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Human placental coated vesicles contain receptor-bound transferrin

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Human placental coated vesicles have been purified by a method involving sucrose-density-gradient centrifugation and treatment with wheat-germ agglutinin. These preparations were free of contamination by placental microvillus fragments. Crossed immunoelectrophoresis demonstrated that the coated vesicles contained a single serum protein, which was identified as transferrin. This transferrin was only observed after the vesicles were treated with a non-ionic detergent, and its behaviour during crossed hydrophobic-interaction immunoelectrophoresis suggested that a large proportion of it was receptor-bound. No other serum proteins, including immunoglobulin G, could be detected in these preparations. Receptor-bound transferrin was the only antigen common to placental coated vesicles and microvilli, implying that other plasma-membrane proteins are excluded from the region of membrane involved in coated-vesicle formation.

The syncytiotrophoblast microvillar plasma membrane is the site at which the human placenta interacts with maternal blood. Studies on preparations of syncytiotrophoblast microvilli have shown that this plasma membrane possesses receptor proteins specific for IgG (McNabb *et al.*, 1976), insulin (Whitsett & Lessard, 1978), transcobalamin II–vitamin B_{12} complexes (Friedman *et al.*, 1977) and transferrin (Wada *et al.*, 1979; Loh *et al.*, 1980). Coated vesicles are found in the syncytioplasm underlying the microvilli, and it has been suggested that these might be involved in the endocytosis of receptor-bound serum proteins, particularly IgG destined for transfer to the foetal circulation. However, when Pearse (1978), using electrophoresis in SDS, examined preparations of coated vesicles isolated from various tissues, including human placenta, she found a remarkable lack of proteins corresponding to material included within the vesicles.

In the present paper we report the results obtained when human placental coated vesicles were examined by the more sensitive techniques of immunoelectrophoresis. We present evidence that they contain receptor-bound transferrin and that other plasma-membrane antigens are excluded from the coated-vesicle membrane.

Materials and general methods

Freshly delivered normal full-term placentae were obtained from Leeds Maternity Hospital and were processed within 15 min of delivery. Transferrin was from Sigma (London) Chemical Co. Ltd., Poole, Dorset, U.K. Sheep antiserum to whole human serum was from Wellcome Reagents Ltd., Beckenham, Kent, U.K. (lot no. K6919). Rabbit antiserum to human transferrin was from Behringwerke A.G., Marburg, Germany (lot no. A5307A). Rabbit antiserum to human spleen ferritin was from Miles Laboratories (U.K.) Ltd., Slough, Berks., U.K. (lot no. R279). Goat antisera to human Fab and Fc fragments of IgG were provided by the Department of Immunology, Royal Hallamshire Hospital, Sheffield, South Yorkshire, U.K. Human placental syncytiotrophoblast microvilli were prepared as previously described (Booth *et al.*, 1980). An antiserum was raised to placental microvilli by immunizing a rabbit with 0.5 mg of microvillar protein in Freund's complete adjuvant intracutaneously. The immunization was repeated at fortnightly intervals in incomplete adjuvant and the animal was bled 1 week after the fourth injection. After 1 month the rabbit was 'boosted' by injecting 2 mg of microvillar protein in 0.15 M-NaCl intravenously, and was bled 1 week later. The sera from the two bleedings were combined. When this antiserum was used as the first antiserum in immunofluorescent staining of sections of frozen placental tissue, fluorescence was restricted to the microvillar region of the syncytiotrophoblast (C. Goodfellow & A. G. Booth, unpublished work). Wheat-germ agglutinin was purified by ion-exchange chromatography and affinity chromatography as described by Bouchard *et al.* (1976), and was stored freeze-dried.

The methods for the solubilization of microvilli,

Abbreviations used: IgG, immunoglobulin G; Mes, 4-morpholine-ethanesulphonic acid; SDS, sodium dodecyl sulphate.

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enzyme and protein assays, immunoelectrophoresis and the staining of immunogels for protein and enzymic activity have been described previously (Booth *et al.*, 1979, 1980). Crossed hydrophobic-interaction immunoelectrophoresis was performed as described by Bjerrum (1978), except that the first-dimension electrophoresis was performed at 5V/cm for 3h, the second dimension at 1V/cm overnight, and the non-ionic detergent used was Emulphogene BC 720. The hydrophobic matrix used was phenyl-Sepharose CL-4B from Pharmacia (G.B.) Ltd., London W.5, U.K. Electrophoresis in SDS was performed by using the buffer system of Laemmli (1970) in gels containing 4–14% (w/v) polyacrylamide gradients. The gels were stained with Coomassie Blue and destained as previously described (Kenny *et al.*, 1976). Samples of coated vesicles, negatively stained with 2% (w/v) uranyl acetate on carbon/formvar-coated grids were examined in a Philips EM300 electron microscope.

Specific methods and results

Purification of placental coated vesicles

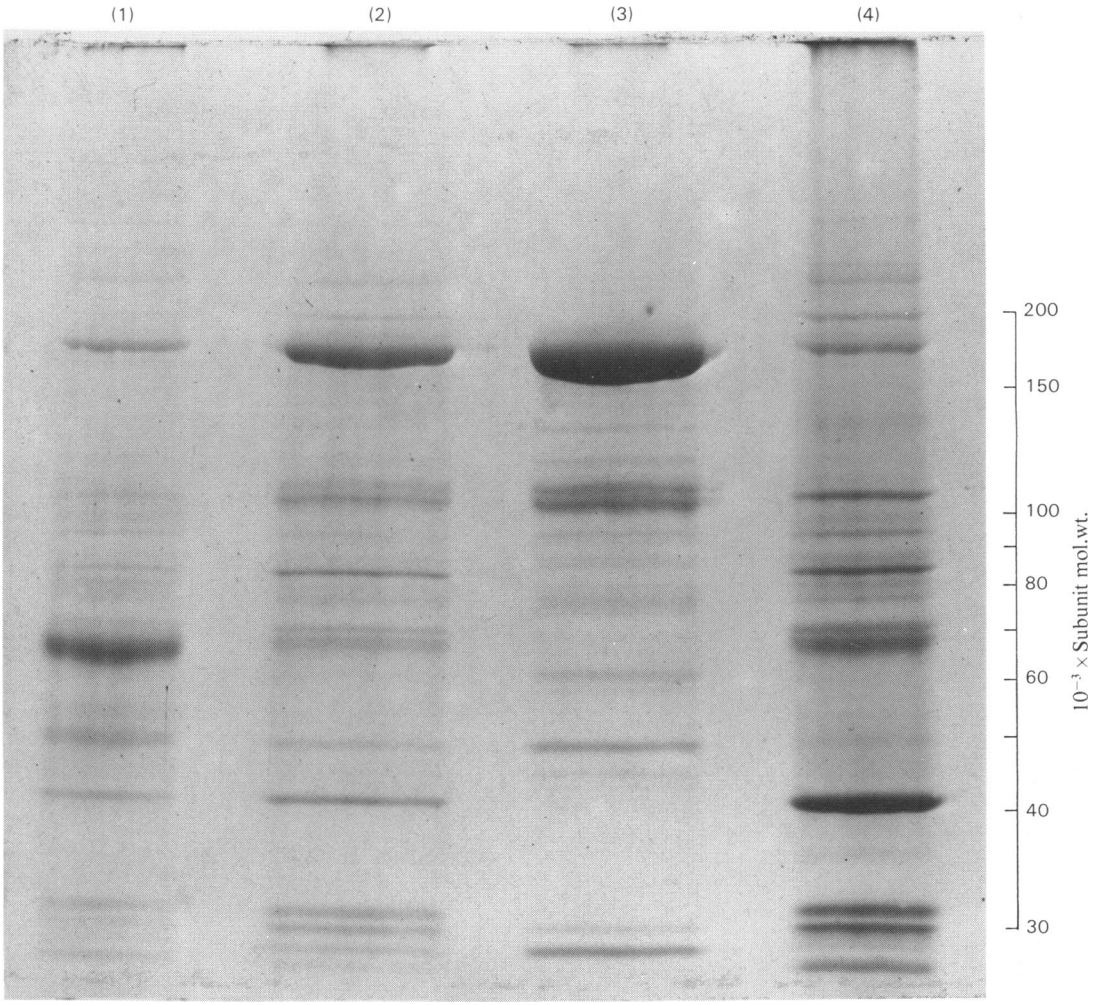
We initially attempted to purify coated vesicles from freshly delivered human placentae by using the method of Pearse (1975) as modified by Keen *et al.* (1979), who purified coated vesicles from bovine brain after two differential-centrifugation and two density-gradient-centrifugation steps. When this technique was applied to human placental tissue, the coated vesicles obtained were always heavily contaminated by larger uncoated membranous structures.

These preparations, when examined after electrophoresis in SDS, contained, in addition to the coated-vesicle protein clathrin, several proteins, including one of subunit mol.wt. 42000, presumed to be actin, which co-migrated with proteins present in preparations of microvilli from term placentae (Plate 1). This suggested that a substantial portion of the contaminating material could be attributed to microvillar membranes. This was confirmed by the presence in this fraction of a substantial activity (1.2μmol/min per mg of protein) of the microvillar marker enzyme alkaline phosphatase. By using the previously obtained specific activity of this enzyme in preparations of purified placental microvilli (Booth *et al.*, 1980), it was calculated that about 30% of the protein in the material recovered from the first sucrose gradient was microvillar in origin.

In order to remove the microvillar contamination, we made use of the observation of Ogbimi *et al.* (1979) that wheat-germ agglutinin, unlike other lectins, binds strongly to human placental microvillar membranes. We found that this lectin would agglutinate placental microvilli optimally at 1mg of lectin:10mg of microvillar protein and that at this ratio all the lectin was removed from solution bound to the agglutinated microvilli. We therefore modified the preparation procedure of Keen *et al.* (1979) by including a treatment with wheat-germ agglutinin designed to remove microvillar contamination. The full method is given below. All procedures were performed at 4°C unless otherwise stated.

Full-term placentae were obtained immediately after delivery and 250g of villus tissue was dissected free of membranes, cord and major blood vessels. The tissue was homogenized with an equal volume of buffer A (1mM-EGTA/0.5mM-$MgCl_2$/NaN_3 (0.2mg/ml)/0.1M-Mes/NaOH, pH 6.5) in a Kenwood blender (model A956A) in three 10s bursts at full speed. The homogenate was centrifuged at 15000*g* for 45min. The supernatant was removed and centrifuged at 85000*g* for 1h. The pelleted material was resuspended in 40ml of buffer A, and 6.5ml portions were layered on to discontinuous sucrose gradients. These were formed in 2.54cm × 8.75cm (1in × 3.5in) tubes and consisted of 4.5ml of 60% (w/v), 4.5ml of 50% (w/v), 9.0ml of 40% (w/v), 9.0ml of 10% (w/v) and 4.5ml of 5% (w/v) sucrose dissolved in buffer A. The gradients were centrifuged in a Beckman SW27 or Kontron TST 28.38 rotor at 25000rev./min (80000*g*) for 75min. The material at the 10%-/40%-sucrose interface was removed, together with most of the 10%- and 40%-sucrose layers, and diluted threefold with buffer A. After centrifugation at 85000*g* for 1h, the pelleted material was resuspended in 9ml of buffer A and a sample was assayed for alkaline phosphatase. The amount of microvillar protein present was calculated by assuming a microvillar specific activity of alkaline phosphatase of 4.06μmol/min per mg of protein (Booth *et al.*, 1980). Wheat-germ agglutinin was then added (1mg/10mg of microvillar protein). After being left at room temperature for 2h or at 4°C for 18h, agglutinated material was sedimented at 20000*g* for 15min. The supernatant was carefully removed and was centrifuged at 165000*g* for 1h. The pellet was resuspended in 1.5ml of buffer A. The yield was generally about 3mg of protein from 250g of villus tissue.

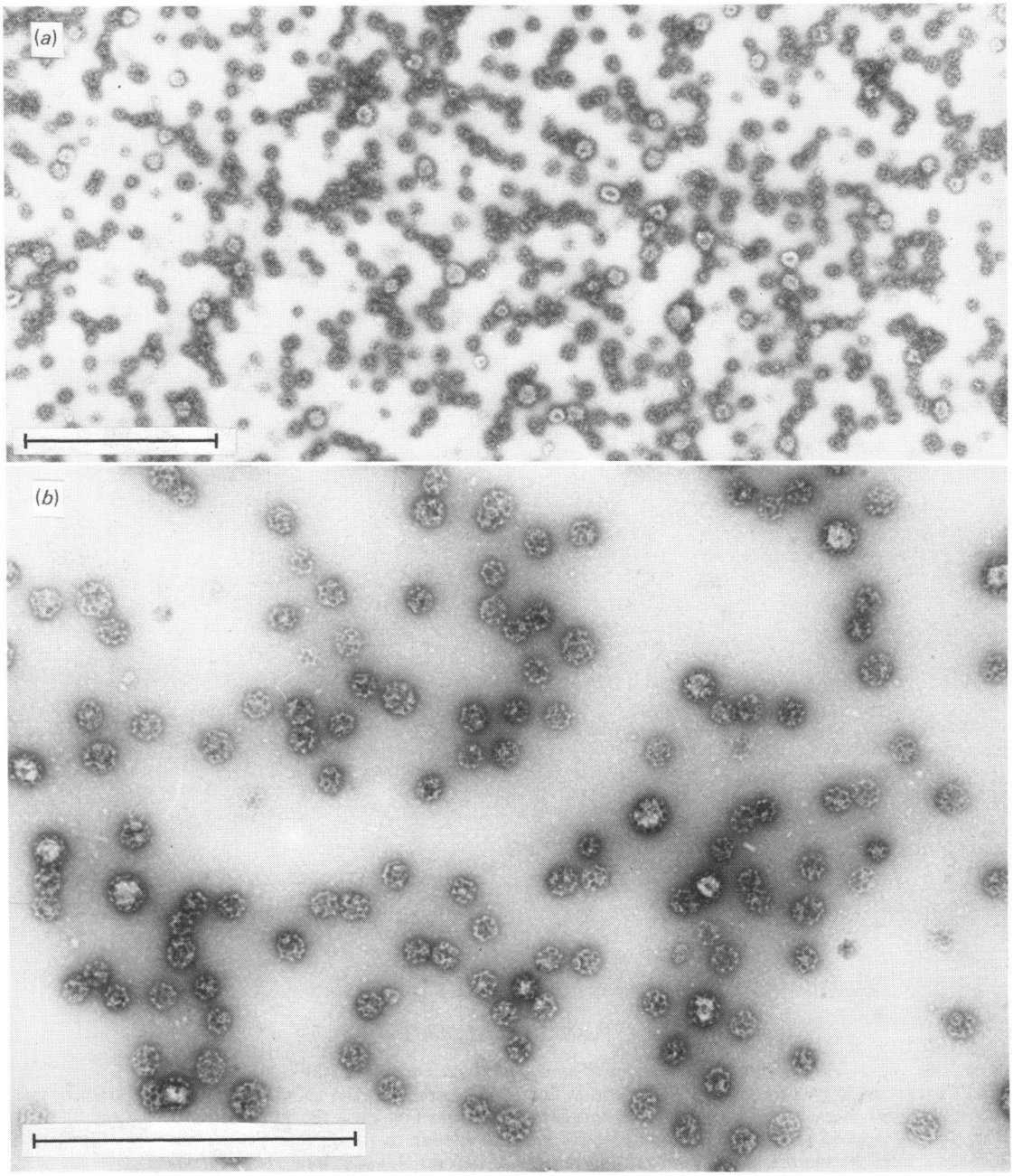
Electron microscopy of the product revealed the remarkable efficiency of the agglutination step [Plates 2(*a*) and 2(*b*)]. All of the uncoated-membranous contamination was removed by this step. The final product consisted almost exclusively of coated vesicles and apparently empty 'baskets'. The only contamination recognized consisted of occasional profiles of ferritin molecules. Polyacrylamide gradient electrophoresis in SDS (Plate 1) confirmed that the agglutination step removed actin and other proteins common to microvilli and the material recovered from the sucrose gradient. The purified coated vesicles showed a pattern of proteins very similar to that published by Pearse (1978). The



EXPLANATION OF PLATE 1

Purification of human placental coated vesicles

(*a*) Polyacrylamide-gradient-gel electrophoresis in the presence of SDS. The gel contained a linear gradient from 4 to 14% (w/v) polyacrylamide, and electrophoresis was performed by using the buffer system of Laemmli (1970). The samples applied (50μg of protein) were: (1) material applied to the sucrose density gradient; (2) material recovered from the sucrose density gradient; (3) purified coated vesicles after treatment with wheat-germ agglutinin (see the Specific methods and results section for details of the preparation method); (4) purified placental microvilli. The dye front was at the bottom of the gel.



EXPLANATION OF PLATE 2

Electron micrographs of purified placental coated vesicles

Samples were negatively stained with 2% (w/v) uranyl acetate. (*a*) Survey picture; (*b*) higher magnification. These preparations contained coated vesicles with various diameters and many apparently empty 'baskets'. The bars represent 1 μm.

most prominent protein was clathrin (subunit mol.wt. 180000). The subunit molecular weights of other prominent proteins were 110000, 103000, 50000, 30000 and 28000. Several minor protein bands were also visible.

Samples of the coated-vesicle preparations were incubated for 30min at room temperature with an equal volume of 0.1M-*N*-acetylglucosamine containing 10% (v/v) Emulphogene BC 720 and then assayed for alkaline phosphatase. Of six preparations examined, two contained no detectable alkaline phosphatase activity. The specific activities of the remaining four samples were in the range 20–55nmol/min per mg of protein.

Coated-vesicle preparations contain transferrin and ferritin but no detectable IgG

Samples of purified coated vesicles (2mg of protein/ml in buffer A) were treated with detergent by adding Emulphogene BC 720 to a final concentration of 5% (v/v). The detergent-treated coated vesicles were examined by crossed immunoelectrophoresis in agarose gels containing 1% (v/v) Emulphogene BC 720. Crossed immunoelectrophoresis against an antiserum raised to whole human serum gave a precipitate corresponding to a single slowly migrating component (Figs. 1*a*–1*c*). This serum protein antigen was not IgG, since crossed immunoelectrophoresis against antisera raised to human IgG (Fc- or Fab-specific) gave no precipitate (Figs. 1*d* and 1*g*). Furthermore, no IgG could be detected in these detergent-treated preparations by using a haemagglutination-inhibition assay, based on the inhibition by IgG of the aggregation of IgG-coated formaldehyde-fixed erythrocytes by an anti-IgG antiserum. On the basis of the detection limit of this assay, IgG, if present, comprised less than 0.05% of the protein in these preparations.

Crossed immunoelectrophoresis against an antiserum raised to human transferrin gave a single precipitate in the same position as the serum protein antigen previously detected (Figs. 1*e* and 1*f*). By performing crossed immunoelectrophoresis against a mixture of the anti-(whole serum) and anti-transferrin sera, both present in the same gel at the concentrations previously used, the serum protein was identified as transferrin. In this case a single precipitate was observed and its 'height' was approximately half that of the precipitates obtained with the single antisera. This indicated that the two antisera were both recognizing the same antigen, i.e. transferrin. The identity of the antigen was transferrin was also confirmed by mixing the detergent-solubilized coated vesicles with authentic transferrin and subjecting the mixture to crossed immunoelectrophoresis against the serum raised to whole human serum. Once again, a single precipitate was observed (result not shown).

The presence of ferritin in the coated-vesicle preparation, previously observed by electron microscopy, was confirmed by crossed immunoelectrophoresis of the detergent-treated coated vesicles against an antiserum raised to human spleen ferritin (Fig. 1*i*).

Coated-vesicle transferrin, but not ferritin, shows latency

The coated-vesicle preparations clearly contained transferrin. This could not be attributed to contamination by plasma, as no other major serum proteins could be detected in these preparations. To examine whether the transferrin was associated with the coated vesicles, or merely present in the medium, samples of untreated and detergent-treated coated vesicle preparations were subjected to 'rocket' electroimmunoassay against the antiserum raised to transferrin in agarose gels devoid of detergent. Transferrin was detected in the detergent-treated samples in amounts equivalent to 0.2–1.0% of the total protein. However, in the untreated samples the amounts of transferrin were consistently less than 10% of those in the detergent-treated samples (0.015–0.08% of the total protein). This latency was not shown by ferritin when the experiments were repeated with the antiserum raised to human ferritin. Untreated and detergent-treated samples of coated-vesicle preparations contained identical amounts of ferritin (0.1–0.15% of the total protein). It would therefore appear that although all of the ferritin in these preparations existed in a free state, more then 90% of the transferrin was latent, being entrapped within membrane vesicles, bound to membranes or both.

Coated-vesicle transferrin is bound to an intrinsic membrane component

To examine whether the transferrin was bound to a membrane component, possibly a receptor protein, detergent-treated coated-vesicle preparations were subjected to crossed hydrophobic-interaction immunoelectrophoresis as described by Bjerrum (1978). In this technique, proteins are separated by electrophoresis in detergent-free agarose gel containing a hydrophobic matrix (phenyl-Sepharose CL-4B). Hydrophilic proteins are unaffected by the hydrophobic matrix and migrate normally. Amphiphilic proteins, such as intrinsic membrane proteins, bind to the matrix and are strongly retarded. Electrophoresis in the second dimension is then performed into agarose gel containing antibodies and non-ionic detergent. Hydrophilic proteins produce immunoprecipitates in a manner identical with conventional crossed immunoelectrophoresis. On the other hand, amphiphilic proteins, eluted from the matrix by the non-ionic detergent (which is driven into the matrix by electroendosmotic flow of the buffer), form precipitates very close to the sample application

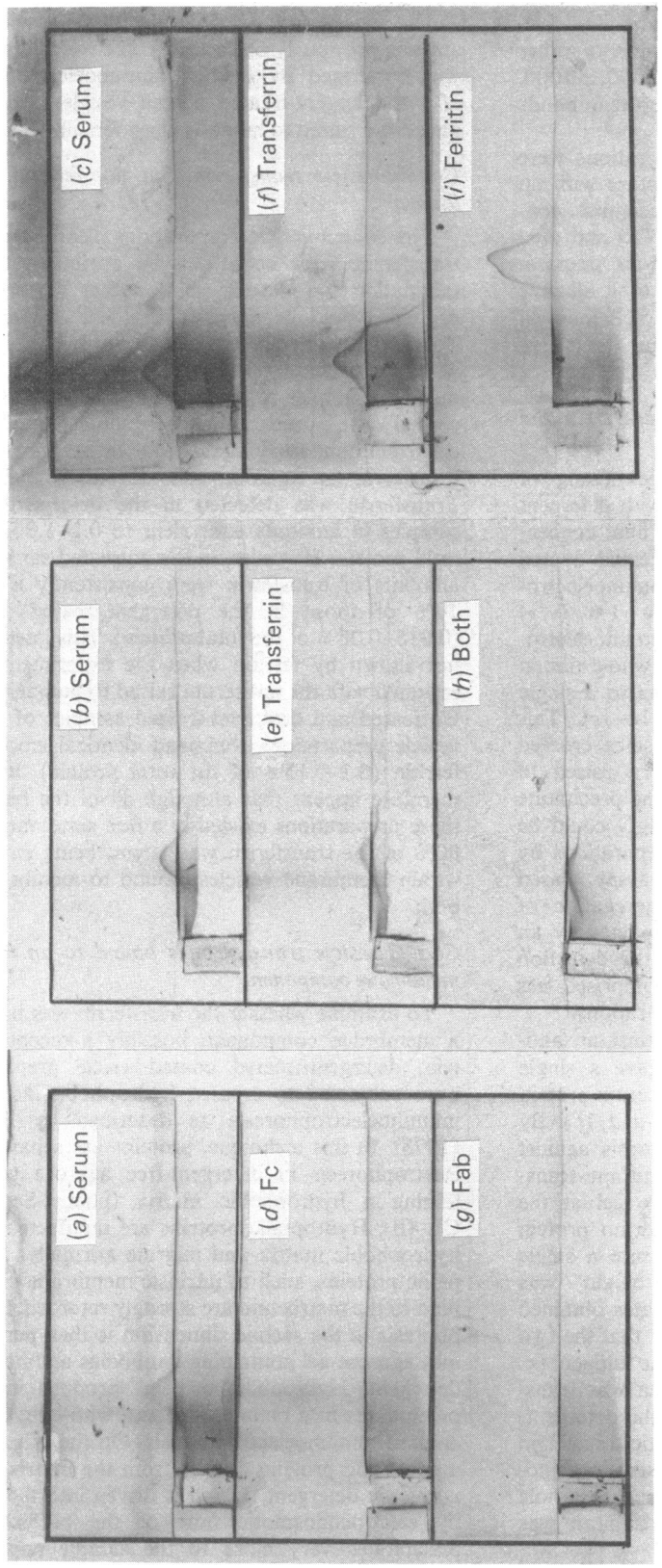


Fig. 1. *Crossed immunoelectrophoresis of detergent-treated coated vesicles*

Electrophoresis was performed in 1% (w/v) agarose containing 1% (v/v) Emulphogene BC 720 at 5 V/cm for 1 h in the first dimension and at 1 V/cm for 18 h in the second dimension. Detergent-treated coated vesicles (72 μg of protein) were applied to each gel. The second-dimension 'windows' contained: (*a–c*) serum, sheep antiserum to whole human serum (0.5 μl/cm^2); (*d*) Fc goat anti-(human Fc) serum (0.2 μl/cm^2); (*g*) Fab, goat anti-(human Fab) serum (2 μl/cm^2); (*e* and *f*) Transferrin, rabbit anti-(human transferrin) serum (0.5 μl/cm^2); (*h*) Both, rabbit anti-(human transferrin) serum (0.5 μl/cm^2) and sheep antiserum to whole human serum (0.5 μl/cm^2); (*i*) Ferritin, rabbit anti-(human spleen ferritin) serum (0.5 μl/cm^2).

point. Control experiments are easily performed by either omitting the hydrophobic matrix or by performing the first-dimension electrophoresis in non-ionic detergent.

Samples of detergent-treated coated vesicles were subjected to crossed hydrophobic-interaction immunoelectrophoresis against the anti-transferrin serum. In control gels, in which 1% (v/v) Emulphogene BC 720 was included in the first-dimension gel, the coated-vesicle transferrin gave a bimodal precipitate, indicating the presence of two forms of the antigen that differed in their electrophoretic mobilities. When the non-ionic detergent was omitted from the first-dimension gel, the slower-migrating form was greatly retarded (Fig. 2). The slowly-migrating form, which comprised 50–80% of the total coated-vesicle transferrin, as estimated from the area under the precipitate, was clearly amphiphilic in nature and was taken to represent transferrin bound to an intrinsic membrane protein receptor.

Receptor-bound transferrin is the only antigen shared by placental microvilli and coated vesicles

When detergent-treated coated vesicles were examined by crossed immunoelectrophoresis against an antiserum raised against purified human placental microvilli, a single precipitate was observed in the same position as the coated-vesicle transferrin (Fig. 3). When the anti-microvillus serum was mixed with anti-(whole serum) or anti-transferrin sera as previously described, a single precipitate of diminished height was observed, indicating that the microvillus antigen recognized in the coated-vesicle preparation was, once again, transferrin. No other microvillar antigens could be detected in the detergent-treated coated-vesicle preparations. The fact

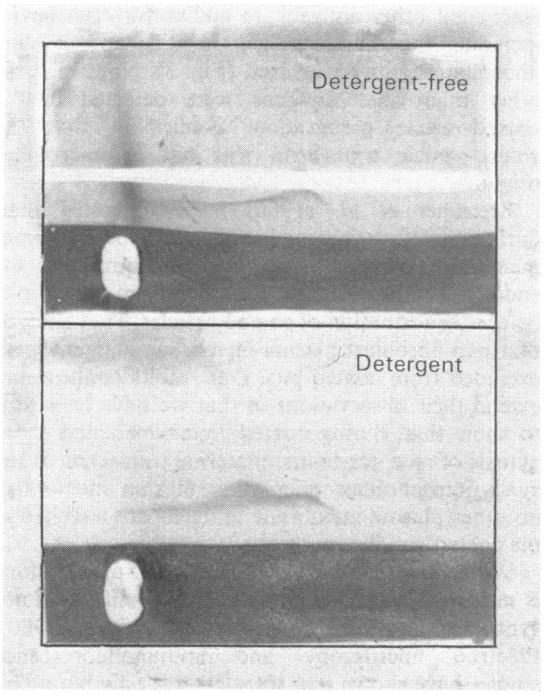


Fig. 2. *Crossed hydrophobic-interaction immunoelectrophoresis of detergent-treated coated vesicles*
Detergent-treated coated vesicles (100 μg of protein) were applied to each gel. Electrophoresis in the first dimension was performed at 5 V/cm for 3 h and in the second dimension at 1 V/cm for 18 h. The first-dimension gel (densely stained) contained phenyl-Sepharose CL-4B in the absence (Detergent-free) or presence (Detergent) of 1% (v/v) Emulphogene BC 720. In both gels the second-dimension gel contained 1% (v/v) Emulphogene BC 720 and 0.5 μl of rabbit anti-(human transferrin) serum/cm^2. The light area in the upper gel is a split caused by the different rates of drying of agarose in the presence and absence of the hydrophobic matrix. Note the extreme retardation of the slowly migrating form of transferrin in the detergent-free hydrophobic matrix.

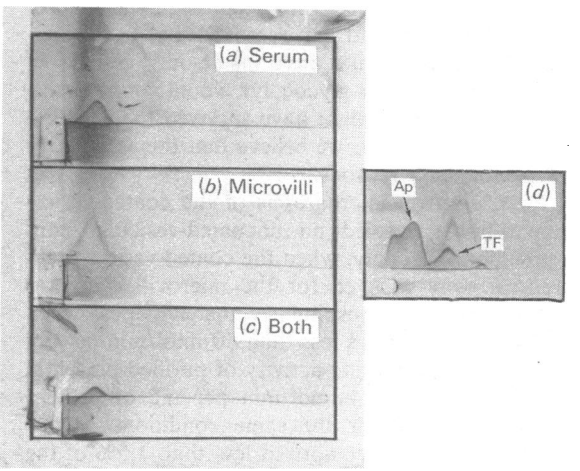


Fig. 3. *Crossed immunoelectrophoresis of placental coated vesicles and microvilli*
Detergent-treated coated vesicles (72 μg of protein) were applied to each of the gels on the left. Conditions of electrophoresis were the same as for Fig. 2. The 'windows' contained: (*a*) Serum, sheep antiserum to whole human serum (0.5 μl/cm^2); (*b*) Microvilli, rabbit antiserum to human placental microvilli (0.5 μl/cm^2); (*c*) Both, 0.5 μl/cm^2 each of the rabbit anti-(whole serum) and anti-microvillar antisera. The gel on the right (*d*) shows the pattern obtained when Emulphogene BC 720-solubilized placental microvilli (50 μg of protein) were subjected to crossed immunoelectrophoresis against the same rabbit anti-microvillus antiserum (1.5 μl/cm^2). In this case, the first dimension was run at 5 V/cm for 2 h. The identified precipitates are transferrin (TF) and alkaline phosphatase (Ap).

that transferrin is a microvillar antigen deserves some comment. Maternal transferrin always co-purifies with human placental microvilli (Smith *et al.*, 1977; Ogbimi *et al.*, 1979). This microvillar transferrin is receptor-bound, and the receptor has recently been isolated (Seligman *et al.*, 1979; Loh *et al.*, 1980; Brown & Johnson, 1981). When rabbits are immunized with purified microvilli, antibodies are raised to several microvillar antigens, including the receptor-bound transferrin (Faulk *et al.*, 1978; Ogbimi & Johnson, 1980).

Discussion

Our preparations of placental coated vesicles contained transferrin. This transferrin displayed latency and a large percentage of it appeared to be receptor-bound. The presence of this serum protein in our preparations could not be attributed to contamination by maternal or foetal plasma, as no other major serum proteins were present. Another possibility is that our preparations were contaminated by fragments of microvilli containing receptor-bound transferrin. 'Right-side-out' microvillar vesicles would be removed by the lectin-aggregation step, but any 'inside-out' vesicle, in which the membrane glycocalyx would not be accessible to the lectin, might have survived the isolation procedure. However, we believe that this is unlikely for the following reasons.

First, electron micrographs of the coated-vesicle preparations revealed no uncoated-vesicular contamination. Secondly, when the coated-vesicle preparations were assayed for the microvillar marker enzyme alkaline phosphatase, the activities, when detected, were always less than 60 nmol/min per mg of protein. The specific activity of purified placental microvilli is about 4 μmol/min per mg of protein when assayed under the same conditions. Hence microvillar protein comprised less than 1.5% of the total protein in the coated-vesicle preparations. Loh *et al.* (1980) estimated that there are approx. 2.5×10^{13} receptors for ^{125}I-labelled transferrin/mg of microvillar protein, i.e. approx. 3.2 μg of ^{125}I-labelled transferrin is bound per mg of microvillar protein at saturation. However, this may be an underestimate. Immunoassays have shown that purified microvilli contain quantities of transferrin in the range 5–15 μg/mg of microvillar protein (Ogbimi *et al.*, 1979), indicating that many receptor sites might be already occupied in these preparations and therefore not accessible to ^{125}I-labelled transferrin. Hence, at saturation, as much as 30 μg of transferrin may be bound per mg of microvillar protein. If we assume that the microvilli contaminating the coated-vesicle preparation contained this amount of transferrin, then the microvillar transferrin would comprise, at most, 0.045% of the total protein, much less than the observed values of 0.2–1.0%. In fact, the proportion of transferrin within coated vesicles is probably considerably greater than this. Coated vesicles comprised about 10% of the observed structures in our preparations, the remainder being apparently empty baskets. The transferrin, being membrane-bound, would be absent from the empty baskets, so that although it represented only 0.2–1.0% of the total protein in the preparations, within the coated vesicles the proportion of transferrin would be expected to be much greater.

Thirdly, the anti-microvillus serum recognized only transferrin in the coated-vesicle preparation. When solubilized microvilli are subjected to crossed immunoelectrophoresis against this antiserum, at least eight other antigens, in addition to transferrin, including the major antigen identified as alkaline phosphatase, are recognized (Fig. 3). None of these other microvillar antigens were detected in the coated-vesicle preparation, confirming that the coated-vesicle transferrin was not of microvillar origin.

Bretscher *et al.* (1980) have suggested that, during coated-vesicle-mediated endocytosis, plasma-membrane proteins other than receptors for the endocytosed material are excluded from coated pits before the formation of coated vesicles. They showed that two fibroblast plasma-membrane antigens were excluded from coated pits. Our results confirm and extend their observations in that we have been able to show that, during coated-vesicle-mediated endocytosis of receptor-bound maternal transferrin at the syncytiotrophoblast microvillar plasma membrane, no other plasma-membrane antigens are included in the coated-vesicle membrane.

We believe that the transferrin in our preparations is maternal in origin. The human placenta does not synthesize transferrin (Galbraith *et al.*, 1980). Electron microscopy and immunofluorescence studies have shown that transferrin is only bound by the syncytiotrophoblast microvillar plasma membrane in contact with maternal plasma (King, 1976; Galbraith *et al.*, 1980) and in coated pits frequently seen between the microvilli. Coated vesicles are found in the region of the syncytiotrophoblast underlying the microvilli, and morphometric studies suggest a functional connection between the roles of these two structures (Ockleford & Whyte, 1977). It would therefore seem reasonable to suggest that maternal transferrin, after binding to the microvillar receptors, concentrates in the region of plasma membrane between the microvilli (as observed by King, 1976) and is then internalized in coated vesicles.

The fate of the coated-vesicle transferrin can only be a matter of conjecture. During pregnancy, iron is transferred unidirectionally from mother to foetus

across the placenta, and the source of this iron is maternal transferrin (Morgan, 1974). However, the transferrin itself does not cross the placenta (Gitlin *et al.*, 1964), so at some stage the iron must be removed from the protein. Under physiological conditions, the release of iron from transferrin by a diffusion-limited mechanism is extremely unlikely, as the binding constants of Fe^{3+}–transferrin complexes are enormous, of the order of $10^{24}\,M^{-1}$ (Aisen & Liebman, 1968). However, the iron is readily released if the transferrin is protonated (Surgenor *et al.*, 1949). In the acidic environment within a lysosome, the iron would be released, and this might represent the destination of the coated-vesicle transferrin. After release of the iron, the iron-depleted transferrin might be degraded. Alternatively, as suggested by studies on reticulocytes (Morgan, 1974), transferrin may be recycled after removal of its iron, in which case the coated vesicles might be carrying iron-depleted transferrin back to the plasma membrane. We cannot say whether the transferrin in our preparations is iron-depleted or not, since the results of any assays for ferric iron would be confused by the presence of ferritin in our preparations.

Studies with electron microscopy have shown that coated vesicles are involved in the transfer of maternal IgG across rabbit yolk sac (Wild, 1979) and neonatal-rat intestine (Rodewald, 1976). There is also a report that coated vesicles are involved in the uptake of IgG by human placental villi cultured *in vitro* (King, 1977), so the apparent lack of IgG in our coated-vesicle preparations deserves comment. There are fewer Fc receptors present on the microvillar membrane than transferrin receptors by a factor of at least ten (McNabb *et al.*, 1976; O. A. Vanderpuye & A. G. Booth, unpublished work). This would be expected to be reflected in the amount of receptor-bound IgG present in coated vesicles. King (1977) and Lin (1980) have suggested that after endocytosis into coated vesicles, the IgG is rapidly transferred to phagolysosome-like structures. These latter structures contain most of the placental IgG and appear to be the main vehicles for its transfer across the syncytiotrophoblast. In this case, the amount of IgG present in coated vesicles at any given time might be relatively low. Ockleford & Whyte (1977) have shown that human placental coated vesicles may lose their clathrin coats soon after pinching off from the plasma membrane. If IgG-containing coated vesicles were to be more susceptible to this loss, then these vesicles would be preferentially lost during the preparation procedure.

In conclusion, although we cannot confirm a role for coated vesicles in the materno-foetal transfer of IgG, we suggest that they may have a role in the transfer of iron by being involved in the receptor-mediated endocytosis or recycling of maternal transferrin.

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