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REVIEW

The up-to-date pathophysiology of Kawasaki disease

Toshiro Hara[1], Kenichiro Yamamura[2] & Yasunari Sakai[3]

[1]Kawasaki Disease Center, Fukuoka Children's Hospital, Fukuoka, Japan
[2]Department of Perinatal and Pediatric Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[3]Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Correspondence
T Hara, Fukuoka Children's Hospital,
5-1-1 Kashiiteriha, Higashi-ku,
Fukuoka 813-0017, Japan.
E-mail: hara.t@fcho.jp

Y Sakai, Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.
E-mail: ysakai22q13@gmail.com

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Abstract

Kawasaki disease (KD) is an acute systemic vasculitis of an unknown aetiology. A small proportion of children exposed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or infected by *Yersinia* reproducibly develop principal symptoms of KD in various ethnic areas, but not in all studies. These microbes provoke a rapid cell-damaging process, called 'pyroptosis', which is characterised by a subsequent release of proinflammatory cellular components from damaged endothelial and innate immune cells. In agreement with these molecular events, patients with KD show elevated levels of damage-associated molecular patterns derived from cell death. In addition, an overwhelming amount of oxidative stress-associated molecules, including oxidised phospholipids or low-density lipoproteins, are generated as by-products of inflammation during the acute phase of the disease. These molecules induce abnormalities in the acquired immune system and activate innate immune and vascular cells to produce a range of proinflammatory molecules such as cytokines, chemokines, proteases and reactive oxygen species. These responses further recruit immune cells to the arterial wall, wherein inflammation and oxidative stress closely interact and mutually amplify each other. The inflammasome, a key component of the innate immune system, plays an essential role in the development of vasculitis in KD. Thus, innate immune memory, or 'trained immunity', may promote vasculitis in KD. Hence, this review will be helpful in understanding the pathophysiologic pathways leading to the development of principal KD symptoms and coronary artery lesions in patients with KD, as well as in subsets of patients with SARS-CoV-2 and *Yersinia* infections.

Keywords: COVID-19, damage-associated molecular patterns, Kawasaki disease, oxidative stress, pyroptosis, vasculitis

INTRODUCTION

Kawasaki disease (KD) is one of the most prevalent vasculitis syndromes in childhood. In addition to the common symptoms of KD associated with systemic inflammation, 25–30% of patients develop coronary artery abnormalities if untreated.[1,2] After introduction of high-dose intravenous immunoglobulin and additional therapies, the incidence of coronary artery lesions

decreases to 5% or less. Despite the therapeutic advances, KD is a leading cause of childhood-onset acquired heart disease in developed countries.[2]

Although over 50 years has passed since the first report, the aetiology of KD remains unknown. Epidemiological studies have suggested that KD may develop in children with genetically predisposing factors when they are exposed to environmental or infectious triggers.[2,3]

A small proportion of children exposed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) develop principal KD symptoms fulfilling the diagnostic criteria for KD in Europe[4–6] and the United States.[7] This is the first virus to be consistently and reproducibly associated with the development of principal KD symptoms in multiple areas even with low rates; for instance, no more than one out of 1000 children exposed to SARS-CoV-2 in Italy developed KD symptoms.[5] Such children have genetic predisposition (high in Hispanic and Black, and low in Asian) and show an older age at onset and atypical symptoms of KD.[4–7] This hyperinflammatory disorder associated with coronavirus disease-2019 (COVID-19), fulfilling full or partial criteria for KD, has been termed 'pediatric inflammatory multisystem syndrome' in the United Kingdom, and 'multisystem inflammatory syndrome in children (MIS-C)' in the United States.[4–7] Patients with MIS-C who met the case definition by Centers for Disease Control and Prevention have shown considerable heterogeneities in the clinical and laboratory features, and only 28 (4.9%) of 570 patients with MIS-C develop principal KD symptoms.[7] There have been several studies on the pathophysiology of MIS-C as a whole,[8–10] but not of a subset of MIS-C with KD symptoms.

Thus, this review focuses on providing an up-to-date pathophysiology of KD, which might help in understanding the pathophysiologic pathways present in subsets of patients with SARS-CoV-2 and *Yersinia* infections fulfilling the diagnostic criteria for KD.

INSIGHTS FROM THREE KD ANIMAL MODELS

There are three representative murine models of KD. In these models, the administration of either *Candida albicans* water-soluble fraction (CAWS), *Lactobacillus casei* cell wall extract (LCWE) or a nucleotide-binding oligomerisation domain-containing protein 1 (NOD1) ligand potently induces coronary arteritis.[11–13] Although none of these models completely capture the clinical features of KD, they serve as useful tools for investigating cellular and molecular mechanisms of KD vasculitis.[14]

Candida albicans water-soluble fraction is an extracellular mannoprotein produced by *C. albicans*. It is a ligand of Dectin-2, a C-type lectin receptor of innate immunity. CAWS induces biphasic cardiac-specific vasculitis, which is characterised by neutrophil and monocyte infiltration, endothelial activation and local production of inflammatory cytokines or chemokines, such as granulocyte–macrophage colony-stimulating factor and C–C motif chemokine ligand 2 (CCL2).[15,16] In addition, CAWS induces vasculitis in nude mice, severe combined immunodeficiency (SCID) mice,[11] *Rag1*- knockout (KO) mice,[17] *Rag*-γc-KO mice[16] and B-cell-deficient ($Igh^{-/-}$) mice.[17] Thus, T cells and B cells are dispensable in the induction of vasculitis by CAWS. The nuclear factor-kappa B (NF-κB) pathway is involved in this process, and mitochondrial reactive oxygen species (ROS)-activated nucleotide-binding oligomerisation domain, leucine-rich repeat and pyrin domain-containing 3 (Nlrp3) inflammasome is responsible for interleukin (IL)-1β production in bone marrow-derived dendritic cells.[18] As etanercept treatment reduces the incidence and severity of CAWS-induced vasculitis, tumor necrosis factor (TNF) also plays an important role in the development of vasculitis.[19] Further, *Candida metapsilosis* water-soluble fraction also induces coronary arteritis and anaphylactoid shock.[20]

In the LCWE murine model, the *Rag1*-, *Myd88*- and *Toll-like receptor (Tlr) 2*-KO mice and the C3H/HeJ mice, harbouring a spontaneous mutation in *Tlr4*, do not develop coronary vasculitis.[12,21–23] These data are consistent with the fact that LCWE contains superantigens and pathogen-associated molecular patterns (PAMPs), both of which are responsible for the phenotypic presentation of this model.[12] Thus, T cells and innate immune cells are indispensable for inducing coronary arteritis in the LCWE model.[12] In this model, $CD11c^{+}$ dendritic cells or macrophages, vascular stromal cells and cytokines, such as TNF, IL-1α and IL-1β, are important in the pathogenesis of coronary arteritis.[24,25] While IL-1α is released from damaged cells as an endogenous damage-associated molecular pattern (DAMP), the

inflammasome-dependent signals are involved in IL-1β production.[26] Endothelial Nlrp3 inflammasome may also contribute to the development of LCWE-induced coronary arteritis.[27] In addition, experimental data in the LCWE model have shown that nitric oxide (NO) and its reactive nitrogen derivative, peroxynitrite, have a role in the development of coronary arteritis and aneurysms. [28] Peroxynitrite is a powerful oxidant with damaging effects on lipids, proteins and DNA, and it can work as a DAMP.[29]

Lactobacillus casei cell wall extract model of KD shares many features with human disease in terms of the clinical course, disease susceptibility in the young and treatment response to drugs.[30] However, the major immune cells within coronary artery lesions in the acute phase of the LCWE model are T cells.[31] This finding is distinct from the cell populations (monocytes/macrophages and neutrophils) present in human-autopsied KD specimens within 2 weeks after the disease onset.[32]

FK565 (a NOD1 ligand) is a synthetic acyltripeptide with a molecular weight of 502.6 and is one of the derivatives of FK156 isolated from *Streptomyces olivaceogriseus*.[14] On binding to NOD1, FK565 functions as a PAMP. In the murine model of KD with FK565, NOD1 ligands supposedly activate the proinflammatory signals in vascular cells of coronary arteries to produce large quantities of chemokines and cytokines, such as CCL2[13] and IL-1β.[33] In response to the increased chemokines and cytokines, circulating monocytes are recruited to the FK565-activated endothelial cells where they subsequently differentiate into cardiac $CD11c^+$ macrophages.[34] In fact, $CD11c^+$ macrophage-deficient mice have revealed an importance of these macrophages in the promotion of the pathogenic process of acute coronary arteritis. We have further reproduced coronary arteritis in SCID- and *Rag1*-KO mice.[13,34] The immunological features of three murine models of KD are summarised in Table 1.

All three models use cell wall components as vasculitis-inducing agents. KD-like vasculitis can be induced by innate immune PAMPs in a T- or B-cell-independent manner in CAWS and NOD1 ligand models.[11,13,16,17,34] In the LCWE and CAWS models, Nlrp3 inflammasome, one of the core molecules firing up the innate immunity, plays a critical role in the induction of vasculitis. In all three models, oxidised molecules appear to serve as innate immune DAMPs, which is observed in humans as well. Finally, the vasculitis-inducing potential of CAWS greatly varies with the structure of mannosyl linkages of *Candida* mannan, which are modulated by the culture conditions.[14,20] Similarly, the pathogenic activities

Table 1. Three representative mouse models of Kawasaki disease

	CAWS model	LCWE model	NOD1 ligand model
Triggers			
PAMPs	CAWS	LCWE	NOD1 ligand (FK565)
DAMPs	Oxidised molecules	Oxidised molecules IL-1α	Oxidised molecules
Others	–	Superantigen	–
PRR	Dectin-2	TLR2	NOD1
Innate immunity	Vascular cells Cardiac macrophages NLRP3 inflammasome	Vascular stromal cells Macrophage, DC NLRP3 inflammasome	Endothelial cells Cardiac macrophages
Acquired immunity	T-cell/B-cell-independent	T-cell-dependent ($CD8^+$T cells)	T-cell/B-cell-independent
Vasculitis	Scid $mice^+$	Scid $mice^-$	Scid $mice^+$
Induction	*Rag1*-KO $mice^+$	Rag1-KO $mice^-$	*Rag1*-KO $mice^+$
Cytokine/chemokine	TNF-α, IL-1β, GM-CSF CCL2	TNF-α, IL-1β, IL-1α	IL-1β, CCL2
Major infiltrating cells[a]	Monocytes/macrophages Neutrophils	T cells, macrophages DC	Monocytes/macrophages Neutrophils

CAWS, *Candida albicans* water-soluble fraction; CCL2, C–C motif chemokine ligand 2; DAMPs, damage-associated molecular patterns; DC, dendritic cell; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; KO, knockout; LCWE, *Lactobacillus casei* cell wall extract; NLRP3, nucleotide-binding oligomerisation domain, leucine-rich repeat and pyrin domain-containing 3; NOD, nucleotide-binding oligomerisation domain-containing protein; PAMPs, pathogen-associated molecular patterns; PRR, pattern recognition receptor; Scid, severe combined immunodeficiency; TLR, Toll-like receptor; TNF, tumor necrosis factor.

[a]The major infiltrating cells in the human coronary arterial lesions of acute Kawasaki disease are monocytes/ macrophages and neutrophils.[32]

of NOD1 ligands differ among the synthetic derivatives of FK565 according to their chemical structures.[13]

INVOLVEMENT OF MICROBES, PAMPS AND DAMPS IN KD

The clinical findings (fever, rash, oral and conjunctival injection and cervical adenitis), unique age distribution (over 80% of the cases occur between the ages of 6 months and 4 years) and epidemiological features (existence of epidemics, community outbreaks and seasonality) of KD[1,2] mimic those of acute infections.

Various microbes and triggers have been reported to be associated with KD, but their causal relationships are yet to be confirmed.[2] Only a few microbes, such as SARS-CoV-2 and *Yersinia*, reproducibly develop principal KD symptoms fulfilling the diagnostic criteria for KD.

With regard to SARS-CoV-2, it infects endothelial cells, immune cells and many other cells using the angiotensin-converting enzyme 2 receptor.[35] The viral elements are detected within endothelial cells, and induction of apoptosis and pyroptosis might have an important role in endothelial injury in patients with COVID-19.[36] Since KD symptoms in COVID-19 typically appear 2–4 weeks after the infection, development of KD symptoms in SARS-CoV-2 seems to be an immune-mediated mechanism rather than a direct consequence of the viral infection.[5,35] Consiglio *et al*.[8] reported a possible involvement of autoantibodies in the pathogenesis of MIS-C, although the autoantibody signals were low and diffuse. Moreover, the role of autoantibodies is not clear in the abovementioned KD murine models and in patients with classic KD.[14] Given the suppressed acquired immunity,[35,37] the autoimmune mechanisms are less likely to be involved in KD-like symptoms in patients with MIS-C.

As for bacteria, *Yersinia pseudotuberculosis* is associated with two unique systemic inflammatory disorders: Far East scarlet-like fever (FESLF)[38] and a subset of KD, in addition to self-limiting gastroenteritis. Superantigen *Y. pseudotuberculosis*-derived mitogen A (YPMa) is implicated in the pathogenesis of FESLF, but not in KD. Patients with FESLF usually show positive anti-YPMa antibodies and increased levels of atypical lymphocytes and activated T cells[38], while patients with KD rarely show such findings.[14,39,40] Furthermore, coronary artery lesions are not documented in FESLF.[38]

In Japan, children infected with *Y. pseudotuberculosis* develop symptoms fulfilling the diagnostic criteria for KD at a frequency of 12–35%,[41] while, in Europe, the incidence of KD increases when the risk of exposure to *Y. pseudotuberculosis* infection is temporally and regionally higher.[42] In line with these studies, 9–10% of the patients hospitalised with KD, in certain areas of Japan, show serological evidence of *Y. pseudotuberculosis* infection.[39] These patients are more likely to develop abdominal symptoms and cardiac sequelae than KD patients without *Y. pseudotuberculosis*.

Children infected with *Yersinia enterocolitica* also develop symptoms fulfilling the diagnostic criteria for KD in Europe, Australia (3%; one out of 32 patients), the United States and Japan (our observation; 10%: two out of 20 patients).[43,44] Most patients show abdominal symptoms and incomplete KD symptoms (five of six patients) without coronary artery lesions.[43,44]

In summary, subsets of patients with *Y. pseudotuberculosis* and *Y. enterocolitica* infections also develop symptoms fulfilling the diagnostic criteria for KD. The incidence of *Yersinia* infection-associated development of KD symptoms varies with the geographical regions. Moreover, *Yersinia* infection triggers the activation of macrophages and caspase-1 *in vivo* and redirects the modality of the host cell death from noninflammatory apoptosis to inflammatory pyroptosis.[45]

There have been reports of nonmicrobial triggers as well. For instance, patients with KD associated with burn and severe sunburn injuries have been reported in Canada, Japan and China.[46–49] In these patients, KD occurred on days 2–5 of the burn or sunburn injury, and seven of the 10 patients showed negative results in their wound cultures. All patients responded to intravenous immunoglobulin administration, and one patient had coronary artery dilatation.[46–49]

Damage-associated molecular patterns including small molecules and bioactive lipids that leak from damaged cells are significantly elevated immediately after a burn injury. Because necrosis is a predominant type of cell death in burns, these molecules contribute to the activation of many monocytes/macrophages, which is a

characteristic pathological finding in patients with burns.[50] An infant with KD, following severe sunburns, showed high serum levels of high-mobility group box protein 1 (HMGB1), one of the DAMPs identified during the acute phase of KD.[47] However, additional host and microbial factors may also be involved in the induction of KD, as burns and severe sunburn injuries are rarely associated with KD.

In summary, pyroptosis during infection with *Yersinia* and SARS-CoV-2, and necrosis in burn or sunburn injuries release proinflammatory cellular contents such as DAMPs. ROS immediately oxidise these molecules, including the membrane phospholipids of the damaged cells.[51] DAMPs including oxidised phospholipids and low-density lipoproteins (LDLs) activate the endothelial and innate immune cells to further produce proinflammatory cytokines and ROS. These processes induce the activation of the NLRP3 inflammasome, resulting in the acceleration of pyroptosis of the endothelial cells and monocytes.[52] S100 proteins, HMGB1, heat-shock proteins, oxidised phospholipids, apoptosis-associated speck-like protein containing a caspase recruitment domain, caspase-1 and gasdermin D are all recognised as cell death-related molecules; these molecules are elevated in the sera of patients with KD.[53–59] Thus, the abovementioned data suggest that proinflammatory cell death (pyroptosis and necrosis) of endothelial and innate immune cells may play a significant role in the development of KD vasculitis (Table 2).

PATHOPHYSIOLOGY OF KD

The initial immune reactions of KD consist of trigger and acute reactive phases. Initial triggers include antigen-driven innate and acquired immune responses to viral and bacterial pathogens. Activation of the innate immune system must be stringently regulated. Otherwise, excessive activation can lead to systemic inflammation and tissue damage. The main pathophysiology of acute phase of KD is associated with innate immune hyperactivation, accompanying a Th17-related immune response and a strong inhibition of most T-cell and B-cell responses[14].

An analogous condition has been observed with SARS-CoV-2. The virus (trigger) initially interacts with the host as a conventional viral antigen. Subsequently, apoptosis and pyroptosis can be provoked by a cytopathic virus, SARS-CoV-2, in endothelial and immune cells in certain individuals.[36] In patients with severe COVID-19 and MIS-C, DAMPs such as HMGB1 and S100A are elevated.[60–62] Thus, it is possible that DAMPs from pyroptosis lead to further activation of innate immune system around a few weeks after the infection,[35,63,64] when KD symptoms might appear in children exposed to SARS-CoV-2.

Table 2. Kawasaki disease and DAMPs released by cell death

DAMPs[26]	Functions	Related cell death	Levels in KD	Reference
ASC	Lysosomal damage IL-1β activation	Pyroptosis	Increased	53
Calreticulin	'Eat me signal' Immunogenicity	Apoptosis	Increased	54
Defensin-α	Antimicrobial Anti-inflammatory	Apoptosis NCD	Increased	55
Heat-shock proteins	Monocyte and neutrophil attraction DC maturation	Necroptosis NCD	Increased	56
HMGB1	DCs and macrophage activation Cytokine activation	Apoptosis necroptosis pyroptosis	Increased	53,57
IL-6	Immune response T-cell differentiation	Necroptosis NCD	Increased	2
Oxidised phospholipids	Proinflammatory Prothrombotic	Necroptosis pyroptosis[52]	Increased	58
S100 proteins	Leucocyte recruitment cytokine induction	Necroptosis NCD	Increased	40,59

ASC, Apoptosis-associated speck-like protein containing a CARD; CD, cluster of differentiation; DAMP, damage-associated molecular patterns; DC, dendritic cell; HMGB1, high-mobility group box 1 protein; IL, interleukin; KD, Kawasaki disease; NCD, necrotic cell death; NLRP3, nucleotide-binding oligomerisation domain, leucine-rich repeat and pyrin domain-containing 3.

Activation of the innate immune system at acute phase of KD

The following clinical and laboratory findings support the concept that the acute phase of KD is driven primarily by the innate immune system. First, the absolute neutrophil and monocyte counts in peripheral blood increase. The majority of the activated T cells in the peripheral blood are γδT cells, one of the innate immune cells.[40] In addition, the major immune cell populations in the coronary arterial lesions are monocytes/macrophages and neutrophils.[32] These innate immune cells express high levels of effector molecules such as elastase and matrix metalloproteinases,[65] thereby suggesting their involvement in the destruction of the elastic lamina of the arterial wall. Neutrophils may contribute to vascular inflammation and cell damage through the enhanced formation of neutrophil extracellular traps.[66] Second, patients with KD have the highest recurrence rate within 12 months following the first episode, and this high-incidence rate of recurrence has been observed in patients who showed cardiac sequelae during the first episode.[67] In addition, patients with a recurrent episode of KD are more likely to have coronary artery abnormalities.[68] Recurrence in a short interval and in a more severe form in patients with KD may be attributed not to the immunological memory of the acquired immune system but to the innate immune memory (trained immunity).[69] Trained immunity is a concept that states that innate immune cells can undergo metabolic and epigenetic reprogramming, resulting in enhanced immune responses to heterologous reinfection or endogenous danger signals.[69] This concept may also be applicable to the cells of nonhaematopoietic origin, such as epithelial cells, endothelial cells and vascular smooth muscle cells.[70] In fact, endothelial cells especially function as sentinel innate immune cells and detect foreign pathogens and endogenous danger signals in the bloodstream.[70,71] Oxidised phospholipids and LDLs, as DAMPs, modify the epigenetic status of monocytes and vascular cells, facilitate memory responses and boost inflammation.[72] Since oxidised phospholipids and LDLs are elevated during the acute phase of KD,[58] we hypothesise that such DAMPs may reprogramme the cellular metabolism and boost hyperinflammation of innate immune cells and vascular cells in patients with KD during its onset and recurrence.[67,68]

Furthermore, the clinical and laboratory data have shown that serum IL-1β levels increase and IL-1 signalling is upregulated in KD.[73,74] These data suggest that the inflammasome, a key component of the innate immune system, is associated with the development of KD vasculitis. Indeed, NLRP3 inflammasome appears to be one of the inflammatory signalling platforms bearing vasculitis in KD.[75]

Soluble innate immune pattern recognition molecules (PRMs) are key players of the humoral arm of innate immunity. They act as opsonins and enhance the clearance of pathogens, dying cells and cellular materials by phagocytic cells; they are also involved in leucocyte recruitment and activation.[76] Soluble PRMs can be classified into four groups: PRMs such as pentraxins,[77] collectins (mannose-binding lectin)[78] and ficolins[79] with the ability to activate the complement system; PRMs such as serum amyloid A that are unable to activate the complement system;[80] the complement components themselves;[81,82] and other molecules such as soluble CD14[83] and natural antibodies.[84] Consistent to our previous finding that some PAMPs/DAMPs from patients with KD bind to immunoglobulin G by column experiments,[85] intravenous immunoglobulin (IVIG) might exert its effect through adsorbing a variety of pattern recognition receptors, including M-ficolin,[79] with the constant regions, and thus clear PAMPs/DAMPs from the blood of patients with KD. The soluble PRMs are shown in Table 3.

Natural antibodies, with specificity for both microbial and self-antigens, act as the first line of defence against infections and promote the clearance of dead cell debris. Oxidation-specific epitopes, serving as DAMPs, are major targets of IgM natural antibodies.[84] IgM natural antibodies recognise the phosphocholine moiety of oxidised phosphatidylcholine present in oxidised LDLs and in membranes of dying cells, as well as other oxidation-specific epitopes (malondialdehyde). Because the generation of oxidation-specific epitopes is associated with cellular death or oxidative damage of molecules, these epitopes represent critical tags that allow innate immunity to distinguish between the viable and damaged or dying cells.[82,84]

Table 3. Soluble pattern recognition molecules in Kawasaki disease

Soluble PRMs[76]	Significance and function	Blood levels in KD	Reference
Pentraxins	Induced by infection, tissue damage and inflammation		
Pentraxin 3	Blood level reflects the extent of tissue damage. Opsonic activity, removal of dead cells	Increased	76,77
C-reactive protein	The prototype of the pentraxin family removal of microbes and dead cells	Increased	76
Collectins	Collagen containing C-type lectins		
Mannose-binding lectin	Opsonic activity, complement activation, removal of dead cells	Increased	78
Ficolins	Have a fibrinogen-like and collagen-like domain		76
M-ficolins	Opsonic activity, complement activation, removal of dead cells, bind to IgG	Increased	79
Serum amyloid A	Interacts with microorganisms and microbe-derived molecules		
Serum amyloid A	Opsonic activity, leucocyte recruitment and local proinflammatory cytokine production	Increased	80
Complement components	Eliminating a great diversity of pathogens and dead cells		
C3	Activates the alternating pathway	Increased	81
C4	Activates the classical pathway	No change	81
Factor H	Regulates the alternative pathway	Decreased	82
Others			
Soluble CD14	Mediates the activation of endothelial cells	Increased	83
Natural antibodies	React to the oxidation-specific epitopes on dead cell phospholipids and in plasma-oxidised LDL	No data available	84

PRMs, pattern recognition molecules.

Abnormalities in the acquired immune system

Kawasaki disease is also regarded as a condition that is associated with acquired immune abnormalities. It is characterised by decreased absolute T-cell counts in the peripheral blood, marked suppression of T-cell receptor/CD3-induced T-cell proliferation,[86] downregulation of T-cell receptor and B-cell receptor signalling pathways,[40,74,87,88] and decreased regulatory T and B cells during the acute phase of the disease.[89,90] The proportion of T helper (Th) 17 cells are significantly elevated during the acute phase of KD.[91]

As oxidised phospholipids and LDLs are markedly elevated in blood obtained from patients with acute KD,[58] these findings suggest that the oxidised phospholipids and LDLs activate the innate immune and endothelial cells, but induce T-cell anergy.[92] The oxidised LDLs lead to a significant elevation of Th17 cells and a reduction in regulatory T cells in a dose- and time-dependent manner.[93]

With respect to B cells, the antibody responses are not generally suppressed in KD.[86] Genetic variants of B-cell-related genes (*CD40*, *CD40L* and *BLK*) increase the susceptibility of children to development of KD.[2,94,95] A difference in the expression levels of CD40/CD40 ligand (L) and B lymphoid tyrosine kinase (BLK) might affect the production rate and type of antibody produced in response to an external stimulus in patients with KD. These studies suggest that B cells and antibodies play a role in the pathogenesis of KD.[96] Furthermore, the difference might also influence oxidised LDL-triggered CD40/CD40L signalling pathway in the endothelial cells to induce inflammation.[97,98] In fact, *CD40L* expression on circulating cells is correlated with the occurrence of coronary artery lesions.[99] In addition to the expression in B cells, *BLK* is also expressed in $\gamma\delta$T cells and dendritic cells that may play a role in the vascular inflammation.[100] From another point of view, natural antibodies against oxidised lipids[101] may be produced and involved in the clearance of apoptotic or pyroptotic cells during the acute and convalescent stages. B cells represent a unique component of the acquired immune system as they express both the B-cell receptor and pattern recognition receptors, such as Toll-like receptors.[102]

In autoimmune aspects of KD, various studies have shown the presence of immune complexes in the peripheral blood of patients with KD, but it remains controversial whether the presence of immune complexes correlates with the severity of KD.[103] Extensive efforts have been made in search

of the deposit of immune complex at vascular lesions of KD in Japan and other countries.[103] However, no immune complex depositions have been detected in KD vasculitis lesions by Japanese and American pathologists.[104,105] In addition, KD vasculitis is characterised by granulomatous inflammation with monocytes/macrophage infiltrations, whereas fibrinoid necrosis rarely occurs.[105] Thus, these pathological findings of KD are also distinct from those of immune complex-associated vasculitis.

This might also be the case with anti-endothelial cell autoantibodies (AECAs). In fact, AECAs are not always increased in patients with KD, and the causal effect of AECAs remains to be determined in different cohorts.[106] KD shows epidemiological (seasonal variation and outbreaks in broad regions), clinical (almost one out of 100 children in Japan have the disease by age 5, self-limitedness, low recurrence rate and no significant association with autoimmune diseases) and laboratory (T-cell suppression) features. Thus, the autoimmune aspect of KD may not be closely associated with the pathogenic mechanisms of vasculitis, but it may reflect a secondary event in the affected patients.[14,30]

In severe COVID-19, inappropriate acquired immune responses (lymphopenia, marked reductions in circulating $CD3^+$, $CD4^+$ and $CD8^+$ T cells) and ineffective activation of cytotoxic $CD8^+$ T cells and NK cells are also observed.[35] In MIS-C, lymphopenia with decreased $CD3^+$, naïve $CD4^+$ and $CD8^+$ T cells is evident.[8] In addition, acquired immune responses against self-antigens may play a role in the immunopathogenesis of MIS-C,[8,62] although the rapid resolution of inflammation in MIS-C goes against this theory.

Identification of possible PAMPs in KD sera

We detected serum KD-associated molecules with high specificity and low sensitivity by liquid chromatography–mass spectrometry (LC-MS) in a previous study.[85] Some serum KD-associated molecules show fragmentation patterns similar to those of PAMPs from biofilm extracts obtained from *Y pseudotuberculosis* and airborne bacteria using liquid chromatography–tandem mass spectrometry. The pathogenic mechanisms of the biofilms include the production of large amounts of bioactive molecules through quorum-sensing mechanism. In fact, KD-associated molecules are produced in large quantities when *Y. pseudotuberculosis* is cultured in an *in vitro* biofilm-forming condition;[85] this is similar to the production of toxic shock syndrome toxin-1, which is 1000-fold higher when *Staphylococcus aureus* is cultured in an *in vitro* tampon sac biofilm condition.[107] In a subsequent high-resolution LC-MS-based lipidomic analysis, we confirmed that the KD- associated molecules possessed structures similar to those found in biofilm extracts from *Y. pseudotuberculosis*.[58]

Oxidative stress and DAMPs in KD

Free radicals in ROS and reactive nitrogen species (RNS) often exert deleterious effects on human health. Intracellular sources of ROS and RNS include the mitochondria, endoplasmic reticulum, lysosome, peroxisome and enzymes in the cytoplasm and plasma membrane.[108] Oxidative stress represents a condition of an imbalance between oxidants and antioxidants, which is in favor of the oxidants.

In cardiovascular diseases, the excessive generation of ROS by nicotinamide adenine dinucleotide phosphate oxidases, uncoupled endothelial nitric oxide synthase (NOS), mitochondria, endoplasmic reticulum and xanthine oxidase has particular relevance.[109] As concurrent messengers with inflammatory signals, the elevated ROS activate the canonical NF-κB pathway and lead to the expression of downstream inflammatory and antioxidant genes, activation of the inflammasome and secretion of cytokines or chemokines.[109] Further, peroxynitrite is an RNS product of the reaction of NO with superoxide, and it can damage various cellular components, as it is a strong oxidising and nitrating agent. Excess ROS and RNS are associated with diverse pathophysiological events in cardiovascular diseases, such as endothelial dysfunction, vascular inflammation and atherosclerosis.[109]

Inflammation can be both a cause and a consequence of increased oxidative stress. At the active sites of inflammation, the inflammatory cells, vascular endothelial cells and smooth muscle cells are all capable of releasing ROS, enzymes and chemical mediators to result in oxidative stress. Oxidative stress also stimulates the NF-κB pathway and expression of cytokines and chemokines to further enhance the inflammation. Thus, inflammation and oxidative stress closely interact and mutually amplify the effects of each other.[108,109]

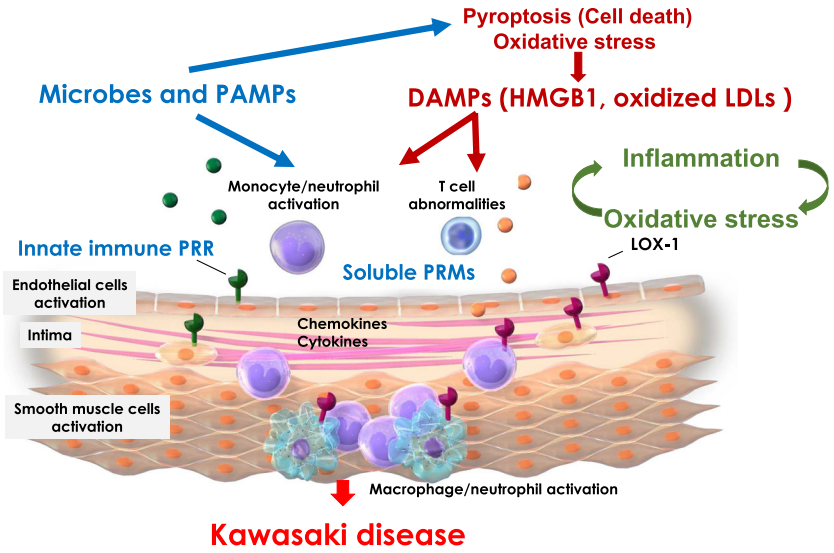

Figure 1. Hypothetical model for the pathophysiology of Kawasaki disease. Innate immune pathogen-associated molecular patterns (PAMPs) from microbes activate proinflammatory signals in innate immune and vascular cells through pattern recognition receptors (PRR), thereby producing large amounts of chemokines and cytokines. Massively produced damage-associated molecular patterns (DAMPs) from cell death and oxidative stress in the circulating blood exert pleiotropic effects on platelets, monocytes, neutrophils, endothelial cells and vascular smooth muscle cells through receptor [lectin-like oxidised LDL receptor-1 (LOX-1)]-mediated and receptor-independent mechanisms. Soluble pattern recognition molecules (PRMs) are also involved in the pathophysiology of Kawasaki disease. In response to these stimulations, monocytes and neutrophils in the peripheral blood are recruited to stimulated vascular cells. Subsequently, monocytes differentiate into macrophages. These macrophages and neutrophils play a pivotal role in the development of acute coronary arteritis. Inflammation and oxidative stress mutually amplify each other, leading to the induction of acute KD. HMGB1, high-mobility group box 1; LDL, low-density lipoprotein.

During the acute phase of KD (Figure 1), majority of the cells infiltrating the coronary arteries are neutrophils and macrophages,[32] which are the primary sources of ROS. The ROS extend the damage to the inflammatory cells themselves and adjacent cells, such as vascular cells.[110–112] Activated neutrophils and monocytes also release a large amount of myeloperoxidase, which is a pro-oxidant enzyme that amplifies the formation of ROS and development of coronary arteritis in KD.[111] In addition, the NOx and NO-derived species (3-nitrotyrosine) levels in plasma, inducible *NOS* mRNA levels in mononuclear cells and amount of NO produced by neutrophils are elevated in patients with acute KD.[111,113,114] Thus, both oxidative and nitrative stresses concur in the acute phase of KD,[110–115] as shown in Table 4.

Secondary products of ROS also contribute to the development of vascular diseases. Lipid peroxidation products such as oxidised phospholipids are implicated in the regulation of NF-κB activation, inflammation, thrombosis, angiogenesis, endothelial function and immune tolerance.[92,109] Intriguingly, oxidised phospholipids or LDLs activate the innate immune system, increase the production of a range of proinflammatory molecules such as cytokines, chemokines, eicosanoids, proteases, ROS and RNS, and lead to immune cell recruitment and arterial wall inflammation.[109] In addition to these proinflammatory functions, the oxidised phospholipids exert prothrombotic effects on a variety of cell types in the vessel walls.[109]

During the acute phase of KD, an overwhelming amount of oxidative stress-associated molecules are generated as by-products of inflammation (Table 4). The lipid peroxidation products include malondialdehyde,[115] F2-isoprostanes,[116] and oxidised phospholipids and LDLs.[58,117] Oxidised phospholipids evoke arterial wall inflammation in humans,[118] and oxidised LDLs induce pyroptosis in vascular endothelial cells.[119] Actually, blood levels of oxidised phosphatidylcholines[58] and oxidised LDLs[117] are associated with the development of coronary arteritis.

Lectin-like oxidised LDL receptor-1 (LOX-1) is a major scavenger receptor for oxidised LDLs in vascular cells[120,121] and is involved in endothelial dysfunction, smooth muscle cell migration and proliferation, inflammation and atherogenesis.[122,123] LOX-1 ligand assay measures

Table 4. Oxidative stress in Kawasaki disease

Oxidative stress-related molecules	Significance and function	Blood levels in KD	Reference
Chemical molecules			
ROS/RNS	Direct and indirect oxidation of various biomolecules	Elevated	110–112
Pro-oxidant enzymes			
Myeloperoxidase	Promote the proinflammatory state	Elevated	111
NO and related molecules			
NO production	Plasma level	Elevated	113
Inducible NOS mRNA	NO synthesis	Elevated	113
3-nitrotyrosine	Oxidative post-translational covalent modification	Elevated	111
Asymmetric dimethylarginine	An endogenous inhibitor of NOS	Decreased	114
Secondary products of ROS Lipid peroxidation products			
Malondialdehyde	End-product of lipid peroxidation	Elevated	115
F2-isoprostanes	Nonenzymatic oxidation product of arachidonic acid	Elevated	116
Oxidised phospholipids	Function as DAMPs	Elevated	58
Oxidised LDLs	Function as DAMPs	Elevated	58,117

DAMPs: damage-associated molecular patterns; F2-isoprostanes, 8-isoprostaglandin F2α; KD: Kawasaki disease; LDL: low-density lipoprotein; NO: nitric oxide; NOS: nitric oxide synthase; ROS/RNS: reactive oxygen species/reactive nitrogen species including superoxide anion, hydroxyl radicals, NO, hydrogen peroxide and peroxynitrite.

the biological activity of apolipoprotein B based on its binding to LOX-1.[124] Using immobilised recombinant human LOX-1, various types of modified LDLs can be detected as LOX-1 ligands in patients with KD.[58] Therefore, LOX-1 ligand assay may better reflect the pathogenic activities of apolipoprotein B than the measurements of oxidised lipids or oxidised LDLs by LC-MS or monoclonal antibodies. LOX-1 ligand assay would be useful in the diagnosis of acute KD and possibly in the diagnosis of MIS-C fulfilling the diagnostic criteria for KD.

CONCLUSIONS

Pathogen-associated molecular patterns and inflammatory cell death-associated DAMPs appear to play a significant role in the development of KD. Subsequently, inflammation and oxidative stress mutually amplify each other, and possibly lead to the induction of KD. KD and a subset of MIS-C fulfilling the diagnostic criteria for KD show some common but distinct pathophysiological features. Therefore, further study will be necessary to find out whether the strategy for the diagnosis and treatment of KD may be useful in the management of a subset of MIS-C fulfilling the diagnostic criteria for KD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Toshiro Hara: Conceptualization; Writing-original draft; Writing-review & editing. **Kenichiro Yamamura:** Writing-original draft; Writing-review & editing. **Yasunari Sakai:** Project administration; Writing-original draft; Writing-review & editing.

REFERENCES

1. Kato H, Koike S, Yamamoto M, Ito Y, Yano E. Coronary aneurysms in infants and young children with acute febrile mucocutaneous lymph node syndrome. *J Pediatr* 1975; **86**: 892–898.
2. McCrindle BW, Rowley AH, Newburger JW *et al*. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the american heart association. *Circulation* 2017; **135**: e927–e999.
3. Nakamura Y, Yashiro M, Uehara R, Oki I, Watanabe M, Yanagawa H. Monthly observation of the number of patients with Kawasaki disease and its incidence rates in Japan: chronological and geographical observation from nationwide surveys. *J Epidemiol* 2008; **18**: 273–279.
4. Toubiana J, Poirault C, Corsia A *et al*. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. *BMJ* 2020; **369**: m2094.

5. Verdoni L, Mazza A, Gervasoni A *et al*. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet* 2020; **395**: 1771–1778.
6. Whittaker E, Bamford A, Kenny J *et al*. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. *JAMA* 2020; **324**: 259–269.
7. Godfred-Cato S, Bryant B, Leung J *et al*. COVID-19-associated multisystem inflammatory syndrome in children - United States, March-July 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**: 1074–1080.
8. Consiglio CR, Cotugno N, Sardh F *et al*. The immunology of multisystem inflammatory syndrome in children with COVID-19. *Cell* 2020; **183**: 968–981.e7.
9. Diorio C, Henrickson SE, Vella LA *et al*. Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS-CoV-2. *J Clin Invest* 2020; **130**: 5967–5975.
10. Lee PY, Day-Lewis M, Henderson LA *et al*. Distinct clinical and immunological features of SARS-CoV-2-induced multisystem inflammatory syndrome in children. *J Clin Invest* 2020; **130**: 5942–5950.
11. Nagi-Miura N, Adachi Y, Ohno N. [Coronary arteritis induced by CAWS (*Candida albicans* water-soluble fraction) in various strains of mice]. *Nihon Ishinkin Gakkai Zasshi* 2008; **49**: 287–292.
12. Lehman TJ, Warren R, Gietl D, Mahnovski V, Prescott M. Variable expression of *Lactobacillus casei* cell wall-induced coronary arteritis: an animal model of Kawasaki's disease in selected inbred mouse strains. *Clin Immunol Immunopathol* 1988; **48**: 108–118.
13. Nishio H, Kanno S, Onoyama S *et al*. Nod1 ligands induce site-specific vascular inflammation. *Arterioscler Thromb Vasc Biol* 2011; **31**: 1093–1099.
14. Hara T, Nakashima Y, Sakai Y, Nishio H, Motomura Y, Yamasaki S. Kawasaki disease: a matter of innate immunity. *Clin Exp Immunol* 2016; **186**: 134–143.
15. Miyabe C, Miyabe Y, Bricio-Moreno L *et al*. Dectin-2-induced CCL2 production in tissue-resident macrophages ignites cardiac arteritis. *J Clin Invest* 2019; **129**: 3610–3624.
16. Stock AT, Hansen JA, Sleeman MA, McKenzie BS, Wicks IP. GM-CSF primes cardiac inflammation in a mouse model of Kawasaki disease. *J Exp Med* 2016; **213**: 1983–1998.
17. Martinez HG, Quinones MP, Jimenez F *et al*. Important role of CCR2 in a murine model of coronary vasculitis. *BMC Immunol* 2012; **13**: 56.
18. Anzai F, Watanabe S, Kimura H *et al*. Crucial role of NLRP3 inflammasome in a murine model of Kawasaki disease. *J Mol Cell Cardiol* 2020; **138**: 185–196.
19. Oharaseki T, Yokouchi Y, Yamada H *et al*. The role of TNF-α in a murine model of Kawasaki disease arteritis induced with a *Candida albicans* cell wall polysaccharide. *Mod Rheumatol* 2014; **24**: 120–128.
20. Tada R, Takano Y, Murakami H *et al*. Vasculitis and anaphylactoid shock in mice induced by the polysaccharide fraction secreted into culture supernatants by the fungus *Candida metapsilosis*. *Microbiol Immunol* 2011; **55**: 357–365.
21. Duong TT, Silverman ED, Bissessar MV, Yeung RS. Superantigenic activity is responsible for induction of coronary arteritis in mice: an animal model of Kawasaki disease. *Int Immunol* 2003; **15**: 79–89.
22. Rosenkranz ME, Schulte DJ, Agle LM *et al*. TLR2 and MyD88 contribute to *Lactobacillus casei* extract-induced focal coronary arteritis in a mouse model of Kawasaki disease. *Circulation* 2005; **112**: 2966–2973.
23. Schulte DJ, Yilmaz A, Shimada K *et al*. Involvement of innate and adaptive immunity in a murine model of coronary arteritis mimicking Kawasaki disease. *J Immunol* 2009; **183**: 5311–5318.
24. Hui-Yuen JS, Duong TT, Yeung RS. TNF-α is necessary for induction of coronary artery inflammation and aneurysm formation in an animal model of Kawasaki disease. *J Immunol* 2006; **176**: 6294–6301.
25. Lee Y, Wakita D, Dagvadorj J *et al*. IL-1 signaling is critically required in stromal cells in kawasaki disease vasculitis mouse model: role of both IL-1α and IL-1β. *Arterioscler Thromb Vasc Biol* 2015; **35**: 2605–2616.
26. Amarante-Mendes GP, Adjemian S, Branco LM, Zanetti LC, Weinlich R, Bortoluci KR. Pattern recognition receptors and the host cell death molecular machinery. *Front Immunol* 2018; **9**: 2379.
27. Chen Y, Li X, Boini KM *et al*. Endothelial Nlrp3 inflammasome activation associated with lysosomal destabilization during coronary arteritis. *Biochim Biophys Acta* 2015; **1853**: 396–408.
28. Adewuya O, Irie Y, Bian K, Onigu-Otite E, Murad F. Mechanism of vasculitis and aneurysms in Kawasaki disease: role of nitric oxide. *Nitric Oxide* 2003; **8**: 15–25.
29. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 2007; **87**: 315–424.
30. Noval Rivas M, Arditi M. Kawasaki disease: pathophysiology and insights from mouse models. *Nat Rev Rheumatol* 2020; **16**: 391–405.
31. Duong TT, Yeung RSM. Update on pathogenesis: lessons learned from animal models of disease. In: Saji BTNJ, Burns JC, Takahashi M eds. Tokyo: Springer Japan, 2017: 45–51.
32. Takahashi K, Oharaseki T, Naoe S, Wakayama M, Yokouchi Y. Neutrophilic involvement in the damage to coronary arteries in acute stage of Kawasaki disease. *Pediatr Int* 2005; **47**: 305–310.
33. Ohashi R, Fukazawa R, Watanabe M *et al*. Characterization of a murine model with arteritis induced by Nod1 ligand, FK565: a comparative study with a CAWS-induced model. *Mod Rheumatol* 2017; **27**: 1024–1030.
34. Motomura Y, Kanno S, Asano K *et al*. Identification of pathogenic cardiac $CD11c^{+}$ macrophages in Nod1-mediated acute coronary arteritis. *Arterioscler Thromb Vasc Biol* 2015; **35**: 1423–1433.
35. Liu PP, Blet A, Smyth D, Li H. The science underlying COVID-19: implications for the cardiovascular system. *Circulation* 2020; **142**: 68–78.
36. Varga Z, Flammer AJ, Steiger P *et al*. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020; **395**: 1417–1418.

37. Tay MZ, Poh CM, Renia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol* 2020; **20**: 363–374.
38. Amphlett A. Far east scarlet-like fever: a review of the epidemiology, symptomatology, and role of superantigenic toxin: *Yersinia pseudotuberculosis*-derived mitogen A. *Open Forum Infect Dis* 2016; **3**: ofv202.
39. Horinouchi T, Nozu K, Hamahira K *et al*. *Yersinia pseudotuberculosis* infection in Kawasaki disease and its clinical characteristics. *BMC Pediatr* 2015; **15**: 177.
40. Ikeda K, Yamaguchi K, Tanaka T *et al*. Unique activation status of peripheral blood mononuclear cells at acute phase of Kawasaki disease. *Clin Exp Immunol* 2010; **160**: 246–255.
41. Sato K, Ouchi K, Taki M. *Yersinia pseudotuberculosis* infection in children, resembling Izumi fever and Kawasaki syndrome. *Pediatr Infect Dis* 1983; **2**: 123–126.
42. Vincent P, Salo E, Skurnik M, Fukushima H, Simonet M. Similarities of Kawasaki disease and *Yersinia pseudotuberculosis* infection epidemiology. *Pediatr Infect Dis J* 2007; **26**: 629–631.
43. Feeney CC, Ajagbe OA, Suryadevara M. *Yersinia enterocolitica* infection presenting as incomplete Kawasaki disease: 2 cases and a review of the literature. *J Pediatric Infect Dis Soc* 2020; piaa016.
44. Marriott DJ, Taylor S, Dorman DC. *Yersinia enterocolitica* infection in children. *Med J Aust* 1985; **143**: 489–492.
45. Bergsbaken T, Fink SL, Cookson BT. Pyroptosis: host cell death and inflammation. *Nat Rev Microbiol* 2009; **7**: 99–109.
46. Ito S, Anze M, Ishikawa A, Aihara Y, Yokota S. Kawasaki disease after burns. *Eur J Pediatr* 2006; **165**: 340–341.
47. Okada S, Hashimoto S, Miyake A *et al*. Kawasaki disease following severe sunburn injury. *Front Pediatr* 2020; **8**: 6.
48. Ueda T, Ota I, Kitazawa Y. A pediatric case of burn associated with Kawasaki disease. *World J Trauma Crit Care Med* 2018; **7**: 8–11.
49. Wong D, Nutting A, Yeung RS, McCrindle BW. Kawasaki disease and scald injuries: a possible association. *Can J Cardiol* 2004; **20**: 1147–1149.
50. Rani M, Nicholson SE, Zhang Q, Schwacha MG. Damage-associated molecular patterns (DAMPs) released after burn are associated with inflammation and monocyte activation. *Burns* 2017; **43**: 297–303.
51. Barnett KC, Kagan JC. Lipids that directly regulate innate immune signal transduction. *Innate Immun* 2020; **26**: 4–14.
52. Zeng C, Wang R, Tan H. Role of pyroptosis in cardiovascular diseases and its therapeutic implications. *Int J Biol Sci* 2019; **15**: 1345–1357.
53. Jia C, Zhang J, Chen H *et al*. Endothelial cell pyroptosis plays an important role in Kawasaki disease via HMGB1/RAGE/cathespin B signaling pathway and NLRP3 inflammasome activation. *Cell Death Dis* 2019; **10**: 778.
54. Abe J, Jibiki T, Noma S, Nakajima T, Saito H, Terai M. Gene expression profiling of the effect of high-dose intravenous Ig in patients with Kawasaki disease. *J Immunol* 2005; **174**: 5837–5845.
55. Truong DML, Lin E. Next generation sequencing in kawasaki disease: a pilot study. *Circulation* 2019; **140**: A15274.
56. Takeshita S, Kawase H, Yamamoto M, Fujisawa T, Sekine I, Yoshioka S. Increased expression of human 63-kD heat shock protein gene in Kawasaki disease determined by quantitative reverse transcription-polymerase chain reaction. *Pediatr Res* 1994; **35**: 179–183.
57. Hoshina T, Kusuhara K, Ikeda K, Mizuno Y, Saito M, Hara T. High mobility group box 1 (HMGB1) and macrophage migration inhibitory factor (MIF) in Kawasaki disease. *Scand J Rheumatol* 2008; **37**: 445–449.
58. Nakashima Y, Sakai Y, Mizuno Y *et al*. Lipidomics links oxidized phosphatidylcholines and coronary arteritis in Kawasaki disease. *Cardiovasc Res* 2019; **117**: 96–108.
59. Foell D, Ichida F, Vogl T *et al*. S100A12 (EN-RAGE) in monitoring Kawasaki disease. *Lancet* 2003; **361**: 1270–1272.
60. Chen R, Huang Y, Quan J *et al*. HMGB1 as a potential biomarker and therapeutic target for severe COVID-19. *Heliyon* 2020; **6**: e05672.
61. Chen L, Long X, Xu Q *et al*. Elevated serum levels of S100A8/A9 and HMGB1 at hospital admission are correlated with inferior clinical outcomes in COVID-19 patients. *Cell Mol Immunol* 2020; **17**: 992–994.
62. Ramaswamy A, Brodsky NN, Sumida TS *et al*. Post-infectious inflammatory disease in MIS-C features elevated cytotoxicity signatures and autoreactivity that correlates with severity. *medRxiv* [Preprint] 2020. https://doi.org/10.1101/2020.12.01
63. Tang D, Comish P, Kang R. The hallmarks of COVID-19 disease. *PLoS Pathog* 2020; **16**: e1008536.
64. Lee S, Channappanavar R, Kanneganti TD. Coronaviruses: innate immunity, inflammasome activation, inflammatory cell death, and cytokines. *Trends Immunol* 2020; **41**: 1083–1099.
65. Biezeveld MH, van Mierlo G, Lutter R *et al*. Sustained activation of neutrophils in the course of Kawasaki disease: an association with matrix metalloproteinases. *Clin Exp Immunol* 2005; **141**: 183–188.
66. Yoshida Y, Takeshita S, Kawamura Y, Kanai T, Tsujita Y, Nonoyama S. Enhanced formation of neutrophil extracellular traps in Kawasaki disease. *Pediatr Res* 2020; **87**: 998–1004.
67. Hirata S, Nakamura Y, Yanagawa H. Incidence rate of recurrent Kawasaki disease and related risk factors: from the results of nationwide surveys of Kawasaki disease in Japan. *Acta Paediatr* 2001; **90**: 40–44.
68. Maddox RA, Holman RC, Uehara R *et al*. Recurrent Kawasaki disease: USA and Japan. *Pediatr Int* 2015; **57**: 1116–1120.
69. Netea MG, Dominguez-Andres J, Barreiro LB *et al*. Defining trained immunity and its role in health and disease. *Nat Rev Immunol* 2020; **20**: 375–388.
70. Shao Y, Saredy J, Yang WY *et al*. Vascular endothelial cells and innate immunity. *Arterioscler Thromb Vasc Biol* 2020; **40**: e138–e152.
71. Opitz B, Forster S, Hocke AC *et al*. Nod1-mediated endothelial cell activation by *Chlamydophila pneumoniae*. *Circ Res* 2005; **96**: 319–326.

72. Di Gioia M, Spreafico R, Springstead JR *et al*. Endogenous oxidized phospholipids reprogram cellular metabolism and boost hyperinflammation. *Nat Immunol* 2020; **21**: 42–53.
73. Maury CP, Salo E, Pelkonen P. Circulating interleukin-1β in patients with Kawasaki disease. *N Engl J Med* 1988; **319**: 1670–1671.
74. Hoang LT, Shimizu C, Ling L *et al*. Global gene expression profiling identifies new therapeutic targets in acute Kawasaki disease. *Genome Med* 2014; **6**: 541.
75. Alphonse MP, Duong TT, Shumitzu C *et al*. Inositol-triphosphate 3-kinase C mediates inflammasome activation and treatment response in Kawasaki disease. *J Immunol* 2016; **197**: 3481–3489.
76. Litvack ML, Palaniyar N. Review: soluble innate immune pattern-recognition proteins for clearing dying cells and cellular components: implications on exacerbating or resolving inflammation. *Innate Immun* 2010; **16**: 191–200.
77. Ching LL, Nerurkar VR, Lim E, Shohet RV, Melish ME, Bratincsak A. Elevated levels of pentraxin 3 correlate with neutrophilia and coronary artery dilation during acute Kawasaki disease. *Front Pediatr* 2020; **8**: 295.
78. Liu W, Liu C, Zhang L *et al*. Molecular basis of coronary artery dilation and aneurysms in patients with Kawasaki disease based on differential protein expression. *Mol Med Rep* 2018; **17**: 2402–2414.
79. Okuzaki D, Ota K, Takatsuki SI *et al*. FCN1 (M-ficolin), which directly associates with immunoglobulin G1, is a molecular target of intravenous immunoglobulin therapy for Kawasaki disease. *Sci Rep* 2017; **7**: 11334.
80. Miwata H, Yamada T, Okada M, Kudo T, Kimura H, Morishima T. Serum amyloid A protein in acute viral infections. *Arch Dis Child* 1993; **68**: 210–214.
81. Salo E, Kekomaki R, Pelkonen P, Ruuskanen O, Viander M, Wagner O. Kawasaki disease: monitoring of circulating immune complexes. *Eur J Pediatr* 1988; **147**: 377–380.
82. Weismann D, Binder CJ. The innate immune response to products of phospholipid peroxidation. *Biochim Biophys Acta* 2012; **1818**: 2465–2475.
83. Takeshita S, Nakatani K, Tsujimoto H, Kawamura Y, Kawase H, Sekine I. Increased levels of circulating soluble CD14 in Kawasaki disease. *Clin Exp Immunol* 2000; **119**: 376–381.
84. Chou MY, Fogelstrand L, Hartvigsen K *et al*. Oxidation-specific epitopes are dominant targets of innate natural antibodies in mice and humans. *J Clin Invest* 2009; **119**: 1335–1349.
85. Kusuda T, Nakashima Y, Murata K *et al*. Kawasaki disease-specific molecules in the sera are linked to microbe-associated molecular patterns in the biofilms. *PLoS One* 2014; **9**: e113054.
86. Kuijpers TW, Wiegman A, van Lier RA *et al*. Kawasaki disease: a maturational defect in immune responsiveness. *J Infect Dis* 1999; **180**: 1869–1877.
87. Ling XB, Lau K, Kanegaye JT *et al*. A diagnostic algorithm combining clinical and molecular data distinguishes Kawasaki disease from other febrile illnesses. *BMC Med* 2011; **9**: 130.
88. Popper SJ, Shimizu C, Shike H *et al*. Gene-expression patterns reveal underlying biological processes in Kawasaki disease. *Genome Biol* 2007; **8**: R261.
89. Furuno K, Yuge T, Kusuhara K *et al*. $CD25^{+}CD4^{+}$ regulatory T cells in patients with Kawasaki disease. *J Pediatr* 2004; **145**: 385–390.
90. Wang GB, Wen P-Q Wang Q, Qi Z-X, Yang J, Li C-R. Changes of regulatory B cells in patients with acute Kawasaki disease and its significance. *Chin J Microbiol Immunol* 2013; **33**: 750–755.
91. Jia S, Li C, Wang G, Yang J, Zu Y. The T helper type 17/regulatory T cell imbalance in patients with acute Kawasaki disease. *Clin Exp Immunol* 2010; **162**: 131–137.
92. Bochkov V, Gesslbauer B, Mauerhofer C, Philippova M, Erne P, Oskolkova OV. Pleiotropic effects of oxidized phospholipids. *Free Radic Biol Med* 2017; **111**: 6–24.
93. Li Q, Wang Y, Chen K *et al*. The role of oxidized low-density lipoprotein in breaking peripheral Th17/Treg balance in patients with acute coronary syndrome. *Biochem Biophys Res Commun* 2010; **394**: 836–842.
94. Onouchi Y, Onoue S, Tamari M *et al*. CD40 ligand gene and Kawasaki disease. *Eur J Hum Genet* 2004; **12**: 1062–1068.
95. Chang CJ, Kuo HC, Chang JS *et al*. Replication and meta-analysis of GWAS identified susceptibility loci in Kawasaki disease confirm the importance of B lymphoid tyrosine kinase (BLK) in disease susceptibility. *PLoS One* 2013; **8**: e72037.
96. Hicar MD. Antibodies and immunity during Kawasaki disease. *Front Cardiovasc Med* 2020; **7**: 94.
97. Li D, Liu L, Chen H, Sawamura T, Mehta JL. LOX-1, an oxidized LDL endothelial receptor, induces CD40/CD40L signaling in human coronary artery endothelial cells. *Arterioscler Thromb Vasc Biol* 2003; **23**: 816–821.
98. Chakrabarti S, Blair P, Freedman JE. CD40-40L signaling in vascular inflammation. *J Biol Chem* 2007; **282**: 18307–18317.
99. Wang CL, Wu YT, Liu CA *et al*. Expression of CD40 ligand on $CD4^{+}$ T-cells and platelets correlated to the coronary artery lesion and disease progress in Kawasaki disease. *Pediatrics* 2003; **111**: E140–E147.
100. Samuelson EM, Laird RM, Papillion AM, Tatum AH, Princiotta MF, Hayes SM. Reduced B lymphoid kinase (Blk) expression enhances proinflammatory cytokine production and induces nephrosis in C57BL/6-lpr/lpr mice. *PLoS One* 2014; **9**: e92054.
101. Sage AP, Tsiantoulas D, Binder CJ, Mallat Z. The role of B cells in atherosclerosis. *Nat Rev Cardiol* 2019; **16**: 180–196.
102. Cerutti A, Puga I, Cols M. Innate control of B cell responses. *Trends Immunol* 2011; **32**: 202–211.
103. Menikou S, Langford PR, Levin M. Kawasaki Disease: the role of immune complexes revisited. *Front Immunol* 2019; **10**: 1156.
104. Rowley AH, Eckerley CA, Jäck HM, Shulman ST, Baker SC. IgA plasma cells in vascular tissue of patients with Kawasaki syndrome. *J Immunol* 1997; **159**: 5946–5955.
105. Takahashi K, Oharaseki T, Yokouchi Y, Hiruta N, Naoe S. Kawasaki disease as a systemic vasculitis in childhood. *Ann Vasc Dis* 2010; **3**: 173–181.
106. Sakurai Y. Autoimmune aspects of Kawasaki Disease. *J Investig Allergol Clin Immunol* 2019; **29**: 251–261.

107. Schlievert PM, Peterson ML. Glycerol monolaurate antibacterial activity in broth and biofilm cultures. *PLoS One* 2012; **7**: e40350.
108. Tejero J, Shiva S, Gladwin MT. Sources of vascular nitric oxide and reactive oxygen species and their regulation. *Physiol Rev* 2019; **99**: 311–379.
109. Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK. Reactive oxygen species in metabolic and inflammatory signaling. *Circ Res* 2018; **122**: 877–902.
110. Niwa Y, Sohmiya K. Enhanced neutrophilic functions in mucocutaneous lymph node syndrome, with special reference to the possible role of increased oxygen intermediate generation in the pathogenesis of coronary thromboarteritis. *J Pediatr* 1984; **104**: 56–60.
111. Straface E, Marchesi A, Gambardella L *et al*. Does oxidative stress play a critical role in cardiovascular complications of Kawasaki disease? *Antioxid Redox Signal* 2012; **17**: 1441–1446.
112. Yahata T, Suzuki C, Hamaoka A, Fujii M, Hamaoka K. Dynamics of reactive oxygen metabolites and biological antioxidant potential in the acute stage of Kawasaki disease. *Circ J* 2011; **75**: 2453–2459.
113. Wang CLWY, Lee CJ. Decreased nitric oxide production after intravenous immunoglobulin treatment in patients with Kawasaki disease. *J Pediatr* 2002; **141**: 560–565.
114. Huang YH, Tain YL, Lee CP, Kuo HC. Asymmetric and symmetric dimethylarginine are associated with coronary artery lesions in Kawasaki disease. *J Pediatr* 2014; **165**: 295–299.
115. Lebranchu Y, Malvy D, Richard MJ, Arnaud J, Favier A, Bardos P. Kawasaki disease and oxidative metabolism. *Clin Chim Acta* 1990; **187**: 193–198.
116. Takatsuki S, Ito Y, Takeuchi D *et al*. IVIG reduced vascular oxidative stress in patients with Kawasaki disease. *Circ J* 2009; **73**: 1315–1318.
117. He YE, Qiu HX, Wu RZ *et al*. Oxidised low-density lipoprotein and its receptor-mediated endothelial dysfunction are associated with coronary artery lesions in Kawasaki disease. *J Cardiovasc Transl Res* 2020; **13**: 204–214.
118. van der Valk FM, Bekkering S, Kroon J *et al*. Oxidized phospholipids on lipoprotein(a) elicit arterial wall inflammation and an inflammatory monocyte response in humans. *Circulation* 2016; **134**: 611–624.
119. Zhaolin Z, Jiaojiao C, Peng W *et al*. OxLDL induces vascular endothelial cell pyroptosis through miR-125a-5p/TET2 pathway. *J Cell Physiol* 2019; **234**: 7475–7491.
120. Sawamura T, Kume N, Aoyama T *et al*. An endothelial receptor for oxidized low-density lipoprotein. *Nature* 1997; **386**: 73–77.
121. Kataoka H, Kume N, Miyamoto S *et al*. Expression of lectinlike oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. *Circulation* 1999; **99**: 3110–3117.
122. Mehta JL, Chen J, Hermonat PL, Romeo F, Novelli G. Lectin-like, oxidized low-density lipoprotein receptor-1 (LOX-1): a critical player in the development of atherosclerosis and related disorders. *Cardiovasc Res* 2006; **69**: 36–45.
123. Chistiakov DA, Orekhov AN, Bobryshev YV. LOX-1-Mediated Effects on Vascular Cells in Atherosclerosis. *Cell Physiol Biochem* 2016; **38**: 1851–1859.
124. Inoue N, Okamura T, Kokubo Y *et al*. LOX index, a novel predictive biochemical marker for coronary heart disease and stroke. *Clin Chem* 2010; **56**: 550–558.