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Immunohistochemical localization of S protein/vitronectin in human atherosclerotic versus arteriosclerotic arteries

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Summary. The localization of S-protein/Vitronectin as deposits in arterial lesions and as a component of extracellular matrices was investigated by indirect immunofluorescence in ten atherosclerotic samples of carotid endarteriectomy compaired with ten arteriosclerotic temporal biopsies. Anti-C5b-9 neoantigens, anti-C3, anti-C3d, anti-H, anti-IgG and anti-IgM antibodies were applied on serial sections. In the atherosclerotic plaque, S-protein deposits were observed as irregular granules and spots in the fibrous cap and the internal part of the media at the vicinity of the plaque; they inconstantly colocalized with C5b-9 neoantigens. When present the SC5b-9 complexes were generally associated with cell remnants in the sclerotic matrix. In the temporal artery biopsies, S-protein was bound exclusively to the internal elastic lamnina in association with C3d, but was absent from the intimal fibrous thickening. S-protein was not detected as a diffuse component of the extra-cellular matrix of either musculo-elastic or muscular medias, but was clearly demonstrated in smaller arteries; this result suggests a differential distribution of S-protein along the aterial tree.

Key words: Atherosclerosis – Arteriosclerosis – Complement – Terminal attack complex – S Protein/Vitronectin

Introduction

S-protein, now known to be identical to Vitronectin is a multifunctional glycoprotein involved in the adhesion of cells to extracellular substrata and in the regulation of the complement and coagulation pathways (Tomasini and Mosher 1986). Activation of the classical and alternative complement sequence to completion on a target cell membrane leads to the formation of a membrane-bound C5b-9(m) complex; C5b-9(m) may induce cell lysis or elicit secondary cellular responses contributing to tissue injury. In the fluid phase, binding of S to C5b-9 leads to the formation of a cytolytically inactive complex, SC5b-9 which is unable to attach to cells (Bhakdi and Tranum-Jansen 1983). Hitherto, S-protein was not demonstrated to be able to bind to C5b-9 membrane inserted complexes although detergent-extracted C5b-9(m) complexes from lyzed cells may bind S-protein when incubated with serum in vitro (Bhakdi and Tranum-Jansen 1982).

It has been suggested that complement activation fragments might play a role in vascular pathology. Both IgG and C3 can be observed in injured endothelium and atherosclerotic lesions (Hansson et al. 1984). The presence of the terminal C5b-9 complexes in the atherosclerotic plaque recently demonstrated by Vlaicu et al. (1985) suggest the pathogenic involvement of complement activation in the chronic progression of the atherosclerotic lesion. However C5b-9 neoantigens identified by the actually available antibodies are expressed either in membrane-bound C5b-9(m) or in SC5b-9 complex. In this study, we have investigated by immunofluorescence means the localization of both C5b-9 neoantigens and S-protein in atherosclerotic plaques and in intimal fibrous thickenings to discriminate between potentially pathogenic C5b-9(m) complexes and inactive SC5b-9 complexes.

In addition, like the other adhesive proteins, S-protein/Vitronectin has been localized in the extracellular matrices of various tissues including vascular walls (Simonton et al. 1985; Hayman et al. 1983; Dahlbäck et al. 1986). This study al-

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lows us to compare S-protein distribution within musculo-elastic (carotid) and muscular (temporal) arterial walls.

Material and methods

Ten atherosclerotic lesions were obtained by carotid endarterectomy. Ten temporal artery biopsies performed for clinical suspicion of giant cell arteritis but showing only arteriosclerotic changes were selected from the files of the department. One half of each arterial fragment was processed for standard light microscopy; histological sections were stained with haematoxylin-eosin, Masson's trichrome and orcein. The other half was snap-frozen in liquid nitrogen and then stored at −80° C. Cryostat sections were serially cut at 6 μm and allowed to air-dry before immunostaining. One cryostat section was stained with haematoxylin-eosin for each sample.

Two frozen kidney biopsies and spleen fragments from necropsy examination of 3 patients were used to test the working dilutions of anti-C5b-9 and anti-S antibodies (Hinglais et al. 1986; Falk et al. 1987).

Monoclonal murine antibodies to C5b-9 neoantigens and S protein were kindly provided by Dr. Bhakdi (Giessen – FRG). Rabbit anti-S-protein antiserum and FITC-conjugated antisera to human IgG, IgM and C3 were obtained from Behring-Werke (Marburg – FRG) and rabbit FITC-conjugated antiserum to human albumin from Dakopatts (Glostrup-Denmark). The anti-C3 antiserum recognizes C3c antigenic determinants expressed on the native C3 molecule and the C3b, C3bi and C3c cleavage fragments of C3. Rabbit anti-human C3d antiserum, which recognizes determinants expressed by native C3, C3b, C3bi, C3dg and C3d was obtained from the Dutch Red Cross (Amsterdam – The Netherlands). Rabbit anti-factor H antiserum was provided by one of us (M.D. Kazatchkine).

Air-dried cryostat sections were incubated for thirty minutes with rabbit FITC-conjugated specific antiserum then washed in PBS pH 7.2 and mounted in aqueous medium for direct immunofluorescence. For indirect immunofluorescence air-dried cryostat sections were incubated for thirty minutes with rabbit specific antiserum or monoclonal murine antibody then washed in PBS pH 7.2. The second incubation was performed respectively with goat FITC-conjugated anti-rabbit Ig antibody (Orthomune Paris – France) or with rabbit FITC-conjugated anti-mouse Ig antibody (Pasteur Paris – France) for thirty min; these two latter antibodies were previously adsorbed on human tissue polymer. After washing in PBS, sections were mounted in aqueous medium. Slides were examined with an epifluorescent Leitz microscope. Negative controls were performed for direct immunofluorescence by preincubating the section with unconjugated rabbit anti-human antibodies and for indirect immunofluorescence by omission of the primary antibody.

Results

In light microscopy, the samples of carotid endarteriectomy showed a range of atherosclerotic lesions. In 5 cases, the atherosclerotic plaque was only fibrous. In the 5 other cases, the plaque was fibro-atheromatous, containing an amorphous and necrotic core with extra in intra cellular lipids between the fibrous cap and the internal part of the media. Calcifications were noted in 4 cases. The plaques were in continuity with intimal thickening. Temporal arteries showed circumferential fibrous intimal thickening in 6 cases and segmental thickening in 3 cases. In one case the intima was nearly normal.

Table 1. Immunofluorescence distribution of specific deposits in atherosclerotic and arteriosclerotic lesions

	Atherosclerosis		Arteriosclerosis	
	+	−	+	−
Albumin	0	10	0	10
IgG	2	8	0	10
IgM	4	6	0	10
C3	6	4	3	7
C3d	7	3	8	2
H	1	9	0	10
C5b-9	6	4	7	3
Sm	3	7	2	8
Sp	9	1	9	1

Sp: polyclonal anti-S-protein antibody
Sm: monoclonal anti-S-protein antibody

Polyclonal anti-S-protein antibody gave positivities of the same topography as monoclonal antibody but these reactions were more numerous (Table 1). On spleen and kidney sections, perimyocytic matrices of arterioles and small muscular arteries were consistently and diffusely stained with anti-S-protein antibodies (Fig. 1). Anti-C5b-9 antibody gave a granular patchy staining in a similar location. In addition, the arteriolar hyaline deposits observed in 2 of the spleen samples demonstrated a constant and bright labelling with both anti-S-protein and anti-C5b-9 antibody.

In the atherosclerotic lesions of carotid arteries S-protein deposits were observed in 9 cases as irregular granules and spots in the fibrous cap and the internal part of the media and/or as large masses binding to the necrotic core. C3 deposits were present in 6 cases (4 fibro-atheromatous plaques and 2 fibrous plaques) and appeared as extra-cellular granules or spots disseminated in the fibrous plaque, in the intimal thickening or in the internal part of the media at the vicinity of the plaque. C3d deposits were observed in 7 cases; they were more abundant as spots and masses in necrotic areas and appeared only as scattered granules or threads in the fibrous plaques. Anti-H staining was positive in only one case as a very few threads in the fibrous plaque. C5b-9 deposits were detected in 6 cases (2 fibro-atheromatous plaques and 4 fibrous plaques) as granules scattered out in the fibrous plaque and the internal part of the media or in one case as spots and threads in the amor-

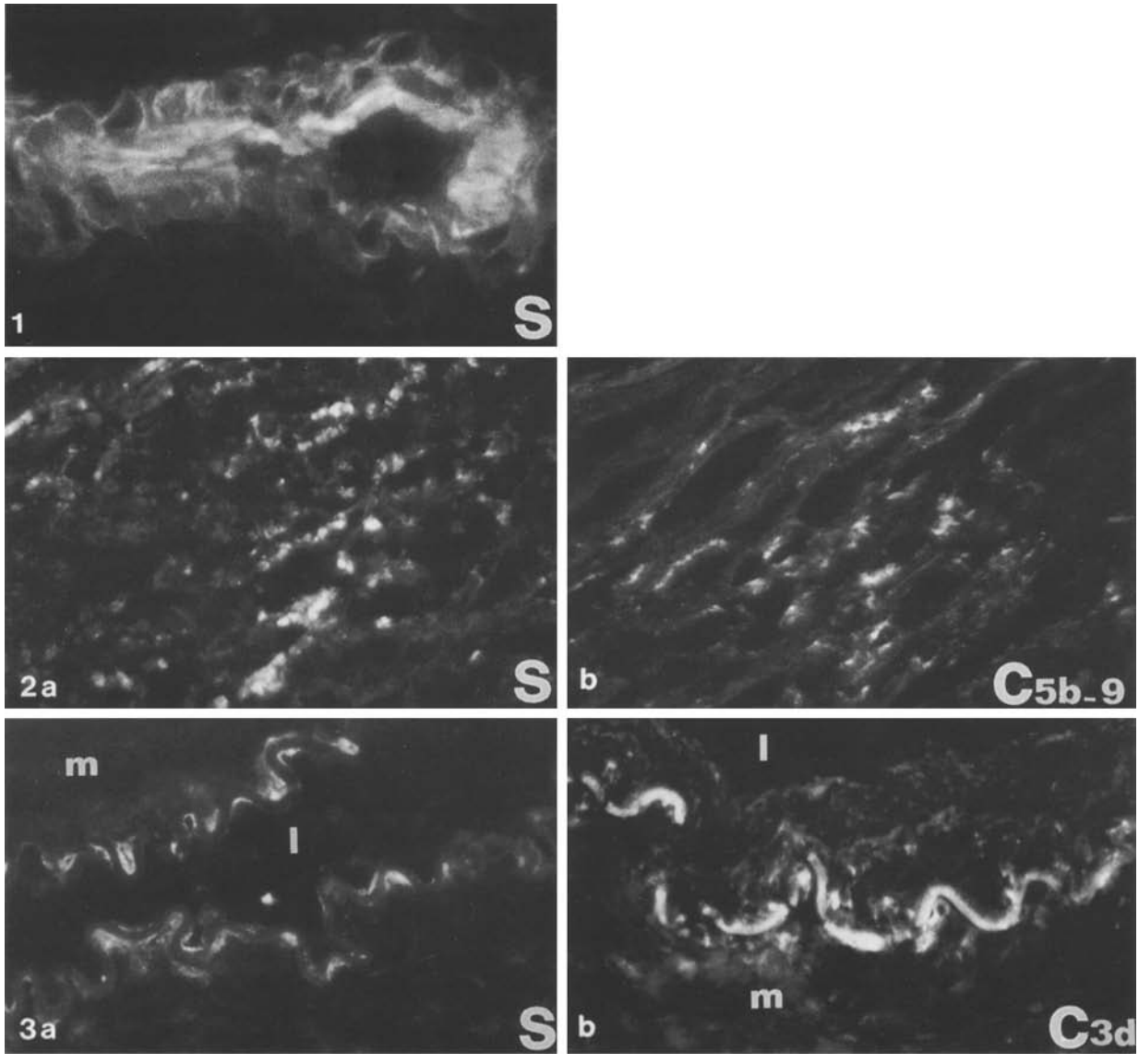

Fig. 1. Staining of the perimyocytic matrice with polyclonal anti-S-protein antibody in a small renal artery. Indirect immunofluorescence, original magnification. ×400

Fig. 2. Colocalization of S-protein (**a**) and C5b-9 neoantigens (**b**) in the fibrous cap of an atherosclerotic plaque. Indirect immunofluorescence, original magnification. ×400

Fig. 3. S-protein deposits (**a**) along the internal elastic limitant in arteriosclerotic temporal artery. C3d deposits (**b**) in the same localization along fragments of the elastic limitant. Indirect immunofluorescence, original magnification ×400, *l:* lumen, *m:* media

phous necrotic area. The colocalization of S-protein and C5b-9 neoantigens was not constant but observed clearly at least in two cases (Fig. 2). In 3 cases, S deposits were noted without associated C5b-9 and in the opposite C5b-9 was found once without associated S-protein. Anti-albumin, anti-IgG and anti-IgM gave a background positivity in the plaque indicative of a diffusion pattern (8 cases). In addition, anti-IgM antibody inconstantly revealed a few deposits as scattered granules or threads in the fibrous plaque.

The inner half or two-thirds of the musculo-elastic media are removed in endarterectomy. The extracellular matrix of this zone exhibit no diffuse

pericellular staining with anti-S-protein antibodies, unlike the matrices of arterioles and small muscular arteries.

In arteriosclerosis S-protein deposits were observed in 9 cases as granules and threads binding exclusively to each side of the internal elastic lamina. In the sections where the arterial wall was nearly normal (with minimal or absent intimal thickening) S deposits were reduced to a very few granular positive areas. In the arteriosclerotic areas, S-protein was more abundant as granules and spots bound to the segmentally fragmented IEL. C3 deposits were observed in only 3 cases as a few granules in the fibrous intima. C3d and to a lesser extent C5b-9 deposits, were noted respectively in 8 and 7 cases in the same topography as S-protein deposits (Fig. 3). Anti-albumin, anti-IgM and anti-IgG were always negative.

No diffuse staining was observed in any case in the perimyocytic matrix of the muscular arterial wall using anti-S-protein antibodies.

Discussion

This immunofluorescence study confirms the presence of S protein and C5b-9 neoantigens in the human atherosclerotic plaque as previously described by Nicolescu et al. (1987). These deposits are not abundant and are localized in the fibrous plaque and in the internal part of the media. S protein is trapped in the necrotic core in three cases and C5b-9 neoantigens are associated to this necrotic material in one case. The colocalization of S protein and C5b-9 was observed clearly in only two samples. The previous immuno-electron microscopic localization of C5b-9 exclusively on cell debris in the atherosclerotic plaque (Rus et al. 1986) and the co-presence of S protein and C5b-9 neoantigens on cell remnants in sclerotic extra-cellular matrices of kidney (Bariety et al., to be published) suggest that this colocalization of S protein and C5b-9 antigens in the atherosclerotic plaque occurs in the same way on cell membrane fragments enmeshed in sclerosis; it could represent either performed SC5b-9 complexes non specifically trapped on cell remnants from the circulation or circulating S protein non-specifically trapped on C5b-9(m) bearing cell debris, since S protein is unable to bind to native membrane-inserted terminal complexes. In all cases, the diffusion mechanism within the plaque is clearly demonstrated by anti-IgM, anti-IgG and anti-albumin staining. The lack of colocalization of C3d and C5b-9 is a supportive argument for the first hypothesis whereas the identification of isolated C5b-9 neoantigens without associated S protein suggest the presence of C5b-9(m) complexes.

In contrast, intimal fibrous thickening of arteriosclerotic temporal arteries does not stain for S protein and C5b-9 neoantigens. However in most cases, S protein and C3d colocalize on both sides of the internal elastic lamina with inconstant association to C5b-9 neoantigens. No deposit was observed along the external elastic structures. The deposits observed in normal arterial segments was more in marked pathological ones. The association of S protein/Vitronectin to elastin was demonstrated in the skin by immunofluorescence and immunoperoxidase techniques (Dahlbäck et al. 1986); the pattern of staining suggest that S protein is associated to the microfibrillar component of elastic fibers. Our personal results with polyclonal anti-S protein antibody using indirect immunofluorescence on frozen skin sections are identical; moreover S protein bound to dermal elastic fibers never colocalize with C5b-9 neoantigens but is also associated to C3d deposits.

In spite of its identity to Vitronectin, an adhesion protein, S protein was not detected in a diffuse pattern within the extracellular matrices of carotid and temporal arteries. In kidney biopsies processed by the same technique, the anti-S protein antibody stained the GBM, the TBM and the perimyocytic matrices of arteriolar and interlobular arterial sections (Falk et al. 1987). Although a systematic study of the exact localization of Vitronectin in human tissues has not been performed, S protein/Vitronectin has been immunolocalized in various tissues: muscle, kidney, embryonal lung and skin (Hayman et al. 1983), vascular smooth muscle in a pericellular location, the capsular surface of all viscera, gastrointestinal muscularis, supporting stroma of intrahepatic portal tracts, splenic cords (Simonton et al. 1985) and skin (Dahlbäck et al. 1986). Gebb et al. (1986) demonstrated that in vitro purified Vitronectin is able to bind to the native form of all collagens (I, II, III, IV, V and VI) and that this binding has characteristics compatible with the occurrence of such an interaction in vivo. In view of these data, the absence of Vitronectin from the wall of the large arteries is a surprizing fact and may reflect a differential distribution within the arterial tree, according either to the diffusion space of plasmatic proteins, or a differential property of local synthesis by the vascular parietal cell.

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