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Immunohistochemical detection of terminal complement complex and S protein in normal and pre-eclamptic placentae

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SUMMARY

Terminal complement complex and S protein were searched for in term placentae obtained from 13 women with normal pregnancy and 15 patients with moderate or severe form of pre-eclampsia. Terminal complement complex was found to localize in the fibrinoid material of the decidua of the basal plate, in the stroma of the chorionic villi and in the vessel walls, as subendothelial deposits. S protein had a quite different distribution, being detected in the syncytiotrophoblast located both in the chorionic villi and in the decidua of the basal plate (DBP) and also on the endothelial cells of fetal stem vessels. Mild deposits of C3 were found in the decidua of the basal plate and also in the stroma and on the basal membranes of the villi. Reactivity for C9 neoantigen was also observed in the cytoplasm of some cells, which were recognized to be macrophages by the presence in their cytoplasm of acid phosphatase and by their reaction with a monoclonal antibody specific for macrophages. Differences in complement deposition in normal and pre-eclamptic placentae were essentially quantitative. Possible mechanisms of complement activation are discussed.

Keywords placenta complement immunohistology pre-eclampsia

INTRODUCTION

The contribution of the complement system to the induction of placental damage in pre-eclampsia (PE) remains a controversial issue (Faulk & Fox, 1982). Substantial amounts of both early and late complement components have been detected in the chorionic villi (Sinha, Wells & Faulk, 1984) and in the walls of uterine spiral arteries of PE patients (Kitzmiller, Watt & Driscoll, 1983; Hustin, Foidart & Lambotte, 1983). However, the relevance of these observations to the onset of placental lesions in PE is somewhat difficult to evaluate since complement components can also be found in normal term and pre-term placentae (Falk *et al.*, 1980; Hsi & Yeh, 1987) as well as in utero-placental arteries in normal pregnancies (Wells *et al.*, 1987), although the deposits are consistently milder than in PE placentae.

The factors responsible for complement activation in normal and pathological pregnancies are unknown, and the mechanism, if any, involved in complement-mediated tissue alterations have not been elucidated. One possibility which has not been explored as yet is that the placental damage in PE may result at least in part from the action of the terminal complement complex (TCC), known to be cytolytic when properly assembled on the cell membrane (Bhakdi & Tranum-Jensen, 1987). Presence of TCC has been documented in various organs and tissues, particularly in immune-mediated lesions, in necrotic areas of human myocardium and in atherosclerotic fibrous plaques, where TCC can be identified either as C5b-9 or, more often, associated with S protein to form SC5b-9 (Mollnes & Harboe, 1987). The amount of TCC detectable in the tissue appears to be correlated with the severity of the lesion (Falk *et al.*, 1987), although mild deposits of TCC have also been observed in normal kidneys (Hinglais *et al.*, 1986). It is therefore tempting to speculate that a similar situation might occur at the placental level and help to differentiate PE from normal pregnancies.

The aim of this study was to examine normal term and PE placentae for the presence and distribution of TCC and S protein and to ascertain whether the pattern and extent of TCC deposition could possibly be related to the placental damage.

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MATERIALS AND METHODS

Study group

Fifteen women, nine nulliparous and six uniparous, with pregnancies complicated by PE were included in this investigation. They all satisfied the following criteria (Maikrantz &

Lindheimer, 1987): systolic blood pressure higher than 140 mm Hg and diastolic blood pressure higher than 90 mm Hg on repeated observations; and proteinuria exceeding 300 mg/24 h. Most patients exhibited a variable degree of oedema localized on the lower limbs. Thirteen women with uncomplicated pregnancies and no history of hypertension served as a control group. The gestational age of women with normal or pathological pregnancies varied from 37 to 40 weeks.

Antibodies

The monoclonal antibody aE11, recognizing C9 neoantigen expressed in the TCC and previously described in detail (Mollnes *et al.*, 1985), was used in this study. The following additional antibodies were also employed: rabbit anti-S protein (Calbiochem, Milan, Italy) and monoclonal antibody to S protein (Cytotech, La Jolla, USA); monoclonal antibody to macrophages and rabbit antisera to fibrinogen and C3d (Dakopatt, Milan, Italy). The goat antibodies to mouse and rabbit IgG were part of universal kits purchased from Ortho Diagnostic (Milan, Italy), which included the PAP reagent.

Placental tissues

Biopsy specimens were obtained from placentae collected from 25 vaginal deliveries and three Caesarean sections, the latter all belonging to the PE group, and were cut from the central area of the maternal surface. Tissue fragments of approximately 1 cm^3 were snap-frozen in liquid nitrogen after embedding in OCT (Miles Laboratory, Milan, Italy) and kept at −80°C until use. Sections of about 6 μm were cut using a cryostat (2800 Frigocut, Pabish, Milan, Italy), air dried, fixed in acetone–chloroform (1:1) for 10 min at room temperature and either used immediately or kept at −80°C wrapped in aluminium foil.

Immunohistochemical procedures

Immunoperoxidase staining was performed on placental sections after rehydration in 50 mM Tris-buffered saline (TBS), pH 7·6, for 10 min using the Ortho Universal mouse or rabbit kit. The protocol of the manufacturer was followed in detail with the following modifications: (i) the primary antibody was incubated with the sections overnight at 4°C at dilutions of 1/300 for aE11, 1/200 for the monoclonal antibody to S protein, 1/150 for the anti-fibrinogen, 1/800 for the polyclonal antiserum to S protein, 1/40 for the monoclonal antibody to macrophage and finally 1/1000 for the anti-C3d antiserum; and (ii) the sections were washed twice with TBS and once with 0·1 M acetate buffer, pH 5·2, prior to incubation with the substrate 3-amino-9-ethylcarbazole, which was limited to 12 min to reduce background staining. The detection system for the mouse antibodies was based on sequential incubations of the tissue sections with goat anti-mouse immunoglobulin and peroxidase-conjugated mouse immunoglobulin. On the contrary, the rabbit antibodies were revealed by sheep antibodies to rabbit immunoglobulin followed by rabbit PAP.

Double labelling for C9 neoantigen and S protein was performed by incubating overnight the placental sections with a mixture of aE11 and rabbit antiserum to S protein used at final dilutions of 1/150 and 1/200, respectively. The Ortho Universal mouse and rabbit kits were then employed to reveal the tissue bound primary antibodies in the following order: (i) the secondary antibodies to mouse and rabbit immunoglobulin were sequentially incubated with the tissue sections at room temperature for 30 min each; (ii) the reaction steps of the mouse Universal Kit were then completed as indicated above except that 1·3 mM 3,3′-diaminobenzidine (DAB) (Sigma, Milan, Italy) was used as substrate for peroxidase; and (iii) the rabbit antibodies were finally revealed using the rabbit PAP reagent and 2·2 mM 4-chloro-1-naphthol (Sigma) as substrate.

Combined immunoperoxidase labelling and histochemistry was used to reveal C9 neoantigen and macrophage acid phosphatase. The immunoperoxidase reaction was employed to detect C9 neoantigen using 4-chloro-1-naphthol as chromogen. After three washes of 15 min each with TBS, the same sections were rinsed in 0·1 M acetate buffer, pH 5·2, and incubated with a solution of naphthol AS BI phosphate (0·5 mg/ml) and fast red TR (1 mg/ml) (Sigma) in 0·2 M acetate buffer, pH 4·2, for 1 h at 37°C.

RESULTS

Localization of TCC and S protein in PE placentae

The analysis of the placental sections obtained from PE patients revealed the presence of C9 neoantigen both in the basal plate and in the chorionic villi. In the basal plate, the C9 neoantigen was located essentially in the part of the decidua of the basal plate (DBP) (Fig. 1a) rich in fibrinoid material, as documented by the specific staining for fibrin with Azan–Mallory and the reaction with anti-fibrinogen antibodies (results not shown). The deposits of C9 neoantigen in this area were intense and widely distributed judging from the reactivity pattern observed when several non-adjacent sections were examined. Staining for S protein in the DBP was generally milder than that for C9 neoantigen and was in any case limited to the syncytiotrophoblastic layer facing the intervillous space, whereas no staining was observed in the fibrinoid area (Fig. 1a). The monoclonal antibody aE11 recognized also some cells in the DBP, most of which had an irregular shape and showed cytoplasmatic reactivity. These cells reacting with aE11 appeared to be macrophages as indicated by the positive staining for acid phosphatase (Fig. 1b) and also by their reactivity with monoclonal antibodies to macrophages (results not shown).

In the chorionic villi C9 neoantigen was located predominantly in the stroma (Fig. 1c), where it was frequently observed in

Fig. 1. Distribution patterns of C9 neoantigen, S protein and C3 in placenta and cellular localization of C9 neoantigen. (a) Decidua of PE placenta with C9 localized in the DBP (brown, big arrows) and S protein on the syncytiotrophoblast layer (violet blue, small arrows); (b) Decidual macrophages positive for C9 neoantigen (violet blue) and acid phosphatase (red); (c) Villi of PE placenta with deposits of C9 neoantigen (brown, big arrows) and S protein (violet blue, small arrows); (d) C9 neoantigen and acid phosphatase in macrophages of the villi (see (c) for details); (e) Syncytiotrophoblast localization of S protein (violet blue) and stromal distribution of C9 neoantiger (brown); (f) Distribution of C3 in PE placenta. Deposits of C3 can be detected both in the DBP and in the villi; (g) Decidua of normal term placenta with mild deposits of C9 in the DBP and S protein on the syncytiotrophoblast layer (see (a) for details); (h) Villi of normal term placenta with deposits of C9 neoantigen and S protein (see (c) for details).

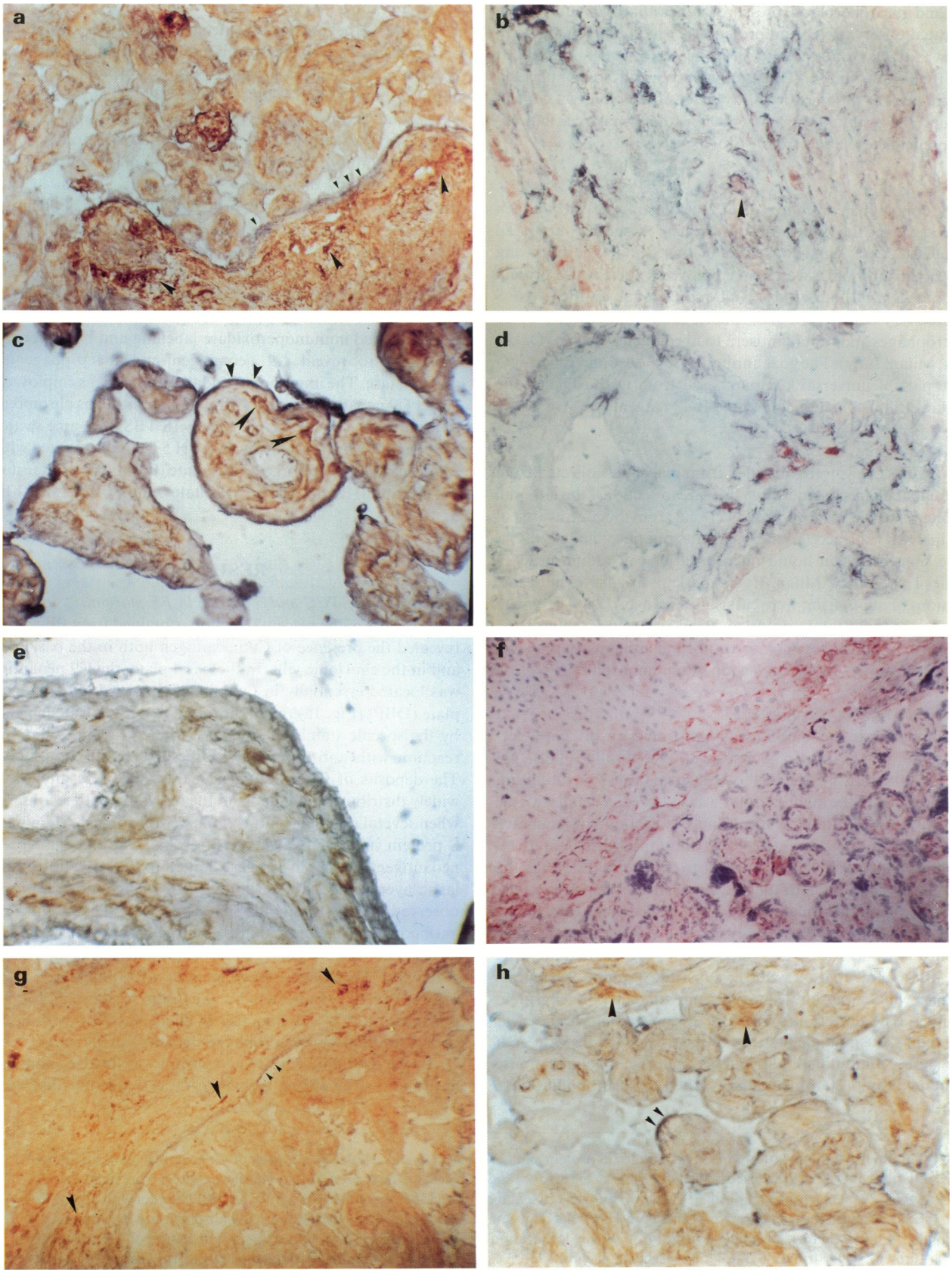
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Table 1. Evaluation of terminal ccomplement complex deposition in normal and pre-eclamptic placentae

	Patients		Controls	
	n	Score	*n*	Score
Decidua of the basal plate	10*	+++	2	++
	5	++	9	+
Villi	13*	++	5	++
	2	+	8	+

* Three samples obtained from Caesarian delivery.
Scores: +weakly positive; ++positive; +++strongly positive.

the cytoplasm of stromal cells with the characteristics of macrophages (Fig. 1d) as were the cells stained with aE11 in DBP. The walls of some fetal stem vessels and all the fibrinoid plaques also showed reactivity for C9 neoantigen, the former with a granular subendothelial pattern. The three placentae obtained by Caesarean delivery were among those with more intense deposits of TCC both in the DBP and in the stroma of the villi.

S protein had a different distribution, being mainly localized along the surface of the villi in close contact with the syncytiotrophoblast layer (Fig. 1e). Co-localization of S protein and TCC was restricted to the fetal stem vessels and to the fibrinoid plaques. Extensive washings of the sections with 0·5 M NaCl in TBS did not appreciably change the intensity of the staining for C9 neoantigen and for S protein, indicating that the deposits could not be attributed to loosely bound proteins.

Staining of the placental sections for C3 showed deposits of C3 both in the DBP and in the villi (Fig. 1f). In the DBP, the staining for C3 was less regularly distributed and generally fainter than that for C9 neoantigen. The deposits of C3 in the villi were localized essentially in the stroma and on the basal membrane.

Distribution of TCC and S protein in normal term placentae

The finding of TCC in the placentae of PE patients is an expression of complement activation occurring in this clinical condition. To establish whether this is restricted to PE or is rather a general phenomenon of normal pregnancy, 13 normal term placentae were examined for the presence of TCC and S protein. The overall distribution pattern of C9 neoantigen and S protein was essentially similar to that observed in PE placentae (Fig. 1 g, h). Thus, S protein was mainly located on the syncytiotrophoblast layers of the villi and of the DBP, whereas TCC was seen predominantly in the fibrinoid area of DBP and in the stroma of the villi. Independent examination of the tissue sections by two observers allowed a quantitative evaluation of TCC and S protein deposits in normal and PE placentae (Table 1). The scores for the deposition of S protein were essentially similar in the two groups of placentae. By contrast, the deposits of TCC in normal placentae were generally less intense in the stroma of the villi and more clearly reduced in the fibrinoid area of DBP, where they also appeared to be less widely distributed.

DISCUSSION

The use of a monoclonal antibody to C9 directed to a neoantigen present in TCC and not in native C9 (Mollnes *et al.*, 1985) allowed the detection of TCC in both normal term and PE placentae. The finding of TCC in normal placentae was not unexpected as both early and late complement components have been reported to be present in term placentae (Faulk *et al.*, 1980), although these studies did not provide conclusive evidence for tissue deposition of the late components in activated form. Similarly, our finding of more intense deposition of TCC in PE placentae confirmed previous observations by Sinha *et al.* (1984) showing higher amounts of complement components in the PE than in the control villi. It is interesting to note that the PE placentae obtained by Caesarean delivery had the highest score of TCC deposits, both in the DBP and in the villi. This may have been caused by factors related to PE, although the contribution of the surgical manipulations cannot be ruled out.

The exact mechanism of complement activation leading to deposition of TCC in normal placenta has not been elucidated yet. One possibility is that complement is activated by cellular and tissue remnants made available locally by tissue turnover. Mitochondrial membranes (Pinckard *et al.*, 1975), lysosomal enzymes (Venge & Olsson, 1975), cytoskeletal intermediate filaments (Linder, Lehto & Stenman, 1979) and red blood cells membranes (Poskitt, Fortwengler & Lunskis, 1973) are examples of potential local activators of complement. It is therefore possible that mild deposits of TCC in normal placenta are an expression of a general phenomenon occurring in normal tissue, as they may also be observed in normal human kidney (Hinglais *et al.*, 1986). Immune complexes formed by the reaction of maternal antibodies and fetal IgG allotypes may further contribute to TCC deposition at the placental level in normal pregnancy (Faulk *et al.*, 1980).

An interesting finding of this study was that the TCC deposited in DBP was not associated with S protein, as was the case of TCC in the villi. This is an unusual situation in most tissues where TCC has been detected in association with S protein (Bhakdi & Tranum-Jensen, 1987) suggesting that the TCC in DBP is still cytolytically active. S protein is in fact a potent complement regulator which binds to the soluble C5b-9 complex (Kolb & Muller-Eberhard, 1975), thereby preventing cellular damage by TCC (Podack & Muller-Eberhard, 1979). In this regard, the selective deposition of S protein on trophoblast may serve the purpose of enabling these cells to resist complement dependent lysis. The biological role of TCC free of S protein is not clear but it may well be that it contributes to the tissue turnover in normal pregnancy. The localization of this complex inside the macrophages is probably an indication that TCC is handled quite efficiently by the phagocytic cells to become harmless or, alternatively, that the macrophages have phagocytosed cells damaged by TCC.

Greater amount of TCC could be identified in the PE placentae, where additional factors are likely to be at work in triggering complement activation. However, the present data do not allow any definite conclusion for a direct role of TCC-mediated placental damage in PE. There is no doubt that TCC can be potentially harmful to tissues not only through a direct lytic effect on cells but also by promoting the release of inflammatory products such as arachidonic acid and reactive

oxygen metabolites (Imagawa *et al.*, 1983; Adler *et al.*, 1986). It remains to be seen whether TCC deposited in the placenta represents an appropriate complex capable of inducing these events.

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