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Kawasaki Disease: Laboratory Findings and an Immunopathogenesis on the Premise of a "Protein Homeostasis System"

Kyung-Yil Lee, Jung-Woo Rhim, and Jin-Han Kang

Department of Pediatrics, College of Medicine, The Catholic University of Korea, Seoul, Korea.

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Corresponding author: Dr. Kyung-Yil Lee,
Department of Pediatrics,
The Catholic University of Korea,
Daejeon St. Mary's Hospital,
64 Daeheung-ro, Jung-gu,
Daejeon 301-723, Korea.
Tel: 82-42-220-9541, Fax: 82-42-221-2925
E-mail: leekyungyil@catholic.ac.kr

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Kawasaki disease (KD) is a self-limited systemic inflammatory illness, and coronary artery lesions (CALs) are a major complication determining the prognosis of the disease. Epidemiologic studies in Asian children suggest that the etiologic agent(s) of KD may be associated with environmental changes. Laboratory findings are useful for the diagnosis of incomplete KD, and they can guide the next-step in treatment of initial intravenous immunoglobulin non-responders. CALs seem to develop in the early stages of the disease before a peak in inflammation. Therefore early treatment, before the peak in inflammation, is mandatory to reduce the risk of CAL progression and severity of CALs. The immunopathogenesis of KD is more likely that of acute rheumatic fever than scarlet fever. A hypothetical pathogenesis of KD is proposed under the premise of a "protein homeostasis system"; where innate and adaptive immune cells control pathogenic proteins that are toxic to host cells at a molecular level. After an infection of unknown KD pathogen(s), the pathogenic proteins produced from an unknown focus, spread and bind to endothelial cells of coronary arteries as main target cells. To control the action of pathogenic proteins and/or substances from the injured cells, immune cells are activated. Initially, non-specific T cells and non-specific antibodies are involved in this reaction, while hyperactivated immune cells produce various cytokines, leading to a cytokine imbalance associated with further endothelial cell injury. After the emergence of specific T cells and specific antibodies against the pathogenic proteins, tissue injury ceases and a repair reaction begins with the immune cells.

Key Words: Kawasaki disease, coronary artery lesions, laboratory parameters, intravenous immunoglobulin, non-responders, pathogenesis, treatment

INTRODUCTION

Kawasaki disease (KD) is a self-limiting systemic inflammatory disease that occurs predominantly in children younger than 5 years of age.[1] Clinical manifestations of KD include prolonged fever (1-2 weeks, mean 10-11 days), conjunctival injection, oral lesions, polymorphous skin rashes, extremity changes, and cervical lymphadenopathy, all of which comprise diagnostic criteria. In addition, arthritis,

aseptic meningitis, anterior uveitis, gall bladder hydrops, urethritis and lung involvement can be seen.[2] Some more severely affected patients show cardiac complications, particularly coronary artery lesions (CALs) such as aneurysms and ectasias, which develop in approximately one quarter of untreated children and 5-10% of intravenous immunoglobulin (IVIG) treated children.[3,4] These diverse systemic inflammations (mainly vasculitis) may be caused by inflammatory mediators with circulating immune cells (neutrophils, lymphocytes, natural killer cells and monocytes), and there may be various immune cell infiltrations in all affected pathologic lesions from affected lymph nodes to skin rashes. Particularly, a larger number of T cells (more CD8 cells than CD4 cells), large mononuclear cells, macrophages and plasma cells, with a smaller number of neutrophils, are observed in various organ tissues of fatal cases of acute KD.[5-8] In addition, peripheral blood analysis of acute KD patients showed T lymphocytopenia with depressed CD8 T cells, increased activated CD4 T cells and depressed CD4+CD25+ regulatory T cells.[9-11] These findings suggest that the majority of circulating T cells move to the pathologic lesions of various tissues in acute KD. Therefore, circulating immune cells, especially T cells, control the inflammation of the majority of the affected regions of KD patients without sequelae, but they also may be involved in the progression of the disease, such as in the case of CALs. Epidemiological and clinical data suggest that KD is an immunological reaction to infectious triggers occurring in genetically susceptible children. Although studies have provided hypothetical explanations for the pathogenesis of KD, the etiologic agents, the immunopathogenesis of the vasculitis, and the mechanism that underlies the predilection for coronary artery involvement in KD are largely unknown.[2,11-16]

Laboratory parameters are used for the diagnosis and evaluation of conditions of patients for any inflammatory disease. As for laboratory findings in KD, many inflammatory indices change throughout the disease process; elevated levels of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), leukocyte count with neutrophilia (lymphopenia), platelet count, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and other inflammation associated enzymes, as well as decreased levels of lymphocytes, albumin, hemoglobin, sodium, potassium and total cholesterol including high density lipoprotein cholesterol (HDL-cholesterol) have been detected.[2] The severity of inflammation in KD is reflected by inflammatory parameters; thus, laboratory findings are helpful for diagnosing incomplete KD and evaluating patients for early prediction of IVIG non-responsiveness. Although some score systems for early detection of IVIG non-responders with a higher risk of CALs have been developed,[17,18] further studies are needed for the early detection and proper treatment of initial IVIG non-responders.

In this article, a brief review of the epidemiologic, clinical and laboratory characteristics of KD, as well as the policy of our institution for initial IVIG non-responders according to the changes in laboratory findings after IVIG infusion are introduced. We also propose a new concept for the immunopathogenesis for KD under the premise of a "protein homeostasis system" of the host.

EPIDEMIOLOGIC AND ETIOLOGIC ASPECTS OF KAWASAKI DISEASE

Since KD was first seen in the early 1960's in Japan,[1] KD has been recognized worldwide. Epidemiological studies in Far East Asian countries including Japan, Korea, Taiwan and China have revealed that KD is a new disease with similar epidemiologic patterns in these countries; after the initial appearance of KD, the incidence of KD showed a gradual increase for a decade up to a nationwide occurrence (becoming an endemic disease), and then KD occurred everywhere with relatively constant but slowly growing rates.[19-21] The appearance and subsequent incidence of KD may be associated with the time of industrialization and westernization of these countries.[13] Therefore, it could be postulated that in the past, KD (infantile polyarteritis nodosa) might have appeared in Western countries around the beginning of the 20th century. Throughout the past decade, KD has appeared in rapidly modernizing countries, including India, where KD is more prevalent in modernized cities of economically higher income.[22] Environmental factors such as improved public hygiene or westernized lifestyles may be associated with the emergence and establishment of KD. In South Korea, although yearly incidence rates show no remarkable spatial (geographical) and temporal (seasonal) differences,[19] recently, clinical features of KD seem to be changing to milder phenotypes with greater numbers of incomplete KD cases.[23] In children of other ethnicities, it was also reported that the clinical features of a disease in an outbreak seem to differ from previous outbreaks.[24,25] These findings suggest that the etiology of KD may not be due to a single agent and raise

the possibility of changing epidemiological and clinical features, including treatment response highlighting the need for enhanced surveillance.

KD has an age predilection for children from 6 months of age to 5 years of age, with a peak incidence in children between 6 and 24 months old. This trait has not changed since the emergence of KD and is reflected in all ethnic groups. This disposition suggests that the maturing immune system in early childhood is involved in the pathogenesis of KD.[13] In general, acute infectious diseases affect children of all ages, although the phenotypes of some infectious diseases are age-dependent. In viral infections such as hepatitis A virus, coronavirus (SARS) and influenza virus as well as in mycoplasma infections, younger children (<5 years of age) show less severe clinical symptoms and signs, compared to older children (>5 years of age) and adults.[26-29] On the contrary, in bacterial infections such as staphylococcal pneumonia and tuberculosis as well as in parasitic infections such as malaria, younger children have a more severe clinical course.[30,31] In KD, older children had prolonged fever and a higher incidence of CALs compared to younger children.[32,33] In addition, there are early childhood immune-mediated hematologic disorders, showing a similar age restriction as that of KD, including transient erythroblastopenia of childhood, autoimmune neutropenia of infancy, childhood immune thrombocytopenic purpura, and transient hypogammaglobulinemia of infancy. These disorders may be associated with infectious insults and have a self-limited clinical nature improving before 5 years of age. These findings suggest that the age of around 5 years old in childhood is the turning point of immune maturation of the host. KD may be regarded as among these disease categories, reflecting immunologic immaturity during early childhood, although KD has an acute immune disturbance.

KD also has a predilection for certain ethnic groups. Asian children, particularly those of Japanese, Korean and Chinese ethnicity have the highest incidence of >10-15 times greater than Caucasians, although KD has been reported in all ethnic groups.[19-21,34] The incidence for Japanese-Americans living in Hawaii is similar to the incidence for Japanese living in Japan, supporting a genetic predisposition for KD.[35] With the development of the study of human genetics and genomics, molecular genetics has become an area of interest for the study of KD. Many genetic studies in different countries have evaluated variants in candidate KD genes, and a number of variants, including inositol 1,4,5 triphosphate 3-kinase (*ITPKC*) and caspase-3, have been implicated.[15,36] Recently as a more precise genetic tool, a genome-wide linkage analysis and follow-up association study (genome-wide associated study) for the potential loci and genes of susceptibility to KD have also been performed in several countries.[37-40] The results of the genetic studies may have some limitations resulting from small sample size, failure to replicate results in subsequent studies, and lack of precise phenotype of KD patients based on CAL outcomes and response to IVIG therapy.[36,41] In Japan, Onouchi, et al.[37,42] reported that the *ITPKC* gene which is a negative regulator of T-cell activation was associated with KD susceptibility and an increased risk of CALs. Although the association of the *ITPKC* gene in replication studies of other populations is controversial,[39,40,43] this finding suggests a relevant clue for the genetic study of KD in which immune reaction of T cells may have a crucial role in the immunopathogenesis of the disease. Recently, the International Kawasaki Disease Genetics Consortium has been organized and has identified many candidate genes potentially related to inflammation, apoptosis and cardiovascular pathology.[38,41] Although susceptibility to KD is polygenic, further studies are necessary to determine relationships between the candidate genes and functional consequences that lead to KD or CALs.

The etiology of KD still remains unknown, despite great efforts to identify the cause for nearly a half a century. The epidemiological characteristics of KD are so unique that it is difficult to find a similar model among acute infectious disease, including newly introduced infectious diseases such as retrovirus infections (acquired immunodeficiency syndrome) or conventional infectious diseases. Although many putative bacterial agents including superantigen producing bacteria, viral agents such as Epstein-Barr virus, retroviruses and coronaviruses, and other agents have been suggested, there was no proven single agent for KD.[44-50] Given the epidemiological and clinical characteristics of KD, we previously postulated that etiologic agents were variants of normal flora produced by environmental changes.[13] The microscopic structures and genomic materials between a pathogen and its related flora are nearly identical except for tiny genetic variations, and some pathogens can change to normal flora after infection in a host. It has been reported that the intestinal microflora in infants are different according to ethnic groups and environments.[51,52] Therefore, environment factors and possibly genetic factors can affect the distribution of microflora and induce the variants of normal flora.

LABORATORY PARAMETERS IN KD

The severity of systemic inflammation in KD varies, resulting in different clinical phenotypes and changes of laboratory parameters among the affected children. A large number of patients have a mild clinical course with shortened fever duration and no CALs, but some severely affected patients show prolonged fever duration of up to 2-3 weeks, multiple coronary artery aneurysms and even death. Laboratory findings reflect the severity of systemic inflammation in KD, with concurrent increase or decrease of laboratory values. To understand the natural course of KD and the changes of laboratory indices during the febrile period is very important for the diagnosis, proper treatment and evaluation of KD patients.

KD is a self-limiting disease. The total duration of fever in the era of non-IVIGs is 1-2 weeks (mean 10-11 days), regardless of treatment with aspirin or corticosteroids.[1,53,54] Therefore, a patient who is expected to have a total fever duration of 11 days reaches a peak in inflammatory processes at the sixth day after fever onset, if the periods of ascent to and regression from the peak are similar (Fig. 1). We previously evaluated the inflammatory indices in KD patients according to the fever duration at presentation. Indeed, the levels of white blood cells (WBC) and neutrophil counts and CRP levels were highest, while the albumin and HDL-cholesterol levels were lowest, at the sixth day, and these indices showed a bell-shaped or U-shaped distribution trend based on these values at the sixth day (the peak) (Fig. 2).[55] These findings suggest that the inflammatory processes of KD progress to a peak, then regress to a convalescent stage during the febrile period. It also suggests that in cases of CALs, the immune cells involved in tissue destruction (endothelial damage) have a predominant role in the early stages and the immune cells involved in tissue repair have a predominant role in the late stages of KD, and the results are reflected by laboratory findings. The transaminases (AST and ALT) appear to be higher in the early days, and markedly decrease after the peak stage of KD. Total cholesterol level may be the lowest in the early days and tends to increase along with the natural course of inflammation. Platelet count is well known to increase in convalescent stages of KD. However, platelet count may begin to increase at the peak stage of KD, suggesting involvement in tissue repair (Fig. 2). These findings may be useful for evaluation of the severity of patients who have the highest risk of CALs. For example, a severely affected patient who is persistently febrile for a week, showing constant low levels of platelet with high levels of AST and ALT in follow-up examination, may still not have reached the peak stage of inflammation, suggesting a higher possibility of more severe CALs.

Laboratory findings are now mandatory for diagnosis of incomplete KD.[2] Patients who do not fulfill conventional diagnostic criteria have been diagnosed as having incomplete KD, with <4 of the principal clinical features of KD.

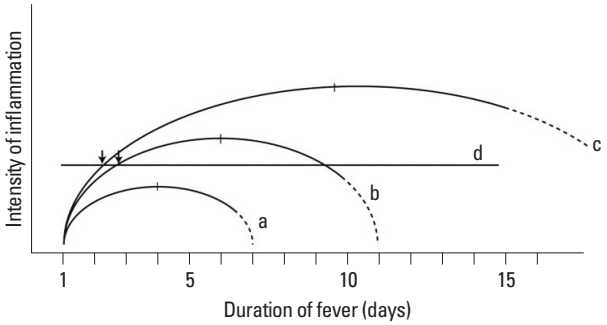

Fig. 1. Inflammatory intensities of KD patients during febrile periods. a, b and c: Inflammatory intensities of the mildly affected patients (a), moderately affected patients (the average of the patients) (b), and the severely affected patients (c). d: An imaginary threshold line of coronary artery lesions. The severely affected patients reach the threshold line earlier, and before the peak in inflammation. KD, Kawasaki disease.

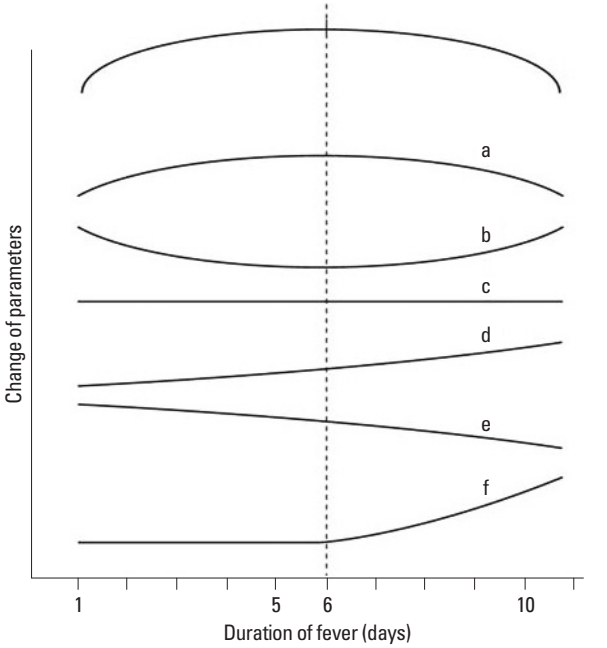

Fig. 2. Suspected patterns in the change of laboratory parameters during febrile period. a: bell shaped pattern; CRP, WBC, neutrophil and CPK, b: U shaped pattern; albumin and HDL-cholesterol, c: little changes; ESR and LDH, d: Steadily decrease; AST, ALT, e: steadily increase: total cholesterol, f: increase after the peak; platelet. CRP, C-reactive protein; WBC, white blood cells; CPK, creatine phosphokinase; HDL, high density lipoprotein; ESR, erythrocyte sedimentation rate; LDH, lactic dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Because incomplete KD is more common in young infants than in older children,[2,32,33] clinicians must make every effort for accurate diagnosis and timely treatment of these young patients. The American Heart Association provided an algorism for diagnosis of incomplete KD in 2004. Patients with fever lasting longer than 5 days with 2 or 3 of the diagnostic signs of KD should be evaluated for systemic inflammation daily if possible. Initially CRP (>3 mg/dL) and ESR (>40 mm/h) levels and complementary laboratory findings including albumin <3.0 g/dL, anemia for age, elevation of ALT, platelets after 7 days >450000/mm^3, WBC count >15000/mm^3, and urine >10 WBC/high-power field, should be reviewed. Patients who fulfill more than 3 of these complementary indices can be treated with IVIG as having incomplete KD followed by echocardiography. Although laboratory findings in KD are non-diagnostic, they may prove useful in the diagnosis of incomplete Kawasaki disease.[2]

It is reported that ~25% of untreated patients and ~5% of IVIG treated patients are affected with CALs.[3,4] Therefore, we were able to determine an imaginary line for the threshold of CALs during the natural course of systemic inflammation in KD, and we assumed that IVIG could lower the peak point of the inflammation curve (Fig. 1). CALs begin at the point where the threshold line and the curve of inflammation intersect, and more severely affected patients reach the threshold line earlier before the peak stage of inflammation (arrows in Fig. 1). Early and extensive treatment with IVIG (2 g/kg) and methylprednisolone pulse therapy (30 mg/kg) may not prevent CALs development in some severely affected patients,[56] and small CALs can progress to huge giant aneurysms after defervescence induced by various treatments. These findings suggest that the severity of CALs is determined in the early period, before the peak stage of KD. Therefore, early IVIG treatment before the peak stage is mandatory to reduce the risk of CALs and the progression of CALs.

Higher severity of systemic inflammation in KD is reflected by prolonged fever duration, a higher incidence of CALs, a higher or lower laboratory values, and a higher rate of IVIG non-responsiveness. In the initial periods of IVIG treatment in Japan and Korea, the score system (Harada score) for evaluation of the severity of KD (a risk of CALs) was used to decide on IVIG treatment; WBC count >12000/mm^3, platelet count <350000/mm^3, CRP >3+, hematocrit <35%, albumin <3.5 g/dL, age <1 year of age, and male sex.[57] KD patients who fulfill ≥4 of the 7 criteria were regarded as having severe inflammation with higher risk of CALs, and were treated with IVIG.[2,57,58] However, now IVIG is recommended for all KD patients. IVIG has a potent anti-inflammatory effect on KD although its mode of action is unknown. It has been reported that approximately 10-15% of KD patients are IVIG non-responders.[17,18,25,59-63] Early detection of IVIG non-responsiveness through laboratory values is a reasonable and simple method for the evaluation of severity of KD and for the selection of severely affected patients who need early intensive treatment. For this purpose, there have been a number of previous reports of putative predictive variables of IVIG non-responders. Laboratory markers including CRP, neutrophil differential including bands, albumin, sodium, hemoglobin, platelets, lactic dehydrogenase (LDH), total bilirubin, γ-GTP, ALT, and AST have been reported to differ significantly between IVIG non-responders and responders before IVIG infusion.[17,18,25,59-63] These different laboratory predictors with the score systems for IVIG non-responsiveness,[17,18] and the earlier Harada score system for risk of CALs may have a limitation resulting from confounding factors; difference of sample size,[64] the intensity of inflammation in individuals, the age of patients,[32] the stage of inflammation response at presentation as shown in Fig. 1,[55] and possibly ethnicity.[25,65] Some studies suggest that earlier IVIG treatment, particularly before day 5 of illness is associated with an increased risk of non-response to IVIG.[17,18,25,63] It is plausible that IVIG non-responders might have more severe inflammation and more florid clinical symptoms and signs, and consequently they may be diagnosed and treated earlier.[18] Because of the limitations of early prediction for IVIG non-responders, some study groups have tried early aggressive treatment for prevention of CALs with IVIG (2 g/kg), aspirin, and methylprednisolone (30 mg/kg) or infliximab. However the results of these studies are controversial.[41,56,66,67] This treatment policy also has some obstacles such as overtreatment of patients with mild clinical course; 85-90% of the patients respond to initial IVIG treatment (2 g/kg). Furthermore it was reported that ~80% of the patients responded to a medium-dose of IVIG (1 g/kg),[68] although high doses of IVIG are more effective in preventing CALs.[69] Thus, early detection of the severely affected patients among all KD patients and prompt further treatment of these patients is necessary because CALs develop and progress before the peak stage of the disease.

IVIG down-regulates nearly all inflammatory laboratory parameters except ESR including total WBC and neutrophil count, CRP, AST, ALT, CPK and LDH in IVIG responders. IVIG increases ESR artificially through interference

with fibrinogen levels.[70] In addition, IVIG has a systemic protein modulation effect *in vivo*; all proteins including albumin, transferrin, apolipoprotein A1, and pro-inflammatory cytokine (TNF-α and IL-6) levels were transiently decreased, but the levels of immunoglobulins (IgA and IgM), electrolytes (sodium, potassium, chloride, calcium and phosphorus) and serum osmolarity were not changed by IVIG infusion.[70-72] Therefore, the beneficial effect of IVIG on KD may, in part, be associated with the control of the proteins which are involved in inflammation in various tissues of the host, although IVIG has multiple modes of action for immune modulation.[11,73]

On the other hand, IVIG non-responders generally have persistently elevated inflammatory parameters, such as neutrophil counts and CRP and lower levels of albumin after IVIG infusion.[59,74] We recently observed that IVIG non-responders have sustained abnormal laboratory parameters following IVIG (within 24 hrs) as well as prior to IVIG compared to IVIG responders; the cut-off values of >13000/μL for total WBC count, >51% for neutrophil differential and <7.2 g/dL for total protein had reasonable sensitivity (91%, 91% and 64%, respectively) and specificity (89%, 76% and 78%, respectively) as independent characteristics of non-response to IVIG.[75] In addition, the kinetics of some inflammatory parameters following IVIG differed markedly between responders and non-responders. Among them, WBC count and CRP in non-responders increased or remained unchanged following initial IVIG infusion (2 g/kg), in contrast to the marked decline in these parameters in responders. Thus, clinicians can use these parameters easily and rapidly for the evaluation of the severity of inflammation in KD. The definition of IVIG non-responsiveness and the treatment modality for initial IVIG non-responders are not clearly determined among the study groups. Many study groups have observed the patients for 36-48 h after termination of IVIG infusion to see whether or not the patients showed defervescence and improvement of clinical signs. The non-responders also consisted of patients with a different severity of inflammation. It was reported that 20-50% of initial IVIG non-responders also did not respond to a second dose of IVIG.[76,77] For these severely affected patients, rapid treatment may lead to a better outcome, because of the possibility of rapid progression of CALs in the early stage of the disease. In our hospital, we defined IVIG non-responders as patients with persistent fever (≥38.0°C) over 24 hrs after termination of IVIG infusion. Our treatment modality for IVIG non-responders is the 2-dose IVIG infusion (initial 2 g/kg, and the second-dose, 1 or 2 g/kg) and intravenous methylprednisolone pulse therapy (10-30 mg/kg, for 3 days). The dose of the second IVIG infusion (1 or 2 g/kg) was assessed on a case-by-case basis. In general, the patients who failed to respond but whose WBC with neutrophil and CRP decreased following the initial IVIG treatment received a further dose of 1 g/kg, whereas those whose WBC count and/or CRP remained unchanged or increased after the initial IVIG treatment received a second IVIG dose of 2 g/kg. Those unresponsive to the second dose of IVIG received intravenous pulsed methylprednisolone (10-30 mg/kg, for 3 days), and the dose was also determined by WBC and CRP levels within 24 h after the second-dose IVIG infusion. We have experienced no patients who remained febrile after termination of this treatment schedule among more than 500 KD patients. We were able to induce defervescence within 12 days from the beginning of the illness in a majority of the IVIG non-responders (~10% of total KD patients), and only three patients were discovered to have giant aneurysms (>8 mm in diameter) after defervescence.[58,75] Since the change of laboratory indices after IVIG therapy appears within 2 hrs after termination of IVIG infusion[71] and the majority of IVIG responders defervesce within 24 hrs of IVIG treatment (2 g/kg), it may be possible to make a decision on commencing the next-step in treatment of IVIG non-responders, earlier than 24 h. With this, further studies in other populations should aim to optimize treatment of IVIG non-responders in KD.

For IVIG non-responders, re-infusion of IVIG (2 g/kg), methylprednisolone pulse therapy (30 mg/kg, for 3 days), infliximab (anti-TNF-α antibodies), and more powerful immune-modulators such as cyclophosphamide or methotrexate, and plasmapheresis have been tried.[78-82] It is believed that severely affected KD patients destined to prolonged fever cannot avoid CALs in the early stage of this self-limited disease, although these treatments can induce defervescence.

A HYPOTHETICAL PATHOGENESIS OF KD

For understanding the pathogenesis of KD, a brief review of resembling diseases may be helpful. Among bacterial infections, scarlet fever/acute rheumatic fever (ARF) may be a representative disease resembling KD. Clinical manifestations of scarlet fever, etiologic agents of which are mainly the group A beta-hemolytic streptococci (GAS), are fever,

strawberry tongue, cervical lymphadenopathy, skin rashes and desquamation of skins after defervescence, mimicking those of KD. Although some patients with GAS infection complain of severe clinical phenotypes including streptococcal toxic shock syndrome and necrotizing fasciitis, scarlet fever is a self-limited disease, with a mean historical fever duration of 6 days without antibiotic treatment.[83] If enough doses of GAS are inoculated to every individual, nearly all individuals who do not have antibodies to toxins would be affected with scarlet fever. The immunopathogenesis of severe GAS infections such as streptococcal toxic shock syndrome and necrotizing fasciitis remains unknown. It is suggested that the streptococcal superantigens, including pyrogenic exotoxins, stimulate an intense proliferation and activation of the immune cells (T cells and macrophages), resulting in the production of large quantities of cytokines. The cytokine imbalance of a local environment may be responsible for tissue injuries and many of the clinical manifestations of severe, invasive GAS infections.[84,85] As nonsuppurative complications, ARF and acute poststreptococcal glomerulonephritis (APSGN) are well-documented after GAS infections.[83]

ARF is an acute febrile disease characterized with prolonged fever, carditis, arthritis, skin rash (erythema marginata) and chorea.[86,87] ARF occurs 2-4 wks after the primary GBS infections of the pharynx. The majority of GAS infected patients recover uneventfully, and only some patients who have genetic susceptibility may be affected with ARF, similar to KD. Although ARF is a disease of which the etiologic agent has been proven, the immunopathogenesis of the disease remains unsolved with speculation as to an immune-mediated disease triggered by infectious insults. One hypothesis suggests that bacterial exotoxins or superantigens may be involved in the cardiac tissue injury through the direct toxic effect on target tissues or through activated cytokines. Others suggest that autoimmune reaction of immune cells that are sensitized to bacterial antigens may attack the target cells that may share epitopes with bacterial antigens ("molecular mimicry").[86,87] The clinical manifestations of ARF can appear without symptomatic GAS infections (pharyngitis) in a third of patients.[86] Antibiotic treatment of scarlet fever rapidly improves clinical symptoms and signs of the disease, but shows no effect in ARF and KD. There may, however, be sites (foci) in which the substances provoking inflammation in ARF or KD as well as in scarlet fever are produced and released to systemic circulation.

At this point, we can deduce that the immunopathogenesis of KD is closer to that of ARF than scarlet fever. In addition, the causes of death in both acute KD and ARF are extensive carditis or complications from distorted cardiac vessels or cardiac valves. There are also rare incidences of a sepsis-like syndrome with multiple organ failure and the fulminating clinical course, which are shown in severely affected patients of GAS infections or any acute infectious diseases, including viral infections. The laboratory findings in both diseases have revealed increased leukocytes, and CRP and ESR values, indicating systemic inflammation with activated immune cells. The characteristic clinical manifestations and pathologic findings in various tissues in scarlet fever, ARF and KD may depend on different inflammatory mediators (proteins) and corresponding immune cells. Pathogens or sole pathogen-associated structural substances have not been identified in the pathologic lesions of KD or ARF (coronary artery, endocardium, and cardiac valve).[86,87] Even in acute GAS infections, bacteria are not found in the affected tissues of toxic shock syndrome or necrotizing fasciitis.[85] Thus, toxic substances and circulating immune cells may be involved in the tissue injuries from these conditions. There are many enigmas as to the pathogenesis of KD. Particularly, as to what the etiologic substance of the systemic inflammation and tissue injury is, how initial inflammation begins, and why only some patients are affected with different clinical severities, as in the case of ARF and APSGN after initial GAS infections, are of concern.

To solve these puzzles, we propose a new hypothesis based on the premise of a "protein homeostasis system" in the host. Mammals, including humans, have evolved through genes which code for proteins. All living activities from embryonic development to the aging process are strictly controlled by a variety of proteins at the molecular level. The numbers and kinds of genes (kinds of proteins) that are activated differ according to the different organ tissue cells and timing of their activation. An organ specific cell produces its own specific proteins. Also, some proteins are produced only during the embryonic stage while some proteins are produce in later stages of the life cycle of mammals. For example, in genetic diseases such as neurofibromatosis or rare genetic prion diseases including fatal familial insomnia, the expression or progression of these diseases appears after 2-5 decades of age.[88] The pathogenesis of these genetic diseases may be associated with transformed proteins, including prions, which may be toxic to nerve cells, and the toxic proteins should be controlled for avoidance of the disease *in vivo*. On the other hand, mammals have also

struggled with external insults in nature. The compositions of various pathogens and animal toxins, such as snake venom and insect venom are mainly proteins of various sizes, shapes and lethal doses. These toxic proteins may have their own affinity to different organ-tissue cells and bind to the receptors on the cells of the host. This toxic protein-receptor binding may induce direct cell injury and/or may produce new proteins through signal transduction pathways in the affected cells. The substances (proteins) from injured cells can be released to systemic circulation and signal other immune cells. Now, it is believed that substances from injured cells of a specific organ tissue can induce an immune reaction and subsequently be toxic to other organ cells or their own tissue cells, if released to the systemic circulation.[89,90] Matzniger proposed an interesting hypothesis for this basic immunological concept, the danger model. Herein, antigen presenting cells (APCs) can be activated by danger/alarm signals (initiators of inflammation) from the injured cells that are caused by pathogens, toxins, mechanical damage, and so forth. The intracellular contents from necrotically died cells could potentially be a danger signal when released, but not that of healthy cells or by cells undergoing physiological deaths (apoptosis).[89] Complete recovery from a systemic infectious disease may be defined as the state in which not only etiologic agents and inflammatory mediators from pathogens but also the substances produced during immune reactions including cytokines and the debris from the injured cells have been completely removed by immune cells, if those substances could be toxic to other tissue cells. We define these toxic proteins as "pathogenic proteins", classifying external pathogenic proteins as those which are of the pathogen origin and internal pathogenic proteins as those which are of host origin, including prions.

A strict protein control system controls the balance of proteins and removes the pathogenic proteins at a molecular level in humans. Serum proteins including albumin, immunoglobulins and various hormones are maintained at constant levels in a steady (healthy) state by unknown protein control systems, and we have named these systems as "protein homeostasis systems". Here, we postulated that the immune system of the host is one of the protein homeostasis systems *in vivo*. The main function of immune cells at a molecular level is to control of a variety of proteins by recognition of size. Innate immune cells (neutrophils and phagocytic monocytes) control larger proteins (microbes and injured cells as a whole), B cells control proteins via antibody production, and T cells control small proteins which cannot induce antibodies, via cytokine production, using T cell receptors (TCR) or via their effect on cell-bound proteins. Macrophages may play the most crucial role in the immune/repair system of the host. Also, they synchronize the communications between innate immune cells and adaptive immune cells in inflammatory reactions, and between immune cells and regenerating tissue cells in repair reactions, via a complex of cytokine (protein) networks. Although the immune system of the mammals have been divided into the separate categories of innate immune system and adaptive immune system, the two immune systems work together against any internal or external insults *in vivo*. It is now accepted that the mediators (proteins) from innate immune reactions affect adaptive immune reactions.[91,92] In infections of various pathogens, pathogen recognition receptors including Toll-like receptors (TLRs) and intracellular sensors which recognize components of pathogens (pathogen-associated molecular pattern) of infected cells and macrophages, induce the production of anti-agent proteins (in case of viruses and interferons) and other proteins including pro-inflammatory cytokines. These proteins may affect the function of adaptive immune reactions such as control of expression of co-stimulatory receptors or activation of specific immune cells.[91,92] B cells (plasma cells) can produce antibodies for any proteins which are recognized by B cell receptors (BCRs). Theoretically, gene recombination of BCR genes can match all internal or external proteins. However, B cells cannot induce antibodies against small foreign proteins and a majority of self serum proteins. Similarly, TCRs of T cells also comprise recombination of variable TCR genes, matching nearly all peptides (proteins) that are constructed with 12-27 amino acids. Despite the more crucial role of T cells than B cells on host defense against invading pathogens (T cell deficiency is more severe compared to B cell deficiency), the role of TCRs is nearly unevaluated except for antigen presentation between T cells and APCs compared to B cell function. Lots of small peptides may exist in inflammatory lesions; however, the metabolism and role of these proteins remain to be evaluated. Indeed, small peptide hormones such as gonadotrophin releasing hormone (GnRH, decapeptide), vasopressin (9 peptide) and oxytocin (9 peptide) exist *in vivo*, and they have crucial roles on systemic hormonal homeostasis.[93] Furthermore in the nucleus of the cells, replication of DNA for cell proliferation and small microsomal RNAs, which regulate gene expression, may also be controlled by proteins, including DNA polymerases and nucleases, suggesting complex mechanisms of protein homeostasis at a cell level.[94]

Superantigens are virulent polypeptides (proteins) that are produced by a variety of infectious organisms including gram positive streptococci and staphylococci. Superantigens can induce activation of many T-cell clones which have specific V_β chains. However, in any human disease including KD, clinical implications of polyclonal activation of T cells have not been clearly explained.[95]

T cells together with other immune cells appear in nearly all pathologic lesions in early stages, before specific T-cells and specific antibodies appear, of infectious diseases, rheumatic disorders including KD and ARF, malignancies as tumor infiltration lymphocytes, tissue rejection, and in the repair processes of tissue injury (keloids). For example, in Mantoux skin tests for diagnosis of tuberculosis, skin tissue injury (positive result) is induced by proteins (purified protein derivatives) and corresponding immune cells, mainly T cells, which were previously sensitized to the proteins from tuberculosis bacilli or *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG). Positive skin reactions can be abolished in states of depressed T cell function or an exhausted number of T cells in cases of corticosteroid treatment, systemic viral infections such as measles, and severe tuberculosis infections.[30] Furthermore, a clean skin injuries such as scar revision in plastic surgery, in which there are no foreign proteins or pathogens, on occasion result in hypertrophic scars or keloids in genetically susceptible patients. The substances (proteins) from injured skin cells and corresponding immune cells, including macrophages and T cells, may also be involved in the pathogenesis of keloids.[96] T cells can be activated by various stimuli such as mitogens and monoclonal antibodies for various receptors on T cells *in vitro*, thus the mechanisms of T cells activation *in vivo* may be different in various pathologic conditions. Although helper T cells can be divided into functional subtypes such as Th1, Th2, Th17 and regulatory T (Treg) cells according to the cytokines produced from the T cells, it is expected that more T cells subtypes, which produce different cytokines, may exist in different pathogenic lesions *in vivo*.[97] Therefore, the functions of T cells are more diverse and complex than we have previously known.

Returning to the immunopathogenesis of KD, it is postulated that systemic inflammation of KD is caused by pathogenic proteins which are associated with an immune reaction of an unknown initial infection. In this infection, a majority of the patients may be asymptomatic. The toxic substances produced during the immune reaction against the initial infection may be removed in a majority of the patients, but some patients who have a genetic defect for this feedback process may lead to foci for which the pathogenic proteins are produced and released into systemic circulation. Although the foci producing pathogenic proteins responsible for KD or ARF are unknown, the secondary lymphoid organs around the initial infection sites (tonsils, lymph nodes or Payer's patches) are primary candidates (Fig. 3A). The pathogenic

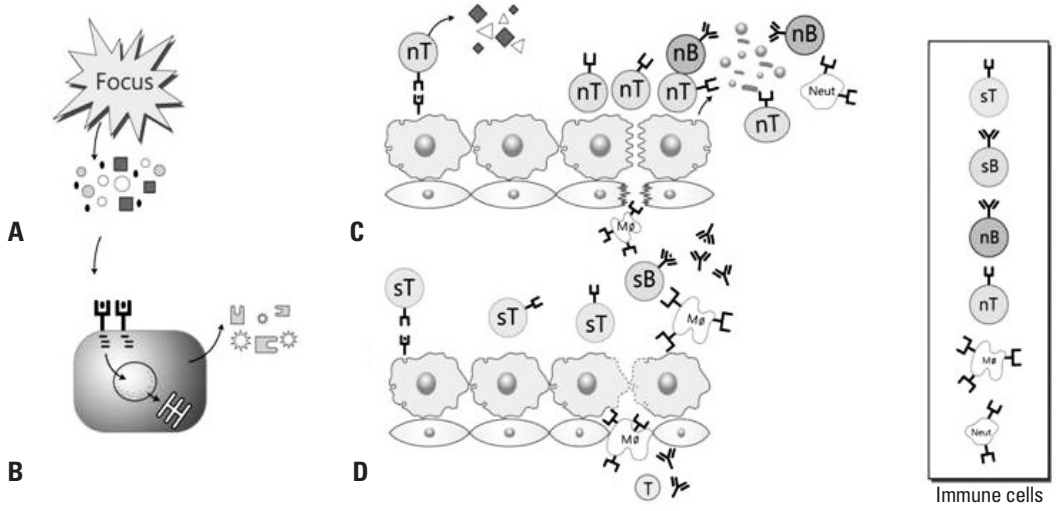

Fig. 3. A hypothetical pathogenesis of KD. After an infection by an unknown KD pathogen(s), substances including pathogenic proteins are produced in a focus (potentially secondary immune organs) (A). The substances spread and reach various tissues via systemic circulation. Immune cells start to control these substances, and clinical symptoms and signs begin to appear. The pathogenic proteins bind to receptors of endothelial cells of coronary arteries, and this process induces cell injury and /or other protein production from endothelial cells (B). Immune cells recruit to the lesions to control the action of the proteins including pathogenic proteins. Initially, non-specific T cells and non-specific antibodies are involved in this reaction, while hyperactivated immune cells produce various inflammatory cytokines and counter-inflammatory cytokines, leading to a cytokine imbalance associated with further endothelial cell injury (C). After emergence of specific T cell clones and specific antibodies for pathogenic proteins, tissue injury ceases and a repair reaction begins with the immune cells (D). KD, Kawasaki disease.

proteins in KD have affinity mainly to endothelial cells of coronary arteries, and bind to the receptors on endothelial cells. This process is directly toxic to the endothelial cells and/or produces new proteins including inflammatory mediators through signal transduction pathways to the nucleus. These mediators from the affected cells are a signal for the recruitment of immune cells (Fig. 3B). To control the pathogenic proteins and/or the new proteins from the injured cells, immune cells, especially T cells for small proteins, are recruited and activated. Because there is a time-gap for the appearance of specific immune cell clones (specific T cells and B cells) in an immune reaction, this reaction may be conducted by non-specific T cells and non-specific antibodies initially, until the specific T cell clones and specific antibodies that can efficiently control pathogenic proteins are produced. The activated immune cells during this immune reaction (in the process of protein control) produce various inflammatory cytokines and counter-inflammatory cytokines, and a cytokine imbalance may be associated with the endothelial cell injury in KD. The substances from the injured cells recruit more immune cells with more cytokine production. Some cytokines such as TNF-α induce other proteins including matrix metalloproteinase (MMP) 9, which is toxic to neighboring cells, and aggravate tissue injuries (Fig. 3C). Other substances released from the initial focus and/or the substances released from the injured lesions and cytokines spread via systemic circulation and induce the initial clinical manifestations of KD, including fever and other diagnostic clinical signs, and other rare manifestations of KD. The substances inducing extra-coronary manifestations including skin rashes and arthritis in KD are controlled by immune cells, and they may not contain the pathogenic proteins which induce a signal to tissue injury. After the emergence of specific T cell clones and specific antibodies for pathogenic proteins, inflammation ceases and a repair reaction begins with the immune cells and regenerating cells (Fig. 3D). Briefly, the genetic determination of KD susceptibility may depend on a defect in the immune cells to detect and remove pathogenic proteins from initial infections. Also, severity of the disease may depend on the amount of pathogenic proteins with corresponding hyperactivity of immune cells and the time-period for emergence of specific immune cells against pathogenic proteins. The immunopathogenesis of ARF or other immune-mediated disease could be explained similarly by this hypothesis. Accordingly, pathogenic proteins, affected target cells, the subset of corresponding immune cells and the kinds of cytokines and other substances including antibodies may be different from KD. We previously used this hypothesis to explain the immunopathogenesis of acute lung injury in *Mycoplasma pneumoniae (M. pneumoniae)* and influenza virus infections.[98,99] For the 2009 H1N1 influenza virus infection, the severity of pneumonia was correlated with lymphocyte counts at presentation, and early immune-modulators (corticosteroids or IVIG) induced dramatic recovery of severe pneumonic consolidations within a day.[99,100] Therefore, it is also acceptable as a new concept that small pathogenic proteins are produced during immune reactions in acute systemic infections (influenza virus or *M. pneumoniae*), not by viruses or mycoplasmas themselves, and can induce cytopathic effects on lung tissues by hyperactivated immune cells, especially T cells.[99] In these infections, like strong natural toxins, extremely small amounts of pathogenic proteins that have affinity to lung tissues can induce severe lung injury leading to death by amplification of a maladjusted immune reaction.

Along with studies for etiologic agents of KD, some investigators have proposed the immunopathogenesis of KD. Recently the study group of Yeung[15] presented an interesting model for the pathogenesis of KD using a mouse model of KD. They created experimental mice which were able to develop coronary arteritis in response to intraperitoneal injections of *Lactobacillus casei* cell wall extract (LCWE). This murine model of KD is similar to human KD including the aspect of massive activation of immune cells, disease susceptibility in the young and a similar pathology of coronary arteritis, although replication of the disease is not perfect. They observed that immune cells began to appear in cardiac tissue at day 3 after LCWE injection, and then more immune cells, mainly T cells, infiltrated around the arteries and peaked at day 28. Also, disruption of the intima and media as well as aneurysm formation were observed at day 42.[15,101] They postulated that T cells have a crucial role in the pathogenesis of KD. A superantigen in LCWE activates massive T-cell clones, and T-cells survive from apoptosis by a co-stimulatory signal on coronary endothelial cells as transformed antigen presentation cells. TLR2 on endothelial cells and a corresponding ligand in LCWE may in part intensify co-stimulation expression on the cells. Activated T cells continued to produce cytokines including TNF-α and IFN-γ, causing a cytokine imbalance in local lesions, that may be responsible for vessel wall injuries. It may be true that T cells have crucial roles in animal KD and in human KD, since adaptive immune deficiency mice (recombination activating gene1 knockout mice) and TCR

α-chain deficiency mice cannot produce vasculitis.[15,102] We previously described similar phenomena in *M. pneumoniae* and influenza virus infections. In these infections, T cell deficiency mice or T cells depressed mice had less severe pneumonia with prolonged survival time and little pathologic findings compared to control mice.[98,99] Recently, Rowley and Shulman[16] proposed a model on the pathogenesis of KD. The agent of KD may be a ubiquitous infectious agent, most probably a single virus or a group of closely related viruses that lead to KD only in a small subset of genetically susceptible children. The initial infection site of the agent may exist in the respiratory tract including ciliated bronchial epithelium, and both innate and adaptive immune responses ensue including B lymphocytes switching to IgA lymphocytes. The KD agents in circulating macrophages reach and infect endothelial cells of the coronary artery as main target cells via systemic circulation. Immune cells, including previously primed CD8 T cells and IgA B cells in initial lymphoid tissues, are re-recruited to infected target tissues and contribute to the inflammatory response within the tissues. Differentiated IgA B plasma cells produce antigen-specific antibodies, and CD8 T cells attack infected cells by cytotoxic mechanisms. The products from infected cells and immune cells including MMPs result in destruction of collagen and elastin fibers, and form an aneurysm. The immune response is ultimately successful in controlling the pathogen, particularly if IVIG is given, because IVIG may have antibodies to KD agents and have a role in antibody dependent cellular cytotoxicity to infected cells.[16]

CONCLUSIONS

KD is the most common cause of acquired heart disease in children in developed countries. Although the etiology of KD remains unknown, the epidemiological and clinical characteristics of KD suggest that etiologic agent(s) are associated with environmental changes of improved public hygiene and/or industrialization. As a self-limiting systemic disease, the clinical course of KD shows progression and regression of the inflammation intensity reflected in laboratory findings. CALs in KD may occur in the early stages of systemic inflammation. Although there are no consensus recommendations for the treatment of initial IVIG non-responders, intensive treatment as soon as possible is mandatory for prevention and progression of CALs. Short-term follow-up (within 24 h) examination of some of the inflammatory parameters may help shorten the fever duration of the severely affected patients. A hypothetical pathogenesis of KD is proposed using the premise of a "protein homeostasis system" of the host. Hyperactive immune cells, especially T cells, with excessive cytokines may be responsible for tissue injury as well as for tissue reconstruction according to this hypothesis. Further developed tools including bioinformatics of candidate genes and proteomics may be needed for establishing more detailed explanations on the pathogenesis as well as developing diagnostic tests and improved treatments for the prevention of KD.

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