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Atherosclerosis 150 (2000) 21–31

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

CD143 in the development of atherosclerosis

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Received 27 November 1998; received in revised form 28 July 1999; accepted 18 August 1999

Abstract

The expression of CD143 (angiotensin-I-converting enzyme, ACE) in cardiovascular diseases may be an important determinant of local angiotensin and kinin concentrations. Much of the experimental and clinical evidence suggests a crucial role for Ang II in fibrogenesis and the development of atherosclerosis. Therefore, we have studied the distribution of CD143 in atherosclerotic and non-atherosclerotic segments isolated from different parts of the human vascular tree, including aorta and coronary, carotid, brachial, renal, iliac and femoral arteries, and staged according to the AHA. Two hundred and thirty native and formalin-fixed specimens of 80 patients were analysed by sensitive APAAP-technique using ten different monoclonal and polyclonal antibodies to human CD143 and several controls. In non-atherosclerotic segments or intimal thickening, CD143 was found almost restricted to the endothelial cells of adventitial arterioles and small muscular arteries. In contrast, a striking accumulation of CD143 was detected in all early and advanced atherosclerotic lesions. This de-novo occurrence of CD143 within the intimal vascular wall was caused by spindle-shaped subendothelial cells with macrophagic/histocytic features, activated macrophages and foam cells. In addition, advanced lesions of atherosclerosis showed a marked neo-expression of CD143 in newly formed intimal microvessels. Hypocellular fibrotic plaques depleted in microvessels and macrophages showed only little CD143. The de-novo occurrence of CD143 was dependent on the stage of atherosclerosis but not on its particular localisation within the vascular system. This early and obligatory CD143 expression at an unusual vascular site may contribute to unusual tissue levels of angiotensins as indicated by co-localisation of immunoreactive Ang II. Thus, it may be an important pathogenetic step in the development of atherosclerosis and an established target for pharmacological prevention.

Keywords: CD143; ACE; Atherosclerosis; Atherogenesis; Angiotensin-II

1. Introduction

Angiotensin-I-converting enzyme (ACE) or kininase II (EC 3.4.15.1), recently clustered as CD143 [1], is a zinc metallopeptidase that acts primarily as a dipeptidylpeptidase, and has a broad specificity capable of hydrolysing a wide range of peptide substrates in vitro. Two isoforms of the molecule are known, a somatic and a testicular one. In contrast to testicular ACE, exclusively expressed by germinal cells, the somatic isoform of ACE is found on different cell types. Somatic ACE plays an important role in the regulation of blood pressure and the control of regional blood flow through the generation of angiotensin II (Ang II) and the inactivation of bradykinin. Both result in strong vasoconstrictory effects, and stimulation of aldosterone secretion, but long-term effects on the vascular wall have also been shown [2,3].

Increased expression of ACE has been demonstrated in many experimental models of hypertension [4,5], cardiac hypertrophy [6–9], intimal thickening after balloon injury [10,11], and atherosclerosis [12–14]. Evidence supports the pathophysiological role of locally produced Ang II in blood vessel regulation in relation to hypertension, cardiac hypertrophy, restenosis after angioplasty and atherosclerosis [15–18]. Ang II has been shown to be a potent stimulator of vascular smooth muscle cell (VSMC) mitosis, growth and migra-

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0021-9150/00/$ - see front matter
PII: S0021-9150(99)00354-8

tion [19–23]. Immunohistochemical studies in animal vascular injury models showed CD143 within the induced lesions supposing a local generation of Ang II [4,5,10,11]. An overexpression of Ang II receptors in these lesions was also demonstrated [12]. In models of experimental atherosclerosis and restenosis, ACE inhibitors and Ang II receptor antagonists were found to reduce the size of lesions in a dose-dependent manner [24–30].

However, two multi-center clinical trials did not demonstrate the beneficial effect of ACE inhibition in preventing restenosis [31,32]. The discrepancy between results in rats and humans may have several explanations (different nature of human atherosclerotic lesions, insufficient doses of ACE inhibitors in clinical trials in comparison with animal models, etc.). Recently we have demonstrated that the pattern of endothelial CD143 distribution along the vascular tree differs greatly in human and rat. In the normal human vasculature the segments within the vascular tree that are most frequently afflicted by atherosclerosis (i.e. aorta, large arteries or epicardial coronary arteries), were found to be virtually free of immunoreactive CD143 in their intimal and medial layers. In contrast to human vessels, endothelial cells of all large vessels of rat demonstrated significant levels of CD143 [33,34].

Several recent studies have shown that in human coronary artery disease (CAD) CD143 was increased within the plaques [35–39]. Thus local overexpression of ACE may act as an important pathogenetic factor in the development of cardiovascular diseases [11,16,18]. However, ACE expression in atherosclerotic human vessels, other than coronary vessels, has not been reported. Therefore we investigated CD143 in different stages of atherosclerotic lesions in a variety of human arteries, using a set of monoclonal antibodies (mAb) to different epitopes of the ACE molecule and using a highly sensitive immunohistochemical technique.

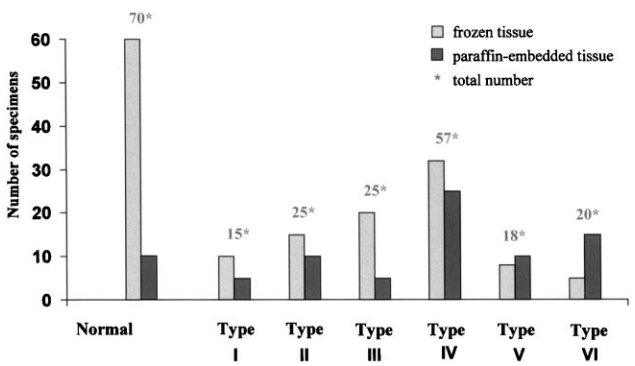

Fig. 1. Survey of analysed specimens and their classification according to AHA-criteria.

2. Materials and methods

2.1. Patients and tissues

For this study two different sources of patients and tissues were used. The first source consisted of 50 deceased patients (mean age: 61, range: 7–85), whose autopsy was performed within 24 h postmortem. The causes of death included trauma, cerebral haemorrhage and sudden death. From each patient, between two and eight tissue specimens were taken from segments with and without macroscopic atheromatous changes. These specimens were immediately placed in liquid nitrogen. The specimens obtained at autopsy ($n = 150$) originated from aorta ($n = 37$), renal artery ($n = 8$), iliac artery ($n = 19$), femoral artery ($n = 14$), brachial artery ($n = 12$), carotid artery ($n = 23$), coronary arteries ($n = 25$), and peripheral systemic vessels ($n = 12$). The second source of atheromatous tissue was obtained from 30 patients during vascular surgery. Here, thrombarteriectomy samples from the carotid artery ($n = 80$) were obtained either frozen or formalin fixed. Altogether 230 specimens from 80 patients were obtained for analysis (Fig. 1).

2.2. Preparation of specimens for immunostaining

Frozen tissues were sectioned at 5 μm by a cryostat microtome (CM 3000, Leica/Jung, Germany). The slides were air-dried at room temperature for 12–24 h and then were either processed directly or stored at −30°C. Formalin fixed, paraffin embedded tissues were sectioned at 2–4 μm (SM 2000 R, Leica/Jung, Germany) followed by drying at 37°C in an incubator overnight. Before immunohistochemical staining, the paraffin sections were dewaxed for 10 min in xylene, followed by 10 min in acetone and 10 min in acetone/Tris buffered saline (1:1). After this treatment the slides were washed in Tris buffered saline (TBS) and placed in microwave-proof tubes (Sigma, Deisenhofen, Germany) containing 0.01 M citrate buffer solution (pH 6.0). The slides were boiled in the tubes for 5 min at 600 W in the microwave (SS 566H, Bosch, Munich, Germany). Evaporated volume was replaced by distilled water and the procedure was repeated four times. After microwave treatment the slides were left to cool down and washed in TBS. Frozen sections were fixed in acetone for 10 min at RT and air-dried afterwards.

2.3. Histochemical staining and classification of atherosclerosis

Histologic classification of the plaque morphology was performed for all specimens by standardised H&E, elastic fiber and Scarlet Red or Oil Red O staining by three independent investigators (R. Metzger, R.M.

Table 1
Antibodies to human CD143, Ang II/III, and other cellular markers

Clone	Immunogen	Concentration (μg/ml)	Source [References]
9B9	Human ACE of lung	0.4	Chemicon, CA [42,43]
i2H5	Human ACE of lung	1.0	Chemicon, CA
3G8	Human ACE of lung	5.0	Chemicon, CA
1A8	Human ACE of lung	33.0	Chemicon, CA
3A5	Human ACE of lung	6.7	Chemicon, CA
5F1	Human ACE of lung	10.0	Chemicon, CA
CG1[a]	Human ACE of kidney	10.0	BMA, Swiss [42]
CG2[a]	Human ACE of kidney	5.0	BMA, Swiss
CG4[a]	Human ACE of kidney	10.0	BMA, Swiss
pAb (Y4)[b]	Human ACE of kidney	2.0	F. Alhence-Gelas [44,45]
pAb[b]	Angiotensin II (synthetic)	Diluted 1:500	Chemicon, CA
IC/70A	CD31	2.3	Dako, Glostrup
10F3	CD54	1.0	Biotract, NY
QBEND/10	CD34	2.0	Dianova, Hamburg
F8/86	vWF	11.6	Dako, Glostrup
CLE-6	Endothelium	10.0	S. Kumar, Manchester [54]
BMA 120	Endothelium	10.0	Behring, Marburg
BNH 9	BUR cell line	pre-diluted	Dianova, Hamburg
HHF35	α and γ actin	0.6	Dako, Glostrup
1A4	α actin	0.6	Dako, Glostrup
KP1	CD68	7.6	Dako, Glostrup
PG-M1	CD68	7.2	Dako, Glostrup
MR12/53[c]	Rabbit Ig	1.0	Dako, Glostrup

[a] Reactive on microwave pretreated formalin fixed and paraffin embedded tissue section.
[b] Polyclonal antibody from rabbit (all others are from mouse).
[c] Used as negative control and as bridging antibody for rabbit pAbs in APAAP-technique.

Bohle, F.E. Franke), according to the recent American Heart Association classification of atherosclerotic lesions and the results are shown in Fig. 1 [40,41].

2.4. Antibodies

Nine well-characterised mouse-monoclonal antibodies (mAb) to human CD143 were used. One set of these mAb (9B9, i2H5, 5F1, 3G8, 1A8, 3A5) has been generated against human lung ACE and recognises the native enzyme [42,43]. The other set (CG1, CG2, CG4) generated against human kidney ACE recognises the slightly denatured molecule [42] and reacts well with CD143 in fixed tissues [33,34]. They recognise nine different epitopes of CD143, most of them are located on the aminoterminal domain of ACE [42,43]. In addition, a purified immunglobulin fraction from polyclonal antiserum to human kidney ACE (pAb Y4, kindly provided by F. Alhenc-Gelas, INSERM U 367, Paris, France), which recognises both the somatic and germinal isoforms of CD143, was used [44,45]. Immunoreactivity for Ang II/III was assessed by a polyclonal antiserum from rabbit immunised by the synthetic Ang II.

To characterise the cellular components of the atherosclerotic plaque, several mAb directed against human endothelial cells (CD31, CD54, Factor VIII, BMA 120, BNH 9, and CLE-6), macrophages/histocytes (CD 68) and smooth muscle cells (α and γ actin) were applied [46–48]. The CLE-6 was kindly provided by S. Kumar, University of Manchester, UK. A mouse anti-rabbit-IgG mAb (MR 12/53) was used as negative control and as bridging antibody for the pAbs from rabbit. The antibody clone, immunogen, source and working dilutions are described in Table 1.

2.5. Immunohistochemistry

The alkaline-phosphatase-anti-alkaline-phosphatase (APAAP) technique was applied according to the method of Cordell et al. [49] on native and formalin-fixed tissue sections. The technique has been applied with the following modifications: To prevent non-specific reactivity, the polyclonal rabbit anti-mouse Ig ('link'-antibody, DAKO, Glostrup, Denmark), was used at a dilution of 1:40. The APAAP-complex (DAKO, Glostrup, Denmark) was used at a dilution of 1:50. Alkaline phosphatase substrate reaction with new fuchsin (100 μg/ml) and levamisole (400 μg/ml) was performed for 20 min at room temperature [8,50]. To confirm the results of immunostaining in double

label experiments a conventional immunofluorescence was performed using a FITC/TRIC based method (DAKO, Glostrup, Denmark). Altogether 1800 immunostained sections were analysed. The analysis of postmortem autolysis on tissue CD143 reactivity has been previously described [51].

3. Results

3.1. CD143 reactivity, effect of postmortem alterations, and particular reactivity of cellular markers

At appropriate concentrations (Table 1), all CD143-mAb showed an identical pattern of staining in different human vessels and organs. No differences were found between those antibodies predominantly reacting on native tissues (mAb 9B9, i2H5, 5F1, 3G8, 1A8, 3A5, pAb Y4) and those predominantly reacting on formalin-fixed tissues (CG1, CG2, CG4). All mAb tested (mAb 9B9, i2H5, 3G8, CG1, CG2, CG4) showed a very stable detection of CD143 in autolysis and the strong molecular resistance of CD143 epitopes to autolysis has recently been described [51–53]. These data show that immunohistochemical analysis of CD143 is possible on routine autopsy specimens and not only on specimens obtained from urgent autopsy or surgery. In contrast to normal vessels and tissues, especially in native material of atherosclerotic lesions, some peculiarities with cell type markers were observed. For example, CD31 and CD54 showed particularly strong expression in inflammatory cells. The FVIII tended to be diffuse and pericellular in its reactivity. The KP1 (CD68) mAb marked fibrohistocytic cells in all layers of the vascular wall and occasionally medial smooth muscle cells. The immunoreactivity with mABs to smooth muscle actin was largely dependent on antibody concentration (not shown).

3.2. CD143 in the non-atherosclerotic vessel wall

In the non-atherosclerotic segments of the analysed arteries we found a very heterogeneous transmural distribution of CD143. No significant immunoreactivity was found in the intimal and medial vessel layers (Fig. 2A, B). CD143 were only seen in endothelial cells of arterioles and small muscular arteries of the vasa vasorum, whereas those of the adventitial capillaries, venules and veins were poorly reactive or completely CD143 negative (Fig. 2A, B). With the exception of some stellate fibrohistocytic cells in the loose adventitial connective tissue and perivascular spaces of adventitial vessels (not shown), no other cell types were found to express CD143 in non-atherosclerotic artery segments.

3.3. CD143 in atherosclerotic lesions of different vascular locations

Structural and functional heterogeneity has to be considered in arterial vessels of different types and location, e.g. between aorta and coronary arteries. Although this heterogeneity might affect or modulate pathogenesis of atherosclerosis, we have not observed principal immunohistological differences in the appearance of atherosclerotic lesions depending on a specific vascular location. Thus, our data on CD143 are presented schematised according to the AHA classification of atherosclerosis.

3.4. CD143 in initial lesions and preatheroma

In 'type I lesions' (initial lesions) with adaptive intimal thickening that do not contain demonstrable macrophages by CD68, no changes in the pattern of CD143 expression were noticed (Fig. 2C) compared to the non-atherosclerotic segments (Fig. 2B). With the appearance of first CD68+ cell forms, foam cells, and in 'type II lesions' (fatty dot or fatty streak), intimal CD143 became detectable in all cases analysed (Fig. 2D). Noteworthy, CD143 was first detectable in spindle-shaped cells of the subendothelial cell layer. Immunoreactivity to Ang II, found to be distributed quite similarly to CD143, even coincided with detection of CD143 in these subendothelial cells (Fig. 3A–C). Endothelial cells were inconspicuous when fine lipid droplets occurred in the extended inner vascular media and intracellular lipids accumulated in actin bearing intimal cells (Fig. 3G–I). The media of the vessel wall continued to be CD143 negative (Fig. 2D, Fig. 3A) and the vasa vasorum of adventitia showed the same expression pattern as in non-atherosclerotic segments (Fig. 2A, B). More advanced stages of 'type II lesions' and 'type III lesions' (intermediate lesion or preatheroma) were associated with necrosis of single intimal cells, extracellular lipid deposits, and an increasing amount of intimal CD143. Double immunofluorescence demonstrated the macrophagic/histocytic character of cells associated with CD143, while endothelial cells of cellular assignment of smooth muscle actin was different on the whole (Fig. 3D–F). Some cells were found to have a CD68+/CD143− or CD68−/CD143+ phenotype, but smooth muscle actin was only occasionally co-localised with CD143 to a small extent (Fig. 3D–F). Interestingly, transient stages between elongated CD68+/CD143+ cells of the subendothelial cell layer, stellate macrophages/histocytes, and macrophagic foam cells occurred (Fig. 3D).

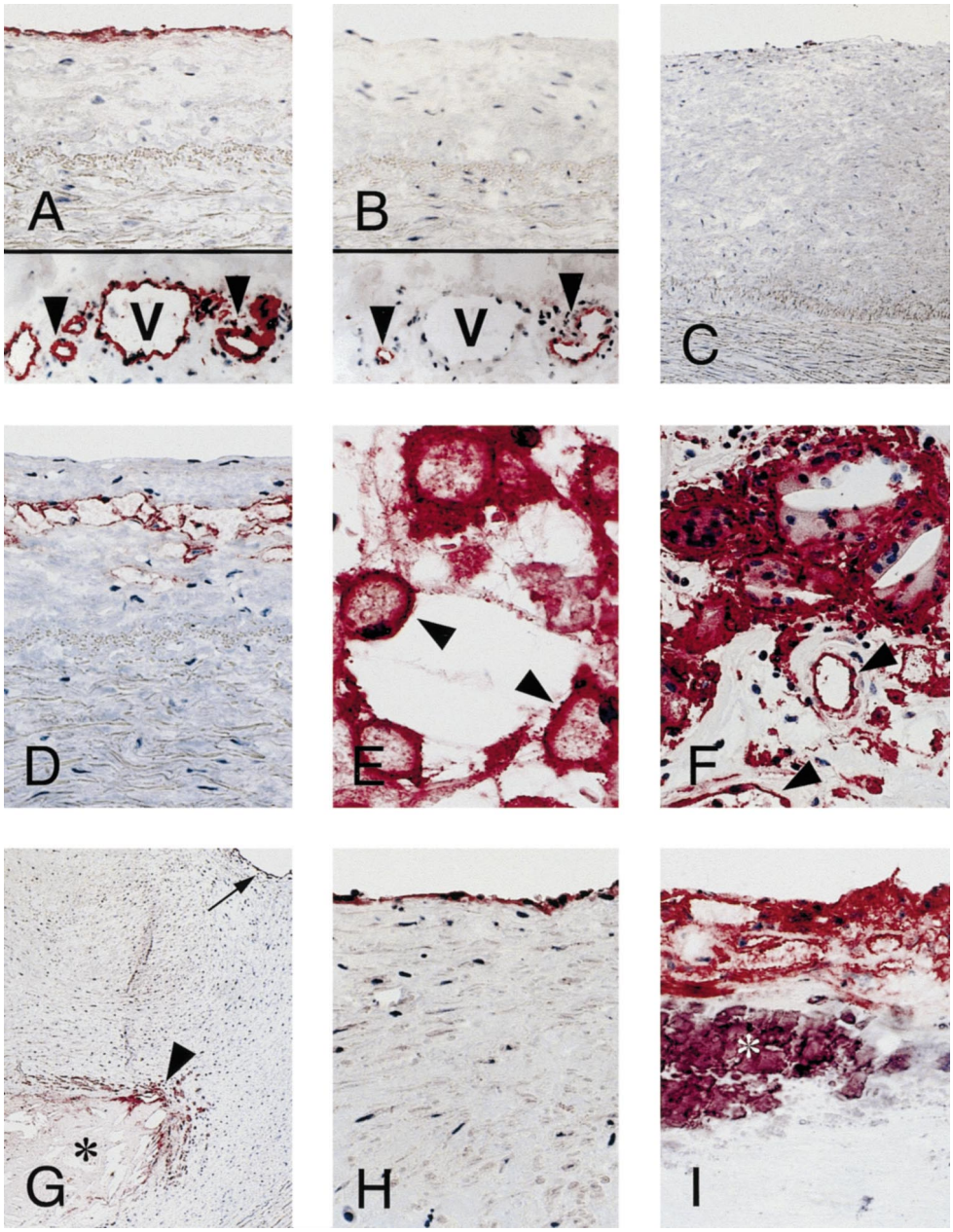

Fig. 2. Localisation of CD143 in the non-atherosclerotic (A, B) and atherosclerotic (C–I) intima. A: Immunostaining of luminal (above) and adventitial (below) endothelial cells in human aorta by anti-PECAM-1 (CD 31) mAb. MAb JC/70A on native tissue, magnification × 150. B: Endothelial cells of arterioles and small arteries of adventitial vessels of human aorta show CD143 immunoreactivity with mAb 9B9 (arrows below), whereas luminal aortic endothelial cells are not labelled (above). Endothelial cells of adventitial veins (V) are negative (below). VSMCs of vascular media or VSMC-like cells of intima are not immunoreactive. Compare the absence of ACE in luminal endothelial cells (B, above) with the homogeneous endothelial labeling by anti-CD31 mAb in A (above). Anti-CD143 mAb 9B9 on native tissue, magnification × 150. C: Pure adaptive intimal thickening shows no change in CD143-immunoreactivity and cell content. Anti-CD143 mAb i2H5 on native tissue, magnification × 70. D: Early lesion of atherosclerosis (AHA type II) demonstrates de-novo occurrence of intimal CD143. This is located on activated macrophages within the subendothelial layers of the intima but not on the luminal endothelial cells. Anti-CD143 mAb 5F1 on native tissue, magnification × 150. E: High magnification shows the ACE expression on the cellular surface of activated macrophagic foam cells (arrowheads), here in a preatheroma lesion. Anti-CD143 mAb CG2 on formalin-fixed, paraffin-embedded tissue, magnification × 600. F: This atheroma lesion shows CD143 expressed by histocytic foreign body giant cells surrounding cholesterol clefts (above) and additionally by endothelium of newly organised microvessels (arrowheads). Anti-CD143 mAb CG1 on formalin-fixed, paraffin-embedded tissue, magnification × 150. G: Decrease of ACE expression in a paucicellular fibrotic lesion. Few macrophages and endothelial cells of the neovascularisation surrounding the lipid core (asterisk) are immunoreactive for CD143 (arrowhead), and also luminal endothelial cells (arrow, see H). Anti-CD143 mAb CG2 on formalin-fixed, paraffin-embedded tissue, magnification × 20. H: Luminal endothelial cells of a fibrotic lesion show neoexpression of ACE. The arrow in G points to the cut-out in H. Anti-CD143 mAb CG2 on formalin-fixed, paraffin-embedded tissue, magnification × 150. I: A calcified region (asterisk) does not show CD143, whereas partially degenerative altered macrophages/histocytes of an adjacent lipid plaque are immunoreactive for CD143. Anti-CD143 mAb 3G8 on native tissue, magnification × 150.

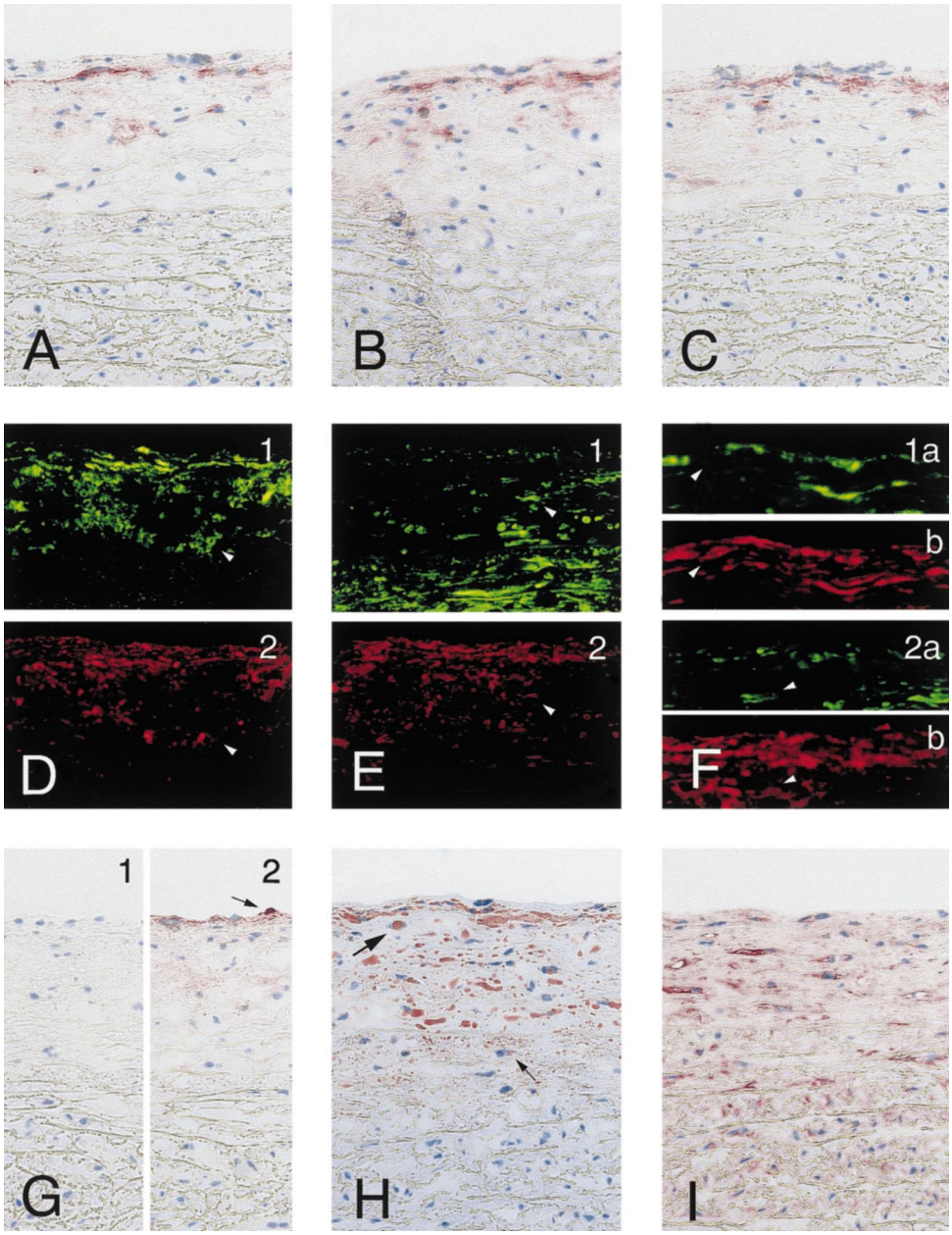

Fig. 3. CD143 (A), Ang II (B), CD68 (C), negative control/endothelial cell marker (G), lipids (H), and smooth muscle actin (I) are demonstrated in an early AHA type II lesion of aorta; double immunofluorescence shows CD68/CD143 (D), smooth muscle actin/CD143 (E), and their distribution in the subendothelial cell layer (F) of a more advanced AHA type II lesion (fatty streak). A: CD143 is first noted in spindle-shaped cell forms of the subendothelial cell layer. Medial VSMCs and VSMC-like cells of intima are not labelled. B: Immunoreactive Ang II co-localises with these cell forms. C: CD68 co-localises with these cell forms. D: The majority of CD143+ cells (2) show co-expression of CD68 (1). Some heterogeneity in the cellular distribution of immunoreactivity occurs (arrowheads at same position). Note the spindle-shaped morphology of expressing cell forms in the subendothelial cell layer and its change to an enlarged and stellate appearance in the deeper intima. E: In the gross, CD143 (2) mismatch with the detection of smooth muscle actin (1). Co-localisation is demonstrated only for few cells or cellular processes (arrowheads at same position). F: CD68 (1a) is shared by most CD143+ cells (1b) of the subendothelial cell layer, although some heterogeneity is apparent (arrowheads at same position). Smooth muscle actin (2a) co-localises rarely with CD143 (2b) and to small extent (arrowheads at same position). G: mAb MR12/53 as negative control (1). An endothelial cell (arrow) and fine cytoplasmic extensions on the luminal surface are demonstrated by mAb CLE-6 (2). H: Fine lipid droplets are visible in the zone of extended inner media with intracellular occurrence (small arrow), and intracellular lipids have been accumulated in intimal cells (large arrow). I: Immunoreactivity to smooth muscle actin is largely distributed in intimal cells but appears most prominent in lipid-laden cells of the VSMC-like type. Anti-CD143 mAb 9B9 (A), anti-Ang II pAb (B), anti-CD68 mAb PG-M1 (C), FITC-labelled anti-CD68 mAb KP1 and TRIC-labelled anti-CD143 pAb Y4 (D1/2, F1a/b), FITC-labelled anti-smooth muscle actin mAb HHF-35 and TRIC-labelled anti-CD143 pAb Y4 (E1/2, F2a/b), mouse-anti-rabbit Ig mAb MR12/53 (G1) and anti-endothelial cell mAb CLE-6 (G2), Oil Red O (H), and anti-smooth muscle actin 1A4 (I). Serial sections of native tissue in the order of A–C (section # 29–31) and G–I (section # 35–38) at the same tissue site (except of G which demonstrates negative control and an endothelial cell one high power field left of the original intimal site). D–F (section # 51, 52) at different tissue sites. Magnifications A–C and G–I × 150, D and E × 70, F × 300.

3.5. CD143 in advanced lesions

In type IV lesions (atheroma lesion), further increase of CD143 occurred within the inflammatory tissue surrounding the lipid core. Comparing sequential sections immunostained for endothelial cells, macrophages, VSMC, lipids and CD143, CD143 immunoreactivity was clearly localised in macrophages/histocytes, macrophagic foam cells, histocytic giant cells, and in endothelial cells of the plaque neovascularisation (Fig. 1E, F). No expression was found in other cell populations of the plaque, either in endothelial cells of the vessel lumen or in VSMCs, lymphocytes, plasma cells or mast cells.

With the increasing amount of fibrous connective tissue combined with a reduction of macrophages/histocytes, macrophagic foam cells and microvessels, the amount of CD143 within type V lesions decreased rapidly (Fig. 1G). In general, immunoreactivity of CD143 was not found in necrotic, fibrotic or calcified regions, but in residual macrophages and microvessels lateral to the lipid core (fibroatheroma lesion, Fig. 1G), or next to calcifications (calcific lesion, Fig. 1I). Interestingly, in type V lesions some endothelial cells of the vascular lumen covering the fibrous plaque showed a de-novo expression of CD143 (Fig. 1G, H). Type VI lesions (complicated lesions) showed, depending on their origin, the CD143 distribution of type IV or type V lesions. In advanced plaques the media was often disarranged and atrophic. Both, the media and the adventitia sometimes had a pronounced inflammatory response to the plaque. Endothelial cells of adventitial vessels, surrounded by a lymphocytic infiltration, in the majority were CD143 negative. Inflammatory infiltrates of the media were almost always expansions from the plaque itself and consisted of lymphocytes and mast cells of CD143 expressing macrophages/histocytes, macrophagic foam cells, and microvessels (not shown).

4. Discussion

Previously we have demonstrated CD143 (ACE) expression in endothelial cells of small muscular vessels and arterioles of the vasa vasorum, while endothelial cells of adventitial capillaries, venules and veins and of the vascular lumen of large human arteries are usually non-immunoreactive [33,34]. This expression pattern, ensured by a set of well-defined mAb to different epitopes of the native as well as the denatured molecule, reflects major quantitative differences in the distribution of CD143 within the human vascular system. Heterogeneity of endothelial CD143 is present in most vasculatures, including the cardiac and gastrointestinal circulation but not in the kidney and lung [33,34]. In contrast, we found that endothelial markers like PECAM-1 (CD31), ICAM-1 (CD54), or CLE-6 antigen [54] were uniformly distributed along the normal vascular tree and also in all endothelial cells of atherosclerotic lesions. These observations support the concept of a structural and functional heterogeneous differentiation of the vascular endothelium [55,56].

In the development of atherosclerosis, minute amounts of CD143 were already found in early lesions, when intracellular lipids accumulate in intimal cells. In these early lesions, the intimal de-novo occurrence of CD143 was first noticed in elongated, spindle-shaped cells of the subendothelial cell layer (Fig. 3). This particular subendothelial tissue site, and coincidence of CD68, hint towards an early macrophagic/histocytic cell differentiation and suggest a relationship to dendritic and the recently classified stellate cells [57]. Furthermore, in all early lesions (AHA types I to III), transitional stages to CD68 + /CD143 + macrophages/histocytes and macrophagic foam cells were morphologically evident, but some heterogeneity in the cellular distribution of immunoreactivity and a few cells with CD68 + /CD143 − or CD68 − /CD143 + phenotypes may occur (Fig. 3). More advanced atherosclerotic lesions additionally showed CD143 on endothelial cells of the intimal neovascularisation (AHA type IV), and on luminal endothelial cells of fibrotic lesions (AHA type V). In synopsis, these changes of CD143 expression were generally found in all atherosclerotic cases and vessels analysed. Based on its early occurrence at a variety of locations, the co-localisation of CD143 at lesion sites suggests that CD143 is obligatorily involved in the pathogenesis of atherosclerosis. The fact that local vascular CD143 increases in advanced stages of atherosclerosis was confirmed in an experimental model using hyperlipidaemic rabbits [14]. Our main finding, that predominantly macrophages/histocytes are causative for this increase, are in agreement with results of other morphological studies which have analysed CD143 in atherosclerotic lesions of coronary arteries so far [37,38].

However, we were not able to detect CD143 in medial VSMCs (so-called differentiated VSMCs), as reported for a part of the analysed material in one of these former studies [37]. Likewise, we were not able to assign CD143 to VSMC-like cells of intima (so-called intimal VSMCs, VSMCs of intermediate or dedifferentiated types), as reported in another study [38]. These discrepancies may have resulted from various technical, immunogenic, histological, pathogenetic, classificatory, and/or biological causes [50,51,58]. Most important, the sole detection of smooth muscle actin does not guarantee a correct cellular assignment to VSMC, either in histological tissue sections or in cell cultures.

Histogenetically, quite different and ubiquitous cell types, like dendritic cells, stellate cells, fibrohistocytes and myofibroblasts, may express actins especially on

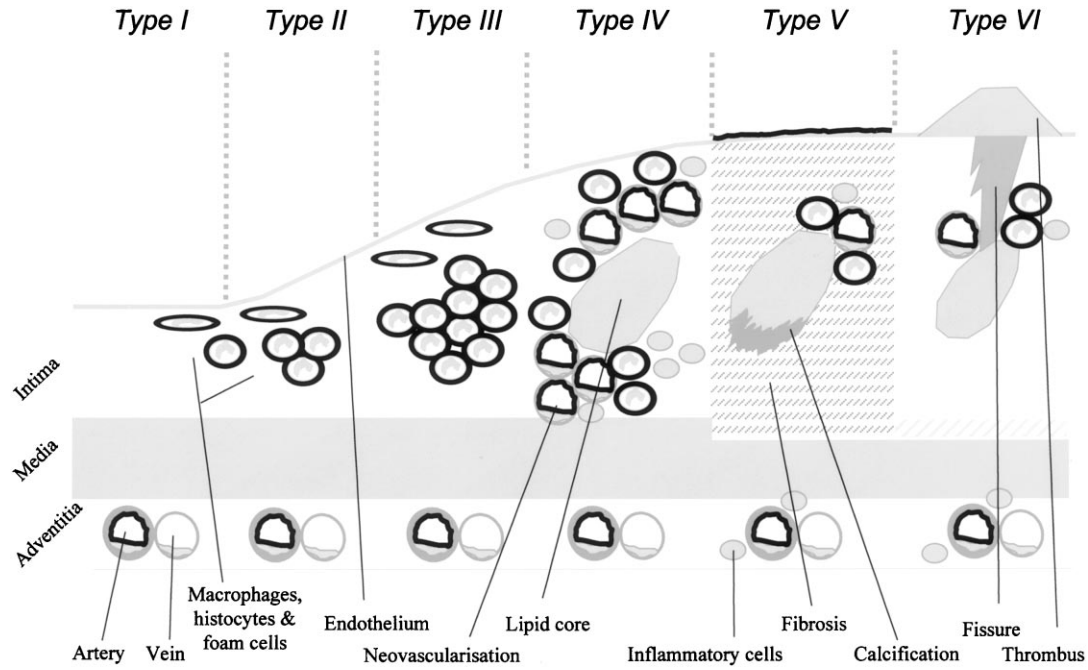

Fig. 4. Schematic summary of CD143 distribution (black) in AHA-stages of atherosclerosis (based on the analysis of 230 specimens from 80 patients and results found to be representative for all large arteries including aorta and coronary arteries). **Initial Lesions and Preatheroma**. With the subendothelial appearance of spindle-shaped CD68+ macrophages/histocytes in Type I lesions and Type II lesions, intimal CD143 (black) became detectable. Luminal endothelial cells and VSMCs of the vessel wall were not immunoreactive to CD143. In more advanced stages of type II lesions (fatty dot or fatty streak) or type III lesions (intermediate lesion or preatheroma) the advancing accumulation of macrophages/histocytes and macrophagic foam cells was associated with increasing amounts of intimal CD143. **Advanced Lesions.** In type IV lesions (atheroma lesion) a marked increase of CD143 occurred within the inflammatory tissue surrounding the lipid core. CD143 was localised in macrophages/histocytes, macrophagic foam cells and additionally in endothelial cells of the plaque neovascularisation. CD143 was not found in luminal endothelial cells, VSMCs, lymphocytes, plasma cells or mast cells of the plaque. In type V lesions (fibrotic lesions) the amount of CD143 decreased with the reduction of macrophages/histocytes, macrophagic foam cells and microvessels, combined with an increasing amount of fibrous matrix. CD143 was not found in necrotic, fibrotic or calcified regions, but in residual macrophages and microvessels lateral to the lipid core (fibroatheroma lesion), or next to calcifications (calcific lesion). Luminal endothelial cells covering the fibrous plaque showed a de-novo expression of ACE. Type VI lesions (complicated lesions) showed, depending on their origin, the CD143 distribution of type IV or type V lesions. Special note: advanced lesions superimposed with PTCA may transiently express additional CD143 in cell-rich areas of tissue repair [36]. In all stages of genuine atherosclerosis, however, we observed no further changes in the CD143-distribution. Endothelial cells of vasa vasorum showed CD143 in small muscular arteries and in arterioles, while those of capillaries and veins were almost negative.

activation, proliferation, and migration (for review see Ref. [57]). Immunohistologically, even the phagocytic uptake [50] of actins, released by necrotic cells or bound to cellular fragments, and possible 'background' detection of shed plasmatic CD143 [59], have also to be considered. On the other hand, proliferating granulation tissues, rich in fibrohistocytes and myofibroblasts, were described to express CD143 in transient stages of tissue repair in a model of myocardial infarction [8], data which correspond well to those obtained after experimental or therapeutic injury of arterial vessels [11,36]. The morphogenesis of naturally occurring atherosclerosis differs remarkably from artificial interventions, provoking proliferative tissue repair from several vascular sites. Medial VSMCs are generally found prone to undergo degeneration and atrophy, while only modest cellular proliferation affect intimal cells arising most probably from the subendothelial cell layer [58]. In line with this concept, we found CD143+ intimal cells only rarely to coincide with significant amounts of actin, however, not sufficient to classify these cells to be of true VSMC or VSMC-like cell origin. In contrast again, the vast majority of CD143+ intimal cells co-expressed clearly CD68, indicating even in early stages of atherosclerosis their differentiation towards macrophages/histocytes.

The appearance of CD143 in atherosclerotic plaques suggests high local tissue levels of Ang II. In coronary arteries, an intimal accumulation of CD143 and a co-localisation of Ang II have already been shown [37]. Although use of pAbs should be interpreted cautiously, we can corroborate this co-localisation of immunoreactive Ang II to the distribution of CD143 even in early atherosclerotic lesions (Fig. 3). However, in addition to Ang II formation, ACE also inactivates bradykinin, a peptide that also inhibits growth of cultured VSMC [60]. Numerous studies suggest that Ang II plays an important role in vascular remodelling and in initiation

or progression of atherosclerosis by stimulating cellular proliferation and migration [19–23], expression of adhesion molecules and plasminogen activator inhibitor [61], and an increasing collagen synthesis with simultaneously suppressed elastin production [62].

In experimental atherosclerosis the inhibition of ACE delayed the formation and progression of intima lesions and restenosis [24–30]. By contrast, clinical studies investigating the effect of ACE inhibitors on the incidence of restenosis in patients undergoing percutaneous transluminal coronary angioplasty (PTCA) do not support the findings in experimental atherosclerosis [31,32]. Several reasons can explain why clinical trails in humans do not reproduce results in rats: (1) compared to the animals the patients were treated with a five to ten times lower ACE inhibitor doses; (2) patients received the treatment only after the PTCA was performed; (3) patients had a relatively short follow-up; and (4) ACE is abundant in the vascular system of rat, while in humans it is normally not expressed in the endothelium of the large vessels [33,34], where restenosis occurs. Although the source of Ang II in restenosis is not exclusively endothelial cells, the fact that distribution and level of ACE expression differs between human and rats may explain the species-specific effect of ACE inhibitors in cardiovascular complications.

In contrast to restenosis, some clinical studies (CONSENSUS, SOLVD, SAVE, AIRE, ISIS-IV and GISSI-III) investigating the properties of ACE inhibitors in the primary and secondary prevention of myocardial infarction (MI), left ventricular failure and left ventricular dilatation report partially positive effects [63,64].

In summary, significant amounts of CD143 were not found in the normal intima of all large vessels studied in humans. First occurrences and accumulations of CD143 were noticed in early lesions of atherosclerosis and were found associated with the appearance of macrophagic/histocytic differentiated cell forms. These findings were representative for all analysed atherosclerotic lesions of human vessels and for different vascular locations and can be summarised in a scheme (Fig. 4). A pathogenetic relation is suggested between the de-novo occurrence of CD143, Ang II tissue accumulation, improper kinin degradation, and the fibrocellular remodelling of vascular intima. Our data support the beneficial effects of a pharmacological ACE inhibition and/or Ang II receptor antagonism on the progression of atherosclerosis, but further clinical studies are still required.

Acknowledgements

The authors thank Professor Dr Andreas Schulz, head of the Department of Pathology, Justus-Liebig University Giessen, for his encouragement and support as well as Mrs Ruocco (Justus-Liebig University Giessen) and Dr Jon Art (University of Illinois at Chicago) for the critical reading of the manuscript. This study was supported by funds from the Justus-Liebig University Giessen.

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