Inflammatory cells in perivascular adipose tissue and the integrity of the tunica media in atherosclerotic coronary arteries

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ABSTRACT

Obstructive coronary artery disease (CAD) is characterized by inflammation within the atherosclerotic coronary arteries. Infiltration of inflammatory cells into muscular media can lead to remodeling and weakening of the arterial wall. We examined the relationship between inflammatory infiltration in perivascular adipose tissue (PVAT), state of the external elastic membrane, and the intensity of inflammatory infiltration in the tunica media of coronary arteries obtained by endarterectomy from symptomatic patients with diffuse CAD. We analyzed endarterectomy sequesters from 22 coronary arteries that contained the intima, media, a part of the adventitia, and PVAT in at least one part of the sequester. The coronary arteries were divided into two groups according to the presence or absence of inflammatory infiltration in PVAT. Staining with hematoxylin-eosin and by the Movat's method showed atherosclerotic changes in the intima and media. Immunohistochemistry (anti-leukocyte common antigen [LCA] antibody) was used for the detection of leukocytes. We found a significant positive correlation between inflammatory infiltration in PVAT and preservation of the external elastic membrane of coronary arteries. Furthermore, we found a significant negative correlation between inflammatory infiltration in PVAT and the intensity of inflammatory infiltration in the media. It seems that the integrity of the external elastic membrane and the proinflammatory properties of PVAT restrain inflammatory cells within PVAT. Both effects may prevent the migration of inflammatory cells into the media and delay the development of CAD.

KEYWORDS: Perivascular adipose tissue; PVAT; atherosclerosis; coronary arteries; endarterectomy; CAD; coronary artery disease; inflammatory infiltration

INTRODUCTION

Coronary artery disease (CAD) is characterized by inflammation within the atherosclerotic coronary vessel wall [1-3]. For decades, the tunica intima and, partly, the tunica media were the focus of research, and perivascular adipose tissue (PVAT) that surrounds coronary arteries (CAs) was considered to be passive and only supportive tissue. Now, it has become evident that PVAT has a crucial role in maintaining the normal function of CAs [4-6], and that it is also involved in the development of CAD [7,8] and hypertension [9]. It has been shown that both vascular smooth muscle and PVAT release a variety of vasoactive factors as well as pro and anti-inflammatory cytokines that modulate vascular function and structure [8,10-14]. Increasing evidence supports the view that inflammatory cytokines released by PVAT may substantially contribute to CAD. On the other hand, PVAT reacts to cytokines released from the arterial wall. Evidently, the interplay between systemic effects such as inflammation, sympathetic tonus, and metabolites results in either pro or antiatherogenic effects.

Here, we present an observation that inflammatory infiltration of PVAT and the intensity of inflammatory infiltration in the media of CAs obtained by endarterectomy from symptomatic patients with diffuse obstructive CAD are inversely reciprocal. Infiltration of the media by inflammatory cells leads to pronounced remodeling and weakening of the vascular wall.

MATERIALS AND METHODS

Statement of ethics

The study and all procedures were pre-approved by the National Medical Ethics Committee (MEC 170/07/13, MEC

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110/03/16). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from each patient included in the study.

Brief description of surgical procedure and sample collection

Endarterectomy was performed in the CAs of 100 symptomatic patients with diffuse obstructive CAD.

Through a 10–15 mm long longitudinal incision, the sclerotic and segmentally calcified intima and media were stripped with fine forceps, clamped using a mosquito clamp, and gently pulled out from the distal end of the CAs. The adventitia was pushed in the opposite direction with the forceps until all the thickened sclerotic and calcified sequesters were removed. The intima and media were removed from the proximal end of the CAs in the same manner as described above [15].

Endarterectomized samples were obtained from the left anterior descending CA, the left circumflex CA, or the right CA. The sequesters were transversally cut into 0.5 cm long pieces. Every other piece was then cut into ten step-serial sections of 5 μm. The step between the two sections was 50 μm thick. In 22 out of 100 patients, the endarterecomized CA samples contained a part of the adventitia and PVAT in at least one step-serial section (Figure 1A). The CAs obtained from the other 78 subjects contained only the intima, media, and very few (if any) tissue of the adventitia and were thus excluded from further analysis (Figure 1B).

Histological analysis

We embedded tissue samples in paraffin and cut them into 5 μm thick step-serial sections. Then, the sections were stained with hematoxylin-eosin (HE) and Movat's pentachrome staining. Immunohistochemistry was used for the detection of leukocytes (leukocyte common antigen [LCA], 1:50, DACO, Glostrup, Denmark) following the manufacturer's instructions.

The CA sections were then divided into two groups according to the inflammatory infiltration in PVAT: group-0 (n= 13) with an absence or very few scattered LCA positive cells in PVAT and group-1 (n = 9) with dense inflammatory infiltrate in PVAT. The external elastic membrane (EM) in the vessel wall was evaluated as either fragmented or preserved. According to our previous studies, we assessed the intensity of inflammation in the media by the number of LCA positive cells counted in the media [1] and designated as absent (0–4), minor (5–9), or major (10 or more LCA positive cells).

Statistical analysis

For statistical evaluation of the data, Mann–Whitney U-test ($p < 0.05$) and Spearman's coefficient of correlation ($p < 0.05$) were calculated using IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp., Armonk, NY).

RESULTS

As shown in Figure 2, the sections of all CAs showed atherosclerotic changes, with fibroproliferation in the intima, disarrangement, and loss of vascular smooth muscle cells and fibrosis in the media. Some of the CAs also showed inflammatory infiltration around vasa vasorum in the media and adventitia (Figure 2A and B). The intensity of inflammatory infiltration in the media was significantly more prominent in group-0 than in group-1 (p = 0.014, Mann–Whitney U-test, Figure 2C).

The internal and external EMs of CAs were either fragmented or preserved (Figure 2E and F). The external EM was more frequently preserved in group-1 than in group-0 (p = 0.004, Mann–Whitney U-test, Figure 2D).

We found a significant positive correlation between inflammatory infiltration in PVAT and preservation of the external EM of CAs (p = 0.002, Spearman's correlation) and a significant negative correlation between inflammatory

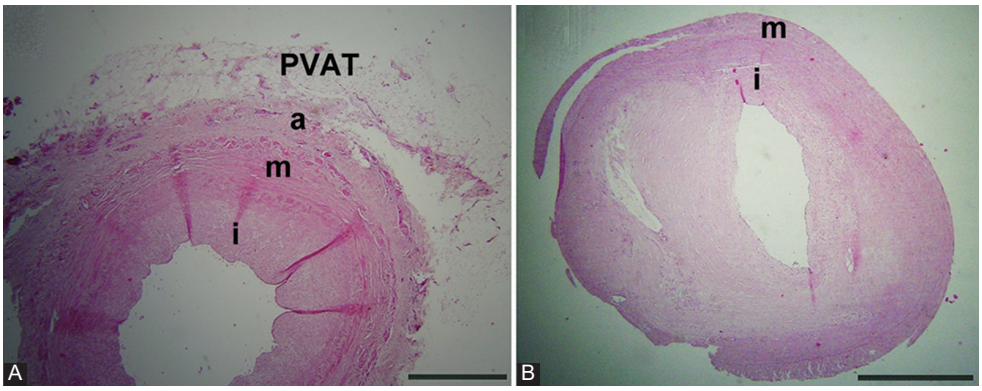

FIGURE 1. Endarterectomy samples (hematoxylin-eosin [HE]). (A) Coronary artery (CA) consists of the tunica intima, media, and a part of the adventitia with perivascular adipose tissue (PVAT). These CAs were included in the study; (B) CA contains only the intima and media. These CAs were excluded from the study. i – intima, m – media, a – adventitia, bars: 1000 μm (A,B).

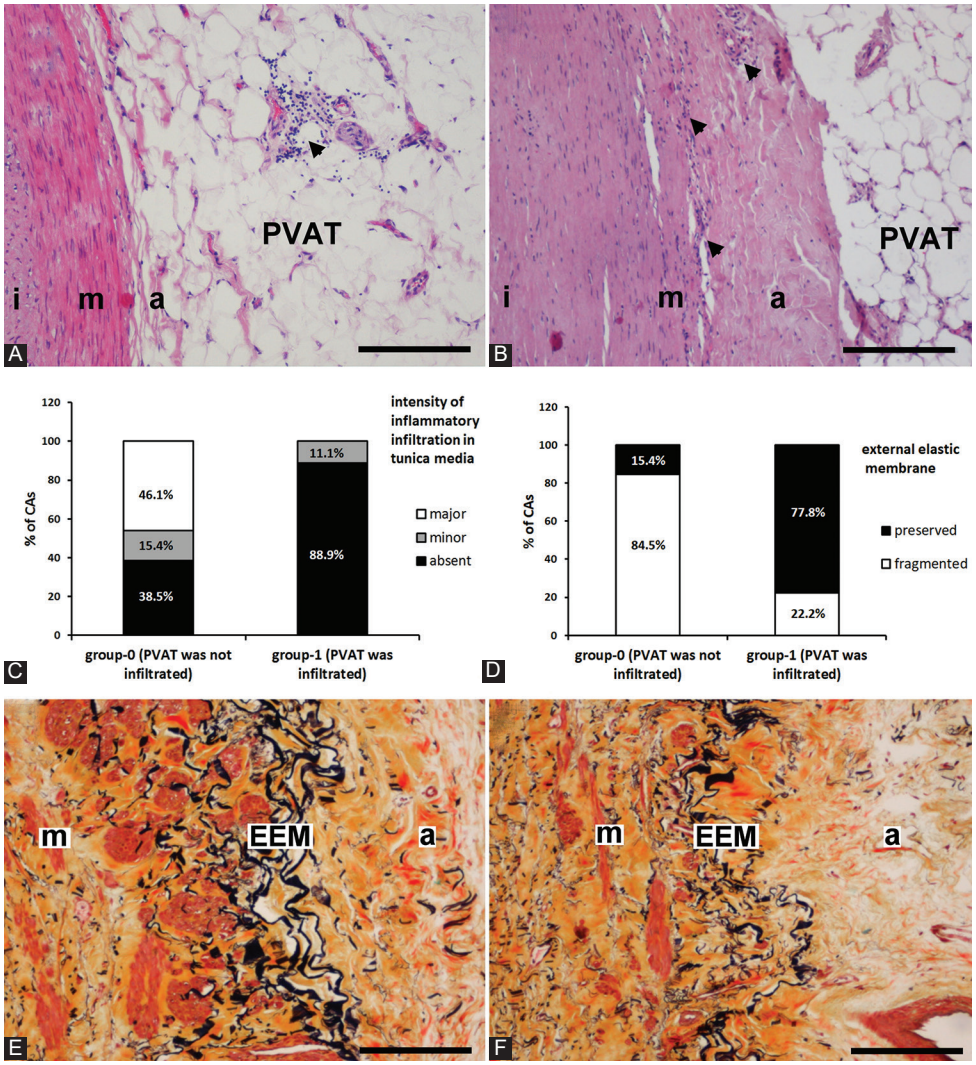

FIGURE 2. The state of the tunica media and perivascular adipose tissue (PVAT) in coronary arteries (CAs). (A) Inflammatory infiltration in PVAT and preserved media in group-1 (hematoxylin-eosin [HE]); (B) absence of inflammatory infiltration in PVAT, media with inflammatory cells in group-0 [HE]; (C) the percentage of CAs with absent, minor and major intensity of inflammatory infiltration in the media in group-0 and group-1 (p = 0.014, Mann–Whitney U-test); (D) the percentage of CAs with preserved or fragmented external elastic membrane (EEM) in group-0 and group-1 (p = 0.004, Mann–Whitney U-test); (E) preserved EEM in group-1 (Movat's pentachrome); (F) fragmented EEM in group-0 (Movat's pentachrome). i – intima, m – media, a – adventitia, arrow – inflammatory infiltration; bars = 200 μm (A, B, E, F).

infiltration in PVAT and the intensity of inflammatory infiltration in the media (p = 0.01, Spearman's correlation).

DISCUSSION

In the current study, we showed that inflammatory infiltration in PVAT negatively affects the intensity of inflammatory infiltration in the media of CAs. The media remained protected from the inflammatory infiltration in the samples with inflammatory infiltration of PVAT. Our findings were unexpected, as several previous studies support the view that PVAT, primarily when infiltrated with inflammatory cells, promotes the development of CAD [7,9,16]. The primary function of the media is to maintain appropriate arterial tone allowing adequate perfusion of tissues and maintaining appropriate blood pressure. Both demands are achieved by the systemic sympathetic control and paracrine vasoactive factors released by the cells of the arterial wall. It has been shown that not only endothelial cells but also adipocytes from PVAT, as well as inflammatory cells, play an essential role in maintaining vascular tone and structure [6,12,16-19]. In healthy adipose tissue, including PVAT, alternatively activated macrophages M2 prevail. M2 macrophages secrete antiinflammatory cytokines and contribute to sympathetic/adrenergic effects on PVAT [20]. Secretion of cytokines and chemokines by PVAT can significantly remodel the CA's media and induce infiltration by inflammatory cells [6,13,21].

On the other hand, the physiology and morphology of PVAT are influenced by inflammatory cytokines, the sympathetic nervous system, and vasoactive peptides [20,22-24]. Despite a growing evidence of the vital role of normal PVAT in maintaining a healthy structure of the arterial wall and a

detrimental effect of diseased PVAT on the development of atherosclerosis, the interplay of all the factors remains to be adequately understood. Our observation of the restraint of macrophages in PVAT and infiltration of the media by macrophages in the absence of infiltration of PVAT is intriguing. One of the possible explanations for this might be that the intact external EM prevents migration of inflammatory cells. Remodeling of the external EM by metalloproteases may allow inflammatory cells to pass into the media. We found that the external EM often remained preserved in the CAs that contained inflammatory infiltration in PVAT. Moreover, we found a positive correlation between inflammatory infiltration in PVAT and the presence of the external EM, and a negative correlation between inflammatory infiltration in PVAT and the intensity of inflammatory infiltration in the media of CAs. This finding may be explained by the immunomodulatory potential of vascular smooth muscle cells. These cells react to stressors by the release of several cytokines and chemokines that affect macrophages, T cells, and other inflammatory cells in the vascular wall, including PVAT [13]. Activated proteases may disrupt the extracellular matrix and the external EM allowing infiltration of the media by inflammatory cells. Inflammatory substances released from the arterial wall reach PVAT, which reacts to those substances by local lipolysis and inhibition of adipogenesis, leading to decreased lipid and increased water content of PVAT. This has already been used as a marker in computed tomography angiography [21]. Our findings are supported by Kralova Lesna et al. [25] who found "a close relationship between the ratio of macrophages in the arterial wall and adjacent perivascular adipose tissue in the coronary heart disease group, but not in the dilative cardiomyopathy group". Evidently, the infiltration of PVAT with immune cells may have different outcomes, depending on the interaction between macrophages in PVAT and cells in the arterial wall.

CONCLUSION

We conclude that the paracrine function of healthy PVAT and preserved external EM may protect the tunica media from infiltration with inflammatory cells and prevent the progress of the atherosclerotic process in CAs. The proinflammatory properties of PVAT may not have an obligatory deleterious role as previously proposed. The integrity of the external EM may be a decisive prognostic factor for the outcome of the atherosclerotic process in CAs, and it may be controlled by the release of proinflammatory substances from the media and PVAT.

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