PLASMA CELL INFILTRATION AND MUCOID DEGENERATION IN THE MEDIA OF ASCENDING AORTA IN PATIENT WITH CORONARY ARTERY DISEASE

TOMOFUMI NAGAREDA
Department of Laboratory Medicine, Pathology Takarazuka Municipal Hospital
Department of Surgical pathology, Hashimoto Municipal Hospital

NOBUAKI HIRATA and YOSHIO KOSAKAI
Department of Cardiovascular Surgery, Takarazuka Municipal Hospital

MASAHIKO OHSAWA
Department of Diagnostic Pathology, Osaka City University,
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Abstract : **Aims** Atherosclerosis results in inflammatory changes in the aortic intima, but little is known regarding medial changes. Atherosclerosis of the ascending aorta coexists with coronary artery disease. The aim of this study was to investigate the atherosclerotic changes in 44 biopsy specimens of media of the ascending aorta associated with coronary artery disease. Plasma cells do not appear in non-inflammatory tissue.

Methods We compared plasma cells, and matrix metalloproteinase (MMP)-2-, -9- and -12-positive cells immunohistochemically, and we also compared mucoid degeneration and fibrosis determined by staining using a point-counting method, for groups with a variable number of coronary stenotic ($\geq$75%) lesions.

Results In patients with one to three coronary stenotic lesions, plasma cells and mucoid degeneration were low in the aortic media. With four to five lesions, both plasma cells and mucoid degeneration increased significantly compared with those in the group with one to three lesions, and MMP-12-positive cells significantly decreased. In patients with six to nine lesions, the number of plasma cells was significantly lower than in patients with four or five lesions, whereas mucoid degeneration significantly increased. There was no change in fibrosis.

Conclusions These findings may help us to better understand and treat atherosclerosis.

Key words : aortic media, atherosclerosis, coronary artery disease, mucoid degeneration, plasma cell infiltration

INTRODUCTION

Atherosclerosis is known to induce chronic inflammation[1,2], and is characterized by intimal thickening, lipid accumulation, and formation of atheromatous plaques, which are raised focal lesions that are covered by a firm, fibrous cap. Atherosclerotic plaque has three principal components: (1) cells, including smooth muscle cells, macrophages, other leukocytes (e.g., T lymphocytes[3,4], and immunological cells (e.g., dendritic cells[5]); (2) extracellular

matrix (ECM), including collagen, elastic fibers, and proteoglycans; and (3) intracellular and extracellular lipids. Local immune inflammation in atherosclerosis is reported to occur within the atherosclerotic plaque[6, 7]. Furthermore, many inflammatory components play roles in the local immune response in the atherosclerotic plaque and have been physiologically detected[8]. Data suggest that the blockade of IL-12 by vaccination reduces atherogenesis in mice[9]. Thus, these factors must have a principal function in atherogenesis. Gelatinase, matrix metalloproteinase (MMP)-9, -2[10] and -12[11], and elastase, are also reported to contribute to mucoid degeneration in atherosclerotic plaque. Mucoid degeneration represents the accumulation of mucin in the connective tissue. Mucins are glycoproteins that contain O-linked oligosaccharides and proteoglycans[12].

The common inflammatory diseases of the aorta and large artery are known to be giant cell arteritis and Takayasu arteritis[13, 14], and atherosclerotic lesions are reported to occur in these pathologies[15]. These diseases display characteristic features of inflammatory cell infiltration and fragmentation of the elastic fibers or fibrinoid necrosis in the media, and they are usually diagnosed by medial changes. It is reported that medial changes in atherosclerosis present as medial thinning and atrophy of the smooth muscle, and that these changes are less pronounced than those of the intima of the atherosclerotic aorta[16].

Activated T-lymphocytes, dendritic cells, macrophages and aberrant MHC class-II expression, which are known to be present in the atheromatous plaque, are also present in the aortic wall of young adults without apparent atherosclerosis[17]. Plasma cells are B-lymphocytes that differentiate into antibody-secreting cells. They produce antibody directed either against persistent antigen in the inflammatory site or against altered tissue component[18]. Plasma cells are not a typical cellular component of normal non-inflammatory connective tissue[19]. Thus plasma cell infiltration is an ideal cellular marker for inflammatory changes. Plasma cell infiltration is predominantly reported in the aorta of some rheumatoid arthritis patients[20]. Autoimmune diseases, such as rheumatoid arthritis[20] and systemic lupus erythematosus[21, 22], are correlated with atherosclerosis. There are reports that plasma cells markedly infiltrate into the media of abdominal aortic aneurysms with atherosclerosis[23, 24]. It was observed that aneurysms with plasma cell infiltration may be a novel type of inflammatory aortic aneurysm due to autoimmune disease because plasma cell infiltration usually appears in autoimmune diseases.

In the current study, we successfully obtained samples of the media from the ascending aorta of patients with coronary artery disease when they underwent coronary artery bypass graft surgery. The orifice of the new graft vessel was usually located at the ascending aorta[25]. There are reports that atherosclerosis of the ascending aorta coexists with coronary artery disease[26, 27]. The severity of atherosclerosis of the ascending aorta may be relevant to the operation procedure and prognosis of coronary artery graft bypass grafting. The aim of the current study was to investigate the atherosclerotic status of the media of ascending aorta with coronary artery disease. Plasma cell infiltration is an ideal cellular marker for inflammatory changes, and MMPs may be related to medial degeneration. Furthermore, we compared the density of plasma cells or MMP-2, -9 and -12-positive cells, and the degree of mucoid degeneration and fibrosis, in the media of the ascending aorta from patients with coronary artery disease, for groups with variable numbers of coronary stenotic lesions.

MATERIAL AND METHODS

Cases

Clinical findings of the 44 patients studied herein are shown in Table 1. All patients underwent coronary artery bypass graft operation, following coronary angiography. These patients consisted of 35 men and 9 women, and their ages ranged from 33 to 79 years (average ± SD, 65.0±8.3 years). Of the 44 patients, 23 (52%) suffered from diabetes mellitus, which was diagnosed according to the criteria of the Guidelines of the Japanese Society of Diabetes Mellitus (i.e. occasional venous plasma glucose concentration ≥200 mg/dl, fasting venous plasma glucose concentration ≥126 mg/dl, or venous plasma glucose concentration ≥200 mg/dl at 2 h following ingestion of 75 g of glucose). Sixteen (36%) patients suffered from hypertension, which was diagnosed according to the criteria of the Guidelines of the Japanese Society of Hypertension (i.e. an average of two or more diastolic blood pressure measurements on one subsequent visit ≥90 mmHg or an average systolic blood pressure of ≥140 mmHg on multiple measurements of blood pressure on several medical examinations after the first visit). Fourteen (32%) patients suffered from hyperlipidemia, which was diagnosed according to the criteria of the Guidelines of the Japan Atherosclerosis Society (i.e. fasting venous plasma low-density-lipoprotein cholesterol concentration ≥140 mg/dl and/or fasting venous plasma triglyceride level ≥150 mg/dl). Six (14%) patients had a smoking habit. All patients had experienced the symptoms of ischemic heart diseases, but no patients had suffered from syphilis or multiple myeloma. Furthermore, the characteristic features of Marfan syndrome were not observed in any patient.

We divided our patients into six groups, depending upon the number of stenotic lesions that accounted for ≥75% stenosis of the coronary arteries. We counted the numbers of stenotic lesions on the coronary angiography of each patient. Groups 1, 2, 3, 4, 5 and 6 had one or two, three, four, five, six, and eight or nine stenotic lesions, respectively. No significant differences in age distributions among the six groups were found.

Table 1 Clinical findings of cases divided into 6 groups according to the number of stenotic lesions of coronary arteries

Group	No of stenotic lesions	No of cases	Age (years) Mean±SD (range)	No of smokers (%)	No of DM cases (%)	No of HT cases (%)	No of HL cases (%)
1	1 or 2	6	62.8±8.5 (54-78)	1 (17)	1 (17)	1 (17)	0 (0)
2	3	12	63.0±12.4 (33-79)	2 (17)	6 (50)	4 (33)	3 (25)
3	4	8	64.9±8.5 (50-78)	2 (25)	4 (50)	3 (28)	4 (50)
4	5	7	67.9±2.4 (64-70)	1 (14)	4 (56)	4 (56)	3 (43)
5	6	8	68.0±2.7 (65-72)	0 (0)	5 (63)	3 (38)	3 (38)
6	8 or 9	3	62.7±4.7 (59-68)	0 (0)	3 (100)	1 (33)	1 (33)
Total		44	65.0±8.3 (33-79)	6 (14)	23 (52)	16 (36)	14 (32)

DM, HT and HL indicate diabetes mellitus, hypertension and hyperlipidemia, respectively. SD indicates standard deviation.

Methods

Forty-four aortic wall biopsy samples were obtained from the ascending aorta at the graft orifices that were constructed at Takarazuka Municipal Hospital. The study took place from 2000 to 2004. Written consent was obtained from each patient prior to the operation, and anonymous use of tissue samples for histological diagnosis and examination was permitted. Tissue samples were fixed in 10% buffered (pH 7.2) formalin and processed for routine paraffin embedding. Sections with 3 to 5 μm thicknesses were made. Several sections were used for staining with hematoxylin and eosin (H&E), Elastica van Gieson's (elastic fibers are dyed black), Azan Mallory (collagen fibers are dyed blue), Periodic acid Schiff (PAS) and Alcian blue (mucin is dyed blue), and colloidal iron (mucin is dyed blue).

The remaining sections were used for immunohistochemistry. Immunohistochemical staining was performed using an automated staining system (Ventana HX system Benchmark/20, Ventana, Tucson, AZ, USA), with the following antibodies: anti-human plasma cell VS38 mouse monoclonal (Clone VS38c[28], 100-fold dilution, Dako, Glostrup, Denmark), anti-human plasma cell CD38 mouse monoclonal CD38[29](100-fold dilution, Novocastra, Newcastle-upon-Tyne, UK), anti-metalloproteinase (MMP)-2 rabbit polyclonal (200-fold dilution, BIOMOL Research Laboratories, PA, USA), anti-MMP-12 rabbit monoclonal (100-fold dilution, BIOMOL Research Laboratories, PA, USA), anti-MMP-9 rabbit polyclonal (100-fold dilution, Thermo Fisher Scientific Anatomical Pathology, CA, USA), and anti-human smooth muscle desmin[30] mouse monoclonal (100-fold dilution, Dako, Glostrup, Denmark).

Density of VS38-positive and MMP-positive cells in the aortic media

We counted VS38-positive and MMP-2-, -9-, and -12-positive cells in 0.01 mm2 grids and the number of grids (mean ± SD, 450.7±224.0), and we calculated the number of VS38- or MMP-positive cells per 1 mm^2 in the aortic media from each section.

Degree of mucoid degeneration and fibrosis in the aortic media

The degree of mucoid degeneration or fibrosis in the aortic media was estimated quantitatively by a point-counting method[31], using an objective lens (10x magnification) and an eyepiece lens (10x magnification), within a 0.04 mm^2 grid. We counted the number of intersecting grid lines and the points stained blue by the colloidal iron stain for mucoid degeneration or by the Azan Mallory stain for fibrosis, and the percentage of stained points was calculated for all samples. The number (mean ± SD) of intersecting points was 62.2±32.8.

Statistical analysis

Statistical significance ($p<0.05$) was determined by Student's t test.

RESULTS

1. CD38-positive plasma cells and desmin-positive smooth muscle cells in the atherosclerotic aorta media from autopsies

To investigate the appearance of plasma cells and smooth muscle cells in the media of the atherosclerotic aorta, we examined three cadavers with known atherosclerosis. The cadavers included were of 91-year-old woman with markedly atherosclerotic aorta, an 87-year-old man with moderately atherosclerotic aorta, and a 47-year-old woman with mildly atherosclerotic aorta. Histology of the aortic wall, CD38-positive plasma cells and

desmin-positive smooth muscle cells from the moderately atherosclerotic aorta, are shown in Fig. 1. Intimal plaque formation and inflammatory cell infiltration in the media are shown in Fig. 1a and b. Fig. 1c shows that morphologically CD38-positive plasma cells have a spindle-like morphology or are pleomorphous in the aortic media, but in the aortic adventitia, the CD38-positive cells appear as normal plasma cells with an oval-shaped cytoplasm and round nuclei (Fig. 1d and e). We were unable to visualize plasma cells in the aortic media with only the H&E staining. No plasma cells were observed in the aortic media of the markedly atherosclerotic case, but a few CD38-positive plasma cells were found in the media of the mildly atherosclerotic case. In the aortic media of all three samples, several desmin-positive smooth muscle cells, which appeared as continuous fibrous cells and slightly larger solitary spindle cells, were observed (Fig. 1f).

2. VS38-positive plasma cells, MMP-positive cells, mucoid degeneration, collagen fibers, and elastic fibers in the aortic media of patients with coronary artery disease

Fig. 2a shows VS38-positive plasma cells that infiltrated into the aortic media. These plasma cells were spindle-shaped, that is CD38-positive plasma cells such as those found in the aortic media of cadavers with atherosclerosis (Fig. 1c). PAS and Alcian blue staining (Fig. 2b) and colloidal iron staining (Fig. 2c) revealed mucoid degeneration in the aortic media, and Azan-Mallory (Fig. 2d) and Elastica van Gieson staining (Fig. 2e) showed collagen fibers and elastic fibers, respectively, in the aortic media. Fig. 2e shows reduced elastic fibers and their fragmentation and irregular morphology. Fig. 2f shows multiple spindle-shaped MMP-9-positive cells.

3. The density of plasma cells and the degree of mucoid degeneration in the aortic media of patients with coronary artery disease

The density of plasma cells and degree of mucoid degeneration in aortic media of each group of patients are shown in Fig. 3. The density of plasma cells in Groups 1 and 2 was low; Groups 3 and 4 displayed the highest density. The density of plasma cells in Groups 5 and 6 was significantly decreased, compared with that in Groups 3 and 4. The degree of mucoid degeneration in Groups 1 and 2 was low and significantly increased in Groups 3, 4, 5 and 6 compared with that in the Groups 1 and 2; furthermore, it was significantly increased in Group 6 compared with that in Group 3.

4. The density of MMP-positive cells in the aortic media of patients with coronary artery disease

Table 2 shows the density of MMP-2-, -12- and -9-positive cells in the aortic media of Groups 1 and 2, Groups 3 and 4, and Groups 5 and 6. The density of MMP-12-positive cells in Groups 3 and 4 was significantly lower than in the other groups. However, no significant difference in the density of MMP-2- or MMP-9-positive cells was observed among these three pairs of groups.

5. The degree of mucoid degeneration and fibrosis in the aortic media of patients with coronary artery disease

The degrees of mucoid degeneration and fibrosis in Groups 1 and 2 as well as Groups 5 and 6 are shown in Table 3. The degree of mucoid degeneration in Groups 5 and 6 was significantly greater than that in Groups 1 and 2. However, no significant difference was found in the degree of fibrosis between these two groups.

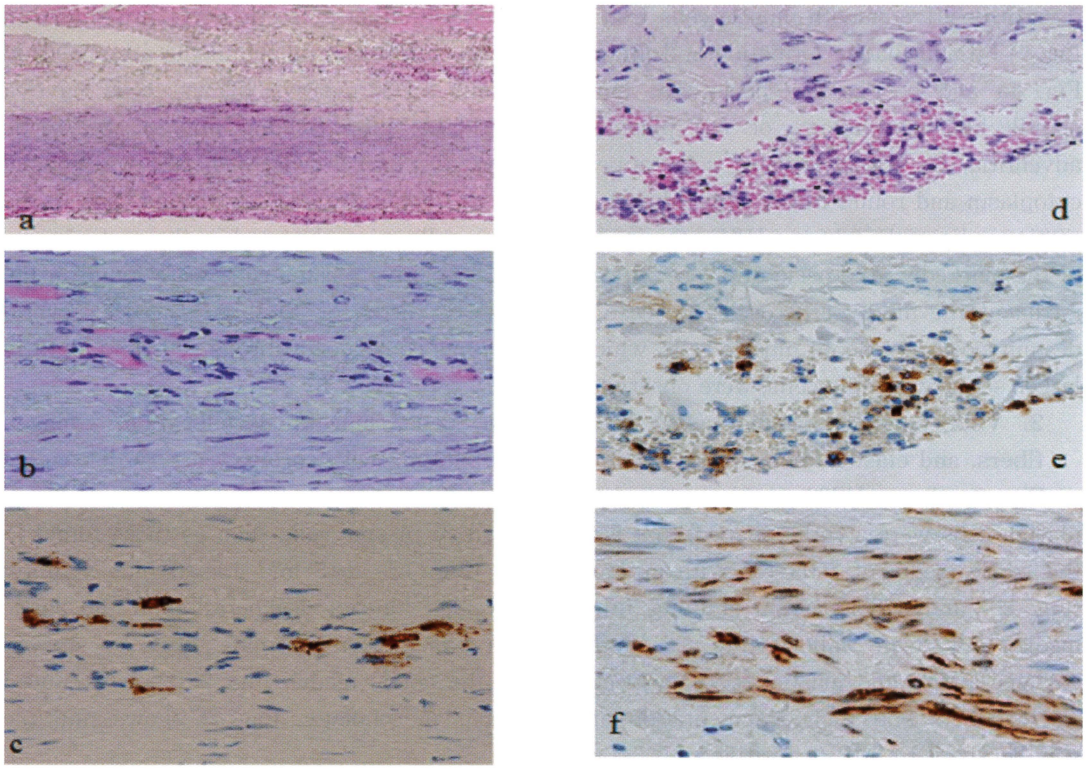

Fig. 1

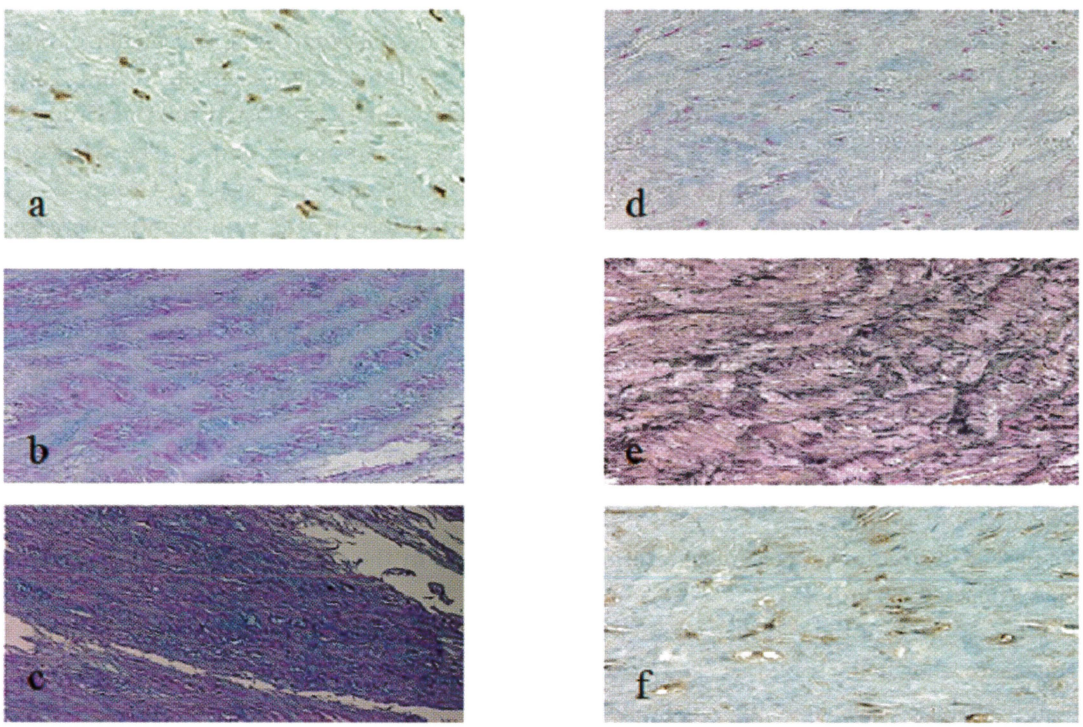

Fig. 2

TITLES AND LEGENDS TO FIGURES

Fig. 1. Histology, and immunohistochemistry of the aorta of an 87-year-old male cadaver with moderate atherosclerosis.

a: Wall of the atherosclerotic aorta. Plaque formation in the intima of the aorta is evident (H&E).

b: Aortic media. Inflammatory cells are evident (H&E).

c: CD38-positive plasma cells in the media.

d: Adventitia of the aorta. Many inflammatory cells are evident (H&E).

e: CD38-positive plasma cells in the adventitia.

f: Desmin-positive smooth muscle cells in the aortic media.

The original magnification of Figs. 1b, c, d, e and f is 200x. Fig. 1a magnification is 40x.

Fig. 2. Immunohistochemical and histochemical staining of the aortic media from patients with coronary artery disease.

a: Immunohistochemical staining with VS38 for plasma cells in the aortic media of a 69-year-old male patient with five coronary stenotic lesions that exhibited 75% stenosis.

b: Periodic Acid Schiff (PAS) plus Alcian blue staining for mucin in the aortic media of a 65-year-old woman with six coronary stenotic lesions.

c: Colloidal iron staining for mucin in the aortic media of a 61-year-old man with eight coronary stenotic lesions.

d: Azan-Mallory staining for collagen fibers in the aortic media of the same individual from Fig. 2b.

e: Elastica van Gieson staining for elastic fibers in the aortic media of the same individual from Fig. 2b.

f: Immunohistochemical staining for matrix metalloproteinase-9 (MMP-9) in the aortic media of a 78-year-old man with three coronary stenotic lesions.

The original magnification of all figures is 200x.

Table 2 The density of MMPs-positive cells in the aortic media

Groups	No of cases	Age (mean±SD)	MMP2-positive cells/mm² (mean±SD)	MMP12-positive cells/mm² (mean±SD)	MMP9-positive cells/mm² (mean±SD)
1 and **2**	18	62.7±10.8	8.2±14.9	9.9±15.0	169.9±59.0
3 and **4**	13	64.8±5.4	2.1±5.1	1.6±2.1#	154.6±72.2
5 and **6**	8	66.9±2.9	4.4±5.9	10.9±14.4	176.8±63.6

#$P<0.05$ versus the value of MMP12 of Groups **1** and **2**, or Groups **5** and **6**. MMP indicates matrix metalloproteinase. SD indicates standard deviation.

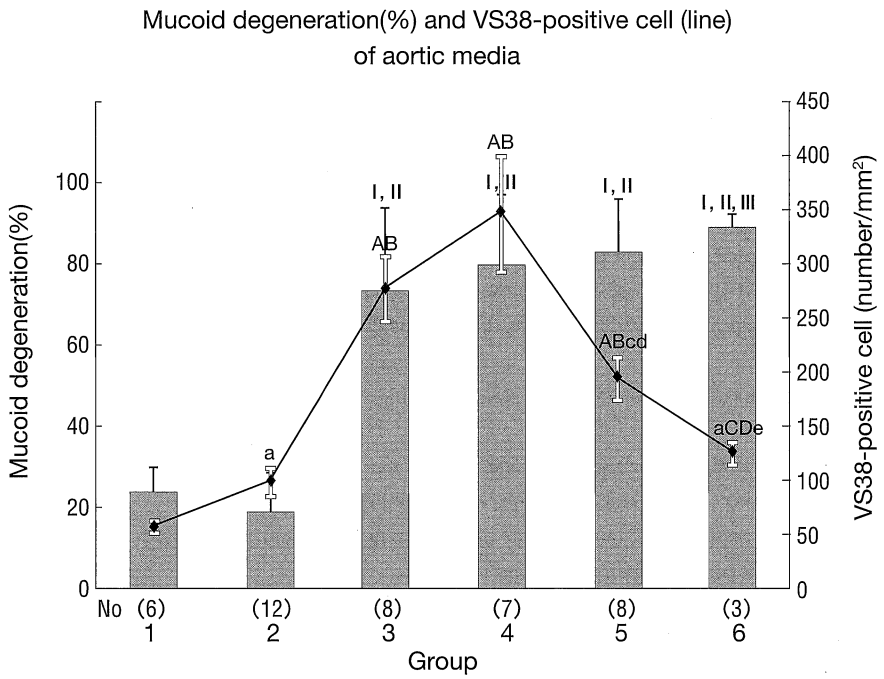

Fig. 3. The density of plasma cells and the degree of mucoid degeneration in the aortic media. The density of VS38-positive plasma cells and the degree of mucoid degeneration are shown by a line and bar, respectively.
a, c, d, e $P<0.05$ versus the value of VS38-positive cell (plasma cell) infiltration in Groups 1, 3, 4, and 5, respectively.
A, B, C, D $P<0.01$ versus the density of VS38-positive cell (plasma cell) infiltration in Groups 1, 2, 3, and 4, respectively.
I, II, III $P<0.05$ versus the value of mucoid degeneration in Groups 1, 2, and 3, respectively.

Table 3. The degree of fibrosis and mucoid degeneration.

Groups	No of cases	Age (Mean±SD)	Mucoid degeneration (%) (Mean±SD)	Fibrosis (%) (Mean±SD)
1 and 2	8	59.6±13.0	23.8±5.1	52.6±31.4
5 and 6	6	66.8±4.3	84.8±6.8*	60.1±28.7

*$P<0.01$ versus the value of mucoid degeneration of Groups1 and 2.
SD indicates standard deviation.

DISCUSSION

Plasma cells are B-lymphocytes that differentiate into antibody-secreting cells and produce antibody directed either against persistent antigen in the inflammatory site or against altered tissue components[18]. Some delay may occur before these antigens or components become effective. Thereafter, the differentiation from B lymphocytes into plasma cells may decrease, followed by the disappearance of persistent antigen or by the repair of antigen-altered tissue components at advanced stage, and then plasma cells may disappear or decrease. This idea is compatible with our results showing that plasma cell infiltration increased at the delayed medial atherosclerotic stage of Groups 3 and 4 and decreased in the advanced stage of Groups 5 and 6. Because plasma cell infiltration appeared in the limited stage, finding it in the aortic media would be difficult. Libby and Hansson reported that a delayed-type hypersensitivity-like reaction may be expected to contribute to chronic inflammation during human atherogenesis, but this claim has not been validated[32]. Our data may not support that study's conclusion, but may support the idea that plasma cell infiltration appeared at the delayed medial atherosclerotic stage. This phenomenon may be partially caused by an autoimmune reaction[20, 21, 22, 23, 24].

Proteoglycans in the aorta are largely synthesized by endothelial cells and by smooth muscle cells[33]. If the gradual increase of mucoid substances in the aortic media of atherosclerosis is only caused by infiltration from the intima or secretion from smooth muscles, then elastic fibers remain intact. However, elastic fibers showed signs of degeneration with mucoid degeneration in the aortic media of atherosclerosis. Elastic fibers in the media have been reported to be coated with type-III collagen, which forms strong interactions with proteoglycans and hyaluronic acid[34], and proteoglycans also partially localize with elastic fibers[35]. The degeneration of the elastic fibers may cause an increase in the mucoid substance in the aortic media. In this sense, the observed increase in mucoid substance in the atherosclerotic aortic media may be related to degeneration of elastic fibers. Mucoid degeneration and elastic fiber degeneration in the aortic media of atherosclerosis may be histologically similar to the features of the aortic media in Marfan's syndrome[36] or Loeys-Dietz syndrome[37]. Furthermore, it has been reported that mucoid degeneration, cystic medial necrosis, elastin fragmentation, and medionecrosis appear in the media of normal, aging aortas[38]. That report suggests that a medial type of atherosclerosis may exist without intimal changes.

MMP-positive cells are reported to be smooth muscle cells of aortic media[11, 39]. Our MMP-9-positive cells had large nuclei and a spindle-shaped morphology, similar to solitary desmin-positive smooth muscle cells in the analyzed cadavers. Thus, MMP-positive cells resemble smooth muscle cells.

MMP-9 and -2 are reported to be associated with mucoid degeneration in the aortic media[10]. Previously, it was demonstrated that the loss of MMP-9 or -12 prevents medial destruction, such as elastin degradation in atherosclerotic mice[40]. Other studies also reported that an increase in the serum level of MMP-9 is associated with a decrease of intracellular MMP-9-positive cells[41]. In our findings, at the delayed stage of Groups 3 and 4, MMP-positive cells decreased, and then recovered at the advanced stage of Groups 5 and 6.

Thus, we hypothesize that at the delayed stage of Groups 3 and 4, the increase of plasma cell infiltration stimulates the smooth muscle cells to secrete MMPs, which extends mucoid degeneration, following the degeneration of the elastic fibers.

Fibrosis is one of the major manifestations in atherosclerotic intima, and type-I collagen is a prominent matrix component in atherosclerotic plaques. Recently, it was reported that type-I procollagen is not detected in the area of media underlying atherosclerotic lesions[42], and our data agree with these findings.

In our study of medial changes in the ascending aorta of patients with coronary artery disease, we hypothesized that three groups of patients: those with one to three, four to five, and six to nine coronary stenotic lesions, represent early, delayed, and advanced medial atherosclerotic stages, respectively. At the early medial atherosclerotic stage, minor inflammatory reactions occurred. At the delayed stage, plasma cell infiltration and mucoid degeneration, increased in the media, and MMP-positive cells decreased. This stage may be due to a delayed reaction or an autoimmune reaction. At the advanced stage, plasma cells rapidly decreased, and mucoid degeneration gradually extended.

Recently, anti-inflammatory agents[43] and antibiotics[44] have been used to treat atherosclerosis. Anti-inflammatory agents may be effective for the suppression of medial degeneration at the delayed stage. If the cause of the delayed medial atherosclerosis stage is found, then an effective therapy to suppress atherosclerotic development can be introduced.

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