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IMMUNOLOGICAL STUDIES OF HUMAN PLACENTAE

BINDING OF COMPLEXED IMMUNOGLOBULIN BY STROMAL ENDOTHELIAL CELLS

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SUMMARY

Endothelial cells of foetal stem vessels in cryostat sections of normal, full-term human placentae bind fluorescein-conjugated heat-aggregated human IgG. Soluble immune complexes of sheep anti-human albumin also bind in a pattern that is similar to that of aggregated human IgG, but native human IgG is not bound by placental endothelial cells. Aggregated IgG binding, unlike soluble immune complexes, is blocked by pretreatment of the sections with antiserum to human IgM. Cross-blocking experiments with aggregated IgG and immune complexes suggest that they may be bound by different receptors.

INTRODUCTION

Several types of mammalian cells have membrane receptors for various forms of immunoglobulin. Monocytes and granulocytes, for example, have receptors for homologous and heterologous IgG (Abramson *et al.*, 1970; Messner & Jelinek, 1970); B lymphocytes bind antigen–antibody complexes (Dickler, 1974; Dickler & Sachs, 1974); aggregated IgG binds to B lymphocytes, thymocytes, activated T lymphocytes, and certain lymphoma cells (Dickler & Kunkel, 1972; Anderson & Grey, 1974; Van Boxel & Rosenstreitch, 1974; Grey, Kubo & Cerottini, 1972), and antibody-sensitized erythrocytes bind to antibody-dependent lymphocyte-mediated cytotoxicity effector cells, activated thymocytes, T and B cell-dependent areas of lymph nodes, reticulum cells in normal liver, and certain neoplastic tissues (Froland, Wisloff & Michaelsen, 1974a; Yoshida & Andersson, 1972; Thunold, Tonder & Wiig, 1973; Tonder, Morse & Humphrey, 1974).

It seems that many of the above reactions involve different receptors, each receptor being specific for one conformational or complexed state of IgG. For example, nylon-fibre column-fractionated lymphocytes form (EA) rosettes with antibody-coated erythrocytes, but do not bind FITC-labelled aggregated IgG (Froland, Natvig & Michaelsen, 1974b). The EA rosette formation is inhibited by certain subclasses of monomeric IgG (Froland *et al.*,

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1974c), and the specificity of this inhibition is similar to that obtained with aggregated IgG subclasses for antibody-dependent lymphocyte-mediated cytotoxicity (Wisloff, Michaelsen & Froland, 1974). On the other hand, the uptake of aggregated IgG by B lymphocytes is not inhibited by monomeric IgG (Dickler & Kunkel, 1972), but is reported to be blocked by immune complexes (Dickler, 1974) as well as by antisera to immune response-associated (*Ia*) gene products (Dickler & Sachs, 1974).

We here report the presence of receptors for aggregated immunoglobulin and immune complexes on vascular endothelial cells of foetal stem vessels in human placentae. This observation is relevant to a concept of receptors on non-immune cells for complexed immunoglobulin, and it offers a possible immunobiological mechanism for protection of the foetus from aggregated immunoglobulin and immune complexes during pregnancy.

MATERIALS AND METHODS

Tissues. Nine normal full-term placentae were used. Several placentae were perfused with chilled Hanks's solution. Blocks (0·5 × 0·5 cm) of tissue from the mid-portion of the central cotyledon were snap-frozen in isopentane cooled liquid nitrogen, embedded in 'Tissue-Tek' O.C.T. compound (Ames Company, Division Miles Laboratories, Indiana), and sectioned on a cryostat. Sections were prepared fresh each day.

Reagents and antisera. Cohn fraction II human IgG (Kabi AB, Sweden) and human serum albumin (HSA) (five times recrystallized) (Sigma Chemical Company, London) was used throughout.

Antisera were obtained from the following sources: Sheep anti-human IgG, sheep anti-human IgA, sheep anti-human IgM, sheep FITC–anti-human IgG, sheep FITC–anti-human IgM, rabbit FITC–anti-sheep immunoglobulin (Wellcome Reagents Ltd, Beckenham, Kent); swine anti-human Fab, goat anti-human Bence–Jones K chain, goat anti-human Bence–Jones L chain (Nordic Diagnostics Ltd, Fraburg, Netherlands); rabbit anti-Fc (μ) antiserum was a gift from Dr M. Papamichail, Taplow (the specificity of this antiserum was checked by immunoelectrophoresis against normal and macroglobulinaemic human serum and IgG preparation from normal human serum). No precipitation reaction was observed with light chains, any other heavy chain apart from μ chain, or any non-immunoglobulin serum protein.

A polyvalent anti-HL-A antiserum was a gift from Dr M. Jeannet, Transplantation Immunology Unit, Department of Medicine, University of Geneva, Switzerland.

Placental eluates were prepared from two normal full-term placentae as described by Faulk *et al.* (1974a).

Preparation of heat-aggregated IgG. Heat aggregation of a 2% solution of human IgG was performed at 63°C for 15 min in phosphate-buffered isotonic saline (PBS), pH 8·0. After cooling to room temperature, the material was centrifuged at 1000 *g* for 15 min to remove insoluble aggregates. The supernatant was then ultracentrifuged at 143,000 *g* for 90 min in a Spinco model L ultracentrifuge, number 40 rotor, at 20°C. The supernatant was discarded and the pellet redissolved in PBS, pH 8·0. This material was then centrifuged at 3000 rev/min for 15 min and the supernatant taken. Analytical ultracentrifugation of one preparation showed that this material predominantly sedimented between 40S and 150S.

Fluorescein conjugation. Conjugation with fluorescein isothiocyanate (FITC) (BDH, Poole), was performed by the method of Johnson & Holborow (1973) and unreacted FITC was removed by Sephadex G-25 gel filtration. Heat-aggregated IgG–FITC was prepared both by directly conjugating a preparation of aggregated IgG and by conjugating native IgG prior to heat aggregation.

Immune complexes. Soluble HSA–sheep anti-HSA immune complexes were prepared after determining the equivalence point of precipitation using sequential antigen dilutions in capillary tubes. Precipitation was then done at equivalence for 1 hr at 4°C in PBS, pH 7·2, washed in PBS, and resolubilized in a ten-fold excess of HSA. The preparation was centrifuged at 1000 *g* for 15 min and the supernatant taken.

Immunofluorescence techniques. Sections were cut at 6 μm in a cryostat and air-dried without fixation. Sections were exposed for 20 min to fluorescein conjugate in a moist chamber and subsequently washed in three changes of PBS, pH 7·2, for 20 min each before mounting in 80% glycerol buffered at pH 8·5. Blocking experiments were performed according to Faulk & Hijmans (1972) by exposure of the sections to neat unlabelled antiserum for 30 min, then washing in three changes of PBS for 1 hr before exposure to the fluorescein conjugate. Indirect immunofluorescence was performed according to McCormick *et al.* (1971)

by exposure of the sections to each layer for 20 min with washing in three changes of PBS for 20 min each between each layer. The stain sections were examined using a Reichert Zetopan microscope equipped with a quartz iodide light source, dark ground condenser, FITC-3 Balzer interference filter and Wratten 12 barrier filter.

RESULTS

Heat-aggregated human IgG–FITC (0·05–0·2 mg/ml) gave a fluorescent pattern on cryostat sections of human placenta as seen in Fig. 1. Bright fluorescence was observed in the apical aspect of endothelial cells of foetal stem vessels and in varying degrees in areas of fibrinoid necrosis. The staining pattern was identical in tissue sections taken from perfused and unperfused placenta. It was found that best results were obtained using freshly prepared heat-aggregated IgG–FITC of OD 495 nm/280 nm ratio of between 0·8 and 1·0. FITC-conjugated native human IgG did not stain.

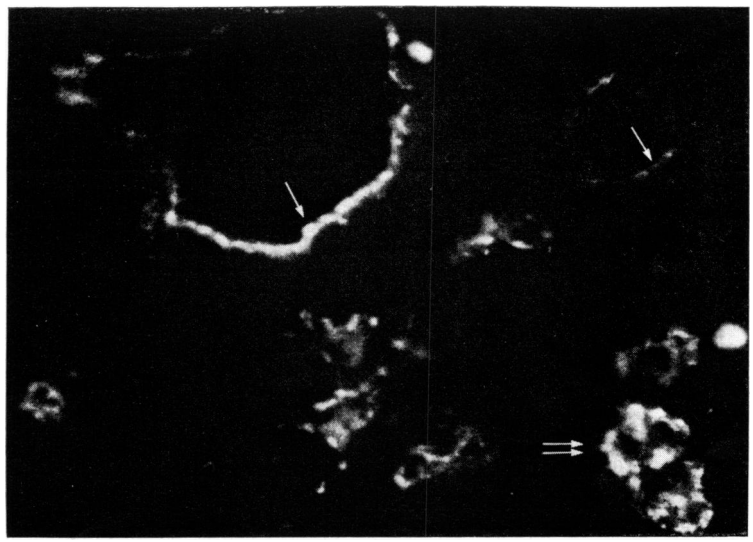

FIG. 1. Cryostat section of normal, full-term, human placenta stained with heat-aggregated human IgG–FITC. There is staining of the apical aspect of endothelial cells of vessels of varying diameter (single arrows) and in areas of fibrinoid necrosis (double arrows). Trophoblasts and trophoblastic basement membrane (not shown) were negative. (Magnification × 280.)

The nature of the binding of heat-aggregated IgG–FITC to placental endothelial cells was investigated by attempting to block the uptake of this material using several unlabelled immunoglobulin preparations and antisera (Table 1). Complete or nearly complete blocking of staining was achieved with unlabelled aggregated IgG, anti-human IgM and anti-human Fc (μ). Antisera to human IgG and IgA failed to block the uptake of heat-aggregated IgG–FITC by endothelial cells, although after incubation with anti-human IgG there was increased fluorescence, notably around the trophoblastic basement membrane and also in some areas of fibrinoid necrosis. Neither polyvalent anti-HL-A serum nor two placental eluate preparations blocked the uptake of heat-aggregated IgG–FITC by endothelial cells.

It was found by indirect fluorescence that a pattern identical to that shown in Fig. 1 was

obtained using unlabelled aggregated IgG followed by sheep FITC–anti-human IgG. To ensure removal of any immunoglobulin aggregates from the conjugate it was necessary to ultracentrifuge the conjugate, diluted 1:12 in PBS, pH 7·2, at 143,000 *g* for 90 min before use at a final dilution of 1:240.

The same pattern of fluorescence as that shown in Fig. 1 was obtained using soluble HSA–sheep anti-HSA immune complexes in antigen excess, followed by staining with rabbit FITC–anti-sheep immunoglobulin. To obtain no placental endothelial cell staining with the conjugate alone, it was ultracentrifuged as described previously and used at a final dilution of 1:100. Similarly, no endothelial cell staining was obtained using non-immune sheep serum, diluted 1:10, or when placental sections were exposed to 10% HSA followed by sheep anti-HSA (1:20) and stained with rabbit FITC–anti-sheep immunoglobulin.

TABLE 1. Effect of blocking agents on placental endothelial cell staining by aggregated IgG–FITC

Blocking agent	Immuno-fluorescence intensity (Scale from − to + + +)	Blocking agent	Immuno-fluorescence intensity (Scale from − to + + +)
PBS	+ + +	Sheep anti-human IgA	+ +
Unlabelled aggregated IgG (5 mg/ml)	−	Normal sheep serum	+ + +
Unlabelled native IgG (5 mg/ml)	+ + +	Rabbit anti-human Fc (μ)	±
Polyvalent anti-HL-A antiserum	+ + +	Normal rabbit serum	+ + +
Placental eluates (0·3–0·9 mg/ml)	+ + +	Goat anti-Bence–Jones K chain	+
Sheep anti-human IgM	±	Goat anti-Bence–Jones L chain	+
Sheep anti-human IgG	+ +	Swine anti-human Fab	+ +

Using indirect immunofluorescence, serial dilutions of heat-aggregated IgG and soluble immune complexes were employed to determine the optimal dilution for endothelial cell staining on sections of human placentae. At optimal dilutions, pretreatment of sections with anti-IgM blocked endothelial cell staining by heat-aggregated IgG, although there was little reduction in the staining obtained using immune complexes. Cross-blocking experiments using aggregated IgG to block the uptake of immune complexes and vice versa showed little blocking at the dilution for optimal staining, but some blocking when aggregated IgG was used at high concentration (5–10 mg/ml).

DISCUSSION

We have demonstrated that endothelial cells of foetal stem vessels in placental tissue can bind immunoglobulin. Since we have shown that these cells bind complexed immunoglobulin (heat-aggregated human IgG and antigen-bound sheep antibody) but do not bind native human IgG, it appears that there is a specificity for 'altered' rather than 'native' homologous and heterologous immunoglobulins. No such endothelial cell staining was achieved with aggregated IgG–FITC on any other tissue sections examined, e.g. human synovium, rat kidney, stomach and liver. Aggregated IgG–FITC was also bound by areas of placental

fibrinoid necrosis, but the mechanism of uptake is unknown since these deposits have been shown by immunohistological studies to contain several serum proteins (McCormick *et al.*, 1971).

Blocking experiments with various antisera (Table 1) suggest that the uptake of heat-aggregated IgG by placental endothelial cells may be related to IgM, presumably of foetal origin. This is in contrast to receptors for aggregated IgG on B lymphocytes that have been shown to be non-immunoglobulin in nature (Dickler & Kunkel, 1972). Epstein, Fong & Tan (1966) have demonstrated IgM haemagglutinins in matched maternal and cord serum that were specific for Bence–Jones proteins and did not react with intact native IgG. We were unable to demonstrate IgM on placental endothelial cells using direct staining with FITC–anti-human IgM, although it is questionable whether this technique would be sufficiently sensitive. Also, by analogy with surface staining for IgM on B lymphocytes, the possibility of a cross-reaction of antisera to the oligosaccharide component of IgM with similar carbohydrate structures on the endothelial cell membrane cannot be overlooked (Merler, Gatien & DeWilde, 1974), especially since anti-Fc (μ) was effective in blocking.

Experiments to determine whether the uptake of heat-aggregated IgG–FITC by placental endothelial cells was blocked by pretreatment with a polyvalent anti-HL-A antiserum were undertaken since Dickler & Sachs (1974) have shown an association of immunoglobulin Fc receptor on murine B lymphocytes with alloantigens determined by the *Ir* region of the H-2 histocompatibility complex, the so-called Ia antigens. However, no blocking was observed with polyvalent anti-HL-A antiserum, suggesting further evidence for the difference between immunoglobulin receptors on placental endothelial cells and those on immune cells such as B lymphocytes. In addition, placental eluate preparations are known to inhibit mixed leucocyte culture reactions (Faulk *et al.*, 1974a, b), but no blocking of the uptake of heat-aggregated IgG–FITC by placental endothelial cells was observed with either of the two eluate preparations used in this study.

The uptake of soluble immune complexes by placental endothelial cells was not substantially blocked by pretreatment with anti-human IgM, suggesting that aggregated IgG and immune complexes may bind to placental endothelial cells by different mechanisms. Cross-blocking experiments also supported this hypothesis. The analogy may be drawn to human B lymphocytes for which there is current controversy as to whether the same cells are capable of binding both aggregated IgG and immune complexes (Dickler, 1974; Froland *et al.*, 1974b).

A physiological role for immunoglobulin receptors on non-immune cells can only be conjectured. Nevertheless, a receptor for complexed immunoglobulin on placental endothelial cells might be implicated in an immunobiological mechanism for the protection of the foetus from aggregated immunoglobulin and immune complexes. Passage of allotypically incompatible immunoglobulin into maternal circulation undoubtedly occurs since maternal antibodies to foetal Gm (Fudenberg & Fudenberg, 1964) and InV (Faulk, van Loghem & Stickler, 1974c) antigens have been described. These and other maternal IgG antibodies to foetal antigens would be expected to form immune complexes with their respective foetal allotype in placentae, most likely in placental stem vessels. Consequently, type III immunopathological lesions would occur in placentae if there were no mechanism to remove immune complexes. It would seem that the receptors on foetal stem vessel endothelium for altered IgG could constitute part of such a mechanism for the removal of immune complexes.

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