Kawasaki disease: a matter of innate immunity

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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.
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, Propionibacterium acnes, Leptospira spp., Streptococcus sanguis, Staphylococcus aureus, Yersinia pseudotuberculosis, retrovirus, 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+ T cells 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 casei [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. casei cell wall extracts (LCWE) also showed that CD11c+ dendritic cells/macrophages and vascular stromal...
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

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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].

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.
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 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 (square) or biofilms (circle) 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].](image-url)
The pathogenesis of Kawasaki disease

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

| Epidemiological features | KD | Other systemic vasculitides |
|--------------------------|----|-----------------------------|
| Age: mostly infants      | Common in older age          |
| Abrupt onset             | Acute-chronic onset          |
| Fever                    | Constitutional symptoms      |
| Self-limited/no recurrence in most cases | Chronic and/or recurrent |

| Clinical and laboratory features | KD | Other systemic vasculitides |
|---------------------------------|----|-----------------------------|
| Autoantibodies: usually absent  | No association with PFAPA syndrome |
| Immune complexes: usually absent| None |
| Association with innate immune disorders | Inflammasome involvement: some + |
| PFAPA syndrome and systemic JIA | no association with PFAPA syndrome |
| Detection of possible PAMPs in sera | no data |

| Pathophysiological features | KD | Other systemic vasculitides |
|----------------------------|----|-----------------------------|
| 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 aetiologies 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 antinuclear antibody (ANCA)-associated vasculitis (AAV) and immune complex SVVs. In AAV, ANCA 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 *ORAII*) 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...
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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References

1 Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. Arerugi 1967; 16:178–222.

2 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; 86:892–8.

3 Galeotti C, Bayry J, Kone-Paut I, Kaveri SV. Kawasaki disease: aetiopathogenesis and therapeutic utility of intravenous immunoglobulin. Autoimmun Rev 2010; 9:441–8.

4 Newburger JW, Takahashi M, Gerber MA et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Circulation 2004; 110:2747–71.

5 Jamieson N, Singh-Grewal D. Kawasaki disease: a clinician’s update. Int J Pediatr 2013; 2013:645391.

6 Yashiro M, Makino N, Nakamura Y, Yanagawa H, Kawasaki T. Results of the 23rd nationwide survey on Kawasaki disease – the highest number of patients ever. In: Proceedings of the 35th annual meetings of the Japanese Society of Kawasaki Disease, 2015:52.

7 Kim KY, Kim DS. Recent advances in Kawasaki disease. Yonsei Med J 2016; 57:15–21.

8 Harada F, Sada M, Komiya T, Yanase Y, Kawasaki T, Sasazuki T. Genetic analysis of Kawasaki syndrome. Am J Hum Genet 1986; 39:537–9.

9 Kottek A, Shimizu C, Burns JC. Kawasaki disease in monozygotic twins, Pediatr Infect Dis J 2011; 30:1114–6.

10 Uehara R, Delay ED. Epidemiology of Kawasaki disease in Asia, Europe, and the United States. J Epidemiol 2012; 22:79–85.

11 Holman RC, Delay ED, Christensen KY, Folksma AM, Steiner CA, Schonberger LB. Hospitalizations for Kawasaki syndrome among children in the United States, 1997–2007. Pediatr Infect Dis J 2010; 29:483–8.

12 Holman RC, Christensen KY, Delay ED et al. Racial/ethnic differences in the incidence of Kawasaki syndrome among children in Hawaii. Hawaii Med J 2010; 69:194–7.

13 Fujita Y, Nakamura Y, Sakata K et al. Kawasaki disease in families. Pediatrics 1989; 84:666–9.

14 Onouchi Y. Genetics of Kawasaki disease: what we know and don’t know. Circ J 2012; 76:1581–6.

15 Onouchi Y, Fukazawa R, Yamamura K et al. Variations in ORAI1 gene associated with Kawasaki disease. PLOS ONE 2016; 11:e0145486.

16 Onouchi Y, Gunji T, Burns JC et al. ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. Nat Genet 2008; 40:35–42.

17 Shimizu C, Jain S, Davila S et al. Transforming growth factor-beta signaling pathway in patients with Kawasaki disease. Circ Cardiovasc Genet 2011; 4:16–25.

18 Karizymono H, Ohno T, Khajoeve V et al. Association of vascular endothelial growth factor (VEGF) and VEGF receptor gene polymorphisms with coronary artery lesions of Kawasaki disease. Pediatr Res 2004; 56:953–9.

19 Breunis WB, Davila S, Shimizu C et al. Disruption of vascular homeostasis in patients with Kawasaki disease: involvement of vascular endothelial growth factor and angiopoietins. Arthritis Rheum 2012; 64:306–15.

20 Rowley AH, Baker SC, Orenstein JM, Shulman ST. Searching for the cause of Kawasaki disease – cytoplasmic inclusion bodies provide new insight. Nat Rev Microbiol 2008; 6:394–401.

21 Pinna GS, Kafetzis DA, Tselkas OI, Skevaki CL. Kawasaki disease: an overview. Curr Opin Infect Dis 2008; 21:263–70.

22 Rowley AH, Baker SC, Shulman ST et al. Cytoplasmic inclusion bodies are detected by synthetic antibody in ciliated bronchial epithelium during acute Kawasaki disease. J Infect Dis 2005; 192:1757–66.

23 Sato K, Ouchi K, Taki M. Yersinia pseudotuberculosis infection in children, resembling Izumi fever and Kawasaki syndrome. Pediatr Infect Dis 1983; 2:123–6.

24 Tahara M, Baba K, Waki K, Arakaki Y. Analysis of Kawasaki disease showing elevated antibody titers of Yersinia pseudotuberculosis Acta Paediatr 2006; 95:1661–4.

25 Vincent P, Salo E, Skurnik M, Fukushima H, Simonet M. Similarities of Kawasaki disease and Yersinia pseudotuberculosis infection epidemiology. Pediatr Infect Dis J 2007; 26:629–31.

26 Rodó X, Ballester J, Cayan D. Association of Kawasaki disease with tropospheric wind patterns. Sci Rep 2011; 1:152.

27 Rodó X, Carcoll R, Robinson M et al. Tropospheric winds from northeastern China carry the etiologic agent of Kawasaki disease from its source to Japan. Proc Natl Acad Sci USA 2014; 111:7952–7.

28 Shulman ST, Rowley AH. Kawasaki disease: insights into pathogenesis and approaches to treatment. Nat Rev Rheumatol 2015; 11:475–82.

29 Abe J, Kotzin BL, Meissner C et al. Characterization of T cell repertoire changes in acute Kawasaki disease. J Exp Med 1993; 177:791–6.

30 Curtis N, Zheng R, Lamb JR, Levin M. Evidence for a superantigen mediated process in Kawasaki disease. Arch Dis Child 1995; 72:308–11.

31 Pietra BA, De Incendio J, Glod MP. Kawasaki disease in a pediatric intensive care unit: a family repertoire and T cell activation markers in Kawasaki disease. Arch Dis Child 1999; 72:308–11.

32 Aparna MS1, Yadav S. Biofilms: microbes and disease. Braz J Infect Dis 2008; 12:526–30.

33 Schlievert PM, Peterson ML. Glycerol monolaurate antibacterial activity in broth and biofilm cultures. PLOS ONE 2012; 7:e40350.

34 Dominguez SR, Friedman K, Seewald R, Anderson MS, Willis L, Glodé MP. Kawasaki disease in a pediatric intensive care unit: a case–control study. Pediatrics 2008; 122:e786–90.

35 Kuijpers TW, Wiegman A, van Lier RAW et al. Unique activation status of T cells in Kawasaki disease: a maturational defect in immune responsiveness. J Infect Dis 1999; 180:1869–77.

36 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; 160:246–55.

37 Li Z, Peres AG, Damian AG, Madrenas J. Immunomodulation and disease tolerance to Staphylococcus aureus. Pathogens 2015; 4:793–815.
38 Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002; 20:197–216.
39 Mai J, Virtue A, Shen J, Wang H, Yang X-F. An evolving new paradigm: endothelial cells – conditional innate immune cells. J Hematol Oncol 2013; 6:61.
40 Furukawa S, Matsubara T, Yabuta K. Mononuclear cell subsets and coronary artery lesions in Kawasaki disease. Arch Dis Child 1992; 67:706–8.
41 Takahashi K, Oharaseki T, Yokouchi Y et al. Kawasaki disease as a systemic vasculitis in childhood. Ann Vasc Dis 2010; 3:173–81.
42 Sato N, Sagawa K, Sasaguri Y et al. Immunopathology and cytokine detection in the skin lesions of patients with Kawasaki disease. J Pediatr 1993; 122:198–203.
43 Foell D, Ichida F, Vogl T et al. S100A12 (EN-RAGE) in monitoring Kawasaki disease. Lancet 2003; 361:1270–2.
44 Ye F, Foell D, Hiroko KI et al. Neutrophil-derived S100A12 is profoundly upregulated in the early stage of acute Kawasaki disease. Am J Cardiol 2004; 94:840–4.
45 Ebihara T, Endo R, Kikuta H et al. Differential gene expression of S100 protein family in leukocytes from patients with Kawasaki disease. Eur J Pediatr 2006; 164:237–31.
46 Hoshina T, Kusuhara K, Ikeda K et al. High mobility group box 1 (HMGB1) and macrophage migration inhibitory factor (MIF) in Kawasaki disease. Scand J Rheumatol 2008; 37:445–9.
47 Broderick L, Tremoulet AH, Burns JC et al. Recurrent fever syndromes in patients after recovery from Kawasaki syndrome. Pediatrics 2011; 127:e489–93.
48 Komatsu H, Tateno A. Failure to distinguish systemic-onset juvenile idiopathic arthritis from incomplete Kawasaki disease in an infant. J Paediatr Child Health 2007; 43:707–9.
49 Dong S, Bout-Tabaku S, Teuter K, Jaggi P. Diagnosis of systemic-onset juvenile idiopathic arthritis after treatment for presumed Kawasaki disease. J Pediatr 2015; 166:1283–8.
50 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; 90:40–4.
51 Ling XB, Lau K, Kanegaye JT et al. A diagnostic algorithm combining clinical and molecular data distinguishes Kawasaki disease from other febrile illnesses. BMC Medicine 2011; 9:130.
52 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.
53 Furuno K, Yuge T, Kusuhara K et al. CD25+CD4+ regulatory T cells in patients with Kawasaki disease. J Pediatr 2004; 145:385–90.
54 Olivetto B, Taddio A, Simonini G et al. Defective FOXP3 expression in patients with acute Kawasaki disease and restoration by intravenous immunoglobulin therapy. Clin Exp Rheumatol 2010; 28:93–7.
55 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; 162:131–7.
56 Rasouli M, Heidari B, Kalani M. Downregulation of Th17 cells and the related cytokines with treatment in Kawasaki disease. Immunol Lett 2014; 162:269–75.
57 Guo MM, Tseng WN, Ko CH, Pan HM, Hsieh KS, Kuo HC. Th17- and Treg-related cytokine and mRNA expression are associated with acute and resolving Kawasaki disease. Allergy 2015; 70:310–8.
58 Nishio H, Kanno S, Onoyama S et al. NOD1 ligands induce site-specific vascular inflammation. Arterioscler Thromb Vasc Biol 2011; 31:1093–9.
59 Tukhatulinen AI, Logunov DY, Gitlin II et al. An in vitro and in vivo study of the ability of NOD1 ligands to activate the transcriptional factor NF-kB. Acta Naturae 2011; 3:77–84.
60 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; 35:1423–33.
61 Duong TT1, 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.
62 Rosenkranz ME, Schulte DJ, Agle LM et al. TLR2 and MyD88 contribute to Lactococcus casei extract-induced focal coronary arteritis in a mouse model of Kawasaki disease. Circulation 2005; 112:2966–73.
63 Hui-Yuen JS, Duong TT, Yeung RS. TNF-alpha is necessary for induction of coronary artery inflammation and aneurysm formation in an animal model of Kawasaki disease. J Immunol 2006; 176:6294–301.
64 Lin IC, Suen JL, Huang SK et al. Dectin-1/Syk signaling is involved in Lactobacillus casei cell wall extract-induced mouse model of Kawasaki disease. Immunobiology 2013; 218:201–12.
65 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; 35:2605–16.
66 Murata H, Naoe S. Experimental Candida-induced arteritis in mice–relation to arteritis in Kawasaki disease. Prog Clin Biol Res 1987; 250:523.
67 Nagi-Miura N, Harada T, Shinohara H et al. Lethal and severe coronary arteritis in DBA/2 mice induced by fungal pathogen, CAWS, Candida albicans water-soluble fraction. Atherosclerosis 2006; 186:310–20.
68 Martinez HG, Quinones MP, Jimenez F et al. Important role of CCR2 in a murine model of coronary vasculitis. BMC Immunol 2012; 13:56.
69 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.
70 Leung DC, Cotran RS, Kurt-Jones E, Burns JC, Newburger JW, Pober JS. Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. Lancet 1989; 2:1298–302.
71 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.
72 Scott DGI, Bacon P. Epidemiology of systemic vasculitis. In: Sharma A, ed. Textbook of systemic vasculitis. London: The Health Sciences Publisher, 2015:3–9.
73 Brogan P, Bagga A. Leukocytoclastic vasculitis: Henoch–Schönlein purpura and hypersensitivity vasculitis. In: Petty RE, Laxer RM, Lindsley CB, eds. Textbook of pediatric rheumatology. Philadelphia, PA: Elsevier, 2016:452–61.
74 Futamura M, Ohya Y, Akashi M et al. Age-related prevalence of allergic diseases in Tokyo schoolchildren. Allergol Int 2011; 60: 509–15.
75 Molodecky NA, Soon IS, Doreen M et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012; 142:46–54.
76 Lloyd AJ, Walker C, Wilkinson M. Kawasaki disease: is it caused by an infectious agent? Br J Biomed Sci 2001; 58:122–8.
77 Weiss PF. Pediatric vasculitis. Pediatr Clin North Am 2012; 59: 407–23.
78 Stojanov S, Lapidus S, Chitkara P et al. Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) is a disorder of innate immunity and TH1 activation responsive to IL-1 blockade. Proc Natl Acad Sci USA 2011; 108:7148–53.
79 Misra DP, Wakhlu A, Agarwal V. Pathogenesis of vasculitis. In: Sharma A, ed. Textbook of systemic vasculitis. London: The Health Sciences Publisher, 2015:40–8.
80 Pryshchep O, Ma-Krupa W, Younge BR, Goronzy JJ, Weyand CM. Vessel-specific Toll-like receptor profiles in human medium and large arteries. Circulation 2008; 118:1276–84.
81 Deng J, Ma-Krupa W, Gewirtz AT, Younge BR, Goronzy JJ, Weyand CM. Toll-like receptors 4 and 5 induce distinct types of vasculitis. Circ Res 2009; 104:488–95.
82 Dillon MJ, Eleftheriou D, Brogan PA. Medium-size-vessel vasculitis. Pediatr Nephrol 2010; 25:1641–52.
83 Prajs K, Bobrowska-Snarska D, Skala M, Brzosko M. Polyarteritis nodosa and Sjögren’s syndrome: overlap syndrome. Rheumatol Int 2012; 32:4019–21.
84 Eleftheriou D, Ozen S. Polyarteritis nodosa. In: Petty RE, Laxer RM, Lindsley CB, eds. Textbook of pediatric rheumatology. Philadelphia, PA: Elsevier, 2016:462–6.
85 Jennette JC, Falk RJ. Pathogenesis of antineutrophil cytoplasmic autoantibody-mediated disease. Nat Rev Rheumatol 2014; 10: 463–73.
86 Cabral DA, Morishita K. Antineutrophil cytoplasmic antibody associated vasculitis. In: Petty RE, Laxer RM, Lindsley CB, eds. Textbook of pediatric rheumatology. Philadelphia, PA: Elsevier, 2016:462–6.
87 Mathew J, Ramya J. Genetics of vasculitis. In: Sharma A, ed. Textbook of systemic vasculitis. London: The Health Sciences Publisher, 2015:10–5.
88 Khor CC, Davila S, Brennis WB et al. Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. Nat Genet 2011; 43:1241–6.
89 Maury CP, Salo E, Pelkonen P. Circulating interleukin-1 beta in patients with Kawasaki disease. N Engl J Med 1988; 319:1670–1.
90 Campbell AJ, Burns JC. Adjunctive therapies for Kawasaki disease. J Infect 2016; 72 Suppl:S1–5.
91 Ramirez GA, Maugeri N, Sabbadini MG, Rovere-Querini P, Manfredi AA. Intravascular immunity as a key to systemic vasculitis: a work in progress, gaining momentum. Clin Exp Immunol 2014; 175:150–66.
92 Kim EH, Park MJ, Park S, Lee ES. Increased expression of the NLRP3 inflammasome components in patients with Behcet’s disease. J Inflamm (Lond) 2015; 12:1.
93 Shah V, Christov G, Mukasa T et al. Cardiovascular status after Kawasaki disease in the UK. Heart 2015; 0:1–10.
94 Noto N, Okada T. Premature atherosclerosis long after Kawasaki disease. In: Atiq M, ed. Recent advances in cardiovascular risk factors. Shanghai: InTech, 2012. ISBN: 978-953-51-0321-9. Available at: http://www.intechopen.com/books/recent-advances-in-cardiovascular-risk-factors/prematureatherosclerosis-long-after-kawasaki-disease. Last accessed: June 4, 2016.
95 Chen S, Lee Y Crother TR, et al. Marked acceleration of atherosclerosis after Lactobacillus casei-induced coronary arteritis in a mouse model of Kawasaki disease. Arterioscler Thromb Vasc Biol 2012; 32:e60–71.
96 Kanno S, Nishio H, Tanaka T et al. Activation of an innate immune receptor, Nod1, accelerates atherogenesis in Apoe−/− mice. J Immunol 2015; 194:773–80.