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Immunological studies of human placentae: complement components in immature and mature chorionic villi

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SUMMARY

The localization and distribution of complement components in term and pre-term normal human placentae have been studied by using haemadsorption and immunofluorescence experiments. The components C1q, C4, C5, C6 and C9 were identified in characteristic locations. Receptors for C3 and C4 were not found. Complement was associated with certain stromal cells, areas of fibrinoid necrosis within the trophoblastic mantle, and in the walls and endothelia of foetal stem vessels. Activation of the complement system on trophoblastic basement membranes (TBM) did not appear to involve the early reacting components of the classical pathway of complement activation, because C1q, C4 and C2 could not be identified on TBM. The C6 component was identified within cytoplasmic granules of foetal stem vessel endothelia, suggesting that it may be synthesized by these cells. These findings put forward the possibility that complement may play an immunobiological role in the materno–foetal relationship during normal human pregnancy.

INTRODUCTION

Both maternal and foetal immunoglobulins (Ig) are found in human placentae, and much information now exists regarding the distribution, class, subclass, genetic type and possible function of placental Ig (McCormick *et al.*, 1971; Faulk *et al.*, 1974; Johnson *et al.*, 1977). In contrast, comparatively little is known about placental complement. Reports describing the deposition of several complement components in normal human placentae have appeared (McCormick *et al.*, 1971; Faulk & Johnson, 1977; Johnson & Faulk, 1978) but no evidence of complement breakdown products has been found in normal pregnancy blood (Thomson *et al.*, 1976). Thus, complement activation may be limited to the placental bed. In an attempt to gain a more comprehensive understanding of the role played by complement in the human materno–foetal relationship, we have studied in detail the deposition sites of many distinct complement components in both term and pre-term placentae.

MATERIALS AND METHODS

Placentae from healthy mothers with uncomplicated pregnancies were collected and immediately placed on ice. Histological examination showed all placentae to be normal. Forty term placentae

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were obtained, thirty by vaginal delivery and ten by Caesarean section. Eighteen pre-term placentae ranging in age from 4 to 14 weeks gestation were obtained by hysterotomy or suction directly into sterile flasks containing chilled RPMI 1640 and 0·1 M EDTA.

Antisera. Rabbit antisera to human C1q were obtained from Professor P. J. Lachmann (MRC Laboratory, Cambridge, England), Dr K. Pondman (Netherlands Red Cross, Amsterdam) and Professor H. Isliker (University of Lausanne, Switzerland). Sheep antiserum to human C2 was a gift from Seward Laboratory, London. Rabbit antisera to human C4 (Batch no. KH 12-7-PO3), C3d (batch no. KH 42-12-PO2) and C5 (batch no. KH 44-02-PO1) were purchased from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service. Rabbit antisera to human C3c (batch no. 5110D) and C9 (batch no. 2602F) were obtained from Behringwerke AG, Marburg, Germany, and goat anti-human C6 sera (lot no. C6-135-001-5) from Atlantic Antibodies, Westbrook, Maine, USA. A rabbit antiserum raised to native human C3 and absorbed with C3 fragments was a gift from Professor P. H. Lambert (University of Geneva, Switzerland). Fluorescein-isothiocyanate (FITC) conjugates of sheep anti-rabbit Ig (F:P ratio, 2·7) and rabbit anti-sheep/goat Ig (F:P ratio, 2·2) were purchased from Wellcome Laboratories, Beckenham, Kent.

Specificity of anti-complement sera was studied using three techniques: immunoprecipitation in gels, haemagglutination and immunofluorescence. The antigen sources were normal human serum (NHS), functionally pure human complement components from Cordis Laboratories (Florida, USA) and partially-purified C3d (Bokish, Dierich & Müller-Eberhard, 1975). Immunoelectrophoresis (IEP) and double radial immunodiffusion showed that each antiserum recognized its homologous antigen and did not precipitate any other complement components, the single exception being a cross-reaction with antisera to C3c and C3d. Specificity studies employing haemagglutination were performed using sheep erythrocytes (SRBC) sensitized by rabbit IgM anti-SRBC antibodies together with either C4 and C2 (EAC4, 2) or C4 and C3 (EAC4,3) (Williams & Chase, 1977). The anti-C4 serum agglutinated both EAC preparations, whereas anti-C3d and anti-C3c agglutinated only EAC4,3. None of the other antisera reacted with these sensitized cells. The EAC4,2 and EAC4,3 preparations were also used in tissue haemadsorption experiments according to Johnson & Matre (1979) for the localization of C4 and C3 receptors. Specificity studies done by immunofluorescence (Faulk & Hijmans, 1972) with anti-complement sera demonstrated that specific immunohistological reactions on cryostat tissue sections of human placentae were abolished following absorption with homologous antigen, but not by absorption with other components. For the FITC-conjugates, specificity studies by IEP and absorption with goat and rabbit IgG prepared by ion-exchange chromatography (Faulk & Pondman, 1969) showed no cross-reactions and that the antisera were specific for Ig antigens. To minimize non-specific fluorescence, the FITC conjugates were passed through a solid-phase immunoadsorbent column containing insolubilized NHS (Galbraith, Galbraith & Faulk, 1979). All antisera were ultracentrifuged weekly for 1 hr at 100,000 *g* to remove Ig aggregates which bind to Fc receptors in cryostat sections of placental tissues (Johnson, Faulk & Wang, 1976).

Immunohistology. Fresh placental tissue was cut into 5-mm cubes and immediately washed in isotonic barbitol-buffered saline, pH 7·4, before being snap-frozen in liquid nitrogen-cooled isopentane. To determine if complement components were loosely or non-specifically trapped in chorionic villi, experiments were performed to study whether the distribution of complement was altered following 3 days of tissue culture (Faulk & Temple, 1976). Fresh cryostat sections (4 μm) were transferred daily to microscope slides and no chemical fixation was employed. Tissue sections were washed either at 4°C with stirring in isotonic phosphate-buffered saline (PBS), pH 7·2, for 1 hr or in a chaotrope (0·5 M ammonium thiocyanate) for 10 min at 20°C to remove blood and other loosely adherent material before beginning specific immunohistological procedures (Faulk *et al.*, 1979).

All immunohistological reactions were performed at 4°C in a humidified chamber. Tissue sections were reacted for 20 min with 20 μl of 1:50 to 1:400 dilutions of anti-complement sera. The preparations were washed twice for 10 min in excess PBS, reacted for 20 min with 20 μl of a 1:60 dilution of FITC-conjugated anti-rabbit Ig or a 1:30 dilution of FITC-conjugated anti-sheep Ig, given three 10-min washes with excess PBS and mounted in PBS-buffered 50% glycerol. Sections were examined by transmitted light from an HBO 50 W high-pressure mercury-arc lamp using a

Zeiss Universal microscope fitted with an FITC interference primary filter, Tiyoda dark-ground condenser housing a toric lens, a 520-nm barrier filter and a × 25 planapo objective. The intensity and distribution of fluorescence for each dilution of anti-complement sera were scored by two independent observers. The dilution of antisera selected for study was one dilution below end-point because at this concentration non-specific reactions were minimal and specific fluorescence was maximal (Faulk & Johnson, 1977).

RESULTS

Clq. The three anti-Clq sera employed produced similar results by immunofluorescence, although variations in reactivity patterns and intensity were noted between placentae of different ages. Term placentae contained a patchy distribution of Clq in the walls of foetal stem vessels, this being more marked in larger vessels (Fig. 1). A granular pattern of fluorescence was also noted in the apical aspect of some endothelial cells regardless of vessel size. Occasionally the cytoplasm of cells located beneath the TBM reacted in a speckled pattern, and similar positive stromal cells were also identified in first-trimester tissues. TBM was rarely reactive with anti-Clq sera. Pre-term differed from term placentae in containing small speckled areas of fluorescence in the very apical aspect of the syncytiotrophoblast for most complement components studied. This is thought to result from the pinocytotic activity of trophoblast plasma membranes of young placentae, because these membranes also contain several other plasma proteins such as fibrinogen, C3 and alpha-2-macroglobulin in a similar apical distribution (Johnson & Faulk, 1978) whereas the same proteins are not found in the term trophoblast (Faulk & Johnson, 1977).

C4 and C2. Unlike Clq, the distribution of C4 was mostly in areas of intervillous fibrin (IVF) where it often was observed closely adherent to the syncytiotrophoblast (Fig. 2), sometimes appearing in pre-term placentae to tunnel beneath as though involved with syncytial shedding (Fig. 3). In term placentae, TBM, mesenchymal stroma and foetal stem vessels were usually negative for C4. Haemadsorption studies with cryostat sections from ten placentae of different ages failed to produce any evidence for placental C4 receptors capable of binding IgM-sensitized, C4-coated SRBC. Antisera to C2 also produced no specific reactions.

C3. The antiserum to native C3 did not react with any placental sections. In contrast, antisera to C3c reacted with segments of TBM and portions of fibrinoid areas in many chorionic villi. Immunofluorescence of TBM was, however, notably more intense using anti-C3d sera (Fig. 4). The reaction of anti-C3d with TBM remained positive in placental tissues following chaotrope washing of tissue sections or 3 days of tissue culture. Some C3d reactivity was also noted in focal, granular clusters within the mesenchymal stroma of term placentae and occasionally in the cytoplasm of stromal cells. In pre-term placentae, C3d-positive segments of TBM appeared laminated and C3d-containing cells were sometimes identified beneath these segments (Fig. 5). Haemadsorption experiments for C3 receptors using IgM-sensitized, C3-coated SRBC were negative.

C5. The pattern of anti-C5 reactivity was similar to anti-C4, but was notably less intense.

C6. The distribution of C6 was the same in both pre-term and term placentae. The most striking feature of anti-C6 reactivity was its presence as small granular cytoplasmic clusters within most endothelial cells of foetal stem vessels (Fig. 6). Less commonly, C6-containing cells were identified in villi and some areas of IVF were reactive with this antiserum.

C9. Like C3d, antisera to human C9 reacted positively with segments of TBM where it usually appeared as a thin line of fluorescence (Fig. 7), whilst that for C3d was often coarse and thick. In addition, anti-C9 often reacted in a speckled pattern with the walls of foetal stem vessels, the apical aspects of endothelium, and stromal cells thought morphologically to be macrophages.

DISCUSSION

This study demonstrates that certain complement components are found in characteristic locations in normal human placentae, suggesting that they may have important biological functions in the

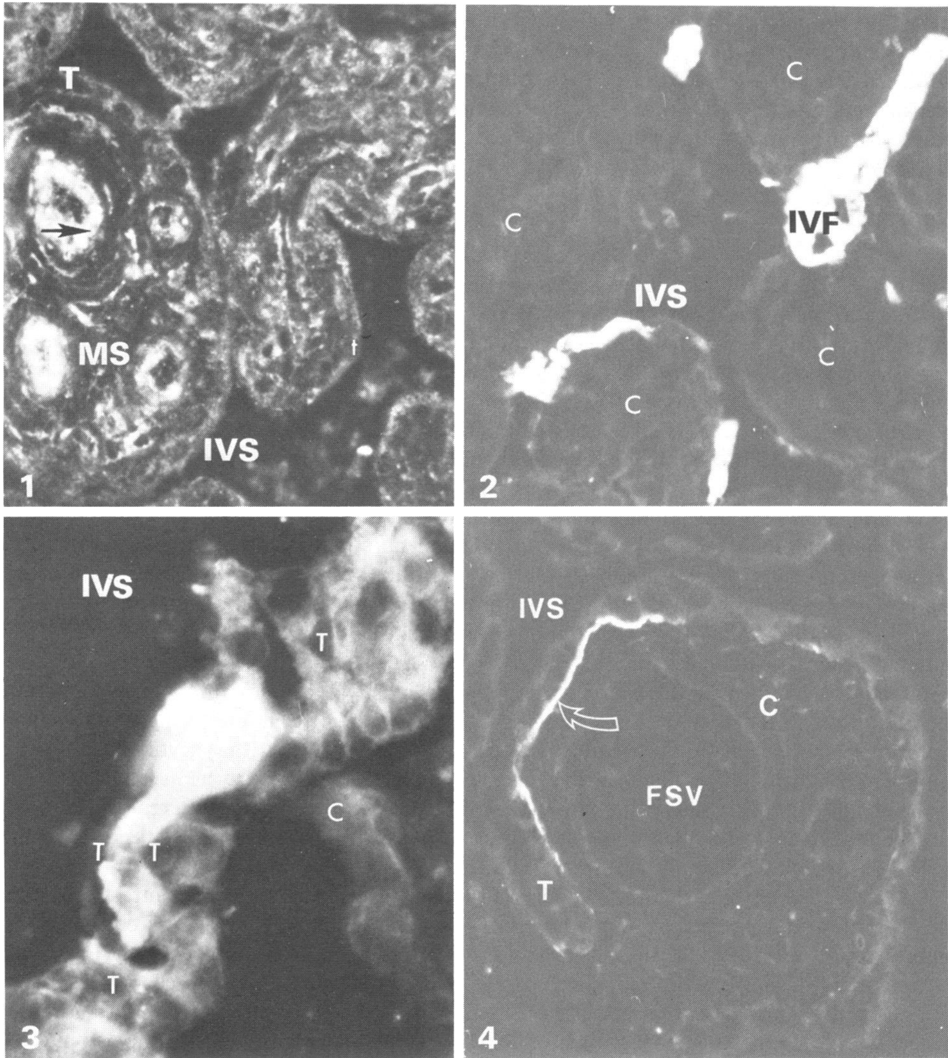

Fig. 1. Complement component C1q in normal term placenta. Dense reactivity in walls of larger vessels (*arrow*). Note absence of fluorescence in the intervillous spaces (IVS), trophoblast (T) and trophoblastic basement membranes. Many cells within mesenchymal stroma (MS) react with anti-Clq serum. (× 60.)

Fig. 2. Complement component C4 in normal term placenta. Reactivity is limited to areas of intervillous fibrin (IVF) in the intervillous spaces (IVS) and none of the structures within the chorionic villi (C) are positive. (× 60.)

Fig. 3. Complement component C4 in normal pre-term placenta. Note C4 reactivity within the trophoblastic mantle (T) at interface of chorionic villus (C) with the intervillous space (IVS). (× 250.)

Fig. 4. Complement component C3d in normal term placenta. Segments of trophoblastic basement membranes (*arrow*) react positively but no other structure within chorionic villi (C) including foetal stem vessels (FVS) and trophoblast (T) react with this reagent. Compare with Fig. 7. (× 150.)

maintenance of the placenta during normal human pregnancies. Although Clq can bind to molecules other than IgG, its presence in vessel walls, endothelium and certain stromal cells of placentae indicates the presence of immune complexes. This interpretation receives support from immunohistological studies with antisera to human IgG allotypes which demonstrated both maternal and foetal IgG in and around placental vessels and within stromal cells (Johnson *et al.*,

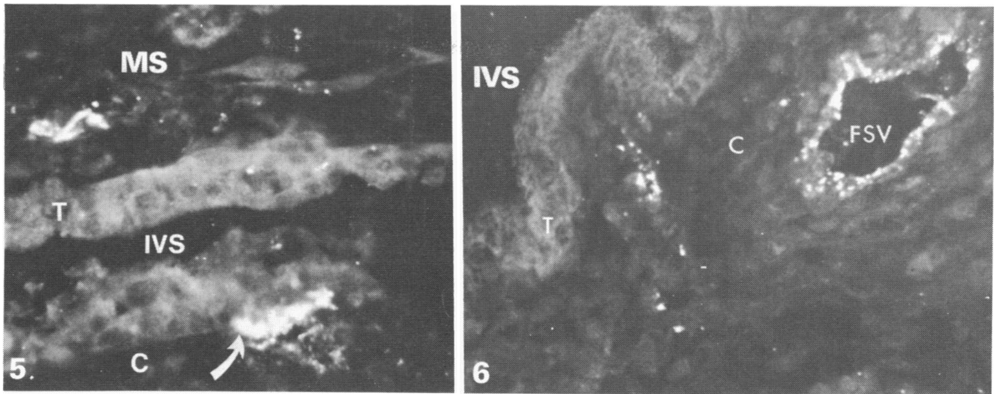

Fig. 5. Complement component C3d in immature placenta. Elective abortion of 8-week human foetus. Note in one chorionic villus (C) the C3d condensation on trophoblastic basement membrane (*arrow*) below the trophoblastic mantle and positive reaction within the mesenchymal stroma (MS) of other villus. The two villi are separated by the intervillous space (IVS). (× 140.)

Fig. 6. Complement component C6 in normal term placenta. Note granular pattern of reactivity within endothelial cells of foetal stem vessel (FSV). Clusters of fluorescence elsewhere in the chorionic villus (C) are localized to the cytoplasm of cells, presumably macrophages. The trophoblast (T) layer is negative. (× 140.)

Fig. 7. Complement component C9 in term placenta. Segments of trophoblastic basement membranes react with anti-C9 serum (*arrows*). Note no reactivity in upper two chorionic villi (C). Unlike C3, some cells within the mesenchymal stroma (MS) and walls of foetal stem vessels (FSV) react positively with anti-C9 serum. (× 250.)

1977). Some of these complexes may involve foetal Gm and Km allotypes since both anti-Gm and anti-Km antibodies with specificity for paternal (foetal) alloantigens have been identified in maternal blood (Fudenberg & Fudenberg, 1964; Faulk, van Loghem & Stickler, 1974).

Because antigens such as HLA, Gm and Km are found in placental mesenchymal stroma, maternal IgG anti-allotype antibodies probably bind their homologous antigens within chorionic villi and consequently never reach the foetus, producing a type of physiological sink for maternal antibody within placentae (Faulk & Johnson, 1980). These complexes offer a readily available substrate upon which complement may be activated. Furthermore, it is unlikely that soluble immune complexes formed within placentae ever enter the foetus, since they would be bound by Fc receptors found on the foetal stem vessel endothelia (Johnson *et al.*, 1976). Maternal IgG antibodies to foetal antigens can thus cause foetal pathology only if the relevant antigen is not present within placental tissues, one such example being blood group antigens (Szulman, 1973; McCormick *et al.*, 1971).

The strong reaction of TBM with anti-C3d sera and absence of reaction with antisera to native C3 indicates that products of activated C3 are present. That these are specifically bound and not passively trapped was substantiated by two observations: firstly, C3d remains on TBM in placental tissues following 3 days of culture and, secondly, chaotrope washing of the tissue sections did not remove C3d activity. The C3 component does not seem to be bound by placental membrane receptor structures, as no detectable immune adherence to placentae was observed either by us or by Johnson & Matre (1979) using C3-coated, IgM-sensitized SRBC. Complement C9 component was found on TBM and also in the walls of foetal stem vessels often in association with Clq. The activation of C3 and subsequent deposition of C9 on TBM might not involve the classical pathway, since C1q, C4 and C2 were seldom identified in this location. The nature of complement-activating material associated with TBM remains unknown, but could conceivably involve tissue-bound glycoproteins or trophoblastic proteases.

The observation of C6 in clustered cytoplasmic granules within foetal stem vessel endothelium in term and pre-term placentae suggests that this component may either be synthesized or stored in these cells. Most products of human placental trophoblast, such as hCG, SP_1 and TA_1 (Faulk *et al.*, 1979) are released into the maternal circulation, but the contents of placental stem vessel endothelium would be discharged into the foetal circulation, putting forward the possibility that the placenta itself may provide certain substances to the foetus. In conclusion, the results of immunohistological studies have shown that complement reproducibly occurs in specific areas of normal placentae, suggesting that it may be relevant to the biology of normal human pregnancy.

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