Remodeling of Coronary Artery Lesions Due to Kawasaki Disease

Comparison of Arteriographic and Immunohistochemical Findings

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SUMMARY

Since the original report of Kawasaki disease in 19671) more than 150,000 cases have been reported in Japan.2) Although there have been no nationwide epidemics in Japan since 1987, more than 6,000 newly diagnosed cases are reported every year, and the number has been increasing year by year despite the decreasing birth rate.2,3) The etiology of the disease is still unknown. High dose intravenous gammaglobulin is currently used during the acute phase in 84 % of the patients in Japan with a concomitant decrease in coronary arterial sequelae.2) However, 7-13 % of the patients still have persistent coronary artery aneurysms after the acute stage.2,3)

The aneurysms are seen mostly in the proximal coronary arteries, and are often associated with aneurysms in the distal coronary artery segments (Figure 1A, 2A). Most of the patients show a decrease in the size of aneurysms soon after the acute phase (Figure 1B). However, the aneurysms may progress to obstructive lesions even after initial regression (Figures 1C, D, 2B).4) Such obstructive lesions may cause sudden death or myocardial infarction. Long term follow-up of coronary artery lesions has revealed several characteristic features, including progressive localized stenosis (Figure 1D), extensive recanalizations (Figure 2D) and development of collateral arteries.4-6) Progressive increases in aneurysm size and the appearance of new aneurysms in the late phase have also been reported.

The basic mechanisms of the coronary arterial remodeling in Kawasaki disease have not yet been elucidated. Only recently has immunohistochemical staining in formalin-fixed specimens become feasible.7) This is a major technical breakthrough since it is almost impossible to obtain fresh frozen specimens of coronary artery lesions of Kawasaki disease.8)

In this paper, we compare immunohistochemical findings in coronary artery lesions with the corresponding coronary angiographic findings, and attempt to make inferences as to the mechanism of remodeling both in early and late phases of the disease based on the expression of vascular growth factors.8) (Jpn Heart J 2000; 41: 245-256)
Key words: Vascular growth, Factors, VEGF, TGFβ, Extracellular matrix, Progressing stenotic lesions

VASCULAR GROWTH FACTORS

Immunohistochemical staining was performed on the specimens of 14 patients who died from 15 days of illness to 19 years after onset of the disease. We classified the specimens into three phases of the disease as follows: An acute group consisting of a specimen from one patient who died on the 15th day of illness; a convalescent group which included specimens of 4 patients who died from 2 months to 13 months after the disease; and a late phase group (9 specimens) which represents deaths occurring longer than 2 years after the disease. The specimens had been preserved in formalin solution for 3 days to 11 years.

We examined four different vascular growth factors: transforming growth factor β1 (TGFβ1), platelet-derived growth factor-A (PDGF-A), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF).

TGFβ1 plays a central role in vascular repair and modulation with effects on cell growth and synthesis of extracellular matrix. PDGF-A stimulates proliferation and migration of vascular smooth muscle cells under the regulation of TGFβ. Basic FGF is a potent smooth muscle cell mitogen and also stimulates endothelial cell growth and angiogenesis. VEGF is another important angiogenic factor. These angiogenic factors play important roles in intimal thickening by maintaining nutrient vascular supply as the intima thickens.

FOLLOW-UP CORONARY ARTERIOGRAPHY

Angiographic comparison studies were based on the arteriograms performed in 1506 patients at the National Cardiovascular Center (Osaka) between July 1978 and August 1998. Four hundred fifty eight of the patients showed coronary arterial lesions. These patients have been followed for an average 9.9 ± 5.4 years (mean ± SD) and underwent 4.1 ± 2.5 arteriographies per patient.

Significant coronary artery obstruction, such as complete occlusion (Figure 2B), recanalized vessels (Figure 2C, D) and localized stenosis of more than 75% luminal narrowing (Figure 1D), occurred in 142 (31%) of the 458 patients with coronary artery lesions. Complete coronary arterial occlusion was observed in 67 (15%) patients. Recanalized vessels...
(also referred to as “segmental stenosis” by the Kawasaki Disease Research committee of the Japanese Ministry of Health and Welfare\(^{13}\)) developed in 64 (14 %) patients. Twelve patients with myocardial ischemia died as of 1999.

In the late phase, an increasing aneurysmal diameter was seen in 2 patients and newly appeared aneurysm combined with localized stenosis was observed in 4 patients.
ANEURYSMS

A histological study has reported that coronary arteritis begins with endothelial cell swelling and subendothelial edema.\textsuperscript{14} All layers of the coronary arteries are then infiltrated by large mononuclear cells accompanied by lymphocytes and plasma cells. Destruction of the internal elastic lamina occurs. After this process takes place, the aneurysm appears. Progressive fibroblastic proliferation occurs, and finally, scar formation is completed in about eight weeks.\textsuperscript{14}

Daily echocardiographic observations in the acute phase revealed aneurysm formation 11.4 days into the illness on average.\textsuperscript{15} Many aneurysms then decrease in size soon after the acute phase. Angiographically 30-60 \% of aneurysms disappear within 1 year after onset, while the remaining aneurysms do not exhibit significant change in size.\textsuperscript{15} A small number of aneurysms showed a very slow decrease in size even several years after onset of the disease.

In an immunohistochemical study, aneurysm walls in the late phase had changed to fibrous scars or calcification, and no significant expression of growth factors was observed, except for a rare type of aneurysm that had a smooth muscle cell rich layer in the intima.\textsuperscript{8} This rare aneurysm with a smooth muscle cell rich area expressing various vascular growth factors may continue the process of intimal proliferation, resulting in a decrease in size of the aneurysm by angiography.

The mechanisms underlying a rare case of increasing aneurysm size in newly formed aneurysms in late phase have not been clarified.

OCCLUSION (COMPLETE OBSTRUCTION OF THE VESSEL)

Sixty-nine coronary artery occlusions occurred in 67 patients. Seventy-eight percent of these occlusions appeared on arteriograms performed within 2 years after onset of the disease. All of these occlusions are thought to be the result of thrombosis of an aneurysm. However, recently we found some occluded lesions due to accumulation of jelly-like extracellular matrix in 4 autopsied cases who died suddenly in the convalescent phase of the disease. Three patients out of the 4 also had ordinal thrombotic occlusions in the other branches.

The remaining 22 \% of the occlusions that occurred more than 2 years after the disease were the result of progressive localized stenosis ultimately resulting in occlusion in late phase. Therefore, at least three different types of occlusions have been observed in this disease.
Occlusion with jelly-like extracellular matrix: In the specimen from a patient who died 2 months after onset of the disease, the occlusion with a jelly-like extracellular matrix showed a few smooth muscle cells which were identified by expression of $\alpha$-actin in the matrix (Figure 3, I-A, B). In the other 3 specimens from patients who died from 7 months to 13 months after the disease, the matrix at occlusion consisted of abundant macrophages, smooth muscle cells, micro-fibers, collagen fibers and neo-micro-vascularizations (Figure 3, II-A, B). Macrophages and smooth muscle cells strongly expressed TGF$\beta$, PDGF-A, bFGF and VEGF. VEGF was especially strongly expressed in the endothelial cells of neo-micro vessels.

Thrombotic occlusion: Thrombotic occlusion of aneurysms usually occurs within a few months after onset of the disease.4,6) After the event, the course of coronary arterial remodeling was changed from intimal proliferation to angiogenesis for recanalization. Therefore, the intima of the occluded aneurysm had not become noticeably thick, and the media had

Figure 3. I-A: The patient died 2 months after onset of the disease and Masson's stain showed occlusion of the right coronary artery with jelly like extracellular matrix. I-B: In the jelly-like extracellular matrix, there were smooth muscle cells identified by staining of anti $\alpha$-actin. II-A: The patient died 7 months after onset of the disease and Masson's stain showed occlusion with extracellular matrix. II-B: In the extracellular matrix, there were large numbers of macrophages, small lymphocytes and endothelial-like cells comprising the rings of the vasculogenesis (HE stain).
remained thin. Some parts of the adventitia were thickened with an abundance of vasa vasorum. The organized thrombus did not express any growth factors but the recanalized vessels showed strong expression of TGFβ1, PDGF, bFGF and VEGF (Figure 4A, B). Thus, markedly active angiogenesis is one of the characteristics of vascular remodeling in Kawasaki disease. The recanalized vessels will be mentioned below.

**Occlusion as a result of progressing stenosis:** Some patients died suddenly due to coronary occlusion as a result of progressive localized stenosis, while others remained asymptomatic or only mildly symptomatic, such as tran-

![Figure 4](image-url)

**Figure 4.** A: Organized thrombotic occlusion and recanalized vessels (duration from the onset to death was 4 years). VEGF is expressed strongly in the smooth muscle cells of recanalized vessels. B: VEGF is expressed not only in the smooth muscle cells of recanalized vessels but also in the endothelial cells of neo-micro angiogenic vessels surrounding the recanalized vessels. C: Masson's stain of localized stenosis (duration from onset to death was 4 years 6 months). In the remarkably thick intima, there are multiple lines of microvessels (arrow heads), smooth muscle cell-rich layers (pink) and fibrous layers (dark blue). The media is very thin (m; black arrow). D: PDGF is expressed strongly in the intimal smooth muscle cells and especially strong in the microvessels (v). E: The cells in the edematous region of the deep intima adjacent to the media express VEGF very strongly and intimal smooth muscle cells also over express VEGF (duration from onset to death was 2 years 6 months). i = intima; m = media; a = adventitia.
sient facial pallor, vomiting and/or abdominal pain. The previously mentioned 22% of newly appearing occlusion by follow-up arteriography in the late phase belonged to this group, and they showed remarkably developed collateral arteries. Well-developed collateral arteries are another characteristic of coronary arterial lesion due to Kawasaki disease.

**RECANALIZATION**

We observed recanalization in 70 patients (15%), involving 76 coronary arteries. Lesions involving the right coronary artery (64 lesions) were significantly more frequently seen than those involving the left coronary artery branches (12 lesions) \((p < 0.01)\). This suggests that the right coronary artery lesions have a greater tendency to be occluded by massive thrombosis or jelly-like extracellular matrix, probably because the blood flow patterns are different in the left and right coronary arteries.

On follow-up angiography, recanalized vessels showed a tendency to gradually dilate (Figure 2). There was a concomitant trend toward an improvement of ischemia as noted by myocardial perfusion scintiscan.\(^6\)

According to histopathological studies, organized thrombotic occlusion contained well-developed recanalized vessels, which were called "arteries within the artery" by Takahashi, et al.\(^{16}\) These newly developed vessels were surrounded by a thick smooth muscle cell layer (Figure 4A, B), which in turn was surrounded by a layer of numerous microvessels (Figure 4B). The lines of microvessels seemed to act as vasa vasorum for the thick smooth muscle cell layer of recanalized vessels. The adventitia of the occluded aneurysm had abundant vasa vasorum, some of which were observed to be connected with newly recanalized vessels. The recanalized vessels appeared in the layer of deep intima adjacent to the media. Arteriography also suggests that the tortuous recanalized vessels run in the deep part of the intimal layer (Figure 2D).

In the immunohistochemical study, TGF\(\beta\), PDGF-A, bFGF, and VEGF were diffusely and strongly expressed in the thick smooth muscle cell layers of the newly recanalized vessels\(^8\) (Figure 4A, C). VEGF was also overexpressed in the endothelial cells of the microvessels in the thrombus (Figure 4C).

These findings indicate very active angiogenesis, even several years after the occlusion, and are consistent with the angiographic follow-up findings of gradually developing recanalized vessels in the late phase.
**LOCALIZED STENOsis**

The most serious problem encountered in the follow-up of the patients is that some of the localized stenoses progress in severity year by year. Until 1998, localized stenoses of greater than 75% were seen in the left coronary arteries of 57 patients (12%) and in the right coronary arteries of 37 patients (8%). Localized stenoses, which had progressed to greater than 90% narrowing, were seen significantly more frequently in the left (32 patients) than in the right coronary arteries (12 patients). In spite of severe stenosis, patients rarely have symptoms of ischemia, and some of them die suddenly. 

Remarkable intimal thickening with expression of various intimal growth factors was observed in a histological study. At the inlet and outlet of aneurysms, there were thick intima, consisting of multiple layers such as linearly arranged microvessels, layers rich in smooth muscle cells and fibrous layers (Figure 4B). Dense smooth muscle cells layers in the intima strongly expressed PDGF-A (Figure 4D), VEGF (Figure 4E), bFGF and TGFβ. Type I TGFβ receptors were strongly expressed in the intimal smooth muscle cells, while type II receptor-positive cells were apparently fewer compared to the type I receptor positive cells in the thick intima. Strong expression of VEGF was observed in the endothelial cells lining the microvessels in the intima. The first line of angiogenetic microvessels always appeared in the deep intimal layer adjacent to the media where an edematous layer appeared in the acute phase (Figure 4E). Multiple layers of thick intima indicate that the intimal thickening had been gradually compacted (Figure 4C). In other words, a line of microvessels was produced because the dense smooth muscular cells, newly migrated from the media to intima and now proliferating there, needed nutrient vessels for facilitating and maintaining the new thick intima. Some parts of the intima had been changed to a layer of fibrosis. Another episode of platelet aggregation or increasing shear stress then stimulated growth factors, which in turn promoted smooth muscle cell proliferation again. Proliferated smooth muscle cells again induce angiogenesis.

Most of the localized stenosis appears at the inlet and/or outlet of aneurysms, where shear stress is known to be high. Exposure of the endothelium to increased laminar shear stress induces the expression of tissue plasminogen activator, which then promotes the conversion of latent TGFβ to the activated form. Platelet aggregation by injured endothelial cells or by activated platelets due to turbulent flow in the aneurysm is
another highly possible factor of activating preserved latent TGFβ.\(^{18}\) It is known that TGFβ usually inhibits proliferation and migration of smooth muscle cells, but recently it was reported that smooth muscle cells from injured vascular lesions respond to TGFβ differently, and TGFβ stimulates these cells to proliferate and to induce enhanced accumulation of extracellular matrix.\(^{19}\) This process is reported to be associated with a decreased ratio of type II / type I receptors.\(^{20}\) It is intriguing that the ratio of type II / type I receptor positive cells in the intima was very likely decreased in Kawasaki disease.\(^{8}\)

Moreover, bFGF was expressed together with PDGF-A in the thick intima of localized stenosis and it was reported that bFGF up-regulates PDGF-receptor α expression and synthesizes smooth muscle cells very potently together with PDGF-AA.\(^{21}\)

**COMPARISON WITH NORMAL CORONARY ARTERY**

Angiographically normal coronary arteries with a history of Kawasaki disease often show abnormally thick intima by intravascular ultrasound.\(^{5}\) In the autopsies of such cases that we examined, all coronary arteries had thick intima from proximal to distal segments. Thus, we could not distinguish the immunohistochemical findings of those Kawasaki patients with coronary artery lesions from those with angiographically normal coronary arteries. In the normal coronary arteries of children who died due to other diseases, the growth factors were seen only in the media. On the other hand, in the coronary arteries of Kawasaki disease children, these growth factors were expressed not only in the thin media but also in the thick intima.\(^{8}\)

There is ongoing controversy that all patients with Kawasaki disease, including those without any history of dilated coronary artery, may have the potential to develop premature atherosclerosis. Our findings support the need for long-term follow-up of these patients using various diagnostic modalities. An immunohistochemical study of a greater number of Kawasaki disease patients may shed more light on this question.

We found that the remodeling of the coronary arterial lesion seen in Kawasaki disease was different from that observed in atherosclerosis.\(^{3,21}\) Based on animal studies, three processes of formation of atherosclerotic lesions were identified\(^{22}\): (1) the proliferation of smooth muscle cells, macrophages and lymphocytes; (2) the formation of extracellular matrix by smooth muscle cells; and (3) the accumulation of lipid. In Kawasaki disease, there was no accumulation of lipid or macrophages. We would
like to propose two main mechanisms for the intimal proliferation in the late phase of Kawasaki disease. One is smooth muscle cell migration, proliferation and synthesis of extracellular matrix in the intima, and the other is remarkably active angiogenesis. The thick intima consisted of dense smooth muscle cells and extracellular matrix. The lamina elastica interna was disrupted where medial smooth muscle cells seem to migrate into the intima. TGFβ1, PDGF-A and bFGF were coexpressed strongly and diffusely in the thick intima, both intracellularly and extracellularly. There were no fatty streaks, and only a few macrophages were seen at the microvessels in the intima in Kawasaki disease. These results suggest that intimal proliferation in the late phase of Kawasaki disease is caused by direct mitogenesis of smooth muscle cells and the accumulation of extracellular matrix in the intima. By contrast, in the early convalescent phase of the disease, the obstruction and intimal thickening were mainly based on the accumulation of soft jelly-like extracellular matrix, which were probably synthesized by smooth muscle cells, and secondly proliferation of smooth muscle cells and fibroblasts seemed to occur in the matrix.

If a means of prohibiting such abundant production of extracellular matrix in the early convalescent phase could be found, we may be able to protect the patient from early ischemic events.

Our research is currently focusing on the mechanisms of arteritis in the acute phase and vascular remodeling in the convalescent phase.

CONCLUSIONS

In our study, it has become clear that there is a difference in remodeling between the early convalescent phase and late phase. The first step of early remodeling appears to be the synthesis of extracellular matrix. The second step involves migration of various cell types, proliferation and angiogenesis in the extracellular matrix. In contrast, the late phase remodeling is mainly based on the smooth muscle cellular proliferation in the intima and migration from the media. It is now clear that coronary arterial lesions continue to undergo an active remodeling process even many years after onset of the disease.

Recently, more than 30 years after Dr. Kawasaki reported this disease, an increasing number of adult patients with coronary arterial lesions either confirmed to be due to or highly suggestive of Kawasaki disease are hospitalized for acute myocardial infarction, aorta-coronary bypass surgery or coronary artery interventions.

The current strategy of long term treatment with anti-platelet agents
and angioplasty may not be sufficient to arrest this remodeling process. A better understanding of the basic mechanism of coronary arterial remodeling may lead us to more innovative and effective treatment.

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