Lessons learned from an animal model of Kawasaki disease

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ABSTRACT

Kawasaki disease is the most common cause of multisystem vasculitis in childhood. Kawasaki disease has been reported throughout the world and affects children of all ethnicity. Coronary artery damage from Kawasaki disease is the leading cause of acquired heart disease in children in the developed world. Diagnostic tests and prognostic markers are lacking, and questions remain unanswered in our understanding of the etiopathogenesis of the disease, thus limiting our ability to improve therapy and coronary outcome. In this article I will review advances made in an animal model of disease, which has helped advance our understanding of the etiology and pathogenesis of this fascinating clinical syndrome.

Introduction

Kawasaki disease (KD), like other idiopathic inflammatory diseases, is a syndrome complex defined by a set of clinical criteria. KD is characterized by multi-system inflammation, presenting as prolonged fever usually greater than 5 days in duration, a polymorphous skin rash, non-exudative bilateral conjunctival injection, oral mucosal inflammation, extremity changes which include redness of the palms and soles and swelling of the dorsum of the hands and feet, and cervical lymphadenopathy typically unilateral and greater than 1.5 cm in diameter. Presence of fever plus 4 out 5 of these clinical features constitutes the diagnosis of typical KD (1, 2). The most common age of occurrence of KD is between 18 months and 5 years although younger children and adults can also be affected. 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.

One of the challenges facing physicians treating children affected with KD, is identification of the child at risk for coronary artery aneurysms. Appropriate early treatment with high dose intravenous immunoglobulin (IVIG) and aspirin still results in coronary artery aneurysms in 5% of affected children. This number increases to 20-25% when corrected for body surface area (3, 4). Determining the risk factors associated with poor coronary outcome have identified clinical and laboratory predictors and the search is on for more robust biomarkers of disease activity. Studies investigating the contribution of clinical phenotype have identified duration of fever as the most powerful predictor of poor coronary outcome (2). Duration of fever may be an indirect measure of the severity of the underlying vascular inflammation. Other surrogate markers of inflammation including platelet count, serum albumin level, and failure to respond to IVIG therapy, are all associated with fever duration, and have been identified as high risk factors for development of coronary artery aneurysms (5). Many obstacles are in the path of finding good prognostic markers. Patient samples are usually taken after the pathogenic process has been occurring for over 5 days, as prolonged fever is one of the diagnostic criteria for KD. Additionally, affected coronary arteries are not readily accessible and the limited number of autopsy samples available represent the very severe end of the disease spectrum. For these and other reasons, an accurate animal model of the disease has been developed to address these questions. Descriptive studies done in children combined with mechanistic studies done in animal models have furthered our understanding of the molecules involved in disease activity leading to vascular damage.

Animal model of KD

Strains of inbred laboratory mice develop coronary arteritis in response to intraperitoneal injections of lactobacillus casei cell wall extract (LCWE). The resultant vascular inflammation is similar to KD in children (6). They share identical histological changes and a similar time course to coronary artery disease. Young mice are much more susceptible to LCWE induced coronary disease compared to older mice, similar to the human disease. Systemic immune activation within hours of disease induction is followed by immune infiltration into cardiac tissue starting at day 3 (6). This inflammatory infiltrate, composed mainly of T-cells intensifies

and peaks at day 28-post injection and is accompanied by elastin breakdown, the hallmark of aneurysm formation at day 42 (7-8), corresponding to the sub-acute phase in childhood KD when coronary artery lesions are maximally seen. Additionally the vascular lesions are responsive to IVIG the same therapeutic agent effective in children.

Etiology of KD

KD has been associated with the many different etiologic agents including both bacteria and viruses. No one causative agent has been consistently demonstrated. Interestingly KD is an endemic disease with interspersed epidemics. The clinical phenotype may be outbreak dependent and contribute to different results from different investigators at different times from different locales (5). KD fits in the spectrum between an infectious disease and a true autoimmune disease with an infectious trigger leading to a prolonged self directed immune response. The longer the search for a single infectious agent the longer the list of diverse infectious organisms identified. Superantigens are a shared property common to multiple infectious agents and may count for many of the features and the many etiologic agents isolated from different KD outbreaks. Superantigens are a group of proteins which share the ability to stimulate a large portion of T-cells compared to a peptide antigen which activates approximately one in a million T cells. T-cell recognition of superantigens does not occur with lock and key specificity like conventional peptide antigens, but the molecular interaction occurs outside of the peptide binding pockets of the TCR, and is specific to a non-variable part of the TCRVβ chain. Since superantigens can bind to several TCRVβ families and humans only have 20-50 TCRVβ families, up to 30% of the entire T-cell repertoire can be activated by a superantigen, initiating a massive immune response with a resultant skewing of the TCRVβ repertoire. Superantigens have been identified in a wide variety of microorganisms including: *Staphylococci, Streptococci, Mycobacteria, Mycoplasma, Yersinia,* EBV and retroviruses, a list similar to the etiologic agents identified in KD outbreaks. (9) We have identified a novel superantigen within LCWE responsible for induction of coronary artery disease in our model of KD (6).

The immune response leading to coronary arteritis in our model of KD is typical for bacterial superantigens. Immune activation after LCWE is Th1 skewed, with rapid production of IFN-γ in the peripheral lymphoid tissue followed by migration locally to the site of end organ damage in the coronary arteries (7). IFNγ is produced de nouveau and detected within the affected coronary vessel wall in a bi-phasic manner, with the first peak coincident with the appearance of the inflammatory infiltrate at day 3 and the second peak occurs at day 42, corresponding to a time of elastin breakdown and aneurysm formation (7). The role of IFN-γ in models of inflammatory disease has been controversial with both pro- and anti-inflammatory/regulatory roles identified. In our model of KD, IFNγ played an important regulatory function, modulating the immune response during disease evolution (7).

Superantigen stimulation also leads to production of TNFα, a key pro-inflammatory cytokine. TNFα and IFN-γ work syngergistically potentiating each other's cellular responses in particular those involved in leukocyte-endothelial interactions and cell cycling. There is rapid production of TNFα in the peripheral immune system after disease induction by LCWE. This immune response becomes site directed with migration to the coronary arteries. (10) Similar to IFNγ, the kinetics of production of TNFα in the heart is coincident with the presence of inflammatory infiltrate at the coronary arteries and persist during the development of aneurysms. But conversely, inflammation and elastin breakdown in the coronary vessels are completely eliminated in the absence of TNFα effector functions. Mice treated with the TNFα blocking agent etanercept, are completely resistant to the development of coronary artery inflammation and elastin breakdown. These TNFα-dependent events are mediated by TNF Receptor (TNFR) 1. Knockout mice with absence of TNFR1 are completely resistant to disease and have the same phenotype as the etanercept-treated wild type mice. In contrast, TNFR2 knock-out mice continue to develop the same incidence of inflammation and elastin breakdown compared to wild-type mice. One of the critical TNFR1 mediated events during development of disease is up-regulation of leukocyte recruitment molecules (10).

Other important TNFα mediated events include regulation of cell cycling and expression of proteolytic enzymes. Unlike the pathogenic process in abdominal aortic aneurysms where apoptosis of smooth muscle cells and extracellular matrix degradation are important pathogenic events, only proteolytic activity appears to contribute to aneurysm formation in our model of KD. Proteolytic activity is markedly elevated, localized and specific, found only around coronary arteries with inflammation (12). In particular, elastolytic matrix metalloproteinases (MMP) have been implicated as the main culprits in arterial aneurysm formation. Although IFNγ and TNFα usually potentiate each other's actions, they work divergently in the regulation of MMP-9, an elastolytic protease. In the absence of MMP-9 activity there continues to be inflammation in the affected coronary artery, but a marked reduction in the incidence of elastin breakdown, dissociating inflammation from vascular damage. Thus, MMP-9 appears to be one of the critical downstream effects of TNFα signaling responsible for aneurysm formation (13).

There is conflicting data on serum levels of MMP-9 in KD patients despite the fact that cases of fatal acute KD have prominent expression of MMP-9 at the site of coronary artery aneurysms. (14) We found no relationship between the serum levels or enzymatic activity of MMP-9 and coronary outcome in affected children (12). Despite evidence supporting the importance of MMP-9 in local proteolytic activity and development of coronary lesions of KD, this local activity is not reflected in the systemic circulation. This may be due, in part, to the tight regula-

tion of MMP activity at the tissue level. When we examined enzymatic activity in the murine model of KD, there was marked enzymatic activity detected by in situ zymography localized to areas surrounding affected coronary arteries. This activity was extremely specific as myocardium from the identical LCWE injected animal did not display any evidence of enzymatic digestion nor did saline injected control animals. Additionally, there was no difference between serum levels of MMP-9 protein or enzymatic activity in mice with or without coronary disease. These results indicated that elevated matrix degrading enzymatic activity is highly localized and specific for disease, found only in affected regions of the heart. Although MMP-9 may be very important pathogenically, circulating peripheral blood protein levels and their corresponding enzymatic activity had no relationship with coronary artery damage (12).

How can we translate some of the lessons learned from the animal model of KD to help us predict coronary outcome in affected children? IFNγ and TNFa play important but divergent roles in the inflammatory response leading to aneurysm formation, TNFα- proinflammatory, and IFNγ - regulatory. They are both non-specific and produced rapidly (hours) and gone rapidly from the peripheral circulation, well before day 5 when most physicians consider the diagnosis of KD, thus not helpful for diagnostic or prognostic purposes. Similarly, circulating levels of MMP-9 may not be useful biomarkers of disease. This is especially relevant to enzymatic activity that is tightly regulated in the local tissue environment.

Understanding the pathogenesis of aneurysm formation can lead to identification and targeting of the pathogenic molecules. TNFα has been identified as a critical pro-inflammatory cytokine responsible for leukocyte recruitment and elastolysis. Blocking TNFα by administration of etaneracept or abolishing TNFα functions by terminating TNFR1 signaling results in complete resistance to both inflammation and elastin breakdown in the coronary arteries of affected animals. TNFα blocking agents are already part of the therapeutical arsenal combating life threatening primary vascular inflammation. Our results suggest that blocking TNFα or its downstream functions such as leukocyte recruitment or matrix degradation may be exciting new options in the battle against coronary artery damage in children with KD. In fact, TNFα blocking agents have been used for rescue therapy in children with recalcitrant or persistent disease failing conventional therapy (15). Children who fail IVIG therapy may be a special subgroup who may benefit from TNF blockade where persistent inflammation is the primary concern and not the infectious trigger. Concurrent infection is present in 33% of children at KD diagnosis thus prohibiting TNF blockade (4). Inhibition of proteolytic activity, specifically elastolysis is also an area of interest. Current therapeutic agents used for children with KD, including IVIG and high-dose aspirin, have both been reported to inhibit MMP-9 expression and enzymatic activity. Administration of ulinastatin, an elastase inhibitor, has been used with success as a second line agent for the treatment of recalcitrant KD in Japan (16). This agent also inhibits MMP-9. Lessons learned from our animal model hold promise for exciting new directions in understanding and managing children with KD.

References

1. KAWASAKI, T, KOSAKI F, OKAWA S: A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. *Pediatrics* 1974; 54: 271-6.
2. 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.
3. DE ZORZI A, COLAN SD, GAUVREAU K, BAKER AL, SUNDEL RP, NEWBURGER JW: Coronary artery dimensions may be misclassified as normal in Kawasaki disease. *J Pediatr* 1998; 133: 254-8.
4. BENSELER SM, MCCRINDLE BW, SILVERMAN ED, TYRRELL PN, WONG J, YEUNG RS: Infections and Kawasaki disease: implications for coronary artery outcome. *Pediatrics* 2005; 116: e760-6.
5. YEUNG RS: Phenotype and coronary outcome in Kawasaki's disease. *Lancet* 2007; 369: 85-7.
6. DUONG TT, SILVERMAN ED, BISSESSAR MV, YEUNG RM: Superantigenic activity is responsible for induction of coronary arteritis in mice: an animal model of Kawasaki disease. *International Immunology* 2003; 15: 79-89.
7. CHAN WC, DUONG TT, YEUNG RS: Presence of IFN-gamma does not indicate its necessity for induction of coronary arteritis in an animal model of Kawasaki disease. *J Immunol* 2004: 173: 3492-503.
8. 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.
9. YEUNG RSM: The Etiology of Kawasaki Disease - A Superantigen-Mediated Process. *Progress in Pediatric Cardiology* 2004; 19: 109-13.
10. HUI-YUEN J, DUONG T, YEUNG R: TNFalpha is Necessary for the Induction of Coronary Arteritis in an Animal Model of Kawasaki Disease. *Arthritis Rheum* 2003; 48: 515.
11. LAU A, DUONG T, CHRISTIE E, HO S, YEUNG R: Matrix Metalloproteinase-9-mediated extracellular matrix degradation leads to coronary artery aneurysm formation in a model of Kawasaki Disease. *Arthritis Rheum* 2005; 52: 223-4.
12. LAU A, ROSENBERG H, DUONGT T, MCCRINDLE BW, YEUNG RSM: Elastolytic Matrix Metalloproteinases and Coronary Outcome in Children with Kawasaki Disease. *Pediatric Research* In Press.
13. LAU A, DUONG T, CHRISTIE E, ITO S, YEUNG R: Matrix Mettaloproteinases-9 activity leads to coronary artery aneurysm formation in a model of Kawasaki disease. *Ann Rheum Dis* 2006; 65: 442.
14. GAVIN PJ, CRAWFORD SE, SHULMAN ST, GARCIA FL, ROWLEY AH: Systemic arterial expression of matrix metalloproteinases 2 and 9 in acute Kawasaki disease. *Arterioscler Thromb Vasc Biol* 2003; 23: 576-81.
15. WEISS JE, EBERHARD BA, CHOWDHURY D, GOTTLIEB BS: Infliximab as a novel therapy for refractory Kawasaki disease. *J Rheumatol* 2004; 31: 808-10.
16. ZAITSU M, HAMASAKI Y, TASHIRO K *et al*.: Ulinastatin, an elastase inhibitor, inhibits the increased mRNA expression of prostaglandin H2 synthase-type 2 in Kawasaki disease. *The Journal of infectious diseases* 2000; 181: 1101-09.