Roles of Intercellular Adhesion Molecule-1 in Hypertensive Cardiac Remodeling

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Abstract—Recently, we have shown that in rats with a suprarenal abdominal aortic constriction (AC), pressure overload induces early perivascular fibro-inflammatory changes (transforming growth factor [TGF]-β induction and fibroblast proliferation) within the first week after AC and then causes the development of cardiac remodeling (myocyte hypertrophy and reactive myocardial fibrosis) associated with diastolic dysfunction. Intercellular adhesion molecule (ICAM)-1 is implicated in the recruitment of leukocytes, especially macrophages, in various inflammatory situations. Thus, we sought to investigate the causal relation of ICAM-1 to macrophage recruitment and cardiac remodeling in AC rats. In AC rats, immunoreactive ICAM-1 was observed transiently on endothelial cells of the intramyocardial coronary arterioles after day 1, with a peak at day 3, returning to baseline by day 7. Also, ED1+ macrophage accumulation was found in the area adjacent to the arteries expressing ICAM-1. Chronic treatment with an anti–ICAM-1 neutralizing antibody, but not with control IgG, remarkably reduced the accumulations of macrophages and proliferative fibroblasts and inhibited the upregulation of TGF-β expression. Furthermore, the neutralizing antibody significantly prevented myocardial fibrosis without affecting arterial pressure and left ventricular and myocyte hypertrophy. In conclusion, ICAM-1 expression was induced by pressure overload in the intramyocardial arterioles, and triggered perivascular macrophage accumulation. In pressure-overloaded hearts, a crucial role in ICAM-1–mediated macrophage accumulation was suggested in the development of myocardial fibrosis, through TGF-β induction and fibroblast activation. (Hypertension. 2003;41[part 2]:819-823.)

Key Words: cell adhesion molecules • fibrosis • hypertension, experimental • macrophages

There is increasing evidence that the inflammatory process is significantly involved in the fibrotic change of various disease situations. In the hypertrophied hearts of spontaneously hypertensive rats (SHR) and experimental renovascular hypertensive rats, inflammatory cells, especially macrophages, are found in the perivascular space and are colocalized with activated fibroblasts showing replication and extracellular matrix production.1-2 Recently, in rats with a suprarenal aortic constriction (AC), we have demonstrated that cardiac hypertrophy develops, associated with marked reactive myocardial fibrosis and diastolic dysfunction.3 In this model, we have shown that pressure overload induces fibro-inflammatory changes within the first week after AC, preceding myocyte hypertrophy and myocardial fibrosis (Figure 1): (1) pressure overload induced fibroblast proliferation in the perivascular space after day 1, with a peak at day 3, returning to lower levels by day 28; (2) transforming growth factor (TGF)-β expression was upregulated after day 3, peaked at day 7, and remained moderately elevated during progression of myocardial fibrosis; and (3) TGF-β–mediated myocardial fibrosis is the major determinant of diastolic dysfunction.3 Adhesion molecules allow cells to adhere to other cells or to extracellular matrix molecules. Adhesion processes are required for cells to interact and to migrate to their destinations. Intercellular adhesion molecule (ICAM)-1 is the major adhesion receptor for monocyte/macrophage attachment to endothelial cells (ECs) at the sites of inflammation and is inducible by various inflammatory stimuli.4,5 Also, in SHR and renovascular hypertensive rats, it has been shown that ICAM-1 is upregulated in ECs of the intramyocardial arterioles, especially in those adjacent to perivascular fibrosis.1,2 Thus, a role of ICAM-1 was suggested in the fibrotic process in hypertensive hearts. A causal relation of ICAM-1 induction to myocardial remodeling, however, has not been determined.

The aim of this study was to clarify the role of ICAM-1 during cardiac hypertrophy induced by hypertension. For this purpose, the effects of ICAM-1 function blocking by an anti–ICAM-1 monoclonal neutralizing antibody (NAb) on the early fibro-inflammatory changes (macrophage infiltration and fibroblast proliferation at day 3; TGF-β expression at day 7) and the late myocardial remodeling (myocyte hypertrophy and myocardial fibrosis at day 28) were investigated in pressure-overloaded hearts of AC rats.
Methods

This study was approved by the institutional committee for the ethics of animal care and treatment. After male Wistar rats (300 to 400 g) were anesthetized intraperitoneally with pentobarbital (50 mg/kg), AC or the sham-operation (sham) was performed. Blood pressure was measured in the unrestricted, conscious state through a heparinized indwelling catheter. Unless otherwise indicated, 6 rats were studied in each group for each time point.

Protocol 1

Tissue Preparation and Morphometry

Rats were killed with an overdose injection of intraperitoneal pentobarbital and were perfusion-fixed with 4% glutaraldehyde in Hanks' solution at 100 mm Hg. The left ventricle (LV) was processed for histological and immunohistochemical studies as described elsewhere. For immunohistostaining for ICAM-1 or ED1, the LV was snap-frozen in acetone/dry ice, embedded in OCT compound, and sectioned with cryostat. To evaluate myocyte hypertrophy and myocardial fibrosis, 3 independent hematoxylin-eosin-stained and 3 Mallory-Azan-stained cross-sections (5 μm in thickness) of each rat were scanned and analyzed using a digital image analyzer, respectively. The shortest transverse myocyte diameter was measured in 50 nucleated transverse sections of the myocytes in each tissue section. The percentage area of myocardial fibrosis was calculated as previously described.

Immunohistostaining

The sections were subjected to immunohistostaining with an antibody for ED-1 (Chemicon International) or ICAM-1 (Santa Cruz) and a commercially available detection system (DAKO). The labeled cells were counted at 200 magnification in 4 independent whole cross-sections of each animal.

RNA Extraction and RT-PCR Analysis

Total RNA was extracted from unfixed hearts as described elsewhere. For quantitative analysis of TGF-β expression, real-time TaqMan RT-PCR was performed with the relative standard curve method. Aliquot (25 ng) of total RNA was reverse-transcribed and amplified in triplicate with TaqMan EZ RT-PCR kit (PE Biosystems). PCR conditions and the nucleotide sequences of PCR primers and TaqMan probes for TGF-β were as previously described. The expression level of TGF-β mRNA was normalized by the GAPDH level in each sample, and the relative changes in TGF-β expression were expressed as an n-fold increase relative to sham rats.

Results

Protocol 2

For ICAM-1 function blocking, rats were treated intravenously with 2 mg/kg per day of NAb (AC+NAb or sham+NAb rats) or subclass-matched control IgG (R&D Systems; AC+IgG or sham+IgG rats) daily from 1 day before the operation to day 28. The NAb (1A29) was a gift from Dr. Masayuki Miyasaka, Osaka University (Osaka, Japan). The ability of this antibody to neutralize ICAM-1 activity was confirmed and described previously. In the present study, the same dose of NAb was used as in the previous study.

Statistical Analysis

Quantitative analysis was performed by a single observer in a blind fashion. One-way ANOVA, followed by the Scheffe F test, was performed for statistical comparisons. A value of P<0.05 was considered significant.

Figure 1. Schematic diagram of the temporal changes in the early fibro-inflammatory responses and myocardial fibrosis in pressure-overloaded hearts. Pressure overload induced fibroblast proliferation in perivascular space after day 1, with a peak at day 3, returning to lower levels by day 28. Myocardial TGF-β expression was upregulated after day 3, peaked at day 7, and remained moderately elevated at day 28. After day 7, myocardial fibrosis progressively extended from the perivascular space into the intermuscular interstitium, and typical features of reactive fibrosis were observed at day 28.

ICAM-1 Expression in Pressure-Overloaded Hearts

Immunoreactivity for ICAM-1 was not found in the hearts of the sham rats (Figure 2A). In AC rats, immunoreactive ICAM-1 was transiently induced on the luminal surface of the
ECs of the intramyocardial arteries after day 1, with a peak at day 3, declining to basal level by day 7.

In sham rats, almost no cells were labeled with ED1 during the observation period (data not shown). In AC rats, at day 3, the accumulation of ED1+ macrophages (Figure 2B) was observed in the perivascular space adjacent to the arteries showing ICAM-1 expression (Figure 2Ac).

Protocol 2
Anti-ICAM-1 NAb or control IgG was administered every day from 1 day before the operation to block ICAM-1 function. MAP was similar in AC+IgG and AC+NAb rats at day 28 (Table). At day 28, LVW/BW was slightly less in AC+NAb than in AC+IgG rats, but the difference was not significant. Control IgG had no effects on these parameters in sham rats (data not shown). Apparently, adverse effects were not observed in rats treated with NAb or IgG.

Macrophage Accumulation and Fibroblast Proliferation
The effects of NAb on the early fibro-inflammatory changes in pressure-overloaded hearts, characterized by macrophage accumulation and fibroblast proliferation, were investigated (Figure 3A). At day 3, the peak numbers of ED1+ macrophages and BrdU+ proliferating fibroblasts were observed in AC+IgG rats. NAb reduced remarkably the pressure overload-induced increases in macrophages and BrdU+ fibroblasts. These counts did not differ between AC and AC+IgG rats (data not shown). Sham rats treated with IgG or NAb and AC rats treated with IgG did not show macroscopic or microscopic abnormalities, as compared with sham and AC rats, respectively (data not shown).

Myocardial TGF-β was induced by AC after day 3, peaked at day 7, and remained moderately elevated by day 28. ICAM-1 blocking remarkably inhibited the AC-induced TGF-β induction at day 7 (Figure 3B).

Myocardial Remodeling
Effects of NAb on the late myocardial remodeling were investigated at day 28 (Figure 3C). AC induced a marked increase in the area of myocardial fibrosis, and the AC-induced fibrosis was markedly prevented by NAb. NAb had no effect on myocyte hypertrophy in response to pressure overload.

Discussion
The present study demonstrated that ICAM-1 expression was transiently induced by pressure overload in ECs of the intramyocardial arteries, beginning within day 1 and reaching a peak at day 3. Macrophage accumulation was found in the perivascular space adjacent to the arteries showing ICAM-1 expression (Figure 2Ac). Furthermore, NAb selectively prevented reactive myocardial fibrosis, but not myocyte hypertrophy, in pressure-overloaded hearts. These effects of NAb appeared independent of arterial pressure.

In the present study, we used the pressure-overloaded hearts of AC rats to investigate the role of ICAM-1 in...
myocardial remodeling in hypertensive hearts because this is a cardiac hypertrophy model characterized by a rapid progression of reactive myocardial fibrosis associated with diastolic dysfunction.\(^1\) In this model, we have shown that pressure overload induces early fibro-inflammatory changes (TGF-\(\beta\) induction and fibroblast activation) within the first week after AC and then develops myocardial remodeling (myocyte hypertrophy and reactive myocardial fibrosis) in the later phase.\(^3\) However, the mechanism which triggers the early fibro-inflammatory changes remained undetermined.

Earlier studies suggested that hypertension enhances responsiveness of ECs to factors that promote monocyte/macrophage adhesion, because cytokine- and endotoxin-stimulated ICAM-1 expression and macrophage infiltration are more intense on ECs derived from SHR compared with cells from normotensive Wistar rats.\(^12\) Also, it was reported that SHR showed increased ICAM-1 expression on the ECs of the arteries, suggesting the role of ICAM-1 in hypertensive end-organ damage.\(^13\) In the present study, ICAM-1 was induced on the ECs of the intramyocardial arterioles in pressure-overloaded hearts of AC rats. It is noteworthy that the AC-induced ICAM-1 induction is an early and transient event, beginning immediately after induction of hypertension and preceding other fibro-inflammatory changes and myocardial remodeling. Moreover, perivascular macrophage accumulation was well colocalized with the ICAM-1–expressing intramyocardial arterioles (Figure 2). These observations may indicate that ICAM-1 induction is a trigger for the inflammatory changes, but is not a consequence of hypertensive tissue damage, in pressure-overloaded hearts.

To further investigate a causal relation of ICAM-1 to the observed changes, we used an anti–ICAM-1 NAb (1A29), because this NAb is an established and effective tool for blocking ICAM-1 function in vivo in various experimental animal models, including vascular remodeling,\(^11\) glomerulonephritis,\(^14\) adjuvant arthritis,\(^15\) and autoimmune thyroiditis.\(^16\) As expected, NAb remarkably attenuated the macrophage accumulation around the arterioles in pressure-overloaded hearts (Figure 3A). Thus, it is plausible that ICAM-1 plays a key role in the recruitment of blood-borne monocytes/macrophages into the perivascular space in response to pressure overload.

Another important finding of this study was that the inhibition of macrophage recruitment by ICAM-1 function blocking not only markedly reduced TGF-\(\beta\) induction and fibroblast proliferation in the early phase (Figures 3A and 3B) but also prevented myocardial fibrosis in the later phase (Figure 3C). Given that the transmigration of macrophages is thought to be the earliest and most significant inflammatory event in vascular lesion formation, such as atherosclerosis,\(^17\) it is suggested that macrophage recruitment plays a key role in triggering the fibro-inflammatory process in pressure-overloaded hearts. Furthermore, these findings raised the possibility that the inhibition of the fibro-inflammatory process in the early phase is an effective strategy for preventing myocardial fibrosis in the later phase, which is implicated in diastolic dysfunction in pressure-overloaded hearts.

In the present study, we have not elucidated the initial mechanism responsible for ICAM-1 induction in response to pressure overload. There is increasing evidence that inflammatory responses lead to alterations of the chemotactic and adhesive property of ECs that support monocyte/macrophage chemotaxis, adhesion, and transmigration into the vessel wall.\(^4\) Recent studies suggested that pressure overload itself is a strong proinflammatory factor because mechanical strain can induce inflammatory cytokines, growth factors, and oxidative stress in the resident cells of the vessel wall, including ECs, smooth muscle cells and inflammatory cell infiltrates.\(^18\) The interplay of these factors may regulate ICAM-1 expression in AC rats. Further studies are needed to address this issue.

**Perspectives**

The present study demonstrated that ICAM-1 is transiently induced on ECs in response to pressure overload and plays a crucial role in macrophage recruitment. Also, ICAM-1 function blocking not only reduced the macrophage accumulation but also attenuated TGF-\(\beta\) induction, fibroblast proliferation, and the subsequent myocardial fibrosis in pressure-overloaded hearts, indicating that ICAM-1 is a key molecule for the initiation of the macrophage-mediated fibro-inflammatory process in hypertensive hearts. Because we cannot deny the possibility that the mechanism, independently of macrophage accumulation, is involved in the observed effects of ICAM-1 function blocking, further investigation is needed to clarify the role of macrophage recruitment in hypertensive myocardial remodeling. Finally, ICAM-1 may be a new molecular target for preventing myocardial remodeling in pressure-overloaded hearts.

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