Universidade Estadual de Londrina

ANA PAULA LOMBARDI PEREIRA

LACTOBACILLUS CASEI CELL WALL EXTRACT (LCWE)-INDUCED MURINE VASCULITIS:

LONG-TERM EVALUATION OF DISEASE PROGRESSION AND POTENTIAL BENEFITS OF TREATMENT WITH SPECIALIZED PRO-RESOLVING LIPIDS

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Thesis submitted to the Health Science Ph.D. Program committee, as partial fulfillment of the requirements for the Ph.D. degree.

Mentor: Waldiceu Verri Jr, PhD.
Co-Mentor: Moshe Arditi, MD.

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"Hey Lord, you know I'm fighting. Hey Lord, you know I'll find it. I don't know when or how today. Hey Lord, I'm on my way." Labrinth.

"I have found that it is the small everyday deed of ordinary folks that keep the darkness at bay.

Small acts of kindness and love."

J. R. R. Tolkien

PEREIRA, Ana Paula Lombardi. ***Lactobacillus casei*** **cell wall extract (LCWE)-induced murine vasculitis:** long-term evaluation of disease progression and potential benefits of treatment with specialized pro-resolving lipids. 2024. 179 p. Graduate Ph.D. Program in Health Science, State University of Londrina, Londrina, 2024.

ABSTRACT

Kawasaki disease (KD) is a febrile syndrome that causes systemic vasculitis in children. It primarily affects the coronary artery (CA), forming aneurysms. Although intravenous immunoglobulin (IVIG) treatment is effective in reducing the incidence of coronary artery aneurysm (CAA), some patients do not respond to the treatment and have a higher risk of developing CAA. Studies have reported that children with a history of KD are morelikely to have cardiovascular complications later in life. However, the underlying mechanisms contributing to this process remain unclear. Unresolved inflammation is believed to be a significant factor in the development of cardiovascular diseases. A novel class of endogenously produced lipid mediators, termed specialized lipid mediators (SPMs), have been identified as critical regulators of the resolution during inflammation. We aimed to investigate the long-term histopathological changes in the murine model of *Lactobacillus casei* wall extract (LCWE)-induced KD vasculitis. We also reviewed the potential cardiovascular protection provided by SPMs in murine models of cardiovascular disease based on the currently available literature. Additionally, we investigated whether treatment with the SPMs could reduce inflammation in the heart vessels in LCWE-induced KD vasculitis. Our results show that LCWE can induce severe inflammation and cardiovascular lesions with ongoing inflammation, tissue scarring, and fibrosis in the cardiac tissue during the long-term evaluation, replicating the findings observed in KD patients. Furthermore, treatment with SPM can reduce inflammation in the heart vessels in the LCWE-induced KD vasculitis model during the acute phase.

Key-words: LCWE; Vasculitis; Inflammation; Resolution.

PEREIRA, Ana Paula Lombardi. **Modelo murino de vasculite induzido pelo extrato da parede celular de *Lactobacillus casei* (LCWE):** avaliação da progressão da doença e o potencial papel protetor do tratamento com mediadores lipídicos pro-resolução. 2024. 180 p. Programa de Pós-graduação em Ciências da Saúde, Universidade Estadual de Londrina, Londrina, 2024.

RESUMO

A doença de Kawasaki (KD) é uma síndrome que causa vasculite sistêmica em crianças afetando principalmente as artérias coronárias resultando na formação de aneurismas. Apesar do tratamento com imunoglobulina ser eficiente em reduzir a incidência de aneurismas nas artérias coronárias (CAAs), uma parte dos pacientes não respondem ao tratamento e apresentam maiores riscos de desenvolver CAA. Estudos demonstram que crianças com histórico de KD também apresentam maior probabilidade de desenvolver complicações cardiovasculares ao longo dos anos, porém, os mecanismos que contribuem para esses eventos tardios ainda não são inteiramente conhecidos. Sabe-se que a inflamação é um fator crucial no desenvolvimento de doenças cardiovasculares. A descoberta de uma nova classe de mediadores lipídicos pro-resolução (SPMs) produzidos de forma endógena, foram identificados como elementos cruciais na regulação da resposta inflamatória. Desta forma, os objetivos do presente trabalho foram investigar as alterações histopatológicas do modelo murino de vasculite induzida por LCWE e revisar o potencial papel cardioprotetor dos SPMs no contexto de doença cardiovasculares em estudos murinos disponíveis na literatura. Além disso, investigamos se o tratamento com SPMs poderia reduzir a inflamação no tecido cardíaco no modelo murino de vasculite induzido por LCWE. Os resultados demonstram que o modelo de vasculite induzido por LCWE induz inflamação severa e lesão cardiovascular com remodelamento e fibrose no tecido cardíaco ao longo do tempo replicando os achados observados em pacientes com KD. O tratamento com SPMs foi capaz de diminuir a inflamação nos vasos do coração no mesmo modelo murino de vasculite durante a fase aguda da doença.

Palavras-chave: LCWE; Vasculite; Inflamação; Resolução.

LIST OF FIGURES

LIST OF TABLES

ABBREVIATION LIST

AHA	American Heart Association
APC	Antigen-presenting cell
ARA	Arachidonic acid
ASA	Aspirin
AST	Alanine transaminase
AT	Aspirin-triggered
BSA	Bovine serum albumin
CAA	Coronary artery aneurysm
CAL	Coronary artery lesion
CAWS	*Candida albicans* water-soluble
COX	Cyclooxygenase
CRP	C-reactive protein
CYP450	Cytochrome P450
DAMP	Damage-associated molecule pattern
DHA	Docosaenoic hydroxy acid
ECG	Echocardiogram
ECM	Extracellular matrix
EKG	Electrocardiogram
EPA	Eicosapentaenoic acid
ESR	Erythrocyte sedimentation rate
GPCR	G-protein coupled receptors
GWAS	Genome-wide association studies
IgG	Immunoglobulin G
IL-1Ra	Interleukin-1 receptor antagonist
IVIG	Intravenous immunoglobulin
KD	Kawasaki disease
KDSS	Kawasaki disease shock syndrome
LC-MS-MS	Liquid chromatography-tandem mass spectrometry
LCWE	*Lactobacillus casei* cell wall extract
LMP	Luminal myofibroblastic proliferation
LOX	Lipoxygenase
LPS	Lipopolysaccharide

LT	Leukotriene
Mar	Maresin
MI	Myocardial infarction
MLNS	Mucocutaneous lymph node syndrome
MMP	Metalloproteinase
MyD88	Myeloid differentiation protein 88
NA	Necrotizing arteritis
NFAT	Nuclear factor of activated T cells
NLRP3	NLR family pyrin domain containing 3
NOD1	Nucleotide-binding oligomerization domain-containing protein 1
PAMP	Pathogen-associated molecule pattern
PD	Protectin
PMN	Polymorphonuclear neutrophil
PRR	Pattern-recognition receptors
PUFA	Polyunsaturated fatty acids
RvD	Resolvin D
RvE	Resolvin E
SA/C	Subacute/chronic
SNP	Single nucleotide polymorphism
SPM	Specialized pro-resolving mediators
TIRAP	TIR domain-containing adaptor protein
TNF-a	Tumor necrosis factor-
Treg	Regulatory T cell
VSMC	Vascular smooth muscle cell

TABLE OF CONTENTS

1 INTRODUCTION

Kawasaki disease (KD) is an acute febrile illness that affects children generally younger than five years old and older than six months. It was first described in 1967 in Japan by the pediatrician Tomisaku Kawasaki (1) and has been reported worldwide in all ethnic backgrounds, ranking as the leading cause of acquired pediatric heart disease in developed countries. KD is a severe vasculitis of medium vessels primarily affecting coronary arteries and can lead to cardiovascular complications stemming from the intense systemic inflammatory response (1,2). KD incidence is higher in children of Asian background, with Japan having the highest reported KD rates (3). Analysis of geographical disparities, seasonal patterns, and clinical and immunological characteristics suggest a potential infectious etiological agent(s) for KD (4,5). However, despite efforts, the specific trigger remains unidentified (3,4).

Principal clinical symptoms include persistent fever, skin rash, lips and oral mucosa erythema, non-purulent conjunctivitis, cervical lymphadenopathy, and changes in the extremities, such as swollen feet and hands (6). Without specific KD biomarkers, KD diagnosis is established based on four or more principal clinical symptoms and persistent fever (6). KD may lead to the development of coronary artery aneurysms (CAA) in 25 to 30% of affected children and can result in myocardial infarction (MI), sudden death, and cardiac ischemia disease within two years of disease onset (7,8). The standard KD therapy is a single high dose of intravenous immune globulin (IVIG) associated with aspirin, significantly reducing CAA incidence and preventing cardiovascular complications (9–11). However, some KD patients are not responsive to this treatment and are at a higher risk of developing CAA and other cardiovascular abnormalities (6,12,13). The pathophysiology of KD is complex and involves both adaptive and innate immune responses, with the overactivation of inflammatory cells and the production of pro-inflammatory cytokines, such as IL-1β (14,15).

Recently, a novel class of endogenously produced lipids, biosynthesized from omega-3 fatty acids, were identified and termed specialized pro-resolving mediators (SPMs). These mediators possess pro-resolution properties and induce a shift in immune

response towards anti-inflammatory and tissue repair mechanisms, thus actively contributing to the resolution of diseases caused by uncontrolled inflammation (14). Leukocytes produce SPMs at the site of inflammation by oxygenating docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are derived from omega-3 fatty acids. These lipids can be converted by lipoxygenases (LOX) or cyclooxygenase 2 (COX-2) into different lipid families, namely E-series resolvins (RvEs), D-series resolvins (RvDs), maresins (Mars), and protectins (PDs) (16,17). Metabolization of arachidonic acid (AA) from omega-6 fatty acids can generate another family of SPMs named Lipoxins (LXs) (18).

SPMs exert their actions by interacting with G protein-coupled receptors (GCPRs) on the surface of leukocytes and structural cells like endothelial cells (19–22). Although their mechanisms are not yet fully understood, research has shown that they can help enhance efferocytosis, limit leukocyte traffic, reduce proinflammatory cytokines at the site of inflammation, and stimulate the release of anti-inflammatory molecules (17,23,24). In studies conducted on animals with atherosclerosis, abdominal aortic aneurysm, ischemic heart disease, and heart reperfusion, researchers have discovered that using SPMs as a treatment can prevent the formation of lesions, reduce existing lesions, decrease necrosis and tissue damage, and improve overall conditions and outcomes, thereby providing cardiovascular protection (25–27). The findings suggest that SPMs have several mechanisms that help induce resolution and repair, including promoting a reparative macrophage phenotype, reducing adhesion molecules for neutrophils and monocytes in endothelial cells, decreasing smooth-muscle cell migration, and reducing proinflammatory cytokines production, amongst other essential actions (24,25). This unique ability of SPMs to balance resolution and inflammation is noteworthy and has prompted investigations for new therapeutic strategies.

In the following chapters, we will review the clinical features, diagnosis, treatment options, and long-term complications of KD. Additionally, the potential therapeutic effects of SPMs on cardiovascular disease will be discussed.

2 LITERATURE REVIEW

2.1 THE FIRST DESCRIPTION OF KAWASAKI DISEASE

In 1967, pediatrician Tomisaku Kawasaki published the first scientific report in a Japanese journal about an unfamiliar and novel syndrome he encountered while practicing at the Japanese Red Cross Medical Center in Tokyo (1). In this study, he carefully described the clinical features observed in 50 patients with this new syndrome (1). Initially, the illness was named mucocutaneous lymph node syndrome (MLNS), but it was later renamed Kawasaki Disease (KD) (1). In 1974, the American Academy of Pediatrics published the original report's English translation (1). Soon after, in 1976, pediatricians Marian Melish and Raquel Hicks at the University of Hawaii reported 12 similar cases of MLNS in the United States, and KD was officially recognized worldwide. By 1979, Japan had already registered over 24,000 KD patients, and the correlation with severe and often fatal cardiac events was well-established (28,29).

2.2 EPIDEMIOLOGY

2.2.1 Kawasaki Disease Incidence Worldwide

Following KD's first report by Dr. Kawasaki, Japanese investigators started to perform nationwide surveys in Japan, releasing epidemiological studies every two years to investigate and monitor the KD's epidemiological data (30). As more information became available, novel guidelines for identification and diagnosis were established and evaluated (6). Reviews of previous reports on infantile polyarteritis nodosa suggest that KD may have been misdiagnosed and mistaken with other pediatric diseases that shared clinical similarities (29). Indeed, KD diagnosis is complicated by the absence of a specific clinical feature, biomarker test, or laboratory finding (6,31).

KD cases are reported in all continents and ethnic groups (**Table 1**). Despite the initial challenges of monitoring the syndrome, epidemiological studies indicate increasing disease incidence (32,33). KD is the most prevalent cause of acquired heart

disease in children in developed countries (33). Epidemiological studies show increased incidence rates of KD in Asian children and children of Asian ancestry, suggesting possible genetic predisposition factors to KD development (33,34).

KD incidence is higher in Asia, with Japan having the highest worldwide incidence and continually increasing over the years, recording a peak in 2018 with 359/100,000 cases (children <5 years old) (35,36). The second highest incidence is in South Korea, with 191.0/100,000 in 2017 (children <5 years old) (37), followed by Taiwan in third place, with 75/100,000 (children <5 years old) reported in 2011 (38,39). Epidemiological data available from China indicates an incidence of 55.1/100,000 (children <5 years old) in Beijing and 104.6/100,000 (children <5 years old) in Shanghai (40,41). In India, a regional study in Chandigarh showed a KD incidence of 4.54/100,00 in children younger than 15 (42).

A study conducted in Hawaii from 1996 to 2006 gathered results from children with KD from different ancestries (43). The overall KD incidence in the studied population was 50/100,000 (children <5 years old); however, when the same data were analyzed according to their ancestry, Japanese children had the highest KD incidence, 210.5/100,000 (children <5 years old), and Caucasian children had the lowest KD incidence 13.7/100,000 (children <5 years old) (43). The same study showed that children from other different Asian backgrounds also exhibit significantly increased incidence varying from 64 to 86/100,000 (children <5 years old) compared with Caucasian children and the continental incidence in the United States (44). The United States and Canada reported similar incidences of 20.8/100,000 and 19.6/100,000 in children under five (43,44).

Epidemiological data from the Middle East and Africa are scarce. A recent study from Iran, using national registration data, detected an incidence of 3.03/100,000 (children <18 years old) in 2019 (45). Israel reported an overall incidence of 6.9/100,000 (children <5 years old) from 2005 to 2009 (46). KD Arab Initiative (Kawarabi) also reported low numbers of KD in Arabic countries (47). Despite the initial belief that KD could be rare in those regions, researchers have reasonably discussed that poor data, lack of training

and awareness of physicians to discern KD cases, and limited access to diagnostic tools, such as echocardiography, could be masking the real incidence numbers (47).

Moreover, information about KD in Latin America is also limited, likely due to the need for more awareness of the disease and the lack of mandatory reporting and national/local databases. The most extensive study conducted in South America to date comes from Chile, which reported an incidence of 8.4/100,000 (children <5 years old) in the period from 2005 to 2007 (48). Raising concerns about the lack of KD epidemiological studies in Latin America led to the creation of the international multicenter study Latin America Kawasaki Disease Network (original acronym REKAMLATINA). The project aims to enhance the management of KD in 20 Latin American countries through collaboration among groups of pediatric infectious diseases specialists. By analyzing clinical, demographic, and epidemiological data, they will conduct collaborative research to improve the understanding and treatment of the disease (49).

Epidemiological studies from Western and Northern Europe indicated varying incidence rates across different countries. The Czech Republic reported the lowest incidence in Europe, with 1.6/100,000 (children <5 years old) in a two-year prospective study (50). Switzerland has an annual average of 8.4/100,000 (children <5 years old) (51), the United Kingdom 9.1/100,000 (children <5 years old) (52), and the highest incidence is from a regional survey in Italy with 17.6/100,000 (children <5 years old) (32,44,53).

KD mainly affects children under five years old, with a heightened risk for those between 6 months and two years old (33). Males are at higher risk of developing KD, with a male-to-female ratio of 1.5:1 among patients (54). Regarding recurrence cases, Japan has reported a recurrence rate of 3.5%, while the United States has a rate of 1.7% (55). Amongst Asian children in the US, the recurrence rate is higher at 3.5% (55). No significant difference has been detected in recurrence rates between females and males (55).

Regarding fatality rates, Japan reported that patients with cardiac sequelae have higher risks of death when compared to patients with no cardiac sequelae (56). The study considered cardiac sequelae as aneurysms, stenosis, coronary artery

occlusion, myocardial infarction, and valvular lesion within one month after KD onset. Patients with cardiac sequelae had a standardized mortality rate of 1.86 (95% confidence interval, 1.02-3.13), and patients without cardiac sequelae had a mortality rate of 0.65 (95% confidence interval, 0.41-0.96). Mortality peaked 15 to 45 days after disease onset when CAA was established (57).

It is considered that most deaths related to KD stem from cardiac complications, which may occur even years after the initial diagnosis into adulthood, regardless of the treatment received. Myocardial infarction (MI) cases in adults, fatal or non-fatal, have been linked to undiagnosed KD during childhood, so-called "missed" KD (4,58).

Table 1. Kawasaki disease incidence.

Country/Region	Incidence[1]	Year	Source
Japan (35,36)	371/188	2019/2020	Nationwide survey
South Korea (37)	191.0	2017	Nationwide survey
Taiwan (39)	75	2011	National Health Insurance Research Database
Shanghai, China (40)	104.6	2017	Regional survey questionnaire
Beijing, China (41)	55.1	2004	Regional survey questionnaire
Chandigarh, India (42)	4.54*	2007	Regional questionnaire
United States (43)	20.8	2006	Nationwide survey
Canada (44)	19.6	2004-2014	Nationwide survey
Iran (45)	3.03*	2019	National Database
Israel (46)	6.9	2005-2009	National Database
Chile (48)	8.4	2005-2007	National Database
Czech Republic (50)	1.6	1997-1999	Hospital Surveillance database
Switzerland (51)	8.4	2013-2017	Hospital Surveillance database
United Kingdom (52)	9.1	2008-2012	National Database

[1] Cases >5 years old (per 100,000)
* Children <15 years old (per 100,000)

2.2.2 Genetic background

The geographical disparities in KD incidence led to further investigation of a possible genetic predisposition for KD susceptibility. Genome-wide association

studies (GWAS) have identified multiple single-nucleotide polymorphisms (SNPs) associated with KD susceptibility. SNPs linked to KD were identified in the following genes: *ITPKC* (Inositol-triphosphate 3-kinase C) (59,60), *CASP3 (Caspase 3)* (61), *BLK* (B lymphocyte kinase) (62), *CD40* (Cluster of differentiation 40) (62) and *FCGR2A* (Fc gamma receptor 2A) (63). Also, variants in three genes in the transforming growth factor β (TGF-β) signaling pathway, *TGFb2 (*transforming growth factor β2), *TGFbR2* (transforming growth factor β receptor 2*),* and *SMAD3* were associated with increased KD susceptibility and contribute to pathogenesis influencing disease outcome and response to treatment (64).

ITPKC acts as a negative regulator of T cell activity by inhibiting the Ca^{2+}/ nuclear factor of activated T cells (NFAT) signaling pathway (59). In a study published by Onouchi *et al.*, an SNP in the *ITPKC* gene was associated with prolonged activation of T cells during the acute phase, leading to KD susceptibility and a greater risk of coronary artery lesions (59,65). A variant in the *CASP3* gene was also identified with increased KD susceptibility and played a role in the same IP_3/Ca^{2+}/NFAT signaling pathway (61,65,66).

B-cells significantly affect the regulation of the immune response and T-cell activity through the interaction between CD40 and its ligand, CD40L. Thus, altered B cell function can affect the inflammation response during KD (67). Earlier studies have associated the presence of polymorphism in *BLK*, a crucial protein for B cell activation and development, and *CD40* with reduced B-cell signaling stimulation (62,68). This alteration contributes to the immunopathogenesis of KD and increases susceptibility (69–71).

The *FCGR2A* gene is responsible for encoding FcγRIIA, which is the Fc receptor portion of immunoglobulin G (IgG) (formerly known as CD32a) and is involved in antibody-dependent response (63). It has been observed that SNPs in *FCGR2A* can impact the effectiveness of IVIG treatment for KD, potentially leading to IVIG resistance (63).

The frequency of alleles among KD patients of different ethnicities can impact genetic association with disease susceptibility. Lee *et al.* demonstrated a discrepancy in the distribution of the *BLK* gene and its association with European descent

compared to Japanese and Taiwanese populations (62,72). *ITPKC* gene variant had no association in the Taiwanese population compared to Japanese and US children with KD (59,73). Interestingly, *CASP3* and *CD40* risk alleles are more frequently found in Europeans than in the Japanese population (67).

2.2.3 Etiology

KD etiological agent(s) remains unidentified despite many years of extensive research. Based on epidemiological data and clinical presentation, many hypotheses have been proposed since the first report of KD (74). Several countries have observed epidemic and seasonal variations in KD incidence, supporting this hypothesis. Japan reported epidemic cases in 1979, 1982, and 1986 (54). In addition, the US also reported increased KD incidence during the winter-spring season (5). The seasonality and geographical outbreaks of KD indicate that this disease could be transmissible among children (5,75). Indeed, in the last quarter of 2020, when strong sanitary measures were implemented to mitigate the spread of COVID-19, KD incidence significantly decreased in Japan (118/100,000 children <5 years old) (76). The incidence of KD cases in children under five years old peaked before the pandemic, with 315-359/100,000 cases from 2016-2019 (76). The notable reduction in KD incidence in 2020 was associated with the lower rate of infectious diseases among the population, possibly due to COVID-19 protective measures such as social distancing and masking, supporting further the infection-triggered pathogenesis hypothesis in KD (4,35,76).

KD clinical features during the acute phase of the disease, such as fever, skin rash, conjunctivitis, cervical lymphadenopathy, and oropharyngeal erythema, also support the infectious trigger theory as similar symptoms are also observed in other types of infectious diseases (33,77). The fact that KD primarily affects children under five years old, an age-restricted population, reinforces the infectious agent(s) theory (4,74). Despite the ability of viruses and bacterial superantigens to initiate a magnified immunological response that could explain KD onset, research groups have not yet demonstrated the association between a specific agent and KD (74,78).

2.3 Clinical signs of Kawasaki disease

KD's diagnosis is based on several clinical signs. The American Heart Association's (AHA) latest guideline considers high persistent fever as the primary symptom and determines disease onset (6). The fever has a remittent characteristic, with high spikes in temperature ranging from 100.4°F (38°C) to 104°F (40°C). It usually begins abruptly without any other sign and is little responsive to antipyretic drugs (6). The principal clinical features for KD diagnosis are skin rash, oropharyngeal erythema, non-purulent bilateral conjunctivitis, cervical lymphadenopathy, and erythema of hands and feet (**Table 2**). Patients with fever and at least four of these clinical features meet the criteria for KD diagnosis and are referred to as complete KD (typical KD) (6). Patients who only partially meet the diagnosis criteria are classified as incomplete or atypical KD (6,79).

Skin rash frequently appears after five days of disease onset, and the most common manifestation is usually extensive and starts in the trunk, moving forward to the arms, legs, and groin region (**Figure 1A, B)** (6,31). Reddening and swelling of the palms and soles of the hands and feet are also commonly present and may be followed by skin desquamation of the fingers and toes (**Figures 1C, D**). Changes in the nails may be visible during the chronic phase with transverse depressions adjacent to the proximal nail fold, known as Beau's line (31,79). The oropharyngeal and lips changes can present with diffuse erythema, dryness, fissures, peeling, or cracking. The tongue can also be affected, acquiring a red and bumpy appearance known as "strawberry tongue" (**Figure 1E**). Bilateral bulbar non-exudative conjunctivitis appears within the first couple of days after fever onset, can last up to 5 weeks, and resolves rapidly (**Figure 1G**) (6,31,79). Cervical lymphadenopathy is the most minor common event out of the five principal clinical signs for KD diagnosis criteria (**Figure 1H)**. It's usually unilateral, confined to the anterior cervical triangle with multiple swollen nodes (≥1.5 cm in diameter). Some patients may experience early and prominent cervical lymphadenopathy that can be misinterpreted as bacterial lymphadenopathy delaying KD diagnosis (6,31,79).

Table 2. American Heart Association diagnosis criteria for Kawasaki Disease.

- Persistent fever for at least five days (≥100.4ºF or 38ºC)
- Presence of at least 4 out of the 5 principal clinical features:
  1. Skin eruptions: maculopapular, scarlatiniform and erythema-multiform-like
  2. Peripheral extremities changes: erythema of palms and soles, edema of hands and feet, and periungual desquamation of fingers and toes
  3. Lips and oral mucosa changes: erythema, dryness, fissuring, peeling, cracking, strawberry tongue, diffuse erythema of oropharyngeal mucosa
  4. Bilateral bulbar conjunctivitis (non-exudative)
  5. Cervical lymphadenopathy (≥1,5 cm diameter)
- Exclusion of other diseases with similar clinical features

Although KD can cause coronary artery lesions and cardiac problems, systemic inflammation during the acute phase can also impact other organs and be responsible for additional symptoms. Common neurological signs include somnolence and irritability (80,81). Gastrointestinal findings are vast and commonly include diarrhea, vomiting, abdominal pain, mild hepatic dysfunction, and gallbladder hydrops (77). Some respiratory findings, such as parabronchial and interstitial infiltrates, can also be present (81).

KD diagnosis should be considered for every child with unexplained prolonged fever and principal clinical signs. Laboratory tests may provide extra support to indicate the best medical approach and detect non-classical KD cases. As the inflammatory response is intense during the acute phase, high counts of white blood cells, or leukocytosis, and granulocytes, mainly neutrophils, are observed (82). Erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) levels are also elevated (83). Thrombocytosis with high platelet count may occur during the second week of disease onset and normalize within six weeks (84,85).

On the other hand, thrombocytopenia during the first few weeks of disease onset, although rare, is associated with disseminated intravascular coagulation that causes severe systemic abnormal blood clotting, leading to higher risks of coronary artery abnormalities (6). Imaging exams like electrocardiogram (EKG) and

echocardiogram (ECG) can help detect cardiac function, coronary lesions, and CAA. Normal results during the first week of disease onset should not exclude KD diagnosis or development of eventual lesions since they are only detectable after the acute period (6).

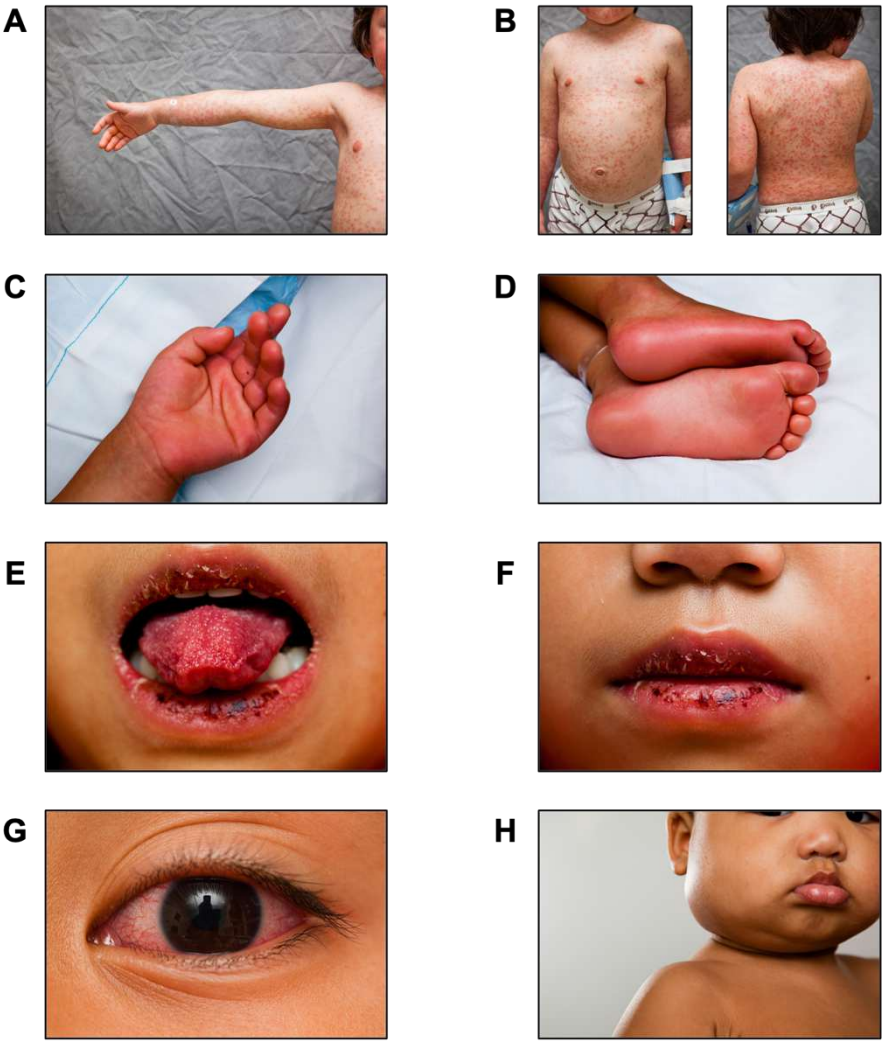

Figure 1. Principal clinical findings in Kawasaki disease patients. A, B. Diffuse maculopapular eruptions in the arms and trunk. C, D. Swollen and erythema of hands with painful induration. E. Strawberry tongue. F. Cracked lips with erythematous appearance. G. non-exudative bilateral conjunctivital injection. H. Unilateral cervical lymphadenopathy. The Kawasaki Disease Foundation, Inc. approved using photographs depicting clinical manifestations.

2.4 KAWASAKI DISEASE TREATMENT OPTIONS

The standard treatment used for KD is a single high dose of IVIG (2 mg/kg) combined with acetylsalicylic acid (aspirin; ASA) that should be administered as

early as possible, preferably within the first ten days of illness onset (6). It is also recommended to follow this protocol in children experiencing unexplained fever for more than ten days and in suspected incomplete KD cases (6,9). Although the specific mechanisms of action of IVIG are unknown, studies have established its effectiveness in reducing systemic inflammation by downregulating the production of inflammatory cytokines and preventing the development of cardiovascular lesions (86,87). However, up to 20% of patients with KD do not respond to IVIG treatment and are at a higher risk of developing complications and coronary artery lesions (88). Nevertheless, IVIG can still improve symptoms such as fever, skin rash, and conjunctivitis in resistant cases, improving overall clinical status (89–91).

ASA is a nonsteroidal anti-inflammatory drug with anti-inflammatory, antipyretic, and antiplatelet properties. It downregulates prostaglandin production and has a crucial antithrombotic effect by irreversibly inhibiting cyclooxygenase, decreasing platelet aggregation, and blocking thrombosis (92,93). ASA has been associated with a lower risk of myocardial infarction, and guidelines recommend aspirin administration as prevention for patients at high risk for cardiovascular events (92–94). The prescribed concentration of ASA during the febrile phase of KD is 80 to 100 mg/kg/d (high dose) and 30 to 50 mg/kg/d (moderate dose) by AHA and the Japanese Society of Pediatric Cardiology and Cardiac Surgery, respectively. A low-dose regime, 3 to 5 mg/kg/d, should be initiated to discontinue ASA treatment (91). Some prospective studies have questioned the appropriate dosage for ASA in KD, and a meta-analysis study showed that high doses of ASA do not reduce CAL incidence. On the other hand, doses of ASA >10 mg/kg/d have the same beneficial outcome as the >30 mg/kg/d regime. However, an optimal dose has not been confirmed (95–97).

Before the advent of IVIG, corticosteroids were the first choice for treating KD. Studies in Japan have reported that additional therapy with a single dose of methylprednisolone (30 mg/kg) or prednisolone (2 mg/kg/d) for five days, combined with standard IVIG and ASA treatment, helped reduce the incidence of CAAs, lower fever duration, decrease systemic inflammation leading to a rapid resolution of the acute phase (98–100).

2.4.1 IVIG-resistance and rescue treatment

Approximately 10-20% of children diagnosed with KD do not respond to IVIG treatment and require additional anti-inflammatory therapy (101,102). AHA defines IVIG-resistant cases as fever persisting or recurring after 36 hours of receiving the first IVIG infusion (6). This non-responsiveness is associated with an increased risk of developing CAA (99,103,104). The higher risk for disease severity associated with IVIG unresponsiveness led to the investigation of a system to aid clinicians in opting for a better course of treatment and follow-up. In 2006, Kobayashi et al. designed a scoring system to help predict IVIG resistance in Japanese KD patients based on laboratory and demographic factors (99). This scoring system is based on the levels of aspartate aminotransferase (AST), CRP, neutrophils percentage, sodium, platelet count, age of diagnosis, and treatment timeframe (99). However, the Japanese model was unsuccessful in predicting resistance in populations of different ethnic backgrounds, and further investigation is required to assess its accuracy in other ethnicities (105).

A combination of a second dose of IVIG that can be associated with alternative anti-inflammatory medications, such as corticosteroids and infliximab, is suggested to treat KD patients with IVIG resistance (6,106). Intravenous methylprednisolone, followed by high-pulsed methylprednisolone, has shown promise as an adjunctive therapy for IVIG-resistant patients (107). The treatment demonstrated a tendency to improve overall symptoms along with reduced rates of fever reoccurrence and coronary abnormalities when used with the second dose of IVIG (108,109). Infliximab is a monoclonal antibody that binds to tumor necrosis factor (TNF)-α, an essential cytokine that participates in inflammation. Studies have shown that infliximab lowers inflammatory markers (CRP and ESR) and reduces neutrophilia and fever duration (106,110).

Echocardiogram follow-up indicated less severe coronary artery lesions with infliximab co-treatment in a study considering infant KD patients aged 2-11 months old (110). The treatment was well-tolerated, and no significant side effects were reported after a single infliximab infusion (110–113). Further treatment is necessary to manage inflammation in cases where the patient does not respond to the second dose of IVIG,

infliximab, and corticosteroids. Some available options are intravenous cyclosporine, a specific inhibitor of calcineurin that blocks the calcineurin/NFAT signaling pathway and downregulates IL-2 transcription, preventing the hyperactivation of T cells that contributes to KD immunopathogenesis (71). Cyclosporin is an immunosuppressive medication, and it is administrated until fever subsides and the CRP levels normalize (71,114).

2.5 CARDIOVASCULAR COMPLICATIONS

Cardiovascular complications can manifest during the acute phase of KD but also years after the initial diagnosis and are the primary cause of mortality associated with KD (6,115,116). CAA is KD's most common cardiac finding, possibly leading to MI (117). Abnormalities in the coronary artery can be present as dilations or aneurysms, a weakened area in an artery that bulges out and may eventually rupture. These coronary abnormalities may vary in size, number, and appearance and are considered a specific criterion for diagnosing atypical KD cases (6).

Approximately 20-25% of untreated patients develop CAAs, which can be reduced to 4% with high-dose IVIG (118–120). Echocardiography is an important imaging tool for assessing coronary artery measures to detect CAA development. In 2017, AHA approved a guideline establishing the z-score system as the standard method for classifying and monitoring CAAs (6). The z-score is a measurement of the internal diameter of the coronary adjusted to the body surface area (BSA) and compared to the average diameter of a person with the same BSA to determine whether the internal diameter of the coronary artery is larger or smaller than average. This normalized measurement allows for a more accurate assessment over time (118). Japan has also implemented the z-score system alongside the conventional method of measuring the absolute coronary artery inner diameter, and a new classification scheme was established (**Table 3)** (6,121,122).

Table 3. Classification of coronary artery aneurysm based on American Heart Association guidelines combined with z-score and absolute dimension of the coronary artery diameter.

Classification of CAA	Z-score
Normal	<2
Dilation	2 to <2.5
Small aneurysm	≥2.5 to <5
Medium aneurysms	5 to <10, and absolute dimension <8 mm
Large or giant aneurysm	≥10, or absolute dimension ≥8 mm

Adapted from McCrindle, et al. Circulation, 2017 (6).

AHA highly recommends that patients with a z-score of ≥2.5 should be further evaluated by coronary angiography (CAG) for better visualization and thrombi detection (6). Most patients will have transient dilatation with a normal z-score (<2) that resolves within 4 to 8 weeks (123). However, 32 to 50% of patients with a normal z-score will show a gradual reduction of the luminal size over follow-up, compared to the first echocardiograph results used as baseline (124,125). Although uncommon, individuals with severe CAAs may also experience aneurysms in other medium-sized arteries that can result in thromboses or ruptures. Common areas for these aneurysms include the axillary, subclavian, brachial, femoral, iliac, splanchnic, and mesenteric arteries (110,113).

Myocarditis is the earliest manifestation of KD and the predominant non-coronary cardiac complication (115,116). Most KD patients will have echocardiography changes during the acute phase, with temporary left ventricle dysfunction, tachycardia, and gallop rhythm indicating sub-clinical and asymptomatic myocarditis. Histological examinations in the early stage reveal interstitial edema, inflammatory infiltration, and dilation of small vessels compatible with myocarditis. Effective therapy with IVIG can lead to complete restoration of myocardium function in mild cases where myocardial cells are still preserved (117,126). Studies on KD have mainly centered around CAA. Still, there is an increasing awareness of the significance of discussing the potential for noncoronary cardiac complications in KD patients over the long term (116,127).

Moreover, cardiovascular collapse and hemodynamic instability with multiple organ involvement is a severe complication that affects 5% of KD children in the US; it is also referred to as KD shock syndrome (KDSS). The episode happens during the acute phase with hypotension and shock and requires extensive medical care. KDSS

cases are usually unresponsive to IVIG treatment and are associated with coagulopathy, high inflammatory parameters, severe CAAs, and myocardial dysfunction (128–130). KDSS patients show impaired diastolic and systolic dysfunction, low ejection fraction, and mitral and aortic regurgitation. Although the z-score appears normal during follow-up, the abnormal ventricular diastolic function is still detected, and long-term consequences must be investigated (128,131,132).

CAAs or CA dilation is prevalent during the acute phase, and most cases are transitory, regressing within the first 3 months and completely resolving in 2 years (133). However, large CAAs have less chance of regression and are associated with higher risks of late cardiovascular complications due to stenosis, thrombosis, and calcifications that can lead to fatal or non-fatal events of MI and stroke over the following years. CAA can also rupture and block regular blood flow of the cerebral and carotid arteries causing ischemic stroke (134). Despite larger CAAs having lower changes of regression, it is not possible to confirm that smaller CAAs are at no risk of developing cardiovascular complications. Regardless of CAA status, KD patients are more likely to experience cardiovascular symptoms such as chest pain, palpitations, and shortness of breath (135).

KD patients have abnormal vascular endothelial function and possibly ongoing vascular inflammation and other intermediate markers for atherosclerosis, such as abnormal lipid profile and elevated CRP serum levels, that may early development of atherosclerosis (136,137). KD patients are also more likely to have other secondary complications like hypertension and peripheral vascular disease (135).

Although the acute febrile phase has a significant clinical impact, the cardiovascular alterations caused by KD can be carried on into adulthood and result in severe, sometimes fatal, events later in life (133,138). A few studies have addressed the long-term consequences and health outcomes of KD patients and reported strong evidence of cardiovascular alterations and tissue remodeling in the months and years following KD diagnosis (138). Myocarditis is an important complication of KD which can still be observed up to 11 years after the initial KD diagnosis and can lead to left ventricular dysfunction (127,139,140). Follow-up studies of KD patients revealed the presence of

histopathological alterations in endomyocardial biopsies with inflammatory infiltration, interstitial fibrosis, myocardium degeneration, abnormal myocardial arrangement, and ventricular hypertrophy up to 14 years after KD onset (141–143). Regardless of CAA presence, myocarditis with intense fibrosis reduces cardiac function and may progress to heart failure, being responsible for deaths associated with KD later in life (132,144). Fibrosis and myocardial ischemia may also lead to arrhythmias, and KD patients can show abnormal electrocardiograms and develop ventricular tachycardia post-MI (133,145).

2.6 Long-term follow-up of Kawasaki disease

Long-term management of KD-related cardiovascular complications requires follow-up surveillance according to the history of coronary artery involvement to determine whether changes in therapy or surveillance plans are necessary (6,31). The focus is to identify changes in the coronary artery that could favor the risks of thrombosis and stenoses, which may lead to ischemic heart events and, eventually, death. Additionally, surveillance helps detect changes associated with myocardial abnormalities and valvular function (6,31). In 2017, the AHA released a recommendation statement for the long-term follow-up of patients based on their CAA status, which is classified using the z-score system. The statement includes two algorithms: one for determining cardiology assessment indications and additional cardiovascular disease risk assessment and another for recommending thromboprophylaxis and medical therapy. The standard cardiology assessment involves thoroughly examining the patient's medical history and physical condition and an echocardiogram and electrocardiography (6).

Repeating the echocardiographic exam after 4 to 6 weeks of KD onset and acute treatment is recommended to reassess the coronary artery involvement. Patients with no lesions (z-score <2) can be discharged from cardiology care between 4 weeks to 12 months. Patients with only dilation (z-score 2 to <2.5) can also be discharged if the z-score decreases to <2 by the 4 to 6 weeks assessment. Otherwise, they should be monitored after six months to 1 year or until total regression. For patients with CAA,

the frequency varies according to the severity and progression of the lesions, ranging from every three months to 5 years based on the risk level classification (6).

To ensure proper care, occasional check-ups every 10 to 20 years are encouraged for any patient with a history of KD (6). Patients with thrombosis, obstructions, and myocardial ischemia symptoms should undergo additional cardiology evaluation, including inducible myocardial ischemia and angiography, to determine the presence of coronary artery stenosis. Regardless of a patient's CAA status, physical activity and age-appropriate reproductive counseling should be available at every health-care visit. It is highly recommended to assess and manage cardiovascular disease risk factors (6). While it is known that KD patients have a higher risk for cardiovascular events and abnormalities later in life, the real impact of CAA, myocardial changes, and cardiac sequelae, including fibrosis/scarring, have yet to be systematically investigated (6).

2.7 Immunopathogenesis of Kawasaki disease

Despite numerous investigations, the etiology of KD remains unknown, including the exact trigger(s) and the immune system response mechanism that initiates the pathological events. Studies analyzing cardiac tissues collected from fatal KD cases to characterize pathological processes involved in KD vasculopathy significantly contributed to the comprehension of the disease (146,147).

2.7.1 Vasculopathy process

Orenstein *et al.* detailed the histological findings and proposed a vasculopathy model with three linked events involved in KD vasculitis in the coronary and non-coronary arteries (147). The three characteristic vasculopathy processes identified were necrotizing arteritis, subacute/chronic vasculitis, and luminal myofibroblast proliferation (**Figure 2**) (147).

During the acute phase of KD, necrotizing arteritis (NA) is the first pathological process, characterized by intense neutrophilic infiltration, which resolves

within two weeks of fever onset. It begins in the endothelial surface of mid-sized arteries, mainly in the coronary arteries. The inflammatory infiltration breaks down collagen and elastic fibers, ultimately destroying the endothelial layer, intima, internal elastic layer, media, external elastic layer, and varying portions of the adventitia, depending on the severity degree. The intense neutrophilic infiltration progressively destroys the structural integrity of the vessel wall, forming saccular aneurysms that may thrombose or rupture. The resolution of NA after the acute phase gave the false impression that cardiovascular damages in KD were limited to the acute phase (147).

The subacute/chronic (SA/C) vasculitis typically starts two weeks after KD onset, initiates in the adventitia tissue, and may progress towards the lumen, leading to luminal myofibroblastic proliferation (LMP), and may last for months (147). Aneurysms form, and mild to severe tissue damage can be observed, eventually leading to thrombosis. In mild cases, the media and elastic laminas are preserved. The initial dilatation may return to normal, remain as a mild fusiform aneurysm, or progress to severe stages. Inflammation occurs in the circumference of the coronary artery and progresses along with LMP. This process, called SA/C-LMP, happens simultaneously until the intra-luminal lesion is complete (147).

As inflammation aggravates, saccular aneurysms are formed with some degree of destruction of both internal and external elastic layers, and the media is also affected. In a severe stage, saccular aneurysms are at risk of thrombotic events. In some cases, media and internal elastic layer components may be preserved and differentiated from NA, where there is a destruction of the internal elastic layer (147).

During the third process, LMP may lead to complete luminal obstruction or stenosis of the affected coronary artery. Smooth muscle cells (SMCs) from the adventitia migrate to the media. During this migration, SMCs transform into myofibroblasts with wound healing phenotype and become more pleomorphic as they approach the lumen. SMC-derived myofibroblasts actively produce collagen and elastic fibers deposited in the intima layer. This intimal process is associated with the SA/C and can persist for months or years, ultimately resulting in complete luminal narrowing (147).

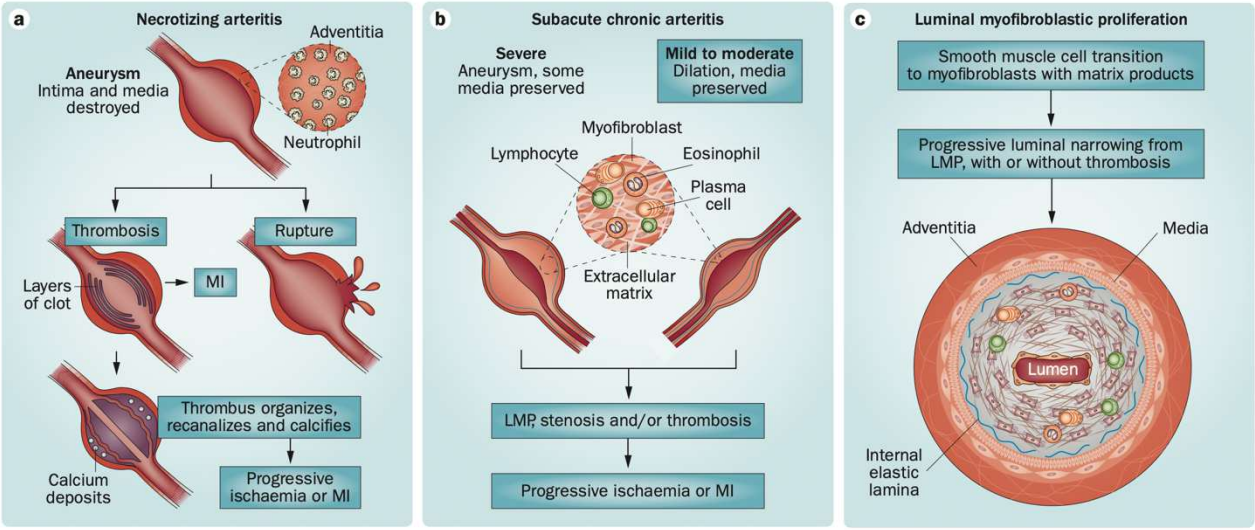

Figure 2. The three-linked vasculopathy process in Kawasaki disease. A. Necrotizing arteritis begins at the luminal endothelium, driven by neutrophil-mediated necrosis of the intima, media, and part of the adventitia. Although necrotizing arteritis resolves within two weeks after fever onset, large saccular aneurysms are formed, which can (rarely) rupture or (more commonly) gradually fill with layers of thrombus, potentially resulting in worsening ischemia or MI. Thrombus layers adjacent to the residual adventitia can calcify, and thrombi might recanalize. Subacute chronic arteritis and LMP are closely linked processes that begin in the first two weeks after fever onset but can persist for months to years. B. Subacute chronic arteritis begins in the adventitia and progresses toward the lumen. The infiltrate consists of lymphocytes, plasma cells, and eosinophils. Subacute chronic arteritis usually leaves some intact media and can result in fusiform arterial dilatation. C. LMP is a unique process involving the proliferation of medial smooth-muscle-cell-derived myofibroblasts and the build-up of their matrix products, potentially resulting in progressive arterial stenosis with or without thrombosis. Abbreviations: LMP, luminal myofibroblast proliferation; MI, myocardial infarction.
Figure from: Shulman S.T. and Rowley A. H. Nature Reviews Rheumatology, 2015 (148).

2.7.2 Immune response

The immune response specific to KD appears complex, involving innate and adaptive responses. The acute phase of KD is characterized by the infiltration of innate immune cells, mainly neutrophils, in the coronary artery and the excessive activation of inflammatory mechanisms with emphasis on the hyperactivation of NLRP3 inflammasome, increased production of IL-1β, and other pro-inflammatory cytokines, including IL-2, IL-6, IL-8, TNF-α, and IFN-γ (149).

Studies have demonstrated that KD is an IL-1-driven disease, which depends on NLRP3 inflammasome activation in both human KD and murine models mimicking the disease (150,151). The NLRP3 inflammasome is a multiprotein complex

responsible for the proteolytic activation of IL-1β and IL-18 and, therefore, is a crucial contributor to the innate immune response. NLRP3 is activated upon recognition of danger signals released by endogenous or exogenous sources, such as microbial, and sensed by damage-associated molecule patterns (DAMPs) and pathogen-associated molecule patterns (PAMPs) from different microbial organisms (152). The inflammasome is composed of an intracellular sensor (NLRP3), an adaptor protein (ASC), and an effector protein (Caspase-1).

The assembling of NLRP3 involves two signals. First, the priming signaling occurs upon stimulation of the upstream sensor TLR. It activates the transcription factor NF-κB that upregulates the expression of NLRP3, IL-1β, and IL-18. The second signal can be transduced by numerous stimuli, such as PAMPs and DAMPs, inducing a common cellular signaling that promotes NLRP3 inflammasome complex assembling and activation of Caspase-1, which in turn cleaves pro-IL-1β and pro-IL-18 into mature IL-1β and IL-18 (**Figure 3**) (152). The activation of the NLRP3 inflammasome is a tightly regulated process, and its dysregulation has been implicated in various inflammatory diseases (153). Studies have found that KD patients have increased serum levels of IL-1β and IL-18 during the acute phase of the disease (154,155).

NLRP3 and IL-1-related genes are upregulated during the acute phase (150,156). Moreover, it was demonstrated that ITPKC regulates NLRP3 activation via intracellular Ca^{2+} stimulation, which leads to the production of IL-1β and IL-18 (154). This finding is consistent with genetic studies identifying an association between ITPKC polymorphism and an increased risk of developing KD and IVIG resistance (60,66).

The strong evidence indicating that IL-1β plays a crucial role in the pathogenesis of KD and the development of cardiovascular lesions has led to investigation of the effects of IL-1 blockage (14). Treatment with Anakinra, an IL-1 receptor antagonist, blocks the binding of IL-1 to its receptor, which helps prevent the pro-inflammatory signal cascade from being triggered (157). Results from Phase II clinical trials demonstrated that Anakinra is safe and effective in reducing cardiovascular

complications in refractory cases of KD (157–163). These successful results encouraged the development of Phase III clinical trials that are currently ongoing (163).

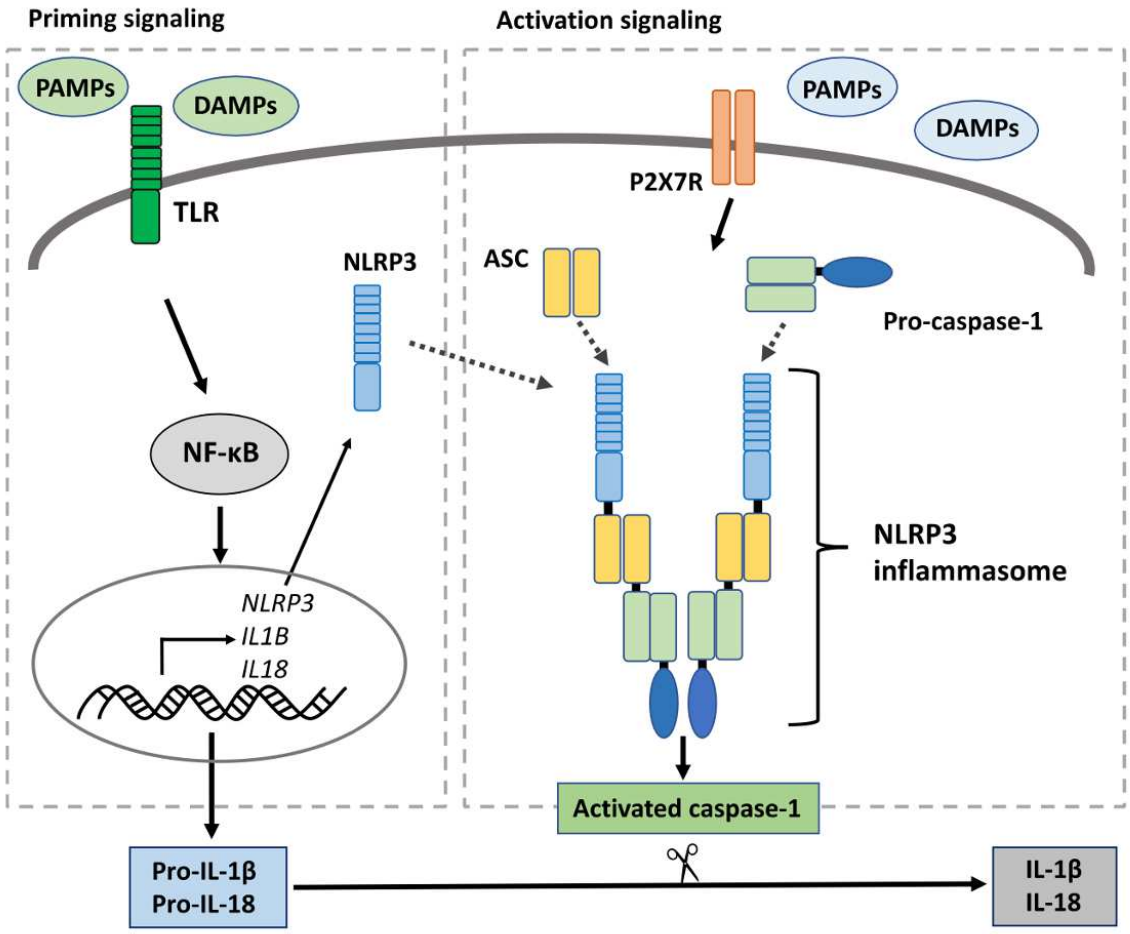

Figure 3. NLRP3 inflammasome activation pathway. NLRP3 inflammasomes are formed by priming and activation signaling. In the priming signaling, several PAMPs or DAMPs bind to the PRRs, such as Toll-like receptors, and activate the NF-κB signaling pathway, which leads to the upregulation of NLRP3, pro-IL-1β, and pro-IL-18. In the activation signaling, various PAMPs, DAMPs, or intracellular changes induce the formation of the NLRP3 inflammasome composed of NLRP3 as a PRR, pro-caspase-1, and adapter proteins such as ASC that connects NLRP3 and pro-caspase-1. Then, the inflammasome complex activates pro-caspase 1. The activated caspase-1 cleaves the pro-IL-1β and pro-IL-18 into IL-1β and IL-18, biologically activated forms.
Abbreviations: PAMPs, pathogen-associated molecular patterns; DAMPs, danger-associated molecular patterns; PRRs, pattern-recognition receptors; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; NLRP3, NOD-like receptor family pyrin domain containing 3; TLR, Toll-like receptor.
Figure from Shimizu, T. et al. International Journal of Molecular Sciences, 2021 (164).

During the acute phase, circulating neutrophils and monocytes secrete alarmins from the S100 protein family (165,166). Alarmins can modulate cyclooxygenase activity and favor pro-inflammatory pathways with further recruitment of leukocytes, production of inflammatory cytokines, matrix metalloproteinases, and reactive oxygen

species in the inflammation site, contributing to tissue damage and ongoing inflammation (167). These two proteins combine to form the complex called calprotectin. There is an increase in plasma levels of S100A8 (calgranulin A) and S100A9 (calgranulin B) during the acute phase, and persistent high levels of calprotectin are associated with giant aneurysms in KD patients (168). Levels of S100A12 (calgranulin C) are also elevated and activate the MAPK/NF-κB pathway leading to IL-1β production (165,169).

A dysfunction and suppression of T cell subset activities contribute to KD immunopathogenesis. The regulatory T (Treg) cells /Th17 cell imbalance can affect the immunopathogenesis of KD. Treg cells play a crucial role in immune regulation by inducing the secretion of anti-inflammatory cytokines such as IL-10 and TGF-β, which help control the immune system's hyperactivation (170). Treg cells and their transcription factor FOXP3 are downregulated in patients with acute KD (171). Furthermore, the expression of the transcription factor ROR-γt expressed by Th17 cells and the frequency of Th17 cells are elevated during the acute phase of KD (170). Th17 T cells produce IL-17, which promotes the recruitment and activation of neutrophils and stimulates monocytes and fibroblasts to produce IL-1, IL-6, IL-8, and TNF-α, thus contributing to inflammation response perpetuation (170).

During the process of SA/C, cellular infiltration in the coronary artery mainly consists of small lymphocytes, particularly $CD8^+$ T cells, eosinophils, and IgA^+ plasma cells. A few scattered $CD68^+$ macrophages may still be present as they were previously recruited for efferocytosis during the NA's neutrophilic infiltration (146,172). TGF-β stimulates fibroblasts to transition into myofibroblasts with wound-healing properties responsible for tissue regeneration and undergoing regulated apoptosis. SMC-derived myofibroblasts are hyper-activated and persist in the lesions, assuming a non-resolving wound healing phenotype. SMC-derived myofibroblasts in vasculitis have a pathological role, and the exact mechanisms involved in this phenotype switch remain unclear (173).

Matrix metalloproteinases (MMPs) are enzymes that degrade extracellular matrix (ECM) and are considered inflammatory markers. Increased levels of MMP3 and MMP9 are detected in acute KD patients with coronary artery lesions (CALs)

and may play an essential role in elastic lamina destruction and LMP (174). Histological evaluation of tissues collected from fatal acute KD cases indicated that IgA^+ plasma cells infiltrate the coronary artery, pancreatic duct, and kidneys (172,175). An experimental mouse model that mimics KD characteristics demonstrated a dysfunctional gut barrier and IgA deposition in vascular tissues (172,175).

2.8 EXPERIMENTAL MURINE MODELS OF KAWASAKI DISEASE VASCULITIS

Research groups worldwide have dedicated arduous work to identify the etiology and the underlying mechanisms involved in KD pathogenesis that would advance diagnosis and therapeutic options. Cardiac tissues from KD patients are scarce and difficult to obtain, limiting the possibility of studying KD pathogenesis. Therefore, mouse models that mimic human KD vasculitis are essential tools that enabled researchers to uncover KD immune-pathological features and identify novel therapies and biological markers to support accurate diagnosis. Three murine models of KD vasculitis are associated with the induction of coronary artery aneurysms and aortitis: the *Lactobacillus casei* cell wall extract (LCWE), the *Candida albicans* water-soluble fraction (CAWS), and the nucleotide-binding oligomerization domain containing 1 (Nod1) ligand models (176).

2.8.1 *Candida albicans* water-soluble model

C. albicans is an opportunistic fungus that usually colonizes the human gastrointestinal tract. It can acquire pathogenic characteristics under certain conditions and cause various degrees of infection and inflammation, particularly in individuals with weakened immune systems (177). In 1979, Murata H. published a study demonstrating that an alkaline extract made from *C. albicans* isolated from the stool of acute KD patients had the potential to induce coronary arteritis in mice (178). Ohno N. later published that water-soluble polysaccharide fractions released by *C. albicans* cultivated in a fully synthetic medium could also cause systemic vasculitis and inflammation in the coronary arteries (177). The main components identified in the CAWS extract are the cell-wall-

related polysaccharides α–mannan and β-glucans, which interact with the immune system and the disease (177,179). Deleting the Dectin-2 receptor, a C-type lectin receptor, protects mice from developing vasculitis in this model. This implies the crucial role of recognition α-mannan present in the extract by the Dectin-2 receptor (180). Histopathological findings share similar characteristics observed in human KD vasculopathy with the destruction of media and internal elastic layer and intimal hyperplasia of the affected coronary artery during the acute phase (180). Neutrophils and macrophages infiltrate the inflamed area, and T lymphocytes can also be observed in the adventitia. This model induces arteritis in medium to large vessels, with a propensity to the aortic root and coronary artery bifurcation after five consecutive intraperitoneal injections of CAWS (178,179).

Studies have shown IL-1β and TNF-α signaling are crucial for developing myocarditis and coronary artery inflammation in the CAWS model (181). IL-1R deficient mice presented acute myocarditis but failed to progress to CAA (181). Whereas administration of an anti-TNF-α drug (etanercept) reduced the incidence and severity of coronary arteritis, genetic deletion of the TNF receptor prevented vasculitis in this model (182). These results suggest that TNF-α is a critical cytokine in this model's onset and progression of vasculitis (180–182). Blocking the IL-1 pathway through genetic deletion of *Il1b*, *Il1r1*, *Nlrp3,* or *Asc* prevents CAWS-induced vasculitis, indicating that NLRP3 inflammasome-driven IL-1β production is required (151). Neutralization of IL-1 using anti-IL-1β antibody also attenuates the vasculitis (183).

T and B cells also contribute to cardiovascular lesions in the CAWS model. Mice lacking T cells can still develop vasculitis, indicating that T cells alone cannot induce the disease in this model (184). Interestingly, *Rag1*$^{-/-}$ mice, lacking both T and B cells, have a lower incidence of cardiovascular lesions, which can be reestablished with wild-type mice but not with *CCR2*$^{-/-}$ mice, suggesting a critical role of CCR2 in CAWS-induced vasculitis (184). Additionally, the CAWS model is distinguished by reduced IL-10 and TGF-β levels, which hinder Treg differentiation, and increased IL-6 levels that facilitate T cell differentiation into Th17 cells, thus creating an imbalance ratio of Treg and Th17

cells and favoring inflammation progression (184). Nagi-Miura et al. showed that IL-10 levels were increased in CAWS-resistant CBA/J mice strain and not in the susceptible strains with predominant Th1 response (185). Nakamura et al. demonstrated that supplementation of IL-10 attenuated inflammation and improved cardiac function in CAWS-induced mice (186). This indicates that IL-10, an essential regulator of the Th1/Th2 response, downregulates the production of proinflammatory cytokines in the context of CAWS vasculitis. Moreover, CAWS components stimulate the Th1 immune response and induce vasculitis in mice with different genetic backgrounds, such as CD-1, C57BL/6N, and C3H/HeN mice strains (179). BALB/c mice exhibit a Th2-dominant immune response and resist CAWS-induced vasculitis (178,179). The susceptible mice strains that develop more severe vasculitis are associated with intense production of IL-1β, TNF-α, and other inflammatory cytokines (179).

2.8.2 Nucleotide-binding oligomerization domain containing 1 ligand model.

Nucleotide oligomerization domain-like receptors (NLRs) are a group of pattern recognition receptors (PRRs) specialized in identifying PAMPs. In the cardiovascular system, endothelial cells constitutively express NLRs and are the first structural cells to recognize endogenous danger signals and exogenous microbial components. The Nod-1 receptor, a member of the NLR receptor family, detects peptidoglycans from gram-negative bacteria and acts as an intracellular sensor. It triggers an inflammatory response via the RIP2 signaling pathway, ultimately activating the NF-κB nuclear factor and producing proinflammatory cytokines (187).

Nishio et al., reported that subcutaneous or oral administration of the synthetic Nod-1 agonist, FK565, in mice primed with lipopolysaccharide (LPS) could induce vasculitis affecting the coronary artery and potentially mimic KD immunopathological features (188). The Nod-1 ligand activates endothelial cells and causes them to express adhesion molecules and secrete chemokines to attract leukocytes to the inflammation site (187). The pathological findings show a site-specific inflammatory infiltration with neutrophils and macrophages and intima hyperplasia in the

coronary arteries, like the acute phase of KD (189). Whereas diffuse inflammation is seen in the aortic root, unlike LCWE or CAWS-induced vasculitis, this model does not cause aneurysms or persistent coronary arteritis (187,190).

In this model, T and B cells may not be required for immunopathogenesis as LPS-primed *Rag1*$^{-/-}$ mice still develop vasculitis after FK565 injection (191). Additionally, Ohashi et al. have demonstrated no difference in TNF-α levels in Nod-1 ligand-induced mice (189). However, the levels of IL-1β were found to be associated with the severity of vasculitis, indicating the critical role of the innate immune response in inducing vasculitis in this model (189). Further investigation is recommended to determine vasculitis's mechanism and histopathological features in this model.

2.8.3 *The Lactobacillus casei* cell wall extract (LCWE) model

The LCWE-induced KD vasculitis model is well-established and a widely accepted murine in the KD research community. This model closely imitates the cardiovascular lesions and histological changes observed in children with KD, including coronary arteritis, aortitis, myocarditis, and cardiac abnormalities (192). Importantly, this model has been instrumental in uncovering the underlying mechanisms of the disease and has provided significant insights into potential therapy targets (176).

Lactobacillus casei is a gram-positive bacterium commonly found in the human and murine gastrointestinal and urogenital systems. In 1985, Lehman et al. described that a single intraperitoneal injection of an extract composed of *Lactobacillus casei* wall fragments could induce coronary arteritis in mice (192). The extract is mainly composed of peptidoglycans and rhamnose. However, the specific mechanism(s) by LCWE to induce vasculitis remains unknown (192).

The previously described KD vasculopathy with three linked processes consisting of NA in the coronary artery with intense inflammatory infiltration in the aortic root, followed by SA/C vasculitis with destruction of the wall vessel and concomitant LMP process culminating in aneurysms and complete coronary artery luminal obstruction are also observed in LCWE-injected mice (147,192). The systemic inflammation caused by

LCWE can impact other arteries, such as the abdominal aorta, renal, and iliac arteries, leading to aneurysms and dilation (193). LCWE-induced vasculitis requires the TLR2 signaling pathway through the participation of the adaptor molecules, myeloid differentiation protein 88 (MyD88) and the TIR domain-containing adaptor protein (TIRAP). The TLR2 interaction activates NF-κB, prompting the transcription of genes related to the expression of pro-inflammatory cytokines (194).

After LCWE injection, TNF-α can be detected in the serum and the inflammation site (195). It leads to the expression of chemokines and adhesion molecules, which facilitate the migration of lymphocytes to the coronary artery, contributing to damage to the vessel wall. TNF-α activity is also associated with elastin disruption, a key factor in aneurysm formation (195). The use of pharmacological blockade agents, such as etanercept and *TNFR1*-deficient mice, abrogate the development of vasculitis after LCWE injection (195).

The critical role of IL-1β in the immunopathogenesis of KD was successfully demonstrated by Lee et al. in the LCWE-vasculitis model (14). The study found that mice deficient in Caspase1 and IL-1R fail to develop coronary arteritis after LCWE injection and that administration of recombinant IL-1β can reestablish the lesions in *Casp1*$^{-/-}$ mice. Importantly, blockage of IL-1R with the administration of IL-1Ra (Anakinra) can prevent LCWE-vasculitis (14). These results provided a significant understanding of the crucial part of IL-1β in KD immunopathogenesis and led to further investigation of novel therapeutics for KD aiming to block the IL-1β signaling pathway.

Serum levels of IL-1β are elevated in mice injected with LCWE, as observed in KD patients during the acute phase (14,154). Porritt *et al*. demonstrated that macrophages, monocytes, and dendritic cells infiltrating the cardiovascular lesions are the primary source of IL-1β (150). Vascular smooth muscle cells (VSMCs) respond to IL-1β signaling, contributing to KD immunopathogenesis progression (150). Moreover, the study showed that inhibition of NLRP3 activation and blockage of IL-1β signaling in VSMCs prevented the development of cardiovascular lesions (150). These findings support that the NLRP3-IL-1β signaling pathway is pivotal in KD immunopathogenesis.

These observations made from studies with human data and the LCWE-induced KD vasculitis mouse model provided valuable insights into the critical involvement of IL-1β in KD immunopathogenesis. LCWE-induced vasculitis also responds to IVIG treatment and IL-1Ra antagonist (Anakinra), significantly reducing IL-1β levels and cardiovascular lesions (14,157). Altogether, these data indicate that IL-1β is a crucial cytokine for KD immunopathogenesis and reiterate the translational importance of the LCWE model (14). In addition, Porritt et al. also reported sex differences in the model, with increased incidence and severity in male mice resembling human data of KD that shows higher incidence and enhanced expression of the IL-1β signaling pathway in male patients (196).

Recently, it was also observed that this model was associated with a dysfunctional intestinal barrier and the deposition of IgA in inflamed vascular tissues (172). Participation of innate and adaptive immune responses is also observed in the LCWE model. According to Schulte et al., T cells contribute to the model since *Rag*$^{-/-}$ mice do not develop any lesions, and positive CD3 staining is found in the lesion, indicating T cell involvement. On the other hand, B cell-null mice develop coronary arteritis similar to WT mice after LCWE injection, suggesting that B cells are not required (15). In addition, $CD4^+$ and $CD8^+$ T cells are present in the lesion area, but depletion of $CD8^+$ with specific antibodies decreases vasculitis development, indicating that $CD8^+$ T cells contribute to LCWE-induced KD vasculitis (197). In KD patients, a higher infiltration of $CD8^+$ T cells than $CD4^+$ T cells is observed in the coronary artery, and genes associated with activated cytotoxic T cells are upregulated (198,199). In LCWE-induced vasculitis, mice exhibit increased expression of genes associated with cytotoxic T cell function in cardiac tissue (197). Although the exact mechanisms by which cytotoxic T cell exert their actions in KD remains obscure, these results support the concept that $CD8^+$ T cells are crucial for KD.

The LCWE-induced vasculitis model also shows functional heart dysfunction, a decreased ejection fraction, and cardiac rhythm abnormalities during the acute phase (157). Myocarditis is also observed in LCWE-injected mice (14,200). Therefore, it appears to be the most promising murine model to investigate acute mechanisms and long-term cardiovascular lesions associated with KD. By closely

reflecting the time course and therapy response, including Anakinra and histopathological features of human KD, the model has proven to be of enormous value, supporting researchers to gain insight into the pathophysiology of KD and explore potential treatment options that ultimately may improve disease management. Nevertheless, it is important to conduct a thorough and detailed investigation to provide a comprehensive description of the long-term histological findings in this model, as KD research has focused on CAA development during the acute phase.

2.9 Resolving inflammation

Inflammation is an essential mechanism that protects the body against unwanted invaders and supports tissue repair when damage occurs. However, the body's response to inflammation can sometimes become too intense and uncontrolled. This excessive inflammatory activation is the leading cause of many diseases, including cardiovascular diseases such as KD (24,199,201). Therefore, understanding the underlying cause of this imbalance is crucial for developing new treatment approaches.

Acute inflammatory response is divided into two phases: the initiation and the resolution phase. Intense recruitment of peripheral leukocytes at the inflammation site occurs during the initiation phase. Neutrophils are usually the first responders and are attracted by a chemoattractant gradient governed by lipids mediators, prostaglandins (PGE_2 and PGI_2), leukotriene (LTB4), and other molecules like cytokines, chemokines, and complement components that aid neutrophils transmigration to the tissue where it can exert its function (202,203). To prevent an excessive inflammatory response, limiting the influx of neutrophils to the site of inflammation and promoting efferocytosis by reparative macrophages to clear cellular debris at the inflammatory site is crucial. Under physiological circumstances, the inflammatory response is limited and switches towards resolution, which supports tissue repair and helps the return to homeostasis without further complications or tissue damage (**Figure 4**) (204,205).

The intriguing process when inflammation response switches towards the resolution phase was once believed to be a passive event or simply a mere consequence

of the declining activation of the proinflammatory response. Recently, the resolution process was recognized as distinct from the anti-inflammatory response by releasing specific molecules that induce resolution (205,206). When investigating an acute inflammatory model in mice, Serhan and collaborators uncovered a novel class of endogenous bioactive molecules in the inflammatory exudate that modulates neutrophil influx and downregulates proinflammatory cytokines, exerting both anti-inflammatory and pro-resolution mechanisms (207). These molecules were termed specialized pro-resolving mediators (SPMs) and are derived from polyunsaturated fatty acids (PUFAs) (16). Their discovery supports the hypothesis that resolution is a programmed event intrinsically orchestrated by these so-called SPMs (204).

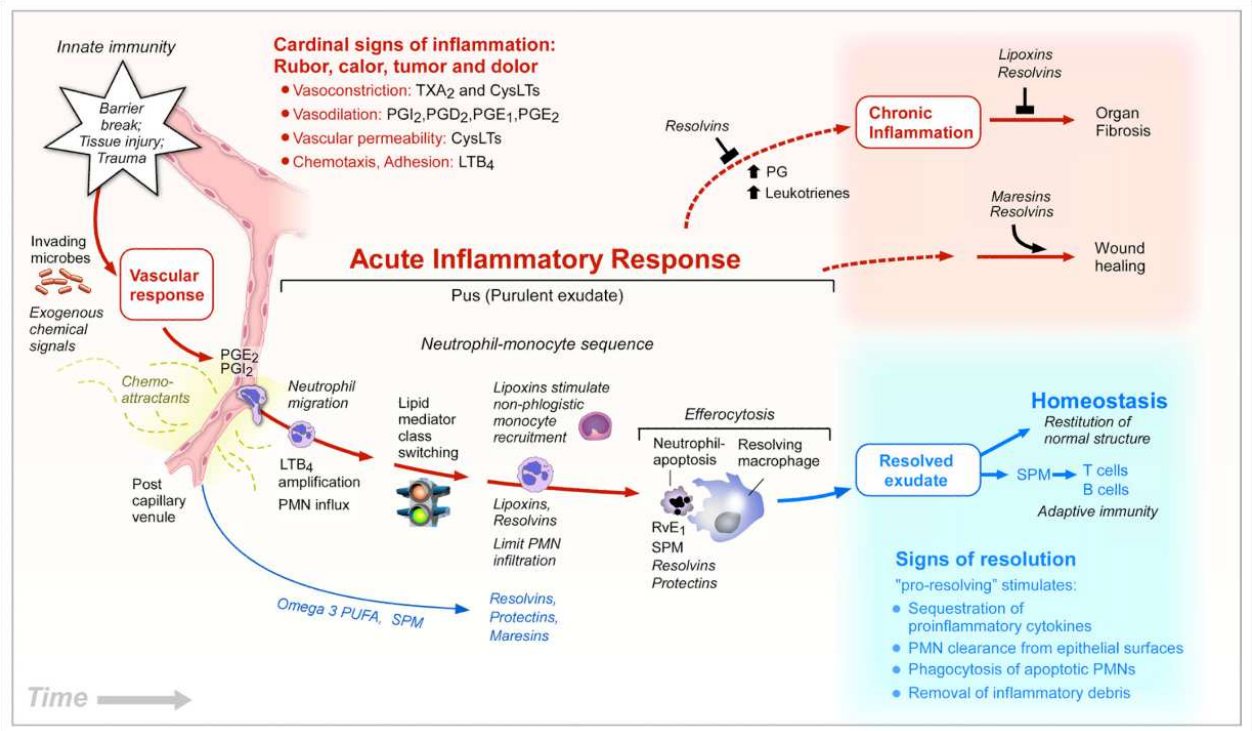

Figure 4. Lipid mediators in the acute inflammatory response, resolution, chronic inflammation, and other outcomes. SPMs play pivotal roles in the vascular response and leukocyte trafficking, from initiation to resolution. Eicosanoids are critical in initiating the cardinal signs of inflammation (upper left). The lipoxins, resolvins, protectins, and maresins are produced in self-limited responses to the initial inflammation stimuli. SPMs stimulate cellular events that counter-regulate pro-inflammatory mediators and regulate PMN, monocyte, and macrophage response, leading to resolution. Depicted are some pro-resolving actions in leukocyte trafficking (neutrophil-monocyte sequence), lipid mediator class switching, and efferocytosis of apoptotic PMN that must occur in resolving exudates for restoration of normal structure and homeostasis. SPMs enhance efferocytosis, stimulate signs of resolution (lower right), and signal adaptive immunity via lymphocytes. Failed resolution may lead to enhanced prostaglandins and leukotrienes, chronic inflammation, and fibrosis. SPMs counter-regulate pro-inflammatory chemical mediators, reducing the magnitude and duration of inflammation and stimulating re-epithelialization, wound healing, and tissue regeneration in model organisms.

Figure from Serhan, C. N. Nature, 2014 (208).

2.9.1 Pro-resolution lipid mediators

Advanced lipidomic studies enabled researchers to identify several SPMs and their intermediates using liquid chromatography-tandem mass spectrometry (LC-MS-

MS)-based metabololipidomics (205). SPMs comprise a family of structurally distinct endogenous lipid mediators, including lipoxins derived from ϖ-6-PUFA arachidonic acid (ARA), ϖ-3-PUFA-derived E-series resolvins from eicosapentaenoic acid (EPA), and D-series resolvins, maresins, protectins derived from docosahexaenoic acid (DHA) (**Figure 5**). These molecules are increased during resolution, and their production and release are perfectly timed and controlled during inflammation. Each SPM has distinct properties and exerts its action by interacting with G-protein coupled receptors (GPRs) present on the surface of leukocytes (207,209). PUFAs are carried to the inflammation site and are converted to SPMs by local leukocytes and some other cells, such as endothelial cells and platelets (22). They are first oxygenated by 12/15-lipoxygenase (LOX) originating lipids intermediates that are enzymatically converted in leukocytes in the inflammation site by 5-LOX to form distinct SPMs. Alternatively, intermediates can be metabolized via cytochrome P450 (CYP450) or by aspirin-acetylated-cyclooxygenase (COX)-2, generating aspirin-triggered (AT) isomers of SPMs (203,210).

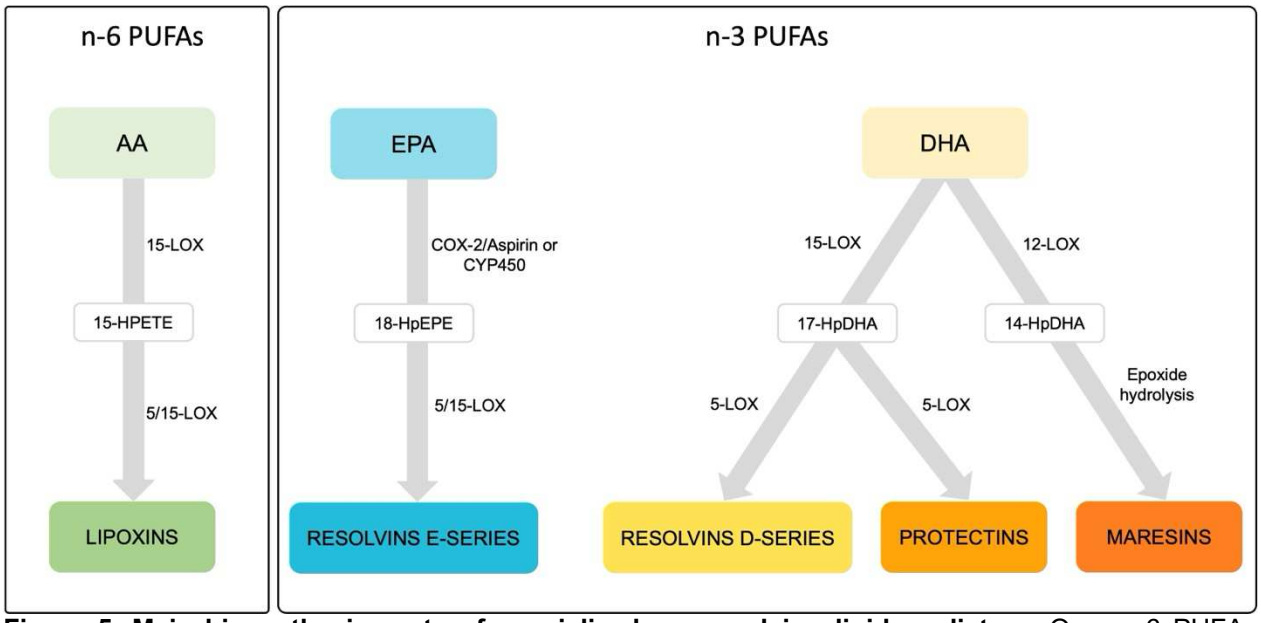

Figure 5. Main biosynthesis route of specialized pro-resolving lipid mediators. Omega-6 PUFAs produce arachidonic acid, which serves as a precursor for lipoxins. Omega-3 PUFAs generate EPA and DHA, the main precursors of most SPMs. EPA is metabolized into resolvin E-series, while DHA can be converted into resolvin D-series, protectins, and maresins.
Abbreviations: PUFAs, polyunsaturated fatty acids; SPMs, specialized pro-resolving mediators; AA, arachidonic acid; EPA, Eicosapentaenoic acid; DHA, docosahexaenoic acid; LOX, lipoxygenase; COX-2/Aspirin, Aspirin acetylates cyclooxygenase-2; CYP450, cytochrome P450; 15-HPETE, 15-hydroxyperoexyeicosatetraenoic acid; 18-HpEPE, 18-(hydroperoxy) eicosapentaenoic acid; 17-HpDHA, 17-hydroperoxyDHA; 14-HpDHA, 14-hydroxyperoxyDHA.

The EPA biosynthesis pathway leads to the formation of E-series resolvins (RvEs). During vascular inflammation, aspirin acetylates COX-2 in leukocytes or endothelial cells, which converts EPA into 18R-hydroxy-EPE (18R-HEPE) (24). Activated neutrophils uptake 18R-HEPE and convert it to aspirin-triggered RvE1 (AT-RvE1). Through metabolization by 5-LOX, the biosynthesis route generates the intermediate 18S-HEPE, further converted into RvE1 and RvE2 in neutrophils. RvE1 can be metabolized via CYP450-oxygenation as well. Eosinophils can convert 18R-HEPE into RvE3 by 12/15-LOX (24). The DHA leads to D-series resolvins, maresins, and protectins. Endothelial cells can metabolize DHA via COX-2 to produce the intermediate 17R-hydroxy-docosahexaenoic acid (17R-HDHA). Neutrophils convert 17R-HDHA using 5-LOX to generate AT-resolvin D1-4. AT-RvD1 can also be produced via an aspirin-independent

pathway through CYP450. Additionally, DHA can be metabolized via 12/15-LOX to generate 17S-HDHA and further be metabolized by 5-LOX to produce RvD1-6 (24).

The Maresins family also derives from DHA. They were initially described as "macrophage mediators in resolving inflammation," but other leukocytes can also produce them. DHA is converted by 12-LOX to form 14-hydroperoxy docosahexaenoic acid (14S-HpDHA), which is further enzymatically converted to form the maresin family. This family comprises maresin-1 (Mar1), maresin-2 (Mar2), maresin conjugate in tissue regeneration (MCTRs), and maresin-like lipid mediators (Mar-Ls). To generate protectin (PD), DHA can also follow metabolization by LOX enzymes or COX-2, generating PD1 or AT-PD1, respectively (208).

Overall, SPMs can limit polymorphonuclear (PMN) leukocyte infiltration and enhance efferocytosis, aiding in microbial and inflammation clearance and tissue repair, ultimately leading to homeostasis (208). These actions are cell-specific, mainly with the direct participation of neutrophils and macrophages. As mentioned, SPMs can interact with immune cells through GPCR interaction. However, other mechanisms and receptors that SPMs use have yet to be further elucidated and identified (24).

Two receptors were reported to interact with RvD1, the lipoxin A4 receptor/formyl peptide receptor 2 (ALX/FPR2) and GPR32 (or DRV1). These receptors are expressed in various types of cells, including leukocytes, vascular cells, and structural cells. In humans, both ALX/FPR2 and GPR32 are involved in the beneficial effects of RvD1, including limiting leukocyte infiltration and enhancing macrophage efferocytosis. However, murine lacks a GPR32 homolog and seems to exert its action through ALX/FPR2 interaction (211). RvD2 can bind with GPR18, stimulating the phagocytosis of debris and apoptotic PMNs (20).

RvE1 interacts with two receptors, Chem23 and BLT1, in human leukocytes. It has several effects, including blocking the transmigration of PMNs through endothelial cells, reducing the production of superoxide, and decreasing the activity of antigen-presenting cells (APCs) by reducing the production of IL-12 through Chem23. BLT1 is also a receptor for LTB4. When RvE1 binds to BLT1, it blocks the activation of

NFκ-B induced by LTB4, acting as a local damper for LTB4 and preventing the propagation of proinflammatory signs exerted by LTB4 (21,212).

Table 4. Receptors identified to interact with specialized pro-resolving lipid mediators.

Receptor	SPMs	Interaction	References
ChemR23 (or ERV1)	RvE1 RvE2	Agonist	Arita, et al. (213) Oh, et al. (214)
BLT1	RvE1 RvE2	Inhibitor	Arita, et al. (213) Oh, et al. (214)
FPR2/ALX	LXA4 RvD1	Agonist	Krishnamoorthy, et al. (19)
GPR18 (or DRV2)	RvD2	Agonist	Chiang, et al. (20)
GPR32 (or DRV1)	RvD1 RvD3 RvD5	Agonist	Arnardottir, et al. (215) Norling, et al. (216) Dalli, et al. (217) Chiang, et al. (218)
LGR6	Mar1	Agonist	Chiang, et al. (219)
TRPV1	Mar1	Inhibitor	Park, et al. (220)
RORα	Mar1	Agonist	Han, et al. (221)
GPR37	PD1	Agonist	Bang, et al. (222)

Abbreviations: Chem23, Chemerin receptor 23; BLT1, Leukotriene B4 receptor 1; FPR2/ALX, N-formyl peptide receptor 2; GPR18, G protein-coupled receptor 18; GPR32, G protein-coupled receptor 32; LGR6, G protein-coupled receptor 6; TRPV1, Transient receptor potential vanilloid 1; Retinoic acid-related orphan receptor-α, ROR-α; G protein-coupled receptor 37, GPR37.

2.9.2 Cardiovascular disease and pro-resolution lipid mediators

Uncontrolled and persistent inflammation, coupled with a failed resolution response, plays a critical role in the pathogenesis and progression of cardiovascular disease. Due to its status as the leading cause of death and comorbidities worldwide, numerous research groups have been devoted to understanding how the immune response regulates cardiovascular inflammation and how impaired resolution mechanisms contribute to disease progression (223).

Research conducted on mouse models of atherosclerosis has shown a notable disparity between pro-inflammatory lipids, such as PGE2 and LTB4, and SPMs (224). Administration of SPMs via intraperitoneal injection has shown promising results in preventing the advancement of atherosclerosis plaques (**Table 3**) (25,224). Treatment of mice with SPMs effectively minimizes the release of pro-inflammatory cytokines, inhibits ROS generation, induces collagen deposition, reduces the adhesion and traffic of leukocytes to the inflammation site, and enhances efferocytosis mechanisms (224).

SPMs have been reported to effectively regulate monocytes and neutrophil infiltration in the inflammation site. Studies have suggested that SPMs can exert local action, inducing macrophage polarization to reparative type and favoring resolution shift. Furthermore, they are also efficient in restricting the infiltration of macrophages and neutrophils into the inflamed tissue. Some of the mechanisms involved in the specific action that SPMs play on the recruitment of inflammatory cells include the regulation of the expression of molecules involved in leukocyte adhesion and the key cytokines involved in signaling cell recruitment. Similarly, SPMs have also been reported to increase efferocytosis during abdominal aorta aneurysm formation (225,226). Other inflammatory vascular disease models also observe reduced pro-inflammatory cytokines, inflammatory infiltration, and increased reparative macrophages (27).

While some studies have demonstrated encouraging results with the administration of SPMs in murine models of cardiovascular diseases like atherosclerosis, myocardial infarction, and aortic aneurysms, no study has yet been conducted to determine their efficacy and effects in treating the KD vasculitis model. It's important to consider certain limitations and challenges when working with SPMs.

Absorption of SPMs is rapid, with peak levels reached within minutes after intraperitoneal or intravenous administration. SPMs are also highly metabolized, leading to a rapid decline in serum levels with a half-life of hours (227–229). This fact has prompted recent investigations into different techniques and approaches to SPM delivery to prolong their half-life and stability. The purity of SPMs and the metabolomics studies should also be considered, as they necessitate sophisticated techniques that can be costly and have limited access for research institutions.

Research groups have conducted clinical trials to elucidate the effects of Omega-3 supplementation on human cardiovascular disease outcomes. Despite these efforts, definitive conclusions regarding the optimal concentration of DHA and EPA within the Omega-3 supplement capsules remain unclear. Furthermore, alongside inherited, there is the additional hurdle to achieving a cost-effective method to obtain the desired concentration and purity of Omega-3 supplementation capsules. These factors have prevented current studies from affirming that Omega-3 supplementation is beneficial for cardiovascular disease outcomes (230–232).

The use of murine models has been instrumental in understanding the mechanisms of action of SPMs. In this context, we have reviewed the effects of SPMs on cardiovascular disease murine models and further investigated whether treating the LCWE-induced KD vasculitis model with SPMs can reduce inflammation and offer cardiovascular protection.

Table 5. Specialized pro-resolving lipid mediators as treatment in murine models of atherosclerosis.

Author	SPM	Treatment	Treatment via	Animals	Key findings
Fredman, G. *et al.* (224)	RvD1 (100 ng/mouse)	Three times per week for 5 weeks	i.p.	*Ldl*$^{-/-}$ male mice on WD for 8 weeks (early lesions) or 17 weeks (advanced lesions)	Suppressed plaque progression and promoted plaque stability. Decreased LTB4 levels in the lesions; enhanced lesional efferocytosis; reduced MMP9 and collagenase; increased fibrous cap
Viola, J. R. *et al.* (25)	RvD2 and MaR1 (100 ng/mouse)	Every other day for 3 months	i.p.	*Apoe*$^{-/-}$ mice on HFD or 4 weeks (early lesions) or 8 weeks (advanced lesions)	Prevented atheroprogression and promoted plaque stability. Reduced necrotic core and macrophage accumulation; increased collagen deposition; stimulated M2 macrophage phenotype
Salic, K. *et al.* (233)	RvE1 (1 or 5mg/kg/day)	Daily for 16 weeks	o.g.	Apoe3*Leiden mice on WD for 9 weeks	Attenuated lesions. Reduced lesion area and necrotic core; down-regulated genes associated with immune cell traffic and inflammation

i.p. (intraperitoneal injection); o.g. (oral gavage); WD (Western-type diet); HFD (high-fat diet)

3 OBJECTIVES

The current dissertation is based on the hypothesis that LCWE-induced KD vasculitis exhibits acute and long-term immunopathological and histological alterations in heart tissues and peripheral blood. Therefore, we first aimed to evaluate the inflammation in the heart vessels and the levels of immune cells of LCWE-induced KD vasculitis in the long-term period of disease progression.

We also hypothesized that SPMs may be beneficial during LCWE-induced vasculitis. Therefore, we first surveyed and summarized the current scientific literature focusing on the beneficial and/or therapeutic effects of SPMs in the context of cardiovascular disease. Next, using the LCWE-induced KD vasculitis mouse mode, we investigated whether treatment with RvD1, RvD2, and MaR1 attenuates the development of inflammation and cardiovascular lesions.

4 SCIENTIFIC PRODUCTION

4.1 PAPER 1

Long-term cardiovascular inflammation and fibrosis in a murine model of Kawasaki disease vasculitis

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Keywords: Kawasaki disease, vasculitis, inflammation, fibrosis, coronary artery aneurysms, aortitis, long-term, abdominal aorta dilations.

ABSTRACT

Kawasaki disease (KD) is a pediatric febrile systemic vasculitis of unknown etiology and the leading cause of pediatric-acquired heart disease in children in industrialized countries. KD affects the coronary arteries (CA) and may result in the development of CA aneurysms (CAA). The standard treatment for KD is a single high dose of intravenous immunoglobulin (IVIG), effectively reducing the incidence of CAA from approximately 25% to 5%. Reports indicate that CAA may persist and progress for months and even years after the acute phase of the disease, resulting in long-term cardiac complications. Therefore, it is necessary to understand and characterize the immune mechanisms and histopathological changes that occur during the long-term of KD. In this study, we used the *Lactobacillus casei* cell wall extract (LCWE) mouse model of KD vasculitis to assess the long-term immune and histopathological changes occurring in cardiovascular lesions of LCWE-induced mice over four months. We show that CAA and abdominal aorta dilations can still be detected up to 4 months after LCWE injection and initiation of vasculitis. We observed alterations in the composition of circulating immune profiles, such as increased monocyte frequencies in the acute phase of the disease and higher frequencies of neutrophils, which persist up to 4 months after disease initiation. Additionally, immunofluorescence analysis of heart tissues indicated the presence of neutrophils in the tissues, mainly localized around the inflamed CA, at all the assessed different time points. We also report a strong positive correlation between neutrophils and inflammatory monocyte counts and the severity of lesions five days after LCWE injection. We also report a deterioration of cardiac function during the acute phase of LCWE-induced KD vasculitis, which worsens over time, and extensive fibrosis within the inflamed

cardiac tissue. Overall, our findings indicate that in LCWE-injected mice, increased neutrophil counts in the peripheral blood are a reliable predictor for disease severity and that long-term cardiac complications stemming from inflammatory cell infiltrations, cardiac dysfunction, and fibrosis can persist over long periods and are still detected up to 4 months after disease initiation.

INTRODUCTION

Kawasaki disease (KD) is a febrile vasculitis that mainly affects children under the age of 5, leading to inflammation in the walls of blood vessels, particularly the coronary arteries (CA) (1). If left untreated, KD can result in serious cardiac complications (2–4). KD is the most prevalent acquired heart disease in children in all developed countries (5,6). The syndrome is characterized by a high fever that persists for at least five days and the apparition of several clinical manifestations, such as changes in the oral mucosa and cracking/fissuring lips, conjunctivitis, skin rash, edema and desquamation of the extremities (hands and feet), and cervical lymphadenopathy (1). Despite significant efforts over the past few decades to determine the cause of KD, its etiology remains unknown, and its pathogenesis is not yet fully understood. It is suggested that an infectious agent(s) causes KD, possibly a virus, which triggers an inflammatory response targeting cardiovascular tissues (7). However, no specific infectious agent has consistently been associated with KD (2).

KD patients commonly experience cardiovascular manifestations, including the development of aneurysms and dilatations in small to medium-sized vessels, predominantly in the CA, and myocarditis (8,9). CA lesions (CALs) are the most prevalent manifestation and may eventually lead to acute myocardial infarction, which in some rare

cases can be fatal (10,11). CALs usually develop during the acute phase of KD (febrile phase), which lasts up to 10 days after fever onset (1). CALs affect approximately 25% of untreated patients and can regress upon proper intervention with approved treatments (11,12). The standard treatment for KD is a single high dose of intravenous immunoglobulin (IVIG) administrated within the first ten days of disease onset (13,14). While IVIG treatment has been proven to effectively reduce the uncontrolled inflammatory response, around 5% of KD-treated patients still develop CALs (14,15). Moreover, approximately 20% of children with KD do not respond to IVIG and are at even higher risk of developing severe cardiovascular complications (16,17). CALs can persist and progress for months and even years following the initial diagnosis, causing long-term cardiac complications (18).

KD vasculopathy develops in three linked pathological processes (19). The first process, necrotizing arteritis (NA), starts at the endothelial layers of the CA. It is followed by subacute/chronic (SA/C) vasculitis and finally by luminal myofibroblast proliferation (LMP) (19). These three-linked processes are complex and involve tissue infiltration by innate and adaptive immune cells. Briefly, NA consists of massive infiltration of polymorphonuclear (PMN) cells, especially neutrophils, which secrete several inflammatory mediators, including cytokines, matrix metalloproteinases, elastase, and other enzymes (19). These mediators destroy the elastic layers and media, progressively causing the structural support of the coronary artery to break down and the subsequent development of aneurysms and dilations (19,20). NA is self-limited, lasting for two weeks, followed by the SA/C vasculitis process that occurs months or years after the acute phase of KD. SA/C vasculitis is an asynchronous non-neutrophilic process with T lymphocytes,

especially $CD8^+$ T cells, IgA plasma cells, eosinophils, and scattered macrophage infiltration within the CA tissue (19,21). SA/C is intimately associated with the progressive LMP process and co-occurs. During SA/C-LMP, myofibroblasts proliferate in the adventitia layer and extend the lesion towards the lumen. Ultimately, persistent and chronic SA/C-LMP processes result in complete arterial wall destruction and the formation of coronary artery aneurysms (CAAs) that can cause coronary artery stenosis, leading to ischemic heart disease, thrombosis or rupture, which may also lead to fatal events (19). Detailed histological studies of cardiac tissue from KD patients have shown critical morphological and histological alterations years after initial diagnosis (10,19). Indeed, cardiovascular remodeling and inflammatory cellular infiltrations in the myocardium and CA may persist over time and lead to cardiac events later in life (10,22,23). A potential link between myocardial infarction deaths and undiagnosed cases of KD has been suggested (18,24). Fatal outcomes related to KD were believed to occur only within 60 days of disease onset and, therefore, were exclusively associated with acute and subacute phases. However, studies have reported that fatal cases due to cardiac complications, with ischemic heart diseases being the most frequent, with an increased likelihood of occurrence during the first year after KD diagnosis and even years after that (10,25).

These observations highlighted the possible long-term complications of KD (18,26). Therefore, investigating potential cardiovascular involvement beyond the acute phase of KD and long-term immunopathogenesis and histological alterations is warranted. However, such studies are challenging due to not only the complexity of the disease but also the limited access to human heart tissue samples (27). The *Lactobacillus*

casei wall extract (LCWE)-induced mouse model of KD vasculitis is a well-described and widely accepted animal model replicating many of the histological changes and immunopathological characteristics observed in KD patients (5,28). This model reproduces the key pathological features of KD, including coronary arteritis, abdominal aorta aneurysms, and cardiac function alterations, as well as treatment response to IVIG and Anakinra (5).

Here, we used the LCWE-induced KD mouse model to investigate the long-term immunological and pathological changes of LCWE-induced cardiovascular inflammation and cardiac function in the long-term phase of the disease.

METHODS

Mice. Five-week-old, Wild-Type (WT) C57BL/6 male mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and housed under specific pathogen-free conditions and used according to the guidelines of Cedars-Sinai Medical Center Institutional Animal Care and Use Committee (IACUC).

LCWE-induced KD vasculitis mouse model. *Lactobacillus casei* (ATCC 11578) cell wall extract (LCWE) was prepared as previously described (5). Briefly, *Lactobacillus casei* was grown in Man-Rogosa-Sharpe (MRS) broth (Sigma-Aldrich, #1.10661) for 48 hours in 37^{o}C incubation, harvested and washed with PBS (1:1 volume). Harvested bacteria were spun down and resuspended in 20 mL of PBS for every 5 grams of bacteria pellet, followed by 2 hours of sonication using a 3/4-inch horn and a garnet tip at maximum power. Samples were maintained in a dry ice/ethanol bath during the sonication procedure to prevent overheat. After sonication, bacteria were centrifuged for 20 minutes

at 12000 rpm and 4ºC. The supernatant was obtained and centrifuged for 1 hour at 38000 rpm and 4ºC. The final supernatant was collected and stored at -80ºC. The total rhamnose content of the cell wall extract was determined by a colorimetric phenol-sulfuric assay as described previously (31). Five-week-old WT male mice were injected with a single dose of 500 µl of either LCWE or PBS intraperitoneally (i.p.) to induce systemic vasculitis, as previously published (5,29,30). At the mentioned time points post-LCWE-injection, either week 2, 4, 8, 12, and 16, blood was collected, and mice were euthanized. Mice were then perfused with PBS, and heart tissues were collected and embedded in a tissue-tek optimum cutting temperature (O.C.T.) compound (Sakura Finetek, catalog #4583). Abdominal aortas were dissected from the level of the left renal artery down to the iliac bifurcation, photographed, and embedded in O.C.T., as previously published (29–32). The maximal abdominal aorta diameter was determined by measuring five different areas separated by 2 mm of the abdominal aorta infra-renal portion (below the left renal artery) with ImageJ (NIH) (31). The infrarenal abdominal aorta area was also measured in ImageJ. Heart tissue sections were stained with Masson's trichrome (Millipore Sigma, catalog #HT15), hematoxylin, and eosin (H&E; Millipore Sigma, catalog #MHS32). Sections were visualized by Keyence’s BZ-9000 microscope, and 10X magnification images were captured with a BZ-II viewer and BZ-II analyzer software (Keyence). Heart tissue histopathological examination and assessment of the severity of cardiovascular lesions (Cas, aortic root vasculitis, and myocarditis) were performed on H&E-stained tissue sections, as previously described (5,32). Briefly, acute inflammation, chronic inflammation, and connective tissue proliferation were each assessed using the following scoring system: 0 = no inflammation, 1 = rare inflammatory cells, 2 = scattered

inflammatory cells, 3 = diffuse infiltrate of inflammatory cells, and 4 = dense clusters of inflammatory cells. Fibrosis was determined using the following scoring system: 0 = no medial fibrosis, 1 = medial fibrosis involving less than 10% of the CA circumference, 2 = medial fibrosis involving 11% to 50% of the CA circumference, 3 = medial fibrosis involving 51% to 75% of the CA circumference, and 4 = medial fibrosis involving more than 75% of the CA circumference. As previously published, all four scores were combined to generate a severity score called the “Heart inflammation score” (5,31–33).

Peripheral blood cell isolation. Mice were anesthetized with isoflurane, and blood was collected by retro-orbital bleeding using a heparinized micro-hematocrit capillary tube (Fisher Scientific, #22-362-566). 40 μL of blood was transferred to a 1.5 mL tube, and red blood cell lysis was performed by adding 200 μL of RBC lysis buffer (eBioscience, catalog #00-4333-57). After 2 minutes, 500 μL of wash buffer (1X PBS and 1% FBS) was added, and samples were centrifuged at 2000 rpm for 5 minutes at 4^{o}C. Pellets were suspended in 50 μl of wash buffer and stained for flow cytometric analysis.

Flow cytometric analysis. Samples were first incubated with an anti-murine CD16/CD32 antibody (Clone 2.4G2; Tonbo Biosciences, catalog #70-0161-M001) for 10 minutes. The following murine antibodies were used for flow cytometric analysis: CD45.2 (1 ug/mL, AF700, clone 104, Biolegend, catalog #109822), CD11b (1 ug/mL, VioletFluor 450, clone M1/70, Tonbo Bioscience, catalog #75-0112), Ly6G (1 ug/mL, APC, clone 1A8, Tonbo Bioscience, catalog # 20-1276) or Ly6C (1 ug/mL, PE, clone HK1.4, Biolegend, catalog #128008). Dead cells were excluded using a fixable viability dye (eFluor 506, Invitrogen, catalog #65-0866-14). Samples were incubated on ice for 30 minutes with the antibodies, then washed two times with buffer (1X PBS and 1% FBS) and fixed with 0.5%

paraformaldehyde (PFA). Before sample acquisition, counting beads were added to each sample (CountBright, Life Technologies, catalog #C36950). Data was acquired on a SONY 3800 Spectral Cell Analyzer (Sony Biotechnology) and analyzed using FlowJo Software (BD Bioscience).

Immunofluorescence. Heart and abdominal aorta tissue cryosections (7 μm) collected from mice injected with either PBS or LCWE were fixed in cold acetone for 5 minutes, washed in PBS, and blocked for 1 hour with anti-goat serum. Samples were stained overnight with the following antibodies: Ly6G (Clone 1A8, BioLegend, catalog #127610), CD3 (Cell Signaling, catalog #78588S), Vimentin (Clone D21H3, Cell Signaling, catalog #5741S). Isotype controls were used as negative controls: Rat IgG2 (Biolegend, catalog #ab400526) and Rabbit IgG (Abcam, catalog #1ab71870). After washing three times with PBS, sections were mounted with DAPI (Abcam, catalog #ab104139). Images were obtained with a Keyence BZ-9000 fluorescence microscope.

Echocardiography. Transthoracic echocardiography was used to evaluate ejection fraction (EF) and left ventricle inner diameter (LVID) by the apical 4-chamber (pulse-wave Doppler mode).

Statistical analysis. Results are presented as mean ± SEM. The normality of data was assessed using the Shapiro-Wilk test. A one-way analysis of variance (ANOVA) was used for multiple comparisons. Tukey post-test analysis was used for normally distributed data, and the Kruskal Wallis test with Dunn's multiple comparisons test was used for non-normally distributed data. A comparison of Kaplan-Meier survival analysis was done using the log-rank (Mantel-Cox) test. Spearman's correlation test was used to analyze two

variables. A value of $p<0.05$ was considered statistically significant. Data were analyzed using GraphPad Prism Software (version 10).

RESULTS

LCWE induces long-term cardiovascular lesions.

We first investigated the long-term pathology of LCWE-induced KD vasculitis by injecting WT mice with either PBS or LCWE and assessing heart vessel inflammation and coronary artery dilation at 2, 4, 8, 12, and 16 weeks after LCWE injection. During the disease course, we observed a trend of increased mortality in LCWE-injected mice compared with control PBS-injected control mice (**Figure 1A**). Inflammation and severity of cardiovascular lesions were assessed by histological evaluation of H&E-stained tissue sections (**Figure 1B, C**). In heart tissues of LCWE-injected mice, we observed extensive infiltrations of inflammatory cells in the area surrounding the CA and several cases of complete stenosis of the CA, even at the earliest time point, at two weeks post-LCWE injection (**Supplementary Figure 1A, B**). Compared with PBS control mice, LCWE-injected mice developed severe heart inflammation (**Figure 1C and Supplementary Figure 1A, B**). LCWE-injected mice also exhibited abdominal aorta dilatations and aneurysms along the supra-renal portion of the abdominal aorta (**Figure 1D-F**). H&E stained cross-sections of the abdominal aorta indicated intimal infiltration and increased aortic wall thickness (**Figure 1D**). However, the severity of cardiovascular lesions reached a plateau, and heart vessel inflammation and the size of abdominal aorta dilations were similar in LCWE-injected mice from week 2 to week 16 post-LCWE injection (**Figure 1C, E, F**). These results indicate that LCWE-induced coronary arteritis and abdominal aorta

dilations do not regress and persist beyond the acute phase of the disease, possibly leading to long-term cardiovascular complications.

Systemic alterations in immune cell composition during the long-term phase of LCWE-induced KD vasculitis.

We next sought to determine the composition of circulating immune cells during the acute and long-term phases of LCWE-induced KD vasculitis. Peripheral blood was collected from either PBS-injected control mice or LCWE-injected mice on day 5, week 4, 8, 12, and 16 after LCWE injection. Samples were analyzed by flow cytometry using specific markers for leukocytes (live $CD45.2^+$ cells), myeloid cells (live $CD45.2^+$ $CD11b^+$ cells) neutrophils (live $CD45.2^+$ $CD11b^+$ $Ly6C^{low}$ $Ly6G^+$ cells), inflammatory monocytes (live $CD45.2^+$ $CD11b^+$ $Ly6G^-$ $Ly6C^{high}$ cells), intermediate monocytes (live $CD45.2^+$ $CD11b^+$ $Ly6G^-$ $Ly6C^{low}$ cells) and patrolling monocytes (live $CD45.2^+$ $CD11b^+$ $Ly6G^-$ $Ly6C^-$ cells) (**Supplementary Figure 2A-D**). We observed that LCWE injection increased the cell number of circulating leukocytes at day 5, with a second peak at week 16 (**Figure 2A, B**). The circulating frequencies and cell numbers of myeloid cells significantly increased at day five and week 12 post-LCWE injection compared to PBS-injected control mice, and cell numbers also increased on week 8 (**Figure 2C, D**). Similarly, frequencies and numbers of circulating neutrophils increased at day five post-LCWE-injection and remained significantly high until week 16 (**Figure 2E, F**). The different populations of monocytes were gated from $CD45.2^+CD11b^+$ $Ly6C^+$ cells and classified according to their Ly6C expression (**Supplementary Figure 2D**). Inflammatory monocytes were selected based on high expression of Ly6C ($Ly6C^{high}$), intermediate monocytes were with intermediate $Ly6C^+$ ($Ly6C^{int}$) expression, and patrolling monocytes identified based on

the low Ly6C ($Ly6C^{low}$) expression (**Supplementary Figures 2D and 3A-F**). The frequencies and numbers of the different circulating monocyte subsets were increased during the acute phase of LCWE-induced KD vasculitis at day five post-LCWE injection (**Supplementary Figure 3A-F**). However, such differences were not observed later in the long-term phase of LCWE-induced vasculitis (**Supplementary Figure 3A-F**). While the frequencies and numbers of inflammatory and patrolling monocytes peaked at day 5 (**Supplementary Figure 3A-D**), only cell numbers of intermediate monocytes peaked at day five post-LCWE (**Supplementary Figure 3E, F**). These results suggest that changes in systemic immune cell composition, particularly in frequencies and numbers of circulating neutrophils and myeloid cells, are still observed during the long-term phase of LCWE-induced KD vasculitis.

Increased circulating levels of neutrophils are associated with the severity of LCWE-induced abdominal aorta dilations and cardiovascular lesions.

We next investigated if increased systemic frequencies and numbers of neutrophils observed at day five post-LCWE injection correlate with the severity of LCWE-induced cardiovascular lesions. Mice were injected with LCWE, blood was collected on day five post-LCWE injection, and circulating neutrophil frequencies and counts were determined by flow cytometric analysis. Heart vessel inflammation and maximal abdominal aorta diameter and area were assessed 14 days post-LCWE injection. Higher neutrophil frequencies and counts strongly positively correlated with enlarged abdominal aorta dilations and the severity of heart vessel inflammation (**Figure 3A-C**). Similarly, higher frequencies and counts of circulating inflammatory monocytes at day five post-LCWE positively correlated with more severe development of abdominal aorta dilations (**Figure**

4A, B). However, we did not find any correlation between heart vessel inflammation and the frequency of inflammatory monocytes **(Figure 4C)**. These data suggest that circulating neutrophil and inflammatory monocyte frequencies and counts are biomarkers indicative of disease severity and the development of LCWE-induced cardiovascular lesions.

Neutrophils infiltrate the inflamed coronary arteries of LCWE-injected mice.

Since the frequencies and numbers of circulating neutrophils increase from day five until week 16 post-LCWE injection and strongly correlate with the development of more severe LCWE-induced cardiovascular lesions (**Figures 2 and 3**), we next determined the presence of neutrophils in cardiac tissues by immunofluorescent (IF) staining. IF staining for Ly6G was performed on heart tissues from LCWE-injected mice at weeks 4, 8, 12, and 16 post-LCWE-injection, and the presence of neutrophils was quantified (**Figure 5A, B**). Surprisingly, neutrophils could still be detected in cardiac tissues in the long-term phase of LCWE-induced KD vasculitis up to 4 months post-LCWE injection (**Figure 5A, B**). Neutrophils concentrated mainly around the inflamed coronary arteries, while no neutrophils were detected in heart tissues from PBS-injected control mice (**Figure 5A, B**). Our results indicate neutrophils can still be detected in heart tissues of LCWE-injected mice up to 4 months post LCWE-induced KD vasculitis and, therefore, may contribute not only to the acute phase of the disease but also to the development of chronic and long-term cardiovascular lesions.

Fibrotic area in the coronary artery and aortic wall.

Inflammatory cells infiltrate the CAs of LCWE-injected mice and can be detected up to 4 months post-LCWE injection (**Figures 6 and Supplementary Figure 4**). We next

sought to determine if LCWE-induced KD vasculitis leads to the development of long-term fibrosis. Heart tissue sections from PBS control mice and LCWE-injected mice at different time points post-LCWE-injection were stained for Masson's trichrome, and the fibrotic area was measured (**Figure 6A, B**). The deposition of collagen fibers was detected around the inflamed CAs and aortic wall, the same area that overlap with inflammatory cell infiltration (**Figure 6A, B**). Control mice injected with PBS showed minimum collagen staining, mainly restricted to the CA and aortic wall (**Figure 6A, B**). Heart tissue fibrosis, as determined by the percentage of trichrome positive area, significantly decreased from week four post-LCWE injection and persisted in the long-term vasculitis phase, up to 4 months post-LCWE injection (**Figure 6B**). Vimentin expression was also evaluated by IF staining. However, no difference was observed for vimentin expression in heart tissues between PBS- and LCWE-injected mice, independent of the time course of the disease **(Supplementary Figure 6A, B)**. We also observed diffuse interstitial fibrosis in the myocardium of mice after 12 weeks of LCWE injection (**Supplementary Figure 5A**). Our data suggest that LCWE-induced KD vasculitis leads to fibrosis in heart tissues, which persists for up to 4 months post-injection.

Long-term Impairment of cardiac function in LCWE-induced KD vasculitis

Next, we aimed to evaluate whether the cardiac functions were affected by the intense inflammation and fibrosis induced by LCWE injection detected previously. We performed transthoracic echocardiogram analysis of PBS control mice and LCWE-injected mice at different time points of the disease. Echocardiography is a non-invasive and accurate tool widely used to assess cardiac function and investigate cardiac problems, including heart failure. We analyzed the ejection fraction (EF) and the left

ventricle internal diameter end-systole (LVIDd) parameters to evaluate cardiac function. EF indicates the oxygenated blood volume the left ventricle can pump to the systemic circulation during each heart contraction. The EF measurement, expressed as a percentage, indicates the current cardiac function and helps determine the risk of heart failure. The LVIDd measures the left ventricle's thickness, which helps determine ventricular hypertrophy. Compared with PBS-injected control mice, cardiac function impairment was already observed in LCWE-injected mice during the acute phase of the disease at two weeks post-LCWE injection (**Figure 7A, B**). Additionally, the decrease in cardiac function following the injection of LCWE not only persisted in the long-term phase of the disease but also worsened over time, at 3 to 4 months post-injection **(Figure 7A, B)**. These results indicate that LCWE-induced KD vasculitis is also associated with long-term dysfunctional cardiac function.

DISCUSSION

The current study demonstrates that LCWE-induced KD vasculitis can lead to long-term cardiovascular, persistent and severe CAA, ongoing inflammatory infiltration, tissue remodeling, and impaired cardiac function. Cardiovascular complications are a significant concern regarding KD management (1). Intense inflammatory cellular infiltrations in the CA destroy the vessel wall and aneurysm formation (8,9). Previously, it was assumed that cardiovascular changes only occurred during the acute phase of the disease. However, histological observations on tissues collected from autopsied KD patients have shown that CAA can develop for several months or even years after diagnosis (19,34,35). While CAA may regress after the acute phase, some lesions, especially the larger ones, can persist beyond that period and silently progress to stenosis or form a thrombus(9,36).

Progressive narrowing and blockage of the CA lead to severe cardiovascular events such as MI or even sudden death in rare cases, as observed in our results. It is necessary to fully describe and comprehend the natural long-term course of KD despite the CAA status of the patients.

Here, we used the LCWE-induced KD vasculitis mouse model, which causes systemic vasculitis and coronary arteritis in mice. LCWE injection also induces inflammation in other vessels, such as the abdominal aorta, brachial, iliac, and renal arteries, which are also affected in KD patients (37). LCWE triggers the destruction of elastic layers, promotes intimal hypertrophy, induces smooth muscle cell (SMC) proliferation towards the lumen and CA stenosis, as well as fibrotic remodeling, reproducing the three linked histopathological processes (necrotizing arteritis, subacute vasculitis and luminal myofibroblastic proliferation), reported in children with KD (5,27,37). However, it is unclear whether this model is suitable for evaluating the long-term effects of KD vasculitis since most studies have only focused on the acute phase of the disease. Suganuma *et al*. observed that maximum intimal thickness and luminal narrowing in the CA occurred 16 weeks after LCWE injection (38). Moreover, Wakita and collaborators showed that abdominal aorta aneurysms (AAA) were still present eight weeks after LCWE injection (30). The current study revealed that LCWE-induced vasculitis can lead to long-lasting cardiovascular lesions that persist for up to 16 weeks and do not regress over time.

Additionally, we found systemic changes in immune cells and positive correlations between frequencies and numbers of circulating neutrophils and inflammatory monocytes during the acute phase of the disease with more severe vasculitis development. We also

observe an extensive fibrotic area around the CA circumference, interstitial fibrosis in the myocardium, and a notable decline in cardiac function. Furthermore, there were substantial infiltrations of inflammatory cells in the CA, with frequent severe or complete stenosis cases up to 16 weeks post LCWE injection. Interestingly, the evaluation of the heart vessel inflammatory score and extension of AAA seemed to plateau around weeks 12 to 16; however, the cardiovascular lesions did not regress and remained stable over time.

Myocardial biopsy results indicate ongoing inflammatory cell infiltration and interstitial fibrosis in patients diagnosed with giant CAA during the acute phase of KD (39). They also reported that their cardiac function, assessed by echocardiograph, ranged from 53-80% during the acute phase of the disease (39). After conducting follow-up assessments, it was found that 25% of patients had asymptomatic CA obstruction and worsening of fibrosis. Cardiac fibrosis has been associated with low ejection fraction (EF) ($\leq$55%) and heart failure amongst KD patients (40). Here, we show that LCWE-treated mice had significantly reduced EF during the acute phase, further decreasing on weeks 12 and 16 of follow-up. We assessed fibrosis around the CA by trichrome staining in the heart tissue, revealing that it undergoes continuous remodeling around the CA circumference with early detection only two weeks after LCWE injection, peaking on weeks 8 and 12. The entire CA area becomes fibrotic, with no trend to revert to its normal state due to the extension of fibrotic tissue.

Vimentin can regulate fibrosis and tissue repair by promoting collagen synthesis, aiding fibroblast migrations and myofibroblast differentiation. It is a broad marker for cardiac fibroblasts and myocardial remodeling (41). Vimentin is upregulated by TGF-β

and is also suggested to be required for NLRP3 inflammasome activation by activating Caspase-1 and maturing IL-1β, a key molecule in KD pathogenesis (42). However, we did not find increased vimentin expression in cardiac tissue in LCWE-injected mice over time.

The exact mechanism by which LCWE triggers vasculitis is unknown. Innate and adaptive immune mechanisms are involved in developing LCWE-induced KD vasculitis (43). TLR2 is required to activate an inflammatory response in a MyD88-dependent manner, activating the NF-κB signaling pathway and the production of several inflammatory cytokines, including IL-1β (28). Neutrophils seem to play an essential role in KD immunopathogenesis. They are among the first cells to reach the inflammation site and play a vital role in the initial innate immune defense, acting quickly to protect the organism from invaders. When neutrophils are activated, they secret enzymes and proteases, such as neutrophil elastase, to neutralize and eliminate the threat. However, overactivation and uncontrolled recruitment can lead to tissue damage, destroying the intima and media layers of the CA wall and contributing to lesion formation. In KD, most circulating neutrophils are activated and are a major source of IL-1β^{+} (44). Takahashi et al. have shown that neutrophils infiltrate the media layer of coronary arteries during the acute phase of KD (45). Additionally, Lech et al. conducted a study that revealed KD patients with giant CAA had increased levels of calprotectin, primarily secreted by neutrophils, years after disease onset (46). Here, we observed that neutrophils are still present in cardiac tissues of LCWE-injected mice up to 16 weeks post-LCWE injection and are mainly concentrated around the inflamed CA.

Alterations in the peripheral levels of neutrophils have been reported, and neutrophilia occurs during the acute and subacute phases of KD (44,47,48). KD patients exhibit elevated levels of neutrophils, with increases of over 80% considered a factor in predicting IVIG unresponsiveness based on a model published by Kobayashi et al. (49). In accordance, our results show that LCWE-injected mice have increased circulating frequencies and counts of leukocyte and neutrophil. We have also established a clear correlation between the severity of abdominal aorta dilations and CAA and the frequencies and counts of circulating neutrophil levels during the acute phase of LCWE-induced KD vasculitis. Furthermore, neutrophil counts and frequencies remained significantly elevated in the long-term phase of the disease, up to 16 weeks post-LCWE injection, which may reflect an ongoing and chronic systemic inflammation.

Increased peripheral monocyte changes have also been reported in the literature during acute KD (50). Monocytes are a heterogeneous cell population of the innate immune system. They are recruited to the inflammation area, where they differentiate into macrophages or dendritic cells, acquiring specific features to counteract the threat. Human monocytes can be classified as classical ($CD14^+$ $CD16^-$), the intermediate ($CD14^+$ $CD16^+$), and the nonclassical ($CD14^+$ $CD16^{++}$) monocytes (51). It's known that cytokines such as IL-1 and TNF-α secreted by activated monocytes during the acute phase of KD exacerbate inflammation and vascular damage, playing a central role in KD immunopathogenesis (5,51–53). Katayama et al. showed higher intermediate $CD14^+$ $CD16^+$ monocyte frequency and count in patients during the acute phase compared to the convalescence phase and control subjects (54). Another study by Kim et al. reported a higher proportion of classical monocytes (55). Moreover, increased peripheral $CD14^+$

monocytes/macrophages levels were found in patients with CAA (56). In addition, treatment with IVIG appears to rapidly decrease the levels of monocytes in responsive patients (54,57). Our flow cytometry analysis of monocyte subpopulations revealed increased levels only during the acute phase of LCWE-induced KD vasculitis.

A significant correlation between late cardiovascular complications and a delay in KD diagnosis and treatment during the acute phase has been established (18). Missed KD diagnosis during childhood will lead to a population of young adults who are unaware of their history of KD. This puts them at a high risk of developing cardiovascular complications, especially events like MI later in life. Therefore, improving the diagnosis and treatment of KD and the follow-up care of affected individuals is crucial to prevent long-term complications (18). Our study demonstrates that LCWE-induced KD vasculitis can lead to long-term histopathological changes, continuous inflammation, and tissue remodeling, severely impacting cardiac function for up to 16 weeks. Our findings suggest that these features persist even after four months of LCWE injection, similar to the long-term effects observed in patients with KD. Based on our findings, we recommend using the murine model of LCWE-induced KD vasculitis to assess the long-term consequences of KD. Furthermore, we encourage further research to gain insights into the histopathological and immune mechanisms of KD, which may aid in developing new strategies for detecting and managing CAA and other cardiovascular lesions, minimizing late cardiac events in KD patients.

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AUTHORS CONTRIBUTIONS

APPL, RP, MNV, and MA conceived the project and designed the experiments. APPL planned and performed the experiments. EA, DPM, and ML assisted with the experiments. TTC helped with flow cytometry data acquisition. TTRM performed echocardiogram. APPL performed statistical analyses. APPL and MNV wrote and reviewed the manuscript. TRC contributed to manuscript revision and editing. WV contributed to the interpretation of the results. MA supervised the project and approved the final manuscript.

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REFERENCES

1. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, et al. Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: A Scientific Statement for Health Professionals From the American Heart Association. Circulation [Internet]. 2017 Apr 25;135(17):e927–99. Available from: https://www.ahajournals.org/doi/10.1161/CIR.0000000000000484
2. Rife E, Gedalia A. Kawasaki Disease: an Update. Vol. 22, Current Rheumatology Reports. Springer; 2020.
3. Ramphul K, Mejias SG. Kawasaki disease: a comprehensive review. Archives of Medical Science – Atherosclerotic Diseases. 2018 Mar 21;3(1):41–5.
4. Burns JC, Kushner HI, Bastian JF, Shike H, Shimizu C, Matsubara T, et al. Kawasaki Disease: A Brief History. Pediatrics [Internet]. 2000 Aug 1;106(2):e27–e27. Available from:

https://publications.aap.org/pediatrics/article/106/2/e27/62795/Kawasaki-Disease-A-Brief-History

5. Lee Y, Schulte DJ, Shimada K, Chen S, Crother TR, Chiba N, et al. Interleukin-1β Is Crucial for the Induction of Coronary Artery Inflammation in a Mouse Model of Kawasaki Disease. Circulation [Internet]. 2012 Mar 27;125(12):1542–50. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L51891216%5Cnhttp://dx.doi.org/10.1161/CIRCULATIONAHA.111.072769%5Cnhttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=00097322&id=doi:10.1161%2FCIRCULATIONAHA.111.072769&atitle=Interl
6. Beom Kim G. Reality of kawasaki disease epidemiology. Vol. 62, Korean Journal of Pediatrics. Korean Pediatric Society; 2019. p. 292–6.
7. Rowley AH. Kawasaki Disease: Novel Insights into Etiology and Genetic Susceptibility. Annu Rev Med. 2011 Feb 18;62(1):69–77.
8. Akagi T, Rose V, Benson LN, Newman A, Freedom RM. Outcome of coronary artery aneurysms after Kawasaki disease. J Pediatr. 1992 Nov;121(5):689–94.
9. Friedman KG, Gauvreau K, Hamaoka-Okamoto A, Tang A, Berry E, Tremoulet AH, et al. Coronary Artery Aneurysms in Kawasaki Disease: Risk Factors for Progressive Disease and Adverse Cardiac Events in the US Population. J Am Heart Assoc. 2016 Sep;5(9).
10. Kato H, Ichinose E, Kawasaki T. Myocardial infarction in Kawasaki disease: Clinical analyses in 195 cases. J Pediatr [Internet]. 1986 Jun;108(6):923–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022347686809283
11. McCrindle BW, Li JS, Minich LL, Colan SD, Atz AM, Takahashi M, et al. Coronary Artery Involvement in Children With Kawasaki Disease. Circulation. 2007 Jul 10;116(2):174–9.
12. Terai M, Shulman ST. Prevalence of coronary artery abnormalities in Kawasaki disease is highly dependent on gamma globulin dose but independent of salicylate dose. J Pediatr [Internet]. 1997 Dec;131(6):888–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022347697700386

13. Phuong LK, Curtis N, Gowdie P, Akikusa J, Burgner D. Treatment Options for Resistant Kawasaki Disease. Vol. 20, Pediatric Drugs. Springer International Publishing; 2018. p. 59–80.
14. Lo MS, Newburger JW. Role of intravenous immunoglobulin in the treatment of Kawasaki disease. Int J Rheum Dis. 2018 Jan 1;21(1):64–9.
15. BURNS JC, CAPPARELLI E V., BROWN JA, NEWBURGER JW, GLODE MP. Intravenous gamma-globulin treatment and retreatment in Kawasaki disease. Pediatr Infect Dis J. 1998 Dec;17(12):1144–8.
16. Son MB, Gauvreau K, Burns JC, Corinaldesi E, Tremoulet AH, Watson VE, et al. Infliximab for Intravenous Immunoglobulin Resistance in Kawasaki Disease: A Retrospective Study. J Pediatr. 2011 Apr;158(4):644-649.e1.
17. Tremoulet AH, Best BM, Song S, Wang S, Corinaldesi E, Eichenfield JR, et al. Resistance to Intravenous Immunoglobulin in Children with Kawasaki Disease. J Pediatr. 2008 Jul;153(1):117-121.e3.
18. Gordon JB, Daniels LB, Kahn AM, Jimenez-Fernandez S, Vejar M, Numano F, et al. The Spectrum of Cardiovascular Lesions Requiring Intervention in Adults After Kawasaki Disease. JACC Cardiovasc Interv. 2016 Apr;9(7):687–96.
19. Orenstein JM, Shulman ST, Fox LM, Baker SC, Takahashi M, Bhatti TR, et al. Three Linked Vasculopathic Processes Characterize Kawasaki Disease: A Light and Transmission Electron Microscopic Study. PLoS One. 2012 Jun 18;7(6):e38998.
20. Shulman ST, Rowley AH. Kawasaki disease: insights into pathogenesis and approaches to treatment. Nat Rev Rheumatol. 2015 Aug 28;11(8):475–82.
21. Rowley AH, Eckerley CA, Jäck HM, Shulman ST, Baker SC. IgA plasma cells in vascular tissue of patients with Kawasaki syndrome. J Immunol. 1997 Dec 15;159(12):5946–55.
22. De Rosa G, Andreozzi L, Piastra M, Castelli B, Rigante D. Acute myocarditis as a revealing clue of complete Kawasaki disease. Reumatismo. 2018 Jul 6;70(2):115–6.

23. Anderson TM, Meyer RA, Kaplan S. Long-term echocardiographic evaluation of cardiac size and function in patients with Kawasaki disease. Am Heart J. 1985 Jul;110(1):107–15.
24. Rizk SRY, El Said G, Daniels LB, Burns JC, El Said H, Sorour KA, et al. Acute myocardial ischemia in adults secondary to missed Kawasaki disease in childhood. American Journal of Cardiology. 2015 Feb 15;115(4):423–7.
25. Robinson C, Chanchlani R, Gayowsky A, Brar S, Darling E, Demers C, et al. Cardiovascular outcomes in children with Kawasaki disease: a population-based cohort study. Pediatr Res. 2023 Apr 15;93(5):1267–75.
26. Burns JC, Shike H, Gordon JB, Malhotra A, Schoenwetter M, Kawasaki T. Sequelae of Kawasaki disease in adolescents and young adults. J Am Coll Cardiol [Internet]. 1996 Jul;28(1):253–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/073510979600099X
27. Lehman TJ, Walker SM, Mahnovski V, McCurdy D. Coronary arteritis in mice following the systemic injection of group B Lactobacillus casei cell walls in aqueous suspension. Arthritis Rheum [Internet]. 1985 Jun;28(6):652–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3924060
28. Rosenkranz ME, Schulte DJ, Agle LMA, Wong MH, Zhang W, Ivashkiv L, et al. TLR2 and MyD88 Contribute to Lactobacillus casei Extract–Induced Focal Coronary Arteritis in a Mouse Model of Kawasaki Disease. Circulation [Internet]. 2005 Nov 8;112(19):2966–73. Available from: https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.105.537530
29. Porritt RA, Zemmour D, Abe M, Lee Y, Narayanan M, Carvalho TT, et al. NLRP3 Inflammasome Mediates Immune-Stromal Interactions in Vasculitis. Circ Res. 2021 Oct 15;129(9).
30. Wakita D, Kurashima Y, Crother TR, Noval Rivas M, Lee Y, Chen S, et al. Role of Interleukin-1 Signaling in a Mouse Model of Kawasaki Disease–Associated Abdominal Aortic Aneurysm. Arterioscler Thromb Vasc Biol [Internet]. 2016 May;36(5):886–97. Available from: https://www.ahajournals.org/doi/10.1161/ATVBAHA.115.307072

31. Marek-Iannucci S, Yildirim AD, Hamid SM, Ozdemir AB, Gomez AC, Kocatürk B, et al. Targeting IRE1 endoribonuclease activity alleviates cardiovascular lesions in a murine model of Kawasaki disease vasculitis. JCI Insight. 2022 Mar 22;7(6).
32. Kocatürk B, Lee Y, Nosaka N, Abe M, Martinon D, Lane ME, et al. Platelets exacerbate cardiovascular inflammation in a murine model of Kawasaki disease vasculitis. JCI Insight. 2023 Jul 24;8(14).
33. Porritt RA, Markman JL, Maruyama D, Kocaturk B, Chen S, Lehman TJA, et al. Interleukin-1 beta-mediated sex differences in kawasaki disease vasculitis development and response to treatment. Arterioscler Thromb Vasc Biol. 2020;802–18.
34. Yonesaka S, Nakada T, Sunagawa Y, Tomimoto K, Naka S, Takahashi T, et al. Endomyocardial Biopsy in Children with Kawasaki Disease. Pediatrics International. 1989 Dec;31(6):706–11.
35. Yutani C, Imakita M, Ueda H, Suzuki J, Kamiya T, Onishi S. Cardiac biopsy in Kawasaki disease. Heart Vessels. 1985 Mar;1(S1):277–277.
36. McCrindle BW, Manlhiot C, Newburger JW, Harahsheh AS, Giglia TM, Dallaire F, et al. Medium-Term Complications Associated With Coronary Artery Aneurysms After Kawasaki Disease: A Study From the International Kawasaki Disease Registry. J Am Heart Assoc. 2020 Aug 4;9(15).
37. Myones BL, Bathoria JM, Lehman TJA, Shulman ST. Human IVIG inhibits Lactobacillus casei-inducible coronary arteritis in a murine model. Kawasaki Disease: Proceedings of the V Inter- national Kawasaki Disease Symposium, Fukuoka, Japan. 1995;252–6.
38. Suganuma E, Sato S, Honda S, Nakazawa A. All trans retinoic acid alleviates coronary stenosis by regulating smooth muscle cell function in a mouse model of Kawasaki disease. Sci Rep [Internet]. 2021;11(1):13856. Available from: https://doi.org/10.1038/s41598-021-93459-3
39. Yonesaka S, Takahashi T, Eto S, Sato T, Otani K, Ueda T, et al. Biopsy-proven myocardial sequels in Kawasaki disease with giant coronary aneurysms. Cardiol Young. 2010 Dec 28;20(06):602–9.

40. Hoshino S, Shimizu C, Jain S, He F, Tremoulet AH, Burns JC. Biomarkers of Inflammation and Fibrosis in Kawasaki Disease Patients Years After Initial Presentation With Low Ejection Fraction. J Am Heart Assoc. 2020 Jan 7;9(1).
41. Ridge KM, Eriksson JE, Pekny M, Goldman RD. Roles of vimentin in health and disease. 2022; Available from: http://www.genesdev.org/cgi/doi/10.1101/gad.349358.
42. dos Santos G, Rogel MR, Baker MA, Troken JR, Urich D, Morales-Nebreda L, et al. Vimentin regulates activation of the NLRP3 inflammasome. Nat Commun [Internet]. 2015 Mar 12;6(1):6574. Available from: https://www.nature.com/articles/ncomms7574
43. Hara T, Nakashima Y, Sakai Y, Nishio H, Motomura Y, Yamasaki S. Kawasaki disease: a matter of innate immunity. Clin Exp Immunol. 2016 Oct 7;186(2):134–43.
44. Zhu YP, Shamie I, Lee JC, Nowell CJ, Peng W, Angulo S, et al. Immune response to intravenous immunoglobulin in patients with Kawasaki disease and MIS-C. Journal of Clinical Investigation. 2021 Oct 15;131(20).
45. Takahashi K, Oharaseki T, Naoe S, Wakayama M, Yokouchi Y. Neutrophilic involvement in the damage to coronary arteries in acute stage of Kawasaki disease. Pediatrics International [Internet]. 2005 Jun;47(3):305–10. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1442-200x.2005.02049.x
46. Lech M, Guess J, Duffner J, Oyamada J, Shimizu C, Hoshino S, et al. Circulating Markers of Inflammation Persist in Children and Adults With Giant Aneurysms After Kawasaki Disease. Circ Genom Precis Med [Internet]. 2019 Apr 1;12(4):e002433. Available from: https://www.ahajournals.org/doi/10.1161/CIRCGEN.118.002433
47. Takeshita S, Sekine I, Fujisawa T, Yoshioka S. Studies of Peripheral Blood Toxic Neutrophils as a Predictor of Coronary Risk in Kawasaki Disease—the Pathogenetic Role of Hematopoietic Colony-Stimulating Factors (GM-CSF, G-CSF). Pediatrics International. 1990 Oct 9;32(5):508–14.
48. Biezeveld MH, Van Mierlo G, Lutter R, Kuipers IM, Dekker T, Hack CE, et al. Sustained activation of neutrophils in the course of Kawasaki disease: an

association with matrix metalloproteinases. Clin Exp Immunol. 2005 Jun 15;141(1):183–8.

49. Kobayashi T, Inoue Y, Takeuchi K, Okada Y, Tamura K, Tomomasa T, et al. Prediction of Intravenous Immunoglobulin Unresponsiveness in Patients With Kawasaki Disease. Circulation. 2006 Jun 6;113(22):2606–12.
50. Matsubara T, Ichiyama T, Furukawa S. Immunological profile of peripheral blood lymphocytes and monocytes/macrophages in Kawasaki disease. Clin Exp Immunol. 2005 Jul 1;141(3):381–7.
51. Cormican S, Griffin MD. Human Monocyte Subset Distinctions and Function: Insights From Gene Expression Analysis. Front Immunol. 2020 Jun 4;11.
52. Furukawa S, Matsubara T, Jujoh K, Yone K, Sugawara T, Sasai K, et al. Peripheral blood monocyte/macrophages and serum tumor necrosis factor in Kawasaki disease. Clin Immunol Immunopathol. 1988 Aug;48(2):247–51.
53. Maury CP, Salo E, Pelkonen P. Circulating interleukin-1 beta in patients with Kawasaki disease. N Engl J Med [Internet]. 1988 Dec 22;319(25):1670–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3264383
54. Katayama K, Matsubara T, Fujiwara M, Koga M, Furukawa S. CD14+CD16+ monocyte subpopulation in Kawasaki disease. Clin Exp Immunol. 2001 Dec 24;121(3):566–70.
55. Kim YS, Yang HJ, Kee SJ, Choi I, Ha K, Ki KK, et al. The “Intermediate” CD14 + CD16 + monocyte subpopulation plays a role in IVIG responsiveness of children with Kawasaki disease. Pediatric Rheumatology. 2021 May 31;19(1):76.
56. Furukawa S, Matsubara T, Yabuta K. Mononuclear cell subsets and coronary artery lesions in Kawasaki disease. Arch Dis Child. 1992 Jun 1;67(6):706–8.
57. Furukawa S, Matsubara T, Jujoh K, Sasai K, Nakachi S, Sugawara T, et al. Reduction of peripheral blood macrophages/monocytes in Kawasaki disease by intravenous gammaglobulin. Eur J Pediatr. 1990 Nov;150(1):43–7.

1 FIGURE AND LEGENDS

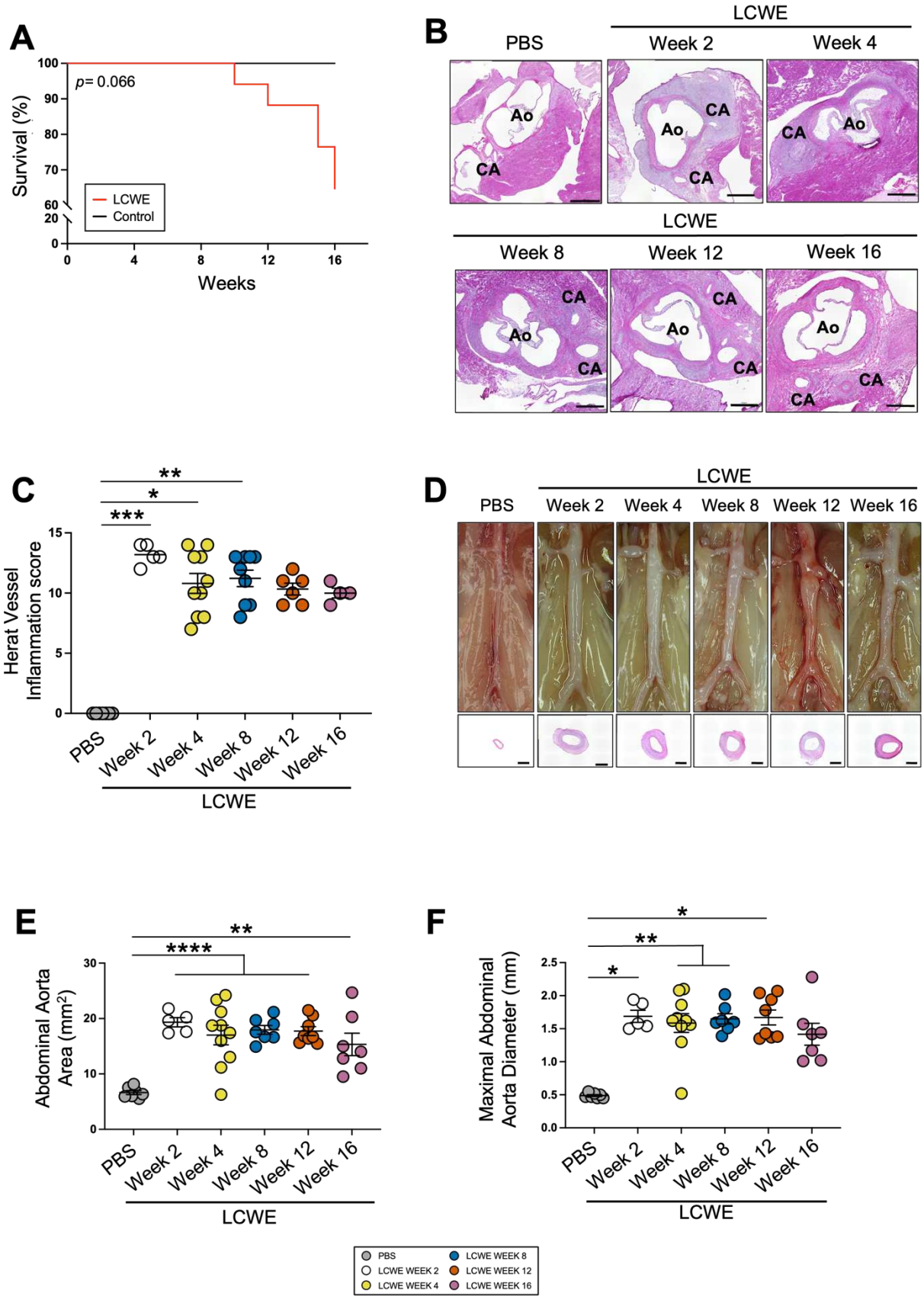
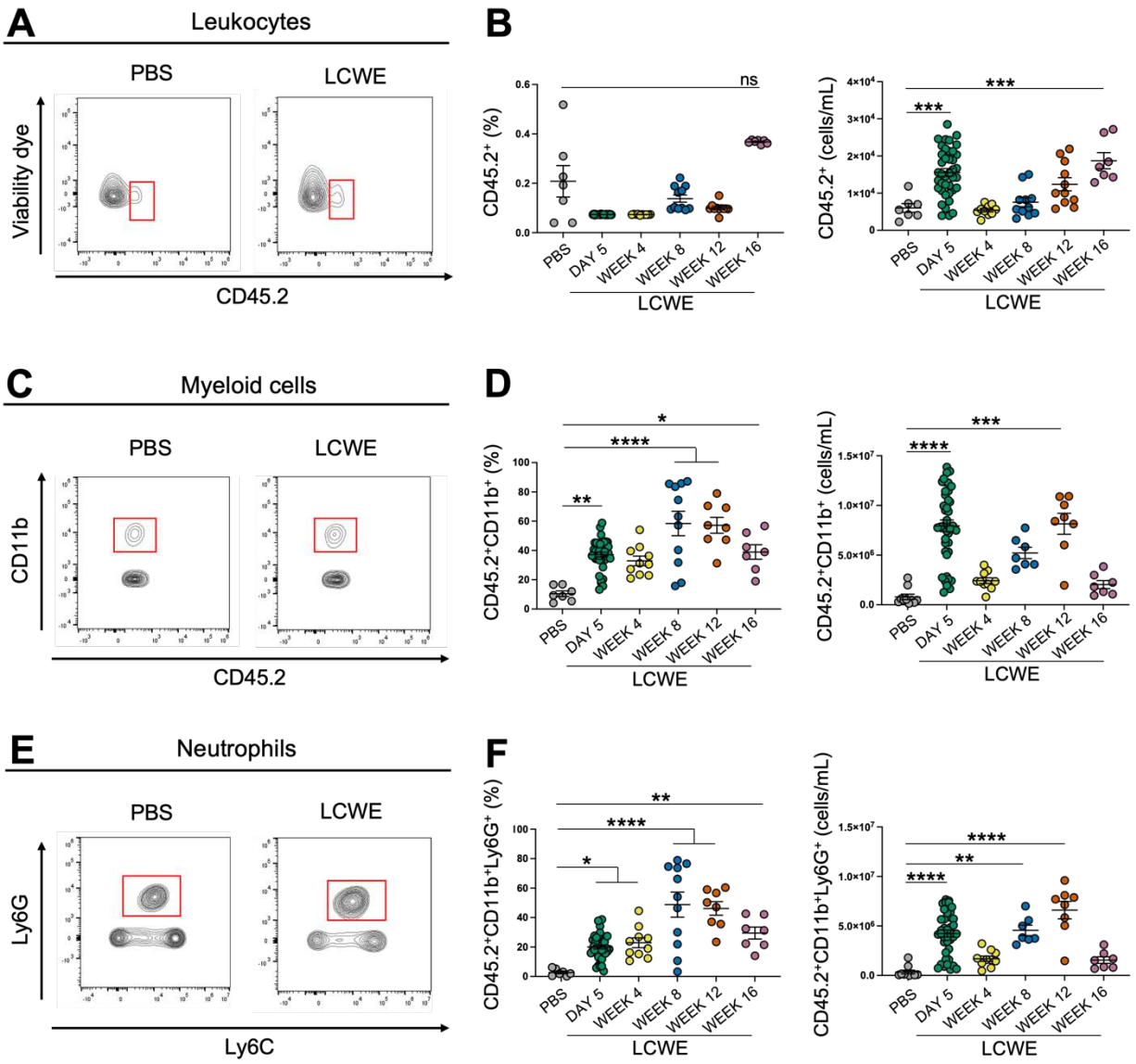


Figure 1. Long-term progression of LCWE-induced KD vasculitis. (A) Survival curves of PBS and LCWE-injected mice (n= 8-17 mice/group). **(B)** Representative H&E-stained heart tissue of PBS or LCWE-injected mice at weeks 2, 4, 8, 12, and 16 post-LCWE-injection. Scale bars: 500 μm. **(C)** Heart vessel inflammation scores of PBS and LCWE-injected mice at 2, 4, 8, 12, and 16 weeks post-LCWE injection (n= 4-10/group). **(D)** Representative pictures of the abdominal aorta area and H&E staining of the abdominal aorta cross-sections. Scale bars: 500 μm. **(E, F)** Abdominal aorta area (E) and maximal abdominal aorta diameter (F) measurements of PBS and LCWE-injected mice at the indicated time points post-LCWE injection (n= 7-9/group). Survival analysis wsa done by Log-rank test (Mantel-Cox) (A). Data are presented as mean ± SEM. * $p<0.05$, ** $p<0.01$, ***$p<0.001$, **** $p<0.0001$ by one-way ANOVA with Tukey post-test (E) or Kruskal-Wallis with Dunn's post-test (C, F). CA indicates coronary artery; and Ao, aorta.



1 **Figure 2. Increased circulating frequencies of immune cells during LCWE-induced**
2 **KD vasculitis. (A, B)** Flow cytometry plots (A), frequencies, and cell numbers (B) of live
3 CD45.2$^+$ cells in the blood of PBS and LCWE-injected mice (n=7-10 per group). (**C, D**)
4 Flow cytometry plots (C), frequencies, and cell numbers (D) of myeloid cells (live CD45.2$^+$
5 CD11b$^+$) in the blood of PBS and LCWE-injected mice (n=7-10 per group). (**E, F**) Flow
6 cytometry plots (E), frequencies, and cell numbers (F) of neutrophils (live CD45.2$^+$
7 CD11b$^+$ Ly6G$^+$) in the blood of PBS and LCWE-injected mice (n=7-10 per group). Data
8 are presented as mean ± SEM. *p<0.05, ** p<0.01, ***p<0.001, **** p<0.0001 by one-

way ANOVA with Tukey post-test or Kruskal-Wallis test with Dunn's post-test for nonparametric data.

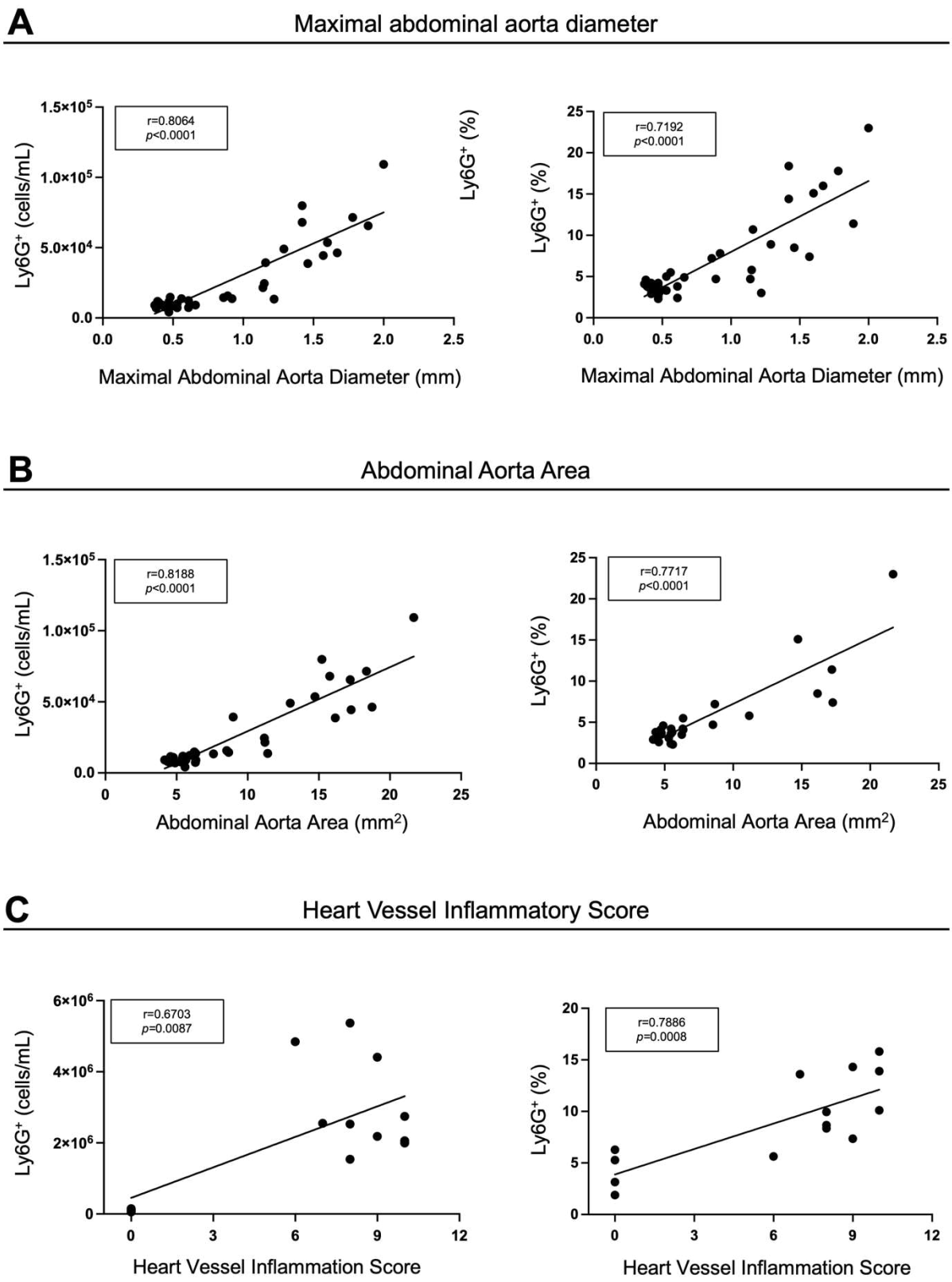


Figure 3. Correlation between heart vessel inflammation and abdominal aorta lesions with neutrophil levels in the peripheral blood five days after LCWE

injection. (A) Spearman Correlation between neutrophils count and frequencies and maximum abdominal aorta diameter at two weeks post-LCWE. **(B)** Spearman Correlation between neutrophils count and frequencies and abdominal aorta area at two weeks post-LCWE**. (C)** Spearman Correlation between neutrophils count and frequencies and heart vessels inflammatory score at two weeks post-LCWE. (n= 4-16 per group). r= correlation coefficient.

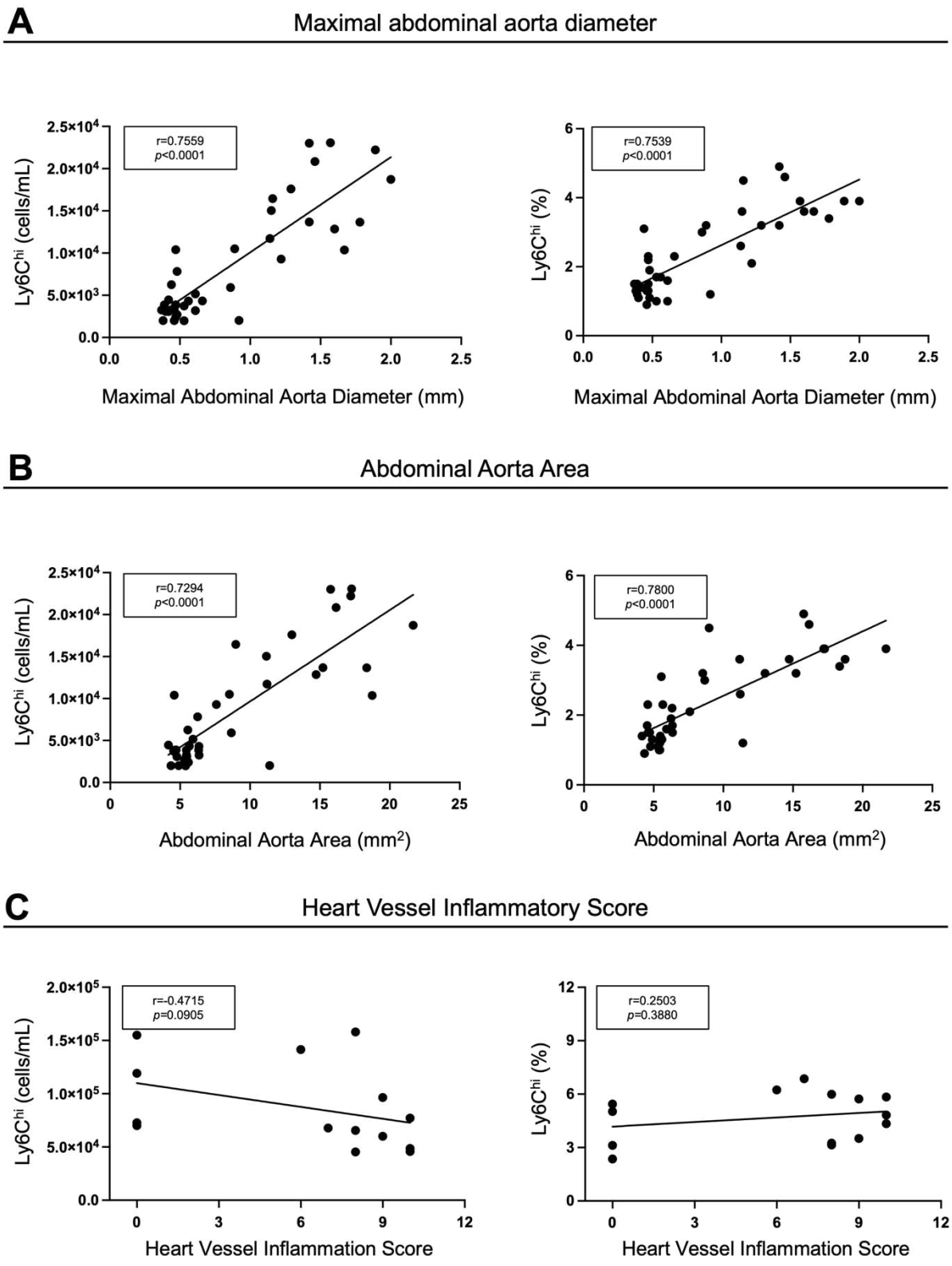
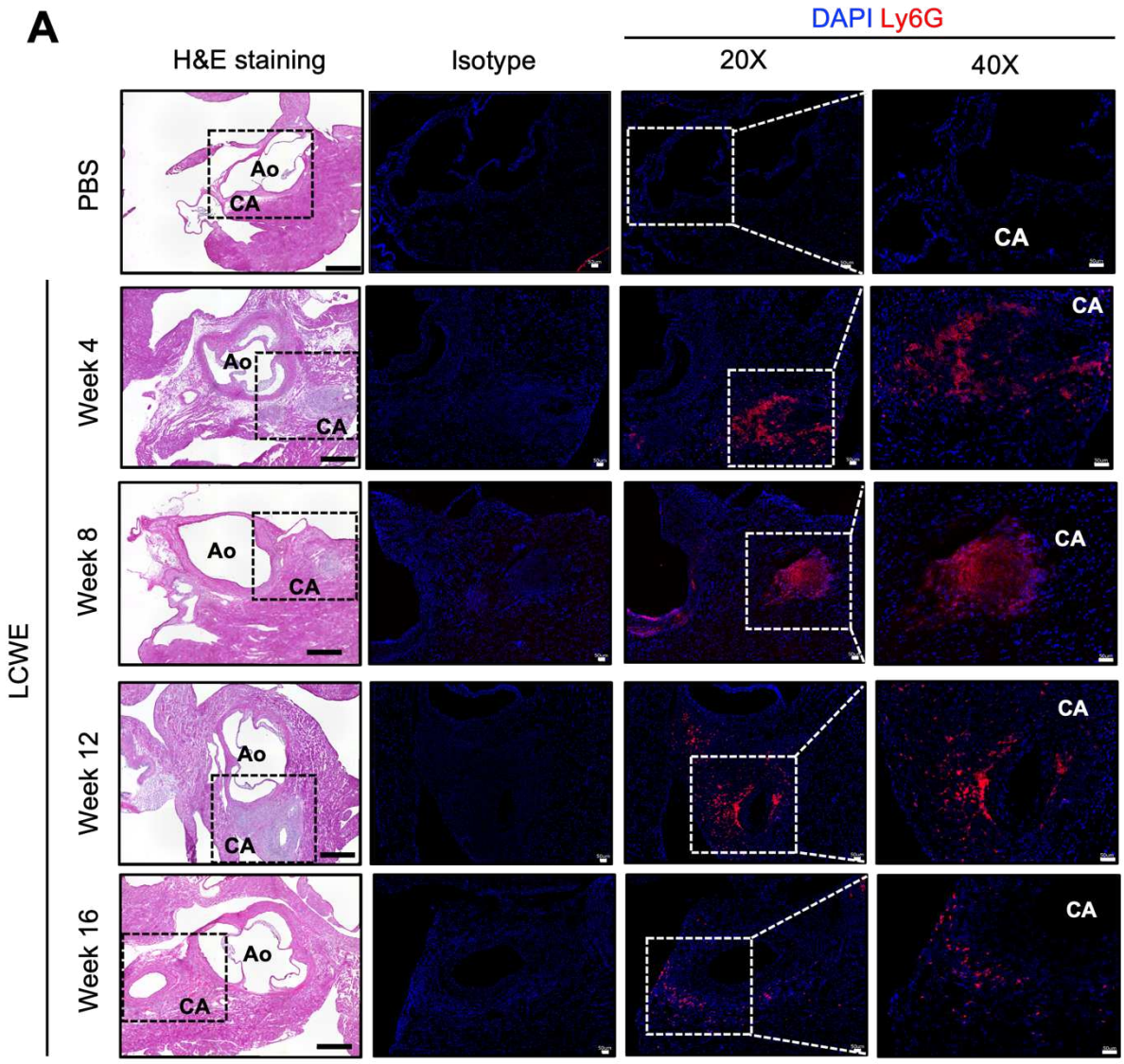


Figure 4. Correlation between heart vessel inflammation and abdominal aorta lesions with inflammatory monocyte levels in the peripheral blood five days after

LCWE injection. (A) Spearman Correlation between inflammatory monocyte count and frequencies and maximum abdominal aorta diameter at two weeks post-LCWE. **(B)** Spearman Correlation between inflammatory monocytes count and frequencies and abdominal aorta area at two weeks post-LCWE. **(C)** Spearman Correlation between inflammatory monocytes count and frequencies and heart vessels inflammatory score at two weeks post-LCWE. (n= 4-16 per group). r= correlation coefficient.


A
H&E staining
Isotype
DAPI Ly6G
20X
40X
PBS
LCWE
Week 4
Week 8
Week 12
Week 16
Ao
CA

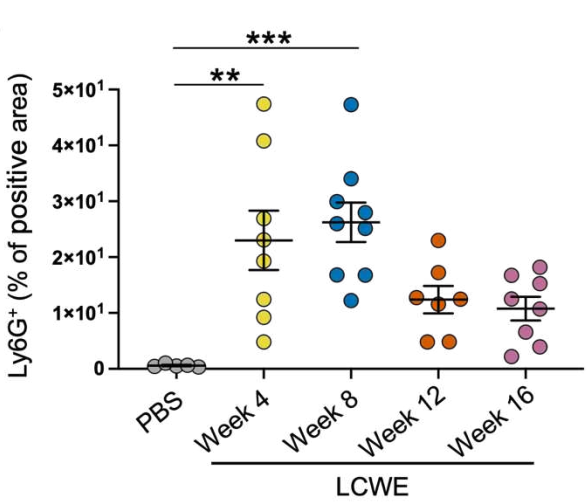
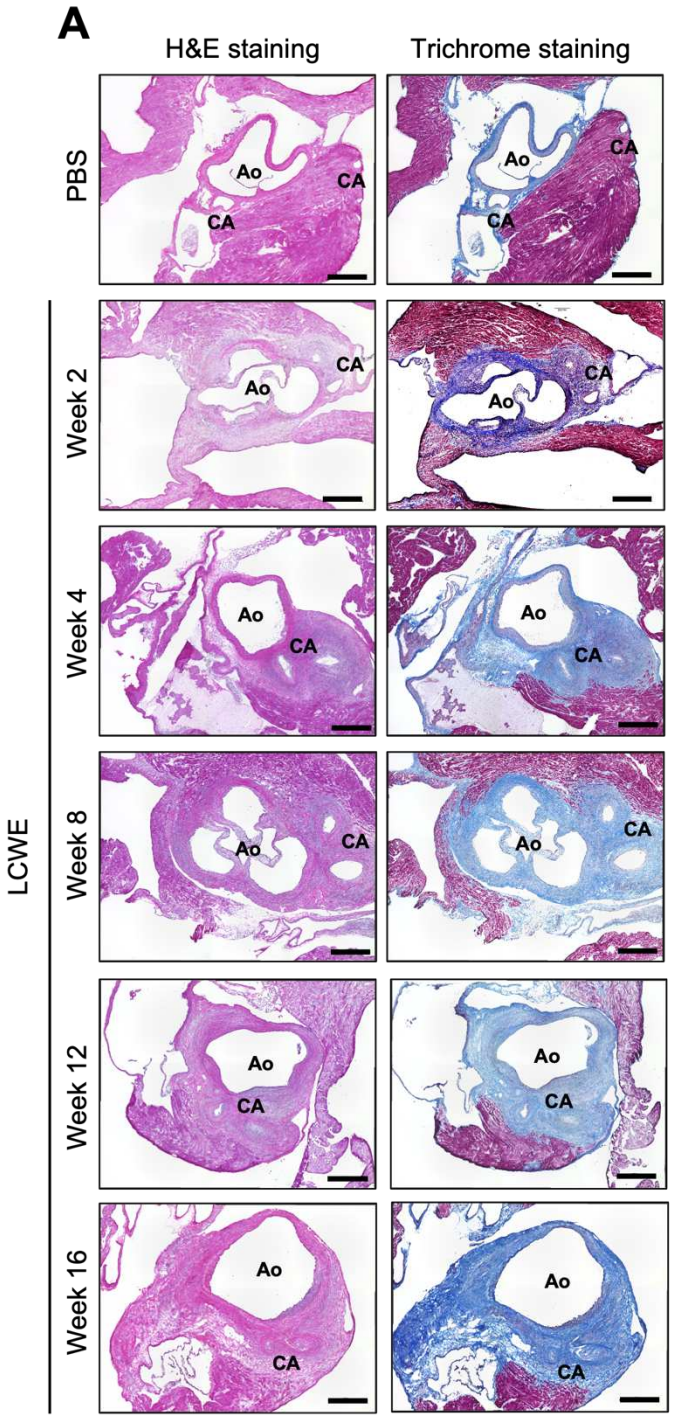
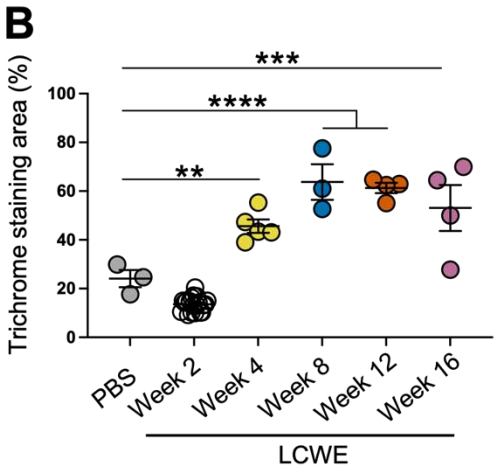

B
***
**
Ly6G+ (% of positive area)
5×10¹
4×10¹
3×10¹
2×10¹
1×10¹
0
PBS
Week 4
Week 8
Week 12
Week 16
LCWE

Figure 5. Long-term neutrophilic infiltrations in heart tissues of LCWE-injected mice. (A) Representative images H&E images and Ly6G (red). Immunofluorescence staining with Ly6G marker of heart tissues of PBS and LCWE-injected mice at different time pots post-LCWE injection (n= 5-9 per group). DAPI (blue) was used to identify cell nuclei. **(B)** Positive Ly6G area in the heart tissue was measured by ImageJ software (n=5-9 per group). The result is expressed in the percentage of heart tissue positive for the Ly6G marker. Scale bar in H&E images: 500 μm. Scale bar in isotype, 20X, and 40X images: 50 μm (A). Data is presented as mean ± SEM. ** $p<0.01$, *** $p<0.001$ by one-way ANOVA with Tukey post-test (n=3-5 per group) (B). CA, coronary artery; Ao; aorta.


A
H&E staining
Trichrome staining
PBS
Week 2
Week 4
Week 8
Week 12
Week 16
LCWE
Ao
CA


B
Trichrome staining area (%)
***
****
**
100
80
60
40
20
0
PBS
Week 2
Week 4
Week 8
Week 12
Week 16
LCWE

1

Figure 6. Long-term fibrosis in heart tissues from LCWE-injected mice. (A) Representative H&E staining and Masson's trichrome staining of heart tissue sections from PBS control mice and LCWE-injected mice at different time points post-LCWE injection. **(B)** Quantification of the fibrotic area (blue) in heart tissues of PBS and LCWE-injected at 2, 4, 8, 12, and 16 weeks post-LCWE. Scale bars: 500 μm. Data is presented as mean ± SEM. *p<0.05, ** p<0.01, by one-way ANOVA with Tukey post-test (n=3-5 per group). CA, coronary artery; Ao, aorta.

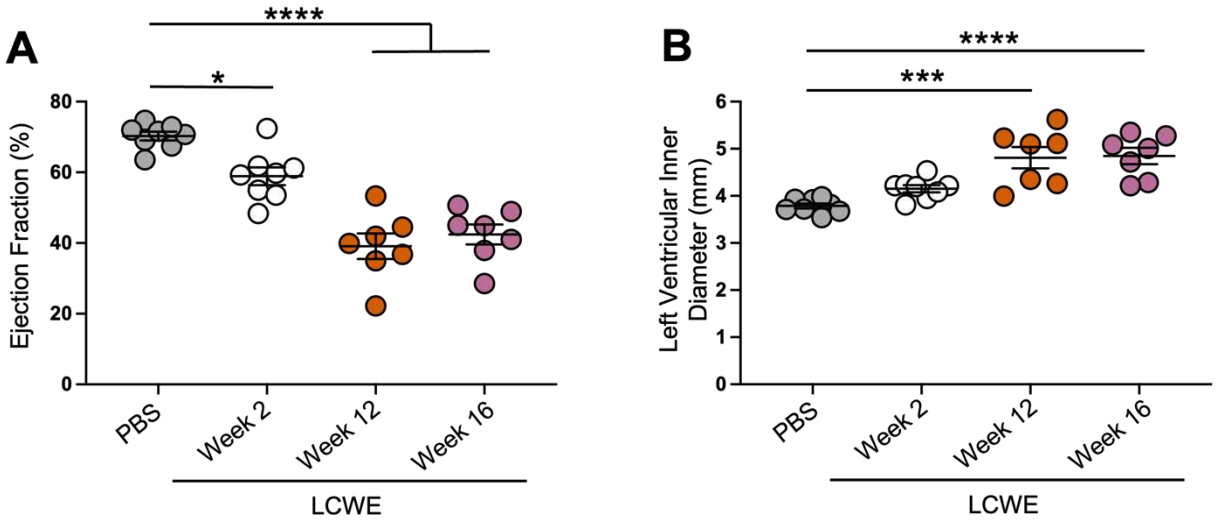
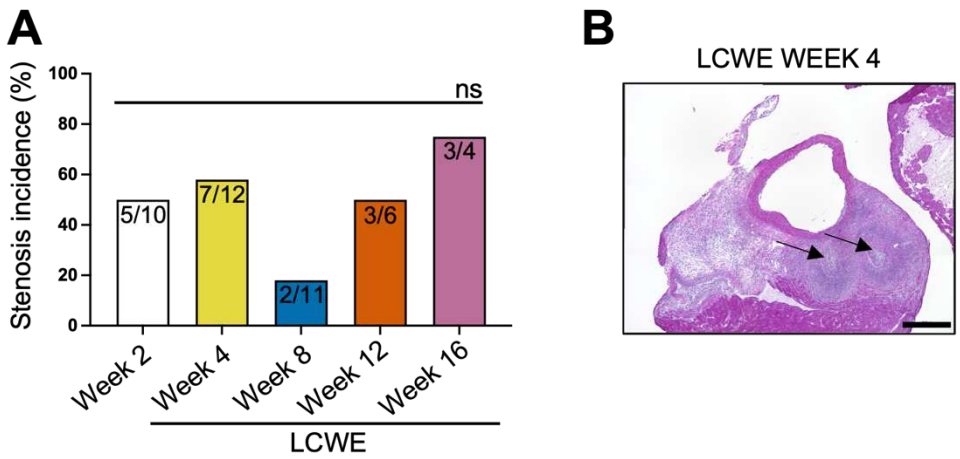
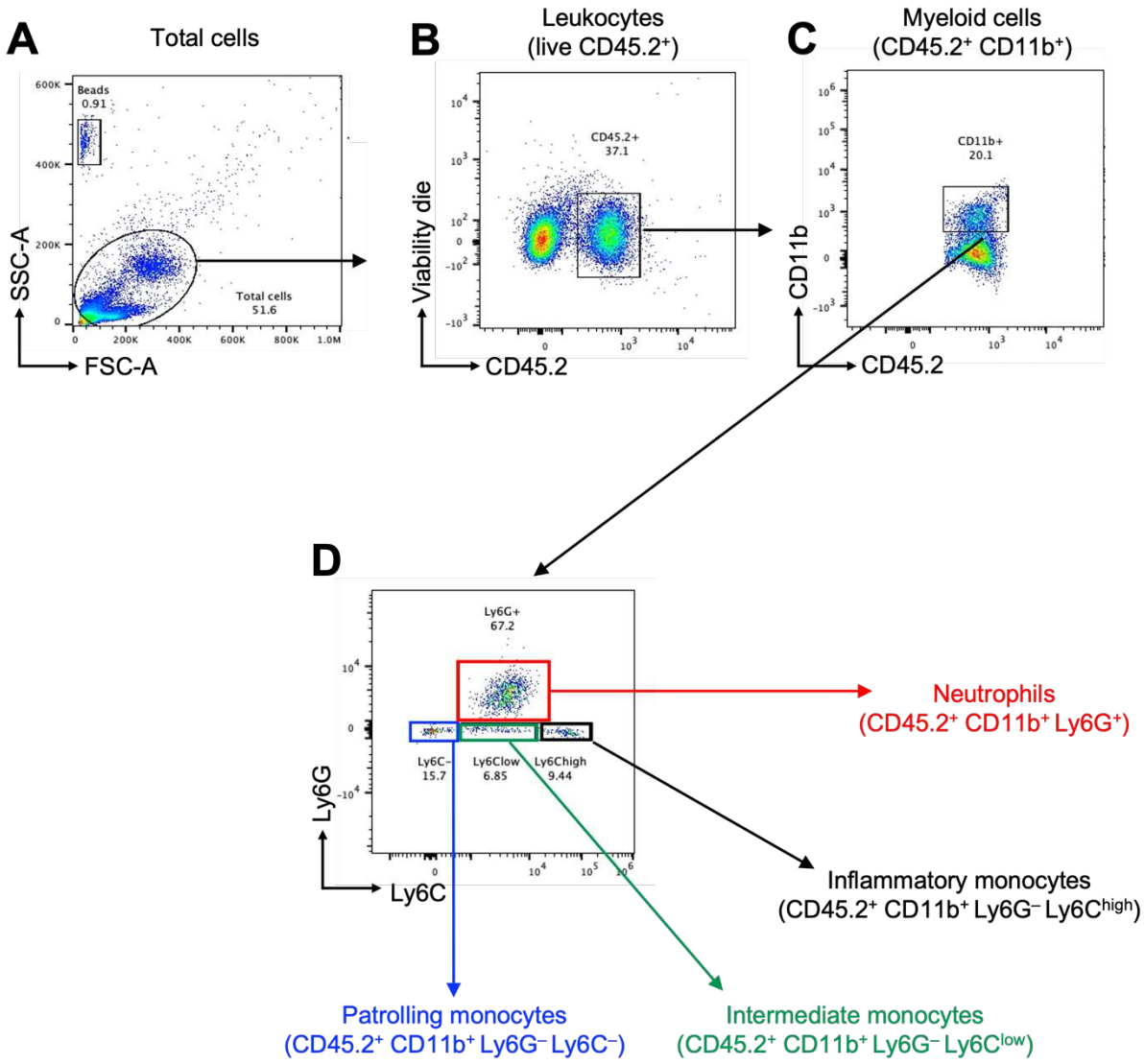


Figure 7. Reduced cardiac function in LCWE-injected mice. (A) The ejection fraction was measured by transthoracic echocardiograph in control PBS and LCWE-injected mice at 2, 12, and 16 weeks post-LCWE injection (n=7-8 per group). **(B). The left ventricle inner diameter was measured by transthoracic echocardiograph in control PBS and LCWE-injected mice at 2, 12,** and 16 weeks post-LCWE injection (n=7-8 per group). Data is presented as mean ± SEM. *p<0.05, **** p<0.0001 by one-way ANOVA with Tukey post-test (n=7-8 per group) (A, B).

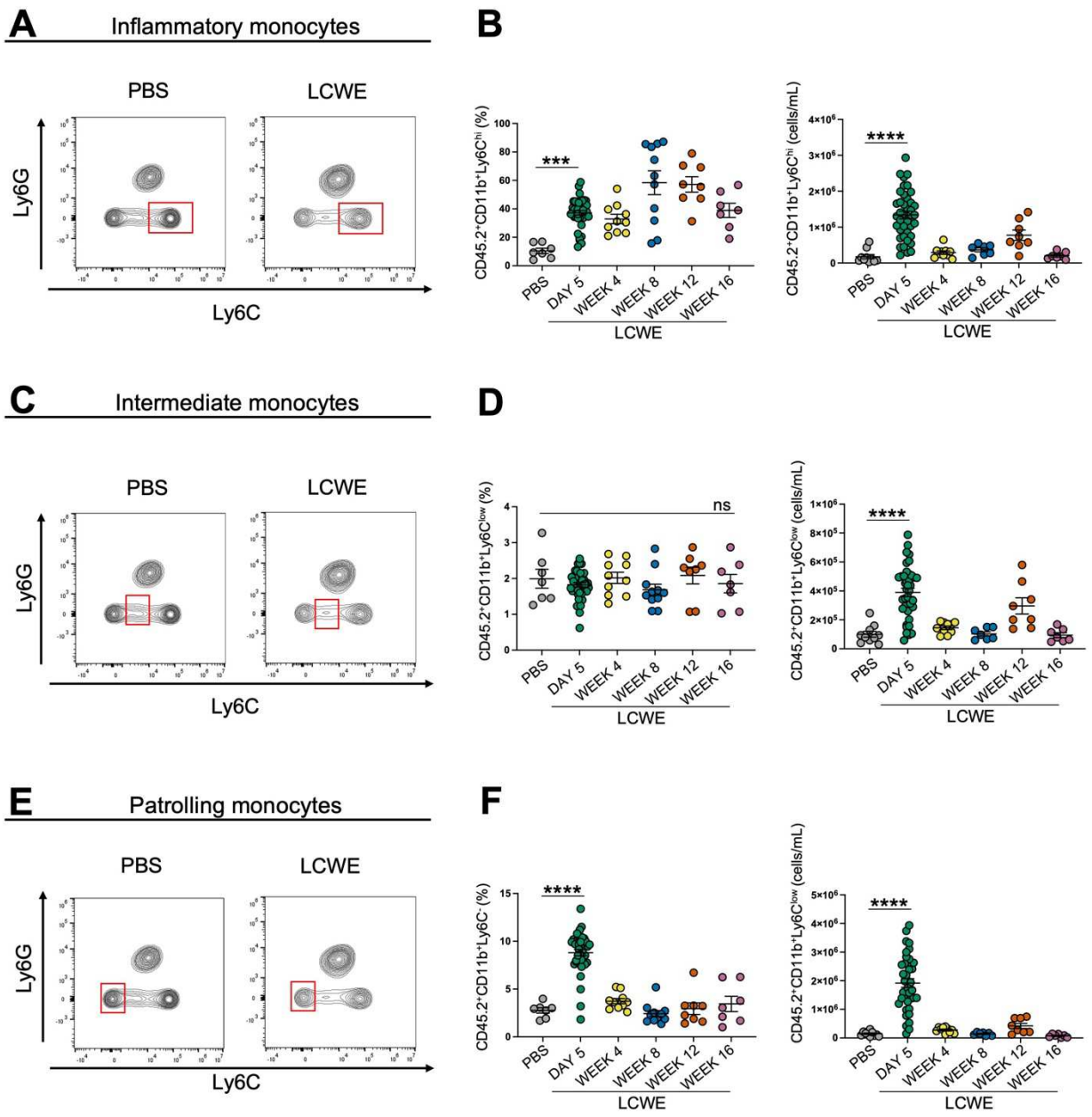


Supplementary Figure 1. Stenosis frequency in the coronary arteries up to 16 weeks after LCWE injection. (A, B) Frequency of coronary artery complete stenosis (A) over 16 weeks post-LCWE injection. Representative H&E staining of heart tissue (B) with complete stenosis event four weeks post-LCWE injection. The arrow indicates CA occlusion. Scale bars: 500 μm. Data is presented as mean ± SEM. One-way ANOVA with Tukey post-test (n= 4-12 per group).



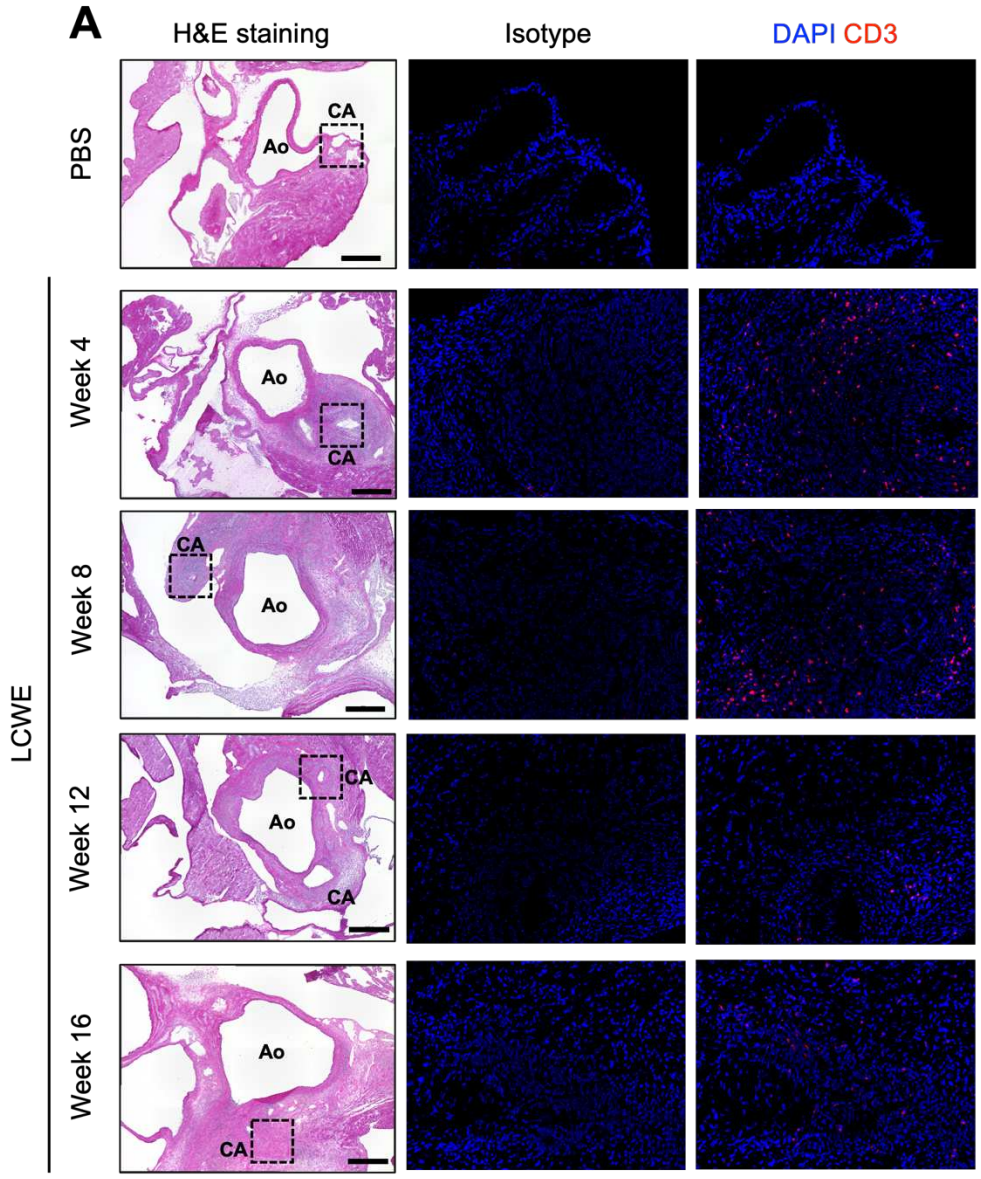
Supplementary Figure 2. Flow cytometric gating strategy and analysis of peripheral blood. (A-D) Representative flow plots showing the gating strategy used to characterize and determine the frequencies of leukocytes (B), myeloid cells (C), neutrophils (D), inflammatory monocytes (D), patrolling monocytes (D), and intermediate monocytes (D) in the blood of PBS and LCWE-injected mice.

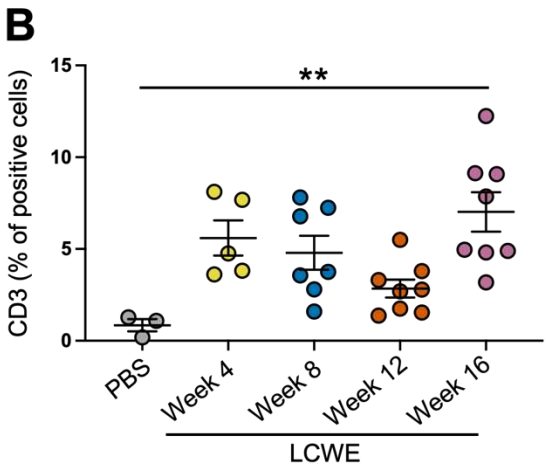
1



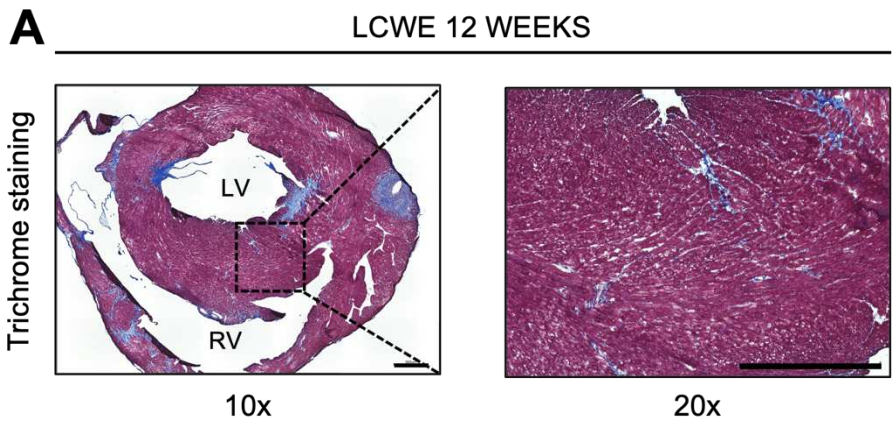
2 **Supplementary Figure 3. Increased circulating frequencies of monocytes during**
3 **acute LCWE-induced KD vasculitis. (A, B)** Flow cytometry plots (A), frequencies and
4 cell numbers (B) of inflammatory monocytes (live CD45.2$^+$ CD11b$^+$ Ly6C^{high}) in the blood
5 of PBS and LCWE-injected mice (n=7-10 per group). (**C, D**) Flow cytometry plots (A),
6 frequencies and cell numbers (B) of intermediate monocytes (live CD45.2$^+$ CD11b$^+$

Ly6C^{low}) in the blood of PBS and LCWE-injected mice (n=7-10 per group). (**E, F**) Flow cytometry plots (A), frequencies and cell numbers (B) of patrolling monocytes (live CD45.2^{+} CD11b^{+} Ly6C^{-}) in the blood of PBS and LCWE-injected mice (n=7-10 per group). Data are presented as mean ± SEM. ***p<0.001, **** p<0.0001 by one-way ANOVA with Tukey post-test or Kruskal-Wallis test with Dunn's post-test for nonparametric data.

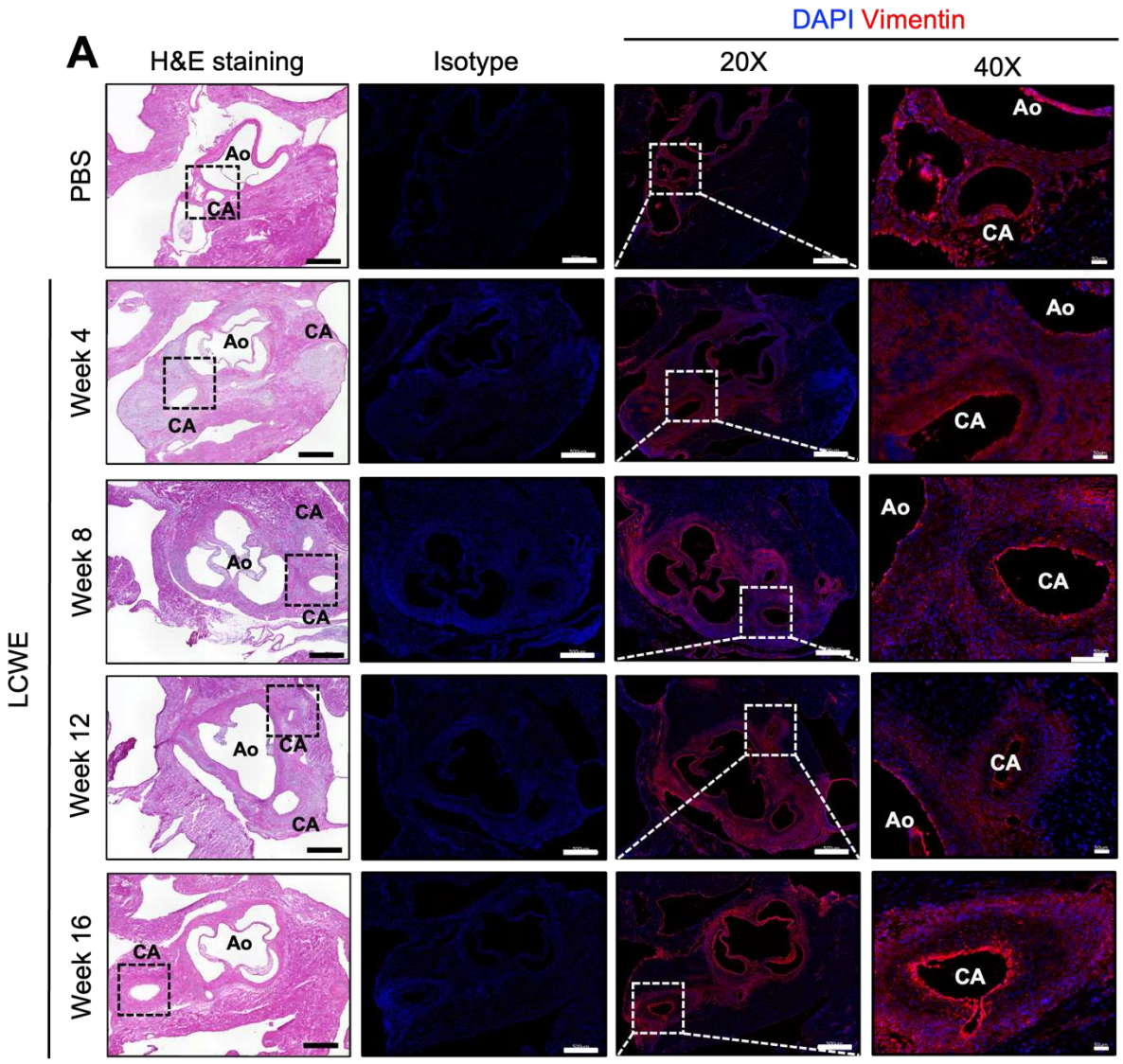

A
H&E staining
Isotype
DAPI CD3
PBS
LCWE
Week 4
Week 8
Week 12
Week 16
CA
Ao

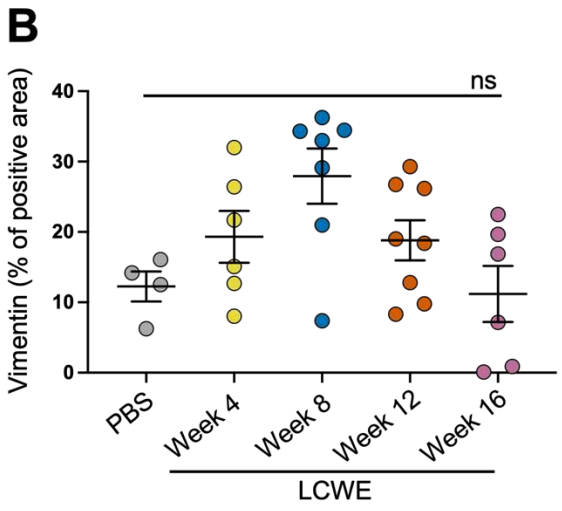

B
CD3 (% of positive cells)
15
10
5
0
**
PBS
Week 4
Week 8
Week 12
Week 16
LCWE

Supplementary Figure 4. Long-term T cell infiltrations in heart tissues of LCWE-injected mice. (A) Representative images H&E images and CD3 (red). Immunofluorescence staining with CD3 marker of heart tissues of PBS and LCWE-injected mice at different time pots post-LCWE injection (n= 3-8 per group). DAPI (blue) was used to identify cell nuclei. **(B)** ImageJ software was used to analyze positive CD3 cells in the heart tissue (n=3-8 per group). The result is expressed in the percentage of CD3 cells out of DAPI within the heart section. Scale bar in H&E images: 500 μm. Data is presented as mean ± SEM. ** $p<0.01$, by one-way ANOVA with Tukey post-test (n=3-8 per group). CA, coronary artery; Ao; aorta



Supplementary Figure 5. Interstitial fibrosis in the myocardium of LCWE-injected mice. (A) Masson's trichrome staining of myocardium tissue section from LCWE mice 12 weeks post-LCWE injection. Scale bars: 500 μm. LV, left ventricle artery; RV, right ventricle.


A
H&E staining
Isotype
DAPI Vimentin
20X
40X
PBS
Week 4
Week 8
Week 12
Week 16
LCWE
Ao
CA


B
Vimentin (% of positive area)
ns
40
30
20
10
0
PBS
Week 4
Week 8
Week 12
Week 16
LCWE

1

Supplementary Figure 6. Vimentin staining in cardiac tissue in LCWE-injected mice. (A) Representative images of immunofluorescence staining for Vimentin (in red) in cardiac sections. **(B)** Vimentin area percentage was calculated considering the entire cardiac tissue out of DAPI (in blue) area proportion (n=4-8 per group). Scale bars for H&E, isotype, and 10X images: 500 μm. Scale bar for 40X images: 50 μm. Data are presented as mean ± SEM. Ns $p>0.05$, by Kruskal-Wallis test with Dunn's post-test for nonparametric data.

4.2 PAPER 2

Protective Role of Omega-3 Derived Pro-Resolving Lipid Mediators in Cardiovascular Disease

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Short title: Resolvins and Maresins and cardiovascular protection.

Conflict of interest: The authors have declared that no conflict of interest exists.

Keywords: Cardiovascular. Inflammation. Resolution. Maresins. Resolvins.

ABSTRACT

Cardiovascular disease (CVD) is the primary cause of death and disability worldwide, and inflammation plays a crucial role in its immunopathogenesis. Although inflammation can be a protective response to harmful events, persistent and uncontrolled inflammation can worsen tissue injury and promote disease progression in the case of CVDs. Previously, resolution in self-limited inflammation was considered a passive event. However, the recent discovery of specialized lipid mediators (SPMs) derived from omega-3 and omega-6 fatty acids has redefined this concept. SPMs are composed of four classes of molecules: Resolvins, Maresins, Protectins, and Lipoxins. These molecules can actively induce the resolution of inflammation, promoting tissue healing and regeneration. Studies have shown that an imbalance of SPMs in CVD could contribute to disease immunopathogenesis, prompting the investigation of new therapeutic approaches using SPMs. Overall, results indicate that SPMs can limit leukocyte infiltration, induce macrophage polarization, enhance efferocytosis, promote inflammation clearance, decrease fibrotic tissue remodeling, and inhibit inflammatory signaling pathways, amongst other actions, in a receptor-specific manner. In this review, we summarized the potential role of SPMs in cardiovascular protection and critical findings of published studies using experimental murine models of the most common CVDs, including atherosclerosis, myocardial infarction, and abdominal aortic aneurysms.

INTRODUCTION

Inflammation is an immune-mediated biological process that responds to harmful stimuli initiated by several factors, such as pathogens, injuries, irradiation, and toxic

substances (1,2). Inflammation is a protective response that aims to eliminate danger, promoting healing and maintaining homeostasis. This response involves interactions between various immune cells, molecules, and the vascular system (3,4). The immune response coordinates the inflammatory process through the release of several immune mediators such as lipids, cytokines, chemokines, components of the complement system, vasoactive amines, and proteolytic enzymes, each of them playing a specific role in inducing cellular, molecular, and vascular alterations (3,5).

Critical events during the initial inflammation process promote the influx of leukocytes in the affected tissue. The first step involves increased vascular permeability and increased expressions of molecules that promote leukocyte arrest and migration from the bloodstream to the affected tissue. The production and release of inflammatory mediators, such as cytokines, chemokines, and leukotrienes, during inflammation are crucial to amplify the inflammatory signal and sustain inflammation to mitigate the stimuli (1,2).

During the inflammation process, the metabolism of cellular membrane-bound phospholipids is critical to produce lipids mediators that will act on the inflammation cascade. Leukocytes and other cells, such as endothelial cells, can uptake polyunsaturated fatty acids (PUFA) at the inflammation site, producing proinflammatory or anti-inflammatory lipid mediators. Usually, inflammation is a self-regulated process involving a balance of anti-inflammatory and pro-resolution mechanisms (5). In this scenario, proinflammatory lipid mediators are critical for boosting the inflammatory process to eliminate the primary cause of injury (4,5). On the other hand, anti-inflammatory lipid mediators promote the resolution of the inflammatory process, such as

tissue healing and homeostasis (6,7). In recent decades, the resolution process of inflammation has gained increased attention following the discovery of a novel family of lipid mediators with anti-inflammatory and resolution properties (8). Previously, the switch from inflammation to resolution was believed to be passive and programmed only by anti-inflammatory mechanisms (7,9). These newly discovered pro-resolving lipid mediators were termed specialized pro-resolving mediators (SPMs) and altered the perspective on the molecular and cellular mechanisms involved in inflammation's natural progression. SPMs are produced locally during inflammation and can coordinate immune responses to induce tissue regeneration and resolve inflammation. Therefore, a dysfunctional resolution process could lead to chronic inflammation and diseases, causing irreparable damage to the host (7,10).

The incidence of cardiovascular diseases (CVDs), a group of disorders affecting the vascular system, has increased dramatically over the decades, affecting almost 500 million people worldwide (11). CVDs are a leading cause of disability and mortality globally, causing 20.5 million deaths in 2021 (12). Ischemic heart disease and stroke are the most common CVDs, representing 85% of deaths (12). Several genetic and lifestyle factors are associated with increased susceptibility and mortality risks to CVD. Unhealthy lifestyle habits such as smoking, high sodium diets, excessive alcohol consumption, and sedentary habits can lead to high blood pressure, elevated glucose and cholesterol levels, diabetes, and obesity, which are strongly associated with the development of CVDs (11). Changes and implementation of healthy lifestyle behaviors significantly reduce the risks of CVD-related mortality and morbidity (12,13).

CVDs are often associated with maladapted and prolonged unresolved inflammation and risk factors associated with CVDs can trigger local and systemic inflammation (11,14). Injury to the endothelial barrier plays a crucial role in developing cardiovascular diseases (CVDs). The damage exposes the adjacent vascular layers to blood components, including platelets, coagulation factors, and leukocytes, which are rapidly recruited to the site, and a complex cascade of events that promote inflammation and thrombotic events are triggered. The production of proinflammatory cytokines and reactive oxygen species (ROS) and the release of proliferative growth factors and adhesion molecules further promote the continuation of inflammation, leading to alterations in the other vascular layers. Consequently, vascular smooth muscle cells (VSMCs) are activated and proliferate, acquiring myofibroblastic characteristics (14–16).

Studies have questioned whether SPMs could harness the resolution process in CVD's immunopathology, as inflammation plays a significant role in the underlying problem of CVDs (16). Indeed, decreased levels of SPMs were associated with severe lesions and plaque instability in murine models of atherosclerosis (17,18). The administration of SPMs improves plaque stability, reduces necrotic core, increases efferocytosis, and decreases the production of proinflammatory cytokines, ultimately promoting the resolution of inflammation and tissue repair (19,20). Overall, SPMs can reduce leukocyte infiltration, enhance efferocytosis, promote inflammatory debris clearance, induce healing mechanisms, and decrease the secretion of inflammatory cytokines (18,21).

In this review, we outline the latest research investigating the functions of omega-3-derived SPMs, which promote the resolution of inflammation and tissue repair in the

context of CVDs. We also discuss the immune mechanisms associated with a dysfunctional resolution of inflammatory processes, which may contribute to cardiovascular immunopathogenesis.

BIOSYNTHESIS OF SPECIALIZED PRO-RESOLVING MEDIATORS

SPMs are classified into Resolvin D series (RvDs), Resolvin E series (RvEs), Maresins (Mars), Protectins (PDs), and Lipoxins (LXs). Various cells, including leukocytes, endothelial cells, and platelets, utilize polyunsaturated fatty acids (PUFAs) to convert them into SPMs. Resolvins E and D series, Maresins, and Protectins derive from Omega-3 PUFAs intermediates, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whereas lipoxins originate from arachidonic acid (AA) from Omega-6 PUFA (**Figure 1**).

Omega-6 PUFA can also produce proinflammatory lipids such as leukotrienes (LTs) and prostaglandins (23). These lipids are found in high concentrations at the site of inflammation in unresolved lesions. Enzymatic conversion of LTs occurs through 5-LOX, while COX converts prostaglandins. These proinflammatory lipid mediators induce the production of proinflammatory cytokines, promote vascular permeability and leukocyte transmigration, and promote inflammation (22).

The enzyme 15-LOX catalyzes the conversion of AA into 15-hydroxyperoexyeicosatetraenoic acid (15-HPETE), which undergoes further conversion into lipoxin A (LXA) and B (LXB) through either 5-LOX in neutrophils or 15-LOX in erythrocytes and reticulocytes (**Figure 1, left panel**) (23). Alternatively, COX-2 can be

acetylated by aspirin, catalyzing the formation of 15-HPETE from AA, which culminates in the generation of aspirin-triggered Lxs (AT-LXs) (23).

EPA is metabolized primarily via two distinct enzymatic pathways, COX-2 and CYP450. EPA is then converted into the intermediate 18-(hydroperoxy) eicosapentaenoic acid (18-HpEPE). This intermediate is then reduced to 18R-HEPE by peroxidase and metabolized by 5-LOX or 15/LOX resulting in the formation of RvEs (**Figure 1, right panel**) (24).

Resolvins D-series (RvD), protectins (PDs), and maresins (Mars) are generated from the metabolism of DHA through a synthesis far more complex than that of other lipids. DHA can undergo lipoxygenation via 15-LOX to form 17S-HpDHA and further oxygenated by 5-LOX to form RvD1-6 (**Figure 1, right panel**) (25). DHA can also generate aspirin-triggered RvDs via aspirin-acetylated-COX-2 and 17R-resolvin D-class via CYP450 metabolization. Protectins are formed from 17S-HpDHA by 15-LOX (**Figure 1, right panel)** (26)**.** DHA can also be metabolized by 12-LOX, generating 14-S-HpDHA that will further be transformed into Maresins by 5-LOX (**Figure 1, right panel)** (27).

RECEPTORS AND SIGNALING PATHWAYS OF SPECIALIZED PRO-RESOLVING MEDIATORS

SPMs can modulate the activity of G protein-coupled receptors (GPCRs) and interact with other receptor types, producing effects in a cell-specific manner. However, the current knowledge about these interactions is limited, and further investigation is needed to understand the downstream signaling cascade (28). In this section, we briefly describe the main receptors reported to be ligands for SPMs.

RvD1 is reportedly to interact with two GPCRs expressed by human leukocytes, FPR2/ALX and GPR32 (29). These interactions boost human macrophages' phagocytic and clearance functions (30). The murine *fpr2* gene is the human ortholog of the FPR2/ALX receptor, and in mice, there is no ortholog for GPR32 (30,31). However, how RvD1 interacts with ALX/FPR2 in mice has yet to be fully understood. Administering RvD1 in *fpr2*-deficient mice eliminates the ability of RvD1 to reduce the recruitment of polymorphonuclear neutrophils (PMNs) during acute peritonitis, indicating that RvD1 possibly interacts specifically with FPR2 in mice (32). RvD2 interacts with GPR18 and is expressed in human and murine PMNs, monocytes, and macrophages (33). Through this interaction, RvD2 stimulates the phagocytosis of apoptotic PMNs and microbial components and contributes to the clearance of infection and resolution of inflammation (33). Moreover, RvD3 and RvD5 can interact with human GPR32, stimulating macrophage phagocytosis of microbial particles (34).

RvE1 binds to the BLT1 receptor, antagonizing BLT1 actions and preventing LTB4-BLT1 interaction, thereby hampering LTB4's inflammatory signals and functioning as a local damper of LTB4 (35). RvE1 can also interact with ChemR23, expressed in human and mouse monocytes, macrophages, and dendritic cells (DCs) (35,36). The interaction of RvE1 with ChemR23 decreases IL-12 production in DCs, limits neutrophil recruitment, and enhances macrophage uptake of apoptotic cells at the inflammation site (37).

Mar1 can interact with LGR6 expressed by immune cells aiding the activation of innate immune response, stimulating phagocytosis and efferocytosis activity by macrophages (38). Interestingly, Mar1 has been reported to interact with a receptor

associated with pain perception, the transient receptor potential vanilloid 1 (TRPV1), blocking its activation and showing important analgesic effects (39). Moreover, Mar1 can also stimulate retinoic acid-related orphan receptor-α (RORα), associated with the transcription of genes involved in lipid metabolism and enhancement of biosynthesis of Mar1 by induction 12-LOX expression, functioning as an autoregulation loop (40).

PD1 was reported to interact with GPR37, which is highly expressed in the brain. The interaction of PD1/GPR37 seems to promote the phenotype switch of macrophages towards pro-resolution (41). PD1 can also enhance the peroxisome proliferator-activated receptor γ (PPAR-γ) transcriptional function promoting anti-amyloidogenic activity in human neuronal-glial cells (42). **Table 1** summarizes the receptors reported to interact with specialized pro-resolving lipid mediators, their specific interactions, and whether they antagonize or block their function.

Table 1. Receptors identified to interact with specialized pro-resolving lipids.

Receptor	SPMs	Interaction	References
ChemR23 (or ERV1)	RvE1 RvE2	Agonist	Arita, et al. (35) Oh, et al. (36)
BLT1	RvE1 RvE2	Inhibitor	Arita, et al. (35) Oh, et al. (36)
FPR2/ALX	LXA4 RvD1	Agonist	Krishnamoorthy, et al. (29)
GPR18 (or DRV2)	RvD2	Agonist	Chiang, et al. (33)
GPR32 (or DRV1)	RvD1 RvD3 RvD5	Agonist	Arnardottir, et al. (31) Norling, et al. (32) Dalli, et al. (34) Chiang, et al. (43)
LGR6	Mar1	Agonist	Chiang, et al. (38)
TRPV1	Mar1	Inhibitor	Park, et al. (39)
RORα	Mar1	Agonist	Han, et al. (40)
GPR37	PD1	Agonist	Bang, et al. (41)

Abbreviations: Chem23, Chemerin receptor 23; BLT1, Leukotriene B4 receptor 1; FPR2/ALX, N-formyl peptide receptor 2; GPR18, G protein-coupled receptor 18; GPR32, G protein-coupled receptor 32; LGR6, G protein-coupled receptor 6; TRPV1, Transient receptor potential vanilloid 1; Retinoic acid-related orphan receptor-α, ROR-α; G protein-coupled receptor 37, GPR37.

SPM IN CARDIOVASCULAR DISEASES

In general, risk factors for CVD are associated with metabolic problems that are directly affected by an unbalanced diet, sedentary lifestyle, poor sleep quality, stress, and tobacco use (44). It is now known that the regulation of metabolism and the immune system are closely interconnected, and any disturbance in their interaction can result in the development of cardiovascular diseases (44). There is a correlation between the increased adoption of the Western-type diet (WD) and the rise in the rates of chronic metabolic disorders (45). The WD is based on the intake of calorie-rich food with high amounts of sugar and saturated fats. Overall, the foods lack nutritional value and do not provide sufficient nourishment. Moreover, they are energy-dense and cause rapid spikes in glucose and insulin levels, resulting in weight gain, altered lipid profiles, metabolic changes, and immune system activation, setting a perfect stage for CVD development (45–47).

As mentioned, SPMs are produced from unsaturated fatty acids, mainly Omega-3 PUFAs, that can be obtained from seafood or nuts through consumption or supplementation. Fish and fish oil are the main sources of DHA and EPA, the precursors for SPMs (48). Although metabolization of Omega-6 can produce Lipoxins, a type of SPMs, they are also the source of pro-inflammatory lipid mediators. The WD presents an unbalanced intake of omega-3 and omega-6 PUFAs in the dietary food choice, and studies have associated a high ratio of omega-6/omega-3 PUFA intake with many chronic diseases (49). The possible link between dietary habits and the prevalence of CVDs has led to several discussions on the real impact of Omega3/Omega-6 consumption in

inflammation, more specifically associated with cardiovascular problems. Researchers have suggested that Omega-3 supplementation could prevent cardiovascular events and improve outcomes (50).

Randomized clinical trials have shown that Omega-3 supplementation, with a total daily intake of 1.08g of EPA and 0.72g of DHA, significantly reduced fatal cardiac events in patients with acute MI on Omega-3 supplementation for at least one year (44,51,52). One of the largest randomized clinical trials published on Omega-3 supplementation and CVD outcomes was conducted by the Italian research group, GISSI-Prevention Study (53). They show a reduction of all-cause mortality in patients with a history of MI within 3 months that were assigned with daily supplementation of 850mg of EPA and DHA and followed for 3.5 years (53). The same study group reported benefits with the same supplementation dosage in heart failure cases, reducing mortality and hospital admissions for cardiac events (54).

Nilsen and collaborators also used with daily supplementation of 850mg of EPA and 882 mg DHA in patients with recent history of MI but found no difference in mortality or outcomes (55). A recent study published by the Risk and Prevention Study Collaborative group randomly assigned individuals with several clinical risk factors for cardiovascular problems to receive a daily placebo containing olive oil or 1g of Omega-3 supplement. However, no evidence was found to support the omega-3 supplementation in CVD cases (56).

Clinical trials have yet to reach a consensus on the beneficial impact of Omega-3 supplementation on the development of CVDs, and results remain controversial (50,56,57). When analyzing and comparing clinical trial results, it's essential to consider

several biases. Factors such as population target, sample size, selection of patients, exclusion criteria, medication intervention, and others can impact the study's outcome. Additionally, some studies didn't use placebos or included dietary habits bias, which could affect Omega-3 levels. It is also important to ensure that the concentration and purity of DHA and EPA in Omega-3 supplement capsules given to the patients are of good standards. Despite the difficulties, Omega-3 supplementation has proven well-tolerable and safe, with minor side effects reported. On this note, based on results from randomized clinical trials, the American Heart Association recommends the daily intake of 1g of EPA/DHA as a secondary prevention in patients with MI history and heart failure (58).

Current immunological and mechanistic insights on the beneficial effects of SPMs in the context of CVDs have been obtained using several different experimental murine models of CVDs. In the following sections, we will provide recent examples of studies conducted with murine models in the context of CVDs, discussing the beneficial effects of omega-3-derived SPMs when used as treatment.

Atherosclerosis

Atherosclerosis is a chronic inflammatory disease characterized by the accumulation of lipids, inflammatory cells, smooth muscle cells, and cellular debris in the walls of large and medium-sized arteries (59). Over time, the accumulation creates a buildup within the vessel wall covered by a fibrous cap, forming an atherosclerotic plaque (or atheroma) (46). The formation of atherosclerotic plaques may begin early in life and is usually asymptomatic until it progresses to advanced stages (59). The plaque grows towards the lumen and can potentially obstruct normal blood flow, causing tissue

ischemia. When this process affects coronary arteries, it causes ischemic heart disease (IHD) or can lead to rupture, forming a thrombus that travels to other organs, including the brain, and can cause ischemic stroke and even death (11). Diagnosis and detection of abnormalities in the arteries can be assessed by non-invasive imaging exams, such as ultrasonography, which measures the intima-media thickness of artery walls and detects early changes (12,13).

The development of atherosclerosis is complex and involves multiple processes with the participation of low-density lipoprotein derived (LDL) from cholesterol and innate and adaptive immune cells (60). Normally, cells take up LDL as needed and regulate the expression of its receptor (LDL-R) to capture it. However, when adopting a Western lifestyle, a high amount of cholesterol is available in the bloodstream, and other factors such as chronic stress, smoking, and toxins can induce ROS generation that oxidases the excess of LDL. The oxidized LDL (ox-LDL) is a more reactive form of LDL and can activate macrophages and endothelial cells.

Damage to endothelial cells and accumulation of ox-LDL in the intimal layer, which can also bind to the extracellular matrix molecules, are initial events that trigger atherosclerosis (14). A series of inflammatory mechanisms are then activated, leading to the recruitment of leukocytes at the injury site. This leukocyte recruitment is mediated by the chemokine monocyte chemoattractant protein-1 (MCP-1), which attracts circulating monocytes that bind to vascular-cell adhesion molecules 1 (VCAM-1) expressed by the activated endothelial cells (61). In the intima layer of the blood vessel, monocytes differentiate into macrophages and bind to oxLDL, progressively engorging and forming

cholesterol foam cells (62,63). The accumulation of these foam cells within the lesion area is considered a hallmark of atherosclerosis (63).

Uptake of ox-LDL uptake by macrophages can also lead to lysosomal damage, activating NLRP3 inflammasome with consequent production of inflammatory cytokines. Proinflammatory cytokines, such as IL-1β, amplify the inflammatory response, resulting in further generation of ROS and the proliferation of vascular smooth muscle cells (VSMCs) (63). In addition, Th1 T cells are also migrating into the inflamed area, releasing inflammatory cytokines, such as IL-1β and TNF-α, activating macrophages, endothelial cells, and VSMCs, thus exacerbating local inflammation. Combined with a compromised efferocytosis process, the buildup of apoptotic cells and foam cells creates a necrotic core surrounded by a fibrotic cap that grows towards the lumen and can potentially rupture (64). Whether it ruptures or not, blood flow may be partially or completely blocked, preventing regular blood supply and resulting in myocardial infarction or stroke (64).

Atherosclerosis lesions may result from persistent inflammatory activation and a failure of resolution mechanisms (65). Endogenously produced SPMs orchestrate the resolution phase of the inflammatory response, playing a significant role in downregulating proinflammatory components and, more importantly, actively stimulating clearance and tissue repair. In contrast to omega-3-derived lipids that may protect against atherosclerosis, omega-6 PUFA metabolism by 5-LOX can also lead to the production of inflammatory lipids such as PGE2 and leukotriene B4, which promote inflammation and exacerbate the disease's development.

Experimental murine models of atherosclerosis have been used to investigate this paradigm (66). Apoe is a protein involved in the mechanisms for LDL uptake by cells.

Therefore, ApoE$^{-/-}$ mice have high levels of LDL and spontaneously develop atherosclerosis (67). The development of atherosclerotic plaque can be sped up by feeding the mice with a Western-type diet. It was shown that ApoE$^{-/-}$ mice have a higher ratio of omega-6/omega-3 FAs in the plasma and the aorta lesions (66).

A study investigated the possibility of endogenously inducing a higher conversion of omega-3 in mice. The group used a Fat-1 transgenic mice, a protein, not encoded by mammals, that can catalyze the conversion of omega-6 PUFA to omega-3 PUFA, therefore, transgenic Fat-1 mice have a higher ratio of omega-3/omega-6 PUFA (61). Crossing *ApoE*$^{-/-}$ mice with *Fat-1* transgenic mice reduced the severity of atherosclerotic lesions associated with a lower omega-6/omega-3 ratio. They also had significantly lower levels of IL-6 and PGE2 in aortic lesions, reduced expression of genes related to inflammation (*ICAM-1*, *MCP1, COX-2*, and *NF-κB)*, and fewer F4/80-positive macrophages were observed in the lesion (68). Together, the results suggest the atheroprotective role of omega-3-derived lipids.

Leukotrienes also participate in the atherosclerosis inflammatory process. They are essential inflammatory lipid mediators derived from the omega-6-PUFA and deoxygenized by 5-LOX, the same enzyme involved in SPM biosynthesis (69). Phosphorylation mechanisms, such as calcium influx and MAPK activation, may result in the translocation of 5-LOX to the nucleus and induce the production of leukotrienes during leukocyte recruitment and activation (70). On the other hand, cytoplasmatic 5-LOX leads to SPM production (71,72). When leukocytes metabolize omega-6-PUFAs, the location of the 5-LOX enzyme can determine whether they produce LTB4 or LXA4. The nuclear location of 5-LOX favors LTB4 production, whereas cytoplasmatic 5-LOX favors LXA4

production. Fredman et al. reported a mechanistic pathway in which RvD1 hampers the production of LTB4 by altering the ratio of nuclear/cytoplasmatic/5-LOX (70). RvD1 binds to its receptor ALX/FPR2 in macrophages, suppressing a key molecule responsible for the intracellular signaling that mediates proinflammatory lipid production (70).

Quantification of lipid mediators in atherosclerotic plaque of human patients and *Ldlr*$^{-/-}$ mice fed a WTD indicated an imbalance between 5-LOX-derived SPMs and leukotrienes in vulnerable human plaques (19). Specifically, RvD1 levels were significantly reduced in regions with higher reactive ROS activity. *In vitro* experiments using human macrophages indicate ROS suppresses RvD1 production (19). SPM intermediates were detected, suggesting that LOX enzymes and COX-2 were active in the lesion area, meaning that SPM's production was unaffected by enzyme inactivation (19).

Atherosclerotic plaques from *Ldlr*$^{-/-}$ mice exhibit decreased levels of RvD1 in severe plaques, and treatment with RvD1 restored the RvD1:LTB4 ratio (19). RvD1 treatment enhanced efferocytosis, which contributed to the reduced necrotic core area, led to a thicker fibrous cap in necrotic areas, enhanced plaque stability, and decreased the expression of collagenase and MMP9 (19). The findings emphasize the significant impact of resolution imbalance on the progression of atherosclerotic plaques.

Hosseini et al. showed that a higher DHA/AA ratio and increased levels of RvD1 at the atherosclerotic plaque were associated with efficient efferocytosis process by macrophages and, therefore, reduced necrotic core (73). Necrotic cells can impair the local biosynthesis of SPMs and induce prostaglandins and thromboxane production, inhibiting SPM production, creating a down-regulation cycle of SPMs. In addition, *in vitro*

studies showed that RvD1 can stimulate macrophages to restore their efferocytic actions (73). This provides further evidence of the pivotal role of RvD1 in the immunopathogenesis of atherosclerosis, particularly through its direct impact on macrophage actions.

In another study, the quantification of SPM levels by LC-MS/MS in the aortas of *Apoe*$^{-/-}$ mice fed with a high-fat diet (HFD) revealed elevated levels of LTB4 and PGE2. In contrast, the levels of RvD2 and MaR1 were decreased in the advanced stages of plaque compared to the early stages (20). When analyzing the parameters of the vulnerability plaque index (VPI), it was found that the concentration of PGE2 and LTB4 in the lesion also correlated with macrophage accumulation and more significant lesions (20). On the other hand, increased RvD2 and MaR1 correlated with SMC activation, collagen deposition, thicker fibrous cap, and a smaller necrotic area favoring plaque stability (20). Treatment with a combination of RvD2 and MaR1 prevented atheroprogression and improved VPI parameters in the atherosclerosis model, resulting in a more stable and less inflamed plaque (20). These findings indicate that atherosclerosis progression in mice is associated with imbalanced resolution and inflammation processes (18,20). In addition, treating atherosclerotic mice with RvD2 and MaR1 also increased plasma levels of TGF-β, but other cytokines and leukocyte count remained unchanged, suggesting a local action of SPMs (20). Immunofluorescence analysis and RNA quantification showed a shift in the macrophage phenotype with more reparative M2 macrophage in the aortic root after treatment (20).

Viola and collaborators also demonstrated that MaR1 and RvD2 treatment increased the number of SMCs responsible for collagen deposition in the fibrous cap (18).

In vitro study with aortic SMCs from *Apoe,*$^{-/-}$ mice in HFD didn't show a direct correlation between the treatment with SPMs and collagen maturation and expression (18). However, when SMCs were treated with the supernatant of TNF-activated macrophages in the presence of RvD2 and MaR1, *Col1a1,* and *Col3a1* mRNA expression and collagen production increased, suggesting collagen production by SMCs (18).

SPMs may promote a reparative macrophage phenotype switch, favoring the resolution of the inflammatory response and healing mechanisms, reducing, and preventing atherosclerosis progression (74). Current therapies for CVD mainly focus on targeting anti-inflammatory mechanisms by antagonizing specific pathways involved in immunopathogenesis (16). However, they may limit tissue repair, potentially leading to immunosuppression and unwanted side effects. Because of their capacity to stimulate the resolution of inflammatory responses and tissue repair mechanisms, SPMs may be a therapeutic strategy to reduce the incidence of atherosclerosis cases and disease severity in patients. However, further investigations are warranted on the optimal SPM concentrations, delivery mode, and purity levels in the context of atherosclerosis.

Table 2. Studies using specialized pro-resolving lipid mediators as treatment in atherosclerosis murine models.

Author	SPM	Treatment regimen	Animals and model	Key findings
Fredman, G. *et al.* (19)	RvD1 (100 ng/mouse)	Three times per week for five weeks; i.p.	*Ldl*$^{-/-}$ male mice on WD for eight weeks (early lesions) or 17 weeks (advanced lesions)	Suppressed plaque progression and promoted plaque stability. Decreased LTB4 levels in the lesions; enhanced lesional efferocytosis; reduced MMP9 and collagenase; increased fibrous cap.
Viola, J. R. *et al.* (20)	RvD2 and MaR1 (100 ng/mouse)	Every other day for three months, i.p.	*Apoe*$^{-/-}$ mice on HFD or four weeks (early lesions) or eight weeks (advanced lesions)	Prevented atheroprogression and promoted plaque stability. Reduced necrotic core and macrophage accumulation; increased collagen deposition; stimulated M2 macrophage phenotype.
Salic, K. *et al.* (75)	RvE1 (1 or 5mg/kg/day)	Daily for 16 weeks; i.p.	Apoe3*Leiden mice on WD for 9 weeks	Attenuated lesions. Reduced lesion area and necrotic core; down-regulated genes associated with immune cell traffic and inflammation.

Abbreviations: i.p. (intraperitoneal injection); o.g. (oral gavage); WD (Western-type diet); HFD (high-fat diet).

Myocardial infarction

Ischemic heart disease (IHD) occurs when the coronary arteries narrow or get blocked. This can lead to myocardial infarction (MI), where the blood flow to the heart suddenly stops and results in persistent ischemic necrosis of the myocardium, requiring medical intervention (76). After MI, leukocytes, mainly neutrophils and macrophages, are rapidly recruited to the infarction area (77). Excessive inflammation with prolonged cell infiltration and accumulation of extracellular matrix can fuel inflammatory response, causing further tissue damage. Inflammatory injury and cardiomyocyte non-regeneration result in the accumulation of fibrous tissues, which disrupts cardiac conduction pathways, limiting cardiac systolic and diastolic functions (78). Over time, this can lead to heart failure, which is characterized by reduced left ventricle function, cardiac hypertrophy, and remodeling of the heart tissue. MI can be fatal, and early diagnosis and treatment can help prevent long-term complications and improve the chances of a full recovery.

Persistent inflammation following MI is the main cause that leads to heart failure, and current treatment options have failed to resolve, highlighting the need for new therapeutic approaches to efficiently resolve inflammation in heart tissue, preventing secondary outcomes and morbidity associated with MI. After MI, the neutrophils are recruited to cardiac tissue and become highly activated, producing proinflammatory mediators, which can lead to the death of cardiomyocytes (79). Resolving inflammation after MI is critical to stop the activation and recruitment of neutrophils.

RvD1 decreases the density of neutrophil infiltration in the cardiac tissue of mice five days post-MI, which was associated with increased expression of the RvD1 receptor, ALX/FPR2, and the enzyme 5-LO in the cardiac tissue (80). RvD1 can also modulate

macrophage-mediated clearance of neutrophils in the infarct area (80). Inflammation in the cardiac tissue can lead to collagen deposition, contributing to tissue stiffness. RvD1 decreased the deposition of collagen and the expression of genes related to the extracellular matrix component (80). Treatment with RvD1 also improves cardiac function and reduces pulmonary edema post-MI in mice (80).

Halade and collaborators also demonstrated that RvD1 ameliorates left ventricle dilation and reduces cardiac edema, thus improving cardiac function in mice after MI induced by coronary artery ligation surgery (80). In addition, they investigated the temporal kinetics of neutrophil infiltration by flow cytometry after MI (81). As expected, neutrophils peaked 24 hours post-MI, and RvD1 didn’t alter neutrophils' immediate infiltration. However, neutrophil clearance on day five markedly decreased in the infarction area with RvD1 treatment. They also addressed the macrophage phenotypes with a higher proportion of reparative macrophages in the lesion of RvD1-treated mice (81). Overall, studies have demonstrated that RvD1 can modulate the resolution of inflammation in the cardiac tissue, improving post-MI conditions.

Similarly, Mar1 has been shown to positively impact cardiac function after MI in mice (82). Treatment with Mar1 after 28 days of MI results in less interstitial fibrosis and lower expression of fibrosis-related markers, including a-SMA, collagen I, and collagen III (82). This suggests that Mar1 can regulate cardiac tissue remodeling. Mar1 also has anti-inflammatory properties, reducing the levels of IL-6 and TNF-a by inhibiting the activation of the TLR4/NF-kB pathway. Additionally, fewer macrophage and apoptotic cells were observed in the lesion (82).

The effects of omega-3 supplementation on morbidity and mortality after a myocardial infarction (MI) event were also investigated in a human cohort (53). The subjects included were patients with recent MI events (≤3 months) randomly assigned to take 850 mg of EPA and 882mg of DHA daily or placebo (53). Follow-up visits were conducted at 6, 12, 18, 30, and 42 months, during which blood samples were taken and clinical assessments were conducted. The results showed no significant changes in the cholesterol profile analysis (total cholesterol, LDL, and HDL), glycemia, and fibrinogen levels compared to the baseline values taken at the beginning of the study. Upon analyzing fatal outcomes in the group supplementing with EPA/DHA, the researchers observed a significant 15% decrease in the risk of non-fatal MI and stroke, which led to a reduction in mortality (53).

Table 3. Sudies using specialized pro-resolving lipid mediators as treatment in myocardial infarction murine model.

Author	SPM	Treatment regimen	Animals and model	Key findings
Kain, V. et al. (80)	RvD1 (3μg/Kg)	Single dose 3 hours post-surgery; i.p.	C57BL/6J 8-12 weeks old mice; coronary artery ligation surgery	Limited neutrophil activation and infiltration in the cardiac tissue; decreased collagen deposition; improved left ventricle function.
Halade, G. V. et al. (81)	RvD1 (3μg/Kg)	Single dose 3 hours post-surgery; i.p.	C57BL/6J 8-12 weeks old mice; coronary artery ligation surgery	Limited neutrophils infiltration; induces macrophage polarization towards reparative phenotype; and improved cardiac function.
Wang, F. et al. (82)	Mar1 (10 ng/g)	Six hours post-surgery and every other day for 28 days; i.p.	C57BL/6J 8-10 weeks old mice; coronary artery ligation surgery	Limited macrophage infiltration, decreased myocardial fibrosis, improved cardiac function; inhibited TLR4/NFKB inflammatory signaling pathway.

Abbreviations: i.p. (intraperitoneal injection).

Abdominal aorta aneurysms

An abdominal aortic aneurysm (AAA) is the dilation of the abdominal portion of the aorta that is enlarged to at least 0.5 to 1 time its original size (83). Risk factors for AAA development include being over 60 years old, male sex, smoking, and hypertension (83). AAA is usually discovered accidentally during an ultrasound, and treatment involves monitoring risk factors like controlling hypertension and dyslipidemia. Surgical repair or stenting may be necessary, depending on the severity of the case. It usually doesn't cause symptoms but can be life-threatening if the AAA ruptures (83). Aneurysm size is the most significant predictor of rupture, and assessing the expansion rate over six months of follow-up can provide further support to evaluate the risks (83).

The development of AAA is primarily caused by inflammation within the aortic wall. Vascular inflammation with intense leukocyte infiltrations and proinflammatory mechanisms are the main pathological features of AAA. However, the specific immunopathology must still be fully comprehended (83).

AAA inflammation is characterized by the infiltration of inflammatory cells in the aortic wall and the intense release of proinflammatory cytokines (83). These events trigger a cascade of processes that ultimately lead to the disruption of the aortic wall and dilation. This disruption includes elastin degradation, SMCs' apoptosis, and dysfunctional tissue remodeling. Similar to the other CVDs, an impaired resolution during AAA progression may significantly contribute to its development. Studies conducted with murine models of AAA indicate that SPM treatment decreases proinflammatory cytokines, preserves elastin, and reduces macrophage infiltration, significantly decreasing aortic dilation.

Elder et al. showed that MaR1 reduces AAA formation in a murine model of elastase-induced AAA (84). Mice were treated either before the induction of AAA (known as the "prevention model") or after its development. The results showed that Mar1 significantly decreased aortic dilation in both treatment approaches, indicating the capacity of Mar1 to prevent and attenuate AAA (84). Mar1 also showed increased preservation of SMC and reduced expression of metalloproteinases in the aortic wall. Interestingly, the same study reported that MaR1 failed to attenuate aortic dilation when blocking the TGFβ2 receptor in SMC of mice in SMCs, indicating a correlation between MaR1 effects and SMC-dependent TGF-β2 signaling (84). Mar1 can also stimulate macrophage uptake of elastase-induced apoptotic SMC (84). Furthermore, the study also shows that Mar1 effects depend on the interaction with LGR6 receptor (84).

The administration of RvD1 and RvD2 is also effective in preventing the formation of AAA and reducing the expression of metalloproteinases (MMP2 and MMP9), elastin degradation, and macrophage infiltrations in a murine model of perfused elastase-induced AAA (85). The treatment also decreased the levels of proinflammatory cytokines in the aortic wall, including MCP-1, IL-1β, and RANTES. RvD2 favored macrophage phenotype shift towards reparative macrophage. Therapy with RvD2 prevented the formation of AAA in the prevention schematic model and attenuated the progression of pre-formed aneurysms when treated after AAA establishment (85). RvD1-treated mice showed decreased T-cell infiltration in aortic tissue, while RvD2 treatment did not affect cellular infiltrations in the abdominal aorta (85). Both SPMs effectively reduced proinflammatory cytokines, including IL-1β (85). However, RvD2 also decreased TNF-α levels (85). These SPMs might exert their action using different signaling pathways (85).

Similar results were obtained in another study, wherein AAA was induced in mice by applying topical elastase (86). RvD1-treated mice had reduced development of aortic aneurysms with elastin preservation and decreased IL-1β and MMP2 levels. Neutrophil infiltrates within the first days of AAA development. RvD1-treated mice exhibited significantly lower levels of neutrophil extracellular traps (NETs) formation on day three after elastase application, indicating that a possible mechanism of RvD1 in promoting resolution in the arterial wall is acting on neutrophils.

In early studies, it was demonstrated that RvD1 exerts its actions through the FPR2/ALX receptor. Upon analyzing aortic tissue from patients with AAA, it was found that there was a decrease in FPR2 mRNA expression and decreased co-expression of FPR2 with macrophages compared to control (87). In addition, $fpr2^{-/-}$ mice had more severe dilation than WT mice in the topical elastase model (87). Overall, $fpr2^{-/-}$ mice elastase-treated showed more inflammation and vascular remodeling with increased leukocyte infiltration within the aortic wall, increased elastic fiber disruption, decreased expression of α-SMA, increased levels of proinflammatory cytokines, including IL-1b, IL-17, IFN-g and TNF-a (87). Treating $fpr2^{-/-}$ mice with RvD1 did not mitigate AAA formation and neither attenuated the expression of proinflammatory cytokines, further demonstrating the crucial role of RvD1/FPR2 signaling via (87).

Table 4. Studies using specialized pro-resolving lipid mediators as a treatment for abdominal aorta aneurysms murine model.

Author	SPM	Treatment regimen	Animals and model	Key findings
Elder, C. T. (84)	Mar1 (4 or 40ng/kg)	Prevention model: on days 1, 3, 5 and 7; i.p. Regression model: on days 7, 9, 11 and 13; i.p.	C57BL/6J 8-12 weeks old mice; topical elastase AAA model	Reduction of aortic diameter; decreased MMP-2 expression; preserved SMC. Increased the uptake of apoptotic SMC by macrophages. Mar1 effects are mediated by LGR6 receptor interaction.
Pope, N. H. (85)	RvD1 (100ng/kg) or RvD2 (100ng/kg)	Prevention model: 3 days prior to elastase perfusion, continuing days 0, 3, 6, 9, and 12. Regression model: on days 3, 6, 9 and 12; i.p.	C57BL/6J 8-10 weeks old mice; elastase perfusion AAA model	RvD2 induces macrophage polarization; decreases proinflammatory cytokine levels in the tissue.
Espinosa, M. (86)	RvD1 (300ng/kg)	Daily, from day 0 to the day of tissue collection (3 or 14)	C57BL/6J 8 weeks old mice; topical elastase AAA model	Attenuation of AAA measurements; elastin preservation, decreased levels of IL-1b, and increased levels of IL-10. Inhibition of NETosis; decreased neutrophil elastase in the tissue.

Abbreviations: i.p. (intraperitoneal injection); AAA (abdominal aorta aneurysm).

POTENTIAL ROLE OF OMEGA-3-DERIVED SPM IN KAWASAKI DISEASE

Kawasaki disease (KD) is a febrile illness that affects young children and is characterized by systemic vasculitis (88,89). The cause of KD is still unknown, and disease diagnosis is based upon specific clinical features, such as persistent fever, skin rash, mucocutaneous edema, cervical lymphadenopathy, non-purulent conjunctivitis, and swallowing hands and feet. The most common complication associated with KD is the development of coronary artery aneurysm (CAA), which can occur in up to 25% of untreated children (90). The current standard treatment is a single high dose of intravenous immunoglobulin (IVIG) that efficiently reduces the incidence of CAA to 5% (91,92). However, about 20% of the patients do not respond to the treatment and are at higher risk of developing CAA (93).

Histological evaluation of heart tissue revealed that CAA formation in KD involves three linked processes: necrotizing arteritis (NA), subacute/chronic vasculitis (SA/C), and luminal myofibroblastic proliferation (LMP) (81). The process starts with intense neutrophilic infiltration in the vessel wall, eventually destroying the elastic layers. The following events disrupt media and adventitia layers, with LMP and migration toward the lumen (94). CAA can lead to stenosis or a thrombotic event leading to ischemic heart disease, such as MI, causing serious cardiovascular complications and even sudden death.

Due to the difficulty in accessing human cardiac samples, murine models that mimic KD vasculitis have been developed. A single intraperitoneal injection of *Lactobacillus casei* wall extract (LCWE) in mice induces the development of cardiovascular lesions similar to the ones observed in KD patients (95). LCWE-induced

vasculitis has been a reliable tool providing valuable insights into treatment options and mechanisms of KD due to its close resemblance to human KD (96–98).

Through their unique ability to promote the resolution of inflammation by reducing leukocyte infiltration and activation, decreasing proinflammatory signaling pathways, and regulating tissue remodeling and fibrotic deposition, which favors tissue function, SPMs show great potential as a treatment option for CVDs. SPMs also improve cardiac function while preventing myocardial fibrosis and promoting clearance of inflammatory infiltration (80–82). The imbalanced levels of SPM detected in the atherosclerosis models highlighted the essential role these molecules play in cardiovascular lesions (19).

We hypothesized that SPMs could attenuate AAA and CAA severity in LCWE-induced KD vasculitis. Based on the literature presented here, we conducted a preliminary study to investigate whether the treatment with RvD1, Mar1, or a combination of RvD1 and Mar1 could impact the main cardiovascular lesions induced by LCWE. Mice were either injected with LCWE or injected with LCWE and treated with either RvD1 (100 ng), Mar1 (100 ng), or a mix composed of RvD1 and Mar1 (100 ng each). Mice were treated every other day, starting one hour after LCWE injection, and the severity of cardiovascular lesions development was assessed at day fourteen. While LCWE injection induced the development of abdominal aorta dilations, we did not observe a beneficial effect of RvD1, Mar1, or RVD1+MAR1 treatment on the severity of LCWE-induced abdominal aorta dilations (**Figure 2**). However, histological examination of heart tissues indicated that treatment with Mar1 alone or the combination of RvD1 and Mar1 significantly reduced heart inflammation (**Figure 3**). Further investigations are required to

confirm this beneficial effect and determine how Mar1 decreases the severity of LCWE-induced KD vasculitis.

As previously mentioned, an alternative biosynthesis route for SPM is the aspirin acetylated-COX-2 that induces the formation of aspirin-triggered SPMs (AT-SPM). The American Heart Association guidelines for KD treatment indicate a high dose of aspirin (80 to 100 mg/kg/d) during the febrile phase and a low dose (3 to 5 mg/kg/d) in the sub/acute and chronic phase as secondary prevention. However, studies failed to determine whether the different concentrations of aspirin impact the development and incidence of CAA (99–101). We speculate that IVIG treatment with SPMs as a co-adjuvant therapy could bring advantageous results similar to the current recommendation of aspirin treatment extending beyond the acute phase as a secondary prevention for long-term cardiac complications. SPMs may enhance the anti-inflammatory effects of IVIG, leading to a decrease in the activation and recruitment of inflammatory cells and likely significantly reducing fibrotic deposition in the heart vessels affected by KD. This could potentially reduce inflammation in cardiovascular tissue, thus preventing further complications and the formation of lesions.

In addition, we briefly touched on the significance of consuming foods with proper nutritional value for maintaining good general health, particularly in the context of CVD. With the growing understanding of the connection between Omega-3-derived SPMs and their pivotal role in resolving inflammation, researchers have increasingly focused on Omega-3 supplementation and its impact on overall bodily health. The gut microbiome is crucial in regulating the body's overall health and is closely related to the immune system. Interestingly, ingesting Omega-3 can balance gut microbiome diversity, enhance mucosal

barrier function, modulate fat metabolism, and improve intestinal immunity (102). Omega-3 may offer protection against intestinal diseases, including KD vasculitis, which affects intestinal immunity and mucosal barrier function.

FINAL CONSIDERATIONS

Chronic inflammation, which underlies many CVDs, can be influenced by an imbalance between proinflammatory and pro-resolution lipids. Recently, endogenous-produced SPMs have been discovered, and their role in regulating inflammatory responses has prompted investigations into their potential therapeutic benefits for a wide range of diseases, including CVDs, which are often associated with maladapted resolution of inflammation or chronic inflammation.

Studies indicate that SPMs might be beneficial by promoting mechanisms involved in resolving inflammatory responses and inhibiting excessive proinflammatory responses and tissue damage, thereby improving disease progression and severity. However, it is noteworthy that studies investigating SPMs and their mechanisms can be quite strenuous. SPM quantification and detection essays are relatively difficult to perform and require specific techniques, such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) used for metabolomics studies. Determining the appropriate concentration, delivery route, and treatment schedule for SPMs can be challenging. It is relevant to mention that one of the major challenges researchers face today is the rapid inactivation of SPMs inside the host. Therefore, the stability and delivery method of SPMs are crucial factors to be considered while developing them as effective therapeutic tools for long-term treatment.

Despite these challenges, the experimental studies conducted on murine models that mimic cardiovascular lesions have shown promising results using SPMs to modulate the inflammatory response. Unlike the prevailing mechanism of current treatment options for CVD that merely suppress the immune response, SPMs stimulate the immune response towards resolution, offering an innovative approach. On that note, considering the strong evidence showing the unique resolution properties of SPMs and their therapeutic potential, they are a promising candidate for adjunct therapy options for CVDs. Many researchers in this field consider this opposite mechanistic idea a turning point, paving the way for future therapeutic approaches.

Conflict of Interest Statement

The authors declare no conflict of interest.

REFERENCES

1. Medzhitov R. Inflammation 2010: New Adventures of an Old Flame. Cell. 2010 Mar;140(6):771–6.
2. Broggi A, Granucci F. Microbe- and danger-induced inflammation. Mol Immunol. 2015 Feb;63(2):127–33.
3. Scotece M, Conde-Aranda J. Inflammation in Health and Disease: New Insights and Therapeutic Avenues. Int J Mol Sci. 2022 Jul 29;23(15):8392.
4. Ahmed AU. An overview of inflammation: mechanism and consequences. Front Biol (Beijing). 2011 Aug 29;6(4):274.
5. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008 Jul 24;454(7203):428–35.
6. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014 Jun 5;510(7503):92–101.

7. Serhan CN. Novel lipid mediators and resolution mechanisms in acute inflammation: To resolve or not? American Journal of Pathology [Internet]. 2010;177(4):1576–91. Available from: http://dx.doi.org/10.2353/ajpath.2010.100322
8. Serhan CN, Chiang N. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. Br J Pharmacol [Internet]. 2009;153(S1):S200–15. Available from: http://doi.wiley.com/10.1038/sj.bjp.0707489
9. Levy BD, Serhan CN. Resolution and Regulation of Inflammation. In: Pathobiology of Human Disease. Elsevier; 2014. p. 332–48.
10. Serhan CN, Chiang N, Dalli J. The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. Semin Immunol. 2015;27(3):200–15.
11. Mariachiara Di Cesare, Honor Bixby, Thomas Gaziano, Lisa Hadeed, Chodziwadziwa Kabudula, Diana Vaca McGhie, et al. World Heart Report 2023: Confronting the World's Number One Killer. World Heart Federation [Internet]. 2023 [cited 2023 Oct 22]; Available from: https://world-heart-federation.org/resource/world-heart-report-2023/
12. Song P, Fang Z, Wang H, Cai Y, Rahimi K, Zhu Y, et al. Global and regional prevalence, burden, and risk factors for carotid atherosclerosis: a systematic review, meta-analysis, and modelling study. Lancet Glob Health. 2020 May;8(5):e721–9.
13. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2016 Aug 1;37(29):2315–81.
14. Mannarino E, Pirro M. Endothelial Injury and Repair: A Novel Theory for Atherosclerosis. Angiology. 2008 Aug 27;59(2_suppl):69S-72S.
15. Shah PK. Inflammation, Neointimal Hyperplasia, and Restenosis. Circulation. 2003 May 6;107(17):2175–7.
16. Kim AS, Conte MS. Specialized pro-resolving lipid mediators in cardiovascular disease, diagnosis, and therapy. Adv Drug Deliv Rev. 2020;159:170–9.

17. Kasikara C, Doran AC, Cai B, Tabas I. The role of non-resolving inflammation in atherosclerosis. Journal of Clinical Investigation. 2018;128(7):2713–23.
18. Fredman G, Spite M. Specialized pro-resolving mediators in cardiovascular diseases. Mol Aspects Med. 2017 Dec;58:65–71.
19. Fredman G, Hellmann J, Proto JD, Kuriakose G, Colas RA, Dorweiler B, et al. An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. Nat Commun [Internet]. 2016;7:1–11. Available from: http://dx.doi.org/10.1038/ncomms12859
20. Viola JR, Lemnitzer P, Jansen Y, Csaba G, Winter C, Neideck C, et al. Resolving Lipid Mediators Maresin 1 and Resolvin D2 Prevent Atheroprogression in Mice. Circ Res. 2016;119(9):1030–8.
21. Liu M, He H, Chen L. Protective Potential of Maresins in Cardiovascular Diseases. Front Cardiovasc Med. 2022 Jul 4;9.
22. Capra V, Rovati GE, Mangano P, Buccellati C, Murphy RC, Sala A. Transcellular biosynthesis of eicosanoid lipid mediators. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2015 Apr;1851(4):377–82.
23. Sharma-walia N, Chandrasekharan J. Lipoxins: nature's way to resolve inflammation. J Inflamm Res. 2015 Sep;181.
24. Serhan CN, Libreros S, Nshimiyimana R. E-series resolvin metabolome, biosynthesis and critical role of stereochemistry of specialized pro-resolving mediators (SPMs) in inflammation-resolution: Preparing SPMs for long COVID-19, human clinical trials, and targeted precision nutrition. Semin Immunol. 2022 Jan;59:101597.
25. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. Journal of Clinical Investigation. 2018 Jul 2;128(7):2657–69.
26. Vidar Hansen T, Serhan CN. Protectins: Their biosynthesis, metabolism and structure-functions. Biochem Pharmacol. 2022 Dec;206:115330.
27. Serhan CN, Dalli J, Karamnov S, Choi A, Park C, Xu Z, et al. Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. The FASEB Journal. 2012 Apr 17;26(4):1755–65.

28. Chiang N, Serhan CN. Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Aspects Med. 2017 Dec;58:114–29.
29. Krishnamoorthy S, Recchiuti A, Chiang N, Fredman G, Serhan CN. Resolvin D1 Receptor Stereoselectivity and Regulation of Inflammation and Proresolving MicroRNAs. Am J Pathol. 2012 May;180(5):2018–27.
30. Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee CH, Yang R, et al. Resolvin D1 binds human phagocytes with evidence for proresolving receptors. Proceedings of the National Academy of Sciences. 2010 Jan 26;107(4):1660–5.
31. Arnardottir H, Thul S, Pawelzik SC, Karadimou G, Artiach G, Gallina AL, et al. The resolvin D1 receptor GPR32 transduces inflammation resolution and atheroprotection. Journal of Clinical Investigation. 2021 Dec 15;131(24).
32. Norling L V., Dalli J, Flower RJ, Serhan CN, Perretti M. Resolvin D1 Limits Polymorphonuclear Leukocyte Recruitment to Inflammatory Loci. Arterioscler Thromb Vasc Biol. 2012 Aug;32(8):1970–8.
33. Chiang N, Dalli J, Colas RA, Serhan CN. Identification of resolvin D2 receptor mediating resolution of infections and organ protection. Journal of Experimental Medicine. 2015 Jul 27;212(8):1203–17.
34. Dalli J, Winkler JW, Colas RA, Arnardottir H, Cheng CYC, Chiang N, et al. Resolvin D3 and Aspirin-Triggered Resolvin D3 Are Potent Immunoresolvents. Chem Biol. 2013 Feb;20(2):188–201.
35. Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN. Resolvin E1 Selectively Interacts with Leukotriene B4 Receptor BLT1 and ChemR23 to Regulate Inflammation. The Journal of Immunology. 2007 Mar 15;178(6):3912–7.
36. Oh SF, Dona M, Fredman G, Krishnamoorthy S, Irimia D, Serhan CN. Resolvin E2 Formation and Impact in Inflammation Resolution. The Journal of Immunology. 2012 May 1;188(9):4527–34.
37. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, et al. Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. J Exp Med. 2005 Mar 7;201(5):713–22.

38. Chiang N, Libreros S, Norris PC, de la Rosa X, Serhan CN. Maresin 1 activates LGR6 receptor promoting phagocyte immunoresolvent functions. Journal of Clinical Investigation. 2019 Oct 28;129(12):5294–311.
39. Park CK. Maresin 1 Inhibits TRPV1 in Temporomandibular Joint-Related Trigeminal Nociceptive Neurons and TMJ Inflammation-Induced Synaptic Plasticity in the Trigeminal Nucleus. Mediators Inflamm. 2015;2015:1–9.
40. Han YH, Shin KO, Kim JY, Khadka DB, Kim HJ, Lee YM, et al. A maresin 1/RORα/12-lipoxygenase autoregulatory circuit prevents inflammation and progression of nonalcoholic steatohepatitis. Journal of Clinical Investigation. 2019 Mar 11;129(4):1684–98.
41. Bang S, Xie YK, Zhang ZJ, Wang Z, Xu ZZ, Ji RR. GPR37 regulates macrophage phagocytosis and resolution of inflammatory pain. Journal of Clinical Investigation. 2018 Aug 1;128(8):3568–82.
42. Zhao Y, Calon F, Julien C, Winkler JW, Petasis NA, Lukiw WJ, et al. Docosahexaenoic Acid-Derived Neuroprotectin D1 Induces Neuronal Survival via Secretase- and PPARγ-Mediated Mechanisms in Alzheimer's Disease Models. PLoS One. 2011 Jan 5;6(1):e15816.
43. Chiang N, Fredman G, Bäckhed F, Oh SF, Vickery T, Schmidt BA, et al. Infection regulates pro-resolving mediators that lower antibiotic requirements. Nature. 2012 Apr 25;484(7395):524–8.
44. Dzau VJ, Antman EM, Black HR, Hayes DL, Manson JE, Plutzky J, et al. The Cardiovascular Disease Continuum Validated: Clinical Evidence of Improved Patient Outcomes. Circulation. 2006 Dec 19;114(25):2850–70.
45. Christ A, Lauterbach M, Latz E. Western Diet and the Immune System: An Inflammatory Connection. Immunity. 2019 Nov;51(5):794–811.
46. Libby P. The changing landscape of atherosclerosis. Nature. 2021 Apr 22;592(7855):524–33.
47. López-Taboada I, González-Pardo H, Conejo NM. Western Diet: Implications for Brain Function and Behavior. Front Psychol. 2020 Nov 23;11.
48. Kris-Etherton PM, Harris WS, Appel LJ. Omega-3 Fatty Acids and Cardiovascular Disease. Arterioscler Thromb Vasc Biol. 2003 Feb;23(2):151–2.

49. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomedicine & Pharmacotherapy. 2002 Oct;56(8):365–79.
50. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association Between Omega-3 Fatty Acid Supplementation and Risk of Major Cardiovascular Disease Events. JAMA. 2012 Sep 12;308(10):1024.
51. Singh RB, Niaz MA, Sharma JP, Kumar R, Rastogi V, Moshiri M. Randomized, Double-Blind, Placebo-Controlled Trial of Fish Oil and Mustard Oil in Patients with Suspected Acute Myocardial Infarction: The Indian Experiment of Infarct Survival—4. Cardiovasc Drugs Ther. 1997;11(3):485–91.
52. Burr ML, Gilbert JF, Holliday RM, Elwood PC, Fehily AM, Rogers S, et al. EFFECTS OF CHANGES IN FAT, FISH, AND FIBRE INTAKES ON DEATH AND MYOCARDIAL REINFARCTION: DIET AND REINFARCTION TRIAL (DART). The Lancet. 1989 Sep;334(8666):757–61.
53. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet. 1999 Aug 7;354(9177):447–55.
54. GISSI-HF investigators. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. The Lancet. 2008 Oct;372(9645):1223–30.
55. Nilsen DW, Albrektsen G, Landmark K, Moen S, Aarsland T, Woie L. Effects of a high-dose concentrate of n−3 fatty acids or corn oil introduced early after an acute myocardial infarction on serum triacylglycerol and HDL cholesterol. Am J Clin Nutr. 2001 Jul;74(1):50–6.
56. The Risk and Prevention Study Collaborative Group. n–3 Fatty Acids in Patients with Multiple Cardiovascular Risk Factors. New England Journal of Medicine. 2013 May 9;368(19):1800–8.
57. Yang WS, Chen YY, Chen PC, Hsu HC, Su TC, Lin HJ, et al. Association between Plasma N-6 Polyunsaturated Fatty Acids Levels and the Risk of Cardiovascular Disease in a Community-based Cohort Study. Sci Rep. 2019 Dec 17;9(1):19298.

58. Siscovick DS, Barringer TA, Fretts AM, Wu JHY, Lichtenstein AH, Costello RB, et al. Omega-3 Polyunsaturated Fatty Acid (Fish Oil) Supplementation and the Prevention of Clinical Cardiovascular Disease. Circulation. 2017 Apr 11;135(15).
59. Björkegren JLM, Lusis AJ. Atherosclerosis: Recent developments. Cell. 2022 May;185(10):1630–45.
60. Borén J, Chapman MJ, Krauss RM, Packard CJ, Bentzon JF, Binder CJ, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2020 Jun 21;41(24):2313–30.
61. Rosenfeld ME. Leukocyte Recruitment Into Developing Atherosclerotic Lesions. Arterioscler Thromb Vasc Biol. 2002 Mar;22(3):361–3.
62. Langer HF, Chavakis T. Leukocyte – endothelial interactions in inflammation. J Cell Mol Med. 2009 Jul 30;13(7):1211–20.
63. Mauersberger C, Hinterdobler J, Schunkert H, Kessler T, Sager HB. Where the Action Is—Leukocyte Recruitment in Atherosclerosis. Front Cardiovasc Med. 2022 Jan 11;8.
64. Silvestre-Roig C, de Winther MP, Weber C, Daemen MJ, Lutgens E, Soehnlein O. Atherosclerotic Plaque Destabilization. Circ Res. 2014 Jan 3;114(1):214–26.
65. Libby P, Tabas I, Fredman G, Fisher EA. Inflammation and its Resolution as Determinants of Acute Coronary Syndromes. Circ Res. 2014 Jun 6;114(12):1867–79.
66. Gisterå A, Ketelhuth DFJ, Malin SG, Hansson GK. Animal Models of Atherosclerosis–Supportive Notes and Tricks of the Trade. Circ Res. 2022 Jun 10;130(12):1869–87.
67. Getz GS, Reardon CA. Animal Models of Atherosclerosis. Arterioscler Thromb Vasc Biol. 2012 May;32(5):1104–15.
68. Wan JB, Huang LL, Rong R, Tan R, Wang J, Kang JX. Endogenously Decreasing Tissue n-6/n-3 Fatty Acid Ratio Reduces Atherosclerotic Lesions in *Apolipoprotein E* –Deficient Mice by Inhibiting Systemic and Vascular Inflammation. Arterioscler Thromb Vasc Biol. 2010 Dec;30(12):2487–94.

69. Haeggström JZ, Funk CD. Lipoxygenase and Leukotriene Pathways: Biochemistry, Biology, and Roles in Disease. Chem Rev. 2011 Oct 12;111(10):5866–98.
70. Fredman G, Ozcan L, Spolitu S, Hellmann J, Spite M, Backs J, et al. Resolvin D1 limits 5-lipoxygenase nuclear localization and leukotriene B_4 synthesis by inhibiting a calcium-activated kinase pathway. Proceedings of the National Academy of Sciences. 2014 Oct 7;111(40):14530–5.
71. Shimizu T, Izumi T, Seyama Y, Tadokoro K, Rådmark O, Samuelsson B. Characterization of leukotriene A4 synthase from murine mast cells: evidence for its identity to arachidonate 5-lipoxygenase. Proceedings of the National Academy of Sciences. 1986 Jun;83(12):4175–9.
72. Dixon RAF, Diehl RE, Opas E, Rands E, Vickers PJ, Evans JF, et al. Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. Nature. 1990 Jan;343(6255):282–4.
73. Hosseini Z, Marinello M, Decker C, Sansbury BE, Sadhu S, Gerlach BD, et al. Resolvin D1 Enhances Necroptotic Cell Clearance Through Promoting Macrophage Fatty Acid Oxidation and Oxidative Phosphorylation. Arterioscler Thromb Vasc Biol. 2021 Mar;41(3):1062–75.
74. Salazar J, Pirela D, Nava M, Castro A, Angarita L, Parra H, et al. Specialized Proresolving Lipid Mediators: A Potential Therapeutic Target for Atherosclerosis. Int J Mol Sci. 2022 Mar 15;23(6):3133.
75. Salic K, Morrison MC, Verschuren L, Wielinga PY, Wu L, Kleemann R, et al. Resolvin E1 attenuates atherosclerosis in absence of cholesterol-lowering effects and on top of atorvastatin. Atherosclerosis. 2016 Jul;250:158–65.
76. Maseri A, Chierchia S, Davies G. Pathophysiology of coronary occlusion in acute infarction. Circulation. 1986 Feb;73(2):233–9.
77. Frangogiannis N. The inflammatory response in myocardial infarction. Cardiovasc Res. 2002 Jan;53(1):31–47.
78. Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. Nat Rev Cardiol. 2014 May 25;11(5):255–65.

79. Zhang S, Dehn S, DeBerge M, Rhee KJ, Hudson B, Thorp EB. Phagocyte–myocyte interactions and consequences during hypoxic wound healing. Cell Immunol. 2014 Sep;291(1–2):65–73.
80. Kain V, Ingle KA, Colas RA, Dalli J, Prabhu SD, Serhan CN, et al. Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function. J Mol Cell Cardiol. 2015 Jul;84:24–35.
81. Halade G V., Kain V, Serhan CN. Immune responsive resolvin D1 programs myocardial infarction-induced cardiorenal syndrome in heart failure. The FASEB Journal. 2018 Jul 13;32(7):3717–29.
82. Wang F, Gong Y, Chen T, Li B, Zhang W, Yin L, et al. Maresin1 ameliorates ventricular remodelling and arrhythmia in mice models of myocardial infarction via NRF2/HO-1 and TLR4/NF-kB signalling. Int Immunopharmacol. 2022 Dec;113:109369.
83. Aggarwal S, Qamar A, Sharma V, Sharma A. Abdominal aortic aneurysm: A comprehensive review. Exp Clin Cardiol. 2011;16(1):11–5.
84. Elder CT, Filiberto AC, Su G, Ladd Z, Leroy V, Pruitt EY, et al. Maresin 1 activates LGR6 signaling to inhibit smooth muscle cell activation and attenuate murine abdominal aortic aneurysm formation. The FASEB Journal. 2021 Aug 28;35(8).
85. Pope NH, Salmon M, Davis JP, Chatterjee A, Su G, Conte MS, et al. D-series resolvins inhibit murine abdominal aortic aneurysm formation and increase M2 macrophage polarization. The FASEB Journal. 2016 Dec 12;30(12):4192–201.
86. Spinosa M, Su G, Salmon MD, Lu G, Cullen JM, Fashandi AZ, et al. Resolvin D1 decreases abdominal aortic aneurysm formation by inhibiting NETosis in a mouse model. J Vasc Surg. 2018 Dec;68(6):93S-103S.
87. Filiberto AC, Ladd Z, Leroy V, Su G, Elder CT, Pruitt EY, et al. Resolution of inflammation via RvD1/FPR2 signaling mitigates Nox2 activation and ferroptosis of macrophages in experimental abdominal aortic aneurysms. The FASEB Journal. 2022 Nov 2;36(11).

88. Sundel RP. Kawasaki Disease. Rheumatic Disease Clinics of North America [Internet]. 2015 Feb 10;41(1):63–73. Available from: http://dx.doi.org/10.1016/j.rdc.2014.09.010
89. Burns JC, Kushner HI, Bastian JF, Shike H, Shimizu C, Matsubara T, et al. Kawasaki Disease: A Brief History. Pediatrics [Internet]. 2000 Aug 1;106(2):e27–e27. Available from: https://publications.aap.org/pediatrics/article/106/2/e27/62795/Kawasaki-Disease-A-Brief-History
90. McCrindle BW, Li JS, Minich LL, Colan SD, Atz AM, Takahashi M, et al. Coronary Artery Involvement in Children With Kawasaki Disease. Circulation. 2007 Jul 10;116(2):174–9.
91. Lo MS, Newburger JW. Role of intravenous immunoglobulin in the treatment of Kawasaki disease. Int J Rheum Dis. 2018 Jan 1;21(1):64–9.
92. Newburger JW, Takahashi M, Beiser AS, Burns JC, Bastian J, Chung KJ, et al. A Single Intravenous Infusion of Gamma Globulin as Compared with Four Infusions in the Treatment of Acute Kawasaki Syndrome. New England Journal of Medicine. 1991 Jun 6;324(23):1633–9.
93. Tremoulet AH, Best BM, Song S, Wang S, Corinaldesi E, Eichenfield JR, et al. Resistance to Intravenous Immunoglobulin in Children with Kawasaki Disease. J Pediatr. 2008 Jul;153(1):117-121.e3.
94. Orenstein JM, Shulman ST, Fox LM, Baker SC, Takahashi M, Bhatti TR, et al. Three Linked Vasculopathic Processes Characterize Kawasaki Disease: A Light and Transmission Electron Microscopic Study. PLoS One. 2012 Jun 18;7(6):e38998.
95. Lehman TJ, Walker SM, Mahnovski V, McCurdy D. Coronary arteritis in mice following the systemic injection of group B Lactobacillus casei cell walls in aqueous suspension. Arthritis Rheum [Internet]. 1985 Jun;28(6):652–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3924060
96. Myones BL, Bathoria JM, Lehman TJA, Shulman ST. Human IVIG inhibits Lactobacillus casei-inducible coronary arteritis in a murine model. Kawasaki

Disease: Proceedings of the V Inter- national Kawasaki Disease Symposium, Fukuoka, Japan. 1995;252–6.

97. Tremoulet AH, Jain S, Kim S, Newburger J, Arditi M, Franco A, et al. Rationale and study design for a phase I/IIa trial of anakinra in children with Kawasaki disease and early coronary artery abnormalities (the ANAKID trial). Contemp Clin Trials [Internet]. 2016 May;48:70–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27080929%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5221485

98. Lee Y, Schulte DJ, Shimada K, Chen S, Crother TR, Chiba N, et al. Interleukin-1β Is Crucial for the Induction of Coronary Artery Inflammation in a Mouse Model of Kawasaki Disease. Circulation [Internet]. 2012 Mar 27;125(12):1542–50. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L51891216%5Cnhttp://dx.doi.org/10.1161/CIRCULATIONAHA.111.072769%5Cnhttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=00097322&id=doi:10.1161%2FCIRCULATIONAHA.111.072769&atitle=Interl

99. Hsieh KS, Weng KP, Lin CC, Huang TC, Lee CL, Huang SM. Treatment of Acute Kawasaki Disease: Aspirin's Role in the Febrile Stage Revisited. Pediatrics. 2004 Dec 1;114(6):e689–93.

100. McFadyen JD, Gardiner EE. An Aspirin a Day…. Arterioscler Thromb Vasc Biol [Internet]. 2022 Oct;42(10):1217–9. Available from: https://www.ahajournals.org/doi/10.1161/ATVBAHA.122.318337

101. Huang YH, Hsin YC, Wang LJ, Feng WL, Guo MMH, Chang LS, et al. Treatment of Kawasaki Disease: A Network Meta-Analysis of Four Dosage Regimens of Aspirin Combined With Recommended Intravenous Immunoglobulin. Front Pharmacol. 2021 Aug 12;12.

102. Fu Y, Wang Y, Gao H, Li D, Jiang R, Ge L, et al. Associations among Dietary Omega-3 Polyunsaturated Fatty Acids, the Gut Microbiota, and Intestinal Immunity. Mediators Inflamm. 2021 Jan 2;2021:1–11.

FIGURES AND LEGENDS

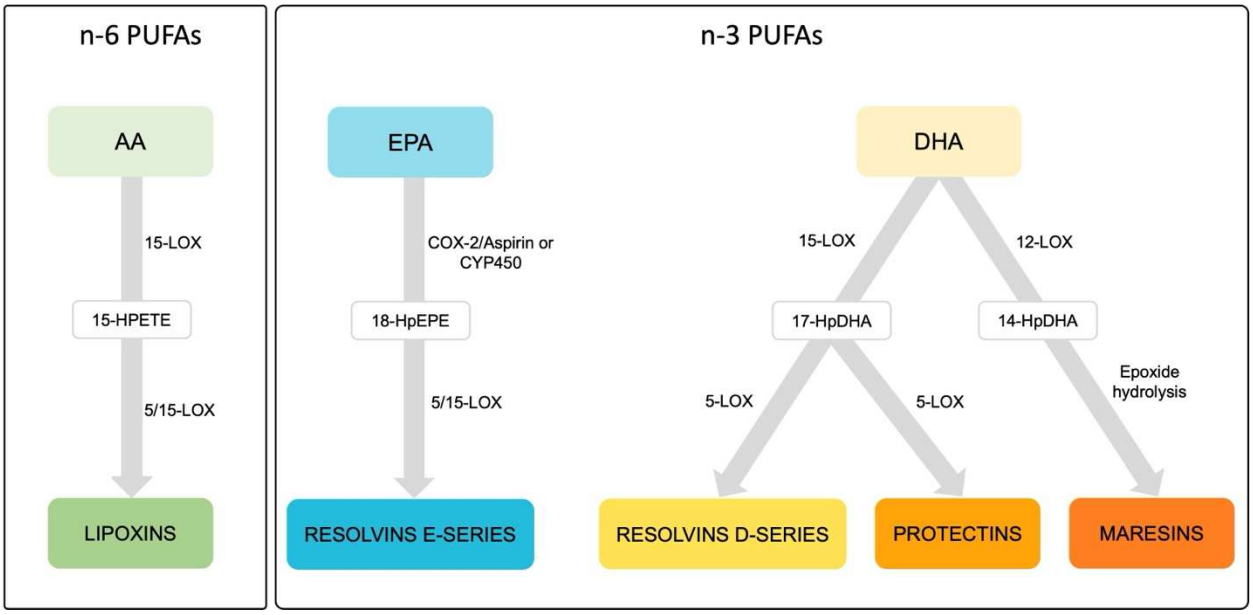

Figure 1. Main biosynthesis route of specialized pro-resolving lipid mediators. Omega-6 PUFAs produce AA, which serves as a precursor for lipoxins. Omega-3 PUFAs generate EPA and DHA, the main precursors of most SPMs. EPA is converted into resolvin E-series, while DHA can be converted into resolvin D-series, protectins, and maresins.

Abbreviations: PUFAs, polyunsaturated fatty acids; AA, arachidonic acid; EPA, Eicosapentaenoic acid; DHA, docosahexaenoic acid; LOX, lipoxygenase; COX-2/Aspirin, Aspirin acetylates cyclooxygenase-2; CYP450, cytochrome P450; 15-HPETE, 15-hydroxyperoexyeicosatetraenoic acid; 18-HpEPE, 18-(hydroperoxy) eicosapentaenoic acid; 17-HpDHA, 17-hydroperoxyDHA; 14-HpDHA, 14-hydroxyperoxyDHA.

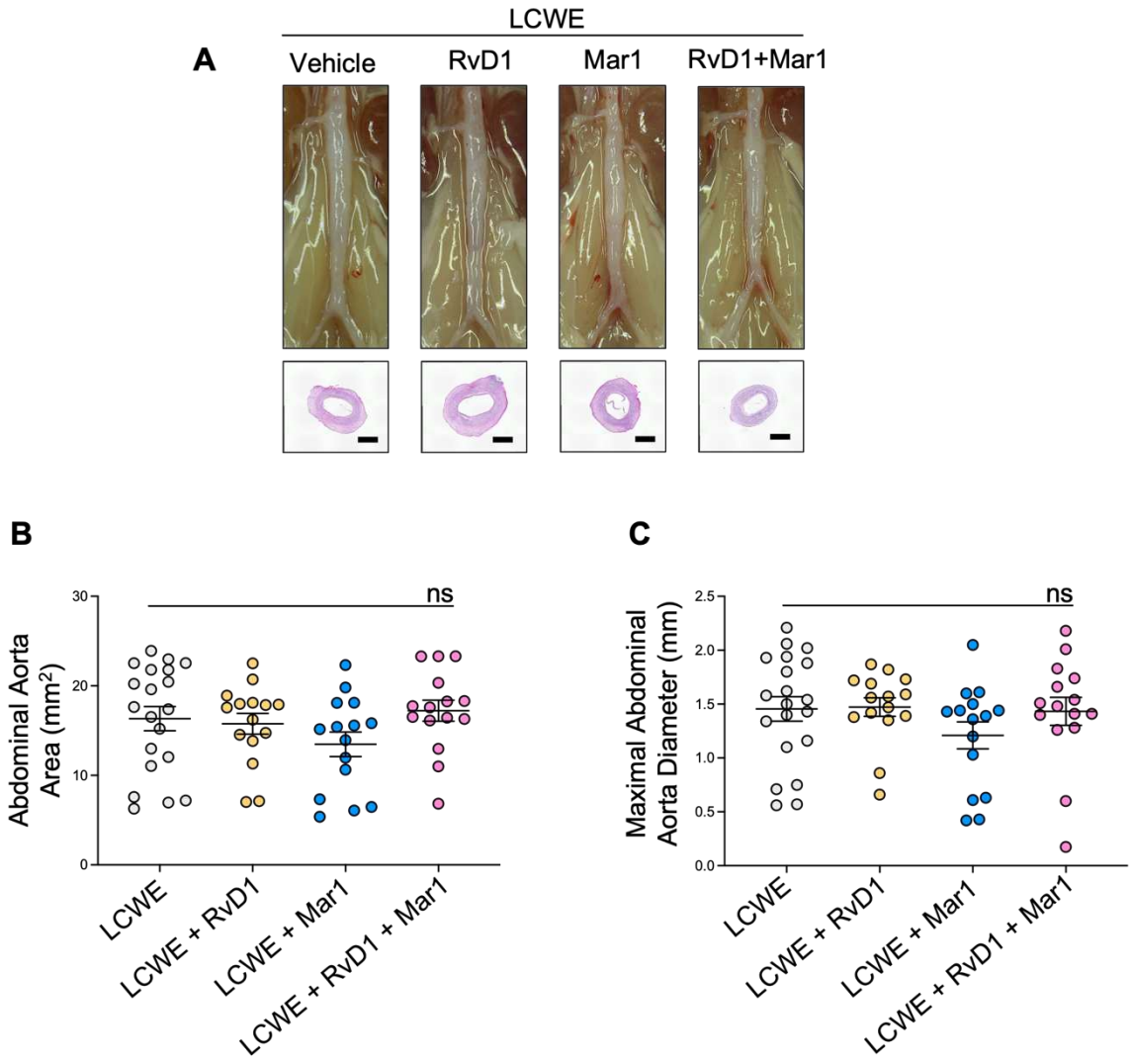

Figure 2. Treatment with RvD1 and Mar1 doesn't attenuate AAA formation in the LCWE-induced KD vasculitis model. (A) Representative pictures of the abdominal aorta area and H&E staining of the abdominal aorta cross-sections. Scale bars: 500 μm. **(B, C)** Abdominal aorta area (E) and maximal abdominal aorta diameter (F) measurements. Mice were injected with LCWE on day 0 and were treated with SPMs one hour after the injection. The treatment groups were divided as follows - RvD1 (100ng/mice), Mar1 (100ng/mice), or RvD1 and Mar1 (100ng/mice of each SPM). The mice were treated on days 2, 4, 6, 8, 10, and 12. Sample collection and analysis were

performed on day 14. Data is presented as mean ± SEM (n= 15-20/group). Kruskal-Wallis one-way ANOVA was used to determine statistical significance (considered $p<0.05$).

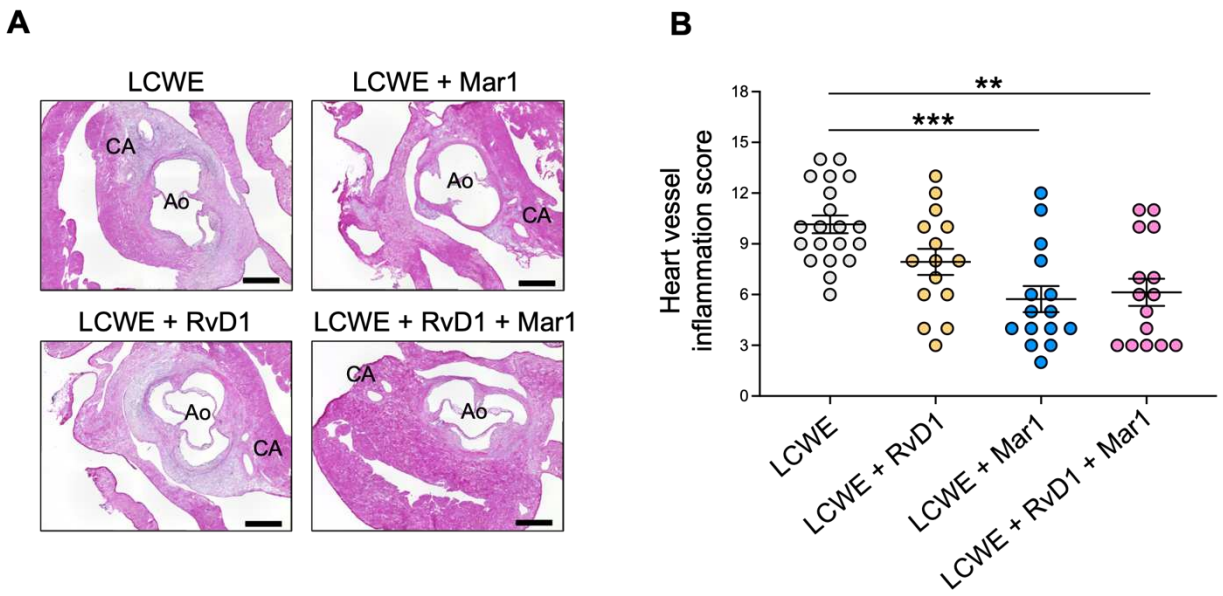

Figure 3. Treatment with Mar1 and Mar1 combined with RvD1 decreases inflammation in the heart vessels in the LCWE-induced KD vasculitis model. (A) Representative H&E-stained heart tissue of 14 days of LCWE injection and **(B)** heart vessel inflammation scores. Mice were injected with LCWE on day 0 and were treated with SPMs one hour after the injection. The treatment groups were divided as follows - RvD1 (100ng/mice), Mar1 (100ng/mice), or RvD1 and Mar1 (100ng/mice of each SPM). The mice were treated on days 2, 4, 6, 8, 10, and 12. Sample collection and analysis were performed on day 14. Data is presented as mean ± SEM (n= 15-20/group). Kruskal-Wallis one-way ANOVA was used to determine statistical significance (** $p<0.01$, ***$p<0.001$). CA indicates coronary artery; and Ao, aorta.

5 CONCLUSIONS

Using the LCWE-induced KD vasculitis murine model, we report long-term histopathological changes in cardiac tissues. Our findings show that cardiovascular lesions are still present even four months after vasculitis induction, with severe inflammatory infiltration and tissue remodeling in the cardiac tissue, leading to impaired cardiac function. We also found a correlation between the severity of the disease during the acute phase and the levels of neutrophils and monocytes in the peripheral blood.

We postulated that SPMs could be an effective treatment for LCWE-induced KD vasculitis based on the results of studies conducted on animal models of atherosclerosis, myocardial infarction, and abdominal aortic aneurysms. These studies have shown that SPMs can reduce inflammation, decrease leukocyte infiltration, enhance efferocytosis, protect tissue from maladaptive fibrosis modulation, and inhibit the production of pro-inflammatory cytokines, among other beneficial effects. Overall, this suggests that SPMs have the potential to improve outcomes in KD vasculitis treatment significantly.

Our results highlight the significant long-term histopathological changes caused by LCWE-induced KD vasculitis, mirroring the observations made in KD patients and presenting it as a reliable tool to understand KD’s long-term consequences further. We also strongly emphasize the criticality of investigating SPMs in the context of KD.

REFERENCES

1. Kawasaki T, Kosaki F, Okawa S, Shigematsu I, Yanagawa H. A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. Pediatrics [Internet]. 1974 Sep;54(3):271–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/4153258
2. Burns JC, Kushner HI, Bastian JF, Shike H, Shimizu C, Matsubara T, et al. Kawasaki Disease: A Brief History. Pediatrics [Internet]. 2000 Aug 1;106(2):e27–e27. Available from: https://publications.aap.org/pediatrics/article/106/2/e27/62795/Kawasaki-Disease-A-Brief-History
3. Nakamura Y, Hirose K, Yanagawa H, Kato H, Kawasaki T. Incidence rate of recurrent Kawasaki disease in Japan. Acta Paediatr. 1994 Oct;83(10):1061–4.
4. Makino N, Nakamura Y, Yashiro M, Sano T, Ae R, Kosami K, et al. Epidemiological observations of Kawasaki disease in Japan, 2013–2014. Pediatrics International. 2018 Jun 1;60(6):581–7.
5. Burns JC, Herzog L, Fabri O, Tremoulet AH, Rodó X, Uehara R, et al. Seasonality of Kawasaki Disease: A Global Perspective. Convertino M, editor. PLoS One [Internet]. 2013 Sep 18;8(9):e74529. Available from: https://dx.plos.org/10.1371/journal.pone.0074529
6. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, et al. Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: A Scientific Statement for Health Professionals From the American Heart Association. Circulation [Internet]. 2017 Apr 25;135(17):e927–99. Available from: https://www.ahajournals.org/doi/10.1161/CIR.0000000000000484
7. Kato H, Sugimura T, Akagi T, Sato N, Hashino K, Maeno Y, et al. Long-term Consequences of Kawasaki Disease. Circulation. 1996 Sep 15;94(6):1379–85.
8. Baer AZ, Rubin LG, Shapiro CA, Sood SK, Rajan S, Shapir Y, et al. Prevalence of Coronary Artery Lesions on the Initial Echocardiogram in Kawasaki Syndrome. Arch Pediatr Adolesc Med. 2006 Jul 1;160(7):686.

9. Lo MS, Newburger JW. Role of intravenous immunoglobulin in the treatment of Kawasaki disease. Int J Rheum Dis. 2018 Jan 1;21(1):64–9.
10. BURNS JC, CAPPARELLI E V., BROWN JA, NEWBURGER JW, GLODE MP. Intravenous gamma-globulin treatment and retreatment in Kawasaki disease. Pediatr Infect Dis J. 1998 Dec;17(12):1144–8.
11. Tremoulet AH, Jain S, Kim S, Newburger J, Arditi M, Franco A, et al. Rationale and study design for a phase I/IIa trial of anakinra in children with Kawasaki disease and early coronary artery abnormalities (the ANAKID trial). Contemp Clin Trials [Internet]. 2016 May;48:70–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27080929%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5221485
12. Friedman KG, Gauvreau K, Hamaoka-Okamoto A, Tang A, Berry E, Tremoulet AH, et al. Coronary Artery Aneurysms in Kawasaki Disease: Risk Factors for Progressive Disease and Adverse Cardiac Events in the US Population. J Am Heart Assoc. 2016 Sep;5(9).
13. Akagi T, Rose V, Benson LN, Newman A, Freedom RM. Outcome of coronary artery aneurysms after Kawasaki disease. J Pediatr. 1992 Nov;121(5):689–94.
14. Lee Y, Schulte DJ, Shimada K, Chen S, Crother TR, Chiba N, et al. Interleukin-1β Is Crucial for the Induction of Coronary Artery Inflammation in a Mouse Model of Kawasaki Disease. Circulation [Internet]. 2012 Mar 27;125(12):1542–50. Available from: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L51891216%5Cnhttp://dx.doi.org/10.1161/CIRCULATIONAHA.111.072769%5Cnhttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=00097322&id=doi:10.1161%2FCIRCULATIONAHA.111.072769&atitle=Interl
15. Schulte DJ, Yilmaz A, Shimada K, Fishbein MC, Lowe EL, Chen S, et al. Involvement of Innate and Adaptive Immunity in a Murine Model of Coronary Arteritis Mimicking Kawasaki Disease. The Journal of Immunology. 2009 Oct 15;183(8):5311–8.
16. Serhan CN, Chiang N. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. Br J Pharmacol [Internet].

2009;153(S1):S200–15. Available from: http://doi.wiley.com/10.1038/sj.bjp.0707489

17. Serhan CN. Novel lipid mediators and resolution mechanisms in acute inflammation: To resolve or not? American Journal of Pathology [Internet]. 2010;177(4):1576–91. Available from: http://dx.doi.org/10.2353/ajpath.2010.100322
18. SCHWAB J, SERHAN C. Lipoxins and new lipid mediators in the resolution of inflammation. Curr Opin Pharmacol. 2006 Aug;6(4):414–20.
19. Krishnamoorthy S, Recchiuti A, Chiang N, Fredman G, Serhan CN. Resolvin D1 Receptor Stereoselectivity and Regulation of Inflammation and Proresolving MicroRNAs. Am J Pathol. 2012 May;180(5):2018–27.
20. Chiang N, Dalli J, Colas RA, Serhan CN. Identification of resolvin D2 receptor mediating resolution of infections and organ protection. Journal of Experimental Medicine. 2015 Jul 27;212(8):1203–17.
21. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, et al. Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. J Exp Med. 2005 Mar 7;201(5):713–22.
22. Chiang N, Serhan CN. Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. Mol Aspects Med. 2017 Dec;58:114–29.
23. Qiu S, Li P, Zhao H, Li X. Maresin 1 alleviates dextran sulfate sodium-induced ulcerative colitis by regulating NRF2 and TLR4/NF-kB signaling pathway. Int Immunopharmacol. 2020 Jan;78:106018.
24. Levy BD, Serhan CN. Resolution and Regulation of Inflammation. In: Pathobiology of Human Disease. Elsevier; 2014. p. 332–48.
25. Viola JR, Lemnitzer P, Jansen Y, Csaba G, Winter C, Neideck C, et al. Resolving Lipid Mediators Maresin 1 and Resolvin D2 Prevent Atheroprogression in Mice. Circ Res. 2016;119(9):1030–8.
26. Fredman G, Ozcan L, Spolitu S, Hellmann J, Spite M, Backs J, et al. Resolvin D1 limits 5-lipoxygenase nuclear localization and leukotriene B_4 synthesis by inhibiting

a calcium-activated kinase pathway. Proceedings of the National Academy of Sciences. 2014 Oct 7;111(40):14530–5.

27. Akagi D, Chen M, Toy R, Chatterjee A, Conte MS. Systemic delivery of proresolving lipid mediators resolvin D2 and maresin 1 attenuates intimal hyperplasia in mice. FASEB Journal. 2015 Jun 1;29(6):2504–13.
28. Melish ME, Hicks RM, Larson EJ. Mucocutaneous Lymph Node Syndrome in the United States [Internet]. Available from: http://archpedi.jamanetwork.com/
29. Landing BH, Larson EJ. Are Infantile Periarteritis Nodosa With Coronary Artery Involvement and Fatal Mucocutaneous Lymph Node Syndrome the Same? Comparison of 20 Patients From North America With Patients From Hawaii and Japan. Pediatrics [Internet]. 1977 May 1;59(5):651–62. Available from: https://publications.aap.org/pediatrics/article/59/5/651/51932/Are-Infantile-Periarteritis-Nodosa-With-Coronary
30. Hirata, Y Nakamura, H Yanagawa S. Incidence rate of recurrent Kawasaki disease and related risk factors: from the results of nationwide surveys of Kawasaki disease in Japan. Acta Paediatr [Internet]. 2001 Jan 1;90(1):40–4. Available from: http://doi.wiley.com/10.1080/080352501750064851
31. Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, et al. Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease. Circulation. 2004 Oct 26;110(17):2747–71.
32. Beom Kim G. Reality of kawasaki disease epidemiology. Vol. 62, Korean Journal of Pediatrics. Korean Pediatric Society; 2019. p. 292–6.
33. Rowley AH, Shulman ST. The Epidemiology and Pathogenesis of Kawasaki Disease. Front Pediatr [Internet]. 2018 Dec 11;6. Available from: https://www.frontiersin.org/article/10.3389/fped.2018.00374/full
34. Taubert KA, Rowley AH, Shulman ST. Nationwide survey of Kawasaki disease and acute rheumatic fever. J Pediatr [Internet]. 1991 Aug;119(2):279–82. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022347605807425
35. Iio K, Matsubara K, Miyakoshi C, Ota K, Yamaoka R, Eguchi J, et al. Incidence of Kawasaki disease before and during the COVID-19 pandemic: A retrospective cohort study in Japan. BMJ Paediatr Open. 2021 Apr 2;5(1).

36. Ae R, Makino N, Kosami K, Kuwabara M, Matsubara Y, Nakamura Y. Epidemiology, Treatments, and Cardiac Complications in Patients with Kawasaki Disease: The Nationwide Survey in Japan, 2017-2018. J Pediatr. 2020 Oct;225:23-29.e2.
37. Kim GB, Han JW, Park YW, Song MS, Hong YM, Cha SH, et al. Epidemiologic features of Kawasaki disease in South Korea: Data from nationwide survey, 2009-2011. Pediatric Infectious Disease Journal. 2014 Jan;33(1):24–7.
38. Lin MC, Lai MS, Jan SL, Fu YC. Epidemiologic features of Kawasaki disease in acute stages in Taiwan, 1997-2010: Effect of different case definitions in claims data analysis. Journal of the Chinese Medical Association. 2015 Feb 1;78(2):121–6.
39. Huang YH, Lin KM, Ho SC, Yan JH, Lo MH, Kuo HC. Increased Incidence of Kawasaki Disease in Taiwan in Recent Years: A 15 Years Nationwide Population-Based Cohort Study. Front Pediatr. 2019 Mar 29;7.
40. Xie L ping, Yan W li, Huang M, Huang M rong, Chen S, Huang G ying, et al. Epidemiologic Features of Kawasaki Disease in Shanghai From 2013 Through 2017. J Epidemiol. 2020 Oct 5;30(10):429–35.
41. Du ZD, Zhao D, Du J, Zhang YL, Lin Y, Liu C, et al. EPIDEMIOLOGIC STUDY ON KAWASAKI DISEASE IN BEIJING FROM 2000 THROUGH 2004. Pediatric Infectious Disease Journal. 2007 May;26(5):449–51.
42. Singh S, Aulakh R, Bhalla AK, Suri D, Manojkumar R, Narula N, et al. Is Kawasaki disease incidence rising in Chandigarh, North India? Arch Dis Child. 2011 Feb 1;96(2):137–40.
43. Holman RC, Belay ED, Christensen KY, Folkema AM, Steiner CA, Schonberger LB. Hospitalizations for Kawasaki Syndrome Among Children in the United States, 1997–2007. Pediatric Infectious Disease Journal. 2010 Jun;29(6):483–8.
44. Manlhiot C, O'Shea S, Bernknopf B, LaBelle M, Chahal N, Dillenburg RF, et al. Epidemiology of Kawasaki Disease in Canada 2004 to 2014: Comparison of Surveillance Using Administrative Data vs Periodic Medical Record Review. Canadian Journal of Cardiology. 2018 Mar 1;34(3):303–9.

45. Shahbaznejad L, Hosseininasab A, Mahboobi L, Mohammadi H, Esmaeili H, Farrokhi Far SM, et al. Epidemiological data of national Kawasaki disease registry in Iran, 2007–2019. Front Pediatr. 2023 Jan 11;10.
46. Bar-Meir M, Haklai Z, Dor M. Kawasaki Disease in Israel. Pediatric Infectious Disease Journal. 2011 Jul;30(7):589–92.
47. Arab Y, Choueiter N, Dahdah N, El-Kholy N, Abu Al-Saoud SY, Abu-Shukair ME, et al. Kawasaki Disease Arab Initiative [Kawarabi]: Establishment and Results of a Multicenter Survey. Pediatr Cardiol. 2022 Aug 27;43(6):1239–46.
48. Borzutzky A, Hoyos-Bachiloglu R, Cerda J, Talesnik E. Rising hospitalization rates of Kawasaki Disease in Chile between 2001 and 2007. Rheumatol Int. 2012 Aug 23;32(8):2491–5.
49. González-Mata A, Ulloa-Gutiérrez R, Brea J, Soza G, Tremoulet AH. Origin and importance of the Latin American Kawasaki Disease Network (REKAMLATINA). Revista chilena de infectología. 2014 Jun;31(3):330–2.
50. Dolezalová P, Telekesová P, Nemcová D, Hoza J. Incidence of vasculitis in children in the Czech Republic: 2-year prospective epidemiology survey. J Rheumatol. 2004 Nov;31(11):2295–9.
51. Gradoux E, Di Bernardo S, Bressieux-Degueldre S, Mivelaz Y, Boulos Ksontini T, Prsa M, et al. Epidemiology of Kawasaki Disease in children in Switzerland: a national prospective cohort study. Swiss Med Wkly. 2022 May 26;152(2122):w30171.
52. Hall GC, Tulloh LE, Tulloh RMR. Kawasaki disease incidence in children and adolescents: an observational study in primary care. British Journal of General Practice. 2016 Apr;66(645):e271–6.
53. Alkanhal A, Saunders J, Altammar F, Huber AM, Lynk A, MacLeod A, et al. Unexpectedly high incidence of Kawasaki Disease in a Canadian Atlantic Province- an 11-year retrospective descriptive study. Pediatric Rheumatology [Internet]. 2023 Apr 3;21(1):30. Available from: https://ped-rheum.biomedcentral.com/articles/10.1186/s12969-023-00805-y
54. Uehara R, Belay ED. Epidemiology of Kawasaki Disease in Asia, Europe, and the United States. J Epidemiol. 2012;22(2):79–85.

55. Maddox RA, Holman RC, Uehara R, Callinan LS, Guest JL, Schonberger LB, et al. Recurrent Kawasaki disease: USA and Japan. Pediatrics International [Internet]. 2015 Dec 1;57(6):1116–20. Available from: https://onlinelibrary.wiley.com/doi/10.1111/ped.12733
56. Nakamura Y, Aso E, Yashiro M, Uehara R, Watanabe M, Oki I, et al. Mortality Among Persons With a History of Kawasaki Disease in Japan Mortality Among Males With Cardiac Sequelae is Significantly Higher Than That of the General Population. Circulation Journal [Internet]. 2008;72(1):134–8. Available from: http://www.jstage.jst.go.jp/article/circj/72/1/72_1_134/_article
57. Burns JC, Shike H, Gordon JB, Malhotra A, Schoenwetter M, Kawasaki T. Sequelae of Kawasaki disease in adolescents and young adults. J Am Coll Cardiol [Internet]. 1996 Jul;28(1):253–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/073510979600099X
58. Yanagawa H, Nakamura Y, Yashiro M, Ojima T, Tanihara S, Oki I, et al. Results of the Nationwide Epidemiologic Survey of Kawasaki Disease in 1995 and 1996 in Japan. Pediatrics [Internet]. 1998 Dec 1;102(6):e65–e65. Available from: https://publications.aap.org/pediatrics/article/102/6/e65/62130/Results-of-the-Nationwide-Epidemiologic-Survey-of
59. Onouchi Y, Gunji T, Burns JC, Shimizu C, Newburger JW, Yashiro M, et al. ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. Nat Genet. 2008 Jan 16;40(1):35–42.
60. Khor CC, Davila S, Shimizu C, Sheng S, Matsubara T, Suzuki Y, et al. Genome-wide linkage and association mapping identify susceptibility alleles in ABCC4 for Kawasaki disease. J Med Genet. 2011 Jul 1;48(7):467–72.
61. Onouchi Y, Ozaki K, Buns JC, Shimizu C, Hamada H, Honda T, et al. Common variants in CASP3 confer susceptibility to Kawasaki disease. Hum Mol Genet. 2010 Jul 15;19(14):2898–906.
62. Onouchi Y, Ozaki K, Burns JC, Shimizu C, Terai M, Hamada H, et al. A genome-wide association study identifies three new risk loci for Kawasaki disease. Nat Genet. 2012 May 25;44(5):517–21.

63. Khor CC, Davila S, Breunis WB, Lee YC, Shimizu C, Wright VJ, et al. Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. Nat Genet. 2011 Dec 13;43(12):1241–6.
64. Shimizu C, Jain S, Davila S, Hibberd ML, Lin KO, Molkara D, et al. Transforming Growth Factor-β Signaling Pathway in Patients With Kawasaki Disease. Circ Cardiovasc Genet. 2011 Feb;4(1):16–25.
65. Onouchi Y, Suzuki Y, Suzuki H, Terai M, Yasukawa K, Hamada H, et al. ITPKC and CASP3 polymorphisms and risks for IVIG unresponsiveness and coronary artery lesion formation in Kawasaki disease. Pharmacogenomics J. 2013 Feb 11;13(1):52–9.
66. Kuo HC, Hsu YW, Wu CM, Chen SHY, Hung KS, Chang WP, et al. A Replication Study for Association of ITPKC and CASP3 Two-Locus Analysis in IVIG Unresponsiveness and Coronary Artery Lesion in Kawasaki Disease. PLoS One. 2013 Jul 24;8(7):e69685.
67. Onouchi Y. The genetics of Kawasaki disease. Int J Rheum Dis [Internet]. 2018 Jan;21(1):26–30. Available from: http://doi.wiley.com/10.1111/1756-185X.13218
68. Chang CJ, Kuo HC, Chang JS, Lee JK, Tsai FJ, Khor CC, et al. Replication and Meta-Analysis of GWAS Identified Susceptibility Loci in Kawasaki Disease Confirm the Importance of B Lymphoid Tyrosine Kinase (BLK) in Disease Susceptibility. PLoS One. 2013 Aug 30;8(8):e72037.
69. Wang CL, Wu YT, Liu CA, Lin MW, Lee CJ, Huang LT, et al. Expression of CD40 Ligand on CD4+ T-Cells and Platelets Correlated to the Coronary Artery Lesion and Disease Progress in Kawasaki Disease. Pediatrics. 2003 Feb 1;111(2):e140–7.
70. Yan J, Wang C, Du R, Liu P, Chen G. Association analysis of CD40 gene polymorphism with acute coronary syndrome. Clin Exp Med. 2010 Dec 2;10(4):253–8.
71. Tremoulet AH, Pancoast P, Franco A, Bujold M, Shimizu C, Onouchi Y, et al. Calcineurin Inhibitor Treatment of Intravenous Immunoglobulin–Resistant Kawasaki Disease. J Pediatr. 2012 Sep;161(3):506-512.e1.

72. Lee YC, Kuo HC, Chang JS, Chang LY, Huang LM, Chen MR, et al. Two new susceptibility loci for Kawasaki disease identified through genome-wide association analysis. Nat Genet. 2012 May 25;44(5):522–5.
73. Chi H, Huang FY, Chen MR, Chiu NC, Lee HC, Lin SP, et al. ITPKC gene SNP rs28493229 and Kawasaki disease in Taiwanese children. Hum Mol Genet. 2010 Mar 15;19(6):1147–51.
74. Rowley AH. Kawasaki Disease: Novel Insights into Etiology and Genetic Susceptibility. Annu Rev Med. 2011 Feb 18;62(1):69–77.
75. Taslakian EN, Wi CIL, Seol HY, Boyce TG, Johnson JN, Ryu E, et al. Long-term Incidence of Kawasaki Disease in a North American Community: A Population-Based Study. Pediatr Cardiol. 2021 Jun 1;42(5):1033–40.
76. Ae R, Makino N, Kuwabara M, Matsubara Y, Kosami K, Sasahara T, et al. Incidence of Kawasaki Disease Before and After the COVID-19 Pandemic in Japan. JAMA Pediatr. 2022 Dec 1;176(12):1217.
77. Baker AL, Lu M, Minich LL, Atz AM, Klein GL, Korsin R, et al. Associated Symptoms in the Ten Days Before Diagnosis of Kawasaki Disease. J Pediatr. 2009 Apr;154(4):592-595.e2.
78. Sakurai Y. Autoimmune Aspects of Kawasaki Disease. J Investig Allergol Clin Immunol. 2019 Apr 2;29(4):251–61.
79. Singh S, Jindal AK, Pilania RK. Diagnosis of Kawasaki disease. Int J Rheum Dis. 2018 Jan;21(1):36–44.
80. Liu X, Zhou K, Hua Y, Wu M, Liu L, Shao S, et al. Neurological involvement in Kawasaki disease: a retrospective study. Pediatric Rheumatology [Internet]. 2020 Dec 14;18(1):61. Available from: https://ped-rheum.biomedcentral.com/articles/10.1186/s12969-020-00452-7
81. Mauro A, Di Mari C, Casini F, Giani T, Sandini M, Biondi L, et al. Neurological manifestations of Kawasaki disease and multisystem inflammatory syndrome in children associated with COVID-19: A comparison of two different clinical entities. Front Pediatr. 2023 Jan 5;10.

82. Tremoulet AH, Jain S, Chandrasekar D, Sun X, Sato Y, Burns JC. Evolution of Laboratory Values in Patients With Kawasaki Disease. Pediatric Infectious Disease Journal. 2011 Dec;30(12):1022–6.
83. Chaudhary H, Nameirakpam J, Kumrah R, Pandiarajan V, Suri D, Rawat A, et al. Biomarkers for Kawasaki Disease: Clinical Utility and the Challenges Ahead. Front Pediatr. 2019 Jun 18;7.
84. Arora K, Guleria S, Jindal AK, Rawat A, Singh S. Platelets in Kawasaki disease: Is this only a numbers game or something beyond? Genes Dis. 2020 Mar;7(1):62–6.
85. Park JH, Choi HJ. Clinical implications of thrombocytosis in acute phase Kawasaki disease. Eur J Pediatr. 2021 Jun 1;180(6):1841–6.
86. Oates-Whitehead RM, Baumer JH, Haines L, Love S, Maconochie IK, Gupta A, et al. Intravenous immunoglobulin for the treatment of Kawasaki disease in children. Cochrane Database of Systematic Reviews [Internet]. 2003 Oct 20;2021(12). Available from: http://doi.wiley.com/10.1002/14651858.CD004000
87. Mori M, Miyamae T, Imagawa T, Katakura S, Kimura K, Yokota S. Meta-analysis of the results of intravenous gamma globulin treatment of coronary artery lesions in Kawasaki disease. Mod Rheumatol [Internet]. 2004 Nov;14(5):361–6. Available from: http://link.springer.com/10.1007/s10165-004-0324-3
88. Tremoulet AH, Best BM, Song S, Wang S, Corinaldesi E, Eichenfield JR, et al. Resistance to Intravenous Immunoglobulin in Children with Kawasaki Disease. J Pediatr. 2008 Jul;153(1):117-121.e3.
89. Dajani AS, Taubert KA, Takahashi M, Bierman FZ, Freed MD, Ferrieri P, et al. Guidelines for long-term management of patients with Kawasaki disease. Report from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Circulation [Internet]. 1994 Feb;89(2):916–22. Available from: https://www.ahajournals.org/doi/10.1161/01.CIR.89.2.916
90. Furusho K, Nakano H, Shinomiya K, Tamura T, Manabe Y, Kawarano M, et al. HIGH-DOSE INTRAVENOUS GAMMAGLOBULIN FOR KAWASAKI DISEASE. The Lancet [Internet]. 1984 Nov;324(8411):1055–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673684915046

91. Terai M, Shulman ST. Prevalence of coronary artery abnormalities in Kawasaki disease is highly dependent on gamma globulin dose but independent of salicylate dose. J Pediatr [Internet]. 1997 Dec;131(6):888–93. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022347697700386
92. McFadyen JD, Gardiner EE. An Aspirin a Day…. Arterioscler Thromb Vasc Biol [Internet]. 2022 Oct;42(10):1217–9. Available from: https://www.ahajournals.org/doi/10.1161/ATVBAHA.122.318337
93. Catella-Lawson F, Reilly MP, Kapoor SC, Cucchiara AJ, DeMarco S, Tournier B, et al. Cyclooxygenase Inhibitors and the Antiplatelet Effects of Aspirin. New England Journal of Medicine [Internet]. 2001 Dec 20;345(25):1809–17. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa003199
94. Antithrombotic Trialists' (ATT) Collaboration. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. The Lancet [Internet]. 2009 May;373(9678):1849–60. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673609605031
95. Durongpisitkul K, Gururaj VJ, Park JM, Martin CF. The prevention of coronary artery aneurysm in Kawasaki disease: a meta-analysis on the efficacy of aspirin and immunoglobulin treatment. Pediatrics. 1995 Dec;96(6):1057–61.
96. Huang YH, Hsin YC, Wang LJ, Feng WL, Guo MMH, Chang LS, et al. Treatment of Kawasaki Disease: A Network Meta-Analysis of Four Dosage Regimens of Aspirin Combined With Recommended Intravenous Immunoglobulin. Front Pharmacol. 2021 Aug 12;12.
97. Hsieh KS, Weng KP, Lin CC, Huang TC, Lee CL, Huang SM. Treatment of Acute Kawasaki Disease: Aspirin's Role in the Febrile Stage Revisited. Pediatrics. 2004 Dec 1;114(6):e689–93.
98. Ogata S, Ogihara Y, Honda T, Kon S, Akiyama K, Ishii M. Corticosteroid Pulse Combination Therapy for Refractory Kawasaki Disease: A Randomized Trial. Pediatrics. 2012 Jan 1;129(1):e17–23.
99. Kobayashi T, Inoue Y, Takeuchi K, Okada Y, Tamura K, Tomomasa T, et al. Prediction of Intravenous Immunoglobulin Unresponsiveness in Patients With Kawasaki Disease. Circulation. 2006 Jun 6;113(22):2606–12.

100. Chen S, Dong Y, Yin Y, Krucoff MW. Intravenous immunoglobulin plus corticosteroid to prevent coronary artery abnormalities in Kawasaki disease: a meta-analysis. Heart. 2013 Jan 15;99(2):76–82.
101. Kibata T, Suzuki Y, Hasegawa S, Matsushige T, Kusuda T, Hoshide M, et al. Coronary artery lesions and the increasing incidence of Kawasaki disease resistant to initial immunoglobulin. Int J Cardiol. 2016 Jul;214:209–15.
102. Burns JC, Roberts SC, Tremoulet AH, He F, Printz BF, Ashouri N, et al. Infliximab versus second intravenous immunoglobulin for treatment of resistant Kawasaki disease in the USA (KIDCARE): a randomised, multicentre comparative effectiveness trial. Lancet Child Adolesc Health. 2021 Dec;5(12):852–61.
103. Durongpisitkul K, Soongswang J, Laohaprasitiporn D, Nana A, Prachuabmoh C, Kangkagate C. Immunoglobulin Failure and Retreatment in Kawasaki Disease. Pediatr Cardiol. 2003 Mar 1;24(2):145–8.
104. Son MBF, Gauvreau K, Ma L, Baker AL, Sundel RP, Fulton DR, et al. Treatment of Kawasaki Disease: Analysis of 27 US Pediatric Hospitals From 2001 to 2006. Pediatrics. 2009 Jul 1;124(1):1–8.
105. Egami K, Muta H, Ishii M, Suda K, Sugahara Y, Iemura M, et al. Prediction of resistance to intravenous immunoglobulin treatment in patients with Kawasaki disease. J Pediatr. 2006 Aug;149(2):237–40.
106. Burns JC, Best BM, Mejias A, Mahony L, Fixler DE, Jafri HS, et al. Infliximab Treatment of Intravenous Immunoglobulin–Resistant Kawasaki Disease. J Pediatr. 2008 Dec;153(6):833-838.e6.
107. Kobayashi T, Saji T, Otani T, Takeuchi K, Nakamura T, Arakawa H, et al. Efficacy of immunoglobulin plus prednisolone for prevention of coronary artery abnormalities in severe Kawasaki disease (RAISE study): a randomised, open-label, blinded-endpoints trial. The Lancet. 2012 Apr;379(9826):1613–20.
108. Furukawa T, Kishiro M, Akimoto K, Nagata S, Shimizu T, Yamashiro Y. Effects of steroid pulse therapy on immunoglobulin-resistant Kawasaki disease. Arch Dis Child. 2008 Feb;93(2):142–6.

109. Ogata S, Bando Y, Kimura S, Ando H, Nakahata Y, Ogihara Y, et al. The strategy of immune globulin resistant Kawasaki disease: A comparative study of additional immune globulin and steroid pulse therapy. J Cardiol. 2009 Feb;53(1):15–9.
110. Tremoulet AH, Jain S, Jaggi P, Jimenez-Fernandez S, Pancheri JM, Sun X, et al. Infliximab for intensification of primary therapy for Kawasaki disease: a phase 3 randomised, double-blind, placebo-controlled trial. The Lancet. 2014 May;383(9930):1731–8.
111. Burns JC, Mason WH, Hauger SB, Janai H, Bastian JF, Wohrley JD, et al. Infliximab treatment for refractory Kawasaki syndrome. J Pediatr. 2005 May;146(5):662–7.
112. Weiss JE, Eberhard BA, Chowdhury D, Gottlieb BS. Infliximab as a novel therapy for refractory Kawasaki disease. J Rheumatol. 2004 Apr;31(4):808–10.
113. Son MB, Gauvreau K, Burns JC, Corinaldesi E, Tremoulet AH, Watson VE, et al. Infliximab for Intravenous Immunoglobulin Resistance in Kawasaki Disease: A Retrospective Study. J Pediatr. 2011 Apr;158(4):644-649.e1.
114. Suzuki H, Terai M, Hamada H, Honda T, Suenaga T, Takeuchi T, et al. Cyclosporin A Treatment for Kawasaki Disease Refractory to Initial and Additional Intravenous Immunoglobulin. Pediatric Infectious Disease Journal. 2011 Oct;30(10):871–6.
115. Takahashi M. Myocarditis in Kawasaki syndrome. A minor villain? Circulation. 1989 Jun;79(6):1398–400.
116. Dahdah N. Not Just Coronary Arteritis, Kawasaki Disease Is a Myocarditis, Too. J Am Coll Cardiol. 2010 Apr;55(14):1507.
117. Harada M, Yokouchi Y, Oharaseki T, Matsui K, Tobayama H, Tanaka N, et al. Histopathological characteristics of myocarditis in acute-phase Kawasaki disease. Histopathology. 2012 Dec;61(6):1156–67.
118. McCrindle BW, Li JS, Minich LL, Colan SD, Atz AM, Takahashi M, et al. Coronary Artery Involvement in Children With Kawasaki Disease. Circulation. 2007 Jul 10;116(2):174–9.
119. Newburger JW, Takahashi M, Burns JC, Beiser AS, Chung KJ, Duffy CE, et al. The Treatment of Kawasaki Syndrome with Intravenous Gamma Globulin. New England Journal of Medicine [Internet]. 1986 Aug 7;315(6):341–7. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM198608073150601

120. Newburger JW, Takahashi M, Beiser AS, Burns JC, Bastian J, Chung KJ, et al. A Single Intravenous Infusion of Gamma Globulin as Compared with Four Infusions in the Treatment of Acute Kawasaki Syndrome. New England Journal of Medicine. 1991 Jun 6;324(23):1633–9.
121. Kobayashi T, Fuse S, Sakamoto N, Mikami M, Ogawa S, Hamaoka K, et al. A New Z Score Curve of the Coronary Arterial Internal Diameter Using the Lambda-Mu-Sigma Method in a Pediatric Population. Journal of the American Society of Echocardiography. 2016 Aug;29(8):794-801.e29.
122. Fukazawa R, Kobayashi J, Ayusawa M, Hamada H, Miura M, Mitani Y, et al. JCS/JSCS 2020 Guideline on Diagnosis and Management of Cardiovascular Sequelae in Kawasaki Disease. Circulation Journal. 2020 Jul 22;84(8):1348–407.
123. van Stijn D, Planken RN, Groenink M, Streekstra GJ, Kuijpers TW, Kuipers IM. Coronary artery assessment in Kawasaki disease with dual-source CT angiography to uncover vascular pathology. Eur Radiol. 2020 Jan 19;30(1):432–41.
124. Dallaire F, Fournier A, Breton J, Nguyen TD, Spigelblatt L, Dahdah N. Marked Variations in Serial Coronary Artery Diameter Measures in Kawasaki Disease: A New Indicator of Coronary Involvement. Journal of the American Society of Echocardiography. 2012 Aug;25(8):859–65.
125. Crystal MA, Manlhiot C, Yeung RSM, Smallhorn JF, McCrindle BW. Coronary artery dilation after Kawasaki disease for children within the normal range. Int J Cardiol. 2009 Jul;136(1):27–32.
126. Pilania RK, Jindal AK, Bhattarai D, Naganur SH, Singh S. Cardiovascular Involvement in Kawasaki Disease Is Much More Than Mere Coronary Arteritis. Front Pediatr. 2020 Sep 24;8.
127. Dionne A, Dahdah N. Myocarditis and Kawasaki disease. Int J Rheum Dis. 2018 Jan 3;21(1):45–9.
128. Kanegaye JT, Wilder MS, Molkara D, Frazer JR, Pancheri J, Tremoulet AH, et al. Recognition of a Kawasaki Disease Shock Syndrome. Pediatrics. 2009 May 1;123(5):e783–9.

129. Lin MT, Fu CM, Huang SK, Huang SC, Wu MH. Population-based Study of Kawasaki Disease Shock Syndrome in Taiwan. Pediatric Infectious Disease Journal. 2013 Dec;32(12):1384–6.
130. Gamez-Gonzalez LB, Moribe-Quintero I, Cisneros-Castolo M, Varela-Ortiz J, Muñoz-Ramírez M, Garrido-García M, et al. Kawasaki disease shock syndrome: Unique and severe subtype of Kawasaki disease. Pediatrics International. 2018 Sep;60(9):781–90.
131. Dominguez SR, Friedman K, Seewald R, Anderson MS, Willis L, Glodé MP. Kawasaki Disease in a Pediatric Intensive Care Unit: A Case-Control Study. Pediatrics. 2008 Oct 1;122(4):e786–90.
132. Gatterre P, Oualha M, Dupic L, Iserin F, Bodemer C, Lesage F, et al. Kawasaki disease: an unexpected etiology of shock and multiple organ dysfunction syndrome. Intensive Care Med. 2012 May 25;38(5):872–8.
133. de La Harpe M, di Bernardo S, Hofer M, Sekarski N. Thirty Years of Kawasaki Disease: A Single-Center Study at the University Hospital of Lausanne. Front Pediatr. 2019 Jan 30;7.
134. Gersony WM. The Adult After Kawasaki Disease. J Am Coll Cardiol. 2009 Nov;54(21):1921–3.
135. Daniels LB, Roberts S, Moreno E, Tremoulet AH, Gordon JB, Burns JC. Long-term health outcomes in young adults after Kawasaki disease. IJC Heart & Vasculature. 2022 Jun;40:101039.
136. Dietz SM, Tacke CE, Gort J, Kuipers IM, de Groot E, Wiegman A, et al. Carotid Intima-Media Thickness in Patients With a History of Kawasaki Disease. Circulation Journal. 2015;79(12):2682–7.
137. McCrindle BW, McIntyre S, Kim C, Lin T, Adeli K. Are Patients after Kawasaki Disease at Increased Risk for Accelerated Atherosclerosis? J Pediatr. 2007 Sep;151(3):244-248.e1.
138. Gordon JB, Daniels LB, Kahn AM, Jimenez-Fernandez S, Vejar M, Numano F, et al. The Spectrum of Cardiovascular Lesions Requiring Intervention in Adults After Kawasaki Disease. JACC Cardiovasc Interv. 2016 Apr;9(7):687–96.

139. Kato H, Ichinose E, Kawasaki T. Myocardial infarction in Kawasaki disease: Clinical analyses in 195 cases. J Pediatr [Internet]. 1986 Jun;108(6):923–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022347686809283
140. Rizk SRY, El Said G, Daniels LB, Burns JC, El Said H, Sorour KA, et al. Acute myocardial ischemia in adults secondary to missed Kawasaki disease in childhood. American Journal of Cardiology. 2015 Feb 15;115(4):423–7.
141. Yutani C, Go S, Kamiya T, Hirose O, Misawa H, Maeda H, et al. Cardiac biopsy of Kawasaki disease. Arch Pathol Lab Med. 1981 Sep;105(9):470–3.
142. Yonesaka S, Nakada T, Sunagawa Y, Tomimoto K, Naka S, Takahashi T, et al. Endomyocardial Biopsy in Children with Kawasaki Disease. Pediatrics International. 1989 Dec;31(6):706–11.
143. YONESAKA S, TAKAHASHI T, MATUBARA T, NAKADA T, FURUKAWA H, TOMIMOTO K, et al. Histopathological study on Kawasaki disease with special reference to the relation between the myocardial sequelae and regional wall motion abnormalities of the left ventricle. Jpn Circ J. 1992;56(4):352–8.
144. Printz BF, Sleeper LA, Newburger JW, Minich LL, Bradley T, Cohen MS, et al. Noncoronary Cardiac Abnormalities Are Associated With Coronary Artery Dilation and With Laboratory Inflammatory Markers in Acute Kawasaki Disease. J Am Coll Cardiol. 2011 Jan;57(1):86–92.
145. Robinson C, Chanchlani R, Gayowsky A, Brar S, Darling E, Demers C, et al. Cardiovascular outcomes in children with Kawasaki disease: a population-based cohort study. Pediatr Res. 2023 Apr 15;93(5):1267–75.
146. Takahashi K, Oharaseki T, Naoe S, Wakayama M, Yokouchi Y. Neutrophilic involvement in the damage to coronary arteries in acute stage of Kawasaki disease. Pediatrics International [Internet]. 2005 Jun;47(3):305–10. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1442-200x.2005.02049.x
147. Orenstein JM, Shulman ST, Fox LM, Baker SC, Takahashi M, Bhatti TR, et al. Three Linked Vasculopathic Processes Characterize Kawasaki Disease: A Light and Transmission Electron Microscopic Study. PLoS One. 2012 Jun 18;7(6):e38998.

148. Shulman ST, Rowley AH. Kawasaki disease: insights into pathogenesis and approaches to treatment. Nat Rev Rheumatol. 2015 Aug 28;11(8):475–82.
149. Hara T, Yamamura K, Sakai Y. The up-to-date pathophysiology of Kawasaki disease. Clin Transl Immunology. 2021 Jan 10;10(5).
150. Porritt RA, Zemmour D, Abe M, Lee Y, Narayanan M, Carvalho TT, et al. NLRP3 Inflammasome Mediates Immune-Stromal Interactions in Vasculitis. Circ Res. 2021 Oct 15;129(9).
151. Anzai F, Watanabe S, Kimura H, Kamata R, Karasawa T, Komada T, et al. Crucial role of NLRP3 inflammasome in a murine model of Kawasaki disease. J Mol Cell Cardiol. 2020 Jan;138:185–96.
152. Swanson K V., Deng M, Ting JPY. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol. 2019 Aug 29;19(8):477–89.
153. Masters SL, Simon A, Aksentijevich I, Kastner DL. Horror Autoinflammaticus: The Molecular Pathophysiology of Autoinflammatory Disease. Annu Rev Immunol [Internet]. 2009 Apr 1;27(1):621–68. Available from: https://www.annualreviews.org/doi/10.1146/annurev.immunol.25.022106.141627
154. Alphonse MP, Duong TT, Shumitzu C, Hoang TL, McCrindle BW, Franco A, et al. Inositol-Triphosphate 3-Kinase C Mediates Inflammasome Activation and Treatment Response in Kawasaki Disease. The Journal of Immunology. 2016 Nov 1;197(9):3481–9.
155. Maury CP, Salo E, Pelkonen P. Circulating interleukin-1 beta in patients with Kawasaki disease. N Engl J Med [Internet]. 1988 Dec 22;319(25):1670–1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3264383
156. Hoang LT, Shimizu C, Ling L, Naim ANM, Khor CC, Tremoulet AH, et al. Global gene expression profiling identifies new therapeutic targets in acute Kawasaki disease. Genome Med. 2014 Nov 20;6(11):541.
157. Gorelik M, Lee Y, Abe M, Andrews T, Davis L, Patterson J, et al. IL-1 receptor antagonist, anakinra, prevents myocardial dysfunction in a mouse model of Kawasaki disease vasculitis and myocarditis. Clin Exp Immunol. 2019 Sep 2;198(1):101–10.

158. Cohen S, Tacke CE, Straver B, Meijer N, Kuipers IM, Kuijpers TW. A child with severe relapsing Kawasaki disease rescued by IL-1 receptor blockade and extracorporeal membrane oxygenation. Ann Rheum Dis. 2012 Dec;71(12):2059–61.
159. Shafferman A, Birmingham JD, Cron RQ. High dose anakinra for treatment of severe neonatal Kawasaki disease: a case report. Pediatric Rheumatology. 2014 Dec 11;12(1):26.
160. Sánchez-Manubens J, Gelman A, Franch N, Teodoro S, Palacios JR, Rudi N, et al. A child with resistant Kawasaki disease successfully treated with anakinra: a case report. BMC Pediatr. 2017 Dec 8;17(1):102.
161. Blonz G, Lacroix S, Benbrik N, Warin-Fresse K, Masseau A, Trewick D, et al. Severe Late-Onset Kawasaki Disease Successfully Treated With Anakinra. JCR: Journal of Clinical Rheumatology. 2020 Mar;26(2):e42–3.
162. Bossi G, Codazzi AC, Vinci F, Clerici E, Regalbuto C, Crapanzano C, et al. Efficacy of Anakinra on Multiple Coronary Arteries Aneurysms in an Infant with Recurrent Kawasaki Disease, Complicated by Macrophage Activation Syndrome. Children. 2022 May 5;9(5):672.
163. Koné-Paut I, Tellier S, Belot A, Brochard K, Guitton C, Marie I, et al. Phase II Open Label Study of Anakinra in Intravenous Immunoglobulin–Resistant Kawasaki Disease. Arthritis & Rheumatology. 2021 Jan 17;73(1):151–61.
164. Shimizu T, Nakamura H, Kawakami A. Role of the Innate Immunity Signaling Pathway in the Pathogenesis of Sjögren's Syndrome. Int J Mol Sci. 2021 Mar 17;22(6):3090.
165. Ye F, Foell D, Hirono K ich, Vogl T, Rui C, Yu X, et al. Neutrophil-derived S100A12 is profoundly upregulated in the early stage of acute Kawasaki disease. Am J Cardiol. 2004 Sep;94(6):840–4.
166. Foell D, Ichida F, Vogl T, Yu X, Chen R, Miyawaki T, et al. S100A12 (EN-RAGE) in monitoring Kawasaki disease. The Lancet. 2003 Apr;361(9365):1270–2.
167. Holzinger D, Tenbrock K, Roth J. Alarmins of the S100-Family in Juvenile Autoimmune and Auto-Inflammatory Diseases. Front Immunol. 2019 Feb 15;10.

168. Lech M, Guess J, Duffner J, Oyamada J, Shimizu C, Hoshino S, et al. Circulating Markers of Inflammation Persist in Children and Adults With Giant Aneurysms After Kawasaki Disease. Circ Genom Precis Med [Internet]. 2019 Apr 1;12(4):e002433. Available from: https://www.ahajournals.org/doi/10.1161/CIRCGEN.118.002433
169. Ebihara T, Endo R, Kikuta H, Ishiguro N, Ma X, Shimazu M, et al. Differential gene expression of S100 protein family in leukocytes from patients with Kawasaki disease. Eur J Pediatr. 2005 Jul 19;164(7):427–31.
170. Jia S, Li C, Wang G, Yang J, Zu Y. The T helper type 17/regulatory T cell imbalance in patients with acute Kawasaki disease. Clin Exp Immunol. 2010 Sep 9;162(1):131–7.
171. Askenasy N, Kaminitz A, Yarkoni S. Mechanisms of T regulatory cell function. Autoimmun Rev. 2008;7(5):370–5.
172. Noval Rivas M, Wakita D, Franklin MK, Carvalho TT, Abolhesn A, Gomez AC, et al. Intestinal Permeability and IgA Provoke Immune Vasculitis Linked to Cardiovascular Inflammation. Immunity. 2019 Sep;51(3):508-521.e6.
173. Shimizu , Chisato, Oharaseki , Toshiaki, Takahashi , Kei, Kottek , Aubri, Franco , Alessandra, Burns JC. The role of TGF-β and myofibroblasts in the arteritis of Kawasaki disease. Hum Pathol. 2013 Feb;44(2):189–98.
174. Tian F, Ma L, Zhao R, Ji L, Wang X, Sun W, et al. Correlation Between Matrix Metalloproteinases With Coronary Artery Lesion Caused by Kawasaki Disease. Front Pediatr. 2022 Feb 11;10.
175. Rowley AH, Eckerley CA, Jäck HM, Shulman ST, Baker SC. IgA plasma cells in vascular tissue of patients with Kawasaki syndrome. J Immunol. 1997 Dec 15;159(12):5946–55.
176. Noval Rivas M, Arditi M. Kawasaki disease: pathophysiology and insights from mouse models. Nat Rev Rheumatol [Internet]. 2020;16(7):391–405. Available from: http://dx.doi.org/10.1038/s41584-020-0426-0
177. Ohno N. Chemistry and Biology of Angiitis Inducer, *Candida albicans* Water-Soluble Mannoprotein-β-Glucan Complex (CAWS). Microbiol Immunol [Internet]. 2003 Jul;47(7):479–90. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1348-0421.2003.tb03409.x

178. Murata H. Experimental Candida-Induced Arteritis in Mice. Microbiol Immunol. 1979 Sep;23(9):825–31.
179. Takahashi K, Oharaseki T, Wakayama M, Yokouchi Y, Naoe S, Murata H. Histopathological features of murine systemic vasculitis caused by Candida albicans extract？an animal model of Kawasaki Disease. Inflammation Research. 2004 Jan 1;53(2):72–7.
180. Oharaseki T, Yokouchi Y, Enomoto Y, Sato W, Ishibashi K, Miura N, et al. Recognition of alpha-mannan by dectin 2 is essential for onset of Kawasaki disease-like murine vasculitis induced by *Candida albicans* cell-wall polysaccharide. Mod Rheumatol. 2020 Mar 3;30(2):350–7.
181. Stock AT, Jama HA, Hansen JA, Wicks IP. TNF and IL-1 Play Essential but Temporally Distinct Roles in Driving Cardiac Inflammation in a Murine Model of Kawasaki Disease. The Journal of Immunology. 2019 Jun 1;202(11):3151–60.
182. Oharaseki T, Yokouchi Y, Yamada H, Mamada H, Muto S, Sadamoto K, et al. The role of TNF-α in a murine model of Kawasaki disease arteritis induced with a *Candida albicans* cell wall polysaccharide. Mod Rheumatol. 2014 Jan 22;24(1):120–8.
183. Hashimoto Y, Fukazawa R, Nagi-Miura N, Ohno N, Suzuki N, Katsube Y, et al. Interleukin-1beta Inhibition Attenuates Vasculitis in a Mouse Model of Kawasaki Disease. Journal of Nippon Medical School. 2019 Apr 26;86(2):108–16.
184. Martinez HG, Quinones MP, Jimenez F, Estrada C, Clark KM, Suzuki K, et al. Important role of CCR2 in a murine model of coronary vasculitis. BMC Immunol. 2012 Dec 17;13(1):56.
185. Miura NN, Komai M, Adachi Y, Osada N, Kameoka Y, Suzuki K, et al. IL-10 Is a Negative Regulatory Factor of CAWS-Vasculitis in CBA/J Mice as Assessed by Comparison with Bruton's Tyrosine Kinase-Deficient CBA/N Mice. The Journal of Immunology. 2009 Sep 1;183(5):3417–24.
186. Nakamura J, Watanabe S, Kimura H, Kobayashi M, Karasawa T, Kamata R, et al. Adeno-associated Virus Vector-mediated Interleukin-10 Induction Prevents Vascular Inflammation in a Murine Model of Kawasaki Disease. Sci Rep. 2018 May 15;8(1):7601.

187. Gatheral T, Reed DM, Moreno L, Gough PJ, Votta BJ, Sehon CA, et al. A Key Role for the Endothelium in NOD1 Mediated Vascular Inflammation: Comparison to TLR4 Responses. PLoS One. 2012 Aug 1;7(8):e42386.
188. Nishio H, Kanno S, Onoyama S, Ikeda K, Tanaka T, Kusuhara K, et al. Nod1 Ligands Induce Site-Specific Vascular Inflammation. Arterioscler Thromb Vasc Biol. 2011 May;31(5):1093–9.
189. Ohashi R, Fukazawa R, Watanabe M, Hashimoto K, Suzuki N, Nagi-Miura N, et al. Characterization of a murine model with arteritis induced by Nod1 ligand, FK565: A comparative study with a CAWS-induced model. Mod Rheumatol. 2017 Nov 2;27(6):1024–30.
190. Cartwright N, Murch O, McMaster SK, Paul-Clark MJ, van Heel DA, Ryffel B, et al. Selective NOD1 Agonists Cause Shock and Organ Injury/Dysfunction *In Vivo*. Am J Respir Crit Care Med. 2007 Mar 15;175(6):595–603.
191. Motomura Y, Kanno S, Asano K, Tanaka M, Hasegawa Y, Katagiri H, et al. Identification of Pathogenic Cardiac CD11c $^{+}$ Macrophages in Nod1-Mediated Acute Coronary Arteritis. Arterioscler Thromb Vasc Biol. 2015 Jun;35(6):1423–33.
192. Lehman TJ, Walker SM, Mahnovski V, McCurdy D. Coronary arteritis in mice following the systemic injection of group B Lactobacillus casei cell walls in aqueous suspension. Arthritis Rheum [Internet]. 1985 Jun;28(6):652–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3924060
193. Wakita D, Kurashima Y, Crother TR, Noval Rivas M, Lee Y, Chen S, et al. Role of Interleukin-1 Signaling in a Mouse Model of Kawasaki Disease–Associated Abdominal Aortic Aneurysm. Arterioscler Thromb Vasc Biol [Internet]. 2016 May;36(5):886–97. Available from: https://www.ahajournals.org/doi/10.1161/ATVBAHA.115.307072
194. Rosenkranz ME, Schulte DJ, Agle LMA, Wong MH, Zhang W, Ivashkiv L, et al. TLR2 and MyD88 Contribute to Lactobacillus casei Extract–Induced Focal Coronary Arteritis in a Mouse Model of Kawasaki Disease. Circulation [Internet]. 2005 Nov 8;112(19):2966–73. Available from: https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.105.537530

195. Hui-Yuen JS, Duong TT, Yeung RSM. TNF-α Is Necessary for Induction of Coronary Artery Inflammation and Aneurysm Formation in an Animal Model of Kawasaki Disease. The Journal of Immunology. 2006 May 15;176(10):6294–301.
196. Porritt RA, Markman JL, Maruyama D, Kocaturk B, Chen S, Lehman TJA, et al. Interleukin-1 Beta–Mediated Sex Differences in Kawasaki Disease Vasculitis Development and Response to Treatment. Arterioscler Thromb Vasc Biol. 2020 Mar;40(3):802–18.
197. Noval Rivas M, Lee Y, Wakita D, Chiba N, Dagvadorj J, Shimada K, et al. CD8+ T Cells Contribute to the Development of Coronary Arteritis in the *Lactobacillus casei* Cell Wall Extract–Induced Murine Model of Kawasaki Disease. Arthritis & Rheumatology. 2017 Feb 28;69(2):410–21.
198. Yilmaz A, Rowley A, Schulte DJ, Doherty TM, Schröder NWJ, Fishbein MC, et al. Activated myeloid dendritic cells accumulate and co-localize with CD3+ T cells in coronary artery lesions in patients with Kawasaki disease. Exp Mol Pathol. 2007 Aug;83(1):93–103.
199. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008 Jul 24;454(7203):428–35.
200. Matundan HH, Sin J, Rivas MN, Fishbein MC, Lehman TJ, Chen S, et al. Myocardial fibrosis after adrenergic stimulation as a long-term sequela in a mouse model of Kawasaki disease vasculitis. JCI Insight. 2019 Feb 7;4(3).
201. Scotece M, Conde-Aranda J. Inflammation in Health and Disease: New Insights and Therapeutic Avenues. Int J Mol Sci. 2022 Jul 29;23(15):8392.
202. Salmi M, Jalkanen S. Cell-surface enzymes in control of leukocyte trafficking. Nat Rev Immunol. 2005;5(10):760–71.
203. Haeggström JZ, Funk CD. Lipoxygenase and Leukotriene Pathways: Biochemistry, Biology, and Roles in Disease. Chem Rev. 2011 Oct 12;111(10):5866–98.
204. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, et al. Resolvins. J Exp Med [Internet]. 2002 Oct 21;196(8):1025–37. Available from: https://rupress.org/jem/article/196/8/1025/39533/Resolvins-A-Family-of-Bioactive-Products-of-Omega

205. Serhan CN, Chiang N, Dalli J. The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. Semin Immunol. 2015;27(3):200–15.
206. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. Journal of Clinical Investigation. 2018 Jul 2;128(7):2657–69.
207. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel Functional Sets of Lipid-Derived Mediators with Antiinflammatory Actions Generated from Omega-3 Fatty Acids via Cyclooxygenase 2–Nonsteroidal Antiinflammatory Drugs and Transcellular Processing. Journal of Experimental Medicine. 2000 Oct 16;192(8):1197–204.
208. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014 Jun 5;510(7503):92–101.
209. Clària J, Nguyen BT, Madenci AL, Ozaki CK, Serhan CN. Diversity of lipid mediators in human adipose tissue depots. American Journal of Physiology-Cell Physiology. 2013 Jun 15;304(12):C1141–9.
210. Serhan CN, Jain A, Marleau S, Clish C, Kantarci A, Behbehani B, et al. Reduced Inflammation and Tissue Damage in Transgenic Rabbits Overexpressing 15-Lipoxygenase and Endogenous Anti-inflammatory Lipid Mediators. The Journal of Immunology [Internet]. 2003;171(12):6856–65. Available from: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.171.12.6856
211. Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee CH, Yang R, et al. Resolvin D1 binds human phagocytes with evidence for proresolving receptors. Proceedings of the National Academy of Sciences. 2010 Jan 26;107(4):1660–5.
212. Hasturk H, Kantarci A, Ohira T, Arita M, Ebrahimi N, Chiang N, et al. RvE1 protects from local inflammation and osteoclastmediated bone destruction in periodontitis. The FASEB Journal. 2006 Feb 22;20(2):401–3.
213. Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN. Resolvin E1 Selectively Interacts with Leukotriene B4 Receptor BLT1 and ChemR23 to Regulate Inflammation. The Journal of Immunology. 2007 Mar 15;178(6):3912–7.

214. Oh SF, Dona M, Fredman G, Krishnamoorthy S, Irimia D, Serhan CN. Resolvin E2 Formation and Impact in Inflammation Resolution. The Journal of Immunology. 2012 May 1;188(9):4527–34.
215. Arnardottir H, Thul S, Pawelzik SC, Karadimou G, Artiach G, Gallina AL, et al. The resolvin D1 receptor GPR32 transduces inflammation resolution and atheroprotection. Journal of Clinical Investigation. 2021 Dec 15;131(24).
216. Norling L V., Dalli J, Flower RJ, Serhan CN, Perretti M. Resolvin D1 Limits Polymorphonuclear Leukocyte Recruitment to Inflammatory Loci. Arterioscler Thromb Vasc Biol. 2012 Aug;32(8):1970–8.
217. Dalli J, Winkler JW, Colas RA, Arnardottir H, Cheng CYC, Chiang N, et al. Resolvin D3 and Aspirin-Triggered Resolvin D3 Are Potent Immunoresolvents. Chem Biol. 2013 Feb;20(2):188–201.
218. Chiang N, Fredman G, Bäckhed F, Oh SF, Vickery T, Schmidt BA, et al. Infection regulates pro-resolving mediators that lower antibiotic requirements. Nature. 2012 Apr 25;484(7395):524–8.
219. Chiang N, Libreros S, Norris PC, de la Rosa X, Serhan CN. Maresin 1 activates LGR6 receptor promoting phagocyte immunoresolvent functions. Journal of Clinical Investigation. 2019 Oct 28;129(12):5294–311.
220. Park CK. Maresin 1 Inhibits TRPV1 in Temporomandibular Joint-Related Trigeminal Nociceptive Neurons and TMJ Inflammation-Induced Synaptic Plasticity in the Trigeminal Nucleus. Mediators Inflamm. 2015;2015:1–9.
221. Han YH, Shin KO, Kim JY, Khadka DB, Kim HJ, Lee YM, et al. A maresin 1/RORα/12-lipoxygenase autoregulatory circuit prevents inflammation and progression of nonalcoholic steatohepatitis. Journal of Clinical Investigation. 2019 Mar 11;129(4):1684–98.
222. Bang S, Xie YK, Zhang ZJ, Wang Z, Xu ZZ, Ji RR. GPR37 regulates macrophage phagocytosis and resolution of inflammatory pain. Journal of Clinical Investigation. 2018 Aug 1;128(8):3568–82.
223. Fredman G, Spite M. Specialized pro-resolving mediators in cardiovascular diseases. Mol Aspects Med. 2017 Dec;58:65–71.

224. Fredman G, Hellmann J, Proto JD, Kuriakose G, Colas RA, Dorweiler B, et al. An imbalance between specialized pro-resolving lipid mediators and pro-inflammatory leukotrienes promotes instability of atherosclerotic plaques. Nat Commun [Internet]. 2016;7:1–11. Available from: http://dx.doi.org/10.1038/ncomms12859
225. Elder CT, Filiberto AC, Su G, Ladd Z, Leroy V, Pruitt EY, et al. Maresin 1 activates LGR6 signaling to inhibit smooth muscle cell activation and attenuate murine abdominal aortic aneurysm formation. The FASEB Journal. 2021 Aug 28;35(8).
226. Pope NH, Salmon M, Davis JP, Chatterjee A, Su G, Conte MS, et al. D-series resolvins inhibit murine abdominal aortic aneurysm formation and increase M2 macrophage polarization. The FASEB Journal. 2016 Dec 12;30(12):4192–201.
227. Sun YP, Oh SF, Uddin J, Yang R, Gotlinger K, Campbell E, et al. Resolvin D1 and Its Aspirin-triggered 17R Epimer. Journal of Biological Chemistry. 2007 Mar;282(13):9323–34.
228. Yellepeddi VK, Parashar K, Dean SM, Watt KM, Constance JE, Baker OJ. Predicting Resolvin D1 Pharmacokinetics in Humans with Physiologically-Based Pharmacokinetic Modeling. Clin Transl Sci. 2021 Mar 30;14(2):683–91.
229. Clish CB, O'Brien JA, Gronert K, Stahl GL, Petasis NA, Serhan CN. Local and systemic delivery of a stable aspirin-triggered lipoxin prevents neutrophil recruitment *in vivo*. Proceedings of the National Academy of Sciences. 1999 Jul 6;96(14):8247–52.
230. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association Between Omega-3 Fatty Acid Supplementation and Risk of Major Cardiovascular Disease Events. JAMA. 2012 Sep 12;308(10):1024.
231. Myung SK. Efficacy of Omega-3 Fatty Acid Supplements (Eicosapentaenoic Acid and Docosahexaenoic Acid) in the Secondary Prevention of Cardiovascular Disease. Arch Intern Med. 2012 May 14;172(9):686.
232. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Lancet. 1999 Aug 7;354(9177):447–55.

233. Salic K, Morrison MC, Verschuren L, Wielinga PY, Wu L, Kleemann R, et al. Resolvin E1 attenuates atherosclerosis in absence of cholesterol-lowering effects and on top of atorvastatin. Atherosclerosis. 2016 Jul;250:158–65.

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