resolution of fever and inflammation in KD patients.

4.2. Overactivation of the inflammasome in monocytes/macrophages

Interleukin-1, a highly inflammatory cytokine, is a central mediator of the innate immunity and is considered to be a master cytokine of local and systemic inflammation [99]. Genetic studies, transcriptome data and measurements of cytokine levels in children with KD as well as studies in the murine model of KD revealed a key role of interleukin-1 signaling in KD vasculitis [25,100–102]. Interleukin-1-receptor blockade prevented the development of vasculitis in the murine model. Three phase I/II clinical trials are currently being conducted to determine the safety and tolerability of interleukin-1 blockade.

Interleukin-1 release depends on the activation of inflammasomes. Inflammasomes are multi-protein complexes in the cytosol consisting of caspase-1, the adaptor protein ASC, and a sensor molecule [103,104]. Nucleotide-binding domain, leucine-rich-repeat-containing family, pyrin domain-containing 3 (NLRP3) is one of the most studied sensors. Activation of the NLRP3 inflammasome requires two separate signals. The priming signal activates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway (e.g. stimulation of Toll-like receptors) and upregulates pro-interleukin-1β and often also NLRP3. The activating signal, which is often a danger signal or a form of cellular stress, then activates the inflammasome. Cytosolic Ca²⁺ can activate the NLRP3 inflammasome [103]. Cytosolic Ca²⁺ promotes the assembly of the inflammasome components [105]. In addition, increased Ca²⁺ transfer at ER-mitochondria contact sites may lead to mitochondrial Ca²⁺ overload, mitochondrial damage, increased production of mitochondrial reactive oxygen species leading to mitochondrial permeability transition, and the release of mitochondrial DNA and externalization of cardiolipin, which then directly activate the NLRP3 inflammasome [103]. Mitochondrial Ca²⁺ accumulation via the mitochondrial Ca²⁺ uniporter and subsequent production of reactive oxygen species have also been shown to be critical signaling components for the induction of the NLRP3 inflammasome underlying inflammatory responses of cystic fibrosis epithelial cells exposed to *Pseudomonas aeruginosa* [106]. Caspase-1 in activated inflammasomes cleaves pro-interleukin-1β into its active form, which is then secreted.

Animal studies revealed a critical role for inflammasome activation in KD. In the *Lactobacillus casei* cell wall extract-induced model of KD, caspase-1-deficient mice were protected from coronary artery lesions, while the injection of interleukin-1β, which is located downstream of caspase-1, restored the ability to cause coronary artery lesions [107,108].

The effect of the genetic polymorphism rs28493229 in the *ITPKC* gene encoding IP₃ 3-kinase C on the NLRP3 inflammasome was studied in Epstein-Barr virus (EBV)-immortalized B cells from children with KD and in cells of healthy controls with the disease-associated *ITPKC* genotype [86]. As mentioned above, SNP rs28493229 resulted in a substitution of a G by a C nucleotide. Cells with the disease-associated CC genotype were compared with cells with the GC and GG genotypes. Cells with the CC genotype expressed less IP₃ 3-kinase C protein, had a higher basal [Ca²⁺]_{cyt} and an exaggerated and longer lasting [Ca²⁺]_{cyt} rise upon stimulation with 1 μM ionomycin in Ca²⁺-free medium (Fig. 2), produced more mitochondrial superoxide, expressed more NLRP3

Fig. 2. *ITPKC* genotype affects [Ca²⁺]_{cyt} in EBV-immortalized B cells from patients with KD before and after addition of ionomycin (1 μM) in Ca²⁺-free conditions. SNP rs28493229 in the *ITPKC* gene encoding IP₃ 3-kinase C resulted in a substitution of a G by a C nucleotide. The C allele is the risk allele. Cells from patients with the CC genotype were compared with cells from patients with the GC and GG genotypes. **p* < 0.001. The figure was taken from Fig. 5A of M.P. Alphonse, T.T. Duong, C. Shumitzu, T.L. Hoang, B.W. McCrindle, A. Franco, S. Schurmans, D.J. Philpott, M.L. Hibberd, J. Burns, T.W. Kuijpers, R.S. Yeung, Inositol-triphosphate 3-kinase C mediates inflammasome activation and treatment response in Kawasaki disease, *The Journal of Immunology* 2016 Nov 1;197(9):3481–3489 [86]. A copyright license to republish this figure has been obtained (Copyright 2016. The American Association of Immunologists, Inc.).

protein, and released more interleukin-1β upon stimulation with lipopolysaccharide and ATP. The elevated levels of circulating interleukin-1β in children with KD correlated with the *ITPKC* genotype, with children with the CC genotype producing the highest levels of this cytokine [86]. These findings on EBV-immortalized B cells from children with KD were confirmed in bone-marrow macrophages from mice deficient for IP₃ 3-kinase C [86]. Macrophages of *Itpkc*-deficient mice showed an increased basal [Ca²⁺]_{cyt} and a more pronounced response to a Ca²⁺ ionophore than cells from control mice. Macrophages from *Itpkc*-deficient mice also expressed more NLRP3 than cells from control mice, both at baseline and after stimulation with *Lactobacillus casei* cell-wall extract. They also secreted more interleukin-1β.

Alphonse et al. [86] proposed a model for the immunopathogenesis of KD in which the role of *ITPKC* is not T-cell mediated, but monocyte/macrophage dependent (Fig. 3). An infectious or environmental agent serves as a priming signal, which through a Toll-like receptor activates the NF-κB pathway and increases pro-interleukin-1β levels in the cytosol. The activating signal, which might be extracellular ATP, increases the [Ca²⁺]_{cyt}. The intronic SNP rs28493229 in the *ITPKC* gene, which results in a substitution of a G nucleotide by a C nucleotide at nine nucleotides from the 5' splice site, resulted in less efficient mRNA splicing, less mature *ITPKC* mRNA and a decreased expression level of IP₃ 3-kinase C. The slower conversion of IP₃–IP₄ will increase the IP₃ concentration, resulting in more Ca²⁺ release from the ER. Ca²⁺ may then directly or indirectly through the mitochondria, stimulate the NLRP3 inflammasome, with the production of more interleukin-1β from its precursor and accordingly a raised inflammatory response.

As discussed above, cyclosporine A is given to children refractory to immunoglobulins to inhibit the Ca²⁺/NFAT pathway in T cells. Cyclosporine A also inhibits the mitochondrial permeability transition by targeting cyclophilin D, an important subunit that is critical for pore opening in response to mitochondrial Ca²⁺ accumulation and oxidative stress [109]. Cyclosporine A may therefore also act via that pathway on the NLRP3 inflammasome.

Fig. 3. Role of monocytes/macrophages in the pathogenesis of KD. The priming signal acting on a Toll-like receptor induces accumulation of pro-interleukin-1 β through the NF- κ B pathway. The activating signal results in an IP₃-dependent [Ca²⁺]_{cyt} rise. Ca²⁺ mobilization mediates the assembly and activation of the NLRP3 inflammasome. Activation of caspase-1 results in the cleavage of pro-interleukin-1 β into the biologically active cytokine interleukin-1 β . ↑: Increased in KD. Abbreviations: IL-1 β interleukin-1 β ; IP₃ inositol 1,4,5-trisphosphate; NF- κ B nuclear factor kappa-light-chain-enhancer of activated B cells; rec. receptor; TLR Toll-like receptor.

4.3. Increased tumor necrosis factor- α production by monocytes/macrophages

Tumor necrosis factor- α , a pleiotropic pro-inflammatory cytokine produced chiefly by activated monocytes/macrophages, is markedly elevated during the acute phase of KD, especially in children that developed coronary artery lesions [110], and is necessary for the development of coronary artery lesions in the *Lactobacillus casei* cell wall extract-induced mouse model of KD [111]. Its secretion requires an increased [Ca²⁺]_{cyt} [112]. Inhibition of the Na⁺/Ca²⁺ exchanger resulted in a slower decrease of the [Ca²⁺]_{cyt} after its peak in macrophages [113], indicating that the exchanger is functional in these cells.

Three SNPs in the *SLC8A1* gene encoding the Na⁺/Ca²⁺ exchanger 1 were associated with KD susceptibility and one SNP was associated with a higher incidence of subsequent coronary artery lesions [28]. These intronic SNPs are located 5' upstream of exon A in an enhancer region of the gene. The encoded protein was amongst others expressed in inflammatory cells in the coronary artery from KD autopsy tissues.

The role of the Na⁺/Ca²⁺ exchanger 1 on the [Ca²⁺]_{cyt} was determined in EBV-transformed B cells from healthy adults genotyped at rs13017968 [28]. Cells with the disease-associated AA genotype had a higher basal [Ca²⁺]_{cyt} than cells with the CA and CC genotype (Fig. 4). Since these experiments were performed in Ca²⁺-free medium, the higher [Ca²⁺]_{cyt} was due to a decreased rate of Ca²⁺ removal from the cytosol in the resting state. Furthermore, when stimulated with 1 μ M ionomycin, a Ca²⁺ ionophore, cells with the disease-associated AA genotype responded with a higher [Ca²⁺]_{cyt} rise than cells with the CA and CC genotype (Fig. 4).

The higher [Ca²⁺]_{cyt} in cells with the disease-associated A allele of rs13017968 in the gene encoding the Na⁺/Ca²⁺ exchanger 1 probably explains the increased secretion of tumor necrosis factor- α . Interestingly, hyponatremia, which occurs in about half of KD patients [114], would further increase the [Ca²⁺]_{cyt} through inhibition of the Na⁺/Ca²⁺ exchanger [4].

As discussed above, cyclosporine A is used in children refractory to immunoglobulins to inhibit the Ca²⁺/NFAT pathway in T cells. Cyclosporine A may in addition decrease the expression of the Na⁺/Ca²⁺ exchanger 1 by inhibiting cyclophilin-mediated protein maturation and folding [115].

Fig. 4. *SLC8A1* genotype is associated with differences in [Ca²⁺]_{cyt} in EBV-immortalized B cells from a healthy adult population before and after addition of ionomycin (1 μ M, at one minute) in Ca²⁺-free conditions. SNP rs13017968 in the *SLC8A1* gene encoding the Na⁺/Ca²⁺ exchanger 1 resulted in a substitution of a C by an A nucleotide. The A allele is the risk allele. Cells with the AA genotype were compared with cells with the CA and CC genotypes. The figure has been taken from Fig. 4 of C. Shimizu, H. Eleftherohorinou, V.J. Wright, J. Kim, M.P. Alphonse, J.C. Perry, R. Cimaz, D. Burgner, N. Dahdah, L.T. Hoang, C.C. Khor, A. Salgado, A.H. Tremoulet, S. Davila, T.W. Kuijpers, M.L. Hibberd, T.A. Johnson, A. Takahashi, T. Tsunoda, M. Kubo, T. Tanaka, Y. Onouchi, R.S. Yeung, L.J. Coin, M. Levin, J.C. Burns; International Kawasaki Disease Genetics Consortium, Genetic variation in the *SLC8A1* calcium signaling pathway is associated with susceptibility to Kawasaki disease and coronary artery abnormalities, *Circulation: Cardiovascular Genetics* 2016 Dec; 9(6):559-568 [28]. A copyright license to republish this figure has been obtained.

4.4. Increased urotensin-2 signaling

The SNPs in the gene for the Na⁺/Ca²⁺ exchanger 1 may also lead to coronary artery damage via the production of urotensin 2 [116]. This peptide is expressed in monocytes, endothelial cells, vascular smooth-muscle cells and fibroblasts. Subjects homozygous for any of the three risk alleles in *SLC8A1* (rs10490051, rs12989852 and rs13017968) had higher plasma levels of urotensin 2 [116]. Urotensin 2 may induce inflammation by attracting monocytes to the vascular wall, either by directly stimulating a urotensin-2 receptor on the monocytes or indirectly by the secretion of monocyte-chemoattractant protein-1 by adventitial fibroblasts. Urotensin 2 in addition induces myofibroblast formation from monocytes [116] and from adventitial fibroblasts [117]. Myofibroblasts recruit pro-inflammatory cells to the arterial wall that then contribute to aneurysm formation and arterial damage [118]. The Na⁺/Ca²⁺ exchanger is important for maintaining the myofibroblast phenotype [119].

4.5. Role of phospholipases C β in activating endothelial cells

Activation of vascular endothelial cells is involved in the occurrence of inflamed and injured vessels during the acute stage of KD [120]. Injured endothelial cells release pro-inflammatory signals that increase the permeability of the vascular wall and recruit leukocytes. In a lipopolysaccharide-induced endothelial-cell inflammation model, phospholipase C β 1 encoded by the *PLCB1* gene regulated the expression of the pro-inflammatory cytokines interleukin-1 β , interleukin-6 and interleukin-8 [69]. Phospholipase C β 1 is one of the four isoforms of phospholipase C β . It hydrolyses phosphatidylinositol 4,5-bisphosphate into IP₃ and diacylglycerol.

Phospholipase-C levels in plasma of patients with KD were related to the *PLCB4/PLCB1* (rs6140791) genotypes [69]. Patients carrying the CC + CG genotype had higher plasma phospholipase-C levels and more

severe inflammation and more risk of developing coronary artery lesions than patients with the GG genotype. The effect of SNP rs6140791 on the $[Ca^{2+}]_{cyt}$ in endothelial cells and the exact mechanism leading to the production of the pro-inflammatory cytokines are unknown.

5. Conclusion

SNPs in various genes encoding proteins with a role in intracellular Ca^{2+} -signaling pathways increase susceptibility to KD and the development of coronary artery lesions. Aberrant cytosolic Ca^{2+} -signaling events may result in hyperactivation of the Ca^{2+} /calineurin/NFAT-signaling pathway in T cells, a hypersecretion of interleukin-18 and tumor necrosis factor- α by monocytes/macrophages, increased urotensin-2 signaling, and more activation of vascular endothelial cells.

Many of the associated genetic variants associated with KD are located in the introns of the genes with no associated molecular function identified [121,122]. The risk C allele of SNP rs28493229 in the *ITPKC* gene encoding the IP_3 3-kinase C is a notable exception because it decreases the expression of this IP_3 -mobilizing enzyme [23,86]. Only two studies directly measured the free $[Ca^{2+}]_{cyt}$ in EBV-immortalized B cells [28,86]. There is therefore an urgent need to measure Ca^{2+} signals in more relevant cell types for the pathogenesis of KD, like human T cells and monocytes, and in various experimental settings. Such studies are needed to definitively establish the exact role of (abnormal) Ca^{2+} signaling in the pathogenesis of KD.

Conflict of interest

None.

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References

- [1] T. Kawasaki, Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children, *Alerugi* 16 (1967) 178–222.
- [2] T. Kawasaki, F. Kosaki, S. Okawa, I. Shigematsu, H. Yanagawa, A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan, *Pediatrics* 54 (1974) 271–276.
- [3] S. Singh, P. Vignesh, D. Burgner, The epidemiology of Kawasaki disease: a global update, *Arch. Dis. Child.* 100 (2015) 1084–1088.
- [4] D. Yeter, R. Deth, H.C. Kuo, Mercury promotes catecholamines which potentiate mercurial autoimmunity and vasodilation: implications for inositol 1,4,5-trisphosphate 3-kinase C susceptibility in Kawasaki syndrome, *Korean Circ. J.* 43 (2013) 581–589.
- [5] F.H. Zhu, J.Y. Ang, The clinical diagnosis and management of Kawasaki disease: a review and update, *Curr. Infect. Dis. Rep.* 18 (2016) 3.
- [6] G.M. Satou, J. Giamelli, M.H. Gewitz, Kawasaki disease: diagnosis, management, and long-term implications, *Cardiol. Rev.* 15 (2007) 163–169.
- [7] H. Kato, S. Koike, M. Yamamoto, Y. Ito, E. Yano, Coronary aneurysms in infants and young children with acute febrile mucocutaneous lymph node syndrome, *J. Pediatr.* 86 (1975) 892–898.
- [8] B.W. McCrindle, A.H. Rowley, J.W. Newburger, et al., Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association, *Circulation* 135 (2017) e927–e999.
- [9] K.A. Taubert, A.H. Rowley, S.T. Shulman, Seven-year national survey of Kawasaki disease and acute rheumatic fever, *Pediatr. Infect. Dis. J.* 13 (1994) 704–708.
- [10] D. Del Principe, D. Pietraforte, L. Gambardella, et al., Pathogenetic determinants in Kawasaki disease: the haematological point of view, *J. Cell. Mol. Med.* 21 (2017) 632–639.
- [11] K.L. Yoon, Update of genetic susceptibility in patients with Kawasaki disease, *Korean J. Pediatr.* 58 (2015) 84–88.
- [12] S.M. Dietz, D. van Stijn, D. Burgner, et al., Dissecting Kawasaki disease: a state-of-the-art review, *Eur. J. Pediatr.* 176 (2017) 995–1009.
- [13] S.A. Cameron, S.M. White, D. Arrollo, S.T. Shulman, A.H. Rowley, Arterial immune protein expression demonstrates the complexity of immune responses in Kawasaki disease arteritis, *Clin. Exp. Immunol.* 190 (2017) 244–250.
- [14] X.L. Qi, L.L. Chen, X.G. Sun, et al., 1,25-Dihydroxyvitamin D₃ regulates T lymphocyte proliferation through activation of P53 and inhibition of ERK1/2 signaling pathway in children with Kawasaki disease, *Eur. Rev. Med. Pharmacol. Sci.* 21 (2017) 3714–3722.
- [15] K. Takahashi, T. Oharaseki, Y. Yokouchi, Histopathological aspects of cardiovascular lesions in Kawasaki disease, *Int. J. Rheum. Dis.* (2017), in press.
- [16] T.J. Brown, S.E. Crawford, M.L. Cornwall, F. Garcia, S.T. Shulman, A.H. Rowley, CD8 T lymphocytes and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease, *J. Infect. Dis.* 184 (2001) 940–943.
- [17] A. Yilmaz, A. Rowley, D.J. Schulte, et al., Activated myeloid dendritic cells accumulate and co-localize with CD3⁺ T cells in coronary artery lesions in patients with Kawasaki disease, *Exp. Mol. Pathol.* 83 (2007) 93–103.
- [18] D.J. Schulte, A. Yilmaz, K. Shimada, et al., Involvement of innate and adaptive immunity in a murine model of coronary arteritis mimicking Kawasaki disease, *J. Immunol.* 183 (2009) 5311–5318.
- [19] J.W. Newburger, M. Takahashi, A.S. Beiser, et al., A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome, *N. Engl. J. Med.* 324 (1991) 1633–1639.
- [20] H.C. Kuo, Y.W. Hsu, C.M. Wu, et al., A replication study for association of *ITPKC* and *CASP3* two-locus analysis in IVIG unresponsiveness and coronary artery lesion in Kawasaki disease, *PLoS One* 8 (2013) e69685.
- [21] H.C. Kuo, Y.W. Hsu, M.S. Wu, S.C. Chien, S.F. Liu, W.C. Chang, Intravenous immunoglobulin, pharmacogenomics, and Kawasaki disease, *J. Microbiol. Immunol. Infect.* 49 (2016) 1–7.
- [22] H.C. Kuo, H.S. Wong, W.P. Chang, et al., Prediction for intravenous immunoglobulin resistance by using weighted genetic risk score identified from genome-wide association study in Kawasaki disease, *Circ. Cardiovasc. Genet.* 10 (2017) e001625.
- [23] Y. Onouchi, T. Gunji, J.C. Burns, et al., *ITPKC* functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms, *Nat. Genet.* 40 (2008) 35–42.
- [24] A.M. Lee, C. Shimizu, T. Oharaseki, et al., Role of TGF- β signaling in remodeling of noncoronary artery aneurysms in Kawasaki disease, *Pediatr. Dev. Pathol.* 18 (2015) 310–317.
- [25] A.H. Tremoulet, Adjunctive therapies in Kawasaki disease, *Int. J. Rheum. Dis.* (2017), (in press).
- [26] M.J. Berridge, The inositol trisphosphate/calcium signaling pathway in health and disease, *Physiol. Rev.* 96 (2016) 1261–1296.
- [27] Y. Onouchi, M. Tamari, A. Takahashi, et al., A genomewide linkage analysis of Kawasaki disease: evidence for linkage to chromosome 12, *J. Hum. Genet.* 52 (2007) 179–190.
- [28] C. Shimizu, H. Eleftherohorinou, V.J. Wright, et al., Genetic variation in the *SLC8A1* calcium signaling pathway is associated with susceptibility to Kawasaki disease and coronary artery abnormalities, *Circ. Cardiovasc. Genet.* 9 (2016) 559–568.
- [29] S. Feske, Calcium signalling in lymphocyte activation and disease, *Nat. Rev. Immunol.* 7 (2007) 690–702.
- [30] A.M. Akimzhanov, D. Boehning, IP_3 R function in cells of the immune system, *WIREs Membr. Transp. Signal.* 1 (2012) 329–339.
- [31] M. Vaeth, J. Yang, M. Yamashita, et al., *ORAI2* modulates store-operated calcium entry and T cell-mediated immunity, *Nat. Commun.* 8 (2017) 14714.
- [32] M. Prakriya, R.S. Lewis, Store-operated calcium channels, *Physiol. Rev.* 95 (2015) 1383–1436.
- [33] I. Frischauf, M. Fahrner, I. Jardin, C. Romanin, The *STIM1*: *orai* interaction, *Adv. Exp. Med. Biol.* 898 (2016) 25–46.
- [34] M. Oh-Hora, M. Yamashita, P.G. Hogan, et al., Dual functions for the endoplasmic reticulum calcium sensors *STIM1* and *STIM2* in T cell activation and tolerance, *Nat. Immunol.* 9 (2008) 432–443.
- [35] O. Brandman, J. Liou, W.S. Park, T. Meyer, *STIM2* is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels, *Cell* 131 (2007) 1327–1339.
- [36] V. Dewaste, C. Moreau, F. De Smedt, et al., The three isoenzymes of human inositol-1,4,5-trisphosphate 3-kinase show specific intracellular localization but comparable Ca^{2+} responses on transfection in COS-7 cells, *Biochem. J.* 374 (2003) 41–49.
- [37] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodeling, *Nat. Rev. Mol. Cell Biol.* 4 (2003) 517–529.
- [38] M. Korthals, K. Langnaese, K.H. Smalla, et al., A complex of neuroplasm and plasma membrane Ca^{2+} ATPase controls T cell activation, *Sci. Rep.* 7 (2017) 8358.
- [39] J. Lytton, Na^{+}/Ca^{2+} exchangers: three mammalian gene families control Ca^{2+} transport, *Biochem. J.* 406 (2007) 365–382.
- [40] E. Donnadieu, G. Bismuth, A. Trautmann, Calcium fluxes in T lymphocytes, *J. Biol. Chem.* 267 (1992) 25864–25872.
- [41] E. Donnadieu, A. Trautmann, Is there a Na^{+}/Ca^{2+} exchanger in macrophages and in lymphocytes, *Pflügers Arch.* 424 (1993) 448–455.
- [42] X. Xie, X. Shi, M. Liu, The roles of genetic factors in Kawasaki disease: a systematic review and meta-analysis of genetic association studies, *Pediatr. Cardiol.* (2017), (in press).
- [43] M.T. Lin, J.K. Wang, J.I. Yeh, et al., Clinical implication of the C allele of the *ITPKC* gene SNP rs28493229 in Kawasaki disease: association with disease susceptibility and BCG scar reactivation, *Pediatr. Infect. Dis. J.* 30 (2011) 148–152.
- [44] H.C. Kuo, K.D. Yang, S.H. Juo, et al., *ITPKC* single nucleotide polymorphism associated with the Kawasaki disease in a Taiwanese population, *PLoS One* 6 (2011) e17370.
- [45] C.C. Khor, S. Davila, W.B. Breunis, et al., Genome-wide association study identifies *FCGR2A* as a susceptibility locus for Kawasaki disease, *Nat. Genet.* 43 (2011) 1241–1246.

- [46] J. Lou, S. Xu, L. Zou, et al., A functional polymorphism, rs28493229, in ITPKC and risk of Kawasaki disease: an integrated meta-analysis, *Mol. Biol. Rep.* 39 (2012) 11137–11144.
- [47] K.Y. Kim, Y.S. Bae, W. Ji, D. Shin, H.S. Kim, D.S. Kim, ITPKC and SLC11A1 gene polymorphisms and gene-gene interactions in Korean patients with Kawasaki disease, *Yonsei Med. J.* 59 (2018) 119–127.
- [48] H. Chi, F.Y. Huang, M.R. Chen, et al., ITPKC gene SNP rs28493229 and Kawasaki disease in Taiwanese children, *Hum. Mol. Genet.* 19 (2010) 1147–1151.
- [49] Q. Peng, C.H. Chen, Q. Wu, et al., Association study of a functional SNP rs28493229 of ITPKC gene and Kawasaki disease in a Chinese population, *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 28 (2011) 644–648.
- [50] Q. Peng, C. Chen, Y. Zhang, et al., Single-nucleotide polymorphism rs2290692 in the 3'UTR of ITPKC associated with susceptibility to Kawasaki disease in a Han Chinese population, *Pediatr. Cardiol.* 33 (2012) 1046–1053.
- [51] M.F. Natividad, C.A. Torres-Villanueva, C.P. Saloma, Superantigen involvement and susceptibility factors in Kawasaki disease: profiles of TCR V β 2 + T cells and HLA-DRB1TNF- α and ITPKC genes among Filipino patients, *Int. J. Mol. Epidemiol. Genet.* 4 (2013) 70–76.
- [52] W. Wang, J. Lou, R. Zhong, et al., The roles of Ca²⁺/NFAT signaling genes in Kawasaki disease: single- and multiple-risk genetic variants, *Sci. Rep.* 4 (2014) 520.
- [53] H.C. Kuo, Y.W. Hsu, M.H. Lo, Y.H. Huang, S.C. Chien, W.C. Chang, Single-nucleotide polymorphism rs7251246 in ITPKC is associated with susceptibility and coronary artery lesions in Kawasaki disease, *PLoS One* 9 (2014) e9111.
- [54] W. Wu, R.S. Misra, J.Q. Russell, R.A. Flavell, M. Rincón, R.C. Budd, Proteolytic regulation of nuclear factor of activated T (NFAT) c2 cells and NFAT activity by caspase-3, *J. Biol. Chem.* 281 (2006) 10682–10690.
- [55] J. Hirota, T. Furuchi, K. Mikoshiba, Inositol 1,4,5-trisphosphate receptor type 1 is a substrate for caspase-3 and is cleaved during apoptosis in a caspase-3-dependent manner, *J. Biol. Chem.* 274 (1999) 34433–34437.
- [56] A. Alam, L.Y. Cohen, S. Aouad, R.P. Sékaly, Early activation of caspases during T lymphocyte stimulation results in selective substrate cleavage in nonapoptotic cells, *J. Exp. Med.* 190 (1999) 1879–1890.
- [57] S. McComb, R. Mulligan, S. Sad, Caspase-3 is transiently activated without cell death during early antigen driven expansion of CD8⁺ T cells in vivo, *PLoS One* 5 (2010) e15328.
- [58] Y. Onouchi, K. Ozaki, J.C. Buns, et al., Common variants in CASP3 confer susceptibility to Kawasaki disease, *Hum. Mol. Genet.* 19 (2010) 2898–2906.
- [59] Q. Peng, C.H. Chen, Q. Wu, Y. Yang, Association of new functional SNP rs72689236 of CASP3 with Kawasaki disease: a meta-analysis, *Zhongguo Dang Dai Er Ke Za Zhi* 15 (2013) 477–483.
- [60] H.C. Kuo, H.R. Yu, S.H. Juo, et al., CASP3 gene single-nucleotide polymorphism (rs72689236) and Kawasaki disease in Taiwanese children, *J. Hum. Genet.* 56 (2011) 161–165.
- [61] S. Feske, Y. Gwack, M. Prakriya, et al., A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function, *Nature* 441 (2006) 179–185.
- [62] H.C. Kuo, Y.J. Lin, S.H. Juo, et al., Lack of association between ORAI1/CRACM1 gene polymorphisms and Kawasaki disease in the Taiwanese children, *J. Clin. Immunol.* 31 (2011) 650–655.
- [63] Y. Onouchi, R. Fukazawa, K. Yamamura, et al., Variations in ORAI1 gene associated with Kawasaki disease, *PLoS One* 11 (2016) e0145486.
- [64] A.A. Khan, M.J. Soloski, A.H. Sharp, et al., Lymphocyte apoptosis: mediation by increased type 3 inositol 1,4,5-trisphosphate receptor, *Science* 273 (1996) 503–507.
- [65] C.C. Mendes, D.A. Gomes, M. Thompson, et al., The type III inositol 1,4,5-trisphosphate receptor preferentially transmits apoptotic Ca²⁺ signals into mitochondria, *J. Biol. Chem.* 280 (2005) 40892–40900.
- [66] G. Bultynck, M. Campanella, Tumor suppressive Ca²⁺ signaling is driven by IP₃ receptor fitness, *Cell Stress* 1 (2017) 73–78.
- [67] T. Oishi, A. Iida, S. Otsubo, et al., A functional SNP in the NKX2.5-binding site of ITPR3 promoter is associated with susceptibility to systemic lupus erythematosus in Japanese population, *J. Hum. Genet.* 53 (2008) 151–162.
- [68] Y.C. Huang, Y.J. Lin, J.S. Chang, et al., Single nucleotide polymorphism rs2229634 in the ITPR3 gene is associated with the risk of developing coronary artery aneurysm in children with Kawasaki disease, *Int. J. Immunogenet.* 37 (2010) 439–443.
- [69] Y.J. Lin, J.S. Chang, X. Liu, et al., Genetic variants in PLCB4/PLCB1 as susceptibility loci for coronary artery aneurysm formation in Kawasaki disease in Han Chinese in Taiwan, *Sci. Rep.* 5 (2015) 14762.
- [70] Y. Onouchi, Y. Suzuki, H. Suzuki, et al., ITPKC and CASP3 polymorphisms and risks for IVIG unresponsiveness and coronary artery lesion formation in Kawasaki disease, *Pharmacogenomics J.* 13 (2013) 52–59.
- [71] R.E. Dolmetsch, K. Xu, R.S. Lewis, Calcium oscillations increase the efficiency and specificity of gene expression, *Nature* 392 (1998) 933–936.
- [72] W. Li, J. Llopis, M. Whitney, G. Zlokarnik, R.Y. Tsien, Cell-permeant caged InsP₃ ester shows that Ca²⁺ spike frequency can optimize gene expression, *Nature* 392 (1998) 936–941.
- [73] E. Smedler, P. Uhlén, Frequency decoding of calcium oscillations, *Biochim. Biophys. Acta* 1840 (2014) 964–969.
- [74] F. Macián, NFAT proteins: key regulators of T-cell development and function, *Nat. Rev. Immunol.* 5 (2005) 472–484.
- [75] L. Sabbagh, M. Bourbonnière, R.P. Sékaly, L.Y. Cohen, Selective up-regulation of caspase-3 gene expression following TCR engagement, *Mol. Immunol.* 42 (2005) 1345–1354.
- [76] F. Macián, F. García-Cózar, S.H. Im, H.F. Horton, M.C. Byrne, A. Rao, Transcriptional mechanisms underlying lymphocyte tolerance, *Cell* 109 (2002) 719–731.
- [77] I. Puga, A. Rao, F. Macián, Targeted cleavage of signaling proteins by caspase 3 inhibits T cell receptor signaling in anergic T cells, *Immunity* 29 (2008) 193–204.
- [78] K. Masuda, S. Takei, Y. Nomura, H. Imanaka, K. Sameshima, M. Yoshinaga, Transient low T cell response to streptococcal pyrogenic exotoxin-C in patients with Kawasaki disease, *Pediatr. Res.* 44 (1998) 27–31.
- [79] G. Elkoreh, V. Blais, E. Béliveau, G. Guillemette, J.B. Denault, Type 1 inositol-1,4,5-trisphosphate receptor is a late substrate of caspases during apoptosis, *J. Cell. Biochem.* 113 (2012) 2775–2784.
- [80] L. Wang, L.E. Wagner 2nd, K.J. Alzayady, D.I. Yule, Region-specific proteolysis differentially regulates type 1 inositol 1,4,5-trisphosphate receptor activity, *J. Biol. Chem.* 292 (2017) 11714–11726.
- [81] Z. Assefa, G. Bultynck, K. Szulcicki, et al., Caspase-3-induced truncation of type 1 inositol trisphosphate receptor accelerates apoptotic cell death and induces inositol trisphosphate-independent calcium release during apoptosis, *J. Biol. Chem.* 279 (2004) 43227–43236.
- [82] K.J. Alzayady, R. Chandrasekhar, D.I. Yule, Fragmented inositol 1,4,5-trisphosphate receptors retain tetrameric architecture and form functional Ca²⁺ release channels, *J. Biol. Chem.* 288 (2013) 11122–11134.
- [83] L. Wang, K.J. Alzayady, D.I. Yule, Proteolytic fragmentation of inositol 1,4,5-trisphosphate receptors: a novel mechanism regulating channel activity?, *J. Physiol.* 594 (2016) 2867–2876.
- [84] T.J. Lehman, S.M. Walker, V. Mahnovski, D. McCurdy, Coronary arteritis in mice following the systemic injection of group B *Streptococcus* casei cell walls in aqueous suspension, *Arthritis Rheum.* 28 (1985) 652–659.
- [85] R.S. Yeung, Lessons learned from an animal model of Kawasaki disease, *Clin. Exp. Rheumatol.* 44 (2007) S69–S71.
- [86] M.P. Alphonse, T.T. Duong, C. Shumitzu, et al., Inositol-trisphosphate 3-kinase C mediates inflammasome activation and treatment response in Kawasaki disease, *J. Immunol.* 197 (2016) 3481–3489.
- [87] M.D. Bootman, C.W. Taylor, M.J. Berridge, The thiol reagent thimerosal, evokes Ca²⁺ spikes in HeLa cells by sensitizing the inositol 1,4,5-trisphosphate receptor, *J. Biol. Chem.* 267 (1992) 25113–25119.
- [88] J.B. Parys, L. Missiaen, H. De Smedt, G. Droogmans, R. Casteels, Bell-shaped activation of inositol-1,4,5-trisphosphate-induced Ca²⁺ release by thimerosal in permeabilized A7r5 smooth-muscle cells, *Pflügers Arch.* 424 (1993) 516–522.
- [89] L. Missiaen, J.B. Parys, I. Sienart, et al., Functional properties of the type-3 InsP₃ receptor in 16HBE14o- bronchial mucosal cells, *J. Biol. Chem.* 273 (1998) 8983–8986.
- [90] G. Bultynck, K. Szulcicki, N.N. Kasri, et al., Thimerosal stimulates Ca²⁺ flux through inositol 1,4,5-trisphosphate receptor type 1, but not type 3, via modulation of an isoform-specific Ca²⁺-dependent intramolecular interaction, *Biochem. J.* 381 (2004) 87–96.
- [91] S.A. Khan, A.M. Rossi, A.M. Riley, B.V. Potter, C.W. Taylor, Subtype-selective regulation of IP₃ receptors by thimerosal via cysteine residues within the IP₃-binding core and suppressor domain, *Biochem. J.* 451 (2013) 177–184.
- [92] D. Yeter, R. Deth, ITPKC susceptibility in Kawasaki syndrome as a sensitizing factor for autoimmunity and coronary arterial wall relaxation induced by thimerosal's effects on calcium signaling via IP₃, *Autoimmun. Rev.* 11 (2012) 903–908.
- [93] Q.J. Yi, C.R. Li, X.Q. Yang, Effect of intravenous immunoglobulin on inhibiting peripheral blood lymphocyte apoptosis in acute Kawasaki disease, *Acta Paediatr.* 90 (2001) 623–627.
- [94] H. Tsujimoto, S. Takeshita, K. Nakatani, Y. Kawamura, T. Tokutomi, I. Sekine, Delayed apoptosis of circulating neutrophils in Kawasaki disease, *Clin. Exp. Immunol.* 126 (2001) 355–364.
- [95] J.V. Chamary, J.L. Parmley, L.D. Hurst, Hearing silence: non-neutral evolution at synonymous sites in mammals, *Nat. Rev. Genet.* 7 (2006) 98–100.
- [96] H. Suzuki, M. Terai, H. Hamada, et al., Cyclosporin A treatment for Kawasaki disease refractory to initial and additional intravenous immunoglobulin, *Pediatr. Infect. Dis. J.* 30 (2011) 871–876.
- [97] A.H. Tremoulet, P. Pancoast, A. Franco, et al., Calcineurin inhibitor treatment of intravenous immunoglobulin-resistant Kawasaki disease, *J. Pediatr.* 161 (2012) 506–512.
- [98] R. Aoyagi, H. Hamada, Y. Sato, et al., Study protocol for a phase III multicentre, randomised, open-label, blinded-end point trial to evaluate the efficacy and safety of immunoglobulin plus cyclosporin A in patients with severe Kawasaki disease (KAICA Trial), *BMJ Open* 5 (2015) e009562.
- [99] G. Schett, J.M. Dayer, B. Manger, Interleukin-1 function and role in rheumatic disease, *Nat. Rev. Rheumatol.* 12 (2016) 14–24.
- [100] L.T. Hoang, C. Shimizu, L. Ling, et al., Global gene expression profiling identifies new therapeutic targets in acute Kawasaki disease, *Genome Med.* 6 (2014) 541.
- [101] J.C. Burns, I. Koné-Paut, T. Kuijpers, C. Shimizu, A. Tremoulet, M. Arditi, Review found in translation: international initiatives pursuing interleukin-1 blockade for treatment of acute Kawasaki disease, *Arthritis Rheumatol.* 69 (2017) 268–276.
- [102] P. Duser, I. Koné-Paut, IL-1 inhibition may have an important role in treating refractory Kawasaki disease, *Front. Pharmacol.* 8 (2017) 163.
- [103] T. Hornig, Calcium signaling and mitochondrial destabilization in the triggering of the NLRP3 inflammasome, *Trends Immunol.* 35 (2014) 253–261.
- [104] D.E. Place, T.D. Kanneganti, Recent advances in inflammasome biology, *Curr. Opin. Immunol.* 50 (2018) 32–38.
- [105] G.S. Lee, N. Subramanian, A.L. Kim, et al., The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca²⁺ and cAMP, *Nature* 492 (2012) 123–127.
- [106] A. Rimessi, V. Bezzerri, S. Patergnani, S. Marchi, G. Cabrini, P. Pinton, Mitochondrial Ca²⁺-dependent NLRP3 activation exacerbates the Pseudomonas aeruginosa-driven inflammatory response in cystic fibrosis, *Nat. Commun.* 6 (2015) 6201.
- [107] Y. Lee, D.J. Schulte, K. Shimada, et al., Interleukin-1 β is crucial for the induction of coronary artery inflammation in a mouse model of Kawasaki disease, *Circulation* 125 (2012) 1542–1550.
- [108] Y. Lee, D. Wakita, J. Dagvadorj, et al., IL-1 signaling is critically required in stromal cells in Kawasaki Disease Vasculitis Mouse Model. Role of both IL-1 α and IL-1 β , *Arterioscler. Thromb. Vasc. Biol.* 35 (2015) 2605–2616.

- [109] J.W. Elrod, J.D. Molkentin, Physiologic functions of cyclophilin D and the mitochondrial permeability transition pore, *Circ. J.* 77 (2013) 1111–1122.
- [110] T. Matsubara, S. Furukawa, K. Yabuta, Serum levels of tumor necrosis factor, interleukin 2 receptor, and interferon-gamma in Kawasaki disease involved coronary-artery lesions, *Clin. Immunol. Immunopathol.* 56 (1990) 29–36.
- [111] J.S. Hui-Yuen, T.T. Duong, R.S. Yeung, TNF-alpha is necessary for induction of coronary artery inflammation and aneurysm formation in an animal model of Kawasaki disease, *J. Immunol.* 176 (2006) 6294–6301.
- [112] N. Watanabe, J. Suzuki, Y. Kobayashi, Role of calcium in tumor necrosis factor-alpha production by activated macrophages, *J. Biochem.* 120 (1996) 1190–1195.
- [113] R.I. Staiano, F. Granata, A. Secondo, et al., Expression and function of Na⁺/Ca²⁺ exchangers 1 and 3 in human macrophages and monocytes, *Eur. J. Immunol.* 39 (2009) 1405–1418.
- [114] T. Watanabe, Y. Abe, S. Sato, Y. Uehara, K. Ikeno, T. Abe, Hyponatremia in Kawasaki disease, *Pediatr. Nephrol.* 21 (2006) 778–781.
- [115] H. Rahamimoff, B. Elbaz, A. Alperovich, et al., Cyclosporin A-dependent down-regulation of the Na⁺/Ca²⁺ exchanger expression, *Ann. N. Y. Acad. Sci.* 1099 (2007) 204–214.
- [116] C.Y. Huang, J.C. Burns, C. Shimizu, Urotensin 2 in Kawasaki disease pathogenesis, *Pediatr. Res.* 82 (2017) 1048–1055.
- [117] Y.G. Zhang, Y.C. Hu, Y.Y. Mao, et al., Transforming growth factor-β1 involved in urotensin II-induced phenotypic differentiation of adventitial fibroblasts from rat aorta, *Chin. Med. J. (Engl.)* 123 (2010) 3634–3639.
- [118] C. Shimizu, T. Oharaseki, K. Takahashi, A. Kottek, A. Franco, J.C. Burns, The role of TGF-β and myofibroblasts in the arteritis of Kawasaki disease, *Hum. Pathol.* 44 (2013) 189–198.
- [119] J.R. Romero, A. Rivera, V. Lança, M.D. Bicho, P.R. Conlin, D.A. Ricupero, Na⁺/Ca²⁺ exchanger activity modulates connective tissue growth factor mRNA expression in transforming growth factor β1- and Des-Arg10-kallidin-stimulated myofibroblasts, *J. Biol. Chem.* 280 (2005) 14378–14384.
- [120] Y.J. Lin, L. Wan, J.Y. Wu, et al., HLA-E gene polymorphism associated with susceptibility to Kawasaki disease and formation of coronary artery aneurysms, *Arthritis Rheum.* 60 (2009) 604–610.
- [121] Y. Onouchi, Genetics of Kawasaki disease: what we know and don't know, *Circ. J.* 76 (2012) 1581–1586.
- [122] J. Kim, C. Shimizu, S.F. Kingsmore, et al., Whole genome sequencing of an African American family highlights toll like receptor 6 variants in Kawasaki disease susceptibility, *PLoS One* 12 (2017) e0170977.