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Expression of vascular cell adhesion molecule-1 (VCAM-1) in the aortae of hypercholesterolemic rabbits with high (HAR) and low (LAR) atherosclerotic response

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Abstract

Recently we have described two strains of rabbits, one with a low (LAR) the other with a high (HAR) atherosclerotic response to dietary hypercholesterolemia. After feeding a cholesterol diet for 12 weeks, HAR rabbits developed atherosclerotic lesions throughout the entire aortic arch and thoracic aorta. In contrast, the lesions in LAR rabbits were mainly confined to the aortic arch. Presently we studied the cellular composition and expression of vascular cell adhesion molecule-1 (VCAM-1) in aortic lesions and in the uninvolved aorta of cholesterol fed HAR and LAR rabbits. Plasma cholesterol levels were 1106 ± 160 and 1152 ± 232 mg/dl in HAR and LAR rabbits, respectively, and the distribution of cholesterol among the lipoprotein fractions was similar after 16 weeks of 0.5% cholesterol feeding. In analogy to our previous findings, in the HAR rabbits more than 70% of the aorta (aortic arch and thoracic aorta) was covered with lesions, whereas in the LAR rabbits the lesions were seen in the aortic arch only and covered less than 20% of the total aortic surface. The cellular composition of aortic lesions was defined using specific antibodies to macrophages, smooth muscle cells, T lymphocytes and Ia expressing cells. All these cellular elements were represented in lesions derived from both strains of rabbits. We also examined the expression of VCAM-1 in the aorta of HAR and LAR rabbits after cholesterol feeding. In the aortic arch, a positive reaction for VCAM-1 was found in lesions from both strains of rabbits. The staining was seen in the endothelium and within the lesion, mainly at its base. In the thoracic aorta of HAR rabbits, VCAM-1 expression was found in all lesions examined. In the thoracic aorta of LAR rabbits, VCAM-1 expression was seen in an occasional very small lesion found at the ostium of an intercostal artery. These results show that the VCAM-1 gene is expressed in the LAR rabbits, but its induction is perhaps attenuated.

Keywords: Atheroma; Adhesion molecules; Endothelium; Macrophages; SMC

1. Introduction

One of the earliest events in human and experimental atherosclerosis is attachment of monocytes to the endothelial surface [1–3]. The adhesion of monocytes to endothelium has been shown to be a multi-step process, involving a series of adhesion molecules [4], among which the vascular cell adhesion molecule-1 (VCAM-1) plays an important role. This adhesion molecule, a member of the immunoglobulin gene superfamily, was shown to be inducible by LPS and cytokines such as IL-1 and TNF [5–7]. In vivo VCAM-1 expression was demonstrated in Watanabe rabbits [7] and it was also induced by cholesterol feeding in New Zealand white rabbits [8]. Focal VCAM-1 expression was observed in

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aortic endothelium after 1 week of cholesterol feeding at a time when no adherent or intimal macrophages were seen. After 3 weeks, VCAM-1 expression was seen in endothelium overlying intimal lesions composed of macrophages [8]. In human coronary arteries, VCAM-1 staining of surface endothelium occurred in 39% of fibrous and 20% of lipid-containing plaques [9]. In another study, VCAM-1 expression in atherosclerotic lesions of human coronary arteries was present also in areas of neovascularization and inflammatory infiltrate at the base of the atherosclerotic plaques [10].

We have characterized two strains of rabbits, one that shows a low atherosclerotic response (LAR) to dietary hypercholesterolemia and the other with the usual high atherosclerotic response (HAR) [11]. Both strains of rabbits when kept on a cholesterol-rich diet develop similar levels of hypercholesterolemia and no differences were found in the lipoprotein distribution and lipid protein composition. Recently, we described that the induction of scavenger receptor by PDGF or phorbol ester was significantly lower in smooth muscle cells (SMC) and fibroblasts derived from LAR rabbits as compared with cells from HAR rabbits [12].

In search of a putative reduced interaction between monocytes and vascular endothelium in LAR rabbits, we examined the expression of VCAM-1 [7–10] in aortae derived from cholesterol-fed LAR and HAR rabbits.

2. Materials and methods

2.1. Animals and diets

New Zealand white rabbits were obtained from two rabbit colonies with low and high atherosclerotic response [11]. Four HAR and four LAR rabbits were fed a diet enriched with 0.5% cholesterol for 16 weeks. Blood was drawn from the main artery of the ear into tubes containing ethylene diamine tetraacetic acid (1 mg/ml) and aliquots of serum were taken for determination of cholesterol, triglycerides, phospholipids and isolation of lipoproteins. The animals were killed by i.v. injection of pentobarbital, the aorta was taken out carefully and opened longitudinally. The native aorta was photographed immediately and eight segments, about 3 mm wide, were removed from sites in the aortic arch and the upper and lower thoracic aorta (Fig. 1). The tissue was embedded in tissue-tek (Miles, Elkhart, IN), snap frozen in isopentane and kept in liquid nitrogen. The extent of atherosclerotic involvement of the aorta was determined planimetrically as described [11] using the photographs taken from the unstained aortae.

2.2. Immunostaining

Serial frozen sections (6 μm thick) were placed on poly-L-lysine coated slides, air dried and fixed in acetone at -20°C for 10 min. Endogenous peroxidases were inactivated with H_2O_2. Non-specific binding was blocked with 20% horse serum for 20 min. Sections were incubated for 10 min with monoclonal mouse IgG1 antibody Rb 1/9, which recognizes VCAM-1. The antibody was a generous gift from Dr Myron Cybulsky, Boston, MA. After washing with PBS, biotinylated horse anti-mouse IgG was applied as secondary antibody and the sections were incubated with avidin-biotin–peroxidase complex according to the instructions of the supplier (ABC-Elite, Vectastain Labs, Burlingame, CA). Peroxidase activity was visualized with 3-amino-9-ethyl-carbazole (Sigma, St. Louis, MO). Sections were counterstained with Mayer's Hemalum (Merck, Darmstadt, Germany). Monoclonal antibody (RAM11 (dilution 1:500) which recognized rabbit macrophages [13] was supplied from Dako (Carpinteria, CA). Monoclonal antibodies against rabbit T-cells (L11/135) and Ia antigen (2C4), derived from hybridoma supernatants were a generous gift of Dr Georg Wick (Innsbruck, Austria). Antibodies against macrophages, T-cells and Ia antigen were visualized as described for VCAM-1. Monoclonal antibody asm-1, which recognizes smooth muscle cell specific α-actin was obtained from Boehringer (Mannheim, Germany). Anti mouse Ig-fluorescein $(F9ab')_2$ fragment (Boehringer Mannheim) was used as secondary anti-

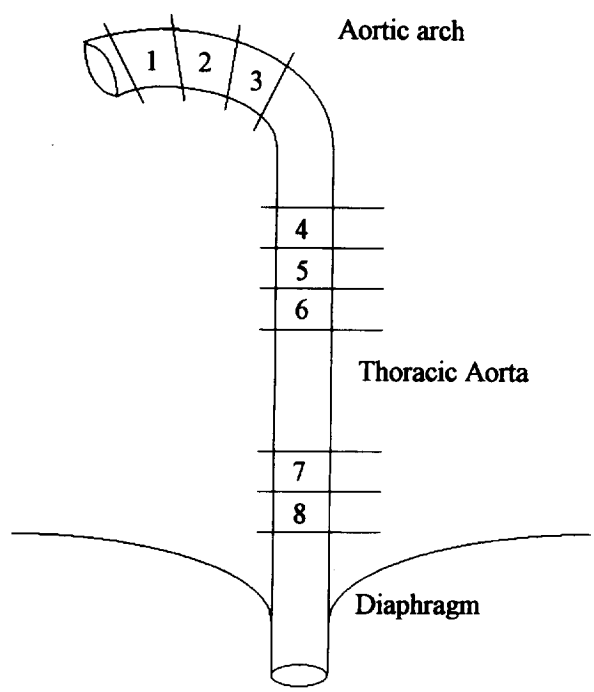

Fig. 1. Schematic representation of sites of sampling of aorta for immunocytochemistry.

Table 1
Plasma cholesterol and triglycerides in HAR and LAR rabbits

	Cholesterol	Triglycerides	VLDL-cholesterol	IDL-cholesterol	LDL-cholesterol	HDL-cholesterol
HAR	1106 ± 160	101 ± 52	586 ± 97	123 ± 13	128 ± 37	20 ± 3
LAR	1152 ± 232	46 ± 11	549 ± 90	183 ± 36	191 ± 56	20 ± 3

Values (mg/dl) are mean ± S.E. of four rabbits in each group fed 0.5% cholesterol for 16 weeks. Lipoprotein fractions were isolated by ultracentrifugation as in Section 2. Plasma phospholipids were 361 ± 66 and 358 ± 58 mg/dl in HAR and LAR rabbits, respectively.

body (dilution 1:10). Staining was performed according to the recommendations of the supplier. Sections were mounted in Moviol (Hoechst, Frankfurt, Germany) and examined with a Leitz-DMRXE-immunofluorescence microscope (Leica, Wetzlar, Germany). The presence of intact endothelium was ascertained by staining with a polyclonal goat anti-human von Willebrand factor (vWf) antibody (goat IgG, Atlantic Antibodies, Stillwater, MN), which shows cross reaction with rabbit vWf [7].

2.3. Plasma lipoproteins

Plasma cholesterol, triglycerides and phospholipids were determined by standard techniques. Plasma lipoprotein cholesterol concentrations were measured at sacrifice. Very low density lipoprotein (VLDL) ($d <$ 1.006 g/ml), intermediate density lipoprotein (IDL) ($d = 1.006–1.02$ g/ml), low density lipoprotein (LDL) ($d = 1.02–1.063$ g/ml) and high density lipoprotein (HDL) ($d = 1.063–1.21$ g/ml) were isolated from rabbit plasma by sequential ultracentrifugation in a fixed angle rotor using a Beckman TL 100 ultracentrifuge [11,14].

3. Results

Plasma cholesterol, triglycerides, VLDL-C, IDL-C, LDL-C and HDL-C levels after 16 weeks of 0.5% cholesterol feeding are shown in Table 1. HAR and LAR rabbits developed hypercholesterolemia of 1106 ± 160 and 1152 ± 232 mg/dl, respectively. It is evident, that all the parameters studied did not show any statistically significant differences between HAR and LAR rabbits. In analogy to previous findings [11,12], the extent of atherosclerotic involvement in HAR rabbit aortae was 70% of the surface area or more, and lesions were seen in both the aortic arch and the thoracic aorta. In the LAR aortae, the atherosclerotic lesions were seen over 20% or less of the total aortic surface, and were limited to the aortic arch. There were only a few spotty lesions in the thoracic aorta, which were localized at the ostia of the intercostal arteries.

Sections taken from the aortic arch of both HAR and LAR rabbits contained atherosclerotic lesions. The cellular elements in these lesions were macrophages, SMC and T-lymphocytes, which were identified by RAM11, anti α-actin and L11/135 (RTC) antibodies, respectively. Qualitatively, the cellular composition of lesions found in aortae of LAR rabbits did not differ from that in HAR rabbits (Fig. 2). The Ia antigen positive cells, visualized by the 2C4 antibody, were also seen in lesions in both strains of rabbits (Fig. 2). In lesions found in the aortic arch of both HAR and LAR rabbits, there was expression of VCAM-1 on the endothelial surface and inside the pathological intima (Fig. 3). In some lesions, the most prominent staining for VCAM-1 was seen at the base of the lesion at the intima–media border (Fig. 3). In adjacent sections stained for α-actin and VCAM-1, it appeared that in the lesions the elements positive for VCAM-1 corresponded to those stained by anti α-actin. No VCAM-1 expression was seen in the media. Sections from upper or lower thoracic aorta from HAR rabbits showed widespread atherosclerotic involvement and VCAM-1 expression (Fig. 4). However, as stated above, there were practically no lesions in the thoracic aortae of LAR rabbits and no VCAM-1 expression in uninvolved regions. Only one very small lesion was found near an ostium of an intercostal artery and the presence of VCAM-1 could be demonstrated (Fig. 4).

4. Discussion

In the present study we examined the possibility that the low atherosclerotic response of rabbits to diet induced hypercholesterolemia could be related to impairment in the induction of VCAM-1. When compared to the HAR rabbits, the formation of atheromas in the LAR rabbits is delayed. Therefore, we chose to examine VCAM-1 expression after 16 weeks of hypercholesterolemia that results in the appearance of lesions in the LAR rabbits, which are limited mainly to the aortic arch. Indeed, we could demonstrate expression of VCAM-1 in sections which included atheromatous lesions, but not in uninvolved regions. The difference between the LAR and HAR rabbits was even more pronounced in sections from the thoracic aorta, as only

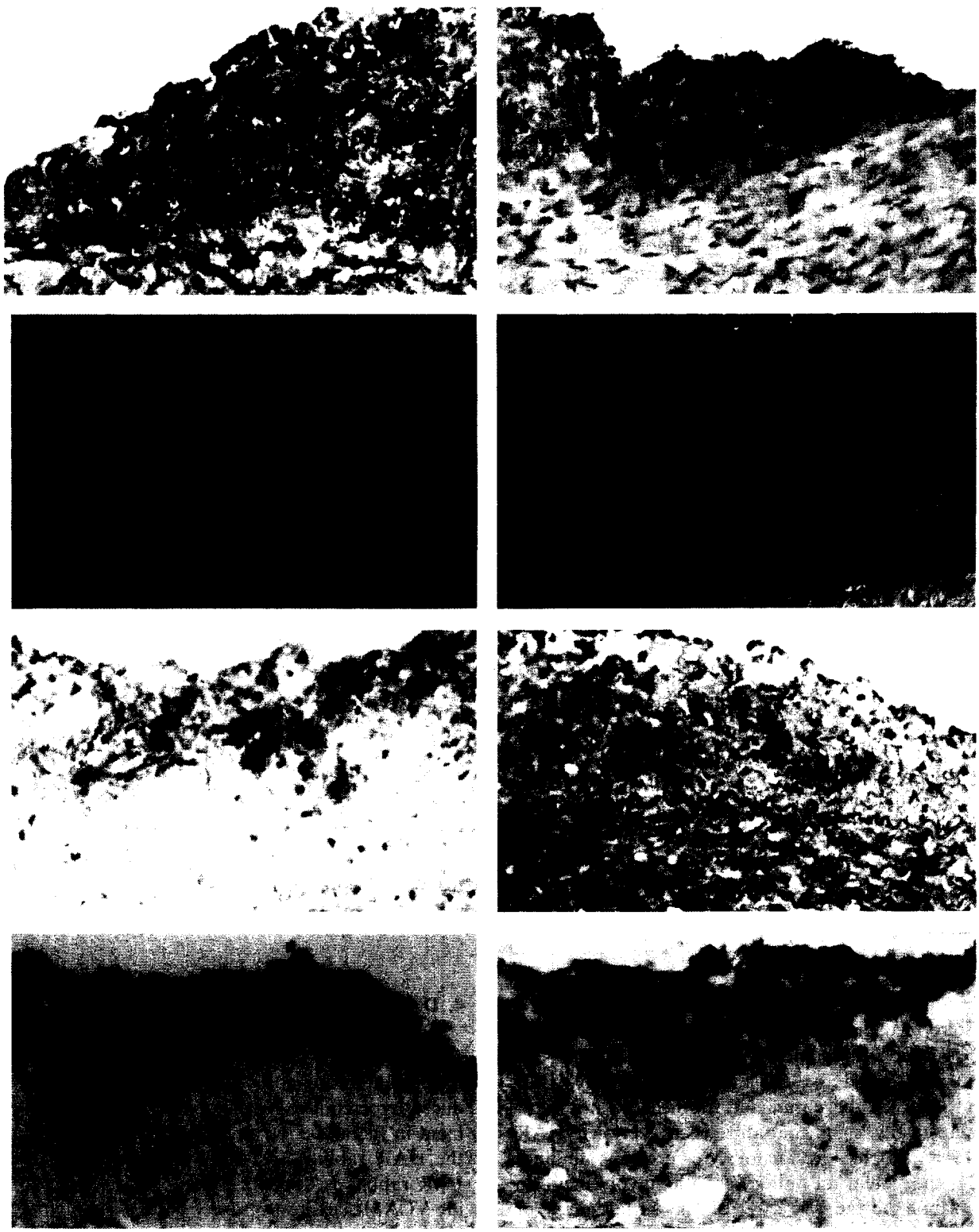

Fig. 2. Cellular elements in lesions from the aortic arch of cholesterol fed HAR (left colum) and LAR (right colum) rabbits. The sections in the first row from top were stained with RAM11 antibody that recognizes macrophages (× 400 left; × 200 right); in the second row with α-actin antibody to identify SMC (× 200); in the third row with RTC antibody to identify T lymphocytes (× 200); in the fourth row with 2C4 antibody that recognizes Ia antigen positive cells (× 200 left, × 150 right). Fluorescein was used to visualize the α-actin antibody, all others were visualized by peroxidase and 3-amino-9-ethyl-carbazole.

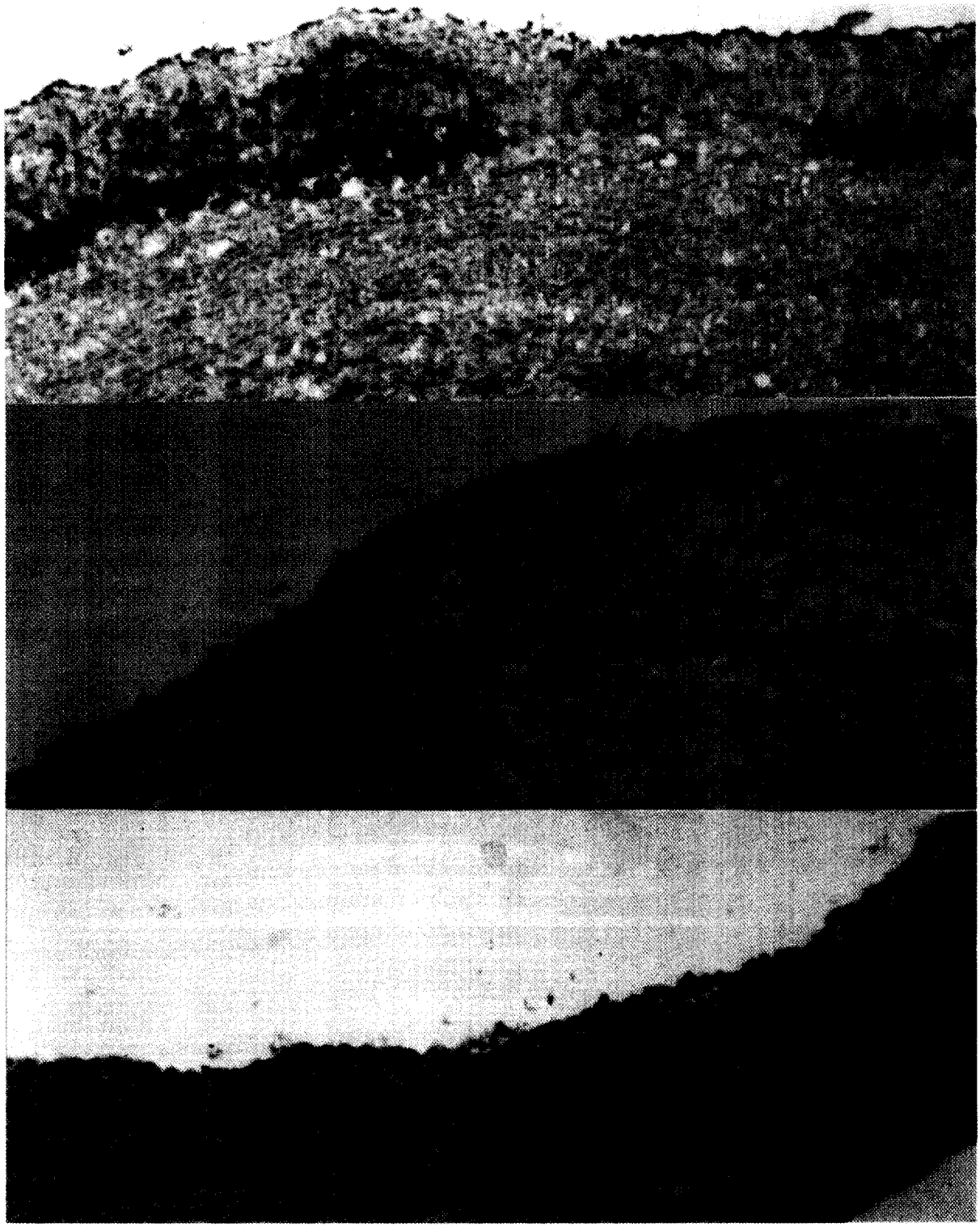

Fig. 3. Expression of VCAM-1 in lesions from the aortic arch of one HAR (top) and two LAR rabbits (middle and bottom) (× 100).

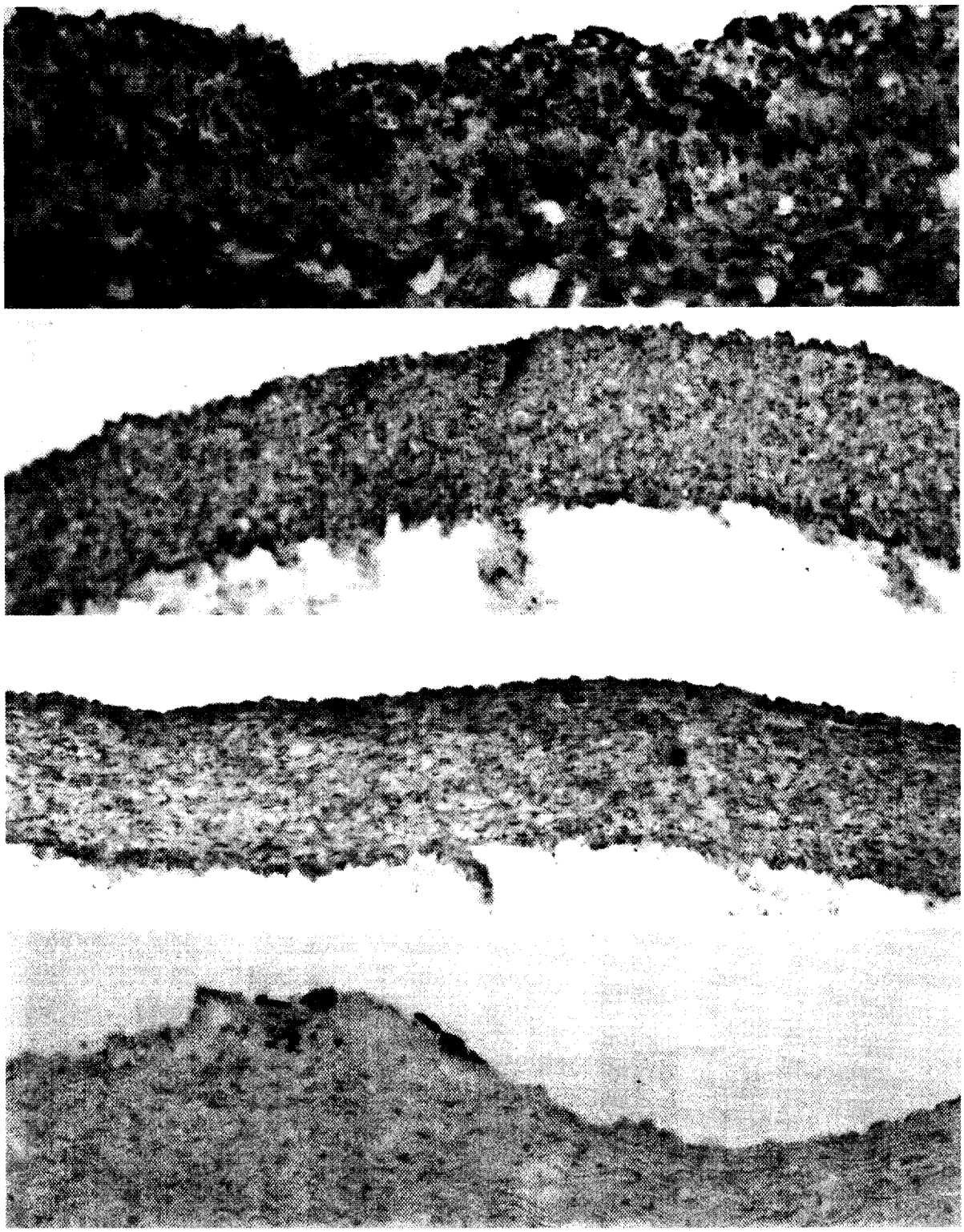

Fig. 4. Expression of VCAM-1 in a lesion from the thoracic aorta of a HAR rabbit (top, × 200), and sections from thoracic aorta of three LAR rabbits; in two aortas (× 100) there was no reaction; in the third aorta, one lesion with VCAM-1 staining was found (bottom × 200).

small focal areas of VCAM-1 could be found in LAR rabbits. These results have provided evidence that the VCAM-1 gene is expressed in LAR rabbits.

Inducibility of VCAM-1 expression by cytokines has been demonstrated in cultures of endothelial cells from human [15,16] and rabbit [5–8,17] aorta, and the main effectors were IL-1, TNF-α and IL-4. VCAM-1 has been demonstrated also in modified smooth muscle cells

in atheromas [10] and its inducibility in cell culture has been verified. It appears, however, that the regulation of VCAM-1 expression in endothelium and SMC occurs via different transcriptional mechanisms [18,19]. There is no general agreement with respect to the effects of some cytokines on SMC; thus, cultured human vascular SMC did express VCAM-1 when stimulated by IL-4 and INF-γ, but not by IL-1 or TNF-α [20,21]. On the other hand, regulation of VCAM-1 expression in human vascular SMC by TNF-α or IL-1β was reported by others [22–25]. VCAM-1 expression in SMC can also be inhibited by cytokines, such as TGF-β [24], a finding that may have important implications for atherogenesis [24]. It seems of interest that high density lipoproteins can also inhibit cytokine induced expression of VCAM-1 [26]. Since HDL levels in LAR rabbits were no higher than in HAR rabbits, this mechanism could not be invoked to explain the different magnitude of the atherosclerotic response.

The atherogenic lipoprotein β-VLDL derived from cholesterol fed rabbits was shown to be chemotactic for monocytes because of its high content of lysophosphatidyl choline (lysoPC) [27]. In addition, lysoPC was also shown to induce VCAM-1 and ICAM-1 in endothelial cells [28]. In our previous study, we found no difference in lysoPC content of β-VLDL isolated from LAR and HAR rabbits [12]. Moreover, both β-VLDL's caused similar stimulation of adhesion of U937 monocytes to endothelium [12]. Therefore, the difference in VCAM-1 expression between LAR and HAR rabbits is probably not due to a different lysoPC content of β-VLDL. Peroxidatively modified lipoproteins have also been shown to participate in induction of VCAM-1 in endothelial cells [29]. More recently, the mechanism of action of oxidized lipoproteins has been linked to cytokine activation [30]. Since peroxidative modification of plasma lipoproteins may occur in the vascular wall, we have compared the peroxidative capacity of aortic SMC and pulmonary macrophages derived from LAR and HAR rabbits towards β-VLDL or LDL [12] and found them to be similar. Therefore, it does not seem likely that the presently observed lesser VCAM-1 expression in the aortae of cholesterol fed LAR rabbits is caused by reduced peroxidative capacity of cellular components of the vessel wall.

Expression of VCAM-1 by SMC in intimal lesions has been considered to be involved in atherogenesis [20] by enhancing the retention of recruited monocytes and facilitation of T cell activation [31,32]. Recently, it was suggested that the vasodilator dysfunction of regenerated endothelium in rabbit iliac arteries is associated with the expression of VCAM-1 [33]. In our search for causes underlying the low atherosclerotic response of LAR rabbits, we have found that induction of scavenger receptor activity by PDGF in cultured SMC, derived from LAR rabbits was highly attenuated when compared to SMC from HAR rabbits [12]. In analogy to the above, one could envisage that the lesser expression of VCAM-1 in the thoracic aorta could have been due to its lower inducibility. However, one cannot rule out that rather than being causative, the decreased expression of VCAM-1 is simply a consequence of fewer lesions.

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