Pathogenesis of Kawasaki disease: the central role of TNF-α

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& Rae SM Yeung

Kawasaki disease is the leading cause of multisystem vasculitis in childhood. The coronary arteries are targets of long-term inflammation and damage, making Kawasaki disease the leading cause of acquired heart disease in children from the developed world. The link between the systemic immune response seen in the acute phase of Kawasaki disease and subsequent damage to the coronary arteries is not clearly understood. Recent work points to TNF-α and its downstream effector molecules as the key players in mediating coronary artery damage. In this article, we will review the evidence pointing to TNF-α in the pathogenesis of disease and the implications for therapy.

Kawasaki disease (KD) is an acute, self-limited vasculitis manifested clinically by the cardinal signs of inflammation – rubor (redness), calor (warmth) and tumor (swelling), which are observed as prolonged fever, polymorphous skin rash, nonprurulent conjunctivitis, oral mucosal and extremity changes, and cervical lymphadenopathy, the diagnostic features of KD [1]. The most common age of occurrence of KD is between 18 months and 5 years, although younger children and adults may also be affected [2]. KD is seen in all ethnic groups and in all regions of the world, but the incidence of disease varies dramatically from region to region and between different ethnic groups. The annual incidence, reported as number per 100,000 children aged under 5 years, ranges from five in Denmark [3], eight in New Zealand [4], 30 in Canada [2], 105 in Korea [5] to over 138 in Japan [6]. It is now the leading cause of acquired heart disease in children from the developed world [7]. Children with KD are at risk of coronary artery aneurysms, myocardial infarction, sudden death and early ischemic heart disease. With treatment, the risk for coronary artery aneurysms is reduced from an incidence of 25% to approximately 5%; however, this may represent an underestimation if coronary artery measurements are adjusted for body surface area [8]. Furthermore, there is also a concern of long-term endothelial dysfunction in the blood vessels of children with KD predisposing them to atherosclerotic disease.

Tomisaku Kawasaki first described the clinical signs of KD 40 years ago [9,10]. Over time, much research has focused on identifying an etiologic agent or agents responsible for the disease, with clinical and epidemiologic features of KD sharing commonalities with that of an infectious illness. However, 40 years later, the list of infections associated with KD continues to grow, favoring less the possibility of a single pathogenic organism. KD is associated with many different etiologic agents ranging from bacteria such as Propionibacterium acnes, Staphylococcus, Streptococcus and Chlamydia, to viruses such as Epstein–Barr, parvovirus and retroviruses [11]. Our group found that a third of patients with typical KD had a documented source of infection, both bacterial and viral, supporting an infectious trigger leading to immune activation in this syndrome complex [12]. There have also been reports of KD temporally associated with immunizations [13,101].

Immune activation
Systemic inflammation is the most striking finding in KD. This is evidenced clinically and biochemically during the acute phase of the illness. Like other syndromes characterized by systemic inflammation, TNF-α is markedly elevated in children during the acute phase of KD. TNF-α is a pleiotropic cytokine critical in the regulation of immune cells and plays a critical role in inflammation. TNF-α is a key player in many autoimmune processes, as well as the principle mediator of the acute inflammatory response to infections [14]. TNF-α is produced by many different cell types. In the immune system, it is produced by activated T cells and macrophages [15]. TNF-α has the ability to bind to two receptors, TNFR1 and TNFR2. TNFRs have no metabolic capability; however, they contain several motifs with functional significance. These motifs interact with intracellular proteins to direct signaling within the cell. The end result of signaling is nuclear
translocation of transcription factors, such as NF-κB, leading to increased expression of many different gene products (Figure 1). These include cell proliferation, expression of leukocyte recruitment molecules, production of other pro-inflammatory cytokines, production of acute-phase reactants and increased expression of proteases, including matrix metalloproteinases (MMPs). Thus, TNF-α acts via NF-κB to regulate transcription of downstream effector molecules, accounting for the long list of proinflammatory effects (Table 1).

TNF-α is elevated in children in the acute phase of KD [16,17]. There is now emerging evidence that TNF-α is critical in the pathogenesis of KD and specifically at the level of the target tissue, the coronary artery [18]. This article will highlight recent exciting advances in unraveling the TNF-α-mediated effects that begin with an acute systemic immune response and end in vascular damage. By understanding the critical ‘players’ and sequence of events in this disease process, we can develop a logical approach to our search for novel biomarkers of disease susceptibility and outcome together with the development of tailored therapeutic agents. We will discuss some key downstream effects of TNF-α signaling and how they may relate to the pathogenesis of KD including leukocyte recruitment and upregulation of matrix-degrading enzymes and proinflammatory cytokines. Understanding how these downstream molecules interplay should pave the way for future discoveries in the study of KD.

Leukocyte recruitment
Leukocyte migration is a highly coordinated process. Chemokines, together with adhesion molecules, are the key controllers of leukocyte traffic [19]. The expression of these chemokines and adhesion molecules is in turn regulated by cytokines including TNF-α. The leukocyte migration cascade follows three steps: leukocyte rolling, firm adhesion and transendothelial migration [20,21]. Leukocyte rolling is the initial tethering of leukocytes in the bloodstream to the endothelium, which is mediated by adhesion molecules with chemotaxis directed by both soluble and immobilized chemokines (step 1). Chemokines synthesized at sites of inflammation mediate rapid integrin activation on leukocytes to initiate firm adhesion (step 2) [19,21]. Activated leukocytes adhering

Figure 1. Pleiotropic effect of TNF-α.

MMP: Matrix metalloproteinase.
to the endothelium then elongate and crawl along the endothelial cell (EC) surface to junctions between adjacent ECs (step 3). This is mediated by immobilized chemokines and adhesion molecules, which facilitate extravasation into inflamed tissues [22]. The vascular endothelium and leukocytes act in concert to produce a local inflammatory response [23]. TNF-α plays a critical role in regulating this response via a number of mechanisms, including upregulation of Th1-associated chemokine-chemokine receptor pairs and adhesion molecule-ligand pairs. These recruitment signals are important components in the pathogenesis of target-organ inflammation in autoimmunity [24,25].

Increased expression of chemokines has been demonstrated in KD in humans. Elevated levels of the Th1-associated chemokines MIP-1β, MCP-1 and RANTES were found in children with KD during both the acute and subacute stages of disease [26]. The adhesion molecules ICAM-1 and E-selectin were elevated at KD diagnosis, but after treatment with intravenous immunoglobulin (IVIG), decreased levels of these same adhesion molecules were detected from skin biopsies [27,28]. Evidence from a mouse model of KD also supports the critical role of TNF-α-mediated expression of leukocyte recruitment molecules in the pathogenesis of disease [18]. TNF-α is rapidly produced and translated in the peripheral immune system after disease induction in the animal model. This is followed by local production of TNF-α at the coronary blood vessel wall. Lymphocyte infiltration into the coronary arteries corresponds temporally with TNF-α production in the affected vessels. One of the key findings in this series of experiments was that the inflammatory response was abrogated in the absence of TNF-α activity, both by blocking TNF-α activity using etanercept, or in disease induction in TNFR1-deficient mice. Interestingly, TNFR1-deficient mice continued to show a normal lymphocyte proliferative response to the disease-inducing superantigen, suggesting that TNF-α-dependent functions extrinsic to the lymphocytes are critical to local inflammation. To determine whether TNF-α-mediated lymphocyte trafficking was this extrinsic factor, mRNA expression of important migratory signals was examined in the cardiac tissue of wild-type mice and TNFR1-deficient mice at time points between and after disease induction. TNFR1-deficient mice failed to upregulate expression of Th1-associated leukocyte migration signals (including ICAM-1 and VCAM-1, RANTES and E-selectin) in the heart during disease induction. Thus, TNF-α is the critical mediator of leukocyte recruitment leading to local inflammation in the target tissue.

Proteolysis

Another important function mediated by TNF-α is induction of enzymatic activity, specifically proteolysis. TNF-α upregulates expression and activity of many members of the MMP family of enzymes. MMPs are a family of zinc-dependent extracellular matrix (ECM)-degrading proteases that share the ability to degrade molecules of the ECM. Elastin is an important ECM component in arterial vessel walls. Breakdown of elastin leads to the loss of structural integrity of the vessel wall and ballooning, the hallmark of aneurysm formation [29]. MMPs play an important role in the degradation of elastin leading to aneurysm formation [30,31].

MMPs are produced by inflammatory cells and vascular tissue [29]. MMP levels and activity are controlled at multiple levels: gene transcription, post-translation modifications and by its natural inhibitor, tissue inhibitor of metalloproteinase (TIMP). Upregulation and activation of MMPs is controlled by many factors, one of which is stimulation by TNF-α [32]. Two MMPs in particular, MMP-2 and MMP-9, have been localized to areas of inflammation and internal elastic lamina degradation in aortic aneurysms [29]. In giant-cell

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**Table 1. TNF-α/NFκB-mediated functions present in Kawasaki disease.**

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<th>NFκB-mediated upregulation</th>
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<td>Acute-phase proteins</td>
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<tr>
<td>C-reactive protein</td>
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<td>Chemokines</td>
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<td>RANTES</td>
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<td>Adhesion molecules</td>
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<td>ICAM</td>
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<td>VCAM</td>
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<td>E-selectin</td>
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<td>Proinflammatory cytokines</td>
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<td>IL-1, IL-2, IL-6</td>
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<td>TNF-α</td>
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<td>Enzymes</td>
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arteritis, MMP-9 appears to play an important pathophysiological role in the degradation of elastic tissue, more so than MMP-2 [33]. In fatal cases of human KD, MMP-9 was expressed in coronary artery aneurysms and unaffected cardiac arterial segments, but not in non-KD control coronary vessels, suggesting a role for MMP-9 in the development of aneurysms [34].

In support of this, MMP-9 activity appears to play an integral role in coronary artery aneurysm formation in our disease model. Using the Lactobacillus casei cell wall extract model of KD, we found increased expression and activity of MMP-9 in smooth muscle cells during the development of coronary artery disease. Increased MMP-9 proteolytic activity coincides with the presence of local inflammation and the presence of elastin breakdown. In the absence of MMP-9 activity, there continues to be inflammatory disease at the coronary arteries, but there is a marked reduction in the incidence of vessel wall damage. Thus, inflammation can be dissociated from end organ damage by the ablation of MMP-9, implying that MMP-9 is one of the critical mechanisms responsible for aneurysm formation [35]. Local production of MMP-9 is dependent on TNF-α activity, as both treatment with the TNF-α-blocking agent etanercept and TNFR1-deficient animals have absence of MMP-9 production in the coronary artery. Therefore, MMP-9 expression may represent a downstream mechanistic link between the local inflammatory process orchestrated by TNF-α and the local damage to the vessel wall.

It is important to point out that while MMP-9 enzymatic activity is important at the coronary artery level, circulating levels of MMP-9 activity have no association with those found in the heart in the mouse model of KD [36]. Similarly, there was no correlation when comparing MMP-2 and MMP-9 protein levels at diagnosis in children who went on to have normal coronary arteries with those who went on to develop lesions [37]. This may be explained by tightly controlled enzymatic activity at the tissue level with peripheral levels failing to predict coronary artery damage.

Proinflammatory cytokines
In addition to playing a critical role in orchestrating leukocyte recruitment and upregulating MMP activity, TNF-α plays an important role in modulating expression of other cytokines and regulating programmed cell death. One such cytokine is IL-6. IL-6 is a proinflammatory cytokine that is upregulated by TNF-α and is elevated in children with KD and thought to be responsible for the thrombocytosis seen in KD [16,36]. IL-6 is produced by a variety of cells (monocytes, T and B cells, fibroblasts and endothelial cells) with a wide range of biologic activities, many of which are seen in KD (Table 2) [38].

Predictors of outcome
There is an urgent need to identify children at risk for developing coronary artery aneurysms. Without these determinants of disease, improvements in individual therapy and outcome are difficult. No clinical or laboratory feature allows us to differentiate KD from other febrile exanthems of childhood, nor are we able to predict coronary outcome in affected children. A number of factors have been identified as part of the high-risk phenotype for poor coronary artery outcome: prolonged fever, younger and older age, low or high platelet count, low albumin and unresponsiveness to IVIG [39]. However, these risk factors are imperfect in predicting coronary artery outcome. The search for novel biomarkers for identification of children at risk for development of disease, as well as a predilection for coronary damage, has led to the search for molecules involved in the immune and vascular response. This would ultimately guide clinical decision making regarding the use of potentially efficacious, but often costly and potentially toxic, therapies. In addition, a diagnostic biomarker may be helpful in establishing the diagnosis in incomplete cases of KD.

It may be that conventional methods of assessing serum levels of cytokines or enzymes thought to be involved in the pathogenic process of KD have been disappointing. The explanation may be found in lessons learned from our disease model. Proinflammatory cytokines, including IFN-γ, TNF-α, and IL-6, are produced very early (by 6 h after exposure to the infectious trigger) in

<table>
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<th>Table 2. IL-6-mediated effects present in Kawasaki disease.</th>
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<td><strong>IL-6-mediated effects</strong></td>
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<tr>
<td>Increased acute-phase proteins</td>
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<td>T-cell activation</td>
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the peripheral immune system, and levels may have decreased by the time the child with KD has received their diagnosis at day 5 of fever. In addition, circulating levels of molecules known to act locally, such as MMPs, may not be useful biomarkers of disease. This is especially relevant to enzymatic activity that is tightly regulated at multiple levels, including the local tissue environment. Although matrix-degrading proteolytic activity was specific for affected mice and localized to inflamed coronary artery segments, the enzymatic activity in the systemic circulation of affected and control mice was not different. Similar to affected children, peripheral blood levels of MMP-9 enzymatic activity did not correlate with coronary artery disease in the animal model [37]. Biologic processes give rise to cascades of potential biomarker fragments from enzyme-generated proteolytic activity. Some of these low-molecular-weight molecules are below the range of detection by conventional techniques requiring mass spectroscopy for their detection [40]. While MMP-9 has not been found to be useful as a biomarker, perhaps early detection of some circulating proteolytic breakdown product could identify the child requiring more intensive therapeutic interventions. While continuing to study KD and search for biomarkers using traditional methods, it is exciting to consider implementing new tools such as molecular imaging and searching for gene variants to address the gap in knowledge of determinants of outcome.

Therapy
Production of TNF-α and subsequent TNF-α-dependent effector functions are central to the pathogenesis of KD. How do our current therapeutic agents perform when measured in this light? The standard of therapy is IVIG and high-dose aspirin in acute KD. The clinical usefulness of IVIG in KD is well established, with its ability to reduce the risk of development of coronary artery aneurysms [41]. IVIG has been used in the treatment of numerous inflammatory and autoimmune diseases, with an extensive list of reported mechanisms of action. Its broad range of activities include modulation of expression and function of Fc receptors, interference with the activation of complement and the cytokine network, provision of anti-idiotypic antibodies, and effects on the activation, differentiation and effector functions of T and B cells [42]. From a theoretical perspective, one can see how IVIG’s numerous effects could dampen the immune response in KD. In vitro it has been shown that IVIG prevents a rapid increase in TNF-α and IL-6 in lipopolysaccharide-stimulated cells, and in vivo a rapid reduction in IL-6 occurred after IVIG therapy in children with KD, with rapid resolution of acute-phase symptoms [43]. Recently, we have demonstrated a dose-dependent inhibition of superantigen-activated T-cell proliferation and TNF-α production with IVIG [44]. The inhibitory activity stops at TNF-α, as IVIG had no effect on modulation of MMP-9 expression or enzymatic activity [Yeung RSM et al. Unpublished Data].

The role of aspirin in preventing coronary artery aneurysms is less clear [44]. However, mechanistically there is a theoretical reason why aspirin might be of benefit in KD. Aspirin, in high concentrations, has the ability to bind IKK-β, an enzyme important in allowing NF-κB to translocate to the nucleus. This disrupts NF-κB’s ability to regulate expression of pro-inflammatory cytokines and other mediators. Moreover, it was shown that the inhibitory concentration (IC50) of aspirin to achieve these effects was higher than the IC50 needed to achieve prostaglandin inhibition, pointing to a potential mechanism-of-action of high-dose aspirin in KD [45]. Some patients, such as those refractory to IVIG or those with evidence of congestive heart failure related to myocarditis, may receive corticosteroids [46]. Corticosteroids exert anti-inflammatory effects via a number of pathways, including inhibition of NF-κB nuclear translocation [42]. In refractory patients, corticosteroids are effective in treating the fever and associated acute features of KD, but their effects on coronary outcome are still uncertain [46].

TNF-α antagonists have recently been used in the treatment of KD. The largest case series reported using infliximab in 16 children (≥10 days into illness) who remained febrile or had persistent arthritis after IVIG and high-dose aspirin [47]. In most, cessation of fever occurred quite dramatically following infliximab treatment, as well as a reduction in C-reactive protein. A high proportion of these patients had coronary abnormalities even prior to instituting anti-TNF-α treatment. The abrupt resolution of fever in these children supports the central role of TNF-α in mediating the inflammatory response during evolution of KD. While disrupting the effects of TNF-α in our disease model very early in disease prevents aneurysms, the risks and benefits of using this powerful biologic agent in the face of an infectious trigger is not clear. In refractory disease, where the immune response is the culprit and not the initial infectious agent, the
benefits appear to outweigh the risks, but in acute disease, where the incidence of concurrent infections is high [12], caution needs to be exercised and the benefit may not be as clear. Although anti-TNF treatment is more directed, this approach (as with IVIG, aspirin and corticosteroids), targets the early molecular players in the pathogenesis of KD.

As more is learned regarding the role of downstream effector molecules in KD, we can direct therapy towards these molecules, perhaps in addition to blocking early triggers of the inflammatory cascade. Furthermore, this approach seems necessary given that, by the time KD is diagnosed in children, the immune activation is well on the way. Although there is a beneficial effect of IVIG, a significant number of children continue to develop coronary artery abnormalities. MMP-9 is one potential therapeutic target. Blocking transcription, proenzyme activation or active-site-directed inhibition are all possible approaches for new therapeutic interventions. For example, in Japan, ulinastatin, a neutrophil elastase inhibitor, is used as a second-line agent in recalcitrant KD. It was recently compared with IVIG for primary therapy of KD. Although ulinastatin appeared inferior to standard IVIG therapy and thus is not recommended as first-line therapy, the principle of protease inhibition may be a fruitful approach to the treatment of KD [48].

Conclusion

TNF-α and subsequent TNF-α-dependent effector functions are critical in the pathogenesis of KD. Further understanding of the downstream effects of TNF-α is central to discovering potential biomarkers that can predict coronary outcome as well as in guiding the development of new treatments.

Future perspective

One critical issue that continues to elude improvements in the care of children with KD is the absence of reliable predictors of coronary outcome. Clinical and laboratory markers have proven insufficient thus far. Central to the quest to identify novel biomarkers is an improved understanding of the immunopathogenesis of KD. TNF-α plays a critical role in orchestrating the inflammatory response in KD (Figure 2). Dissecting TNF-α-mediated effector functions will help us understand the mechanisms linking inflammation and coronary artery damage. These downstream molecules may themselves serve as good biomarkers predictive of coronary outcome; however, as we have learned, they may be non-specific, or their levels in the peripheral circulation may poorly reflect biologic activity in the heart. Genetic variations in these molecules may be helpful in discovering high-risk individuals and furthering our understanding of pathogenesis. Breakdown products of tissue damage, such as elastin, may provide a clue to identifying children at risk for development of aneurysms at a time when intervention is still possible. New imaging modalities, such as molecular imaging, may aid in directly visualizing pathogenic events occurring in the heart. The ultimate goal is to improve the outcome in children affected with KD.

Financial & competing interests disclosure

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Executive summary

TNF-α plays a critical role in the pathogenesis of Kawasaki disease

- TNF-α is rapidly produced in the peripheral circulation in Kawasaki disease, followed by localization to the coronary arteries.
- TNF-α-mediated leukocyte recruitment is necessary for coronary artery inflammation.
- TNF-α upregulates MMP-9, which in turn plays a critical role in elastin breakdown and aneurysm formation, providing a link between the inflammation and vessel wall damage.
- Infliximab has been used successfully in patients with refractory Kawasaki disease.

Predictors of coronary outcome are needed

- Clinical and laboratory features fail to adequately identify patients at increased risk of poor coronary outcome.

Future perspective

- Novel molecular imaging modalities may facilitate visualization of pathogenic events at the coronary artery, bypassing the need to sample peripheral blood.
- Pathogenic molecules will be targeted for specific inhibition.
- Genetic variants controlling expression of pathogenic molecules may be candidate biomarkers, providing the ability to tailor treatment based on genetic risk of poor coronary outcome.
Pathogenesis of Kawasaki disease: the central role of TNF-α – REVIEW

Figure 2. Pathogenic process in Kawasaki disease.

ASA: Aspirin; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; IVIG: Intravenous immunoglobulin; KD: Kawasaki disease.

ASA: Aspirin; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; IVIG: Intravenous immunoglobulin; KD: Kawasaki disease.

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Papers of special note have been highlighted as either of interest (+) or of considerable interest (+++) to readers.


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