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# Detection of receptors for immunoglobulin on human placenta by EA rosette formation

E. J. JENKINSON, W. D. BILLINGTON & JOANNA ELSON *Reproductive Immunology Group, Department of Pathology, University of Bristol*

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SUMMARY

Direct evidence for the existence of Fc receptors on the surface of first trimester and term human placental cells has been obtained by the use of an antibody-coated red cell (EA) rosette assay. The modification of a Ficoll density gradient separation procedure for placental cell populations in conjunction with dye uptake experiments, cytocentrifuge preparations and cytological analysis has enabled an identification of the rosette-forming cells in the mature placenta as predominantly, if not entirely, syncytiotrophoblastic.

The significance of these findings, together with those demonstrating the presence of cell-surface Fc receptors on the chorionic membrane, are considered in relation to the transmission of immunoglobulin from mother to foetus and to the protection of the foetus as an intra-uterine allograft.

## INTRODUCTION

The tissues forming the foeto-maternal interface in the uterus of the pregnant mammal play an important role both as an immunological barrier preventing rejection of the foetal allograft and in the regulation of macromolecular exchange between mother and foetus. In particular, maternal IgG can be transferred selectively to the foetal circulation in many species, including man. Although the cellular basis of the transport mechanism underlying this process has yet to be fully elucidated there is considerable support for the hypothesis proposed by Brambell (1966, 1970) that attachment of IgG to receptors with affinity for the Fc component of the immunoglobulin molecule is involved. Evidence of a circumstantial nature has come from studies in the rabbit on the preferential transfer of labelled Fc as compared to Fab fragments from the maternal to foetal circulation (Brambell *et al.*, 1960; Hemmings, 1974) and from studies on the kinetics of transport across the suckling mouse gut, where selective transport of IgG is also known to take place (Morris, 1964).

More direct attempts to investigate the existence of Fc receptors have involved examination of IgG binding to cell membrane preparations from the rabbit yolk sac (Hemmings & Williams, 1974) and to intact formalin-fixed rabbit yolk sac membranes *in vitro* (Sonoda & Schlamowitz, 1972), although it is not clear whether this reflects receptors of the type envisaged in the Brambell hypothesis or a more general effect based on charge relationships (Wild, 1974). Recently, however, the use of a rosetting technique has provided direct evidence for the existence of Fc receptors on the surface of yolk sac endoderm and placental cells in the mouse (Elson, Jenkinson & Billington, 1975).

Correspondence: Dr E. J. Jenkinson, Reproductive Immunology Group, Department of Pathology, The Medical School, University of Bristol, Bristol BS8 1TD.

In man the kinetics of immunoglobulin transfer are also consistent with the existence of a specific saturable carrier system for IgG (Gitlin, 1974). Unlike the rabbit and mouse, however, the yolk sac of the human embryo is not known to be involved in this process and the placenta is considered to be the primary route of transfer. It might therefore be expected that Fc receptor-bearing elements would be detectable in this organ. Whilst it is generally assumed that these are likely to be located on the syncytiotrophoblast, this has yet to be established and the involvement of other cell types in the structurally complex placenta has not been assessed.

The present report is concerned with the detection and identification of Fc receptor-bearing cells in preparations of human placentae of different gestational ages, using rosette formation by antibody-coated red cells (EA rosettes) as an indicator system (Berken & Benaceraff, 1966). The findings are discussed in relation to immunoglobulin transfer and foeto-maternal immunological interaction.

## MATERIALS AND METHODS

*Placental preparations.* Human placental material was obtained either from therapeutic abortions at 9–12 weeks or from term deliveries. Terminal tips of placental villi were carefully dissected from the main placental mass, gently teased apart, washed three times in cold PBS and incubated in 0·25% trypsin for 30 min at 37°C. The resultant cell suspension was filtered through silk gauze, washed and resuspended in medium RPMI + 10% FCS at $10^6$/ml for subsequent use in the rosette test. Examination of these suspensions showed that the syncytiotrophoblast was disrupted by this procedure to yield a mixture of elements containing from one to four nuclei, together with some larger aggregates. Samples of the chorion laeve, the outermost foetal membrane, were also taken from the term conceptus and cell suspensions prepared in a similar manner.

*Indicator cells.* Antibody-coated indicator red cells (RBC) were prepared by incubating one volume of packed, washed, human group O Rhesus (D) positive RBC with an equal volume of human anti-D antiserum diluted 1/2 in PBS for 1 hr at 37°C. After coating, RBC were washed in PBS to remove excess antiserum and resuspended in RPMI + 10% FCS at a concentration of 2%. Non-coated RBC for use as controls were similarly washed and resuspended at the same concentration.

*Rosette test.* Rosette tests were performed by incubating 0·5 ml aliquots of placental or chorionic cell suspension with an equal volume of appropriate indicator RBC in small glass test-tubes. Experimental tubes received antibody-coated RBC whilst control tubes were incubated with non-coated RBC. After 1 hr incubation at room temperature under 5% $CO_2$ in air, cultures were gently resuspended and samples taken for counting of rosette-forming cells and for total cell counts using a haemocytometer. Cytocentrifuge preparations of representative aliquots were also examined.

*Identification of rosette-forming cells.* (a) *Cell labelling.* To facilitate identification of the cells potentially involved in rosette formation, samples of isolated intact placental villi from 12-week material were pre-incubated for 5 min in a 0·25% solution of neutral red in culture medium. Some of this material was fixed immediately in neutral buffered formalin for histological examination and identification of cells which had taken up neutral red, whilst the remainder was trypsinized for use in the rosette test as described above. Cytocentrifuge preparations of these suspensions were made and examined for the presence of neutral red in the rosette-forming cells.

(b) *Cell separation.* To assess the involvement of elements from the outer syncytiotrophoblast layer of the mature placenta in rosette formation, fractionation of cell preparations from term placentae was carried out using a density gradient centrifugation procedure based upon that described by Zembala & Asherson (1970). Cell suspensions were prepared as detailed above, spun down, resuspended in 3·4 ml of 25% Ficoll 400 in PBS and transferred to a 15 ml test-tube. Further layers of 3·4 ml of 21%, 4·4 ml of 16% and 5·3 ml of 12% Ficoll were then added sequentially and the preparation centrifuged at 20,000 *g* for 1 hr. The four resultant fractions A, B and C at each interface and the pellet D were collected using a fine glass pipette, washed three times in PBS and resuspended in medium RPMI + 10% FCS for use in the rosette test. Smears of each fraction were also prepared for histological examination.

*Histology.* Samples of material from each placenta used were fixed in neutral formalin, wax embedded, sectioned at 5 $\mu$m and stained with haematoxylin and eosin for comparative purposes. Material pre-incubated in neutral red was processed through cellosolve avoiding the use of alcohol both in the dehydration of the material for embedding and in the preparation of slides. Sections of this material were examined either unstained or after staining with haematoxylin alone.

## RESULTS

The results are summarized in Tables 1 and 2. As indicated in Table 1, Fc receptor-bearing cells are present in comparable numbers in both 9–12 week and term placentae and constitute between 6 and 18% of the total placental cell population. The chorionic membrane also possesses receptor-bearing cells, although in this case there is rather more variation in the number of rosettes formed from one preparation to another.

TABLE 1. EA rosette formation by human placental cells and chorionic membrane

| Source of material | Gestational age (weeks) | Percentage rosettes: Ab-coated RBC | Percentage rosettes: Uncoated RBC |
|---|---|---|---|
| Early placenta | 9 | 6·3 | n.d. |
| | 10 | 12·0 | <0·32 |
| | 10 | 18·4 | <0·15 |
| | 12 | 14·9 | <0·14 |
| Term placenta | 38 | 9·9 | <0·19 |
| | 39 | 12·5 | <0·09 |
| | 40 | 7·2 | <0·35 |
| | 40 | 17·3 | <0·32 |
| | 40 | 7·4 | <0·04 |
| Chorion | 38 | 4·9 | <0·74 |
| | 40 | 1·4 | <0·17 |
| | 40 | 21·3 | <0·74 |
| | 40 | 7·3 | <0·03 |

n.d. = Not determined.

TABLE 2. Effect of Ficoll fractionation on the rosetting characteristics of human placental cell preparations

| Fraction | Percentage rosettes: Experiment 1 | Percentage rosettes: Experiment 2 |
|---|---|---|
| Whole placental preparation | 11·3 | 7·2 |
| A. Few mononuclear cells | 2·2 | 3·3 |
| B. Syncytiotrophoblast elements (mainly one to four nuclei) plus | 40·8 | 13·5 |
| C. a few cytotrophoblast and/or Hofbauer cells (<5%) | 34·4 | 24·4 |
| D. Few syncytiotrophoblast elements, leucocytes, RBC and other placental elements | 2·0 | 0·3 |

At 12 weeks of gestation a typical placental villus consists of an outer syncytiotrophoblast component underlain by a less continuous layer of cytotrophoblast which together enclose an inner mesenchymal core containing the foetal blood vessels (Fig. 1). The structure of the mesenchyme is uniform except for scattered vacuolated cells, the cells of Hofbauer. Examination of cytocentrifuge preparations from a 12-week placenta revealed that many of the

rosette-forming elements were mononuclear. Similar preparations from villi pre-incubated in neutral red showed that the majority of rosette-forming cells had taken up the dye, whilst histological sections of such villi revealed dense accumulations of neutral red in the syncytiotrophoblast, cytotrophoblast and Hofbauer cells. No dye was seen in the mesenchyme although some cells of the blood vessel endothelium showed faint staining. On this basis it would seem unlikely that mesenchymal cells are involved in the rosette formation observed but that syncytiotrophoblast, cytotrophoblast and Hofbauer cells are all potential contributors. The identity of the rosette-forming elements is considered further in the discussion.

In the case of term material, as shown in Table 2, separation on the particular Ficoll gradient employed gave four fractions, A, B, C and D, of which fractions B and C showed enrichment of the rosette-forming elements as compared to the unfractionated preparation, whereas fractions A and D were considerably depleted in this respect. Examination of smears prepared from these fractions revealed that B and C consisted of a mixture of small fragments

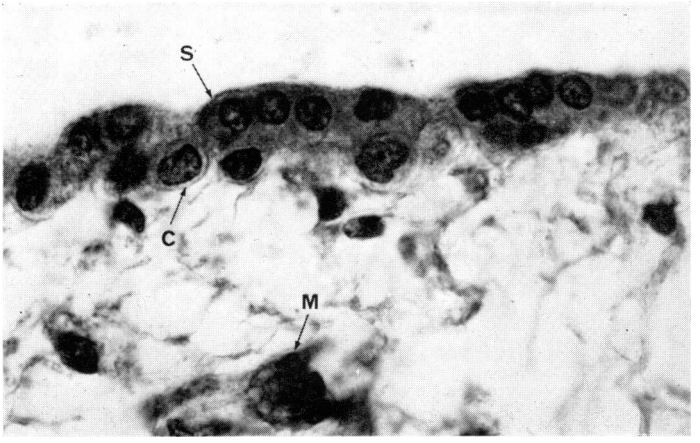


FIG. 1. Transverse section of part of a chorionic villus from a 10-week human conceptus. The main cellular elements consist of an outer layer of syncytiotrophoblast (S), an underlying discontinuous layer of cytotrophoblast cells (C), and mesenchymal cells (M) in a loose connective tissue core, in which scattered Hofbauer cells may be present. In the mature placenta the cytotrophoblastic component is very considerably reduced, and may disappear completely near term. (H & E; magnification $\times 945$.)

of syncytiotrophoblast containing one to four nuclei (approximately 40% mononuclear) easily recognized by their marked basophilia, some larger aggregates of syncytiotrophoblast and a few ($<5\%$) more lightly staining single cells possibly cytotrophoblast or Hofbauer cells. Fraction A contained very few identifiable cells whilst fraction D consisted of some syncytiotrophoblast, the remaining placental elements, leucocytes, RBC and debris. It can therefore be concluded that the syncytiotrophoblast of the mature placenta is the principal source of the rosette-forming elements observed in preparations of this material and hence possesses Fc receptors.

## DISCUSSION

Studies in experimental animals have provided considerable circumstantial support for the proposal by Brambell (1966) that attachment of IgG to Fc receptors associated with the placenta or foetal membranes plays a fundamental role in the transfer of this molecule from mother to foetus. The involvement of a similar mechanism in man has also been suggested, for example by the ability of IgG to bind to placental homogenates (Gitlin & Gitlin,

1973) and by the recent report that intact antibody to tetanus toxoid can enter the foetus whereas Fab fragments of the same antibody cannot (Börner *et al.*, 1974). The present study now provides direct evidence for the presence of cell-surface Fc receptors in the human placenta, a finding consistent with the existence of a receptor-dependent mechanism for materno-foetal immunoglobulin transport in this organ.

In view of the complex nature of the placenta any consideration of the role of Fc receptors in immunoglobulin transport must take into account both the identity and location of the receptor-bearing cells. In functional terms it might be expected that any receptors involved in the uptake of protein would be located on the outermost layer of the placental villi, the syncytiotrophoblast, which is bathed directly by the maternal blood. That this is the case is clearly indicated by the rosetting ability of purified syncytiotrophoblast obtained by Ficoll fractionation of placental cell preparations, as well as by the ultrastructural characteristics of the rosette-forming elements (unpublished observations). It is perhaps worth noting that the use of Ficoll separation procedures may be valuable for other studies on placental immunobiology, which previously have also been complicated by the presence of non-trophoblastic elements.

Apart from the syncytiotrophoblast, the use of neutral red as a tracer indicates that, at least in the 12-week placenta, both the cytotrophoblast and the Hofbauer cells may contribute to rosette formation. However, since the Hofbauer cells are relatively few in number it would seem reasonable to conclude that the majority of rosetting elements are in fact trophoblastic. Unlike the mature placenta, there is considerably more cytotrophoblast in the early placental villi and the relative contribution of this trophoblast type has yet to be assessed. As far as the Hofbauer cells themselves are concerned, the phagocytic properties which they display and the possibility that they possess Fc receptors might suggest that they have a role in combating infection within the placenta, as recently suggested for macrophage-like cells in the mouse placenta which have antigen processing capabilities (Ptak, Prjyma & Moskalewski, 1974).

Although the presence of Fc receptors on the syncytiotrophoblast of the mature placenta can be related to the transfer of IgG, the functional significance of Fc receptor-bearing cells at the earlier gestational age is more difficult to interpret. Small amounts of IgG are transmitted to the foetus at this time but it is not until after the 22nd week that transfer rises to significant levels (Gitlin, 1974). It has been suggested that there may be a sudden activation of the carrier mechanism for IgG at about 22 weeks (Gitlin, 1974). On the basis of the present results it would seem that factors other than the presence of Fc receptors are limiting, and it is not until the complete system for transfer, of which Fc receptors constitute only one part, is established that transfer can occur. Alternatively, it may be that at the earlier stages Fc receptors are confined to a population of cytotrophoblast stem cells which are not in a position to participate in transport and it is not until the outer syncytial layer is sufficiently populated by the progeny of these cells that full-scale transfer is possible. This might be resolved by the use of fractionation procedures on cell suspensions of the earlier material, and this is currently under investigation.

The role of the chorion in immunoglobulin uptake by the foetus is not yet clear. It has been suggested that IgG may pass across the chorion into the amniotic fluid, followed by selective uptake across the foetal gut when this fluid is swallowed, although experimental evidence has not so far supported this proposition (see Wild, 1974). The present demonstration of the existence of Fc receptors on the chorion suggests the alternative possibility that this membrane might contribute to IgG uptake in a manner analogous to that of the yolk sac in the mouse, which is considered to be an important route of transfer and has also been shown to possess Fc receptors (Elson *et al.*, 1975). In this case uptake from the uterine fluid would take place at the level of the chorion and immunoglobulin would pass into the foetal circulation via the chorionic blood vessels.

In addition to their involvement in the transmission of passive immunity the presence of Fc receptors in the placenta may have other implications for the foeto-maternal immunological relationship. The pregnant female is known to possess antibodies to paternally derived histocompatibility (and other) antigens of the foetus and the existence of a mechanism for IgG transport across the placenta might, as well as facilitating the transfer of passive immunity, provide the means whereby these potentially deleterious antibodies could gain access to the foetus in effective quantities. With the exception of Rhesus disease, the extent to which this takes place is at present unknown, but the possibility of such a transfer may necessitate the existence of a mechanism(s) within the foetus, such as complement deficiency, to prevent any consequent damage.

One of the main contributory factors in the survival of the foetal allograft is currently believed to be the presence of specific blocking factors in the maternal serum, composed of foetal antigen–maternal antibody complexes operating in a manner similar to that described for tumour enhancement (Hellström & Hellström, 1974). Although there are a number of ways in which these complexes may achieve their blocking effect, in the pregnancy situation there is the additional possibility that complex binding to Fc receptors on the exposed syncytiotrophoblast may contribute to the immunoresistance of this tissue by interfering with maternal lymphocyte recognition of its surface determinants. On the other hand, in pregnancy toxaemia, where immune complex production may be excessive, saturation of the trophoblast surface receptors might lead to disturbances of normal physiological exchange, with serious consequences for foetal well-being.

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