Ben Tsutomu Saji · Jane W. Newburger
Jane C. Burns · Masato Takahashi
Editors

Kawasaki Disease

Current Understanding of the Mechanism and Evidence-Based Treatment

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Dedication

It was almost 45 years ago, in November 1969, when I met Dr. Kawasaki. I was working in the Department of Pathology at the Japanese Red Cross Medical Center (formerly: Red Cross Central Hospital). I had little knowledge of Kawasaki disease but suddenly became interested when I conducted autopsies of Kawasaki disease patients. I have been deeply involved with this mysterious disease ever since.

Dr. Kawasaki is a great doctor, who proved from daily observations of patients that Kawasaki disease has very peculiar features. He has always been straightforward, like an innocent child, and I have been on friendly terms with this "Extraordinary child" for a long time.

Admiring the Glory of Dr. Tomisaku Kawasaki and his family.

Shiro Naoe, M.D., Ph.D.
Professor Emeritus
Toho University

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I feel greatly privileged to have the opportunity to write this dedication to honor Dr. Tomisaku Kawasaki, my dear friend for the past 31 years. Tomi has devoted his life to the care of children as Pediatrician Extraordinaire*! This is one of life's greatest opportunities and responsibilities. In addition, Tomi's outstanding clinical skills led him in the 1960s to recognize the important illness that should forever bear his name. For many decades Dr. Kawasaki has tirelessly and effectively devoted himself to fostering research within Japan and internationally to help solve the mysteries surrounding this disorder, always being most supportive, gracious, and collaborative, focusing on advancing understanding of the disease to improve patient care and outcomes.*

My wife Claire and I have been so fortunate for the friendship of Reiko and Tomi Kawasaki since the First US–Japan Workshop on Kawasaki Disease at Makaha, Hawaii, organized by Marian Melish in January 1984. We have traveled together in Kyushu, Honshu, Hawaii, Pisa, Amsterdam, Taiwan, Manila, Chicago, and other locales.

The French embryologist Jean Rostand stated: "What a profession this is—this daily inhalation of wonder." Since caring for his first Kawasaki patient in 1961, Tomisaku Kawasaki has lived by these words.

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Virginia H. Rogers
Professor of Pediatric Infectious Diseases
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***

When the first research committee on MCLS [Kawasaki disease was called Muco-Cutaneous Lymph Node Syndrome (MCLS) at that time] was organized in 1970, the first nationwide epidemiologic survey of Kawasaki disease was conducted with the collaboration of the late Professor Itsuzo Shigematsu, Head of the Department of Epidemiology at the National Institute of Public Health. Since the first survey, I was in charge of the surveys until handing over responsibility to my successor, Professor Yoshikazu Nakamura, Department of Public Health, Jichi Medical University.

I met Professor Tomisaku Kawasaki when I was a young staff member in the department and was assigned to the survey by Professor Shigematsu. I feel quite fortunate to have had this unexpected encounter with Professor Kawasaki and to have had the chance to come into contact with his personality.

He has always said, "Be strict in medical research and be warm in medical practice." His attitudes in clinical practice and in scientific research have always been consistent with his principles. I am convinced that the discovery of Kawasaki disease was a logical consequence of his serious attitude.

I firmly believe that Professor Kawasaki is the most important person in the support and leadership for those involved with research on Kawasaki disease. I sincerely hope for his continued good health and happiness.

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Almost half a century has passed Prof. Tomisaku Kawasaki's first description of a unique disease. He saw the first 4-year-old patient with fever and rash in 1961. At that time he described the diagnosis "unknown". This "unknown diagnosis" may be an important key to his serendipitous discovery of this new disease, based on his deep insight into clinical observations and scientific considerations. His first English article reported the detailed clinical findings, and the epidemiology of Kawasaki disease (KD) in Japan and was surprising because it described the very small number of patients who died from myocardial infarction (Kawasaki: Pediatrics 1974). In 1973, I introduced coronary angiography for patients who recovered from acute KD and found that a certain number of them had silent coronary aneurysms even if they were free from cardiac symptoms or normal ECG findings (Kato: J Pediatr 1975). Prof. Kawasaki appreciated our study and since then has always encouraged our research. My personal communication with Prof. Kawasaki started and then, and I resolved to continue KD research at that time. During 40 years of contact with Prof. Kawasaki, I have been honored to learn from him the clinician's careful observations and deep considerations, the pediatrician's warm and gentle way with children, and the scientist's deep insight and careful discretion.

KD is not only great interest in pediatric medicine but also has a great impact in various fields of clinical medicine. In pediatric cardiology, KD is now a leading cause of acquired heart disease among children in Japan and North America. In the past, pediatric cardiologists knew little about the coronary arteries, but now must learn about coronary artery disease. Furthermore, patients in an earlier era already carried the disease to adulthood, and for a certain number of them coronary artery sequelae developed into adult coronary artery disease manifesting as a new coronary syndrome and premature atherosclerosis. These presented long-term problems in the areas of adult cardiology and vascular biology.

The incidence of KD rapidly increased since 1960s and continuing to increase, particularly in Japan? This mystery likely depends on the etiology of the disease. The epidemiology suggests that KD develops in susceptible children exposed to a common infectious agent or agents. However, the question remains as to why KD has rapidly increased in incidence in Japan. Some additional environmental factor(s) may be involved. Elucidation of the etiology of the disease is an urgent issue, and must certainly be Prof. Kawasaki's wish.

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Foreword

In January 1961, I saw my first case of what is now known as typical Kawasaki disease. The 4-year-old boy had unique symptom complexes, ones that I had never experienced in my 10-year pediatric career. I could not help but classify it as

"diagnosis unknown" when he was released from the hospital. In February 1962, one year later, a 2-year-old boy with suspected sepsis was admitted to the emergency room of our hospital. When I saw him, I immediately remembered the undiagnosed case I had seen the previous year. By the end of 1966, I had experienced 50 cases that fell into the same category. I reported these cases in an article, "Acute Febrile Muco-Cutaneous Lymph Node Syndrome: Clinical Observation of 50 cases," which was published in the Japanese journal *Allergy* in 1967.

With research funds obtained from the Ministry of Health and Welfare in 1970, we organized a research committee, drew up a diagnostic guideline, and conducted the first nationwide survey. Although the committee had thought that the prognosis for the disease was favorable, cases of sudden death had been reported. Kawasaki disease is now classified as a systemic vasculitis that can result in coronary artery lesions. It requires not only the services of pediatric internists but also those of professionals from a wide range of disciplines, from the basic sciences to social medicine.

One of four untreated patients develops coronary artery aneurysms, which can persist after the acute period and result in myocardial infarction or sudden death. Although intravenous immunoglobulin therapy, the standard therapy for acute Kawasaki disease, has reduced the incidence of coronary artery aneurysms, some patients are resistant to this therapy. Effective therapy is necessary for these patients. Unfortunately, the etiology of Kawasaki disease remains unknown despite the efforts of many researchers.

It has been 53 years since I saw my first KD case, and 47 years have passed since the original paper on Kawasaki disease was published in Japanese. This is the first reference work on Kawasaki disease and provides current information regarding basic research, epidemiology, medical treatment, diagnosis, examination, interventions, and surgical treatment of Kawasaki disease. I hope it will be useful for all medical professionals, from those in basic research to those in clinical practice.

Tomisaku Kawasaki

Preface

Kawasaki Disease: Current Understanding of the Mechanism and Evidence-Based Treatment has been a monumental undertaking and is an appropriate tribute to the genius of Dr. Tomisaku Kawasaki.

Many are familiar with the story of his first encounter with mucocutaneous lymph node syndrome, later renamed Kawasaki Disease, as told by Dr. Kawasaki himself. In January 1961 he encountered a 4-year-old boy who presented with what we now recognize as the classic clinical signs. After meticulously recording and pondering the child's presentation and laboratory test results, Dr. Kawasaki could not recognize the clinical signs as those of any known childhood illness. Nevertheless, with improvised treatments of intravenous hydration, penicillin, and a few doses of prednisone, the child's condition improved over a few weeks and he was discharged without a specific diagnosis. It was not until Dr. Kawasaki saw the second patient with similar clinical features a year later that he suspected that he was seeing a new disease. Over the next 6 years, he made meticulous notes on 50 similar cases that formed the basis of his landmark Japanese publication in 1967 [1]. It was not until a committee convened by the Japanese Ministry of Health conducted a nationwide survey that it was recognized that the "benign, self-limited syndrome" could be associated with coronary artery aneurysms, thrombosis, and myocardial infarction in a subset of children. The expanded syndrome was first presented to Western audiences in the 1974 publication in *Pediatrics* entitled "A new infantile acute mucocutaneous lymph node syndrome prevailing in Japan" [2].

In 2001, the Japan Kawasaki Disease Research Center re-published his original 1967 paper in booklet form with a side-by-side, page-by-page English translation. In its Preface, Dr. Kawasaki quoted a message from Dennis Burkitt (of lymphoma fame) to his medical students: "You should not despair if you do not have access to sufficient research funds or facilities: what is most important in conducting outstanding studies are steady observation and logical deduction." Like Burkitt before him, Dr. Kawasaki has a penchant for detailed observation and intellectual honesty that has earned universal respect from scholars and clinicians throughout the world. And yet Dr. Kawasaki remains genuinely friendly and humble. He always addresses himself as *boku* (僕) ("servant"), and lends his ear to anyone with any topic. He

attributes his success to having able co-workers and being at the right place at the right time. Without Dr. Kawasaki's missionary zeal to influence so many scholars from infectious disease to cardiology, from epidemiology to pathology, from all corners of the globe, we could not have made such strides in research efforts in the areas of genetics, pathology, pathogenesis, treatment, and psychosocial concerns, and certainly this kind of book could not have been written.

We, the undersigned, join all the contributors to this book in saying a heart-felt "Thank you" to our friend and *sen-sei* ("teacher"), Dr. Tomisaku Kawasaki.

Seattle, WA, USA — Masato Takahashi
San Diego, CA, USA — Jane C. Burn
Boston, MA, USA — Jane W. Newburger
Tokyo, Japan — Ben Tsutomu Saji

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Contents

Part I
Basic Research

The History of Kawasaki Disease: A Personal Perspective

Marian E. Melish

Abstract To understand the history of Kawasaki Disease (KD) one has to examine three periods of time: (A) the period from 1960 through the mid-1980s, (B) back to the 1870s, and (C) forward to the present. Dr. Kawasaki's seminal paper, published in the Japanese-language journal *Arerugi* (Japanese Journal of Allergy) (Kawasaki, Arerugi [Japanese J Allergy] 16:178–222, 1967) in 1967, is the best starting point, because of its remarkably complete delineation of the clinical features of KD in living children, which is the basis for the diagnostic criteria we use today. The more than 100 years of pathologic records from the first description of fatal KD, by Samuel Gee in 1871, (Gee, St Barth Hosp Rep 7:148, 1871) to Kawasaki's report includes the era of growing interest in and elegant description of the autopsy diagnosis that came to be called "infantile periarteritis nodosa". (Munro-Faure, Pediatrics 23:914–926, 1959; Roberts and Fetterman, J Pediatr 63:519–529, 1963) The most recent period, from the mid-1980s to the present, marks the period of international cooperation, heightened interest, and scientific progress in the understanding of this still enigmatic disease. The following chapters will outline the many areas of scientific progress. The history of KD is very personal to me as I have had the special privilege to be a participant in all these eras of discovery.

Keywords Tomisaku Kawasaki • Infantile periarteritis nodosa • Mucocutaneous lymph node syndrome • Mucocutaneous ocular syndrome

Introduction

To understand the history of Kawasaki Disease (KD) one has to go through three periods of time: (A) the period from 1960 through the mid-1980s, (B) back to the 1870s, and (C) forward to the present. Dr. Kawasaki's seminal paper, published in the Japanese-language journal *Arerugi* (Japanese Journal of Allergy) [1] in 1967, is the best starting point, because of its remarkably complete delineation of the

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clinical features of the disease in living children, which is the basis for the diagnostic criteria we use today. The more than 100 years of pathologic records from the first description of fatal KD, by Gee in 1871, [2] to Kawasaki's report includes the era of growing interest in and elegant description of the autopsy diagnosis that came to be called "infantile periarteritis nodosa" (IPN). [3, 4] The most recent period, from the mid-1980s to the present, marks the era of international cooperation, heightened interest, and scientific progress in the understanding of this still enigmatic disease. The following chapters will outline the many areas of scientific progress. The history of KD is very personal to me as I have had the special privilege to be a participant in all these eras of discovery.

The Era of Clinical Description and Pathologic Recognition: 1960–1984

I came of age in medicine in the 1960s, already fascinated by the elegant case series of IPN presented by Munro–Faure, and Roberts and Fetterman, when I saw my first two patients with KD in 1967, as a pediatric resident in Rochester, New York. Unable to reach a diagnosis, we presented the first case at Grand Rounds to a fascinated audience. Our colleagues were as puzzled as we were, so, like Kawasaki when he saw his first case in 1960, we filed these two cases away in our minds under "God only knows". [5] I had the good fortune to come to Hawai'i as an Assistant Professor in 1972. I began to see more puzzling cases and met my rheumatologist colleague, Dr. Raquel Hicks, at the bedside of febrile children with persistent fever, conjunctivitis without exudate, stomatitis, rash, arthritis, and urethritis. As newly minted academic physicians we had trouble believing that we were seeing a new or previously undescribed disease. We were sure that these children had the same unique disease and were toying with the idea that they might have a childhood version of Reiter syndrome, because of the triad of conjunctival involvement, arthritis, and urethritis common to most of our patients. We also became aware that our pathologist colleague, Dr. Eunice Larson, had diagnosed IPN in a child who died in our Children's Hospital in the year before our arrival. The clinical features he had before his sudden death—bilateral thrombosed aneurysms on the 22nd day of illness—were very similar to those in the children we were seeing. In 1973, at the time of our 12th case, we had lunch with Dr. Fumio Kosaki, a colleague of Dr. Kawasaki at the Japan Red Cross Hospital, who was on the final stop of a personal tour of North American Children's Hospitals. He showed us photographs of children with mucocutaneous lymph node syndrome (MCLS). He had shown these photographs and described Kawasaki's diagnostic criteria at all the medical centers he had visited and no one recalled seeing any similar cases. We said that we were not only very familiar with the symptom complex but indeed had a compatible patient in the hospital on that same day. Dr. Kosaki visited the patient with us and agreed that it was Kawasaki's MCLS. We were momentarily chagrined that we

were not the first to recognize a new disease but pleased to know that we were correct in our suspicion that it was a unique entity. We began corresponding with Dr. Kawasaki in Japanese, grateful that we had many nurse and housekeeper colleagues who were fluent in Japanese. We presented our experience at the May 1974 meeting of the North American Society for Pediatric Research and published our series of 16 cases in 1976 [6].

Meanwhile, events had been progressing rapidly in Japan since 1967. Kawasaki's presentations and publication were initially met with skepticism as to whether his cases were a newly recognized disease entity or a variant of scarlet fever, Stevens-Johnson syndrome, or erythema multiforme. There were reports and small case series from other Japanese physicians of what was called mucocutaneous ocular syndrome (MCOS), which did not fully match Kawasaki's criteria but would now fall under the expanded KD umbrella. The pathologist at the Japan Red Cross Hospital, Dr. Noboru Tanaka, autopsied a child who Kawasaki had diagnosed as having MCOS. That child died suddenly and unexpectedly and had post-mortem findings of coronary thrombosis [7].

The presence of cardiac involvement in living children meeting MCLS and MCOS characteristics at another Tokyo hospital was reported in a paper published in 1968 by Takajiro Yamamoto [8]. The controversy and interest regarding MCLS/MCOS led to the formation of a research group and the First National Survey of MCLS just 3 years after Kawasaki's first paper. This survey was supported by the Japanese Ministry of Health and was headed by an epidemiologist, Dr. Itsuzo Shigematsu. A questionnaire using Kawasaki's diagnostic guidelines and photographs of the major features in a brochure were sent to children's hospitals with over 100 beds. It included questions about cardiac complications encountered.

The First National Survey validated Kawasaki's description of a new clinical entity and demonstrated an astonishing prevalence for a previously unrecognized entity: 415 hospitals (43 % response rate) reported 3140 cases during the decade 1961–1970. There was a monomodal age distribution with a peak in the second year of life, a male:female ratio of 1.5–1, and a seasonal distribution of cases, with clusters in winter and spring [9].

Until that time Kawasaki believed that MCLS was severe and often prolonged but ultimately benign and self-limited. He was surprised by the results of the First National Survey, which reported 10 cases of sudden death in children meeting his diagnostic criteria [10] The first two national surveys demonstrated an annual case-fatality rate of 1.7 %. Four of the first 10 cases were autopsied; all had thrombosed coronary artery aneurysms. The histologic appearance was similar to the pathologic diagnosis generally reported as infantile periarteritis (or polyarteritis) nodosa (IPN) [11]. Initially, Japanese pathologists disagreed about how similar these cases of fatal MCLS were to IPN. Controversy aside, the reports of sudden death due to coronary disease focused attention on the cardiovascular system of all children with MCLS. This was explored elegantly by the coronary angiography studies of Dr. Hirohisa Kato, which revealed coronary dilation and aneurysms in 12/20 MCLS patients with no cardiac symptoms [12]. The period 1970–1984 was one of active research and discovery in Japan with ever increasing yearly KD incidence,

introduction of noninvasive echocardiography allowing for universal screening and monitoring of coronary abnormalities, more complete discovery of the natural history of KD (namely, the findings that coronary aneurysms occur in 20–25 % of patients and peripheral aneurysms in 5 % of patients), and the first nationwide epidemics, in 1979 and 1982 [13]. Japanese physicians and scientists held regular research meetings and established collaborations. Because of the usefulness of anti-inflammatory doses of aspirin in acute rheumatic fever, this therapy became standard for KD. Toward the end of this period came the seminal collaborative study of Dr. Kensi Furusho and colleagues from several institutions, which demonstrated that 1.6 g of intravenous immunoglobulin (IVIG) plus aspirin (n = 40) was more effective than aspirin alone (n = 45) in reducing transient (incidence: 15 % vs. 42 %, respectively) and persistent (31 % vs. 8 %) coronary artery abnormalities [14].

During this period interest in KD was building in North America and Europe but active investigation in those regions was far less robust than in Japan. The opportunity for investigators to meet and collaborate was very limited. Review of the histories and pathologic findings of cases diagnosed as IPN in continental North America and fatal KD cases in Hawai‘i and Japan confirmed that they were indistinguishable but different from adult or classical periarteritis nodosa [15]. Echocardiography and angiography findings became established and reported [16, 17]. Community-wide epidemics were reported in several regions [18, 19]. A racial difference in incidence was established: children of Japanese ancestry had a markedly elevated risk as compared with children of European ancestry living in the same multiethnic community [19, 20].

Back to the Future: 1871–1984 and Other Riddles of KD

> Disease is very old, and nothing about it has changed. It is we who change, as we learn to recognize what was formerly imperceptible. *(De l'expectation en médecine*. Jean Marie Charcot 1825–93)

Gee's report of the death, in 1871, of an English boy with thrombosed coronary aneurysms and Malet's report in the Lancet in 1887 show that fatal KD was present in Europe in the nineteenth century [2]. At its emergence in Japan in the 1960s, KD had a case fatality rate of 1.7 %. But where were the 98 % of non-fatal cases? Starting in the 1930s there were many single case reports of IPN in the United States, all diagnosed at autopsy and most with clinical features we would now recognize as KD. Roberts and Fetterman were able to develop an autopsy series, but no clinician had encountered and followed more than one IPN patient in his/her lifetime. Monro–Faure, and Roberts and Fetterman, recognized, and very nearly described, the principal diagnostic features of KD retrospectively from the case reports [3, 4]. While alive these patients had received diagnoses of Stevens–Johnson syndrome, erythema multiforme, scarlet fever, and hypersensitivity reaction, as had the early KD patients identified in the First National Survey in Japan. It

seems likely that KD had sometimes been misdiagnosed as measles, adenovirus, or enterovirus infection among patients in North America [21]. The presence of these confounding conditions explains how KD was "hiding in plain sight" in North America and Europe, where we know it existed from reports of fatal cases in the pathologic record. In Japan, however, KD may have emerged, as both a pathologic and clinical entity, in the 1950s. The search for evidence of convincing KD autopsy and clinical cases before 1950 has been unrewarding [22]. Fatal cases with autopsy findings consistent with KD were seen in the Annual of Pathologic Autopsy Cases from 1960 to 1970, and had received varied clinical diagnoses. From 1970 through 1982 there was a striking increase in the number of autopsy KD cases, and a steady fall from the mid-1980s, due to better diagnosis and treatment. Unfortunately, the autopsy registry began in 1958 [23]. A review of clinical records at Tokyo University Hospital from 1940 to 1965 identified diagnoses that could mimic KD and revealed 10 apparent KD cases from 1950 to 1967 but no convincing cases from 1940 to 1950 [24, 25]. Among the many unsolved mysteries of KD is the microbial or environmental agent that may have triggered the sudden emergence of the still expanding epidemic of KD cases among the uniquely susceptible child population of Japan [26].

The Era of Enhanced Discovery and International Collaboration: 1984–2015

In contrast to their colleagues in Japan, the relatively small number of clinicians and researchers interested in KD in North America and Europe were isolated and not fully aware of the progress being made in Japan. I organized what would become the First International Kawasaki Disease Symposium (IKDS), which was held at Makaha Hawaii in January of 1984. At that meeting the North American colleagues learned much more from the leading Japanese researchers than we contributed. Nevertheless, we established productive and personal relationships that have endured and produced many advances in the understanding of KD. Members of the US contingent organized the first US Multicenter Trial of the Efficacy of IVIG in the Treatment of Kawasaki Syndrome at that meeting [27]. The following IKDS conferences have been held in Asia or the US every 3–4 years, most recently, the 11th IKDS in Honolulu Hawai'i in February 2015. These meetings invite abstracts and have research poster and oral presentations with invited lectures focusing on new directions. The major sessions at the 11th IKDS were on epidemiology, genetics, etiology and pathogenesis, animal models, clinical studies in diagnosis/biomarkers, therapy, imaging, natural history, and long-term prognosis, with invited lectures on the conduct of clinical trials and a session on collaborative research. KD is now recognized among children of all racial and ethnic groups in all continents. Major advances in clinical care have reduced the mortality rate and severity of vascular disease, helped define the natural history and long-term

prognosis, and increased interest in the genetics of susceptibility in various populations. KD cases are seen worldwide, but the disease remains underdiagnosed and undertreated in most areas with low prevalences and in locations where it has only recently been diagnosed for the first time. The etiologic agent has not been discovered, and a sensitive and specific diagnostic test is not available. IVIG, the current standard therapy, is expensive, requires hospitalization for administration, and fails to control inflammation in 15–20 % of cases. Serious coronary vascular lesions still develop, with no proven effective adjunctive therapy. Despite our historical progress there is much work to be done to remove this threat to the children of the world.

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Histopathology of Coronary Arteritis in Acute Kawasaki Disease and Murine Systemic Vasculitis Induced by *Candida Albicans* Cell Wall Polysaccharide

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Abstract This chapter describes the characteristics of Kawasaki disease vasculitis and a *Candida albicans* cell wall polysaccharide–induced murine vasculitis model. Kawasaki disease vasculitis and murine vasculitis have a number of similarities, namely, (1) vasculitis readily develops at bifurcations of medium-sized arteries, (2) inflammatory cell infiltrate mainly comprises neutrophils and macrophages; fibrinoid necrosis is rare, (3) vasculitis follows the typical course of acute inflammation, (4) proinflammatory cytokines such as tumor necrosis factor α are closely associated with vasculitis onset, and (5) vasculitis shows some response to IVIG therapy and anti–tumor necrosis factor α therapy.

Keywords Kawasaki disease • Arteritis • Pathology • *Candida albicans* • Pathogen-associated molecular patterns (PAMPs)

Introduction

Kawasaki disease (KD) is an acute febrile disease of children and is classified as a vasculitis syndrome. Relapse and recurrence are rare, but most cases follow the typical course of acute inflammation. This is a major difference between KD and other types of vasculitis, such as polyarteritis nodosa and Takayasu arteritis.

This chapter outlines the histopathological features of coronary arteritis in acute KD, describes the characteristics of a KD vasculitis model, and explains the similarities between this model and KD vasculitis.

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Histopathological Characteristics of Coronary Arteritis in Acute KD

KD is characterized by a high incidence of infiltration of epicardial coronary arteries. The origins of coronary arteries are particularly prone to aneurysm development. Occasionally, a bead-like aneurysm forms along the entire length of the coronary artery outside the muscle layer, starting at the origin [1–4].

Histologically, the earliest changes are seen on the sixth to eighth day of illness, starting with edematous changes in the media and progressing to neutrophil and macrophage infiltration of the intima and adventitia. By the 10th day of illness, inflammation in the intima and adventitia merges, forming panvasculitis across all layers of the vessel wall. The lesion exhibits inflammatory cell infiltration by macrophages and neutrophils and proliferative changes of fibroblasts, among other changes [5]. Inflammation rapidly worsens and becomes panvasculitis involving all layers of the vessel wall. Even at the peak of inflammation, fibrinoid necrosis like that seen in polyarteritis nodosa is rare. If the internal and external elastic lamina and smooth muscle cells in media are damaged by severe inflammation, the artery becomes unable to withstand the pressure of the blood and dilates; thus, aneurysm formation is complete by about the 12th day of illness. Thrombi readily form inside the aneurysm and are a cause of ischemic heart disease (Fig. 1). Therefore, completion of treatment by the 10th disease day is important in preventing aneurysm formation.

Severe inflammatory cell infiltration persists until around the 25th day of illness, after which it usually gradually subsides. Infiltration is usually almost gone by about the 40th day of illness. Therefore, KD vasculitis generally exhibits the typical course of acute inflammation. Scarring remains if the vessel wall undergoes a certain degree of destruction. If a giant aneurysm persists, long-term antithrombotic and anticoagulant therapy is required.

A recent study of KD vasculitis histology reported findings that differed from previously reported results [6]. The authors described a subacute/chronic vasculitis

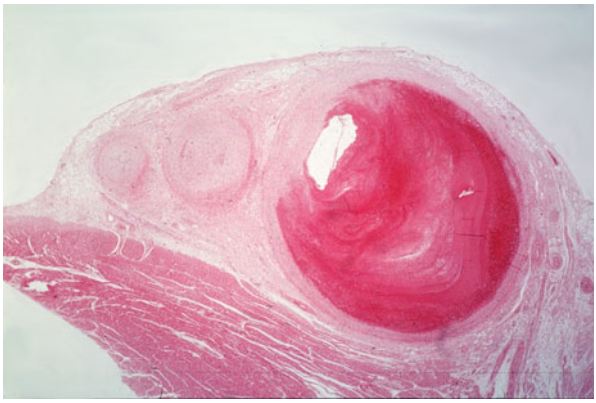

Fig. 1 Histology of coronary aneurysm with thrombotic occlusion in acute KD

that is usually observed several months to years after KD onset. Although this may represent a new disease pattern, it is necessary to clarify the clinical characteristics of the patients studied. Our group has examined more than 100 KD autopsy cases but has never found evidence of chronic vasculitis.

The *C. Albicans* Cell Wall Polysaccharide–Induced Murine Vasculitis Model

Mice [7–9], rabbits [10], and swine [11] have been used as models of KD vasculitis. Here, we describe systemic vasculitis induced in mice by a *C. albicans* cell wall polysaccharide.

Model Development and Description of the Vasculitis-Inducing Agent

This vasculitis model was originally reported by Murata, in 1979 [7]. He ascertained that the amount of *Candida* in stool was significantly greater in children with KD than in healthy control children and that anti–*Candida* antibody titers were higher than in patients with scarlet fever. As an inflammatory agent, Murata initially used a polysaccharide component extracted from *C. albicans* with alkali. However, it was later found that a similar vasculitis could be induced with a polysaccharide released into the supernatant when *Candida* was cultured in a completely synthetic medium [12]. The inflammatory agent is a complex of mannan, beta-glucan, and protein [13]. Interestingly, the structure of the polysaccharide varies with the culture conditions, resulting in differences in vasculitis-inducing activity [14]. The receptor for the inflammatory substance was identified as dectin-2, which is believed to be involved in innate immunity in the onset of vasculitis [15].

Histopathological Characteristics of C. Albicans *Polysaccharide–Induced Murine Vasculitis*

In this model, the coronary bifurcation and aortic root are the most frequent sites of vasculitis (Fig. 2a). In addition to the coronary arteries, vasculitis develops in renal arteries, common iliac arteries, at bifurcations of medium-sized arteries such as the intercostal arteries, and the aorta. In all vascular lesions, the main infiltrating inflammatory cells are neutrophils and macrophages (Fig. 2b) [16]. Small numbers of T lymphocytes are seen in the adventitia but almost no B lymphocytes. This

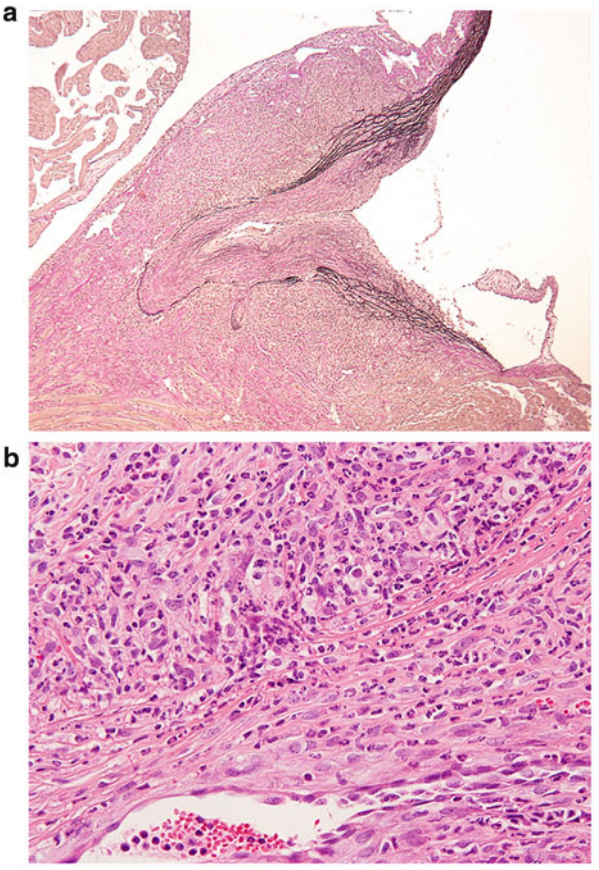

Fig. 2 Histology of murine coronary arteritis induced by polysaccharide extracted from *Candida albicans* with alkali. (**a**) Low-power view (elastica van Gieson stain), (**b**) High-power view (hematoxylin and eosin stain)

vasculitis follows a typical course of acute inflammation, ie, inflammation gradually disappears and lesions become scar tissue.

Relationship of Mouse Genetic Background to Cytokines and Vasculitis

The incidence of vasculitis development in this model differs among mouse strains, which indicates that genetic factors are involved in vasculitis development [16]. Although the disease-associated genes have not yet been identified, two chromosomal regions have been reported to be associated with vasculitis [17]. Numerous genes related to inflammation are clustered in those regions.

Cytokine production in response to exposure to *Candida*-derived polysaccharide also differs between mouse strains. Splenocytes obtained from a high-incidence mouse strain produced proinflammatory cytokines such as IL-1β, IL-6, and TNF-α,

whereas low-incidence-strain cells did not and instead produced IL-10, an anti-inflammatory cytokine [18].

Response of C. Albicans *Polysaccharide–Induced Vasculitis to Treatment*

The vasculitis in this model was suppressed by administration of a high-dose human immunoglobulin [19].

In recent years, anti-TNF-α agents have been used as additional therapy for IVIG nonresponders. Anti-TNF-α agents potently suppress vasculitis in this murine *C. albicans* polysaccharide–induced vasculitis model, and TNF-α is thus believed to be closely associated with vasculitis development [20].

There is still no animal model that exhibits all the clinical symptoms of KD. However, the *C. albicans* cell wall polysaccharide–induced murine vasculitis model described here is similar to KD vasculitis in histopathological characteristics and vasculitis course, the principal feature of KD.

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Histopathological Characteristics of Noncardiac Organs in Kawasaki Disease

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Abstract Kawasaki disease (KD) causes inflammation in medium-sized muscular arteries throughout the body, including the coronary artery, and is thus classified as a systemic vasculitis syndrome. In this chapter we review the histopathology of noncardiac organs, with a focus on vascular lesions. The main histopathological characteristic of KD vasculitis is proliferative inflammation consisting of marked accumulation of monocytes/macrophages. Vasculitis throughout the body starts at disease onset, rapidly reaches an inflammatory peak, and then slowly subsides and heals with scarring. KD vasculitis is thus a monophasic inflammatory process.

Keywords Kawasaki disease • Systemic vasculitis syndrome • Pathology • Macrophages

Introduction

Histopathological observation of Kawasaki disease (KD) has focused on the coronary artery because coronary arterial lesions are directly associated with mortality and long-term outcomes. However, noncardiac lesions must also be considered when describing KD pathology and etiology. In this chapter, we review the histopathology of noncardiac organs in KD, with a focus on vascular lesions.

Brief Overview of Systemic Vascular Lesions in KD

In the 1980s systemic vasculitis in KD was histologically evaluated in the body by Amano et al. [1], Hamashima et al. [2], Naoe et al. [3], and Landing et al. [4]. They reported that, although the incidence was highest for coronary arteritis, vasculitis developed at various other sites in the body (Table 1). Amano et al. [1] and

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Hamashima et al. [2] reported that vasculitis started in arterioles, venules, and capillaries, and inflammation disseminated to larger arteries, including the coronary artery. Naoe et al. [3] reported that KD vascular lesions started in the tunica interna and externa of medium-sized muscular arteries, such as the coronary artery. The size of vessels in which inflammation starts is unclear, but researchers agree that the histological characteristic of KD vasculitis is proliferative inflammation consisting of markedly accumulating monocytes/macrophages—fibrinoid necrosis is rare—and that vasculitis in KD starts at disease onset, rapidly reaches an inflammatory peak, and then slowly subsides and heals with scarring. Thus, KD vasculitis is a monophasic inflammatory process. However, Landing et al. [4] observed vasculitis scars in about one-third of arteries in patients who died during the acute stage (within 2 weeks after onset) and acute inflammation in about half of arteries even at 3 months after onset. These findings show that vasculitis during the acute and cicatricial phases is mixed in KD. Our observations indicate that the course of KD vasculitis is synchronous throughout the body [5]. The mixed presence of acute-phase and cicatrical-phase vasculitis is a histological feature of polyarteritis nodosa (PAN). Patients with PAN during childhood might have been included in the survey reported by Landing et al.

Histological Changes in Noncardiac Organs in KD

Kidney The incidence of panarteritis in kidney varies [6–8]. Asaji et al. [6] observed panangiitis or its resultant scarring in kidney arteries during autopsy in 75 % of KD patients who died 6 days to 11 years after KD onset. Arteritis developed in a patient who died on the 13th illness day, and proliferative inflammation was noted in patients who died on days 17–28. Inflammation resolved after day 30. Panangiitis is localized in the interlobar arteries and rarely develops in arcuate and interlobular arteries [Fig. 1]. Renal aneurysm is a known complication, and renal hypertension due to renal arterial stenosis has been reported [9]. Regarding glomerular lesions, the presence of segmental or global glomerulosclerosis has been frequently reported, but such changes are considered to be physiological changes occurring with childhood development, ie, infantile glomerulosclerosis. Focal segmental mesangial proliferation is another reported glomerular change in KD [7, 10]. Tubular changes were reported in 8 % of cases [6].

Liver Liver dysfunction is a frequent complication of acute KD. Tanaka et al. [11] performed liver biopsies of 19 patients at 7–36 days after KD onset and observed fatty and edematous degeneration of hepatocytes and severe inflammatory cell infiltration in the portal area in most of them. Vascular inflammation in the portal area was unclear, and hepatic changes were assumed to be caused by toxicity rather than by circulatory impairment. Ohshio et al. [12] reported frequent inflammatory cell infiltration in the portal area during acute KD and that, in the portal area, cholangitis and pericholangitis were more noticeable than vasculitis.

Table 1 Incidence of arteritis in various organs

	Amano [1]	Hamashima [2]	Naoe [3]	Landing [4]
Aorta	100 %	82 %	+	41 %
Carotid A	75 %		+	23 %
Subclavian A	71 %	67 %	+	
Celiac A	79 %	63 %	+	
Iliac A	100 %	93 %	+	
Coronary A	**100 %**	**95 %**	**95 %**	**100 %**
Renal A	80 %	64 %	73 %	55 %
Mesenteric A	79 %	86 %	+	27 %
Hepatic A	76 %	44 %	+	23 %
Intercostal A	58 %	60 %	+	
Spleen	50 %		11 %	50 %
Gastrointestinal tract			10 %	18 %
Paratrachea			+	36 %
Pancreas/peripancreas			31 %	36 %
Adrenal/periadrenal			+	32 %
Spermatic cord			+	41 %
Testis		67 %	15 %	18 %
Vagina			+	9 %
Uterus			+	5 %
Skeletal muscle				27 %
Meninges		36 %	1 %	5 %
Pulmonary A	71 %	50 %	59 %	32 %

Pancreas and Spleen Vascular lesions in the pancreas developed in 30 % of autopsy patients examined. The lesions were located at sites up to the pancreatic interlobular arteries. Vasculitis started on the 10th illness day, reached an inflammatory peak at about day 28, and then healed, although fibrous intimal thickening remained [13]. Yoshioka et al. [14] reported that inflammatory cell infiltration of the pancreatic duct and surrounding tissue and vasculitis were characteristic findings and that inflammation was marked in the pancreatic duct during acute KD. Regarding the spleen, arteritis was noted in the hilar and trabecular regions of arteries, and histological changes were similar to those in the pancreas [13].

Gallbladder Masuda et al. [15] histopathologically investigated gallbladders that were surgically excised after a diagnosis of cholecystitis in four patients with acute KD and observed characteristic nonspecific acalculous cholecystitis. Regarding vascular changes, perivascular cell infiltration was present, but panangiitis was noted in only one of the four patients, and panangiitis was noted in an artery in the subserosal layer. Gallbladder inflammation improved as KD resolved, which indicates that surgical excision of a swollen gallbladder is unnecessary in patients with KD.

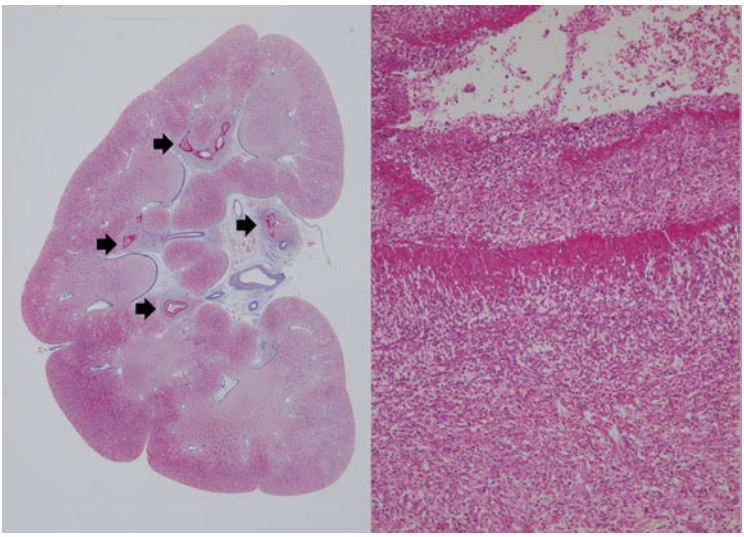

Fig. 1 Arteritis in the kidney. Panarteritis is localized in the interlobar arteries in the renal hilar region (*arrow*). The lesions show proliferative inflammation consisting of markedly accumulating macrophages. (*left*: Azan-Mallory stain; *right*: H & E stain)

Gastrointestinal Tract Vasculitis was noted in 10 % of KD patients at autopsy [16] but was exclusively localized in arteries in the subserosa and not present between the mucosal and muscular layers. Ulcers were present in three patients, and reactive hyperplasia of lymphoid follicles was often noted in mucosa at the end of the ileum. Nagata et al. [17] immunohistochemically investigated biopsy specimens of small-intestinal mucosa and hypothesized that the antigen that activates CD4-positive cells in intestinal mucosa and intestinal epithelial cells is associated with KD development.

Skin Changes in the skin are a principal clinical finding in KD diagnostic guidelines, and many pathological observations have been reported. These reports can be summarized as follows [18, 19]: (1) skin lesions are characterized by markedly inflammatory edema accompanied by vasodilatation in the dermal papillary layer and fibrin exudation; (2) endothelial cells are enlarged and surrounded by infiltrating monocytes/macrophages and CD4-positive T cells, although very few neutrophils and B cells were present; and (3) panangiitis is absent. Immunohistological studies showed that IL-1α and TNF-α are strongly positive in the acute phase but negative during recovery [20]. These changes are marked in BCG vaccination scars, and granulomatous inflammation was noted in some patients [21].

Lymph Nodes Cervical lymph node swelling is also a principal clinical finding in KD diagnostic guidelines and is present in 70 % of acute cases. Yokouchi et al. [22] reported that histological changes occur not only in the cervical region but also in lymph nodes throughout the body. Most lymphadenopathy is nonspecific and is

caused by sinus expansion and paracortical zone enlargement, but there are also necrotic lesions of various sizes that are likely due to ischemic changes in some lymph nodes. Necrotic foci start to develop immediately below the capsule and are accompanied by fibrin thrombi in small vessels and perivascular nuclear debris. Especially in cases of cervical lymph nodes with necrosis, a high degree of nonpurulent inflammation develops in the lymph node capsule and surrounding connective tissue.

Lung Panvasculitis developed in the pulmonary artery in 59 % of patients within 60 days after onset, and inflammation was localized to the elastic pulmonary artery at sites up to the fourth branching [23]. The earliest change in the pulmonary artery was edematous dissociation of the tunica media in a patient who died on the 13th illness day. The condition progressed to severe panarteritis on the 25th–30th illness day. After day 30, inflammation started to subside, and scars formed in patients who died at 3 months. No aneurysm or arterial dilatation was noted in the pulmonary artery, perhaps due to low blood pressure. During acute KD, some patients have interstitial lung shadows. On autopsy, interstitial changes were observed in 31 % of patients who died on the 29th–57th illness day. Histologically, the changes corresponded to diffuse alveolar damage [24].

Central Nervous System Aseptic choriomeningitis and/or leptomeningitis was noted in about half of KD autopsy cases, and mild or moderate inflammatory cell infiltration by lymphocytes, monocytes/macrophages, and a few neutrophils was observed. Edema in perivascular or perineuronal areas and a localized spongy state were occasionally noted. Regarding cerebral blood vessels, perivascular inflammatory cell infiltration was observed, but panangiitis was not [25].

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Identification of Novel Kawasaki Disease Susceptibility Genes by Genome-Wide Association Studies

Yoshihiro Onouchi

Abstract Completion of the Human Genome Project has helped in identifying disease genes, particularly with regard to mapping high-density single nucleotide polymorphisms and development of high-throughput genotyping platforms, which have considerably advanced research on complex disorders. Genome-wide searches are now practical and led to identification of genetic variations within previously unexamined genes relevant to diseases. In a genome-wide linkage study, the author and colleagues discovered that *ITPKC* and *CASP3* are common susceptibility genes for Kawasaki disease. This prompted examination of the Ca^{2+}/NFAT pathway and a subsequent continuous series of newly identified Kawasaki disease susceptibility genes. The recent identification of the *FCGR2A*, *BLK*, *CD40*, and *HLA class II* gene regions in genome-wide association studies has shed new light on the pathogenesis of Kawasaki disease.

Keywords Kawasaki disease • Susceptibility gene • Single nucleotide polymorphism • Genome-wide association study

Introduction

Although clinical and epidemiological features suggest the presence of infectious triggers in Kawasaki disease (KD) pathogenesis, genetic components appear to have important roles. KD is thus a multifactorial disease, and its pathogenesis involves both environmental and genetic factors. These two elements must therefore be unraveled before the cause of KD is fully understood.

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History of the Genetic Study of KD

Until the draft sequence of the human genome was released, genetic studies of complex diseases were based on limited information of sequence variation in genes of interest. Most studies investigated several known polymorphisms in candidate genes (e.g., human leukocyte antigen [HLA] and cytokine genes) [1]. Unfortunately, these candidate gene studies could not identify a susceptibility gene that was repeatedly found to be associated with KD.

Genomic Studies of KD in the Post-Genomic era

Completion of the Human Genome Project, along with single nucleotide polymorphism (SNP) and haplotype mapping by the International HapMap project and the development of high-throughput genotyping platforms, has enabled genome-wide scans for susceptibility genes of complex diseases. In particular, establishment of a method for genome-wide association studies (GWAS) has dramatically improved such studies. Today, six susceptibility genes/loci for KD were found to have significant associations in GWAS (Table 1).

ITPKC

A SNP located in intron 1 of the inositol 1,4,5-trisphosphate 3-kinase C (*ITPKC*) gene, in the 19q13.2 region, was found to be significantly associated with KD susceptibility [2]. A positive linkage signal had been identified in an earlier genome-wide linkage study [3]. ITPKC is a kinase of inositol 1,4,5-trisphosphate, the second messenger molecule in the Ca^{2+}/NFAT signaling pathway, which transduces signals from various surface receptors (Fig. 1). ITPKC is believed to negatively regulate this pathway, and the associated SNP allele (C allele of rs28493229) reduces expression of *ITPKC* mRNA in peripheral blood mononuclear cells (PBMCs). The association of rs28493229 with KD has been replicated in several populations [4, 5].

CASP3

A positional candidate gene study of the 4q34–35 region, where a positive linkage signal was reported [3], identified SNPs around the caspase-3 (*CASP3*) gene that were significantly associated with KD [6]. CASP3 is an effector caspase that directly cleaves cellular proteins and triggers apoptosis. rs113420705, one of the

Table 1 Functions of KD susceptibility genes and possible roles in disease pathogenesis

Location	Gene	Gene product function	Effect of susceptibility allele on gene function	Possible influence of susceptibility allele on KD pathogenesis
1q23	*FCGR2A*[a]	IgG Fc receptor	Increased binding affinity of the protein to IgG2 isotype	Enhanced neutrophil/macrophage activation
4q34–q35	*CASP3*	Executioner of cellular apoptosis	Decreased mRNA expression	Increased longevity of activated immune cells
6p21.3	Undetermined (*HLA* or non-*HLA* genes)	Antigen presentation (HLA)	Unknown	Unknown
8p23–p22	*BLK* or *FAM167A*	BLK: nonreceptor protein tyrosine kinase FAM167A: function unknown	Decreased (*BLK*) or increased (*FAM167A*) mRNA expression[b]	Unknown
19q13.2	*ITPKC*	Kinase of inositol 1,4,5-trisphosphate	Decreased mRNA expression	Enhanced activation of inflammatory cells and vascular endothelial/smooth muscle cells
20q12–q13.2	*CD40*	Receptor of CD40L	Increased protein expression	Enhanced activation of inflammatory cells and vascular endothelial/smooth muscle cells

[a]It is possible that other variants within neighboring *FCGR* genes confer KD susceptibility
[b]Observation in B lymphoblastoid cell lines from a European population [14]

associated SNPs located in exon 1 of *CASP3*, affects *CASP3* mRNA expression, and the risk allele (A) expresses less *CASP3* mRNA, as compared with the opposite allele (G), in PBMCs [6]. CASP3 is pivotal in the apoptosis of immune cells; thus, reduced CASP3 expression likely facilitates sustained activation of immune cells and progression of KD inflammation. The results of several replication studies and a meta-analysis of these studies support an association of rs113420705 with KD [7].

FCGR2A

SNPs near the Fc gamma receptor (*FCGR*) gene cluster on chromosome 1q23 were associated with KD in a GWAS of a European population [5]. The strongest significant association was for a functional SNP of the Fc fragment of IgG, low affinity IIa, receptor (*FCGR2A*) gene, and this association has been confirmed in different ethnic groups [8, 9]. FCGR2A is expressed on neutrophils and

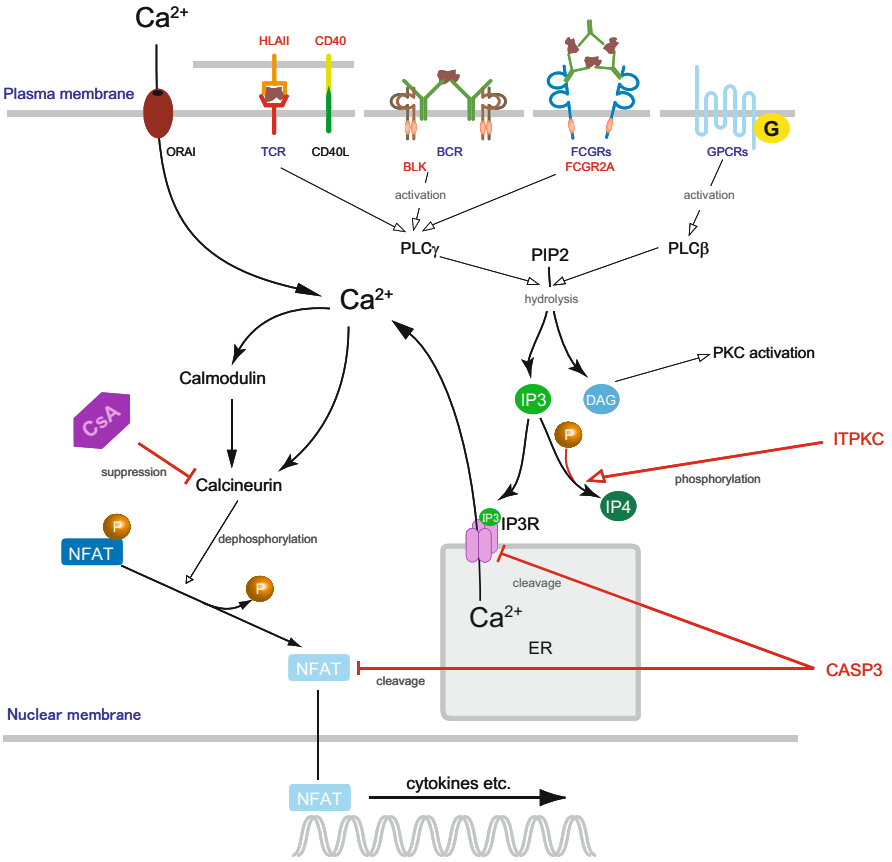

Fig. 1 Possible roles of KD susceptibility genes in the Ca^{2+}/NFAT pathway. *TCR* T-cell receptor, *BCR* B-cell receptor, *FCGRs* Fc gamma receptors, *GPCRs* G-protein-coupled receptors, *PLC* phospholipase C, *PIP2* phosphatidylinositol 4,5-bisphosphate, *IP3* inositol 1,4,5-trisphosphate, *IP3R* inositol 1,4,5-trisphosphate receptor, *IP4* inositol 1,3,4,5-tetrakisphosphate, *DAG* diacylglycerol, *PKC* protein kinase C, *ITPKC* inositol 1,4,5-trisphosphate 3-kinase C, *CASP3* caspase-3, *ER* endoplasmic reticulum, *NFAT* nuclear factor of activated T-cells, *CsA* cyclosporine A

macrophages and transduces the activation signal when ligated with immune complexes and clustered on the cell surface. The associated allele (A) of the SNP (rs1801274 A/G) changes the 131st amino acid from arginine to histidine and enhances its binding affinity to the IgG2 subclass.

BLK

Two independent GWAS, in Japan [9] and Taiwan [10], identified significant associations of SNPs in the 8p23–p22 region. The association peak at this locus was located in the intergenic region between the B lymphoid kinase (*BLK*) and family with sequence similarity 167, member A (*FAM167A*) genes. SNPs in this area have been associated with multiple autoimmune diseases. Because BLK is expressed mainly in B cells and is involved in B cell receptor signaling, *BLK*, but not *FAM167A* (which has not been characterized functionally), is considered a susceptibility gene because of the pivotal roles of B cells in autoimmunity.

CD40

CD40, also known as TNF receptor superfamily member 5 (*TNFRSF5*), is located on chromosome 20q12–q13.2. It is expressed on the cell surface of antigen-presenting cells and vascular endothelial cells and is stimulated when ligated with CD40L, which is expressed on activated CD4 T cells and platelets and transmits activation or differentiation signals into cells. A significant association of the SNPs around *CD40* with KD susceptibility was reported in the abovementioned two GWAS [9, 10]. The associated SNPs were in linkage disequilibrium with a known functional SNP that alters the efficiency of CD40 protein translation (rs1883832 C/T), and the C allele, which corresponds to higher CD40 protein expression, is linked with the SNP alleles conferring susceptibility to KD in this area. As with *BLK*, *CD40* is a common autoimmune disease susceptibility gene.

HLA class II

SNPs in the HLA class region were significantly associated with KD in a GWAS of Japanese KD patients [9]. This association peaked in the intergenic region between *HLA-DQB2* and *HLA-DOB*. Unfortunately, extended linkage disequilibrium and numerous genes with high sequence homology and densely distributed variations in this area complicate identification of the true susceptibility gene and variant. However, a better understanding of this association might help elucidate the contribution of HLA to KD susceptibility, which has long been controversial.

Current Understandings and Recognition

Although a number of the identified susceptibility genes appear to be related to immune function (Table 1), many have not been investigated as potential candidate genes in KD, and the exact functions of their products in KD pathogenesis are not understood. However, the existing evidence has provided several new insights. The association of the SNPs of *ITPKC* and *CASP3* (described in the next section "Recent Advances") has shed light on a new treatment strategy that targets the Ca^{2+}/NFAT signaling pathway [11, 12]. The robust association of the SNP in the *FCGR* gene cluster suggests the involvement of immune complexes in KD pathogenesis. Although the involvement of autoimmunity in KD has not been conclusively demonstrated, genetic components (*BLK* and *CD40*) shared with systemic lupus erythematosus and rheumatoid arthritis suggest a common pathophysiological mechanism between KD and other diseases. However, *ITPKC* and *CASP3* variants have not been associated with any other inflammatory/infectious disorders in GWAS and might reflect conditions highly specific to KD. It is clear there are many more unidentified susceptibility genes because the present evidence cannot fully account for differences in incidence rates among ethnic groups or for observed familial aggregation.

Recent Advances

Onouchi et al. reported that KD patients with susceptibility alleles of both *ITPKC* and *CASP3* had an increased risk for resistance to intravenous immunoglobulin therapy and coronary artery lesion formation [13]. This finding, together with previous knowledge of IP3R and NFATc2 cleavage in T cells by CASP3, suggests that CASP3 also acts as a negative regulator of the Ca^{2+}/NFAT pathway in KD pathophysiology (Fig. 1). Cyclosporine, a calcineurin inhibitor that specifically suppresses this signal transduction pathway, has received attention as an effective drug for refractory KD [11, 12].

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Immunological Abnormalities and Use of Biomarkers and Cytokines to Predict the Severity of Kawasaki Disease

Jun Abe

Abstract Although the cause of KD remains unknown, understanding of its pathogenesis has increased. An overt immune reaction triggered by unknown infectious agents may cause systemic vasculitis. The mediators of this reaction are mainly inflammatory cytokines such as TNF-α, IL-1β, and IFN-γ. Several genetic factors differentially affect susceptibility to KD in various ethnic groups. However, the mechanisms of overt immune reaction during acute KD and the cytokines/biomarkers that are best able to predict KD severity are not well understood. Knowledge of the systems biology of complex cytokine networks is essential for the development of new diagnostic and therapeutic strategies to prevent CAL formation in KD.

Keywords Biomarkers • Proinflammatory cytokines • Interleukins • G-CSF • Systems biology

Introduction

Kawasaki disease (KD) is an acute systemic vasculitis associated with fever, cervical lymphadenopathy, skin rash, conjunctival injection, strawberry tongue, and induration of hands and feet. Although the cause of KD remains unknown, evidence regarding its pathogenesis is increasing. It is now known that an overt immune reaction triggered by unknown infectious agents is responsible for systemic vasculitis. The mediators of this reaction are mainly inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and interferon (IFN)-γ. In addition, genetic factors differentially influence susceptibility to KD in various ethnic groups. The recent success of biologic therapy, such as infliximab, in the treatment of intravenous immunoglobulin (IVIG)-nonresponsive KD suggests that TNF-α has a central role in KD pathogenesis [1].

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IVIG, the standard initial therapy for KD, reduces systemic inflammation and the incidence of coronary artery lesions (CAL). However, when KD is suspected in patients with fewer clinical symptoms, clinicians must weigh the possibility of unnecessary IVIG treatment against that of delayed diagnosis of KD. In addition, about 20–30 % of KD patients do not respond to IVIG, and develop CAL more frequently than do IVIG responders. Therefore, it is essential to identify risk factors associated with IVIG nonresponse, to allow rescue therapy to be started before coronary artery aneurysms develop.

This review will provide a current overview of cytokine storm, an important immunological abnormality in KD, and discuss the possibility of using cytokines and the other biomarkers as prognostic indicators of IVIG responsiveness and the risk of CAL formation.

KD and Hypercytokinemia

Cytokines are small proteins released by cells. They affect other cells by means of transfer signals relating to proliferation, differentiation, metabolism, and motility of the target cells. Cytokines include interleukins, chemokines, colony-stimulating factors, and interferons. The inflammatory cytokines, which have roles in innate immune response, have received most of the attention in KD pathogenesis. A pioneering study by Leung et al. noted that the monokines IL-1 and TNF made cultured vascular endothelial cells more susceptible to lysis by antibodies circulating during KD [2]. Later, researchers thought that overt secretion and consumption of inflammatory cytokines such as TNF-α and IFN-γ by T cells were important in KD pathogenesis because of the resemblance of clinical symptoms in KD and toxic shock syndrome, which is caused by *Staphylococcus aureus* infection. Such infection produces a superantigen, TSST-1, and is associated with cytokine storm [3]. Subsequently, a variety of cytokine and chemokine genes, such as IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), and vascular endothelial growth factor (VEGF), were cloned and measured in the plasma of KD patients, using ELISA [4, 5]. Today, the plasma levels of more than 30 cytokines are known to be elevated in KD (Table 1).

A characteristic of hypercytokinemia in KD is that innate immune cells such as neutrophils, macrophages, and dendritic cells—as well as endo/epithelial cells—are important in the production of inflammatory cytokines. In innate immune response, a variety of cells recognize and respond to infection by pathogens, and to injuries caused by burns, irradiation, and chemical exposures, and release inflammatory mediators responsible for acute inflammation (Fig. 1). These cytokines induce or suppress their own synthesis or that of other cytokines in other target cells and regulate the extent of inflammatory responses, so as to limit injury to the host, reduce inflammation, and eventually re-establish immune homeostasis (cytokine network). On the basis of this homeostatic perspective, cytokines are often classified as pro- and anti-inflammatory. Plasma levels of pro- and anti-inflammatory

Table 1 Hypercytokinemia reported in Kawasaki disease

Year of publication	Cytokine/Chemokine
1988	Tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interferon (IFN)-γ
1989	IL-6
1990	Soluble IL-2 receptor-α
1991	IL-2
1992	IL-8
1994	Soluble TNF-α receptors
1996	IL-4, IL-10
1997	Regulated on activation, normal T cell expressed and secreted (RANTES), monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1β
1998	Thrombopoietin, vascular endothelial growth factor (VEGF)
1999	Macrophage-colony stimulating factor (M-CSF), granulocyte-colony stimulating factor (G-CSF)
2002	Hepatocyte growth factor (HGF)
2003	IL-15, IL-17, CD40 ligand, IP-10, S100A12
2004	IL-18
2005	S100A8, S100A9
2006	Stromal cell-derived factor (SDF-1)
2007	Macrophage migration inhibitory factor (MIF)
2008	High mobility group box 1(HMGB1)
2010	IL-23, Transforming growth factor (TGF)-β[a]
2011	Brain natriuretic peptide (BNP)
2012	Resistin, hepcidin
2013	B-cell activating factor (BAFF)

[a]Decreased level

cytokines are elevated during acute KD [6]. Neonatal innate responses differ from those in adults. In response to most TLR ligands, neonatal immune cells produce less IL-12p70, IFN-γ, and TNF-α and more IL-1β, IL-6, IL-23, and IL-10 [7, 8]. This may explain the simultaneous elevation of TNF-α, IL-6, and IL-10 levels in the very early phase of KD inflammation.

Which Cytokines/Biomarkers Best Predict KD Severity?

The introduction of IVIG has led to better control of systemic inflammation in KD and decreased the prevalence of CAL from 20 to <5 %. However, fever and KD symptoms persist after IVIG in some patients, and this is associated with increased risk for CAL. Numerous studies have attempted to identify risk factors associated with IVIG nonresponse and have investigated patient baseline clinical and laboratory parameters. Among the factors studied, sex, age, white blood cell and

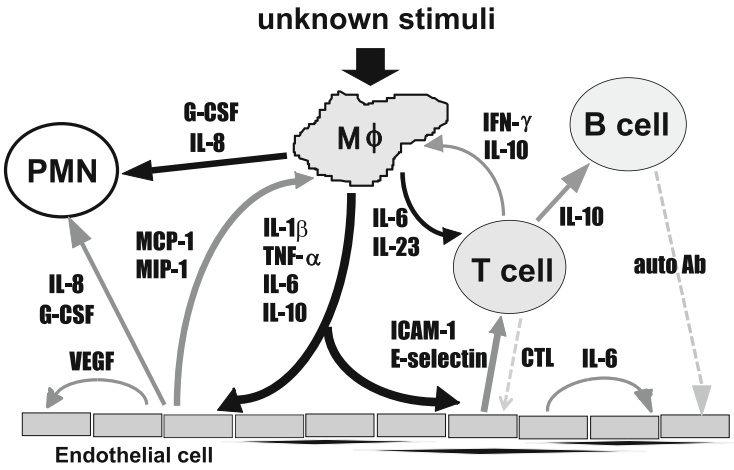

Fig. 1 Interactions between immune cells and endothelial cells in KD (Abbreviations: *PMN* polymorphonuclear leukocyte, *ICAM* intercellular adhesion molecules; *auto Ab* auto-antibody)

neutrophil counts, and serum aspartate aminotransferase and C-reactive protein (CRP) levels were frequently shown to be useful in devising a risk classification instrument [9–11]. (For a detailed review of risk scoring methods, see chapter "Scoring Systems to Predict Coronary Artery Lesions and Nonresponse to Initial Intravenous Immunoglobulin Therapy".) However, because these factors were selected by retrospective statistical analysis of medical records, the precise mechanisms underlying the relations of these factors with clinical outcomes remain uncertain. Moreover, it is unclear whether a particular risk classification is valid in all populations. Sleeper et al. reported that the sensitivity of three risk scoring systems used to predict IVIG resistance in Japan was low (33–42 %) in patients from North America [12]. They and another research group suggested that genetic differences between cohorts influence the effectiveness of these scoring systems.

Despite these limitations, some laboratory variables, such as neutrophil count and percent bands and plasma concentrations of CRP, appear to be higher in patients with severe KD. Tremoulet et al. reported that higher percent bands and CRP were strongly associated with IVIG nonresponse in 362 patients in San Diego [13]. In addition, DNA microarray studies conducted by the present author and colleagues and another group showed that neutrophils in IVIG nonresponders were more numerous and qualitatively different in their expression of an immature granulocyte-specific marker, polycythemia rubra vera 1 (PRV-1, CD177) [14, 15]. Similarly, serum granulocyte colony-stimulating factor (G-CSF) levels were higher in IVIG nonresponders than in responders. These findings suggest that G-CSF is overproduced by inflamed vascular endothelial cells in patients with severe KD and is involved in the expansion and premature egress of granulocytes from bone marrow. In addition, high-dose IgG specifically and completely inhibited overproduction of inflammatory cytokines such as G-CSF, IL-6, and IL-1β by cultured human coronary artery endothelial cells [16].

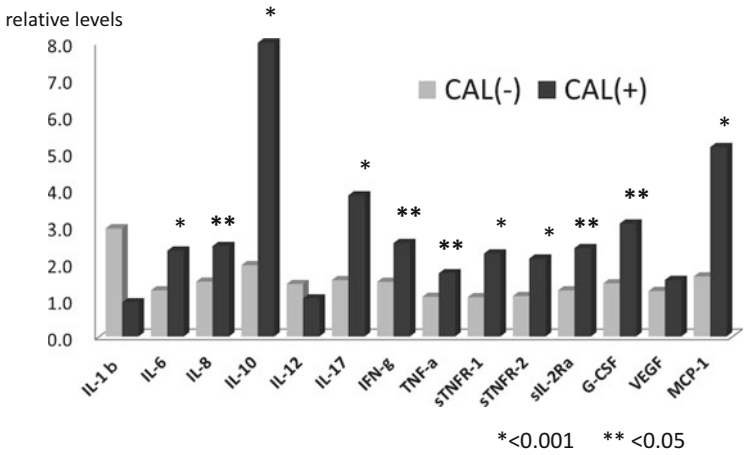

Fig. 2 Cytokines and chemokines that are elevated in IVIG-nonresponsive KD patients

In addition to G-CSF, a variety of pro- and anti-inflammatory cytokines (see Table 1) are overproduced in patients with KD. Moreover, plasma levels of most of these cytokines are higher in patients with more severe KD. However, it is not clear which of these cytokines can be used as clinical biomarkers to predict IVIG response and risk of CAL formation. Recently developed techniques in quantitative suspension array may help answer this question by analyzing patterns and correlations among cytokines/chemokines (cytokine profiling). In 2012, Wang et al. used this type of assay to analyze serum levels of IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ in 143 KD patients [17]. They found that IL-6 and IL-10 were particularly elevated, before and after IVIG treatment, in patients who later developed CAL. Our preliminary analysis using a multiplex bead assay system indicated that, among 14 cytokines studied in 273 KD patients before IVIG treatment, 8 proinflammatory cytokines (TNF-α, IL-6, IL-8, IL-17, IFN-γ, G-CSF, MCP-1, and sIL-2Rα) and 3 anti-inflammatory cytokines (IL-10, sTNFR1, and sTNFR2) were simultaneously elevated in patients who later developed CAL (Fig. 2) [18]. Moreover, levels of some of these cytokines were strongly correlated, particularly TNF-α, IL-10, sIL-2Rα, sTNFR1, and sTNFR2. These results suggest that both pro- and anti-inflammatory cytokines are relevant to KD severity and prognosis.

Conclusions

The use of newly developed methods such as quantitative suspension array technology and proteomics analysis of blood and urine is increasing our nascent understanding of the immune pathogenic mechanisms of KD [19, 20]. However, we have not yet identified the cytokines and biomarkers best suited for predicting

KD severity. Cytokine profiling shows that pro- and anti-inflammatory cytokine levels are simultaneously elevated in patients with more severe KD, which indicates that not every biomarker is an appropriate therapeutic target. An improved understanding of the systems biology of the complex cytokine networks is essential to the development of new diagnostic and therapeutic strategies to prevent CAL formation in KD.

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Pathophysiology of Kawasaki Disease

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Abstract Kawasaki Disease (KD) vasculopathy, which most significantly affects the coronary arteries, is characterized by three linked pathological processes: necrotizing arteritis, subacute/chronic (SA/C) vasculitis, and luminal myofibroblastic proliferation (LMP). Necrotizing arteritis (NA), initiated at the endothelial luminal surface, leads to giant aneurysms that can rupture or thrombose. SA/C vasculitis begins in the adventitia and is closely associated with LMP. LMP consists of actively proliferating smooth muscle cell-derived myofibroblasts and their matrix products, and can result in progressive arterial luminal stenosis. All three processes begin in the first 2 weeks after fever onset. NA subsides in the first 2 weeks, while subacute/chronic vasculitis and LMP can persist for months or years. The clinical and epidemiological features of KD are best explained by infection with an as-yet-unidentified ubiquitous agent, likely a virus entering via the respiratory route. Recent advances in genomics and RNA sequencing are beginning to reveal specific immune response dysfunction in KD that could lead to new diagnostics and therapeutics for this important childhood illness.

Keywords Necrotizing arteritis • Subacute/chronic vasculitis • Luminal myofibroblastic proliferation • Pathology • Gene expression

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