Kawasaki disease: pathophysiology and insights from mouse models

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Abstract | Kawasaki disease is an acute febrile illness and systemic vasculitis of unknown aetiology that predominantly afflicts young children, causes coronary artery aneurysms and can result in long-term cardiovascular sequelae. Kawasaki disease is the leading cause of acquired heart disease among children in the USA. Coronary artery aneurysms develop in some untreated children with Kawasaki disease, leading to ischaemic heart disease and myocardial infarction. Although intravenous immunoglobulin (IVIG) treatment reduces the risk of development of coronary artery aneurysms, some children have IVIG-resistant Kawasaki disease and are at increased risk of developing coronary artery damage. In addition, the lack of specific diagnostic tests and biomarkers for Kawasaki disease make early diagnosis and treatment challenging. The use of experimental mouse models of Kawasaki disease vasculitis has considerably improved our understanding of the pathology of the disease and helped characterize the cellular and molecular immune mechanisms contributing to cardiovascular complications, in turn leading to the development of innovative therapeutic approaches. Here, we outline the pathophysiology of Kawasaki disease and summarize and discuss the progress gained from experimental mouse models and their potential therapeutic translation to human disease.

Kawasaki disease is a systemic vasculitis that affects infants and young children1–5. Kawasaki disease is now the leading cause of acquired heart disease among children in North America, Europe and Japan6–9. The cardiovascular sequelae resulting from childhood Kawasaki disease are increasingly recognized to extend into adulthood, and the disease is no longer considered self-limiting10–12. The triggering agents for Kawasaki disease remain unidentified; however, results from our laboratory10,11 and others12,13 are consistent with the interpretation that a conventional antigen is probably responsible. Coronary arteritis and predominantly coronary artery aneurysms (CAAs) occur in up to 30% of untreated children, although this rate is reduced to 5–7% in children treated with high-dose intravenous immunoglobulin (IVIG)14,15. IVIG treatment leads to CAA regression in 60–75% of patients with Kawasaki disease16,17. However, the exact mechanisms by which IVIG reduces the rate of cardiovascular complications are unknown16. Up to 15–20% of patients with Kawasaki disease do not respond to IVIG treatment, and these individuals have an increased rate of CAA development18–21.

Kawasaki disease is associated with infiltration of the coronary artery wall by a broad variety of innate and adaptive immune cells. Immunohistochemical analysis of human post-mortem tissues shows accumulation in the arterial wall of monocytes, macrophages and neutrophils22,23, and the presence of activated CD8+ T cells24 as well as IgA+ plasma cells25,26. The release of pro-inflammatory cytokines, such as TNF and IL-1β, by infiltrating immune cells promotes vascular endothelial cell damage and the development of CAAs27,28.

However, understanding of Kawasaki disease pathophysiology is limited by the low availability of human tissues of the disease, failure to identify specific aetiological agents triggering the disease, and incomplete understanding of the molecular and cellular mechanisms leading to cardiovascular sequelae. Therefore, experimental animal models mimicking the human features of Kawasaki disease and their translational utility have been invaluable to investigation of this disease. In this Review, we discuss advances from human and mouse studies that have contributed to an improved understanding of Kawasaki disease pathophysiology and the cellular and molecular circuits involved in disease development. We also outline how evidence obtained from experimental mouse models of Kawasaki disease vasculitis has paved the way for the development of new efficient therapeutics to treat human Kawasaki disease.

Aetiological agents

The causative agents initiating the disease have still not been identified >50 years after the first description of Kawasaki disease. However, the trigger is suspected to be of viral origin and to enter the body through the
Key points

- Kawasaki disease is a childhood systemic vasculitis leading to the development of coronary artery aneurysms; it is the leading cause of acquired heart disease in children in developed countries.
- The cause of Kawasaki disease is unknown, although it is suspected to be triggered by an unidentified infectious pathogen in genetically predisposed children.
- Kawasaki disease might not be a normal immune response to an unusual environmental stimulus, but rather a genetically determined unusual and uncontrolled immune response to a common stimulus.
- Although the aetiological agent in humans is unknown, mouse models of Kawasaki disease vasculitis demonstrate similar pathological features and have substantially accelerated discoveries in the field.
- Genetic and transcriptomic analysis of blood samples from patients with Kawasaki disease and experimental evidence generated using mouse models have demonstrated the critical role of IL-1β in the pathogenesis of this disease and the therapeutic potential of targeting this pathway (currently under investigation in clinical trials).

mucosal surfaces in the lung[29] (Fig. 1). This hypothesis is supported by the seasonality of Kawasaki disease outbreaks, which is similar to that of other respiratory infections. In Japan, two seasonal peaks have been observed, one in winter and another in summer, whereas in the USA, the incidence peaks are observed during spring and winter[30]. Development of Kawasaki disease is age specific, with children from 6 months to 5 years of age at greatest risk[20,21,22], which suggests a protective maternal passive immunity against the causative agent from birth to 6 months of age and the importance of immune system maturation in children 26 years of age[23].

The clinical features of Kawasaki disease, such as high fever, skin rash and peeling, conjunctivitis and intense release of pro-inflammatory cytokines, are reminiscent of other infectious diseases such as staphylococcal and streptococcal toxic shock syndromes[24]. Some studies have shown that, compared with healthy control individuals, patients with Kawasaki disease have a skewed Vβ T cell repertoire and increased frequencies of circulating Vβ2 and Vβ8.1+ T cells, leading to the early suggestion that a superantigen toxin might have a role in triggering Kawasaki disease[25–27,28]. However, similar results were not reproduced in later studies[29,30], leading to the more generalized hypothesis that the development of Kawasaki disease might be triggered by multiple conventional antigens.

Several early studies showed reduced prevalence of antibodies to the Epstein–Barr virus (EBV) capsid antigen in Japanese children with Kawasaki disease compared with age and sex-matched control patients[31–35], suggesting the involvement of an abnormal immune response to EBV in disease development. However, this difference in EBV antibody seropositivity could not be reproduced in other studies[36,37,38]. A human coronavirus was detected more frequently in respiratory secretions of patients with Kawasaki disease than in control individuals[39], although, again, other studies could not replicate this finding[40,41], indicating that the original association might have been coincidental. The possibility that a retrovirus is the triggering agent for Kawasaki disease has also been proposed, owing to detection of retrovirus-specific reverse transcriptase activity in the

SNPs influencing susceptibility

Although Kawasaki disease has been observed around the world and in multiple ethnic groups, geographical differences exist in incidence. The highest incidence is in Asian countries such as Korea and Japan, where it has increased over the past decades and is now 10–20 times more prevalent than in North America and Europe[42]. This increased susceptibility in Asian children, as well as in children with Asian ancestry living in North America, indicates that genetic components predispose to disease susceptibility. In Japan, siblings of children with Kawasaki disease are at increased risk of developing the disease[43]. Single nucleotide polymorphisms (SNPs) in multiple genes have been associated with increased susceptibility to Kawasaki disease (Fig. 1); however, mechanisms linking those SNPs with Kawasaki disease progression are not yet well understood and require more investigation.

Calcium signalling pathway. Inositol 1,4,5-trisphosphate 3-kinase C (ITPKC), a kinase that phosphorylates inositol 1,4,5-trisphosphate (IP₃), is involved in many signalling processes in a wide array of cells. In T cells, IP₃ is released after T cell receptor stimulation, thus increasing levels of intracellular Ca²⁺ through IP₃ receptors expressed on the endoplasmic reticulum and leading to nuclear translocation of nuclear factor of activated T cells (NFAT). Although no significant association between ORAI1 polymorphisms and Kawasaki disease...
Increased numbers of have been proposed as triggering agents for Kawasaki disease; however, none has been corroborated, and the aetiological agent remains unidentified. Different aetiological agents, from viruses to environmental toxins, are implicated in the development of Kawasaki disease. Single nucleotide polymorphisms in the immunoglobulin genes have been associated with susceptibility or IVIG treatment response. CD40 engagement is associated with cell survival, activation, proliferation and cytokine production. Compared with control patients with other febrile illnesses, patients with Kawasaki disease have increased CD40L expression on CD4+ T cells and platelets, which correlates with increased development of coronary artery lesions and is reduced by IVIG treatment. An SNP in CD40L has been reported in Japanese patients with Kawasaki disease and is more frequent in male patients with coronary artery lesions than in female patients. This polymorphism was not observed in a cohort of Taiwanese patients; however, another SNP in the CD40 gene has been reported in an independent cohort of Taiwanese patients and is associated with increased susceptibility to Kawasaki disease and development of coronary artery lesions. These results indicate a role of the CD40–CD40L pathway in the development and severity of Kawasaki disease and highlight this pathway as a potential therapeutic target.

**Mannose-binding lectin.** Mannose-binding lectin (MBL), a pattern recognition molecule of the innate immune system, binds the surface of pathogenic organisms and activates the complement pathway. A polymorphism in MBL2 was found to be an age-related risk factor for development of coronary artery lesions in a Dutch cohort of patients. Another study in a cohort of Japanese patients with Kawasaki disease showed that codon 54 variants in MBL2 are significantly associated with susceptibility to Kawasaki disease. Interestingly, in the Candida albicans water-soluble fraction (CAWS) mouse model of Kawasaki disease vasculitis, MBL-A and MBL-C deposition are observed in the aortic root, suggesting involvement of the MBL-dependent lectin pathway in this experimental model. However, further studies are required to understand the pathogenic roles of those two proteins as well as their potential as therapeutic targets.

**Fcy receptors.** Polymorphisms in genes encoding the receptors for the Fc portion of immunoglobulins, Fcy receptors (FcyRs), have been associated with the development of autoimmune and infectious diseases. As Kawasaki disease is considered an infectious disorder, several studies have investigated the potential association of FcyR SNPs with Kawasaki disease susceptibility and the development of coronary artery lesions. In a cohort of Dutch patients, no difference in FcyR SNP distribution was observed between healthy individuals and patients with Kawasaki disease, and no association was noted between SNPs in FcyR genes and Kawasaki disease susceptibility. However, a later study with >2,000 patients with Kawasaki disease and 9,000 control patients from multiple independent cohorts across different populations highlighted a Kawasaki disease-associated polymorphism in the FCGR2A locus, which encodes FcyRIIA (CD32a), a member of the family of IgG receptors. This polymorphism has important implications as the standard of care for Kawasaki disease is IVIG, a pool of plasma IgG that interacts with FcyRs on immune cells. Interestingly, 15–20% of patients with Kawasaki disease have IVIG-resistant disease and

**Fig. 1 | Environmental and genetic factors implicated in the development of Kawasaki disease.** Different aetiological agents, from viruses to environmental toxins, have been proposed as triggering agents for Kawasaki disease; however, none has been corroborated, and the aetiological agent remains unidentified. Increased numbers of IgA+ plasma cells have been detected in the pancreas, the kidneys, the coronary artery wall and the respiratory tract of patients with Kawasaki disease. Patients with Kawasaki disease have increased concentrations of secretory IgA in their serum, indicative of defective intestinal barrier function and increased intestinal permeability. Changes in the gut microbiota composition (dysbiosis) have also been suggested to have a role in the development of Kawasaki disease. Single nucleotide polymorphisms in the genes listed have been associated with susceptibility to Kawasaki disease and disease severity. The current understanding is that Kawasaki disease is triggered in genetically predisposed children by a ubiquitous environmental stimulus that typically would not result in an uncontrolled immune response and development of vasculitis.
require another round of IVIG treatment or the use of adjunctive therapies\(^{15,19,20,80}\). The exact mechanisms by which IVIG mediates its therapeutic effect and how IVIG resistance develops remain unknown, and the potential involvement of this FcγRIIA polymorphism in IVIG resistance requires further investigation.

**Pathophysiology of Kawasaki disease**

*The innate immune response.* The immune response associated with Kawasaki disease is complex and involves the activation and infiltration of the coronary artery wall by both innate and adaptive immune cells (Fig. 2). On the basis of studies of post-mortem tissue from patients with Kawasaki disease, Kawasaki disease vascular pathology has been classified into three sequential linked pathological processes\(^{81}\). Necrotizing arteritis develops in the first 2 weeks of the disease and is associated with neutrophilic infiltration, which gradually destroys the intima, media and some portions of the adventitia of the coronary artery. CD8\(^+\) T cells, IgA\(^+\) plasma cells, monocytes and macrophages compose the inflammatory infiltrate during subacute chronic arteritis. These cells release pro-inflammatory cytokines such as IL-1\(\beta\) and TNF, which contribute to luminal myofibroblast proliferation, in which myofibroblasts, mainly derived from smooth muscle cells, and their matrix products progressively obstruct the coronary lumen.

Matrix metalloproteinases. Matrix metalloproteinases (MMPs; zinc-dependent endopeptidases that degrade extracellular matrix components) are known to have an important role in both inflammation and tissue remodelling processes\(^{86}\). Increased expression and activity of a diverse set of MMPs has been demonstrated in acute Kawasaki disease\(^{87-89}\). The expression levels of MMP3 and MMP9, both known to mediate vascular smooth muscle cell migration and neointimal formation\(^8\), are increased in patients with Kawasaki disease\(^{88}\), and the circulating levels of these MMPs correlate with the development of CAAs in these patients\(^7\). MMP3 SNPs are also associated with the development of CAAs\(^8\), and...
this protease is considered to be a driving factor allowing IL-1-induced signalling to lead to migration of vascular smooth muscle cells and their transition to proliferating myofibroblasts97-99. Whereas MMP9 has been studied and implicated in elastin breakdown in the Lactobacillus casei cell wall extract (LCWE)-induced Kawasaki disease mouse model96,97, information about the role of MMP3 in this mouse model is lacking.

**MicroRNAs.** MicroRNAs (miRNAs; a class of small non-coding RNAs that regulate mRNA expression) are emerging as critical gene regulators in a host of cellular processes, including inflammation94. Of human coding genes, 60–70% are estimated to be regulated by miRNAs95. Several studies attempting to discover Kawasaki disease biomarkers have found that the miRNA profiles of serum exosome or coronary artery tissues are associated with acute Kawasaki disease100-104. These miRNAs include miR-23a105-108, miR-27b100, miR-223 [REFS100-103], and miR-145 [REF 105]. These miRNAs might provide clues as to the molecular mechanisms involved in the development of the cardiovascular lesions associated with Kawasaki disease. For example, miR-145 is highly expressed in vascular smooth muscle cells and has been reported to promote their switching to neointimal proliferating cells106,107 and to regulate the transforming growth factor-β signalling pathway108. Increased levels of miR-23a contribute to cardiomyocyte apoptosis and may promote inflammatory responses by blocking macrophage autophagy activity107,109. However, improved understanding and characterization of the molecular and cellular mechanisms underlying the different roles of miRs during Kawasaki disease require further studies with animal models.

**Myocarditis.** Most attention in Kawasaki disease research and clinical practice has focused on the development of CAAs and long-term complications of coronary artery stenosis and ischaemia106. However, the subacute and chronic inflammation of Kawasaki disease is also associated with the development of myocarditis110-112. Myocarditis has been described as the ‘hidden face of the moon’ in Kawasaki disease110. Reports indicate that myocarditis occurs frequently during acute Kawasaki disease111, and serial myocardial biopsy studies have documented that histological myocarditis develops in the majority of patients with Kawasaki disease, even in the absence of coronary aneurysms113-114. More recent data indicate that myocardial inflammation can be documented in 50–70% of patients using gallium citrate (^{^6}Ga) scans and technetium-99 (^{99m}Tc)-labelled white blood cell scans115. Another study has shown that myocardial inflammatory changes and myocardial oedema in Kawasaki disease occur even before coronary artery abnormalities and without concurrent ischaemic damage116.

Myocarditis in Kawasaki disease tends to develop early, and acute left ventricular dysfunction is generally transient and responds readily to anti-inflammatory treatment116. However, Kawasaki disease myocarditis might be associated with fatal arrhythmias in infants, and in certain cases might lead to long-term complications including myocardial fibrosis117-119. Therefore, myocarditis during Kawasaki disease and its potential consequences deserve serious investigation, and long-term studies into late adulthood are needed.

**Complement and immune complexes.** Kawasaki disease affects small and medium sized vessels, particularly the coronary arteries; however, dilatations and aneurysms can occur systemically, including in the axillary, subclavian, brachial, renal and iliac arteries as well as the abdominal aorta118-120. Post-mortem findings have revealed that 73% of patients with Kawasaki disease have renal artery involvement and acute kidney injury121 involving glomerulonephritis with intracapillary changes and deposition of immune complex composed of IgA and complement component 3 (C3)122,123. These findings are comparable to those in two other human vasculitis diseases, IgA vasculitis (IgAV) and IgA nephropathy (IgAN), which are similarly characterized by IgA immune complexes with C3 deposition in kidney glomeruli (see below). Increased concentrations of circulating IgA and secretory IgA (sIgA) have been reported in the serum of children with Kawasaki disease during the acute phase124. IgA+ plasma cells are present in the coronary artery wall and in non-vascular tissues, such as the kidney, trachea and pancreas of patients with Kawasaki disease125-128. This IgA response is oligoclonal, seems to be antigen driven and might be caused by Kawasaki disease-triggering agents125-128.

**The IL-1 signalling pathway.** Evidence from mouse models of Kawasaki disease129,130, as well as transcriptionome analysis performed on whole blood of patients with Kawasaki disease during the acute or convalescent phase131,132, demonstrate the involvement of innate immune cells and inflammasome overactivation throughout the acute phase of the disease. In vitro cultured PBMCs isolated from patients with Kawasaki disease spontaneously release IL-1β into the supernatant, and this process is substantially reduced after IVIG treatment133. Serum concentrations of both IL-1β and IL-18 are also higher in children with acute Kawasaki disease than in control patients with other febrile illnesses, and markedly decrease during the convalescent phase134, supporting the concept of activation of the NLRP3 inflammasome complex. Similarly, IL-1 and NLRP3-related gene transcripts are upregulated in PBMCs from patients with acute Kawasaki disease and are decreased during the convalescent phase of the disease135, and an IL1B-gene-related signature is associated with acute phase disease and IVIG resistance135. Furthermore, a study has shown that differential expression of IL-1β and related signalling genes might have a role in mediating the sex-based differences seen in patients with Kawasaki disease136. In the LCWE mouse model of Kawasaki disease, the activation of caspase 1, IL-1α and IL-1β is key to the development of coronary arteritis, aneurysms, myocarditis and abdominal aorta aneurysms137,138. IL-1 has the capacity to expand and promote the differentiation of antigen-specific CD8+ T cells139, and indeed the frequencies of circulating CD4+ and CD8+ T cells are increased in patients with Kawasaki
Infiltrations of mature dendritic cells as well as activated cytotoxic CD8+ T cells have been reported in arterial layers of coronary aneurysms. Therefore, blocking the NLRP3–IL-1β pathway seems to be a valid therapeutic option in Kawasaki disease.

**Role of the gastrointestinal tract**

**Intestinal permeability.** The intestinal barrier has a critical role in maintaining intestinal homeostasis and health by preventing harmful organisms and luminal antigens from entering the circulation. A dysfunctional intestinal barrier, characterized by increased intestinal permeability, is recognized as a pathogenic factor in many inflammatory diseases. In Kawasaki disease, abdominal pain, diarrhoea and vomiting are often observed at the onset of acute illness, affecting up to 60% of diagnosed patients and indicating that the gastrointestinal tract is also affected. A multicentre study of >300 patients revealed that gastrointestinal manifestations at onset of disease complicate diagnosis, delay adequate treatment and correlate with IVIG resistance and severity of CAAs. Immunohistochemical studies have revealed higher numbers of activated CD4+ T cells and macrophages along with lower numbers of CD8+ T cells in the jejunum lamina propria in patients with Kawasaki disease than in control patients with diarrhoea from cows’ milk protein intolerance. However, these cellular abnormalities are specific to the acute phase of the disease and return to normal during the convalescent phase. IgA+ plasma cells have also been observed in a variety of different vascular and non-vascular tissues in patients with Kawasaki disease, and patients with Kawasaki disease also have increased concentrations of sIgA, which is produced at the intestinal mucosal surface, in their serum. These studies indicate that the gastrointestinal tract is affected during Kawasaki disease and that mucosal immune activation might compensate and protect from defective intestinal barriers.

The role of gut-related immunity in the induction of inflammation in organ systems distant from the gut has been the subject of intensive investigation. We have observed increased intestinal permeability and a disregulated intestinal immune response characterized by increased numbers of IgA+ B cells in the Peyer’s patches in the LCWE-induced mouse model of Kawasaki disease (Fig. 3). In this model, the excessive IL-1β release associated with LCWE injection acts on intestinal epithelial cells to open tight junctions, and administration of IVIG or pharmacological agents that block intestinal permeability significantly reduces disease development. Altogether, these observations link increased intestinal permeability and defective intestinal barrier function with systemic IL-1β release in Kawasaki disease.

**The intestinal microbiome.** Despite the strong connection between the intestinal microbiome and development of cardiovascular diseases, only a few studies have investigated the role of the intestinal microbiome during development of Kawasaki disease or treatment resistance. Microbiological culture-based methods demonstrated that, compared with healthy control individuals, patients with Kawasaki disease have a different intestinal microbiota composition characterized by a lower incidence of the Lactobacillus genus and increased Streptococcus and Staphylococcus species. Lactobacilli have been reported to prevent diarrhoeal disorders and to improve intestinal barrier function by increasing the expression of intestinal tight junctions, enhancing the intestinal mucus layer and modulating the intestinal microbiota composition. Lactobacilli have also been shown to boost innate and immune functions against a variety of bacterial infections, and their disappearance during acute Kawasaki disease might lead to the blooming of other bacterial pathogens, which might further promote intestinal barrier dysfunction and inflammation. Intriguingly, a retrospective study of 364 patients with Kawasaki disease showed that children who received microbiome-altering antibiotics in the week before Kawasaki disease diagnosis were substantially more likely to have IVIG-resistant disease than those who did not receive antibiotics. Antibiotics alter the abundance, taxonomic richness and diversity of the bacterial as well as fungal intestinal microbiome, and those alterations might persist from weeks to years after treatment discontinuation. A longitudinal metagenomic study of faecal samples derived from patients with Kawasaki disease showed a marked increase of five Streptococcus spp. during the acute phase of Kawasaki disease; however, all patients in that study were treated with antibiotics in the early stage of disease, therefore this observation might be reflective of antibiotic-induced dysbiosis and not Kawasaki disease itself. Nonetheless, how this intestinal dysbiosis occurs and how its effect on intestinal permeability affects the development of cardiovascular lesions during Kawasaki disease vasculitis remains unknown and under-appreciated.

**Link with IgA vasculitis**

IgAV, or Henoch–Schönlein purpura, is an IgA-mediated necrotizing vasculitis resulting in fibrinoid destruction of the affected small vessels. Renal involvement, characterized by IgA deposition in the kidney glomeruli, is also observed in IgAV. IgAV nephritis is closely related to another glomerular disease, IgAN, wherein accumulation and deposition of IgA and IgA immune complexes in the kidney glomerular mesangium drive glomerular inflammation. IgA is mainly found at mucosal surfaces, a ‘gut–kidney axis’, influenced by a mix of genetic, microbial and dietary factors, has been suggested to be involved in the development of both IgAN and IgAV in paediatric and adult patients. We have demonstrated that the LCWE-induced mouse model of Kawasaki disease vasculitis is associated with the deposition of IgA and IgA–C3 immune complexes in vascular tissues, such as the inflamed coronary artery and abdominal aorta. Deposited IgA and IgA–C3 immune complexes might result in overactivation of the immune cells present in the cardiovascular lesions and subsequent amplification of inflammation. Substantial evidence indicates that immune complexes might promote vascular damage during human Kawasaki disease through the
activation and aggregation of platelets, the release of vasoactive mediators, and the subsequent recruitment of neutrophils and leukocytes to the site of inflammation (reviewed elsewhere). Interestingly, we have also observed IgA and C3 deposition in the kidney glomeruli of LCWE-injected mice developing Kawasaki disease, and immune complex-mediated nephropathy has also been observed in patients with Kawasaki disease, and intestinal dysbiosis might contribute further to the inflammatory process. LCWE injection is also associated with a dysregulated intestinal immune response characterized by increased numbers of IgA+ B cells in the gastrointestinal tract and elevated secretory IgA (sIgA) concentrations. Intestinal barrier dysfunction results in sIgA leakage to the systemic circulation and pathogenic IgA–C3 immune complex deposition in the vascular tissues.

IgAN also shares pathological features with Kawasaki disease, such as increased intestinal permeability, low to moderate intestinal inflammation associated with activation of inflammatory cells in the small intestinal mucosa and colocalization of sIgA–complement in the glomerular mesangium. Moreover, a polymorphism in the promoter of the lipopolysaccharide (LPS) receptor CD14 (CD14/159) is associated with coronary artery abnormalities in patients with Kawasaki disease and has been linked to progression of IgAN to more severe renal disease. IL-1β has a key pathogenic role during Kawasaki disease and also seems to be implicated in renal complications related to IgAV and IgAN. Altogether, given that Kawasaki disease shares clinical features and pathological mechanisms with both IgAV and IgAN, it is possible that Kawasaki disease is a form of IgAV.

Similarly, treatments that have shown efficacy in Kawasaki disease, such as anakinra and IVIG, might be suitable and useful for treating IgAV and IgAN.
**Mouse models of Kawasaki disease**

The lack of identification of specific aetiological agents and incomplete understanding of the molecular mechanisms involved in Kawasaki disease cardiovascular pathology have delayed the development of targeted and effective treatment options for this disease. In addition, the limited availability of tissue samples from patients with Kawasaki disease has considerably impeded progress in understanding the pathogenesis of the disease, making the availability of relevant animal models of Kawasaki disease extremely valuable. Kawasaki disease vasculitis can be induced in mice by injection of cell wall components from *L. casei* or *C. albicans* or nucleotide-binding oligomerization domain containing 1 (Nod1) ligand. These mouse models of Kawasaki disease have accelerated research and have enhanced understanding of the pathogenesis of this disease. However, no animal model perfectly recapitulates human disease. Particularly in the context of Kawasaki disease, given that the aetiology remains unknown, researchers must exercise caution in interpreting results based on experimental models and confirm findings in patient cohorts. Nevertheless, even though the extrapolation of preclinical mouse data to humans is far from straightforward, mouse models are still invaluable tools to study certain pathological aspects of human inflammatory diseases and gain mechanistic insights.

**The LCWE mouse model.** *L. casei* is a Gram-positive bacteria that colonizes the gastrointestinal and urogenital tracts of both human and animals. More than 35 years ago, Lehman et al. demonstrated that a single intraperitoneal injection of LCWE induces a dose-dependent and chronic polyarthritis in rats. However, when injected into mice, LCWE induces instead a focal coronary arteritis. How and which element of LCWE triggers Kawasaki disease vasculitis is unknown. LCWE is mainly composed of peptidoglycans, contains high levels of rhamnose and is resistant to lysozyme degradation.

The cardiovascular lesions induced in mice by LCWE are histologically similar to those observed in human disease. LCWE-induced Kawasaki disease vasculitis is characterized by infiltration of inflammatory cells in the aortic root, development of necrotizing arteritis in the coronary artery followed by luminal obstruction due to LMP that can lead to complete coronary artery stenosis, recapitulating the three pathological processes of human Kawasaki disease described above. In children with Kawasaki disease, thrombotic occlusion of the inflamed coronary artery leads to ischaemic heart disease and, similarly, occluding organizing thrombus in the coronary artery can be observed in LCWE-injected mice. Acute myocarditis and chronic scarring of the coronary arteries with the formation of stenotic fragments are also observed in LCWE-induced Kawasaki disease vasculitis, even long after the acute phase, which is similar to the fibrotic lesions that might lead children with Kawasaki disease to develop long-term cardiovascular sequelae in adulthood. MRI and echocardiography in LCWE-injected mice demonstrate the presence of electrocardiographic changes (as observed in human Kawasaki disease) and myocardial dysfunction, which are responsive to anakinra therapy.

The LCWE-induced Kawasaki disease vasculitis in mice is dependent on intact TLR2 and MyD88 signalling and the subsequent release of pro-inflammatory cytokines, including IL-1β, IL-6 and TNF. Genetic depletion of the TNF receptor or pharmacological blockade of the TNF signalling pathway (with infliximab (monoclonal antibodies to TNF) or etanercept (soluble TNF receptors)) protects mice from LCWE-induced Kawasaki disease vasculitis. This model is also T cell dependent, as Rag1−/− mice develop fewer cardiovascular lesions. CD8+ T cells are specifically required for LCWE-induced Kawasaki disease vasculitis as treatment of LCWE-injected mice with an anti-CD8-depleting antibody prevents the development of vasculitis. This finding correlates with human disease, in which infiltrations of CD3+ T cells, and particularly CD8+ T cells, are detected in the CAA.

The LCWE model has also confirmed the importance of CD8+ T cells in the development of CAA.

**Table 1 | Comparison of the three mouse models of Kawasaki disease**

| Characteristic | Lactobacillus casei cell wall extract | Candida albicans water-soluble fraction | Nod1 ligand (FK565) |
|---------------|-------------------------------------|--------------------------------------|---------------------|
| Induction     | Single intraperitoneal injection     | Repeated intraperitoneal injections  | Priming with LPS and Nod1 ligand intraperitoneal injection |
| Pathology     | Aortic root inflammation; coronary arteritis; epicardial coronary arteritis; luminal myofibroblast proliferation; development of abdominal aorta aneurysms | Aortic root inflammation; coronary arteritis; inflammation focally extending to coronary arteries; development of abdominal aorta aneurysms | Aortic root inflammation; coronary arteritis |
| Immune characteristics | MyD88−/−TLR2-dependent; NLRP3 inflammasome-dependent; innate immune cell dependent (neutrophils and macrophages); T cell dependent | Dectin-2 receptor-dependent; increased antineutrophil cytoplasmic antibodies; innate immune cell dependent (neutrophils and macrophages); T cell dependent | CD11c+ macrophage-dependent; T cell-independent |
| Therapy       | IVIG; anakinra; IL-1α antibody; IL-1β antibody; TNF antibody | IVIG; IL-1β antibody; GM-CSF antibody | NA |

GM-CSF, granulocyte–macrophage colony-stimulating factor; IVIG, intravenous immunoglobulin; LPS, lipopolysaccharide; NA, not available.
of the ITPKC pathway in Kawasaki disease development and demonstrated that ITPKC deficiency is associated with increased Ca\(^{2+}\) flux and levels of IL-1\(\beta\) in vitro\(^{59}\). Interestingly, the relatively mild development of coronary arteritis in LCWE-injected CBA/N mice—which are characterized by a defective B cell maturation process and poor humoral immune responses—suggests that the humoral immune response might participate in amplification of the disease\(^{186}\). IgA\(^{+}\) plasma cells infiltrate vascular and non-vascular tissues during the acute phase of Kawasaki disease\(^{25,26}\), resulting in the development of an oligoclonal IgA response in the coronary artery\(^{125,126}\). Interestingly, we have observed increased numbers of IgA\(^{+}\) plasmablasts in the spleen, Peyer’s patches and abdominal aorta draining lymph nodes of LCWE-injected mice, as well as increased concentrations of circulating IgA and IgA deposition in heart tissues, abdominal aorta and kidney glomeruli\(^{143}\).

Mouse models also provide a useful opportunity to evaluate the efficacy of therapeutic regimens on the development and healing of cardiovascular lesions. When given up to 5 days after LCWE injection, IVIG substantially decreases the severity of cardiovascular lesions in mice\(^{187}\), mirroring the effects of IVIG treatment in humans. As described above, IL-1\(\beta\) signaling is higher in patients with Kawasaki disease than in age-matched control patients with other febrile illnesses\(^{11,103}\), and studies using the LCWE model helped lead to the discovery of the importance of this pathway in the pathogenesis of the disease and the therapeutic potential of IL-1 blockade. Depletion of macrophages or blocking the IL-1 pathway either genetically using IL1R\(^{-/-}\), IL1a\(^{-/-}\) or IL1\(\beta\)\(^{-/-}\) mice or with antibodies targeting IL-1\(\alpha\) or IL-1\(\beta\), or anakinra (IL1Ra), strongly reduces cardiovascular lesion development as well as myocardial dysfunction in LCWE-injected mice\(^{130,132,184}\).

**The CAWS mouse model.** *C. albicans* is a harmless commensal fungus normally present in the human gastrointestinal tract that can transition into a pathogen capable of inducing inflammation in immune-impaired hosts. In 1979, Murata demonstrated that an alkaline extract made from *C. albicans* isolated from faeces from a patient with Kawasaki disease induced coronary arteritis in mice\(^{177}\). CAWS is composed of polysaccharides, mainly \(\beta\)-glucans and \(\alpha\)-mannan proteins of the yeast cell wall\(^{189}\), and needs to be injected intraperitoneally for five consecutive days in the first week of the disease to induce vasculitis in the aortic valves and the coronary arteries\(^{189,190}\). In this model, recognition of \(\alpha\)-mannan proteins by the dectin-2 receptor seems to be essential, as CAWS-injected Dectin-2\(^{-/-}\) mice do not develop vasculitis\(^{191}\).

The CAWS model shares some histological similarities with human Kawasaki disease pathology in that inflammation affects both the aortic root and the proximal region of the coronary arteries\(^{190}\). Inflammation can also affect non-coronary artery sites in 25% of CAWS-injected mice and can be observed in the lymph nodes, the kidneys and the liver\(^{190,192}\). CAWS-induced coronary artery lesions resemble those of human Kawasaki disease and are typically proliferative, granulomatous and characterized by intimal thickening with destruction of the elastic lamina and media\(^{190}\).
Echocardiography in CAWS-injected mice indicates a marked decrease of cardiac function, which can be restored by IL-10 supplementation\(^{191}\). IL-10 is a potent anti-inflammatory cytokine that might improve the outcome of CAWS-induced vasculitis by inhibiting the release of pro-inflammatory mediators, such as TNF and IL-1β, from tissue-infiltrating innate immune cells\(^{194}\). Interestingly, CAWS-induced Kawasaki disease vasculitis is also strain dependent, as CAWS injections lead to a high incidence of vasculitis in CD-1, C3H/HeN, DBA/2 and C57BL/6N mice, but the CBA/J/N strain is resistant to coronary arteritis\(^{190,195}\). The DBA/2 strain is the most sensitive, with the highest mortality rate resulting from a more intense coronary arteritis\(^{190}\). The sensitivity of DBA/2 mice is associated with increased production of the pro-inflammatory cytokines TNF, IL-6 and IFNγ\(^{195,196}\), whereas resistance of CBA/J/N mice is explained by increased levels of IL-10 production in that strain\(^{197}\).

Despite the presence of T cell and B cell infiltration in the inflamed coronary artery, mice lacking T cells still develop moderate to typical cardiac inflammation, indicating that T cells might not be required in the development of Kawasaki disease vasculitis in this particular model\(^{198,199}\). Absence of both T cells and B cells in Rag1\(^{−/−}\) mice leads to lower incidence of CAWS-induced Kawasaki disease vasculitis; reconstitution of Rag1\(^{−/−}\) mice with wild-type, but not CCR2\(^{−/−}\), T cells and B cells restores cardiovascular lesions, suggesting roles for both T cells and B cells and the modulation of disease development by CCR2 expression\(^{200}\). The innate immune response also participates in vasculitis development; resident macrophages recognize the CAWS antigens through the dectin-2 receptor, leading to their activation, release of CCL2, and recruitment of neutrophils and inflammatory monocytes producing IL-1β in the aortic root\(^{201}\).

CAWS-induced vasculitis is also associated with the rapid production of granulocyte–monocyte colony-stimulating factor in the heart, which subsequently drives inflammatory myocarditis by activating tissue macrophages and promoting recruitment of neutrophils and monocytes\(^{195}\). TNF is also produced during the acute phase of CAWS-induced Kawasaki disease vasculitis and is essential for the development of acute myocarditis, as TNF receptor-deficient mice are protected from the development of CAWS vasculitis\(^{202}\). IVIG administration substantially reduces CAWS-induced heart vessel inflammation\(^{203}\). Like the LCWE model, the CAWS model is also dependent on the IL-1 pathway, as IL1R\(^{−/−}\), IL1β\(^{−/−}\), Asc\(^{−/−}\) and Nlrp3\(^{−/−}\) mice are protected from induction of vasculitis, and treatment with anti-IL-1β agents substantially attenuates CAWS vasculitis\(^{202,204,205}\).

**The Nod1 ligand mouse model.** Endothelial cells are equipped to sense microbial components through Toll-like receptors and nucleotide-binding oligomerization domain-containing protein like receptors. Subcutaneous injection or oral delivery of FK565, a specific synthetic Nod1 ligand, in mice primed with LPS results in a diffuse cellular inflammation of the aortic root and transmural infiltration of inflammatory cells in the coronary artery wall\(^{174,206}\). Other arteries, such as the iliac and renal arteries, also show signs of inflammation associated with a thickening of the intima\(^{208}\).

The mechanisms by which FK565 induces coronary arteritis in mice remain unknown. When administered orally, FK565 does not induce intestinal mucosa inflammation, but specifically activates vascular cells to produce a diverse array of pro-inflammatory cytokines, including IL-1β\(^{208}\), and chemokines such as CCL2, resulting in the recruitment of inflammatory cells in the tissues\(^{174}\). This model seems to be independent of T cells, B cells and natural killer T cells, as LPS-primed Rag1\(^{−/−}\) mice still develop aortitis and coronary arteritis after FK565 injection\(^{207}\). The inflammatory infiltrates observed around the inflamed aortic root and coronary arteries mainly comprise neutrophils and CD11c+cardiac macrophages; their specific depletion considerably reduces the development of FK565-induced Kawasaki disease vasculitis\(^{178,207}\). The concentration of circulating IL-1β is substantially increased in the serum of FK565-injected mice compared with control or CAWS-injected animals, and higher IL-1β levels correlate with a larger inflammation area\(^{208}\). However, specific studies further investigating the role of IL-1β in this model are needed.

**Treatment of Kawasaki disease**

### Traditional and novel therapies in humans

The current standard of care for Kawasaki disease is the use of high-dose IVIG together with aspirin. If given during the first 10 days of the disease, IVIG reduces the risk of development of coronary arteritis and aneurysms from about 30% to 5–7%\(^{14,15}\). The mechanisms by which IVIG treatment reduces the inflammatory responses are still unknown; however, IVIG is suspected to have a wide spectrum of action targeting multiple arms of the immune response\(^{16}\). IVIG has been shown to inhibit IL-1β production from in vitro stimulated macrophages and to stimulate the production of IL-1Ra\(^{208,209}\). During Kawasaki disease, IVIG reduces production of inflammatory cytokines and chemokines, and decreases the activation and number of circulating neutrophils, monocytes, macrophages and activated T cells by saturating Fc receptors\(^{18}\). The majority of patients with Kawasaki disease who are treated with IVIG improve and do not develop coronary artery damage; however, up to 20% of children with Kawasaki disease do not respond to treatment or have fever recurrence after initial IVIG treatment, and these patients are at the highest risk of developing coronary artery lesions\(^{2,20,210}\).

The involvement of pro-inflammatory cytokines in the acute phase of Kawasaki disease suggests that combinational therapy, composed of IVIG associated with TNF inhibitors, steroids, calcineurin inhibitors or anakinra, might be useful to treat patients with IVIG-resistant disease. The use of TNF inhibitors in combination with IVIG has had mixed results thus far. Infliximab was associated with decreased fever duration and reduced markers of inflammation (C-reactive protein and neutrophil counts), suggesting a possible improvement of coronary artery outcomes\(^{211}\); however, etanercept treatment
resulted in a substantial reduction in IVIG resistance only in patients > 1 year old\(^\text{217}\).

An important area of research is the use of biomarkers to predict IVIG resistance in Kawasaki disease. The Kobayashi scoring system, based on a combination of laboratory test results (for example, C-reactive protein levels, neutrophil percentages, platelets counts and levels of aspartate and alanine aminotransferase) and demographic variables (sex, age and number of days of illness before the start of the treatment) has been successfully used to predict IVIG-resistance in Japanese patients\(^\text{213}\), but not in North American children with Kawasaki disease\(^\text{214}\). The combination of prednisolone and IVIG to treat Japanese patients with Kawasaki disease predicted to have IVIG-resistant disease according to the Kobayashi score (RAISE study) resulted in more rapid fever resolution, reduced development of CAAs and lower incidence of additional rescue treatment\(^\text{215}\) compared with IVIG alone.

As discussed above, Kawasaki disease susceptibility and increased coronary artery lesion risk are associated with an SNP in \(ITPKC\)^\(^6\) that results in a lack of NFAT regulation and activation of the T cell compartment owing to increased IL-2 production\(^\text{216}\). CD8\(^+\) cytotoxic T cells are present in the inflamed arterial wall during Kawasaki disease\(^\text{213,215}\); therefore, targeting T cell expansion might be an efficient approach to preventing CAAs during Kawasaki disease. A combination treatment of IVIG and ciclosporin, a calcineurin inhibitor that suppresses IL-2 production and T cell activation, was tested in a clinical trial in Japanese patients with Kawasaki disease predicted to have IVIG-resistant disease based on the Kobayashi score (KAICA trial)\(^\text{219}\). In this trial, the combination treatment was shown to be safe and associated with a lower incidence of CAAs; however, treatment was linked with increased risk of relapse\(^\text{217}\). Furthermore, the scoring system used to identify IVIG-non-responders is poorly predictive in European children with Kawasaki disease, limiting the conclusions of this study.

The important role of the IL-1β–IL-1 receptor pathway in Kawasaki disease development has been demonstrated in both human patients\(^\text{27,28,129,130}\) and mouse models\(^\text{227,132,202,204}\). Therefore, clinical trials investigating IL-1 pathway inhibition by using anakinra, which blocks both IL-1α and IL-1β, have been initiated in North America (ANAKID; ClinicalTrials.gov identifier NCT02179853)\(^\text{218}\) and Europe (Kawakinra; European Clinical Trials number 2014-002715-4)\(^\text{219}\). Already, multiple case reports exist of the successful use of anakinra to treat patients with IVIG-resistant Kawasaki disease\(^\text{219–223}\), indicating the promise of this second-line therapy.

**Therapeutic insights from mouse models.** Although no animal model can fully mimic human disease, the LCWE-induced Kawasaki disease mouse model has been accepted by many in the research community as a reliable experimental model providing novel insights that can be tested in patients. For example, IVIG efficiently prevents coronary arteritis development in LCWE-injected mice\(^\text{177}\) as well as in the CAWS mouse model of Kawasaki disease\(^\text{228}\).

The effects of the calcineurin inhibitors ciclosporin and tacrolimus have been investigated in the Nod1 ligand-induced mouse model of Kawasaki disease vasculitis\(^\text{225}\). This approach was rational given the established role of T cells and calcium signalling in Kawasaki disease. However, contrary to the expected outcome, these inhibitors exacerbated the coronary arteritis\(^\text{225}\). Notably, however, this result was probably related to the choice of mouse model, as the Nod1 ligand-mediated mouse model of Kawasaki disease vasculitis has previously been shown to be T cell-independent\(^\text{226}\). Indeed, in an independent study using the CAWS mouse model, which is T cell dependent, ciclosporin suppressed CAWS-induced vasculitis\(^\text{228}\), emphasizing the importance of model selection in preclinical studies. Most importantly, results in human studies bear out the therapeutic potential of calcineurin inhibition, as the Japanese phase III trial (KAICA trial) showed that adding ciclosporin to IVIG in patients with Kawasaki disease who were at high risk of IVIG resistance was beneficial in diminishing overall incidence of CAAs\(^\text{177}\).

The role of TNF has been investigated in both the LCWE and the CAWS mouse models of Kawasaki disease vasculitis\(^\text{185}\). Initially, etanercept treatment or genetic deletion of TNF receptor 1 was shown to protect mice from LCWE-induced coronary arteritis\(^\text{227,228}\). Infliximab treatment also prevented the development of both LCWE-induced coronary arteritis and myocarditis\(^\text{131}\). Similar results were obtained in the CAWS mouse model of Kawasaki disease vasculitis, in which etanercept\(^\text{206,229}\) suppressed the incidence and decreased the severity of vasculitis. Mechanistically, TNF has been proposed to be produced by myeloid cells in the acute phase and to promote myocarditis and recruitment of immune cells by acting on cardiac stromal cells\(^\text{225}\). However, infliximab and etanercept might not directly target the TNF signalling pathway, and their observed effects might be indirect. Indeed, infliximab is not able to bind mouse TNF\(^\text{227,224}\); therefore, the anti-inflammatory effect of infliximab might be attributable to the binding of Fc receptors at the surface of activated cells\(^\text{225,230}\).

The overwhelming evidence for the critical role of IL-1β in promoting LCWE-induced Kawasaki disease vasculitis in mice\(^\text{27,128,132}\) led to the initiation of clinical trials testing the effect of anakinra for blocking IL-1β as a second therapy option to treat children with IVIG-resistant Kawasaki disease. Multiple case reports now outline the successful use of anakinra to treat patients with IVIG-resistant Kawasaki disease\(^\text{221–224}\). Alternatively, direct inhibition of the NLRP3 inflammasome might be a more targeted therapeutic strategy to treat Kawasaki disease, as it would affect several pathways beyond IL-1β, including IL-1α and IL-18. Several NLRP3 inhibitors have been identified\(^\text{231}\) and tested in mouse models of inflammatory diseases, such as experimental autoimmune encephalomyelitis and cryopyrin-associated periodic syndrome\(^\text{232}\). It would be interesting to determine if such drugs could be used to prevent and reduce the cardiovascular complications in mouse models of Kawasaki disease vasculitis.
Conclusions

Over the past 40 years, research has improved our understanding of Kawasaki disease pathology and the development of coronary vasculitis. However, some questions still remain unanswered, such as the identification of the aetiological agents, how the disease is triggered, and the specific immune pathways associated with coronary vasculitis development and IVIG resistance. Owing to the rarity of human tissues from patients with Kawasaki disease, the use of animal models reproducing human Kawasaki disease features is invaluable. Many advances have been made over the decades by combining biological observations in human samples with mechanistic insights from experimental animal models. This 'bench to bedside' approach successfully led to the identification of the critical role of IL-1β in Kawasaki disease and resulted in the development of clinical trials in which anakirina is being used to treat children with IVIG-resistant Kawasaki disease.

LCWE-injected mice exhibit a dysfunctional intestinal barrier, and the increased IgA response and elevated sIgA levels in both LCWE-injected mice and children with Kawasaki disease reveal the existence of a 'gut–vascular' axis. In evaluating this model system and the role of IgA, it should not be forgotten that injection of identically prepared LCWE induces chronic polyarthritis in selected inbred rat strains. This observation implies that a common immunogenetic pathway might underlie a variety of autoimmune illnesses, with disease expression moderated not by the inducing agent, but rather by host genetics. The fact that cell wall fragments of common gut bacteria can produce varying disease manifestations in the face of inflammation-induced increased gut permeability suggests that some autoimmune diseases might not in fact be induced by the normal response to an unusual agent, but rather an unusual response (genetically determined) to a common environmental stimulus. This hypothesis has major implications for understanding the aetiology and pathogenesis of not only Kawasaki disease but also IgA-mediated diseases and perhaps others. In addition, it strongly suggests that inhibition of IL-1β might be effective for the many chronic inflammatory diseases in which IgA deposition is a key finding.

Published online 26 May 2020
Kawasaki disease in the Taiwanese population.

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