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A novel mouse model of coronary stenosis mimicking Kawasaki disease induced by *Lactobacillus casei* cell wall extract

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Abstract: Kawasaki disease (KD), a febrile systemic vasculitis in infants associated with coronary aneurysm, is a major cause of cardiac sequelae such as myocardial infarction (MI) and sudden death. These events are caused by coronary stenosis due to intimal proliferation or thrombotic formation; however, histological evaluation is limited to autopsy cases of human KD. We therefore investigated the histological features of coronary artery (CA) stenosis in mice induced by *Lactobacillus casei* cell wall extract (LCWE). LCWE-induced coronary inflammation gradually progressed in a time-dependent manner and expanded to all layers of the vessel wall over 28 days. In addition, frequent elastin degradation was observed and abundant α-smooth muscle actin (SMA)-positive vascular smooth muscle cells (VSMCs) infiltrated into the intima. Furthermore, most VSMCs were positive for proliferating cell nuclear antigen (PCNA) following staining, suggesting that VSMCs likely exhibited a proliferative phenotype. In conclusion, we show a novel mouse model of coronary stenosis induced by LCWE that is characterized by coronary stenosis with severe coronary vasculitis and elastin degradation. In addition, VSMC proliferation plays an important role in the formation of coronary stenosis. This model is an appropriate model of KD coronary stenosis.
Key words: coronary stenosis, elastin degradation, intimal proliferation, Kawasaki disease, vascular smooth muscle cell

Introduction

Kawasaki disease (KD), an acute systemic vasculitis that mainly affects young children [6], was first reported by Dr. Tomisaku Kawasaki in 1967 [7]. Approximately 25% of untreated patients with KD who develop coronary arterial aneurysm (CAA) are at risk for cardiac events such as myocardial infarction (MI) and sudden death [6]. Although multitarget anti-inflammatory agents, including readministered intravenous immunoglobulin (IVIg) [26], steroids [8], infliximab [23] and cyclosporin A [5], are currently available, the incidence of coronary artery (CA) involvement is still 2.3% at 1 month after the onset of KD [13]. In a nationwide survey of patients with giant coronary aneurysm secondary to KD, the incidence of MI and the mortality rate were 18% and 3.4%, respectively [3]. Although MI is assumed to cause luminal stenosis due to neointimal and/or thrombus formation, the underlying mechanism of coronary stenosis is not well understood.

A study by Orenstein *et al.* showed that the following three linked processes are responsible for KD vasculopathy: necrotizing arteritis, subacute chronic vasculitis, and luminal myofibroblastic proliferation (LMP). In particular, the third process, LMP, involves smooth muscle-derived myofibroblasts and their associated matrix products deposited in a concentric mass within the vessel wall. Activated myofibroblasts can persist and

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lead to progressive coronary stenosis [21]. Thus, LMP plays a crucial role in progressive coronary stenosis followed by cardiac sequelae.

Several mouse models of coronary arteritis have been reported. Coronary arteritis was induced in these mouse models through the intraperitoneal injection of *Candida albicans* water-soluble fractions (CAWS) [17, 20] or *Lactobacillus casei* cell wall extract (LCWE) [9, 10, 12, 24] and the subcutaneous injection or oral administration of nucleotide-binding oligomerization domain-containing protein 1 (NOD1) ligands [15, 16]. However, an experimental model focusing on CA stenosis has not been established because there is no suitable animal model that histologically resembles human CA stenosis. Herein, we show a novel mouse model of coronary stenosis mimicking KD induced by LCWE.

Materials and Methods

Mice and experimental protocol

Wild-type (C57BL/6J background) male mice at 4 weeks of age were purchased from CLEA Japan (Tokyo, Japan) and kept in a 12 h light/12 h dark environment under specific pathogen-free conditions. The animal care and experimental procedures were in accordance with Saitama Children's Medical Center animal care facility guidelines and approved by the Animal Experimental Committee of Saitama Children's Medical Center (Saitama, Japan). The mice were sacrificed on days 3, 7, 14 and 28 after the intraperitoneal injection of LCWE (n=4–6 in each group) or PBS (n=3 in each group). Cardiac tissue was harvested for histological assessment.

LCWE preparation

LCWE (ATCC 11578; American Type Culture Collection, Manassas, VA) was prepared as previously described [24]. Briefly, *L. casei* was cultured in MRS broth (BD Difco, Franklin Lakes, NJ, USA) for 48 h at 37°C, harvested and washed with PBS. The cells were disrupted in 2 packed volumes of 4% sodium dodecyl sulfate (SDS) overnight at room temperature. Cell wall fragments were extensively washed 8 to 10 times with PBS to remove any residual SDS. The SDS-treated cell wall fragments were sonicated (5 g of packed wet weight in 15 ml of PBS) for 2 h using a Q500 sonicator with a 1/2" diameter probe at a setting of 60 to 70% amplitude (QSONICA, LLC, Newtown, CT, USA). During sonication, the cell wall fragments were maintained by cooling in a dry ice/ethanol bath. The supernatant was centrifuged for 1 h at 20,000 ×g at 4°C, and the supernatant containing the cell wall extract was administered to the mice. The concentration of LCWE dissolved in PBS was determined based on the rhamnose content, which was measured by a colorimetric phenol-sulfuric assay and adjusted to 1,000 μg/0.1 ml of LCWE. To induce aortic-coronary inflammation in mice, 1,000 μg of LCWE preparation was intraperitoneally injected per mouse.

Histology

At the time of sacrifice, blood was collected by puncture of the left ventricles of mice under isoflurane anesthesia. In order to remove circulating blood from the mice, the mice were slowly perfused with 5ml PBS through the left ventricle. Upper cardiac tissue containing the bilateral CAs of the mice was fixed with 4% paraformaldehyde and embedded in paraffin. Then, 2.5-μm sections of cardiac tissue were stained with hematoxylin and eosin (H&E) and Elastica van Gieson (EVG).

Histological analysis of murine cardiac tissue

For histological analysis, we selected 5 consecutive sections slightly distal to the bifurcation of the bilateral CAs. The intensity of coronary arteritis was scored with the following four stages: 0, no inflammation; 1, inflammatory cells in only the intima; 2, inflammatory cells in both the intima and adventitia; 3, panvasculitis as previously described [27]. The intensity of CA inflammation is expressed as the total score from 5 sections per individual animal. Elastin breaks were defined as interruptions in the elastin fiber together with reappearance of the fiber and are expressed as the number of elastin breaks per mm^2 of medial area as described previously [25].

Immunohistochemistry

Paraffinized sections (2.5 μm-thick) from LCWE-injected mice were stained with the following antibodies: anti-mouse CD3 (1:200, monoclonal mouse anti-human CD3 clone F7.2.38, Dako, Santa Clara, CA, USA), anti-CD20 (1:400, L26 monoclonal mouse antibody, Nichirei Bioscience, Inc., Tokyo, Japan), anti-CD68 (1:500, monoclonal mouse anti-human CD68 clone PG-M1, Dako), anti-CD163 (1:2000, anti-CD163 antibody, Abcam, Cambridge, UK), α-smooth muscle actin (SMA, 1:1000, SMA clone 1A4, Dako), and anti-PCNA (1:800, anti-PCNA antibody, Abcam). In each experiment, negative controls without the primary antibody were included and showed no staining.

Quantification of coronary stenosis

The intimal area (μm^2), medial area (μm^2), and luminal area (μm^2) of each CA were measured with NIS-Elements AR Ver5.11.00 (Nikon Instruments, Inc, Tokyo, Japan). Coronary stenosis was expressed as %

stenosis=100×[intimal area/(luminal area + intimal area)]. The I/M ratio was expressed as the intimal area (μm^2)/medial area (μm^2). The final score was the total of the scores of 5 sections per mouse.

Statistical analysis

All values are presented as the mean ± SEM. Statistically differences between mean values were determined using a two-tailed Mann-Whitney test (*P<0.05, **P<0.001). Differences in which P<0.05 were considered statistically significant. IBM SPSS Statistics for Windows Version 24.0 (SPSS Japan, Tokyo, Japan) was used to analyze the data.

Results

Inflammation of the aorta and CA

We first examined pathological changes in cardiac cross-sections including the CAs of LCWE-injected mice at 28 days after injection. Although none of the control mice injected with PBS developed any histological changes (Fig. 1a), extensive inflammation of the CA and aortic wall was observed in mice that received a single injection of LCWE (Fig. 1b). Next, we focused on the time course of coronary arteritis and examined histological changes at days 3, 7, 14 and 28 after LCWE administration (Fig. 2a). There were no histological changes at 3 days after LCWE injection. However, inflammatory cells infiltrated only the adventitial side of the CA. The inflammation further extended in the intima and adventitia of the CA at 14 days after LCWE injection. Furthermore, on day 28, mice injected with LCWE exhibited extensive coronary vasculitis and frequent interruption of elastic fibers in the tunica media. The coronary inflammatory score gradually increased in a time-dependent manner in mice injected with LCWE, whereas this increase in the score was not observed in the control mice (Fig. 2b, left panel). Similarly, the number of elastin breaks in LCWE-treated mice increased rapidly with time, but only a slight increase in elastin breaks was detected in the control mice (Fig. 2b, right panel). In the current experimental protocol, no evidence of aneurysm formation was observed in LCWE-injected mice.

Coronary stenosis

In addition to the progression of coronary arteritis, mice injected with LCWE exhibited intimal proliferation at 14 days after injection. Severe luminal stenosis of the CA was observed on day 28. Furthermore, thickened intima, namely, “neointima”, consisting mainly of α-SMA-positive vascular smooth muscle cells (VSMCs) was histologically observed (Fig. 3d). In addition, little positive staining cells for CD3, CD20 and CD68 in the neointima was observed, and CD163-positive cells were not observed (Figs. 4a–d). The incidence of coronary stenosis on days 14 and 28 was 20% (1/5) and 67% (4/6), respectively (Table 1). α-SMA-positive spindle-shaped cells (myofibroblasts) were present in the intima and adventitia (arrows in Fig. 5a–d) but not in the CA media. Notably, most of these observed cells stained positive for PCNA (Fig. 5e–f), suggesting the VSMCs likely exhibited a proliferative rather than a contractile phenotype.

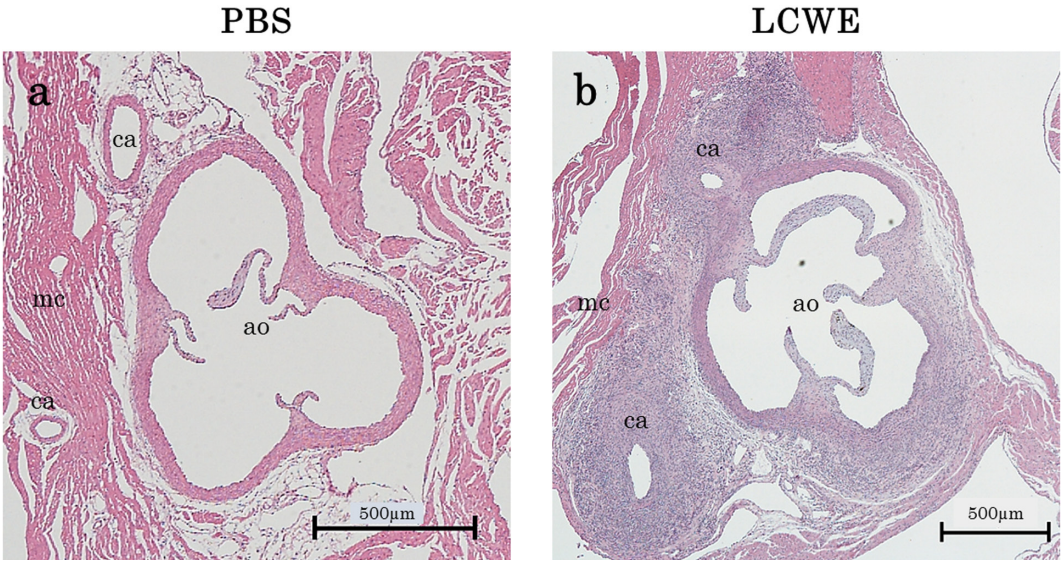

Fig. 1. Aortitis and coronary arteritis. Representative photographs showing the cross-section of an aortic lesion containing the bilateral coronary arteries from mice 28 days after the administration of PBS (a) and LCWE (b). Hematoxylin & eosin (H&E) staining. ao, aorta; ca, coronary artery; mc, myocardium

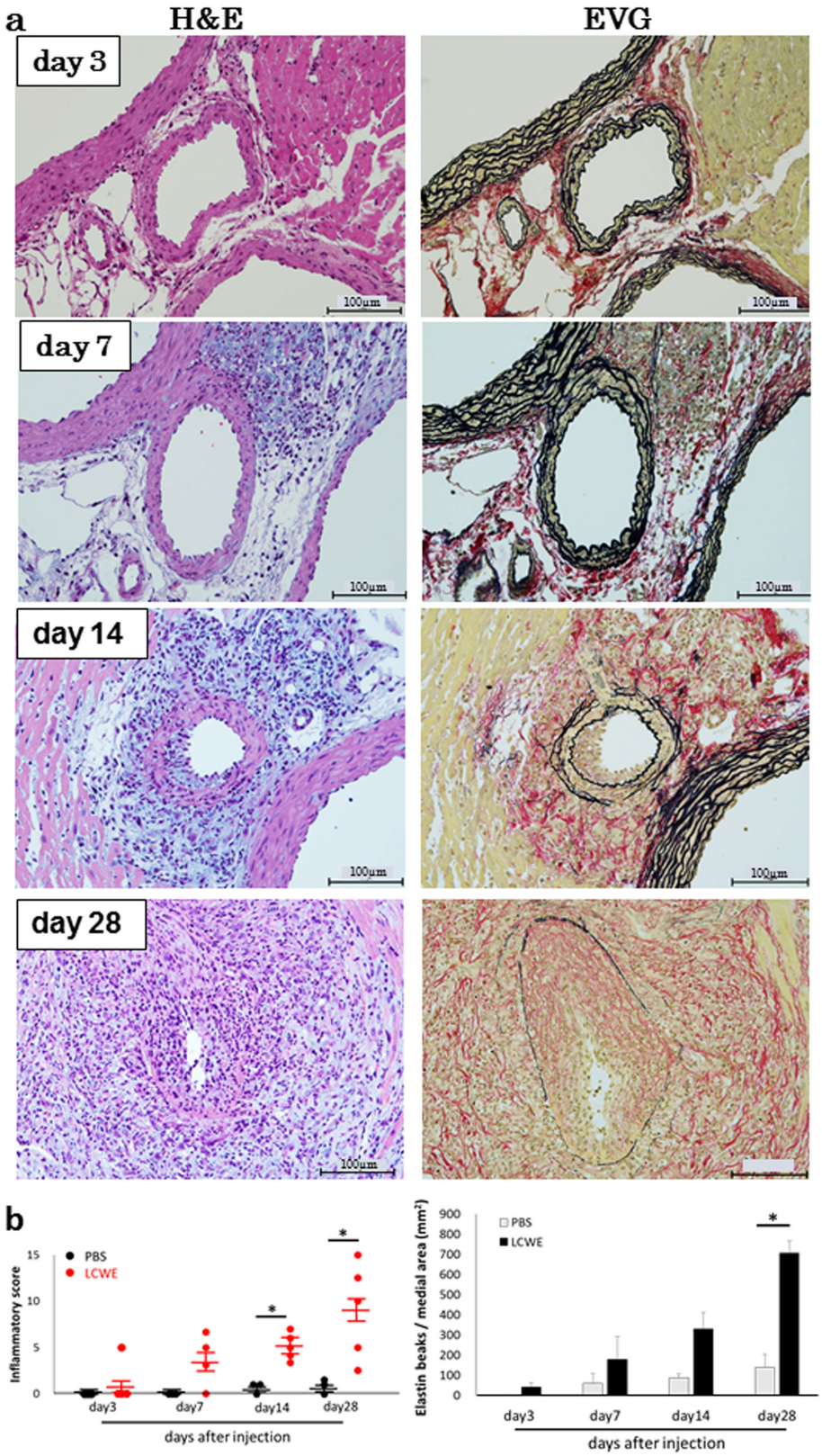

Fig. 2. Time course of LCWE-induced coronary arteritis and elastin degradation. a. Representative photographs showing H&E and Elastica van Gieson (EVG) staining of the mouse CA at 3, 7, 14 and 28 days after LCWE injection. b. The inflammatory score indicates the severity of coronary arteritis. 0, no inflammation; 1, inflammatory cells limited to only the intima; 2, inflammatory cells localized to the intima and adventitia; 3, panvasculitis. The inflammatory score is expressed as the total scores of 5 sections per individual animal (left panel). Elastin breaks are defined as interruptions in the elastin fiber together with reappearance of the fiber and expressed as the number of elastin breaks per mm^2 of medial area (right panel). Representative graphs compering the LCWE- and PBS-treated groups at various time points. * indicates a significant difference from control mice ($P<0.05$, Mann-Whitney test).

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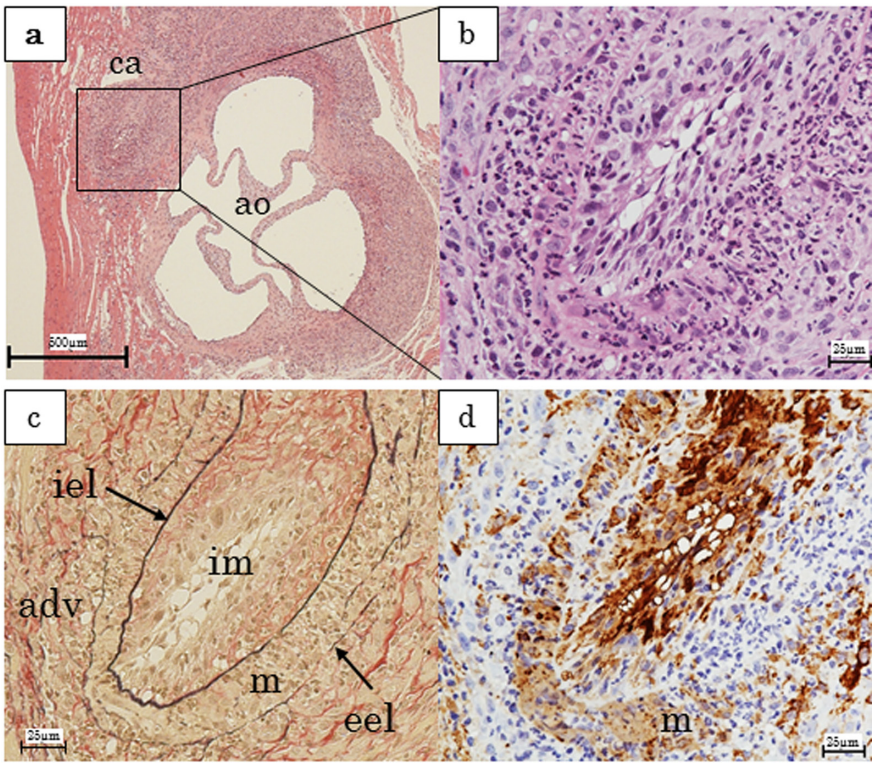

Fig. 3. Immunohistochemical analysis of coronary arteritis and stenosis. Coronary arteries from mice injected with LCWE exhibited coronary stenosis and severe inflammatory cell infiltration into all layers of the vessel wall. Microphotographs showing H&E staining (a: low magnification, b: high magnification), EVG staining (c), and α-SMA (d). The α-SMA-positive cells (brown) strongly infiltrated the intima (d). ca, coronary artery; ao, aorta; im, intima; m, media; adv, adventitia; iel, internal elastic lamina; eel, external elastic lamina.

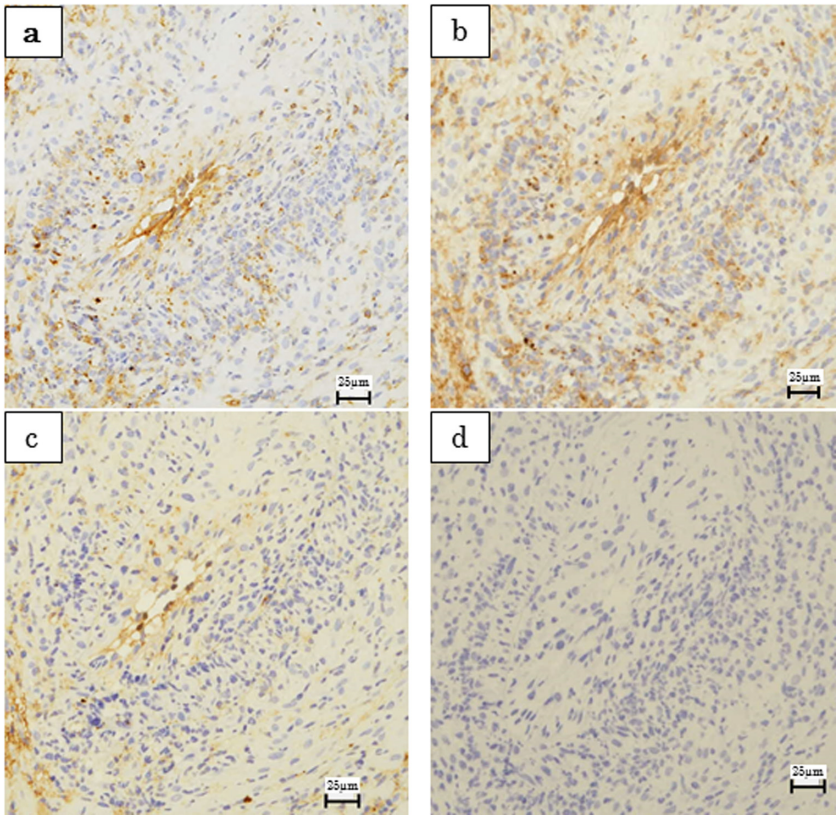

Fig. 4. Immunohistochemical staining for CD3 (a), CD20 (b), CD68 (c) and CD163 (d) in the thickened intima of CAs. Cells positive for all stains are indicated by a brown color.

Table 1. Time-dependent stenotic changes in coronary arteries

	Incidence of neointima % (n)	% stenosis	I/M ratio
Day 3	0 (0/5)	0	0
Day 7	0 (0/4)	0	0
Day 14	20 (1/5)	2.8 ± 2.3	0.02 ± 0.01
Day 28	67 (4/6)	35 ± 14	0.17 ± 0.1

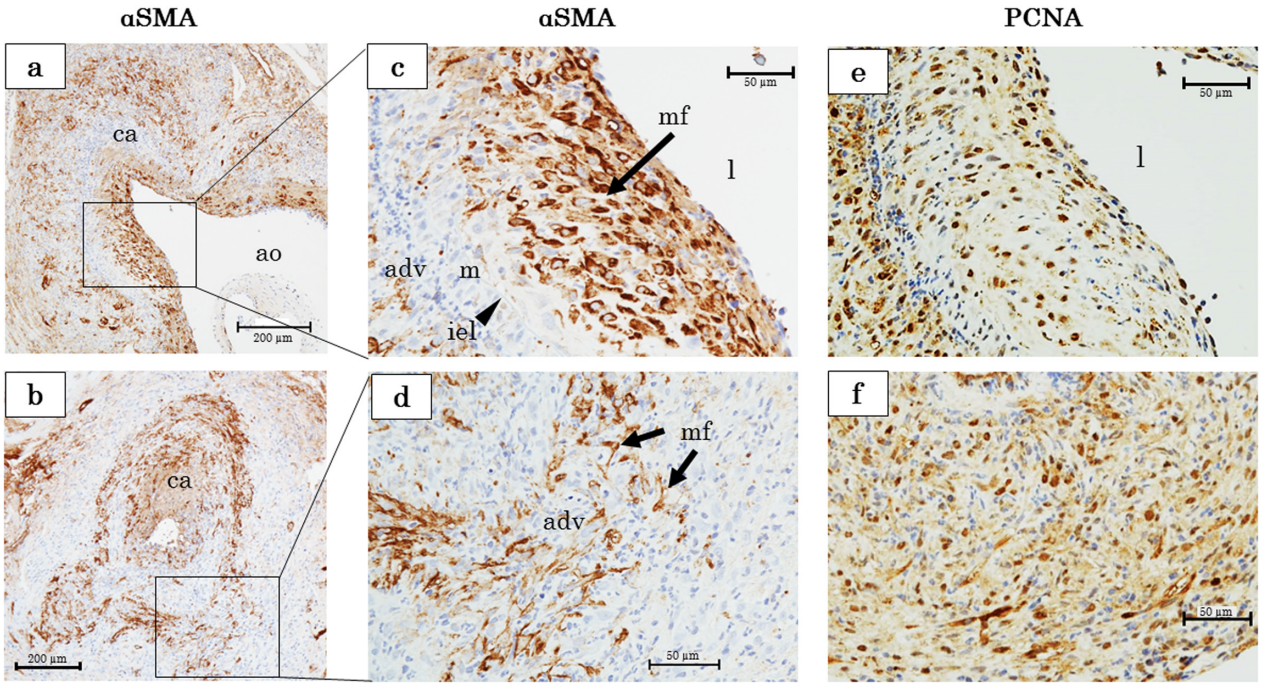

Fig. 5. Coronary stenosis and luminal myofibroblastic proliferation (LMP). The thickened intima consists of mainly α-SMA-positive spindle-shaped cells (myofibroblasts) at 28 days after LCWE-injection (a-d). Myofibroblastic proliferation observed in both the intima and adventitia of CAs (c, d). Most of these cells are PCNA-positive (e,f). Cells positive for all stains are indicated by a brown color. ca, coronary artery; ao, aorta; m, media; adv, adventitia; mf, myofibroblast; l, lumen.

Discussion

In this study, we show a mouse coronary stenosis model induced by LCWE. The pathological changes of this model were characterized by severe coronary vasculitis along with intimal proliferation, leading to coronary stenosis. Recently, Fujii *et al.* reported a rabbit model of coronary vasculitis induced by the administration of horse serum. In this model of coronary vasculitis, coronary arteritis was followed by intimal thickening via the accumulation of proteoglycans and the proliferation of VSMCs [2]. According to previous reports [10], intimal proliferation often caused the narrowing of the vascular lumen of the CA that was observed at least 56 days after LCWE-injection. Therefore, coronary stenosis may have been previously induced by LCWE injection. However, the frequency and intensity of CA stenosis and its pathological features were not fully understood. Our present study clearly and specifically focused on the pathology of coronary arteritis and coronary stenosis. To the best of our knowledge, our study is the first to focus on coronary stenosis in mice, which was characterized by coronary stenosis, severe coronary vasculitis and elastin degradation, histological features similar to those found in KD patients with coronary aneurysm [21, 28]. However, there are several differences between human KD and mouse vasculitis. In this mouse model, all processes occurred simultaneously and at a relatively early stage of illness, whereas human coronary arteritis usually begins 6 to 8 days after the onset of KD and persists for up to 25 days. After inflammation of the CAs has been alleviated, luminal stenosis due to intimal thickening may occur in late-stage KD [28]. Furthermore, none of the LCWE-injected mice developed thrombotic occlusions in the coronary vessel, which are often observed in patients with KD (data not shown). Therefore, there may be species-dependent differences in the structure of the vessel wall or coagulation between humans and rodents.

Table 2 describes three types of KD vasculitis models,

Table 2. Comparison of three KD vasculitis mouse models

	administration	inflammed artery (pathological findings)	immunological involvement	related cytokine	references
CAWS *Candida albicans* water-soluble fraction	i.p (5 days)	coronary, aorta renal, common iliac	dectin-2, TLR4, CCR2	TNF-α, IL-10 CCL2, IL-1β	[14, 17–19, 29]
synthetic NOD1 ligand (FK565)	oral/s.c (2 days)	coronary	NOD1, $CD11c^+$ macrophage vascular tissue	IL-6, IL-8 CCL2, IL-1β	[15, 16]
LCWE *Lactobacillus casei* cell wall extract	i.p (single)	coronary, aorta (epicarditis, myocarditis AAA, coronary stenosis)	macrophage, TLR2/MyD88 dectin-1/syk	IL-1β, TNF-α IL-6	[9, 10, 12, 22, 24]

i.p., intraperitoneal; s.c., subcutaneous; AAA, abdominal aortic aneurysm; TLR, Toll-like receptor; CCR2, C-C chemokine receptor type 2, NOD1, nucleotide-binding oligomerization domain-containing protein 1.

comparing the characteristics of each mouse model. Histopathological findings showed that CAWS- and LCWE-induced vasculitis primarily caused CA and aorta as well as small and medium arteritis, epicarditis and myocarditis [10, 17, 24, 29]. On the other hand, nucleotide-binding oligomerization domain-containing protein 1 (NOD1) ligand-induced vasculitis was characterized by inflammation specific to the CAs [16]. All models were thought to be involved in pathways mediated by innate immunity such as dectin-2, NOD1 and Toll-like receptor 2 (TLR2)/MyD88 [15, 16, 18, 22]. In addition, similar inflammatory cytokines (IL-1β, IL-6 and TNF-α) played an important role in the development of vasculitis in each type of model [9, 12, 14, 15, 19]. However, the only model among the three previous reported models that showed coronary stenosis was the LCWE-induced model [10].

Recently, the VSMC phenotype was demonstrated to be important in deterioration during other cardiovascular diseases such as atherosclerosis [1]. More specifically, phenotypic switching in VSMCs directly promotes atherosclerosis via cell proliferation, migration, and the secretion of various extracellular matrix (ECM) proteins and cytokines. In addition, processes during ECM degradation such as elastin disruption also promote phenotypic switching and facilitate both cell proliferation and migration [4, 11, 25]. Consequently, it is speculated that switching of the SMC phenotype promotes sustained inflammation and destruction of the arterial wall structure. Similarly, in autopsy patients with KD, LMP was shown to cause progressive arterial stenosis and thrombosis, and unique SMC-derived pathogenic myofibroblasts was observed [21]. In our mouse model, a large number of VSMCs located in the intima were PCNA-positive and spindle-shaped, suggesting that the VSMC phenotype had switched to a proliferative phenotype. Thus, we believe that VSMC proliferation and elastin degradation played an important role in the development of CA stenosis in our mouse model.

This study had some limitations. The first limitation was the relatively short duration of the experimental period. We focused on histological changes in the CA for up to only 28 days after LCWE administration. In addition, thus far, we have not been able to observe coronary aneurysm in this mouse model. Therefore, the coronary arteritis and stenosis of this mouse model are pathologically different from coronary stenosis after coronary aneurysm seen in human autopsy patients with KD. The second limitation was that we were unable to elucidate the underlying mechanism by which LCWE affects VSMC phenotypic modulation and the progression of coronary stenosis. Future prospective research will require in vitro experiments to clarify changes in the SMC phenotype and the associated cytokines and chemokines. The third limitation was a change in pathological characteristics that depended on the LCWE concentration. In a previous study, the LCWE dose was adjusted to 20 mg/kg (approximately 300 μg per mouse) [10]. However, in current study, LCWE was administered at a 3-fold higher dose (1,000 μg per mouse) to induce more vasculitis. Interestingly, epicarditis and myocarditis were observed at low doses and were not observed in the high dose group. Finally we were able to obtain the pathological stenosis of the CA 28 days after LCWE injection. In addition, in a previous study reported by Lehman *et al*. [10], significant stenosis of inflamed coronary vessels due to intimal proliferation was observed 56 days after LCWE injection when the LCWE concentration was adjusted to 500 μg per mouse. Therefore, it is necessary to clarify the pathological changes caused by the concentration of LCWE in the future. The present study is novel because in addition to coronary vasculitis, it reproduced CA stenosis with pathology that closely resembled the pathology of KD in humans. Additionally, the establishment of this model will be useful for the investigation of the mechanism of coronary stenosis as well as for development of a new drug to prevent coronary stenosis.

Conclusion

We have described the pathological features of a mouse model of coronary stenosis induced by LCWE. The pathological changes in this model were characterized by coronary stenosis with severe coronary vasculitis and elastin degradation. In addition, LMP was shown to play an important role in the formation of coronary stenosis. This model is an appropriate model of KD coronary stenosis.

Acknowledgments

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References

1. Bennett, M.R., Sinha, S. and Owens, G.K. 2016. Vascular Smooth Muscle Cells in Atherosclerosis. *Circ. Res.* 118: 692–702. [Medline] [CrossRef]
2. Fujii, M., Tanaka, H., Nakamura, A., Suzuki, C., Harada, Y., Takamatsu, T. and Hamaoka, K. 2016. Histopathological Characteristics of Post-inflamed Coronary Arteries in Kawasaki Disease-like Vasculitis of Rabbits. *Acta Histochem. Cytochem.* 49: 29–36. [Medline] [CrossRef]
3. Fukazawa, R., Kobayashi, T., Mikami, M., Saji, T., Hamaoka, K., Kato, H., Suzuki, H., Tsuda, E., Ayusawa, M., Miura, M., Ebata, R., Kobayashi, T., Yashiro, M. and Ogawa, S. 2017. Nationwide Survey of Patients With Giant Coronary Aneurysm Secondary to Kawasaki Disease 1999-2010 in Japan. *Circ. J.* 82: 239–246. [Medline] [CrossRef]
4. Fukumoto, Y., Deguchi, J.O., Libby, P., Rabkin-Aikawa, E., Sakata, Y., Chin, M.T., Hill, C.C., Lawler, P.R., Varo, N., Schoen, F.J., Krane, S.M. and Aikawa, M. 2004. Genetically determined resistance to collagenase action augments interstitial collagen accumulation in atherosclerotic plaques. *Circulation* 110: 1953–1959. [Medline] [CrossRef]
5. Hamada, H., Suzuki, H., Onouchi, Y., Ebata, R., Terai, M., Fuse, S., Okajima, Y., Kurotobi, S., Hirai, K., Soga, T., Ishiguchi, Y., Okuma, Y., Takada, N., Yanai, M., Sato, J., Nakayashiro, M., Ayusawa, M., Yamamoto, E., Nomura, Y., Hashimura, Y., Ouchi, K., Masuda, H., Takatsuki, S., Hirono, K., Ariga, T., Higaki, T., Otsuki, A., Terauchi, M., Aoyagi, R., Sato, T., Fujii, Y., Fujiwara, T., Hanaoka, H., Hata, A., KAICA trial Investigators 2019. Efficacy of primary treatment with immunoglobulin plus ciclosporin for prevention of coronary artery abnormalities in patients with Kawasaki disease predicted to be at increased risk of non-response to intravenous immunoglobulin (KAICA): a randomised controlled, open-label, blinded-endpoints, phase 3 trial. *Lancet* 393: 1128–1137. [Medline] [CrossRef]
6. Kato, H., Sugimura, T., Akagi, T., Sato, N., Hashino, K., Maeno, Y., Kazue, T., Eto, G. and Yamakawa, R. 1996. Long-term consequences of Kawasaki disease. A 10- to 21-year follow-up study of 594 patients. *Circulation* 94: 1379–1385. [Medline] [CrossRef]
7. Kawasaki, T. 1967. [Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children]. (in Japanese). *Arerugi* 16: 178–222. [Medline]
8. Kobayashi, T., Saji, T., Otani, T., Takeuchi, K., Nakamura, T., Arakawa, H., Kato, T., Hara, T., Hamaoka, K., Ogawa, S., Miura, M., Nomura, Y., Fuse, S., Ichida, F., Seki, M., Fukazawa, R., Ogawa, C., Furuno, K., Tokunaga, H., Takatsuki, S., Hara, S., Morikawa, A., RAISE study group investigators 2012. Efficacy of immunoglobulin plus prednisolone for prevention of coronary artery abnormalities in severe Kawasaki disease (RAISE study): a randomised, open-label, blinded-endpoints trial. *Lancet* 379: 1613–1620. [Medline] [CrossRef]
9. Lee, Y., Schulte, D.J., Shimada, K., Chen, S., Crother, T.R., Chiba, N., Fishbein, M.C., Lehman, T.J. and Arditi, M. 2012. Interleukin-1β is crucial for the induction of coronary artery inflammation in a mouse model of Kawasaki disease. *Circulation* 125: 1542–1550. [Medline] [CrossRef]
10. Lehman, T.J., Walker, S.M., Mahnovski, V. and McCurdy, D. 1985. Coronary arteritis in mice following the systemic injection of group B *Lactobacillus casei* cell walls in aqueous suspension. *Arthritis Rheum.* 28: 652–659. [Medline] [CrossRef]
11. Li, D.Y., Brooke, B., Davis, E.C., Mecham, R.P., Sorensen, L.K., Boak, B.B., Eichwald, E. and Keating, M.T. 1998. Elastin is an essential determinant of arterial morphogenesis. *Nature* 393: 276–280. [Medline] [CrossRef]
12. Lin, I.C., Suen, J.L., Huang, S.K., Huang, S.C., Huang, H.C., Kuo, H.C., Wei, C.C., Wang, F.S., Yu, H.R. and Yang, K.D. 2013. Dectin-1/Syk signaling is involved in *Lactobacillus casei* cell wall extract-induced mouse model of Kawasaki disease. *Immunobiology* 218: 201–212. [Medline] [CrossRef]
13. Makino, N., Nakamura, Y., Yashiro, M., Kosami, K., Matsubara, Y., Ae, R., Aoyama, Y. and Yanagawa, H. 2019. Nationwide epidemiologic survey of Kawasaki disease in Japan, 2015-2016. *Pediatr. Int. (Roma)* 61: 397–403. [Medline] [CrossRef]
14. Martinez, H.G., Quinones, M.P., Jimenez, F., Estrada, C., Clark, K.M., Suzuki, K., Miura, N., Ohno, N., Ahuja, S.K. and Ahuja, S.S. 2012. Important role of CCR2 in a murine model of coronary vasculitis. *BMC Immunol.* 13: 56. [Medline] [CrossRef]
15. Motomura, Y., Kanno, S., Asano, K., Tanaka, M., Hasegawa, Y., Katagiri, H., Saito, T., Hara, H., Nishio, H., Hara, T. and Yamasaki, S. 2015. Identification of Pathogenic Cardiac CD11c+ Macrophages in Nod1-Mediated Acute Coronary Arteritis. *Arterioscler. Thromb. Vasc. Biol.* 35: 1423–1433. [Medline] [CrossRef]
16. Nishio, H., Kanno, S., Onoyama, S., Ikeda, K., Tanaka, T., Kusuhara, K., Fujimoto, Y., Fukase, K., Sueishi, K. and Hara, T. 2011. Nod1 ligands induce site-specific vascular inflammation. *Arterioscler. Thromb. Vasc. Biol.* 31: 1093–1099. [Medline] [CrossRef]
17. Murata, H. 1979. Experimental candida-induced arteritis in mice. Relation to arteritis in the mucocutaneous lymph node syndrome. *Microbiol. Immunol.* 23: 825–831. [Medline] [CrossRef]
18. Oharaseki, T., Yokouchi, Y., Enomoto, Y., Sato, W., Ishibashi, K., Miura, N., Ohno, N. and Takahashi, K. 2019. 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.* 29: 1–8. [Medline] [CrossRef]
19. Oharaseki, T., Yokouchi, Y., Yamada, H., Mamada, H., Muto, S., Sadamoto, K., Miura, N., Ohno, N., Saji, T., Naoe, S. and Takahashi, K. 2014. The role of TNF-α in a murine model of Kawasaki disease arteritis induced with a Candida albicans cell wall polysaccharide. *Mod. Rheumatol.* 24: 120–128. [Medline] [CrossRef]
20. Ohno, N. 2008. A murine model of vasculitis induced by fungal polysaccharide. *Cardiovasc. Hematol. Agents Med. Chem.* 6: 44–52. [Medline] [CrossRef]
21. Orenstein, J.M., Shulman, S.T., Fox, L.M., Baker, S.C., Takahashi, M., Bhatti, T.R., Russo, P.A., Mierau, G.W., de

Chadarévian, J.P., Perlman, E.J., Trevenen, C., Rotta, A.T., Kalelkar, M.B. and Rowley, A.H. 2012. Three linked vasculopathic processes characterize Kawasaki disease: a light and transmission electron microscopic study. *PLoS One* 7: e38998. [Medline] [CrossRef]
22. Rosenkranz, M.E., Schulte, D.J., Agle, L.M., Wong, M.H., Zhang, W., Ivashkiv, L., Doherty, T.M., Fishbein, M.C., Lehman, T.J., Michelsen, K.S. and Arditi, M. 2005. TLR2 and MyD88 contribute to *Lactobacillus casei* extract-induced focal coronary arteritis in a mouse model of Kawasaki disease. *Circulation* 112: 2966–2973. [Medline] [CrossRef]
23. Saji, T., and Kemmotsu, Y. 2006. Infliximab for Kawasaki syndrome. *J. Pediatr.* 149: 426, author reply 426. [Medline] [CrossRef]
24. Suganuma, E., Niimura, F., Matsuda, S., Ukawa, T., Nakamura, H., Sekine, K., Kato, M., Aiba, Y., Koga, Y., Hayashi, K., Takahashi, O. and Mochizuki, H. 2017. Losartan attenuates the coronary perivasculitis through its local and systemic anti-inflammatory properties in a murine model of Kawasaki disease. *Pediatr. Res.* 81: 593–600. [Medline] [CrossRef]
25. Suganuma, E., Babaev, V.R., Motojima, M., Zuo, Y., Ayabe, N., Fogo, A.B., Ichikawa, I., Linton, M.F., Fazio, S. and Kon, V. 2007. Angiotensin inhibition decreases progression of advanced atherosclerosis and stabilizes established atherosclerotic plaques. *J. Am. Soc. Nephrol.* 18: 2311–2319. [Medline] [CrossRef]
26. Sundel, R.P., Burns, J.C., Baker, A., Beiser, A.S. and Newburger, J.W. 1993. Gamma globulin re-treatment in Kawasaki disease. *J. Pediatr.* 123: 657–659. [Medline] [CrossRef]
27. Takahashi, K., Oharaseki, T., Yokouchi, Y., Miura, N.N., Ohno, N., Okawara, A.I., Murata, H., Naoe, S. and Suzuki, K. 2010. Administration of human immunoglobulin suppresses development of murine systemic vasculitis induced with Candida albicans water-soluble fraction: an animal model of Kawasaki disease. *Mod. Rheumatol.* 20: 160–167. [Medline] [CrossRef]
28. Takahashi, K., Oharaseki, T. and Yokouchi, Y. 2018. Histopathological aspects of cardiovascular lesions in Kawasaki disease. *Int. J. Rheum. Dis.* 21: 31–35. [Medline] [CrossRef]
29. Takahashi, K., Oharaseki, T., Wakayama, M., Yokouchi, Y., Naoe, S. and Murata, H. 2004. Histopathological features of murine systemic vasculitis caused by *Candida albicans* extract--an animal model of Kawasaki disease. *Inflamm. Res.* 53: 72–77. [Medline] [CrossRef]