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Kawasaki disease: a matter of innate immunity

T. Hara,*†‡ Y. Nakashima,† Y. Sakai,† H. Nishio,† Y. Motomura†‡ and S. Yamasaki‡

*Fukuoka Children's Hospital, †Department of Pediatrics, Graduate School of Medical Sciences, and ‡Division of Molecular Immunology, Research Center for Infectious Diseases, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

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Correspondence: T. Hara, President, Fukuoka Children's Hospital, Professor Emeritus, Kyushu University, 5-1-1, Kashiiteriha, Higashi-ku, Fukuoka, 813-0017, Japan.
E-mail: harat@pediatr.med.kyushu-u.ac.jp

Summary

Kawasaki disease (KD) is an acute systemic vasculitis of childhood that does not have a known cause or aetiology. The epidemiological features (existence of epidemics, community outbreaks and seasonality), unique age distribution and clinical symptoms and signs of KD suggest that the disease is caused by one or more infectious environmental triggers. However, KD is not transmitted person-to-person and does not occur in clusters within households, schools or nurseries. KD is a self-limited illness that is not associated with the production of autoantibodies or the deposition of immune complexes, and it rarely recurs. Regarding the underlying pathophysiology of KD, innate immune activity (the inflammasome) is believed to play a role in the development of KD vasculitis, based on the results of studies with animal models and the clinical and laboratory findings of KD patients. Animal studies have demonstrated that innate immune pathogen-associated molecular patterns (PAMPs) can cause vasculitis independently of acquired immunity and have provided valuable insights regarding the underlying mechanisms of this phenomenon. To validate this concept, we recently searched for KD-specific PAMPs and identified such molecules with high specificity and sensitivity. These molecules have structures similar to those of microbe-associated molecular patterns (MAMPs), as shown by liquid chromatography-tandem mass spectrometry. We propose herein that KD is an innate immune disorder resulting from the exposure of a genetically predisposed individual to microbe-derived innate immune stimulants and that it is not a typical infectious disease.

Keywords: Kawasaki disease, innate immunity, liquid chromatography-mass spectrometry, pathogen-associated molecular patterns

Introduction

Kawasaki disease (KD) is an acute self-limiting systemic vasculitis of early childhood that was first described by Tomisaku Kawasaki in 1967 [1]. It affects predominantly the coronary arteries and causes coronary artery abnormalities in 25–30% of untreated patients [2]. After the introduction of intravenous immunoglobulin (Ig), the incidence of coronary artery lesions (CALs) decreased to fewer than 5% [3]. Nonetheless, KD is the most common cause of acquired childhood heart disease in developed countries [4,5]. The incidence of KD is still increasing, according to the most recent nationwide survey (2013–14) in Japan [6].

Although almost 50 years have passed since its initial description, the aetiology of KD remains unknown. KD is usually diagnosed by clinical symptoms and signs, because no specific diagnostic tests are available. The clinical and epidemiological features of KD suggest strongly that the disease results from the exposure of a genetically predisposed individual to an unidentified, possibly infectious environmental trigger [5,7]. This review will focus on the genetic, environmental and immunological aspects of KD in an attempt to elucidate its aetiology.

KD aetiology

Genetic background

Twin studies have revealed that the concordance rates of KD were 14·1% (11 of 78) and 13·3% (four of 30) for monozygotic and dizygotic twins, respectively, in Japan

[8]. In the United States, the concordance rate of KD for monozygotic twins was 25% (one out of four) [9]. For a single-gene disorder with complete penetrance, the expected concordance rate should be 100% for monozygotic twins, while it should be lower for dizygotic twins. For conditions that are determined completely by environmental factors, the concordance rates for monozygotic and dizygotic twins should be essentially equal and depend upon the shared environment in which the twins live. Thus, these twin studies suggest that environmental factors contribute more to the development of KD than genetic factors among individuals with the same ethnicity.

However, a genetic predisposition to KD has been proposed in various epidemiological studies. For example, the incidence of KD is highest in Asian populations, especially Japanese populations. The incidence among Japanese individuals is 10–15 times higher than that among Caucasians [10]. The incidence of KD among Japanese American children in Hawaii is as high as that among Japanese children, while the incidence among Caucasian children in Hawaii is as low as that among Caucasian children in the continental United States [11,12]. The idea of genetic susceptibility to KD is supported further by the fact that a higher relative risk of KD exists within families [13].

Linkage analysis and genome-wide association studies (GWASs) have identified KD susceptibility alleles for the following genes: *ITPKC*, *CASP3*, *BLK*, *CD40*, *HLA* and *FCGR2A* [14]. Recently identified genetic variations of *ORAI1* may explain the aforementioned Asian susceptibility to KD [15]. Although inositol 1,4,5-trisphosphate 3-kinase C (ITPKC) was described initially as a negative regulator of T cell activation [16], ITPKC is a ubiquitous molecule that is present in innate and acquired immune cells, as well as endothelial cells. ITPKC, caspase-3 (CASP3) and calcium release-activated calcium channel protein 1 (ORAI1) may play important roles in KD development by regulating the calcium/nuclear factor of activated T cells (NFAT) pathway [14–16]. The candidate gene approach showed that KD is linked to genetic variations (*TGFB2*, *TGFBR2* and *SMAD3*) in the transforming growth factor beta (TGF-β) signalling pathway that are important in inflammation and vascular remodelling [17]. *VEGFA*, *KDR* and *ANGPT1* have also been reported to be associated with KD. These data suggest that dysregulation of vascular endothelial growth factor (VEGF) and angiopoietins contributes to the disruption of vascular homeostasis in KD [18,19].

Environmental factors

The clinical picture of KD supports the notion that microbes or infectious organisms are capable of triggering onset of the disease, as do the following facts: (a) children are affected mainly between 6 months and 5 years of age, and the peak age of disease onset coincides with the period during which children are most susceptible to common pathogens; (b) KD is characterized by an acute onset and follows a self-limited clinical course; and (c) KD shows epidemics, community outbreaks and seasonality [3–5,7].

Many microbes or microbe-derived substances are believed to cause KD, including Rickettsia-like agent, *Propriobacterium acnes*, *Leptospira* spp., *Streptococcus sanguis*, *Staphylococcus aureus*, *Yersinia pseudotuberculosis*, retroviruses, Epstein–Barr virus, cytomegalovirus, coronavirus, parvovirus B19, human bocavirus, undetermined RNA viruses and staphylococcal or streptococcal superantigens [20,21]. Rowley *et al.* detected cytoplasmic inclusion bodies containing virus-like particles in the bronchial epithelium of a patient with acute KD, using synthetic antibodies [20,22]. However, no causative viruses have been identified thus far.

It is well known that approximately 10% of patients with *Y. pseudotuberculosis* infection develop Kawasaki syndrome in Japan [23] and that Kawasaki syndrome patients with known *Y. pseudotuberculosis* infection show a higher tendency to develop CALs [24]. In addition, epidemiological data indicate that higher incidences of KD have been observed in populations at high risk for *Y. pseudotuberculosis* infection [25]. Rodó *et al.* suggested that a wind-borne environmental trigger induces KD [26,27]. However, no such agents have been identified [7,28].

Regarding superantigens, Vβ-restricted T cell expansion or activation in patients with KD has been observed by some researchers [29,30]. However, other research groups failed to detect such T cell expansion [31]. Because only a small proportion of KD patients have shown Vβ-restricted T cell activation [29–31], and there are no differences in KD symptoms or signs between patients with and without T cell activation (our unpublished observation), superantigen-induced T cell activation may be an epiphenomenon rather than a necessity for the development of KD. Biofilms form as a result of interactions between microbes and the environment and are capable of producing large amounts of various bioactive molecules, including superantigens, through quorum-sensing mechanisms [32]. For example, when *S. aureus* was cultured in biofilms adhering to tampon sacs, the level of toxic shock syndrome (TSS) toxin-1 in these bacteria was more than 1000-fold higher than that in bacteria cultured via conventional methods [33]. Although TSS caused by TSS toxin-1 exhibits clinical features similar to those of KD [34], TSS is characterized by superantigen-induced excessive T cell activation. In contrast, most cases of KD are characterized by T cell suppression, as well as endothelial cell/innate immune cell activation [35,36]. KD and TSS rarely develop in association with isolated sepsis or bacteraemia [37], in which bacteria grow under planktonic conditions. Thus, as is the case for TSS, the pathogenesis of KD may be evoked not by microbes themselves but by bioactive molecules that are produced by microbes under biofilm-like conditions.

Immunological aspects

Innate immunity *versus* acquired immunity. The innate immune system has both cellular and humoral components. The cellular components include neutrophils, eosinophils, monocytes, macrophages, dendritic cells, γδT cells, natural killer cells and natural killer T cells [38]. In addition, endothelial cells function as sentinel innate immune cells and detect foreign pathogens and endogenous danger signals in the bloodstream [39].

The following clinical and laboratory evidence suggests that the acute phase of KD is driven primarily by the innate immune system: (a) the absolute neutrophil and monocyte counts in the peripheral blood are increased [40]; (b) the majority of the activated T lymphocytes in the peripheral blood are γδT cells [36]; (c) the majority of the cells infiltrating the coronary arteries and skin lesions are macrophages [41,42]; (d) the levels of damage-associated molecular patterns (DAMPs), such as S100 proteins and high mobility group box 1 (HMGB1), are elevated in the sera of KD patients during the acute phase [43–46]; (e) KD is sometimes associated with disorders characterized by hyperactive innate immunity, such as periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome [47] and systemic juvenile idiopathic arthritis (JIA) [48,49]; and (f) KD patients have the highest recurrence rate within 12 months following the first episode [50], which may be attributed to the fact that the innate immune system lacks immunological memory.

KD is also regarded as a condition associated with acquired immune dysfunction that is characterized by (a) decreased absolute $CD3^+$, $CD4^+$ and $CD8^+$ T cell counts in the peripheral blood [40]; (b) marked suppression of T cell receptor/CD3-induced T cell proliferation [35]; (c) down-regulation of T cell receptor and B cell receptor signalling pathways, as shown by microarray studies [36,51,52]; and (d) suppression of regulatory T cells during the acute phase of the disease [53,54]. Whether T helper type 17 (Th17) cells contribute to the development of KD remains controversial, because a previous study showed that the levels of Th17 cells were only slightly elevated in the peripheral blood of KD patients [55], and inconsistent results were obtained by two other studies [56,57]. Based on currently available evidence KD is unlikely to have an autoimmune cause, as it is not associated with autoantibody production, resolves spontaneously and rarely recurs [5].

KD animal models. The clinical and laboratory features of KD suggest strongly that innate immunity plays a critical role in the development of coronary vasculitis in patients with KD. We therefore performed *in-vitro* studies regarding the proinflammatory effects of innate immune ligands to corroborate this hypothesis, using human coronary artery endothelial cells (HCAECs). HCAECs have been shown to produce interleukin (IL)-6 and IL-8 when treated with ligands for Toll-like receptors (TLR)−2 and −4 and nucleotide-binding oligomerization domain-containing proteins (NOD)1 and 2 [58]. To validate this finding *in vivo*, we injected various innate immune ligands into wild-type C57Bl/6 mice and found that a NOD1 ligand, FK565, was a more potent inducer of coronary arteritis than any other ligand [58]. Similar results were also obtained via oral administration of FK565, as shown in Fig. 1a [58]. Using severe combined immunodeficient (SCID) mice, we demonstrated that NOD1 ligands induce coronary arteritis in the absence of functional T and B cells (Fig. 1c).

FK565 is a synthetic acyltripeptide (heptanoyl-γ-D-glutamyl-meso-diamino- pimelyl- D-alanine) with a molecular weight (MW) of 502·6. By binding to NOD1, FK565, which harbours diaminopimelic acid within its structure, functions as a pathogen-associated molecular pattern (PAMP). Diaminopimelic acid is also the active constituent of other environmental PAMPs. For example, dipeptide γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP, MW: 319·3), L-alanyl-γ-D-glutamyl-meso-diaminopimelic acid (MW: 390·39) and lauroyl-γ-D-glutamyl-meso-diaminopimelic acid (MW: 501·61) are components of peptidoglycan in the cell walls of Gram-negative and certain groups of Gram-positive bacteria [58,59].

Regarding the molecular and cellular pathophysiology of FK565-induced coronary arteritis, we propose that NOD1 ligands activate proinflammatory signals in vascular endothelial cells, thereby producing large amounts of chemokines. In response to these chemokines, monocytes in the peripheral blood are recruited to FK565-stimulated endothelial cells and differentiate subsequently into cardiac $CD11c^+$ macrophages [60]. Genetically manipulated mice lacking $CD11c^+$ macrophages present with milder coronary vasculitis after administration of FK565, indicating that $CD11c^+$ macrophages play a pivotal role in the pathogenesis of acute coronary arteritis (Fig. 2). We also verified that FK565 reproducibly induces acute coronary vasculitis in SCID and *Rag1*-knock-out mice [58,60]. These data provide new insights into the pathogenic mechanisms of vasculitis in humans and demonstrate that innate immunity (PAMPs) can cause vasculitis independently of acquired immunity.

To date, two other animal models of KD coronary arteritis have been established. These mouse models showed that crude microbe-associated molecular patterns (MAMPs)/PAMPs from *Lactobacillus caseii* [61–65] and *Candida albicans* [66–68] induce acute coronary vasculitis (Table 1). Thus, these animal studies confirmed that stimulation of innate immunity with molecules such as NOD1 ligands induces vasculitis that mimics the coronary artery lesions of KD. In agreement with our study, an animal study using *L. caseii* cell wall extracts (LCWE) also showed that $CD11c^+$ dendritic cells/macrophages and vascular stromal

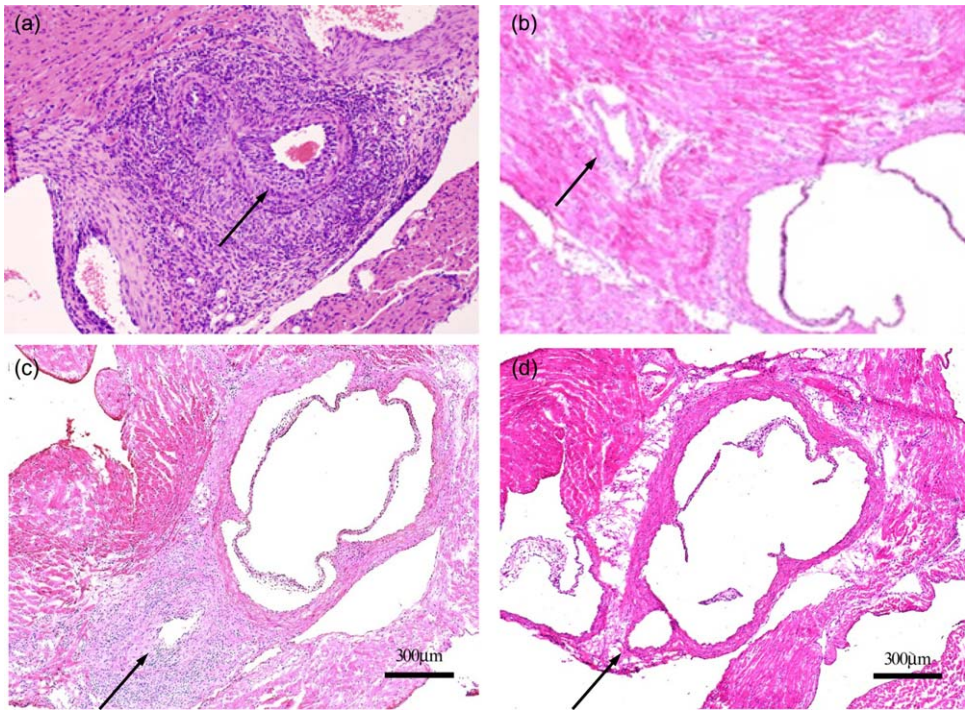

Fig. 1. Nucleotide-binding oligomerization domain-containing protein (NOD1) ligand (FK565)-induced coronary arteritis. (a) Administration of FK565 (100 μg/day p.o. for 2 weeks) induces coronary arteritis. This Kawasaki disease (KD) model is characterized by panarteritis with dense inflammatory cell infiltration involving neutrophils and macrophages, but is not associated with fibrinoid necrosis. This histopathology recapitulates the coronary artery lesions of KD. (b) Control solvent: no arteritis. (c) FK565-induced coronary arteritis in a severe combined immunodeficient (SCID) mouse. This panel shows that FK565 also induces a milder form of coronary arteritis in SCID mice than that induced in wild-type mice. Most of the infiltrating inflammatory cells are neutrophils and macrophages, as is the case in wild-type mice. These data show that the coronary artery lesions of KD are mediated by the innate immune system (PAMPs) and develop independently of acquired immunity. (d) Absence of vasculitis in a NOD1-knock-out mouse from Nishio *et al.* [58].

cells with cytokines (IL-1α and β) are important in the pathogenesis of coronary arteritis [65]. In addition, a recent study has demonstrated that the activation of endothelial Nlrp3 inflammasome, a key component of the innate immune system, may contribute to the development of coronary arteritis induced by LCWE [69].

Searching for KD-specific molecules in humans. A seminal study detected endothelial cell-activating antigens in skin biopsy samples from KD patients [70]. To confirm these findings, we searched for unknown ligands that may activate NOD1 or other vasculitis-inducing pathways. We first prepared whole extracts and fractionated samples from the

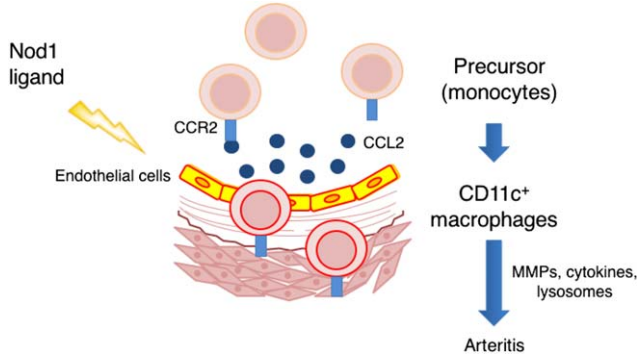

Fig. 2. Schematic representation of the molecular and cellular mechanisms underlying nucleotide-binding oligomerization domain-containing protein (NOD1)-induced arteritis. A NOD1 ligand, FK565, activates endothelial cells which produce large amounts of chemokines, including CCL2. In response to CCL2 and other chemokines, CCR2 (chemokine receptor)-expressing precursor cells (monocytes) in the peripheral blood are recruited to FK565-activated endothelial cells. This process subsequently induces the differentiation of cardiac $CD11c^{+}$ macrophages, which play a pivotal role in the pathogenesis of acute coronary arteritis. MMP = matrix metalloproteinase.

Table 1. Animal models of Kawasaki disease (KD)-like vasculitis

Microbe-derived or synthetic substances	Innate immunity	Acquired immunity		Important cytokine/chemokine
		T cell	B cell	
LCWE (*Lactobacillus casei* cell wall extract) [61–65,69]	$CD11c^+$ Macrophages/DC Vascular stromal cells	superantigen+++	+/–	IL-1β IL-1α
Crude PAMP	TLR2/MyD88 Dectin-1/Syk Nlrp3 inflammasome			TNF-α
CAWS (*Candida albicans* water-soluble fraction) [66–68]	Inflammatory monocytes	++ Th17	+/–	IL-6
Crude PAMP		Regulatory T cell		
NOD1 ligand (FK565) [58,60]	Nod1 in endothelial cells	+/–	+/–	CCL2
Pure PAMP (synthetic)	$CD11c^+$ macrophages			

CCL = chemokine (C-C motif) ligand; DC = dendritic cells; IL = interleukin; MyD = myeloid differentiation primary response; Nlrp3 = nucleotide-binding, leucine-rich repeat containing family pyrin domain containing 3; NOD1 = nucleotide-binding oligomerization domain-containing protein; TNF = tumour necrosis factor; Syk = spleen tyrosine kinase.

sera of KD patients and found that KD sera contain bioactive substances that induce production of IL-6 and IL-8 in HCAECs [71]. However, no NOD1-activating ligands were detected in these KD samples by cell-based reporter systems or liquid chromatography-mass spectrometry (LC-MS) analysis [71]. Thus, these results suggest that other innate immune receptor(s) may be associated with the development of KD vasculitis. Alternatively, the sensitivities of cell-based reporter system and LC-MS analysis may not be high enough to detect NOD1 ligands in KD serum samples.

To exclude the possibility of cytokine and chemokine contamination in the aqueous fractions of sera from KD patients, we analysed the lipophilic fractions of the above-mentioned 117 samples. We detected novel molecules between 227·1 m/z and 1487·8 m/z with a specificity of 100% and a low sensitivity ranging from 9·3 to 48·8% [71]. We defined them as 'KD-specific molecules'. Then, we investigated whether these KD-specific molecules had structures similar to those of MAMPs from *Y. pseudotuberculosis* [23–25] and airborne bacteria [26,27], using liquid chromatography-tandem mass spectrometry (LC-MS/MS). We used various types of culture media, as well as different temperatures, durations of shaking and incubation and supplemental nutrients, to stimulate bacterial growth. Lipid extracts from three bacterial culture components (cell, supernatant and biofilm) were subjected to LC-MS/MS analysis [71]. The serum KD-specific molecules showed MS/MS fragmentation patterns that were similar to those of MAMPs in the biofilm extracts from *Y. pseudotuberculosis* and airborne bacteria; however, these patterns were not similar to those of MAMPs in the other two extracts (cell and supernatant). Production of MAMPs similar to serum KD-specific molecules was enhanced markedly under biofilm-forming conditions in the presence of butter [71]. HCAEC-stimulatory activities (IL-6 and IL-8 production from HCAECs) also tended to be much higher in the biofilm extracts from *Y. pseudotuberculosis* and airborne bacteria in cultures supplemented with butter, as shown in Fig. 3.

More recently, we used modified extraction and analysis methods in a nationwide collaborative study. KD-specific molecules were detected in the sera of affected patients with a specificity of 100% and a sensitivity of almost 100% (Nakashima *et al.* 2016, manuscript in preparation). In this study, we confirmed that KD-specific molecules possessed structures similar to those of MAMPs found in biofilm

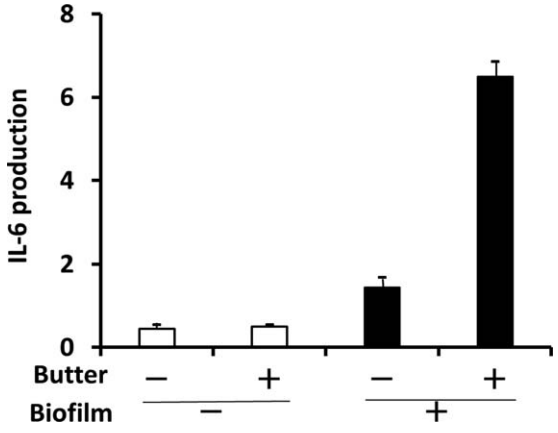

Fig. 3. Human coronary artery endothelial cells (HCAECs)-stimulatory activities of biofilm extracts from *Yersinia pseudotuberculosis*. HCAECs-stimulatory activities of *Y. pseudotuberculosis* extracts were measured by interleukin (IL)-6 production from HCAECs. *Y. pseudotuberculosis* extracts were prepared from culture supernatants (□) or biofilms (■) of *Y. pseudotuberculosis* cultured in the presence (+) or absence (–) of butter. Medium alone, ethyl acetate alone or ethyl acetate extract from glass slides cultured in the absence of microbes was used as a negative control. FK565 (10 μg/ml) was used as a positive control. Data are expressed as the fold change in induction of IL-6 production compared to positive control levels. Modified from the data of Kusuda *et al.* [71].

Table 2. Kawasaki disease (KD) pathogenic features in light of those of other systemic vasculitides

	KD	Other systemic vasculitides
Epidemiological features	Epidemics +, community outbreaks +	No epidemics, no outbreaks
	seasonality +	usually no seasonality
		(IgA vasculitis: seasonality +)
Clinical and laboratory features	Age: mostly infants	Common in older age
	abrupt onset	acute-chronic onset
	acute infection-like symptoms	constitutional symptoms
	self-limited/no recurrence in most cases	chronic and/or recurrent
		(IgA vasculitis: usually self-limited)
	Autoantibodies: usually absent	autoantibodies: some +
	immune complexes: usually absent	immune complexes: some +
	association with innate immune disorders,	no association with PFAPA syndrome
	PFAPA syndrome and systemic JIA	and systemic JIA
Pathophysiological features	Inhibited T cell receptor signalling pathway	none
	Inflammasome involvement +	Inflammasome involvement: some +
	hypercytokinaemia (IL-1, IL-6, TNF-α, etc.)	hypercytokinaemia: some +
	Detection of possible PAMPs in sera	no data

IL = interleukin; JIA = juvenile idiopathic arthritis; PAMPs = pathogen-associated molecular patterns; PFAPA syndrome = periodic fever, aphthous stomatitis, pharyngitis and adenitis syndrome; TNF = tumour necrosis factor.

extracts from *Y. pseudotuberculosis* and airborne bacteria (Nakashima *et al.* 2016, manuscript in preparation).

A comprehensive view of KD pathogenesis in the context of systemic vasculitides. The epidemiological features (existence of epidemics, community outbreaks and seasonality) of KD suggest that the disease is caused by one or more infectious environmental triggers [5,7,72]. Among other systemic vasculitides, only IgA vasculitis exhibits seasonality in the absence of outbreaks or epidemics [73], as shown in Table 2. The incidence of KD, as well as those of allergies and non-infectious inflammatory bowel disease, has increased [6,74,75], while those of infectious diseases have decreased continuously in Japan. These facts suggest that KD is not a typical infectious disease.

The unique age distribution (more than 80% of cases occur between the ages of 6 months and 4 years) of KD is reminiscent of paediatric infectious diseases [3–5,7]. However, as KD is not transmitted person-to-person and does not occur in clusters within households, schools or nurseries, it does not possess the characteristics of an infectious disease [76]. Conversely, the peak age of IgA vasculitis onset is between 4 and 6 years, and those of the onset of other systemic vasculitides with possible autoimmune **a**etiologies are much higher [77].

The clinical symptoms and signs (fever, injection of the eyes or oropharynx, rash and cervical lymphadenopathy) of KD mimic those of acute infections, while those of other systemic vasculitides usually do not [77]. Innate immune disorders, such as PFAPA syndrome [78], and superantigen-induced diseases, such as TSS, have clinical features that are indistinguishable from those of infectious diseases. As superantigen-induced αβT cell activation is usually not observed in most patients with KD [30,31,36], it is possible that KD is an innate immune disorder.

KD is a self-limited illness that is not characterized by autoantibody production or immune complex deposition and it rarely recurs, which suggests that it is unlikely to be an autoimmune disease [5]. In contrast, large-vessel vasculitides are considered PAMP (TLR-ligands)-triggered T cell-mediated autoimmune disorders [79–81]. The immunopathogenic process of polyarteritis nodosa (PAN), a medium-vessel vasculitis, is associated with both innate and acquired immunity, although the exact pathogenic mechanisms remain unknown [82–84]. The small vessel vasculitides (SVVs) comprise antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and immune complex SVVs. In AAV, ANCAs seem to play an indispensable role in the development of vasculitis by activating primed neutrophils and monocytes, triggering a subsequent inflammatory amplification loop in the vessel wall [85,86]. Among immune complex SVVs, IgA vasculitis is considered to be a predominantly IgA-mediated immune disorder [73,79].

The genetic background of KD differs from that of other systemic vasculitic syndromes. GWASs have demonstrated an association between the genes (*ITPKC*, *CASP3* and *ORAI1*) of the calcium/NFAT pathway and KD [14–16]; however, these genes do not appear to be associated with other vasculitic syndromes [87]. Only the variant alleles of *FCGR2A* [88] have been linked to susceptibility to KD and Takayasu's arteritis [87].

Regarding the pathophysiology of KD, T cell suppression [35], and down-regulation of T cell receptor and B cell receptor signalling pathways [36,51,52] in KD have not been documented in other systemic vasculitides. Furthermore, animal models [65,69], as well as the clinical and laboratory features (increased serum IL-1β levels, IL-1

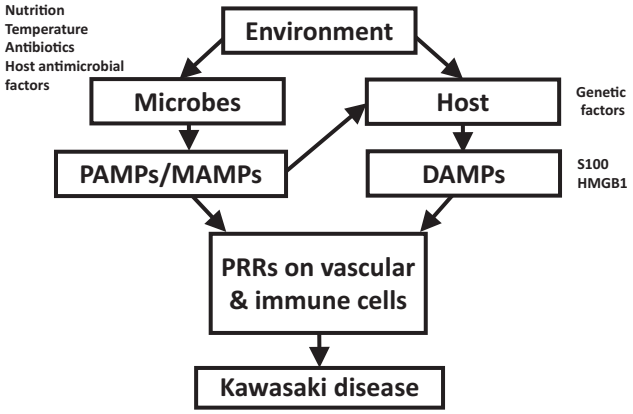

Fig. 4. Innate immune-mediated vasculitis as a pathogenic model for Kawasaki disease (KD). Microbes produce massive amounts of pathogen-associated molecular patterns /microbe-associated molecular patterns (PAMPs/MAMPs) under certain biofilm-like conditions. PAMPs/MAMPs induce damage-associated molecular pattern (DAMP) [S100 proteins and high mobility group box 1 (HMGB1)] production by and release from host cells. These molecules activate endothelial and immune cells co-operatively through innate immune pattern recognition receptors (PRRs). Recruitment of immune cells to activated endothelial cells and destruction of vascular structures result in the development of KD vasculitis and aneurysms. These molecular scenarios may be even more prominent in genetically predisposed individuals.

signalling pathway up-regulation and anti-IL-1β treatment effectiveness) of KD [52,89,90], suggest that the inflammasome (a key component of the innate immune system) is associated with the development of KD vasculitis. Inflammasome activation may also be associated with the development of other systemic vasculitides, including autoinflammatory disease-associated systemic vasculitis and Behçet disease [91,92]. Serum levels of a variety of cytokines are elevated in KD [3] as well as in several other systemic vasculitides [77,85]. In addition, we have identified possible PAMPs in KD sera with high specificity and sensitivity by LC-MS/MS (Nakashima *et al.* 2016, manuscript in preparation). Further study is necessary to identify such molecules in other systemic vasculitides linked closely to infections. KD is also associated with innate immune disorders (PFAPA syndrome and systemic JIA) [47–49]; however, no other systemic vasculitides have been linked to these innate immune disorders.

In contrast to the animal models of other systemic vasculitides, our KD animal model has provided new insights regarding the mechanisms underlying the disease and shown that PAMPs associated with innate immunity can cause vasculitis independently of acquired immunity [58,60]. The possible presence of PAMPs and DAMPs, such as S100 proteins and HMGB1, in KD patient sera [43–46,70] support the hypothesis that PAMPs/MAMPs, together with DAMPs, activate endothelial and immune cells co-operatively through innate immune pattern recognition receptors (PRRs), as shown in Fig. 4. Recruitment of immune cells to activated endothelial cells and destruction of vascular structures lead to the development of KD vasculitis and aneurysms. These molecular scenarios may be even more prominent in genetically predisposed individuals. Although vasculitis can occur independently of T cell- and B cell-mediated immunity, acquired immunity is undoubtedly associated with the vasculitides mediated by the innate immune system in humans. Regarding late KD vasculopathy [93] and the premature development of atherosclerosis in patients with a prior history of KD [94], further studies are necessary to elucidate the mechanisms underlying these phenomena. They may result from persistent exposure to small amounts of vasculitis-inducing molecules produced by endogenous microbes or from acquired immunity-mediated vascular inflammation [95,96].

Conclusions

Based on the results of epidemiological, clinical, laboratory and animal studies, we have concluded that KD is not an infectious disease but an innate immune disorder. We propose that KD results from the exposure of a genetically predisposed individual to PAMPs from microbes growing under biofilm-like conditions, as well as DAMPs.

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Disclosure

The authors declare that there are no disclosures.

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