J. Anat. (1996) 188, pp. 43–51, with 10 figures Printed in Great Britain

Behaviour of two IgG subclasses in transport of immunoglobulin across the human placenta

L. C. MONGAN AND C. D. OCKLEFORD

Department of Preclinical Sciences, University of Leicester Medical School, UK

(*Accepted 23 May 1995*)

ABSTRACT

The human IgG subclasses are a family of highly related yet distinct molecules. Each of these four subclasses performs a discrete function within the human immune system. Previous studies have shown that one of these molecules, hIgG2, may be discriminated against in transport across the human placenta. We have aimed to elucidate the mechanism of this discrimination in order to gain a more comprehensive understanding of the process of transport of immunoglobulin across the human placenta. We have used a combination of immunocytochemical localisation and biochemical analysis to detail the behaviour of hIgG2. Confocal laser scanning microscopy was used to compare the localisation of hIgG1 (chosen as representative of the efficiently transported subclasses) and hIgG2 in term and first trimester chorionic villi. Complementary evidence was provided from immunoblot analysis of isolated placental coated vesicles. The data presented here suggest that hIgG2 is transported into the syncytiotrophoblast and appears to accumulate in the stroma of the villi. This leads us to the hypothesis that the fetal capillary endothelium is the cellular impediment to the transport of hIgG2 into the fetal circulation.

Key words: Placenta; immunoglobulin; chorionic villi; syncytiotrophoblast; IgG subclasses.

INTRODUCTION

The inefficiency of the fetal immune system is compensated for by antibodies passively derived from the mother during pregnancy, (Grubb & Laurel, 1956; Linnet-Jepson et al. 1958; Brambell, 1970). Transport of immunoglobulin across the placenta is selective: Franklin & Kunkel (1958) have shown that of the four classes of immunoglobulin in the human, only one, the IgG class, is transported to the fetal circulation. The increased concentration of IgG in cord serum over maternal levels (Kohler & Farr, 1966) suggests an active transport mechanism involving receptor mediated endocytosis by receptors specific for the Fc portion of the molecule. Biochemical evidence (Pearse, 1982) from analysis of isolated placental coated vesicles, and study of the histological route by autoradiographic methods (Ockleford & Clint, 1980), support a receptor mediated endocytotic mechanism of internalisation. Receptors locate in clathrin coated pits which then invaginate to form clathrin coated vesicles (Goldstein et al. 1979). Any transfer of IgG antibody molecules from the maternal to fetal circulation must take place across the chorionic villi of the placenta which present, at term, two cellular barriers: the syncytiotrophoblast epithelium and the endothelium of the fetal capillaries. Examination of the behaviour of these subclasses in the transport process could lead to a deeper understanding of the mechanism by dissecting this complex process into its component steps.

Human IgG subclasses differ in their physicochemical properties (Grey & Kunkel, 1964; Frangione et al. 1966; Hamilton, 1987) and in function within the immune system (Natvig & Kunkel, 1973).

Morell et al. (1971) showed transplacental transport (expressed as a ratio of concentration in cord blood to the corresponding concentration in maternal blood) of hIgG1 exceeds that of hIgG2. On the other hand, Wang et al. (1970) could detect hIgG2 in trace amounts only in the fetal circulation. These studies both indicated discrimination against this subclass in

Correspondence to Dr C. D. Ockleford, Department of Preclinical Sciences, University of Leicester, Leicester LE1 9HN, UK.

transport. Discrepancies in the rates of transport of the IgG subclasses cannot be attributed to differences in the metabolic characteristics of these molecules: hIgG3 has the shortest biological half-life and the highest fractional catabolic rate (Morell et al. 1970), but is transported more efficiently than hIgG2.

In this study, the in situ localisation of the IgG subclasses in term chorionic villi was examined using hIgG1 as representative of the transported subclasses and hIgG2, the inefficiently transported subclass. In addition, it was decided to examine the localisation of IgG subclasses in immature tissue to correlate with observations in term tissue. Receptor mediated endocytosis is a cellular mechanism of selectively concentrating particular molecules during uptake. In order to give complementary information to the data collected from in situ localisation of the IgG subclasses, isolated placental coated vesicles were analysed by immunoblotting. Thus we aimed to define differences in the transplacental transfer of two IgG subclasses using immunofluorescence and immunoblotting techniques.

MATERIALS AND METHODS

Immunocytochemical localisation of IgG subclasses

Term placentae (n = 13) and first trimester placental tissues (n = 11) were obtained from Leicester Royal Infirmary Maternity Hospital within minutes of normal delivery or termination of pregnancy. Chorionic villi were dissected from the placentae, washed briefly in ice-cold phosphate buffered saline (PBS) to remove maternal blood, and snap-frozen at −73 °C in Tissue Tek OCT tissue embedding medium (Miles Inc.) over a hexane/dry ice slush. Frozen sections of 10 μm thickness were cut using a Kryostat cryomicrotome (Leitz), and melted onto Multitest 10 well slides (Flow Laboratories). An indirect immunofluorescence localisation protocol was then carried out using a panel of mouse monoclonal antibodies: clone numbers SG-16, HP6012 and HP6091 to localise hIgG1, and clone numbers HP6014 and HP6002 to localise hIgG2. HP6013 and HP6091 recognise epitopes in the Fc region of hIgG1, while HP6002 recognises the Fc portion of hIgG2; SG-16 and HP6014 recognise epitopes in the Fab regions of hIgG1 and hIgG2 respectively). Ascites fluid was diluted to give a final total protein concentration of 0.1 mg/ml applied to each tissue section. Fluorescent labelling was achieved using rabbit antimouse immunoglobulin F(ab′)$_2$ fragment FITC conjugate (ST-AR 9A) to minimise nonspecific binding to endogenous Fc receptors. Purified mouse immunoglobulin of the same isotype as the primary antibody was used as control. All immunoreagents were obtained from Serotec (Oxford, UK), with the exception of monoclonal antibodies clone numbers SG-16 and HP6002, which were obtained from Sigma Immunochemicals (Poole, UK). The appropriate dilutions were determined by titration, and the isotype control was matched to the protein concentration of the monoclonal antibody used in the experimental labelling. As a blocking agent, 10% normal rabbit serum was routinely included in all dilutions.

The sections were gently fixed in 3% formaldehyde in PBS for 10 min, washed and then permeabilised using 0.05% Triton X-100/PBS for 10 min prior to incubation with the primary antibody. Incubation was overnight at 4 °C in the case of the primary antibodies and for 1 h at 37 °C for the secondary antibody. Preparations were mounted in antiphotobleach mountant (Citifluor) and viewed both by conventional epifluorescence microscopy using a Zeiss standard epifluorescence microscope, and confocal laser scanning microscopy, using a BioRad MRC 600 confocal laser scanning attachment for a Zeiss Axiovert 10 inverted epifluorescence microscope.

Isolation of placental coated vesicles

Clathrin coated vesicles were isolated by isotonic extraction in a method after Pearse (1982). Briefly, placentae were transported to the laboratory on ice, where the membranes and cord were removed and the chorionic villus tissue was minced and rinsed in ice cold PBS. The tissue was then homogenised in 10 mM Hepes, 0.15 M NaCl, 1 mM EGTA, 0.5 mM $MgCl_2$, 0.02% NaN_3 and 0.2 mM phenyl methyl sulphonyl fluoride, pH 7.2 Hepes 'buffer', for 30 s in a blender, followed by a further 30 s in an Ultra Turrax (Janke & Kunkel, Ika Werke). The homogenate was then centrifuged at 1000 *g* for 30 min in a Sorvall RC 5B refrigerated superspeed centrifuge, using a GSA rotor. Bovine pancreatic RNase was added at 10 units/ml for 30 min at room temperature to dissociate polyribosomes. The supernatant was then centrifuged at 44000 *g* for 1 h in a type 70Ti rotor for the Beckman L8–80M ultracentrifuge. The pellet was resuspended in Hepes buffer, loaded onto 10–90% ^2H_2O gradients, and centrifuged at 45000 *g* for 30 min. All fractions above the pellet were collected, pooled and a crude coated vesicle preparation was generated by pelleting at 100000 *g* for 1 h. Crude coated vesicles were then further purified by loading the preparation onto a continuous 9% ^2H_2O/2% Ficoll–90% ^2H_2O/20%

Ficoll gradient which was centrifuged at 80000 *g* for 16 h.

Biochemical analysis of vesicular contents: immunoblotting

The proteins of purified coated vesicle preparations from (n = 5) term placentae were routinely separated by SDS PAGE under reducing conditions (Laemmli, 1970), then electrophoretically transferred to Hybond C transfer membranes (Amersham). Efficacy of transfer was checked by reversible staining with a 0.2% Ponceau S solution in 3% trichloroacetic acid. After blocking with fat free milk (5%)/Tween 20 (0.1%), the membranes were incubated with mouse monoclonal antibodies directed against hIgG1 and hIgG2 (as before); bands were visualised using an alkaline phosphate conjugated second step with BCIP/NBT (5-bromo, 4-chloro, 3-indolyl phosphate/nitro blue tetrazolium) as substrate. In order to study complete molecules, the contents of the isolated coated vesicles were separated under nonreducing conditions (that is, in the absence of β-mercaptoethanol), leaving all proteins in a more natural state. The separated proteins were then subjected to immunoblot analysis as before. In order to calculate the molecular weights of any resultant bands, a calibration curve was generated in each case using a BioRad broad range molecular weight marker kit.

Semiquantitative evaluation of fluorescence intensity localisations

A random sample of 10 images for each of hIgG1 and hIgG2 localisations were selected from the data obtained from all term placentae studied (n = 13). From these images, a random sample of intensity values was collected both for hIgG1 and hIgG2 localisation in term placenta. Each value was then collated with respect to its corresponding region of the tissue, that is, syncytiotrophoblast, endothelium or stroma. Finally, the values were subjected to statistical analysis.

RESULTS

Term chorionic villi

Significant differences in the localisation patterns of hIgG1 and hIgG2 are apparent. False colour has been used as an aid to visualise varying fluorescence intensity. The look up tables (LUT) are explained in the figure legends.

In term placenta, hIgG1 localises in the syncytiotrophoblast (Fig. 1*a*). Interestingly, immunoreactivity for hIgG1 is also detected in the endothelial cells of the fetal capillaries. The stroma of the villi, which consist of extracellular matrix and its secreting cells, shows a pattern of fluorescence consistent with cellular localisation when probed for hIgG1. In contrast, a micrograph demonstrating the localisation pattern of hIgG2 in term chorionic villi shows a low level of fluorescence overall, with greater intensity of localisation in the syncytiotrophoblast (Fig. 2*a*). The pattern for hIgG2 is more diffuse than is the case for hIgG1. The endothelium does not appear to be strongly positive for hIgG2: it may be said that the endothelium is not favoured above any other location in the villus structure with respect to hIgG2 concentration, as appears to be the case for hIgG1.

The chorionic villi shown in Figures 1*a* and 2*a* are stem villi, which are characterised by a compact fibrous stroma and centrally located arterioles and

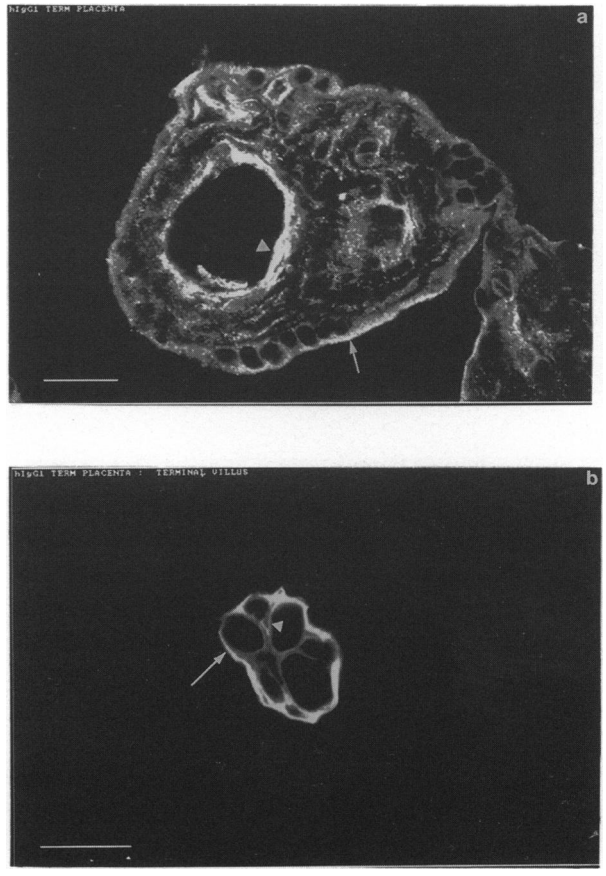

Fig. 1. (*a*) hIgG1 localisation in a human term stem chorionic villus. hIgG1 localises in the syncytiotrophoblast (arrow) and the fetal capillary endothelium (arrowhead). The stroma of the villus shows a cellular localisation pattern. Bar, 25 μm. (*b*) hIgG1 localisation in a human terminal chorionic villus. hIgG1 is localised in the endothelium, as is the case in the stem villus in (*a*) (arrowhead) and also in the 'vasculosyncytial membrane' (arrow) specialised for maternofetal transfer. Bar, 25 μm.

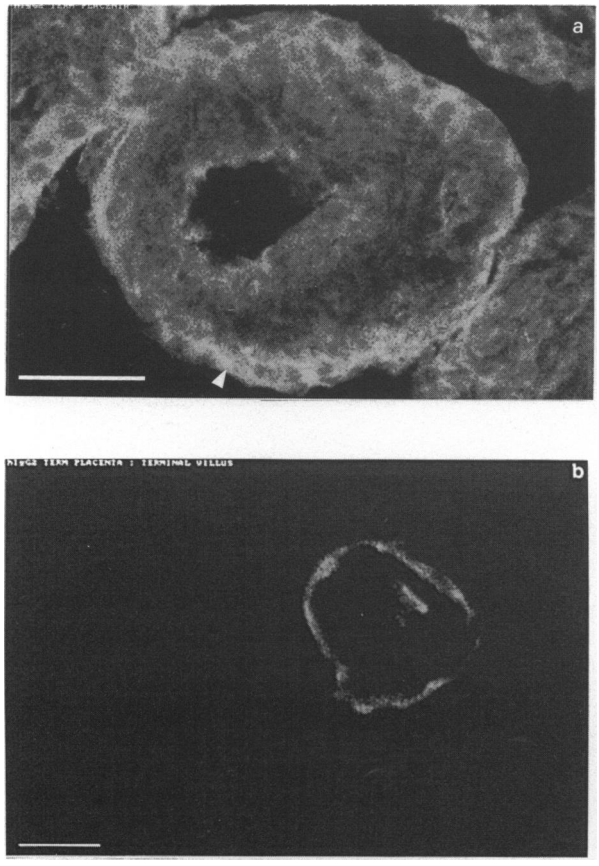

Fig. 2. (*a*) hIgG2 localisation in a human term stem chorionic villus. The pattern for hIgG2 localisation differs considerably from that for hIgG1. The syncytiotrophoblast again shows significant intensity of localisation (arrowhead). The endothelium does not appear to be strongly positive for hIgG2. Bar, 50 μm. (*b*) hIgG2 localisation in a human terminal chorionic villus. There is no strong localisation in the endothelium as is seen for stem chorionic villi. Bar, 50 μm.

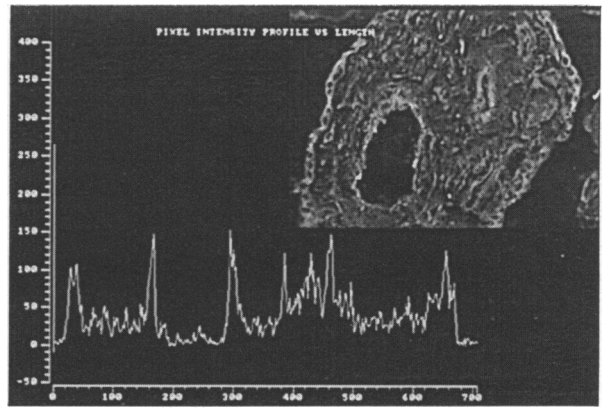

Fig. 3. Plot of pixel intensity versus length for hIgG1 localisation. A clear peak of pixel intensity represents syncytiotrophoblast associated fluorescence. The fluorescence levels then fluctuate across the stroma, and another peak occurs corresponding to the fetal capillary endothelium associated fluorescence (y axis shows pixel intensity, the X axis shows distance along the line in μm).

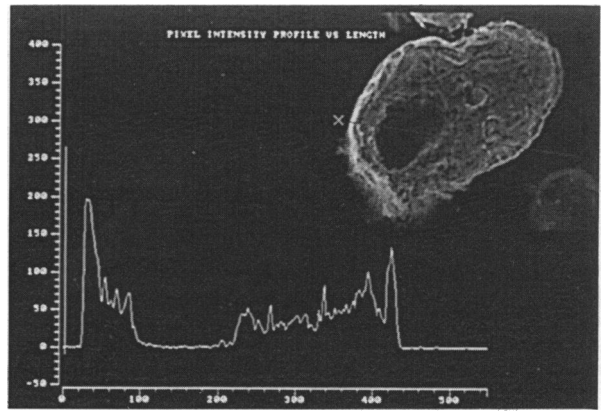

Fig. 4. Plot of pixel intensity versus length for hIgG2 localisation. A peak corresponding to the syncytiotrophoblast is again apparent, following which the pixel intensity values level out into a plateau (y axis shows pixel intensity, the x axis shows distance along the line in μm).

venules. These villi are believed to give mechanical strength to the villous tree. In contrast the villus shown in Figure 1*b* is a terminal villus. These villi show a high degree of capillarisation, and vasculosyncytial membrane which is believed to have an important role in maternofetal exchange, frequently occurs. The patterns of localisation of hIgG1 and hIgG2 are similar both in stem and terminal villi.

Figures 3 and 4 demonstrate graphically the differences in the patterns of localisation of hIgG1 and hIgG2. A plot is generated to depict the variation in the levels of fluorescence across a nonrandom line drawn to incorporate the same tissue locations for both hIgG1 and hIgG2. Thus in the case of hIgG1, a clear peak of pixel intensity represents syncytiotrophoblast-associated fluorescence. The fluorescence intensity levels then drop considerably across the stroma, and another peak occurs corresponding to the fetal capillary endothelium associated fluorescence.

When a similar plot is generated from an image of hIgG2 localisation (Fig. 4), the resulting profile reflects the diffuse pattern of fluorescence; while a peak exists corresponding to syncytiotrophoblast associated fluorescence, there are no other outstanding peaks of intensity but instead there is a plateau of one level of fluorescence across the stroma and endothelium.

Statistical analysis of fluorescence intensity profiles

Intensity values for each of 3 discrete locations in the tissue were randomly sampled (as described in Materials and Methods) from 10 samples each for each of hIgG1 and hIgG2 localisations in term

Table 1. *Descriptive statistics for the fluorescence intensity profile of hIgG1*

Location	n	Mean	S.E.M.	Min	Max
Syncytiotrophoblast	37	134.28	5.77	80.47	229.65
Endothelium	28	143.88	7.99	90.18	222.57
Stroma	17	60.31	2.22	16	120.4

Table 2. *Descriptive statistics for the fluorescence intensity profile of hIgG2*

Location	n	Mean	S.E.M.	Min	Max
Syncytiotrophoblast	51	106.44	5.4	44	205.05
Endothelium	29	58.52	3.69	26.02	94.22
Stroma	145	64.64	1.52	30.73	115.6

n, the number of values obtained by a random sample method for each location in the tissue; S.E.M., standard error of the mean; Min, minimum value measured; Max, maximum value measured.

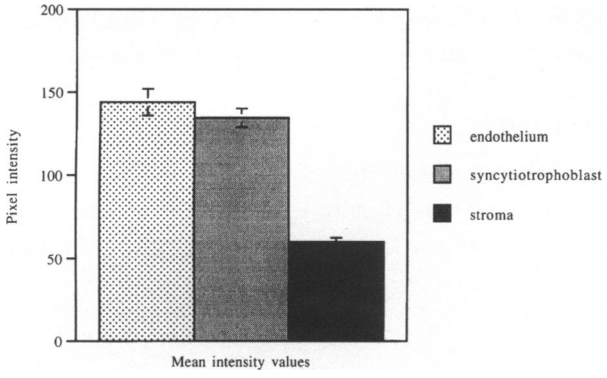

Fig. 5. Histogram demonstrating the mean fluorescence intensity for syncytiotrophoblast, endothelium and stroma for hIgG1 localisation in term chorionic villi.

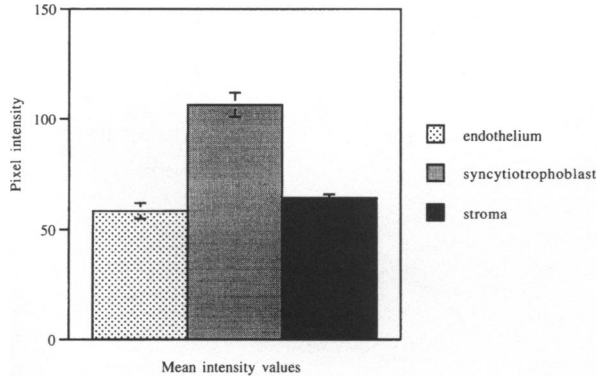

Fig. 6. Histogram demonstrating the mean fluorescence intensity for syncytiotrophoblast, endothelium and stroma for hIgG2 localisation in term chorionic villi.

chorionic villi. Mean values for syncytiotrophoblast, endothelium and stroma were then calculated and plotted as histograms. Tables 1 and 2 display the descriptive statistics for hIgG1 and hIgG2 localisations respectively. The intensity values are on an arbitrary scale of units of intensity from 0–255.

Table 3. *Two sample t test results at 95% confidence level*

Condition	t	*P*	DF
hIgG1: syn = endo	−0.097	0.33	51
hIgG2: syn = endo	7.33	0	77

syn, syncytiotrophoblast-associated fluorescence; endo, endothelium-associated fluorescence.

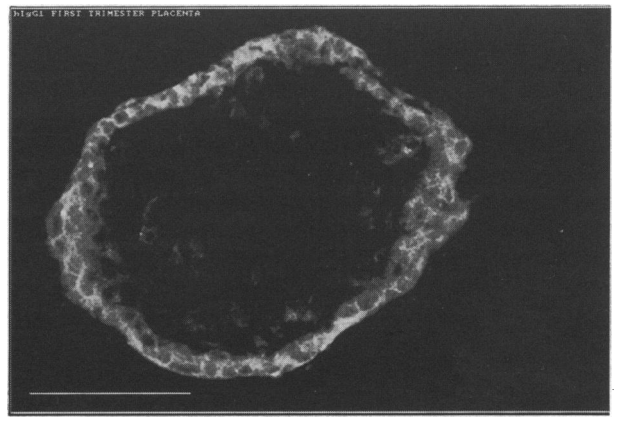

Fig. 7. hIgG1 localisation in first trimester chorionic villi. hIgG1 is localised strongly in the syncytiotrophoblast (arrow). Bar, 100 μm.

The histogram in Figure 5 shows the fluorescence intensity profile for hIgG1, where the value for the endothelium approximates that of the syncytiotrophoblast. In contrast, for hIgG2 localisation (Fig. 6), the value for the endothelium is significantly lower than that for the syncytiotrophoblast. The values for the stroma are included for comparison.

The mean values for endothelium and syncytiotrophoblast were compared by a 2 sample, 2-tailed Student t test to test the significance of these data. The statistics are tabulated in Table 3, the null hypothesis in this instance being that the mean value for syncytiotrophoblast is equal to the mean value for endothelium (see Discussion for interpretation of the statistics.)

First trimester chorionic villi

During the first 3 months of gestation, transport of immunoglobulin does not occur to any great extent (Pitcher-Wilmott et al. 1980), i.e. between d 28 and wk 16, fetal serum IgG is 5–8% of the value in the serum of adults (Gitlin & Biasucci, 1969). As yet the process of maturation of the transport mechanism is not completely understood.

Unlike the patterns of localisation in hIgG1 and hIgG2 in human term chorionic villi, when these subclasses are localised in immature tissue, there are

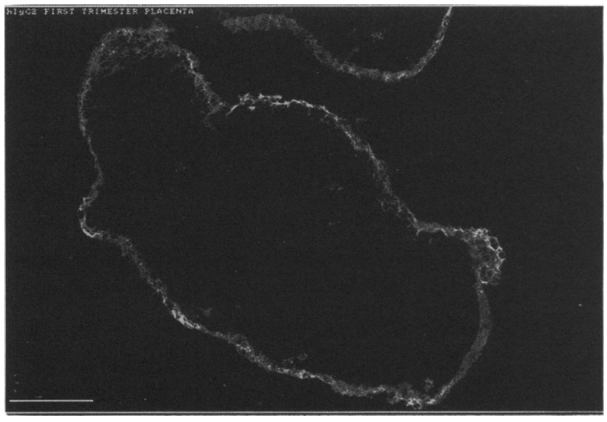

Fig. 8. hIgG2 localisation in first trimester chorionic villi. Again hIgG2 is strongly localised in the syncytiotrophoblast. Bar, 100 μm.

no apparent differences (Figs 7, 8). Both are localised in the syncytiotrophoblast epithelium. A residual amount of fluorescence is apparent in the stroma, but it seems to be a cellular association of immunoglobulin; it therefore seems likely that this is immunoglobulin detected in the form of immune complexes, that is, immunoglobulin reactive with fetal antigens, captured before entry into the fetal circulation.

Analysis of coated vesicle contents

When purified placental coated vesicles are separated by SDS PAGE prior to electrophoretic transfer to nitrocellulose membranes a major protein present is clathrin with a molecular weight of 180 kDa. Figure 9*a* shows SDS polyacrylamide gel electrophoresis, under reducing conditions, of coated vesicles purified from human term placenta. Figure 9*b* shows the transferred reduced proteins probed with anti-hIgG1 monoclonal antibody, and Figure 9*c* shows similar immunoblot analysis using an anti-hIgG2 monoclonal antibody. Molecular weight standards are indicated and the molecular weights of the resultant immunoreactive bands in both figures are calculated to be at 50 kDa, the molecular weight of hIgG heavy chain.

Figure 10*a* shows the electrophoretic profile of isolated placental coated vesicle proteins under nonreducing conditions. As expected, the resolution under nonreducing conditions is not as sharp as that achieved when proteins are separated as single polypeptide chains. Figure 10*b*, *c* shows immunoblot analysis of native coated vesicle proteins and contents using monoclonal anti-hIgG1 and anti-hIgG2, respectively. The resultant bands are at 150 kDa, the molecular weight of complete hIgG molecules.

DISCUSSION

It has been evident for some time that hIgG2 is discriminated against with regard to the transport of immunoglobulin across the human placenta (Wang et

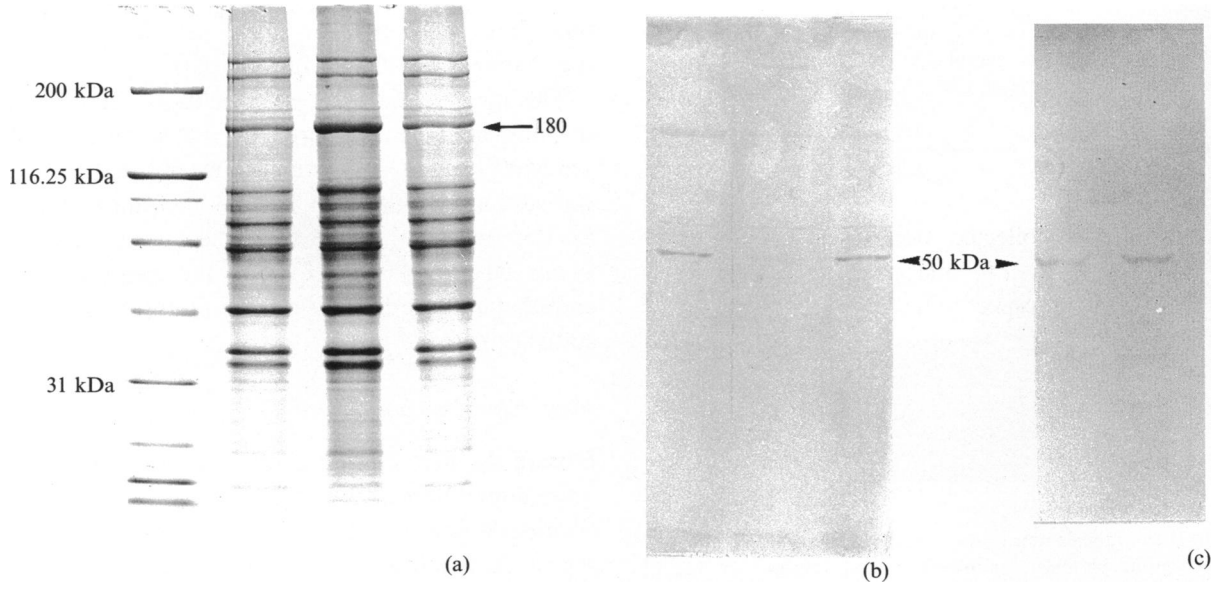

Fig. 9. (*a*) SDS polyacrylamide gel electrophoresis showing separation of isolated placental coated vesicle constituent proteins. The predominant protein present is clathrin with a molecular weight of 180 kDa. Lanes from left: (1) molecular weight markers. Lanes 2–4 are isolates of human placental coated vesicle proteins from three separate placentae. (*b*) Immunoblot of isolated placental coated vesicle proteins probed with anti-hIgG1 (Fc) monoclonal antibody. A band was visualized at 50 kDa, the molecular weight of the heavy chain of hIgG1. The two lanes are immunoblots of isolates from two separate placentae. (*c*). Immunoblot analysis of isolated placental coated vesicle proteins probed with anti-hIgG2 (Fc) monoclonal antibody. Similarly, a band at 50 kDa was visualised. The two lanes are immunoblots of isolates from two separate placentae.

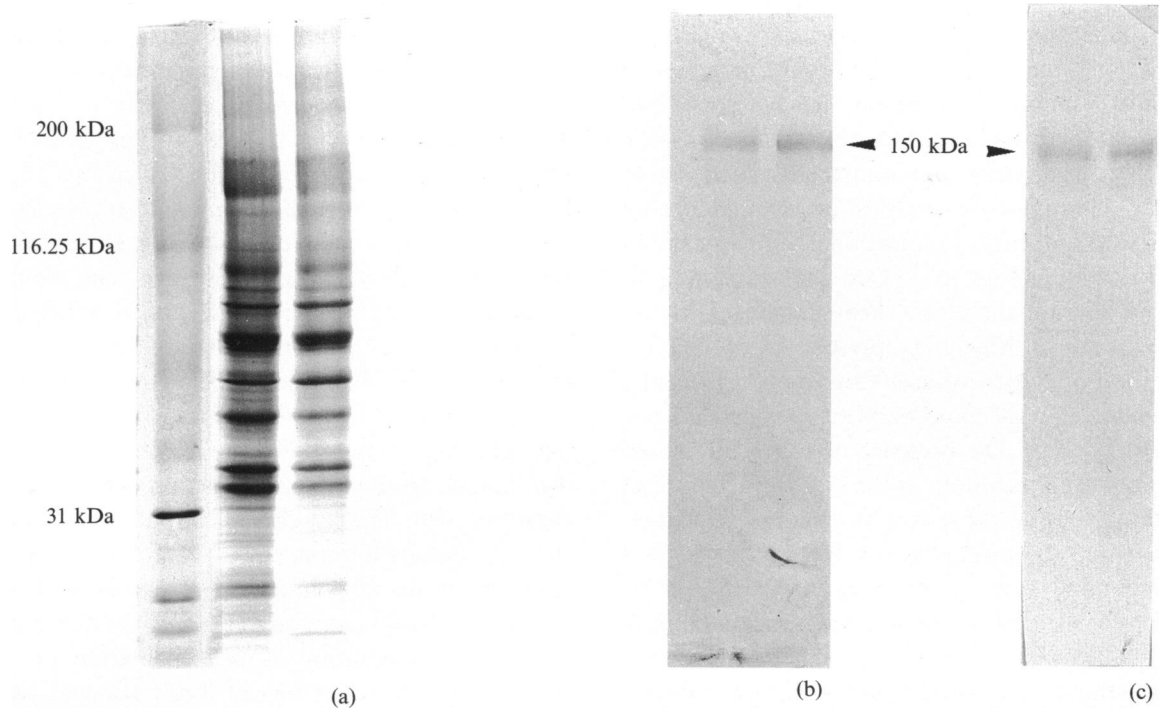

Fig. 10. (*a*) SDS PAGE electrophoresis of isolated placental coated vesicles separated under nonreducing conditions. Molecular weight standards are indicated. Lanes from left: (1) molecular weight markers. Lanes 2 and 3 are isolates of proteins from two separate placentae. (*b*) Immunoblot analysis of isolated placental undernatured vesicle proteins separated in the coated state: hIgG1 reactivity is seen at 150 kDa molecular weight. The two lanes contain immunoblots of protein from two separate placentae. (*c*) As for (*b*): hIgG2 reactivity is also seen at 150 kDa molecular weight. The two lanes contain immunoblots of protein from two separate placentae.

al. 1970; Morell et al. 1971). However, the molecular mechanism for this has yet to be defined and, similarly, the point at which this discrimination occurs has not been elucidated. We have aimed to describe the behaviour of these subclasses in order to attain a deeper understanding of the transport process active in the human placenta. Relevant information has been obtained from the in situ localisation of the molecules studied here. At term, hIgG1 is concentrated in syncytiotrophoblast, but interestingly also in the fetal capillary endothelium (Figs 1*a*, 3, 5). The cellular localisation pattern described in the Results section may represent hIgG1 bound in the form of immune complexes at the scavenger cells of the stroma. It seems that the localisation pattern of hIgG1 in fetal capillary endothelium is a cellular association rather than paracellular, suggesting that an active uptake system is operative at this level in addition to that at the syncytiotrophoblast epithelium. In contrast, hIgG2 is also concentrated in the syncytiotrophoblast epithelium (Fig. 2*a*), but appears to accumulate in the connective tissue of the villous stroma. The relatively lower level compared to that for hIgG1 localisation can be explained to some extent by the fact that these intensities reflect the relative amounts of hIgG1 and hIgG2 in maternal blood (hIgG1 is present in almost four times the amount of hIgG2 in adult human serum). No obvious concentration in the endothelium is observed when hIgG2 is localised in human term placenta (Figs 4, 6).

The statistics given in Table 3 show that there is no significant difference between the intensity values for syncytiotrophoblast and endothelium when hIgG1 is localised, while when hIgG2 is localised a significant difference in the intensity values for the syncytiotrophoblast and the endothelium is observed. This appears to suggest that hIgG2 is impeded by an undefined mechanism at the level of the fetal capillary endothelium. It would therefore appear that the rate limiting step with respect to hIgG2 transport occurs after transport across the syncytiotrophoblast is complete. Support for these data is provided by the results from both the immunocytochemical localisation of the hIgG subclasses in immature tissue and biochemical analysis of the known transport process of receptor mediated endocytosis. Both subclasses are present in the syncytiotrophoblast of first trimester chorionic villi, which indicates that hIgG2 is in fact transported into the syncytium.

Clathrin coated vesicles isolated from human placenta will have their origin from a variety of locations in the tissue, i.e. any given preparation will contain a complement of vesicles from the syncytiotrophoblast epithelium, the fetal capillary endothe-

lium, and from the various cell types of the villous stroma. Therefore, when the constituent proteins are probed using anti-hIgG1 or anti-hIgG2 monoclonal antibodies in immunoblot analysis, it is not possible to make an absolute statement as to the class of vesicle which is contributing immunoreactivity to either molecule. Immunoblot analysis of isolated coated vesicles with anti-hIgG2 monoclonal antibody shows a band corresponding to 50 kDa molecular weight, the molecular weight of the heavy chain of hIgG2 (Fig. 9*c*). Since hIgG2 is present in a purified preparation of coated vesicles from human placenta, it is probable that the molecule is involved in an active transport process. The presence of hIgG2 in coated vesicles confirms its uptake at the syncytiotrophoblast surface, and while there is a possibility of sequestration within the trophoblast, it would suggest that discrimination is likely to occur after hIgG2 is transported successfully across the syncytiotrophoblast.

There remains a possibility that substantial degradation of hIgG takes place as it passes through the placenta. In an attempt to provide evidence that data shown represent localisation of whole hIgG molecules, undernatured electrophoretic preparations were subjected to immunoblot analysis. This approach showed whole hIgG1 and whole hIgG2 to be present in isolated placental coated vesicles (Fig. 10*b*, *c*). Furthermore, we have used a panel of monoclonal antibodies, which recognise a range of corresponding epitopes (namely in the Fab and Fc regions) on each of the hIgG1 and hIgG2 molecules, in immunocytochemical localisation studies. It is difficult to determine precisely from these light microscopic images, but it is possible to speculate that the cellular impediment to hIgG2 transfer to the fetal circulation is the endothelium of the fetal capillaries. These two highly homologous molecules appear to be treated quite differently by a selective transport process. In one respect this is not surprising because these subclasses do perform discrete functions within the immune system, and hence behave as distinct entities.

Human FcγRII is the most widely distributed human Fc receptor for IgG (Anderson & Looney, 1986). The molecule exists as multiple isoforms (Grundy et al. 1989; Qiu et al. 1990). The IIa isoform exists in allelic forms termed HR and LR (for high and low responder respectively) (Tax et al. 1984; Van de Winkel & Anderson, 1991). Individuals may be homozygous and express one form only, or be heterozygous and express both forms. Interestingly from the point of view of this study, Warmerdam et al. (1991) found that the FcγRIIa isoform is also polymorphic with respect to its ability to bind hIgG2. Only the LR form of the hFcγRIIa is capable of binding hIgG2. It is therefore possible that one of the molecular mechanisms involved in hIgG2 discrimination in transplacental transfer of immunoglobulin may be the expression of an allelic form of the FcγRII receptor at the final cellular barrier to transport.

In summary, we have shown hIgG1 to localise primarily in syncytiotrophoblast and fetal capillary endothelium. The stroma of the villus is not strongly immunoreactive. On the other hand, hIgG2 is present in syncytiotrophoblast and appears to accumulate in the stroma. Thus it may be hypothesised from these data that hIgG1 and hIgG2 are treated differently by the immunoglobulin transport process active in chorionic villi. The hIgG2 subclass is present in the stroma; the rate limiting step in its transport process therefore occurs after transport across the syncytium is complete. This is supported both by the detection of hIgG2 in a preparation of isolated placental coated vesicles, and by the results of localisation of hIgG1 and hIgG2 in first trimester tissue where both subclasses are easily demonstrable in syncytiotrophoblast (but not in the stroma), so it is only as the transport process matures that the differences become apparent. Hence hIgG2 is transported into the syncytiotrophoblast and is discriminated against thereafter, possibly at the level of the fetal capillary endothelium.

ACKNOWLEDGEMENTS

This work was supported by a Research Studentship to L. M. from the Anatomical Society of great Britain and Ireland, and by an equipment grant from the Medical Research Council of Great Britain to J. Cavieres, C. Ockleford and G. Boulnois. The authors would like to thank the midwives, and obstetricians and gynaecologists of the Leicester Royal Infirmary NHS Trust for clinical coordination. We would also like to thank Simon Byrne, Neil Cockcroft and Chris d'Lacey for their skilled technical assistance, and J. L. Beckett for advice on statistical analysis.

REFERENCES

ANDERSON CL, LOONEY RJ (1986) Human leukocyte IgG Fc receptors. *Immunology Today* **7**, 264–266.

BRAMBELL FWR (1970) Transmission of immunity in man and the monkey. In *The Transmission of Passive Immunity from Mother to Young* (ed. F. W. R. Brambell), pp. 234–276. Amsterdam: North Holland.

FRANGIONE B, FRANKLIN EC, FUDENBERG HH, KOSHLAND ME (1966) Structural studies of human γG myeloma proteins of different antigenic subgroups and antigenic specificities. *Journal of Experimental Medicine* **124**, 715–732.

FRANKLIN EC, KUNKEL HG (1958) Comparative levels of high molecular weight (19S) γ globulin in maternal and umbilical cord sera. *Journal of Laboratory and Clinical Medicine* **52**, 724–727.

GITLIN D, BIASUCCI A (1969) Development of γG, γA, γM, $\beta 1_C/\beta 1_A$, c′ esterase inhibitor, ceruloplasmin, transferrin, hemopexin, haptoglobin, fibrinogen, plasminogen, α1-antitrypsin, orosomucoid, β-lipoprotein, α2 macroglobulin, and prealbumin in the human conceptus. *Journal of Clinical Investigation* **48**, 1433–1446.

GOLDSTEIN JL, ANDERSON RGW, BROWN MS (1979) Coated pits, coated vesicles and receptor mediated endocytosis. *Nature* **279**, 679–685.

GREY HM, KUNKEL HG (1964) H chain subgroups of myeloma proteins and normal 7S γ globulin. *Journal of Experimental Medicine* **120**, 253–266.

GRUBB R, LAURELL AB (1956) Hereditary serological human serum groups. *Acta Pathologica et Microbiologica Scandinavica* **39**, 390–398.

GRUNDY HO, PELTZ G, MOORE KW, GOLBUS MS, JACKSON LG, LEBO RV (1989) The polymorphic Fcγ receptor II gene maps to human chromosome 1q. *Immunogenetics* **29**, 331–339.

HAMILTON RG (1987) Human IgG subclass measurement in the clinical laboratory. *Clinical Chemistry* **33/10**, 1707–1725.

KOHLER PF, FARR RS (1966) Elevation of cord over maternal IgG immunoglobulin: evidence for active placental IgG transport. *Nature* **210**, 1070–1071.

LAEMMLI UK (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

LINNET-JEPSON P, GALATIUS-JENSEN F, HAUGE M (1958) On the inheritance of the Gm serum group. *Acta Genetica (Basel)* **8**, 164–167.

MORELL A, TERRY WD, WALDMAN T (1970) Metabolic properties of IgG subclasses in man. *Journal of Clinical Investigation* **49**, 673–680.

MORELL A, SKVARIL F, VAN LOGHEM E, KLEEMOLA M (1971) Human IgG subclasses in maternal and fetal serum. *Vox Sanguinis* **21**, 481–492.

NATVIG JB, KUNKEL HG (1973) Human immunoglobulins: classes, subclasses, genetic variants and idiotypes. *Advances in Immunology* **16**, 1–59.

OCKLEFORD CD, CLINT JM (1980) Uptake of IgG by human chorionic villi: a correlated autoradiographic and wide aperture counting study. *Placenta* **1**, 91–111.

PEARSE BMF (1982) Coated vesicles from human placenta carry ferritin, transferrin and immunoglobulin G. *Proceedings of the National Academy of Sciences of the USA* **79**, 451–455.

PITCHER-WILMOTT RW, HINDOCHA P, WOOD CBS (1980) The placental transfer of immunoglobulin G subclasses in human pregnancy. *Clinical and Experimental Immunology* **41**, 303–308.

TAX WJM, HERMES FF, WILLEMS RW, CAPEL PJA, KOENE RAP (1984) Fc receptors for mouse IgG1 on human monocytes: polymorphism and role in antibody induced T cell proliferation. *Journal of Immunology* **147**, 1731–1734.

QIU WQ, DE BRUIN B, BROWNSTEIN BH, PEARSE R, RAVETCH J (1990) Organisation of the human and mouse low affinity FcγR: duplication and recombination. *Science* **248**, 732–735.

VAN DE WINKEL JGJ, ANDERSON CL (1991) Biology of human immunoglobulin Fc receptors. *Journal of Leukocyte Biology* **49**, 511–524.

WANG AC, FAULK WP, STUCKEY MA, FUDENBERG HH (1970) Chemical differences of adult, fetal, and hypogammaglobulinemic IgG immunoglobulins. *Immunocytochemistry* **7**, 703–708.

WARMERDAM PAM, VAN DE WINKEL JGJ, GOSSELIN EJ, CAPEL JA (1990) Molecular basis for a polymorphism of human Fcγ receptor II (CD32). *Journal of Experimental Medicine* **172**, 19–25.

WARMERDAM PAM, VAN DE WINKEL JGJ, VLUG A, WESTERDAAL NAC, CAPEL PJA (1991) A single amino acid in the second immunoglobulin like domain of the human Fcγ receptor II is critical for IgG2 binding. *Journal of Immunology* **147**, 1338–1343.