Histol Histopathol (2008) 23: 127-136

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Medial and adventitial macrophages are associated with expansive atherosclerotic remodeling in rabbit femoral artery

A. Yamashita[1], K. Shoji[1], T. Tsuruda[2, 4], E. Furukoji[3], M. Takahashi[1], K. Nishihira[4], S. Tamura[3] and Y. Asada[1]

[1]Department of Pathology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan, [2]Department of Nutrition Management, Faculty of Health and Nutrition, Minami-Kyushu University, Japan, [3]Department of Radiology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan and [4]Division of Circulatory and Body Fluid Regulation, Department of Internal Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

Summary. Expansive vascular remodeling is considered a feature of vulnerable plaques. Although inflammation is upregulated in the media and adventitia of atherosclerotic lesions, its contribution to expansive remodeling is unclear. We investigated this issue in injured femoral arteries of normo- and hyperlipidemic rabbits fed with a conventional (CD group; n=20) or a 0.5% cholesterol (ChD group; n=20) diet. Four weeks after balloon injury of the femoral arteries, we examined vascular wall alterations, localization of macrophages and matrix metalloproteases (MMP)-1, -2, -9, and extracellular matrix. Neointimal formation with luminal stenosis was evident in both groups, while expansive remodeling was observed only in the ChD group. Areas immunopositive for macrophages, MMP-1, -2 and -9 were larger not only in the neointima, but also in the media and/or adventitia in the injured arterial walls of the ChD, than in the CD group. Areas containing smooth muscle cells (SMCs), elastin and collagen were smaller in the injured arterial walls of the ChD group. MMP-1, -2 and -9 were mainly localized in infiltrating macrophages. MMP-2 was also found in SMCs and adventitial fibroblasts. Vasa vasorum density was significantly increased in injured arteries of ChD group than in those of CD group. These results suggest that macrophages in the media and adventitia play an important role in expansive atherosclerotic remodeling via extracellular matrix degradation and SMC reduction.

Key words: Atherosclerosis, Expansive remodeling, media, Adventitia, macrophage

Offprint requests to: Atsushi Yamashita, MD, PhD, Department of Pathology, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan. e-mail: atsushi@fc.miyazaki-u.ac.jp

Introduction

Plaque growth initially induces expansive remodeling in coronary arteries. Although expansive remodeling allows the preservation of blood flow, it is currently considered a feature of vulnerable plaques (Glagov et al., 1987; Varnava et al., 2002). Although the prevalence and clinical presentation of expansively remodeled lesions may differ among vascular beds, all types of expansive atherosclerotic vessels have inflammatory cells and subsequent protease activities (Pasterkamp et al., 2004). Matrix metalloprotease (MMP)s belong to a group of zinc- and calcium-dependent proteases, and regulate not only matrix turnover but also tissue remodeling, inflammation and various other aspects of cell biology (Galis and Khatri, 2002). Areas immunostained for macrophages, MMP-1, MMP-2 and MMP-9 tend to be more prevalent in plaques of expansively, than constrictively remodeled segments (Pasterkamp et al., 2000; Orbe et al., 2003). Aikawa et al. (1998) clearly demonstrated an inverse relationship between MMP expression by macrophages and collagen content using balloon injured, high-cholesterol fed rabbit. The results of one animal study suggest that macrophages facilitate expansive arterial remodeling through increased matrix degradation by MMPs (Ivan et al., 2002).

Atherogenesis involves not only the intima but also the media and adventitia. Features of expansively remodeled arteries include medial atrophy and the expansion of external elastic lamina (Clarkson et al., 1994; Burke et al., 2002). Increasing evidence shows that inflammation in the media and adventitia is upregulated underneath advanced atherosclerotic plaques, and closely associated with ischemic heart disease (Kohchi et al., 1985; Moreno et al., 2002). These findings suggest that medial and adventitial inflammation plays a critical role in the remodeling of

atherosclerotic vessels. However, its role is not understood in detail. We investigated this issue in the injured arteries of normo- and hyperlipidemic rabbits.

Materials and methods

Experimental design

The Animal Care Committee of Miyazaki University approved our animal research protocols. This investigation also conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (1996).

Forty male Japanese white rabbits weighing 2.5 to 3.0 kg were fed with a conventional diet (CD group; n=20) or a 0.5% cholesterol diet ChD group; (n=20) for 1 week before and 4 weeks after balloon injury. All surgical procedures proceeded under aseptic conditions and general anesthesia was applied through an intravenous injection of pentobarbital (25 mg/kg, body weight). One week after starting the diets, an angioplasty balloon catheter (2.5-mm diameter, 9-mm length; QUANTUM, Boston Scientific, Galway, Ireland) was inserted via the carotid artery into the left femoral arteries under fluoroscopic guidance. The catheter was inflated to 1.5 atm (balloon-to-artery ratio; 1.1:1 to 1.2:1) and retracted by 5 cm three times to denude the endothelium (Yamashita et al., 2004). Immediately and 4 weeks after balloon injury, the femoral arteries were imaged by conventional angiography, and the luminal diameters were measured at the most stenotic portion. Thirty minutes and 4 weeks after procedures, the rabbits were injected with heparin (500 U/kg, i.v.) and then killed with an overdose of pentobarbital (60 mg/kg, i.v.) 5 minutes later. The animals were perfused with 50 ml of 0.01 mol/L phosphate buffered saline and then perfusion-fixed with 4% paraformaldehyde for histological and immunohistochemical evaluation.

Serum lipid marker sampling and analysis

Blood samples were collected from the peripheral ear arteries of the rabbits after a 12-h fast and before starting the cholesterol diet, and again 4 weeks after injury. Serum total cholesterol (TC) and triglyceride (TG) levels were measured using the Eiken T-CHO (Eiken Kagaku, Tokyo, Japan) and Triglyceride G test (Wako Chemical, Osaka, Japan) kits, respectively.

Light microscopy and immunohistochemistry

The femoral arteries were fixed in 4% paraformaldehyde for 24 hours at 4°C, cut into 3 sections, and embedded in paraffin. Sections (3 μm thick) were morphologically evaluated by staining with hematoxylin and eosin/Victoria blue dye. Collagen or elastin density was determined by staining with Sirius red or Victoria blue, respectively (Wohlrab, 1991; Nakamura et al., 2004). The serial sections were immunohistochemically examined using the following primary antibodies: mouse monoclonal anti-smooth muscle actin (HHF35, Dako, Glostrup, Denmark), mouse monoclonal anti-macrophage (RAM11, Dako), mouse monoclonal anti-MMP-1 (DAIICHI FINE CHEMICAL, Takaoka, Japan), mouse monoclonal anti-MMP-2 (DAIICHI FINE CHEMICAL, Takaoka, Japan), mouse monoclonal anti-MMP-9 (DAIICHI FINE CHEMICAL), and goat anti-von Willebrand factor (VWF) (Santa Cruz Biotechnology, Santa Cruz, CA), an endothelial marker. The sections were stained with Envision (Dako) or donkey anti-goat IgG secondary antibody (Jackson ImmunoResearch, Baltimore, MA). The activity of horseradish peroxidase was visualized using 3,3'-diaminobenzidine tetrahydrochloride, and the sections were faintly counterstained with Meyer's hematoxylin. Immunostaining controls included non-immune mouse IgG1 or goat serum instead of the primary antibodies. Lumen (μm^2), intimal, internal elastic lamina (IEL), and external elastic lamina (EEL) areas (μm^2) were measured at the most stenotic section using an image-analysis system (Axio Vision 2.05, Carl Zeiss, Munich, Germany). IEL and EEL areas were defined as inside areas of IEL and EEL. Vasa vasorum densities in rabbit femoral arteries before and 4 weeks after balloon injury were expressed as the numbers of VWF immunopositive lumen per 10,000 mm^2. Areas of collagen, elastin, and positive immunostaining in the intima, media, and adventitia were analyzed using a color imaging morphometry system (Win Roof, Mitani, Fukui, Japan). Briefly, the commands for the extraction of specified colors were used to extract the Sirius red positive, Victoria blue positive, or immunopositive areas. Extraction of each color was carried out using specific protocols based on the hue, lightness, saturation (HLS) color parameters. Data were expressed as percentage of the Sirius red positive, Victoria blue positive, or immunopositive areas in corresponding areas (Yamashita et al., 2006). Adventitia was defined as fibrous connective tissue outside the EEL. Two investigators (K. S. and M. T.) who were blinded to the treatment assignments performed the morphological analyses.

Zymographic MMP activity

Zymographic MMP activities were determined as described (Tsuruda et al., 2002). Non-fixed femoral arteries from both groups 4 weeks after balloon injury were homogenized in protein lysis buffer with proteinase inhibitor cocktail (Sigma, Saint Louis, MO, USA). Homogenate (50 mg protein) concentrated by filter centrifugation (Microcon, Millipore, Bedford, MA, USA), was mixed with Laemmli sample buffer (Bio-Rad, Hercules, CA, USA) for 10 minutes at room temperature. These samples were then loaded onto 10% Tris-glycine gels with 0.1% gelatin. The gels were stained with 0.5% (w/v) Coomassie Brilliant Blue (Bio-Rad) and destained with Coomassie R-250 destaining solution and then zymographic MMP abundance was densitometrically analyzed (ChemiDoc XRS, Bio-Rad).

We confirmed that the lytic banding represented Ca^{2+}-dependent MMP abundance by soaking a separate set of gels in developing buffer containing 20 mmol/L ethylenediaminetetraacetic acid, a Ca^{2+} chelator (data not shown).

Statistical analysis

All data are presented as median and range or interquartile range. Differences for individual groups were tested using the U-test of Mann-Whitney (StatView; SAS Institute Inc., Berkeley, CA, USA). A P value less than 0.05 was considered statistically significant (n indicates the number of animals).

Results

Serum concentrations of TC and TG

The serum concentration of TC was significantly higher in ChD, than in CD rabbits (madian; 677 mg/dl, range; 510-887 mg/dl vs. 51 mg/dl, 21-96 mg/dl, $p<0.001$; n=8). The serum TG level was significantly higher in the ChD than the CD rabbits (median; 272 mg/dl, range; 129-375 mg/dl vs. 34 mg/dl, 3-101 mg/dl, $p<0.001$; n=8). The weight of both groups of rabbits 4 weeks after balloon injury did not differ (CD vs. ChD groups: median; 3.7 kg, range; 3.0-4.0 kg vs. 3.5 kg , 3.0-4.2 kg, p=0.37; n=8).

Luminal stenosis 4 weeks after balloon injury

Conventional angiograms of the femoral arteries obtained 4 weeks after balloon injury (left femoral artery) revealed luminal stenosis in both groups of rabbits (Fig. 1A-C). However, the degree of luminal stenosis in the injured arteries was significantly smaller in ChD than in CD rabbits (Fig. 1C).

Morphological characteristics

Figure 2 shows the histological characteristics of

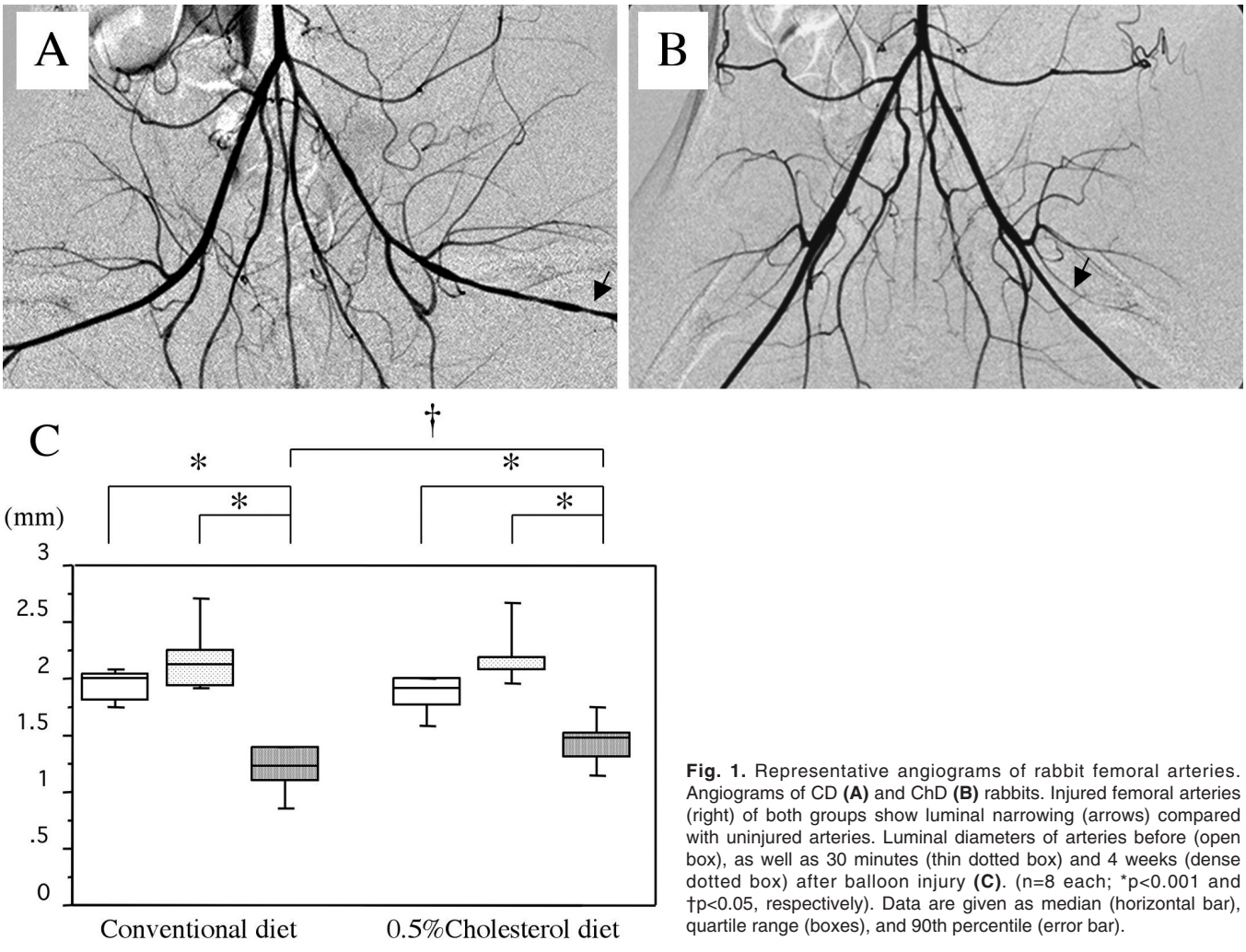

Fig. 1. Representative angiograms of rabbit femoral arteries. Angiograms of CD **(A)** and ChD **(B)** rabbits. Injured femoral arteries (right) of both groups show luminal narrowing (arrows) compared with uninjured arteries. Luminal diameters of arteries before (open box), as well as 30 minutes (thin dotted box) and 4 weeks (dense dotted box) after balloon injury **(C)**. (n=8 each; *$p<0.001$ and †$p<0.05$, respectively). Data are given as median (horizontal bar), quartile range (boxes), and 90th percentile (error bar).

uninjured and injured femoral arteries. Neointimal formation with luminal stenosis was obvious in the injured arteries of both groups. The IEL and EEL areas of injured arteries in the ChD group were significantly larger at 4 weeks, than at 30 minutes after balloon injury whereas those of CD group did not significantly differ. Four weeks after balloon injury, the areas of the lumen, intima, IEL and EEL were significantly larger in the ChD, than in the CD group. More IEL were disrupted per cross-section in the ChD, than in the CD group (median; 2, range; 0-5 vs. 0, 0-1 $p<0.01$; n=8).

Zymographic MMP-2 and MMP-9 activity in injured arteries

Lytic bands appeared at a molecular mass corresponding to each of MMP-2 (72 and 68 kDa) and MMP-9 (83 and 92 kDa) (Fig. 3A). The abundance of

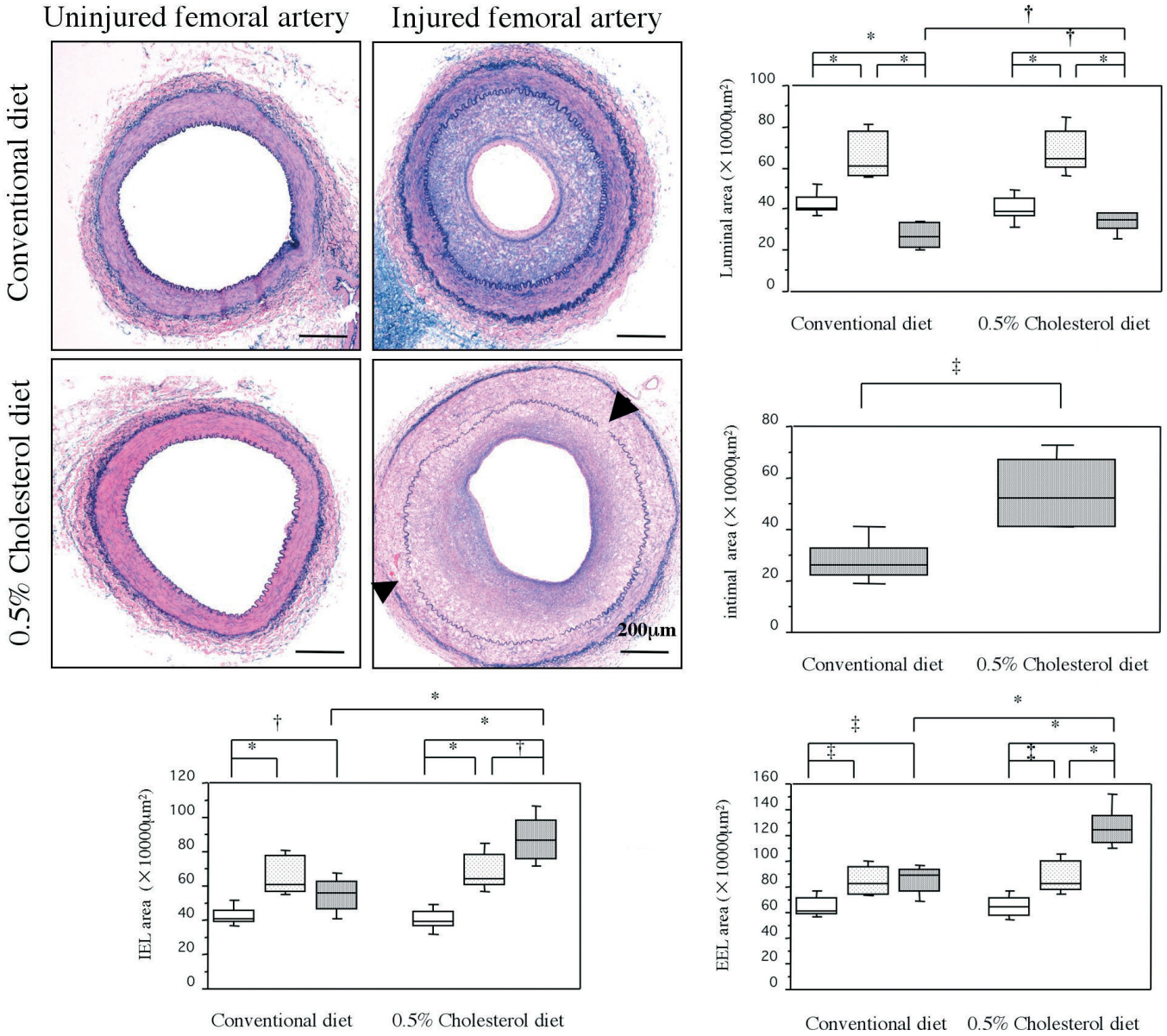

Fig. 2. Representative microphotographs and histological parameters of rabbit femoral artery. Injured arteries (right) show luminal narrowing by neointimal formation compared with uninjured arteries (left). Injured artery in ChD rabbits shows more intimal formation, IEL disruption (arrows), and expansive remodeling. Luminal, intimal, IEL and EEL areas of femoral arteries before (open box), 30 minutes (thin dotted box) and 4 weeks (dense dotted box) after balloon injury (n=8 each; *$p<0.001$, †$p<0.05$ and ‡$p<0.01$, respectively). Data are given as median (horizontal bar), quartile range (boxes), and 90th percentile (error bar).

zymographic MMP-2 and MMP-9 in injured arteries was significantly increased in ChD rabbits compared with the CD group (Fig. 3B).

Localization of macrophages, MMP-1, MMP-2 and MMP-9 in injured arteries

Figure 4 shows the areas that were immunopositive for smooth muscle cells (SMCs), macrophages, MMP-1, MMP-2 and MMP-9 in arteries 4 weeks after balloon injury. The neointima and media of the injured femoral artery in the CD group was diffusely immunopositive for SMC, and immunoreactivity for macrophages, MMP-1, MMP-2, and MMP-9 was slight or mottled. In contrast, the whole wall of the injured femoral artery in the ChD group was more immunopositive for macrophages and less so for SMCs. MMP-1, MMP-2, and MMP-9 were mainly localized in macrophages (Fig. 4A), whereas some of SMCs and adventitial fibroblasts were positive for MMP-2 (Fig. 4B). The areas that were positive for macrophages, MMP-1, MMP-2 and MMP-9 were significantly larger in the ChD, than in the CD group, except for MMP-1, MMP-2 in the adventitia. Replacing primary antibodies with non-immune mouse IgG1 resulted in no immunoreaction (data not shown).

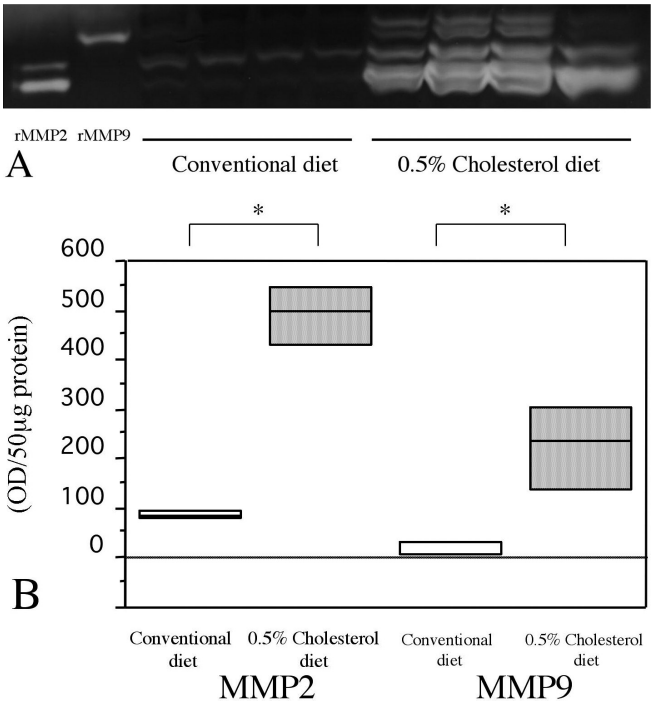

Fig. 3. Representative zymographic MMP abundance and densitometric findings of femoral arteries 4 weeks after balloon injury. **A.** Zymogram showing gelatinase activity in homogenates (50 μg protein); rMMP2, recombinant MMP-2; rMMP9, recombinant MMP-9. **B.** Densitometric findings of MMP-2 and MMP-9 abundance in 50 μg protein (n=4 per group; *p<0.05). Data are given as median (horizontal bar), quartile range (boxes), and 90th percentile (error bar).

Matrix density in injured femoral artery

Figure 5 shows representative images of arteries stained with Sirius-Red (Fig. 5A-D) and Victoria blue (Fig. 5E-F) as well as the density of the extracellular matrix. Sirius-Red staining detected significantly less collagen density in the media and adventitia (Fig. 5G) in the ChD, than in the CD groups of rabbits. Victoria blue staining detected significantly less elastin density in the neointima and media of rabbits in the ChD, than in the C group (Fig. 5H).

Vaso vasorum density in injured femoral artery

Figure 6 shows representative immunohistochemical microphotographs and vaso vasorum density in injured femoral arteries. Immunohistochemistry for VWF revealed vaso vasorum in CD group (A) and ChD group (B). Vasa vasorum density was significantly increased in injured femoral arteries of ChD group than those in injured femoral arteries of CD group (C). Replacing primary antibodies with non-immune goat serum resulted in no immunoreaction (data not shown).

Discussion

The present results showed that the femoral arteries of hypercholesterolemic rabbits 4 weeks after balloon injury became expansively remodeled, suggesting that infiltrating macrophages, MMP-1, MMP-2 and MMP-9 played a significant role in this process not only in the intima but also in the media and adventitia.

Expansive arterial remodeling is considered a natural response that compensates for plaque formation and it is initially beneficial as it preserves the lumen. However, increasing experimental and clinical evidence has revealed that such remodeling would ultimately increase the likelihood of plaque instability and rupture (Schoenhagen et al., 2000). Therefore, the underlying mechanisms of expansive remodeling should be understood. Since medial SMCs, elastic fibers and interstitial collagens in the media and adventitia determine much of the structural integrity and stability of arteries, the excessive loss of medial SMCs and extracellular matrix from the media and adventitia is considered to significantly contribute to expansive remodeling (Pasterkamp et al., 2004). Although their triggers and clinical presentation may differ, inflammatory responses are prevalent in expansive remodeling as well as aneurysm formation (Freestone et al., 1997; Pasterkamp et al., 2000). The obvious inflammatory infiltrates as well as decreased amounts of collagen and SMCs in the caps and shoulders of atheromas that are hallmarks of plaque instability, are associated with expansive remodeling (Pasterkamp et al., 1998). The present study found more neointimal formation with luminal stenosis, and more significant expansion of IEL and EEL in the femoral arteries 4 weeks after balloon injury in the ChD, than in the CD

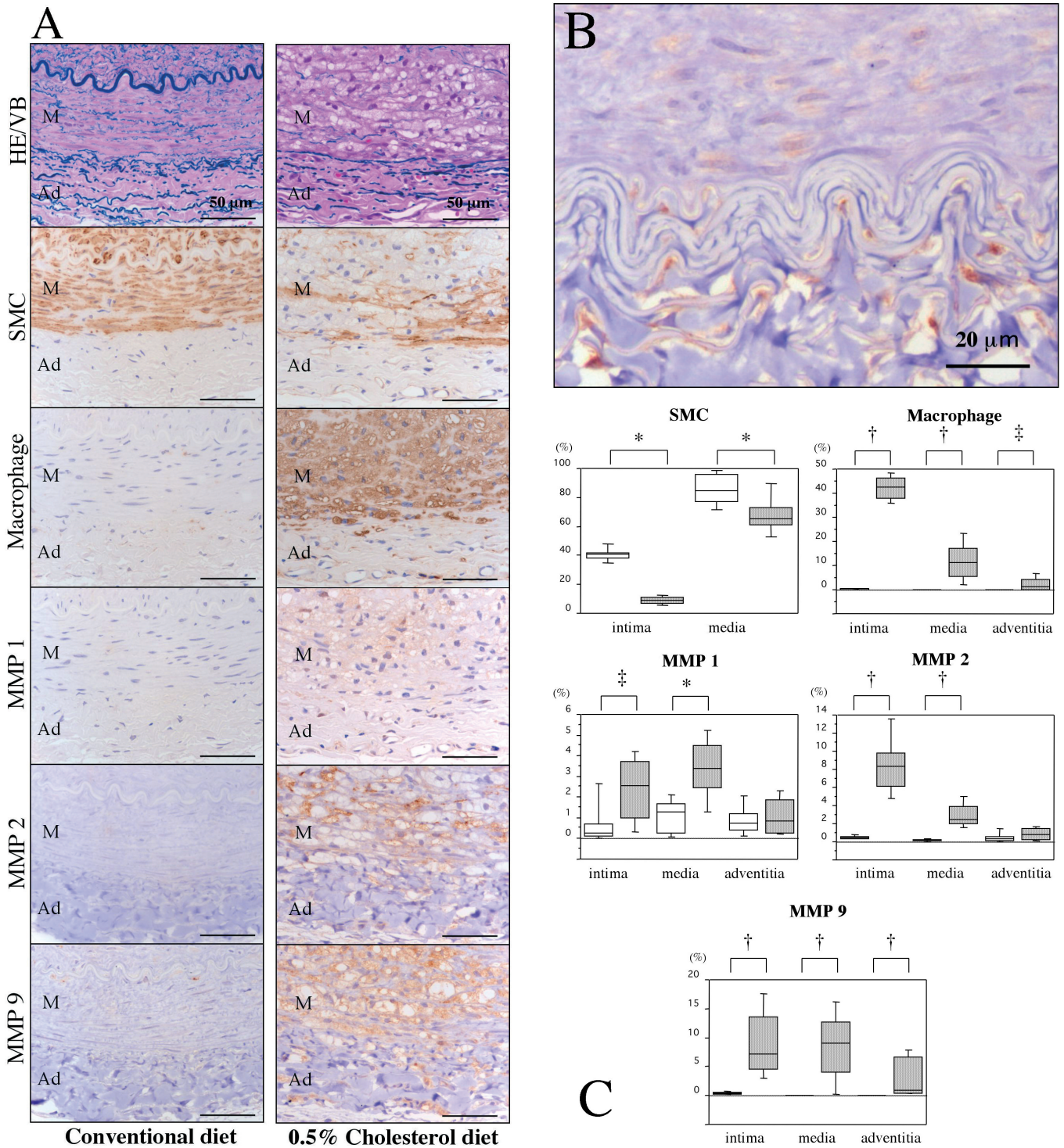

Fig. 4. Representative immunohistomicrographs and immunopositive areas in femoral arteries 4 weeks after balloon injury. Injured femoral artery of CD rabbit: diffuse SMC immunoreactivity in neointima and media, and slight or spotty immunoreactivity for macrophages, MMP-1, MMP-2 and MMP-9. Injured femoral artery of ChD rabbit: closely localized macrophage, MMP-1, MMP-2 and MMP-9 in whole wall (**A**). M, media; Ad, adventitia. Adventitial fibroblasts and SMCs are also positive for MMP-2 (**B**). M, media; Ad, adventitia. **C.** Areas immunopositive (%) for smooth muscle cells, macrophages, MMP-1, MMP-2 and MMP-9 in rabbit femoral arteries of CD group (open box) or ChD group (dotted box) 4 weeks after balloon injury (n=8 per group; *p<0.01, †p<0.001 and ‡p<0.05, respectively). Data are given as median (horizontal bar), quartile range (boxes), and 90th percentile (error bar).

group. The expanded arteries had more macrophage infiltrate, MMP-1, MMP-2 and MMP-9 in the wall, not only in the neointima, but also in the media and/or adventitia. These findings are compatible with those of human atherosclerotic vessels with expansive remodeling (Galis and Khatri, 2002; Pasterkamp et al., 2004).

Inflammation is upregulated in the medial and adventitial regions of advanced atherosclerotic lesions as well as in the intima (Kohchi et al., 1985; Burke et al., 2002; Moreno et al., 2002). Both macrophages and MMP expression have been associated with the expansive enlargement of atherosclerotic arteries. In this study, medial and adventitial macrophages were increased concomitant with MMP activation as well as decreased collagen and elastin density in the injured femoral arteries of the ChD group. The close localization of macrophages, MMP-1, MMP-2 and MMP-9 immunoreactivity suggests that the medial and adventitial macrophages contribute to expansive remodeling through extracellular matrix degradation. In addition to macrophages, SMCs and fibroblasts of the injured vessels in the ChD group tended to be more immunopositive for MMP-2. One report indicates that some cytokines released from monocytes induce SMCs to secrete MMP (Lee et al., 1995). These findings imply that interactions among monocytes, SMCs and fibroblasts promote MMP production, and that macrophages and MMPs expressed not only in the neointima, but also in the media and adventitia, affect expansive remodeling by modulating such intercellular interaction.

Although both MMP-2 and MMP-9 share substrates, their effects are not always identical during the remodeling process. Animal studies have revealed that a deficiency of MMP-2 reduces SMC migration and inhibits neointimal formation, and that the absence of MMP-9 protects against atherosclerotic plaque

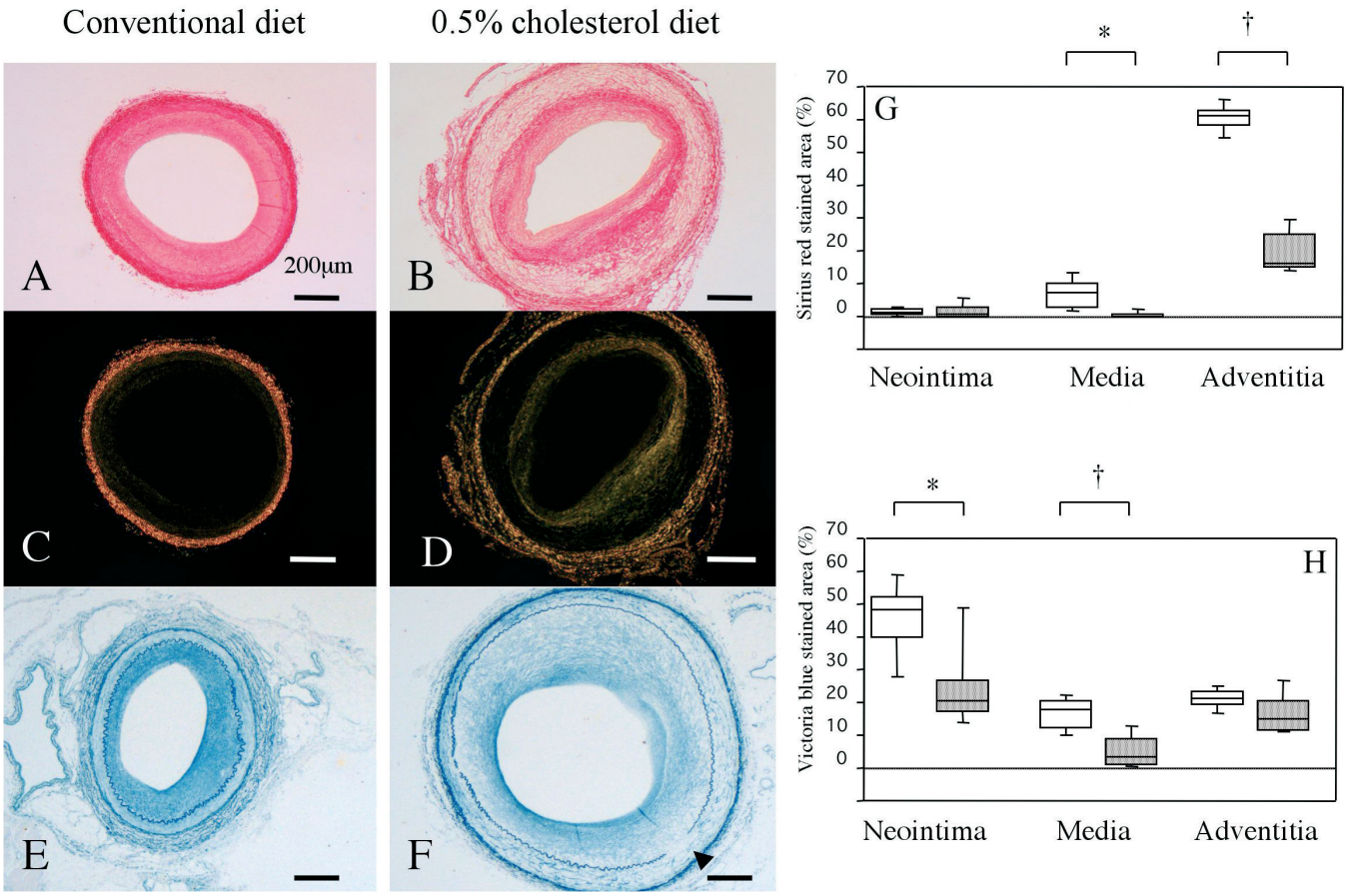

Fig. 5. Representative staining and density of matrix in rabbit femoral arteries 4 weeks after balloon injury. Injured femoral arteries were stained with Sirius red **(A-D)** and Victoria blue **(E, F)**. Intense refraction under polarized light in adventitia of injured femoral artery from CD rabbits **(C)**, and less collagen density in ChD group **(D)**. Elastin is less dense and IEL is more disrupted (arrow) in injured femoral artery of ChD rabbits **(F)**. **G.** Sirius red stained areas (%) in CD (open box) and ChD (dotted box) groups (n=8 per group; *p<0.01 and †p<0.001, respectively). **H.** Victoria blue stained area (%) in CD (open box) and ChD (dotted box) groups (n=8 per group; *p<0.05 and †p<0.001, respectively). Data are given as median (horizontal bar), quartile range (boxes), and 90th percentile (error bar).

formation, medial destruction and ectasia (Kuzuya et al., 2003; Lutten et al., 2004), whereas MMP-9 overexpression affects vessel diameter (Mason et al., 1999). The expression and activation of MMP-2 and MMP-9 is increased during flow-induced arterial remodeling (Karwowski et al., 1999; Tronc et al., 2000). These findings suggest that the contributions of medial and adventitial MMP-2 and MMP-9 to expansive remodeling overlap, but they are not identical.

The roles of MMPs other than MMP-1, MMP-2 and MMP-9 in vascular remodeling have been described (Galis and Khatri, 2002). Our results might represent one aspect of MMP involvement in positive remodeling. Because of their enzymatic nature, areas immunopositive for MMPs do not necessarily mean that these enzymes are active. All secreted MMP zymogens require further proteolytic processing to exert matrix-degrading potential. In fact, the collagen density of the neointima of rabbits fed with dietary cholesterol tended to increase, becoming even more immunopositive for MMPs. However, the medial and adventitial localization of MMPs with a concomitant decrease in matrix density suggest that MMPs and inhibitors are unbalanced in the media and adventitia of this model.

It is generally considered that vasa vasorum plays an important role in development and progression of atherosclerotic lesions (Williams et al., 1988; Moreno et al., 2004). Vaso vasorum density was significantly increased in injured femoral arteries of ChD group than those of CD group. Whether it is a cause and/or

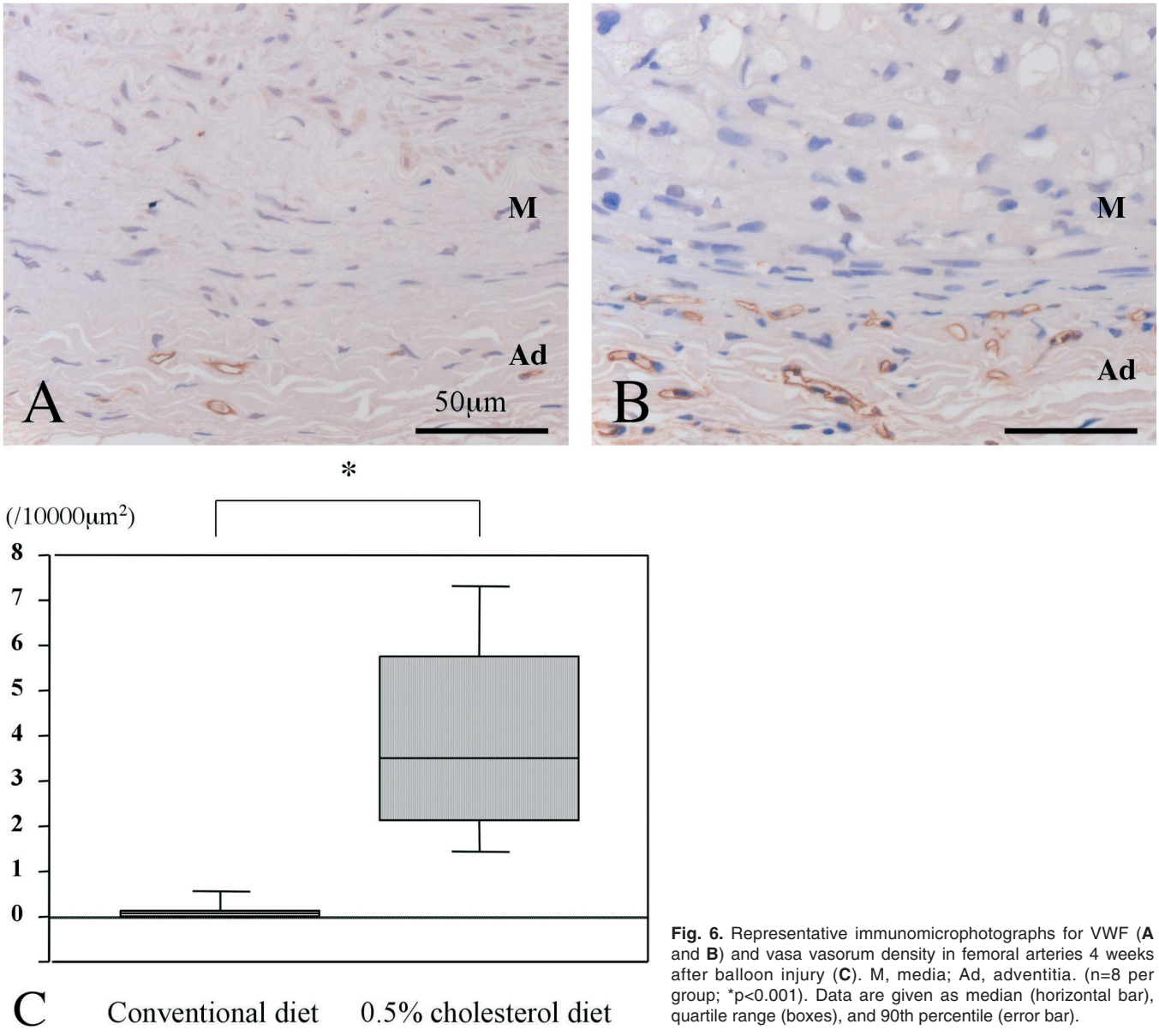

Fig. 6. Representative immunomicrophotographs for VWF (**A** and **B**) and vasa vasorum density in femoral arteries 4 weeks after balloon injury (**C**). M, media; Ad, adventitia. (n=8 per group; *p<0.001). Data are given as median (horizontal bar), quartile range (boxes), and 90th percentile (error bar).

consequence remains still unclear. Vasa vasorum enhanced the recruitment of macrophages in atherosclerotic lesion (Moulton et al., 2003). Moreover, activated macrophages can secrete inflammatory cytokines, growth factors, and angiogenic stimuli, which might enchance inflammatory response and development of vasa vasorum (Ross 1999). Our results suggest that vasa vasorum plays an important role in arterial expansive remodeling as well as atherogenesis.

The amounts of medial and adventitial extracellular matrix, collagen and elastin were decreased in expansively remodeled rabbit femoral arteries. The decreased collagen density in the arteries seems questionable, because adventitial fibrosis is a consistent feature of expansive positive remodeling such as that associated with human abdominal aortic aneurysms (AAA) (Freestone et al., 1995). Inflammatory cell infiltration and MMP activity are more intense in AAA than in the non-aneurysmal aorta (Lopez-Candales et al., 1997). An adventitial response might differ between aneurismal and non-aneurysmal vessels. Matrix turnover and fibrosing processes might differ in the adventitia between the human aorta and the rabbit femoral artery, and anatomical differences (muscular vs. arterial elasticity) might also exert an effect.

There is a limitation in the present animal model. Because the arterial lesions were induced by balloon injury and cholesterol diet, the lesions are not always identical to human atherosclerotic lesions. Moreover, the hyperlipidemic state in blood does not always reflect that of the arterial wall. To investigate the mechanisms of vascular remodeling of atherosclerotic vessels, further studies using animal models with more complicated lesions are needed.

In summary, the present findings suggest that macrophages not in the intima, but in the media and adventitia play an important role in expansive atherosclerotic remodeling via matrix degradation and SMC reduction.

Acknowledgements. This study was supported in part by Grants-in-Aid for Scientific Research and for the 21st COE Research (Life Science) from the Ministry of Education, Science, Sports and Culture, Japan, and by University of Miyazaki, Project Research 2005.

References

Aikawa M., Rabkin E., Okada Y., Voglic S.J., Clinton S.K., Brinckerhoff C.E., Sukhova G.K. and Libby P. (1998). Lipid lowering by diet reduces matrix metalloproteinase activity and increases collagen content of rabbit atheroma: a potential mechanism of lesion stabilization. Circulation 97, 2433-2444.

Burke A.P., Kolodgie F.D., Farb A., Weber D. and Virmani R. (2002). Morphological predictors of arterial remodeling in coronary atherosclerosis. Circulation 105, 297-303.

Clarkson T.B., Prichard R.W., Morgan T.M., Petrick G.S. and Klein K.P. (1994). Remodeling of coronary arteries in human and nonhuman primates. JAMA 271, 289-294.

Freestone T., Turner R.J., Coady A., Higman D.J., Greenhalgh R.M. and Powell J.T. (1995). Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. Arterioscler. Thromb. Vasc. Biol. 15, 1145-1151.

Freestone T., Turner R.J., Higman D.J., Lever M.J. and Powell J.T. (1997). Influence of hypercholesterolemia and adventitial inflammation on the development of aortic aneurysm in rabbits. Arterioscler. Thromb. Vasc. Biol. 17, 10-17.

Galis Z.S. and Khatri J.J. (2002). Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ. Res. 90, 251-262.

Glagov S., Weisenberg E., Zarins CK., Stankunavicius R. and Kolettis G.J. (1987). Compensatory enlargement of human atherosclerotic coronary arteries. N. Engl. J. Med. 316, 1371-1375.

Ivan E., Khatri J.J., Johnson C., Magid R., Godin D., Nandi S., Lessner S. and Galis Z.S. (2002). Expansive arterial remodeling is associated with increased neointimal macrophage foam cell content: the murine model of macrophage-rich carotid artery lesions. Circulation 105, 2686-2691.

Karwowski J.K., Markezich A., Whitson J., Abbruzzese T.A., Zarins C.K. and Dalman R.L. (1999). Dose-dependent limitation of arterial enlargement by the matrix metalloproteinase inhibitor RS-113,456. J. Surg. Res. 87, 122-129.

Kohchi K., Takebayashi S., Hiroki T. and Nobuyoshi M. (1985). Significance of adventitial inflammation of the coronary artery in patients with unstable angina: results at autopsy. Circulation 71, 709-716.

Kuzuya M., Kanda S., Sasaki T., Tamaya-Mori N., Cheng X.W., Itoh T., Itohara S. and Iguchi A. (2003). Deficiency of gelatinase a suppresses smooth muscle cell invasion and development of experimental intimal hyperplasia. Circulation 108, 1375-1381.

Lee E., Grodzinsky A.J., Libby P., Clinton S.K., Lark M.W. and Lee R.T. (1995). Human vascular smooth muscle cell-monocyte interactions and metalloproteinase secretion in culture. Arterioscler. Thromb. Vasc. Biol. 15, 2284-2289.

Lopez-Candales A., Holmes D.R., Liao S., Scott M.J., Wickline S.A. and Thompson R.W. (1997). Decreased vascular smooth muscle cell density in medial degeneration of human abdominal aortic aneurysms. Am. J. Pathol. 150, 993-1007.

Luttun A., Lutgens E., Manderveld A., Maris K., Collen D., Carmeliet P. and Moons L. (2004). Loss of matrix metalloproteinase-9 or matrix metalloproteinase-12 protects apolipoprotein E-deficient mice against atherosclerotic media destruction but differentially affects plaque growth. Circulation 109, 1408-1414.

Mason D.P., Kenagy R.D., Hasenstab D., Bowen-Pope D.F., Seifert R.A., Coats S., Hawkins S.M. and Clowes A.W. (1999). Matrix metalloproteinase-9 overexpression enhances vascular smooth muscle cell migration and alters remodeling in the injured rat carotid artery. Circ. Res. 85, 1179-1185.

Moreno P.R., Purushothaman K.R., Fuster V. and O'Connor W.N. (2002). Intimomedial interface damage and adventitial inflammation is increased beneath disrupted atherosclerosis in the aorta: implications for plaque vulnerability. Circulation 105, 2504-2511.

Moreno P.R., Purushothaman K.R., Fuster V., Echeverri D., Truszczynska H., Sharma S.K., Badimon J.J. and O'Connor W.N. (2004). Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. Circulation. 110, 2032-8.

Moulton K.S., Vakili K., Zurakowski D., Soliman M., Butterfield C., Sylvin E., Lo K.M., Gillies S., Javaherian K. and Folkman J. (2003).

Inhibition of plaque neovascularization reduces macrophage accumulation and progression of advanced atherosclerosis. Proc. Natl. Acad. Sci. USA. 100, 4736-41.

Nakamura R., Kato J., Kitamura K., Onitsuka H., Imamura T., Cao Y., Marutsuka K., Asada Y., Kangawa K. and Eto T. (2004). Adrenomedullin administration immediately after myocardial infarction ameliorates progression of heart failure in rats. Circulation 110, 426-431.

Orbe J., Fernandez L., Rodriguez J.A., Rabago G., Belzunce M., Monasterio A., Roncal C. and Paramo JA. (2003). Different expression of MMPs/TIMP-1 in human atherosclerotic lesions. Relation to plaque features and vascular bed. Atherosclerosis 170, 269-276.

Pasterkamp G., Schoneveld A.H., van der Wal A.C., Haudenschild C.C., Clarijs R.J., Becker A.E., Hillen B. and Borst C. (1998). Relation of arterial geometry to luminal narrowing and histologic markers for plaque vulnerability: the remodeling paradox. J. Am. Coll. Cardiol. 32, 655-662.

Pasterkamp G., Schoneveld A.H., Hijnen D.J., de Kleijn D.P., Teepen H., van der Wal A.C. and Borst C. (2000). Atherosclerotic arterial remodeling and the localization of macrophages and matrix metalloproteases 1, 2 and 9 in the human coronary artery. Atherosclerosis 150, 245-253.

Pasterkamp G., Galis Z.S. and de Kleijn D.P. (2004). Expansive arterial remodeling: location, location, location. Arterioscler. Thromb. Vasc. Biol. 24, 650-657.

Schoenhagen P., Ziada K.M., Kapadia S.R., Crowe T.D., Nissen S.E. and Tuzcu E.M. (2000). Extent and direction of arterial remodeling in stable versus unstable coronary syndromes: an intravascular ultrasound study. Circulation 101, 598-603.

Ross R. (1999). Atherosclerosis--an inflammatory disease. N. Engl. J. Med. 340, 115-126.

Tronc F., Mallat Z., Lehoux S., Wassef M., Esposito B. and Tedgui A. (2000). Role of matrix metalloproteinases in blood flow-induced arterial enlargement: interaction with NO. Arterioscler. Thromb. Vasc. Biol. 20, E120-126.

Tsuruda T., Boerrigter G., Huntley B.K., Noser J.A., Cataliotti A., Costello-Boerrigter L.C., Chen H.H. and Burnett J.C. Jr (2002). Brain natriuretic Peptide is produced in cardiac fibroblasts and induces matrix metalloproteinases. Circ. Res. 91, 1127-1134.

Varnava A.M., Mills P.G. and Davies M.J. (2002). Relationship between coronary artery remodeling and plaque vulnerability. Circulation 105, 939-943.

Williams J.K., Armstrong M.L. and Heistad D.D. (1988) Vasa vasorum in atherosclerotic coronary arteries: responses to vasoactive stimuli and regression of atherosclerosis. Circ. Res.62, 515-23.

Wohlrab F. (1991). Victoria blue: staining properties in orthology and pathology. Acta. Histochem. 91, 51-57.

Yamashita A., Furukoji E., Marutsuka K., Hatakeyama K., Yamamoto H., Tamura S., Ikeda Y., Sumiyoshi A. and Asada Y. (2004). Increased vascular wall thrombogenicity combined with reduced blood flow promotes occlusive thrombus formation in rabbit femoral artery. Arterioscler. Thromb. Vasc. Biol. 24, 2420-2424.

Yamashita A., Sumi T., Goto S., Hoshiba Y., Nishihira K., Kawamoto R., Hatakeyama K., Date H., Imamura T., Ogawa H. and Asada Y. (2006). Detection of von Willebrand factor and tissue factor in platelets-fibrin rich coronary thrombi in acute myocardial infarction. Am. J. Cardiol. 97, 26-28.

Accepted July 6, 2007