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Immunological studies of human placentae: the distribution and character of immunoglobulins in chorionic villi

P. M. JOHNSON, J. B. NATVIG, U. A. YSTEHEDE & W. P. FAULK *Institute of Immunology and Rheumatology, and Department of Obstetrics and Gynaecology, Rikshospitalet University Hospital, Oslo, Norway, and Department of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina, Charleston, South Carolina, U.S.A.*

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SUMMARY

All four human IgG subclasses, and both κ and λ light chains, were detected by immunofluorescence in similar distributions in chorionic villi of human placentae. IgG1 and IgG3 were the predominant subclasses. No evidence was obtained for local enzymatic digestion of IgG during placental transfer. Most of the IgG on the trophoblastic basement membrane (TBM) was loosely bound and could be removed by prolonged washing, although some appeared to be more tightly bound to small segments of the TBM. IgM, but not IgA, was present in small amounts in placental villous structures. Immunoglobulin was never observed within the syncytiotrophoblast. Antisera to IgG genetic (Gm) markers were used to locate IgG thought to be of foetal or maternal origin. The presence of paternal Gm markers not carried by the mother was taken as evidence for foetal IgG. Foetal (paternal) Gm markers were observed in placentae, although maternal IgG was the major immunoglobulin present in placental villi. Both maternal and foetal IgG were demonstrated in fibrinoid deposits, vessel walls and the cytoplasm of some stromal cells. Only foetal IgG was definitively observed in the immunoglobulin that is tightly bound to the TBM.

INTRODUCTION

Studies on immunoglobulins in human placentae have contributed to the understanding of the mechanism of transfer of IgG from mother to foetus, and of the specificity and consequences of maternal antibody responses to foetal alloantigens. Previous immunofluorescence studies of human placentae have demonstrated IgG in the villous stroma, particularly in a perivascular location, as well as in fibrinoid deposits and on the trophoblastic basement membrane (TBM) (McCormick *et al.*, 1971; Faulk & Johnson, 1977). Immunoglobulins obtained by acid elution of extensively washed human placental tissue homogenates have been shown to bind to fibrinoid deposits and areas of TBM (McCormick *et al.*, 1971; Faulk *et al.*, 1974), and the $F(ab')_2$ fragment of such eluate–IgG preparations also inhibits *in vitro* assays of lymphocyte reactivity (Faulk *et al.*, 1974). The specificity of this blocking antibody activity is not yet fully established (Revillard *et al.*, 1976; Jeannet *et al.*, 1977). Furthermore, receptor activity for the Fc region of IgG has been reported on human trophoblastic tissue (Matre, Tønder & Endresen, 1975; Jenkinson, Billington & Elson, 1976) and on placental endothelium (Johnson, Faulk & Wang, 1976a). Hofbauer cells (macrophages) in the mesenchymal stroma of placental villi may also express Fc receptor activity, although there is little experimental data on this.

All maternal IgG subclasses and allotypes are thought to cross the human placenta, but there is little, if any, transfer of IgM or IgA (Gitlin *et al.*, 1964; Hay, Hull & Torrigiani, 1971; Mellbye & Natvig, 1973). The human foetus is itself capable of immunoglobulin synthesis: foetal IgM has been identified by 11 weeks of gestation (Gitlin & Biasucci, 1969), and foetal IgG by the 20th week (van Furth, Schuit &

Correspondence: Dr P. M. Johnson, Department of Immunology, University of Liverpool, P.O. Box 147, Liverpool, L69 38X.

Hijmans, 1965). Cord blood obtained at delivery may contain IgG expressing genetic markers of the neonate's phenotype, but the bulk of the IgG is of maternal phenotype (Mårtensson & Fudenberg, 1965; Mellbye & Natvig, 1973).

The present study was initiated to further define and characterize the immunoglobulins present in human placentae by the antigenic identification of immunoglobulin isotypes and subfragments. Genetic (Gm) markers on human IgG were also used to locate IgG thought to be of foetal or maternal origin within chorionic villi.

MATERIALS AND METHODS

Human sera. Sera were collected from pregnant mothers, and the respective fathers, close to the time of the delivery. Sera containing anti-nuclear antibody activity, used for some control studies, were selected at random from those received for routine autoantibody determinations. All sera were from Caucasian individuals. Gm typing of sera for Gm(a), Gm(g), Gm(f) and Gm(b) markers was performed by haemagglutination inhibition (Natvig & Kunkel, 1968, 1973).

Tissue sections. Twenty full-term human placentae were studied: each placenta was judged to be normal by gross and microscopic examination. Small pieces of tissue from the central cotyledon were obtained immediately after delivery, washed at 4°C with gentle rotation in Hanks' medium, and then snap-frozen. Tissue sections, prepared fresh each day, were cut at 4 μm in a cryostat: sections were either air-dried without fixation, or fixed with 1% fresh paraformaldehyde in 0·1 M phosphate buffer, pH 7·2, containing 7·5% sucrose, for 30 min, followed by three washes of 15 min each in phosphate-buffered saline (PBS), pH 7·2.

Tissue from six human placentae of between 11 and 14 weeks of gestation, obtained from induced abortions, were processed for immunofluorescence in the same manner.

Antisera. Anti-Gm and anti-IgG subclass sera were raised in rabbits by immunization with isolated human IgG myeloma proteins and absorbed with excess amounts of IgG lacking the appropriate subclass or Gm antigen (Natvig & Kunkel, 1968, 1973; Faulk & Johnson, 1977). Rabbit antisera against Fab, $F(ab')_2$, Fc and pFc' fragments of human IgG, and sheep antiserum against human γ chains, were raised as described previously (Natvig & Turner, 1971; Faulk & Johnson, 1977). An antiserum specific for the pepsin site of IgG was made by absorption of a rabbit anti-human $F(ab')_2$ serum with excess pooled human IgG (Kabi AB, Sweden). The resultant antiserum agglutinated human OR_1R_2 erythrocytes coated by the chromic chloride technique (Gold & Fudenberg, 1967) with the $F(ab')_2$ fragment of pooled human IgG to a titre of 16, whereas no agglutination was observed when cells were coated with an intact human IgG1 myeloma protein. In contrast, both $F(ab')_2$- and IgG-coated erythrocytes were agglutinated by the rabbit anti-human $F(ab')_2$ serum to titres of 512 or greater. Other antisera were obtained from the following sources: rabbit anti-human γ-chain serum and anti-human κ and λ light chain sera (Dakopatts A/S, Denmark); sheep anti-human IgG; anti-human IgA and anti-human IgM sera (Wellcome Reagents, Beckenham, Kent, England). The specificity of each antiserum was confirmed by double radial immunodiffusion and electrophoretic analyses.

Because of a lack of appropriate negative immunofluorescence controls on human placentae using anti-Gm(f) and anti-Gm(b) sera, each anti-Gm serum was also used to screen ten human sera with strong anti-nuclear antibody activity of the IgG class. The Gm markers detected in the immunofluorescent anti-nuclear antibody staining given by each serum on mouse liver tissue sections were compared with those found in the test serum by independent study using haemagglutination inhibition. Five of these sera expressed a Gm(a+, g+, f+, b+) phenotype, three were Gm(a−, g−, f+, b+) and two were Gm(a+, g+, f−, b−). A selection of Gm markers in immunofluorescent anti-nuclear antibody staining was observed in some cases, as has been reported previously (Munthe & Natvig, 1971). Positive anti-nuclear antibody staining was never observed using an antiserum to a Gm marker that was not detected in the test serum by haemagglutination inhibition.

Immunofluorescence techniques. Indirect immunofluorescent staining of tissue sections was performed as described previously by Faulk & Johnson (1977). The optimal dilution of each unconjugated antiserum was determined by titration of the antiserum on both air-fixed and formaldehyde-fixed placental tissue sections. Fluorescein-conjugated goat or sheep anti-rabbit immunoglobulin sera and rabbit anti-sheep immunoglobulin serum were obtained from Wellcome Reagents, Beckenham, Kent, England, and Behringwerke AG, Marburg, Germany.

Tissue sections were examined for immunofluorescent staining using a Leitz Orthoplan microscope equipped with an HBO 200 high-pressure mercury-vapour burner light source. In determining the distribution of immunoglobulin antigens in human placentae by immunofluorescence, representative villi from separate areas of each cryostat section were examined. Fluorescence intensities were graded from − to +++. Photomicrographs were taken with an Orthomat camera using epi-illumination through a Leitz vertical illuminator. Gaf 500 high-speed 35 mm film was used throughout.

Formaldehyde fixation of human placental tissues has previously been shown to destroy the Fc receptor activity of placental endothelium (Johnson, Faulk & Wang, 1976b). We therefore included this procedure in order to exclude the possibility that any aggregates or immune complexes in our antisera may bind to endothelium and give positive fluorescence. Thus, air-fixed and formaldehyde-fixed tissues were studied in parallel. For well-washed tissues, the mode of fixation did not affect the immunofluorescence pattern for immunoglobulin antigens, except that formaldehyde-fixed tissues often expressed slightly less IgG in areas of foetal stem vessel endothelium. Consequently, all results presented in this study refer to formaldehyde-fixed placental tissue.

RESULTS

Immunoglobulins in full-term human placentae

All anti-γ-chain sera consistently gave positive immunofluorescent staining of fibrinoid necrotic areas, endothelium and perivascular tissue, as well as some staining of stromal connective tissue and the cytoplasm of some scattered mononuclear cells within the mesenchymal stroma. Fibrinoid deposits and foetal stem vessels were generally most strongly stained, although marked variation was observed from tissue to tissue. There was no staining of syncytiotrophoblast using any of the antisera to human immunoglobulin antigens that were employed in this study. Placental tissue that had been washed for only short periods of time prior to being snap-frozen exhibited clear staining for IgG on the TBM in some villi (Fig. 1a). However, in contrast to other positively stained areas, the staining on the TBM progressively diminished as the washing time was extended up to a period of 6 hr. Tissues washed for

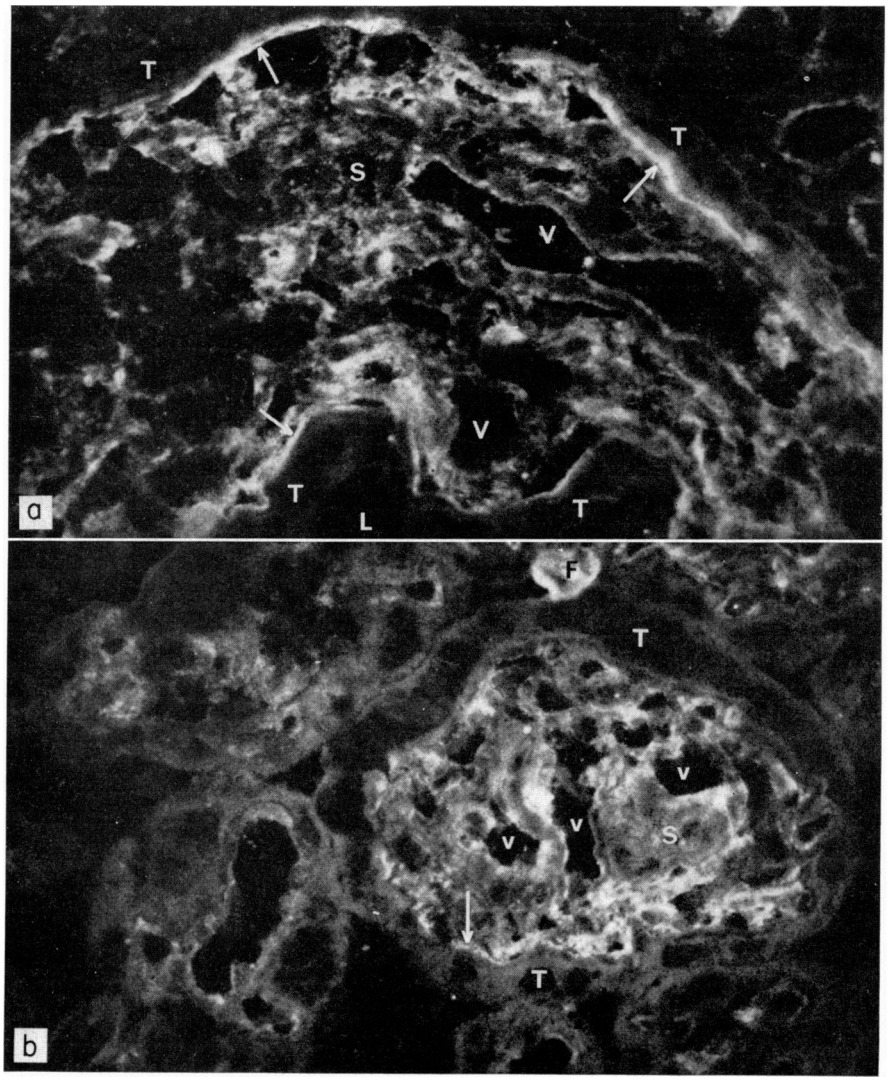

FIG. 1. Indirect immunofluorescent staining for IgG on full-term human placental tissue fixed with 1% paraformaldehyde. (a) Tissue washed for 30 min prior to snap-freezing. (Magnification ×490.) (b) Tissue washed for 8 hr prior to snap-freezing. (Magnification ×490.) L is maternal lake; S is villous stroma; T is trophoblasts; V is placental vessel and F is area of fibrinoid necrosis. Arrows point to positively stained TBM.

6 hr or more consistently exhibited staining for IgG on only small scattered segments of TBM (Fig. 1b). It thus appears that the bulk of IgG is loosely bound to the TBM and can be removed on prolonged washing, although a small amount is too firmly bound to be removed in this way. All subsequent data described here will refer to placental tissues washed for 6 hr or more.

All four subclasses of human IgG exhibited a similar immunofluorescent staining distribution to that seen with anti-γ-chain sera, although the IgG2 and IgG4 subclasses appeared the most sparingly represented. Antisera to Fab, $F(ab')_2$, Fc and pFc' fragments of human IgG, and to κ and λ light chains, also gave closely similar staining patterns. IgA was not identified within chorionic villi, but was often observed in intervillous fibrin deposits. The distribution of IgM was similar, except that weak staining of intravillous fibrinoid areas and foetal stem vessel endothelium could also be seen. Anti-pepsin site serum gave faint staining of fibrinoid deposits, endothelial cells, some stromal cells and very limited segments of TBM. This antiserum did not stain any connective tissue structures.

Immunoglobulins in first trimester human placentae

IgG was only faintly represented in stromal connective tissue and vessel walls within first trimester chorionic villi, but consistent cytoplasmic staining of some mononuclear cells in the mesenchymal stroma was observed. Areas of fibrinoid necrosis often demonstrated strong staining for IgG, although negative areas of intervillous fibrin deposition could occasionally be noted. IgG was never identified in syncytiotrophoblast, or in the underlying discontinuous layer of cytotrophoblast, and was very rarely identified on the TBM. When IgG appeared present on isolated segments of TBM, the staining was diffuse, weak and extended into the stromal connective tissue beneath the TBM. All four subclasses of human IgG, and both κ and λ light chains, were demonstrated in similar distributions within first trimester placental villous structures. IgA and IgM were not identified within first trimester chorionic villi: however, IgM could be observed in intervillous fibrin deposits, whereas IgA was rarely seen in these areas.

Studies on the distribution of maternal and foetal IgG using genetic (Gm) markers

The Gm(a) and Gm(f) markers of IgG1, and the Gm(b) and Gm(g) markers of IgG3, behave in caucasians as being determined by alleles within the genes coding for the constant region of these IgG subclasses (Natvig & Kunkel, 1973). From the occurrence of Gm(a), Gm(g), Gm(f) and Gm(b) markers in twenty pairs of maternal and paternal sera, we have deduced the possible Gm alleles that the foetus may inherit (Table 1). The Gm(a) and Gm(g) markers, as well as the Gm(f) and Gm(b) markers, are inherited in Caucasians as closely-linked alleles, and were always either both absent or both present in the sera studied. Thus, the data given in Table 1 for the Gm(a) and Gm(f) markers apply also to the Gm(g) and Gm(b) markers, respectively. Certain combinations of parental Gm alleles offered a possibility of using anti-Gm sera to differentiate between maternal and foetal IgG in FITC-staining of the corresponding full-term placenta. Three groups of such combinations could be distinguished, according to the Gm allotypic differences between mother and foetus (Table 1). The staining patterns within chorionic villi using anti-Gm sera in such situations are summarized in Table 2. Immunofluorescent staining was always performed and recorded prior to collection of data into the three groups. No staining of syncytiotrophoblast was ever observed using anti-Gm sera. Also, there was never a significant difference between anti-Gm(a) and anti-Gm(g), or between anti-Gm(f) and anti-Gm(b), in the staining distribution on each placenta studied.

In group 1, both parents of each child were homozygous for different Gm alleles, and, in these examples, all children must inherit the Gm(f,b) allotypic markers from the father only (Table 1): thus the distribution of the Gm(f,b) markers in the corresponding placenta will represent only IgG of foetal origin (group 1, Table 2). Such foetal IgG was manifest by clear staining of fibrinoid deposits, the apical aspect of foetal stem vessels and the cytoplasm of some mononuclear cells scattered within the mesenchymal stroma, as well as diffuse staining of limited segments of the TBM. There was very little staining of any stromal connective tissue. A representative example of the staining distribution for foetal IgG is given in Fig. 2(a).

TABLE 1. Combinations of maternal and paternal Gm(a) and Gm(f) alleles allowing differentiation between maternal and foetal IgG by FITC-staining of corresponding full-term placentae using anti-Gm sera

	Combinations of Gm alleles* (maternal × paternal)	Number of matings in group	Gm alleles that the foetus may inherit	Comments to origin of Gm allotypes in corresponding placentae
Group 1	*a,a* × *f,f*	3	*a,f* only	Gm(f) must be foetal only
Group 2	*a,a* × *a,f*	1	*a,f* or *a,a*	If foetus is *a,f*, then Gm(f) must be foetal only
	f,f × *a,f*	2	*a,f* or *f,f*	If foetus is *a,f*, then Gm(a) must be foetal only
Group 3	*a,f* × *a,a*	1	*a,a* or *a,f*	If foetus is *a,a*, then Gm(f) must be maternal only
	a,f × *f,f*	5	*f,f* or *a,f*	If foetus is *f,f*, then Gm(a) must be maternal only
	a,f × *a,f*	5	*a,a, f,f* or *a,f*	If foetus is *a,a*, then Gm(f) must be maternal only: if foetus is *f,f*, then Gm(a) must be maternal only

* Three matings are not included since they were *f,f* × *f,f* matings, and gave no possibility of differentiating between maternal and foetal IgG: these matings were used as negative controls for staining with anti-Gm(a) and anti-Gm(g) antisera. Other combinations of Gm alleles were not encountered in the matings studied.

TABLE 2. Immunofluorescence staining patterns in full-term human placentae in situations where the separate distributions of maternal and foetal IgG may be determined using anti-Gm sera

	Number of matings in group	Placental tissue component giving positive fluorescence (scale: − to +++)*					Notional designation for staining pattern (see text)
		TBM	Villous stroma	Stromal cells	Vessels	Fibrinoid deposits	
Group 1	3	+	(+)	+	+(+)	++(+)	Foetal IgG
Group 2	(a) 1	+	(+)	+	+(+)	++(+)	Foetal IgG
	(b) 2	−	−	−	(+)	+	Non-specific background
Group 3	(c) 4	−	++	+	++	++(+)	Maternal IgG
	(d) 12	+	++	+(+)	++(+)	+++	Maternal plus foetal IgG

* Values in table indicate the mean observed over all placentae studied in each group.

In group 2, the parental Gm allotypes are such that the foetus has a 50% chance of inheriting paternal Gm allotypes not present in the mother (Table 1). Two separate immunofluorescent patterns were observed in group 2. One pattern (line (a), Table 2), noted in a single tissue, was the same as previously seen for Gm (f,b) in group 1 and was therefore considered to represent the distribution of foetal IgG. The second pattern (line (b), Table 2), observed in two tissues, manifested only very faint staining of those placental components commonly associated with considerable amounts of IgG. This distribution was also noted for the Gm(a) marker in three placentae derived from $Gm^fGm^f \times Gm^fGm^f$ matings that were used as negative controls. This exceptionally weak staining was therefore considered to represent non-specific background in placentae expressing no IgG containing the Gm allotypic marker under study.

No such clear definition of maternal IgG could be achieved by these means. However, group 3 represented pregnancies in which the mother carried Gm markers that might not necessarily be inherited by the foetus (Table 1). Two separate immunofluorescent patterns were observed for this group. The larger subgroup (line (d), Table 2) represented a distribution in full-term placentae closely similar to that seen using anti-γ-chain sera. The same degree of variability as without anti-α-chain sera was also noted from placenta to placenta, but anti-Gm sera commonly gave a more diffuse staining of positive segments of the TBM. Furthermore, fifteen additional combinations of parental Gm(a,g) or Gm(f,b) allotypes

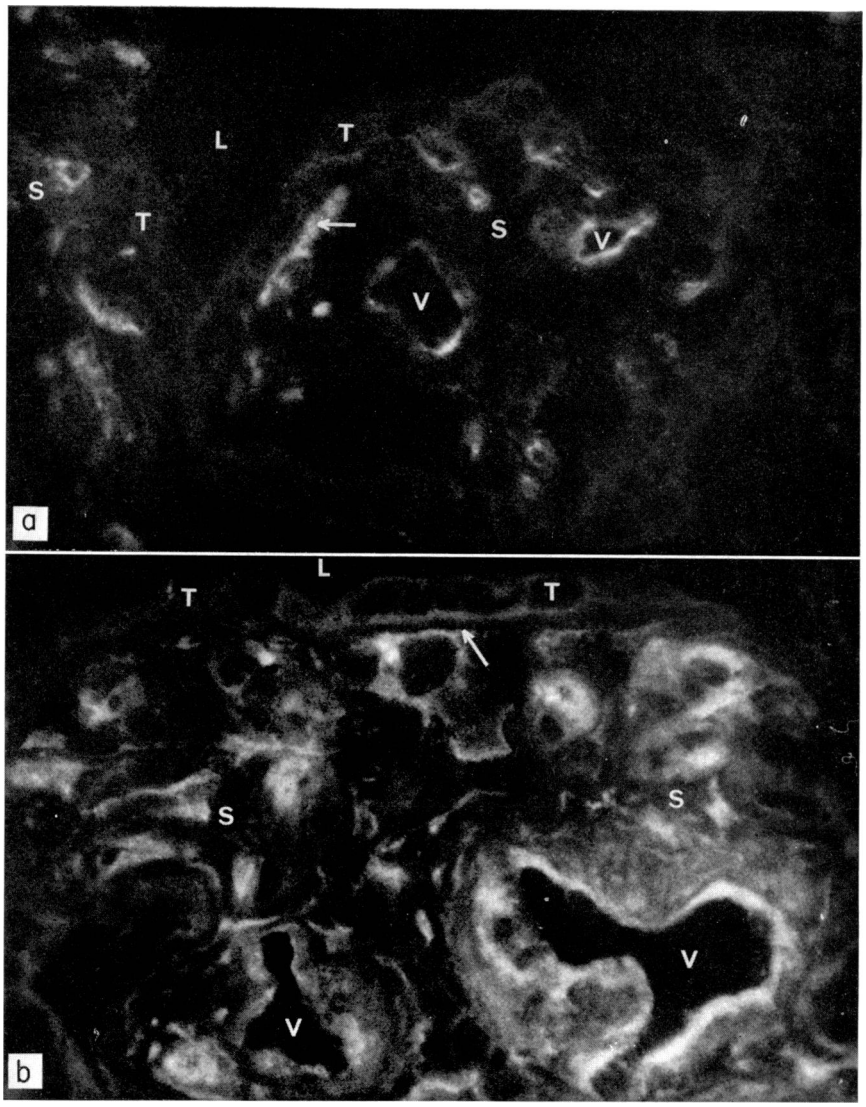

FIG. 2. Indirect immunofluorescent staining with anti-Gm antisera on two separate full-term human placental tissues fixed with 1% paraformaldehyde. Code as Fig. 1. (a) anti-Gm(f): representative on this placenta of staining for IgG of foetal origin (arrow points to positively-stained TBM). (Magnification × 630.) (b) anti-Gm(g): representative on this placenta of staining for IgG thought to be of maternal origin (arrow points to unstained TBM). (Magnification × 630.)

were such that the foetus must inherit only Gm markers that were also carried by the mother. In these situations, the immunofluorescent staining using antisera to these Gm markers was always comparable to that of the larger group 3 subgroup (line (d), Table 2). This pattern was thus considered to represent IgG of both maternal and foetal origin. However, a smaller subgroup of group 3 (line (c), Table 2) could also be distinguished. This subgroup demonstrated a similar degree of staining in stromal connective tissue, but slightly reduced staining of foetal stem vessels and fibrinoid deposits, and lacked definitive TBM staining. By comparison with that for foetal IgG (group 1), this latter distribution (line (c), Table 2) was considered to represent IgG of solely maternal origin. A representative example of the staining thought to represent maternal IgG is given in Fig. 2(b).

DISCUSSION

Insight into the possible biological role of various proteins in human placentae has been provided by immunofluorescence studies (Faulk & Johnson, 1977). A detailed investigation of the distribution and character of immunoglobulins has now been undertaken using the same approach. The amount of immunoglobulins detected in first trimester placentae was very limited: this is in accord with the observations in the first trimester of pregnancy that comparatively small amounts of maternal IgG are transported to the foetus (Gitlin, 1974), and that the amount of foetal immunoglobulin synthesis is very low (van Furth *et al.*, 1965; Gitlin & Biasucci, 1969). IgA was detected only in intervillous fibrin deposits (i.e. deposits bathed in maternal blood), whereas IgM could also be seen weakly on placental endothelium and in intravillous fibrinoid areas in full-term placentae. These observations are compatible with the lack of significant placental transfer of maternal IgA and IgM, and a small amount of foetal synthesis of IgM but not IgA at term (Gitlin *et al.*, 1964; van Furth *et al.*, 1965). Similarly, all four human IgG subclasses, and both κ and λ light chains, were detected in similar distributions in full-term or first trimester chorionic villi. Furthermore, the Gm markers of each maternal phenotype were always present in the corresponding full-term placental tissue. These observations support studies on cord sera indicating that all maternal IgG subclasses and allotypes are transferred across the placenta from mother to foetus (Hay *et al.*, 1971; Mellbye & Natvig, 1973). Furthermore, all human IgG fragments were also detected in similar distributions, arguing against possible local enzymatic digestion during placental transfer that might affect the expression of biological activities associated with the Fc region of IgG.

There is support for the hypothesis (Brambell, 1970) that selective placental transfer of maternal IgG may be associated with binding of the Fc region to membrane receptors and subsequent pinocytotic vesicle formation. In line with this hypothesis, Fc receptors have been described on trophoblastic tissue (Matre *et al.*, 1975; Jenkinson *et al.*, 1976). It would therefore be expected that, at any time, some maternal IgG should be present in trophoblasts, but no such IgG was demonstrated in the present investigation. This might be related to a possible highly efficient exo- and endocytosis ability of these cells (Faulk & Johnson, 1977), and to the failure to demonstrate trophoblastic Fc receptors by immunofluorescence techniques (Johnson *et al.*, 1976a; Matre & Johnson, 1977).

The variable amount of IgG found in foetal stem vessels in placenta may reflect the extent of alloantigenic incompatibility between mother and foetus, since the endothelial Fc receptors may bind immune complexes formed within chorionic villi by maternal antibody and foetal alloantigens (Johnson *et al.*, 1976a). For example, maternal antibody to foetal (paternal) HLA antigens can be detected in placental eluates but not in neonatal sera, while the converse is true for maternal anti-HLA antibody resulting from a previous pregnancy or transfusion and lacking specificity for the present pregnancy (Doughty & Gelsthorpe, 1976; Jeannet *et al.*, 1977). Fibrinoid deposits show a variability of staining for IgG similar to that observed in vessel walls: it has previously been suggested (McCormick *et al.*, 1971) that fibrinoid deposits may represent the result of immunological reactions. Positive staining for IgG has also been observed on the TBM and in scattered cells within placental villi. Most of the IgG appears loosely bound to the TBM and can be removed by prolonged washing, although a small amount is firmly bound and may represent antibody to antigens expressed on the TBM. The nature of the cells exhibiting cytoplasmic staining in placental villous stroma is unknown, although their appearance and low numbers suggests that they could be Hofbauer cells. Weak staining was also observed with anti-pepsin site sera in occasional stromal cells, vessels, limited segments of TBM and in fibrinoid deposits. This underlines the possible presence of immune complexes in these areas, since anti-pepsin site sera can also react with immune complexes (Mellbye & Natvig, 1970).

Antisera to Gm markers on human IgG have been used to identify IgG thought to be of maternal or foetal origin within chorionic villi. The presence of paternal Gm markers that were not carried by the mother was taken as evidence for IgG of foetal origin. Furthermore, the distinction of two separate staining patterns in that group of matings where the mother carried a Gm marker not necessarily inherited by the foetus was considered sufficiently pronounced to indicate the distribution of IgG of maternal origin. The distribution of maternal IgG differed from that for foetal IgG. Maternal IgG was

more extensively distributed, and contributed the majority of generalized staining throughout the connective tissue villous stroma. This pattern may represent the bulk of maternal IgG in transit from mother to foetus. Both maternal and foetal IgG were found in fibrinoid deposits, vessel walls and in the cytoplasm of some stromal cells. Only foetal IgG was definitively observed on limited segments of the TBM, and this was noted in all cases where foetal IgG could be identified exclusively.

The observation that foetal IgG contributes to the immunoglobulin tightly bound to the TBM is of interest, since placental acid-eluate IgG both inhibits *in vitro* assays of lymphocyte reactivity and demonstrates binding to the TBM (McCormick *et al.*, 1971; Faulk *et al.*, 1974; Revillard *et al.*, 1976). The absence of β_2 microglobulin, HLA- and Ia-like antigens on human trophoblast and TBM (Faulk & Temple, 1976; Faulk, Sanderson & Temple, 1977) suggests that further specificities may be involved in the 'blocking antibody' activity in placental eluate IgG, other than the maternal antibodies against HLA or B-cell alloantigens that can be found in pregnancy (Winchester *et al.*, 1976; Doughty & Gelsthorpe, 1976). Also, eluate IgG preparations without demonstrable anti-HLA or B-cell alloantibody activity have been reported to inhibit mixed lymphocyte culture (MLC) reactions, albeit less strongly than those with such antibodies (Jeannet *et al.*, 1977). Since some placental IgG has been shown to be of foetal origin, the possibility that foetal IgG can contribute to part of the blocking antibody activity should also be considered. In this regard, it is interesting that eluate IgG does not show a clear specificity for either the corresponding maternal or paternal lymphocytes in inhibition of one-way MLC reactions (Jeannet *et al.*, 1977). However, previous investigations involving Gm typing have indicated a maternal origin for eluate IgG (Faulk *et al.*, 1974), although such preparations are difficult to Gm type since excess maternal IgG in the eluate, not necessarily with all the blocking antibody activity, may mask the presence of foetal IgG. Clearly, further studies are needed to elucidate the full blocking antibody activity in placental eluates, notably investigations of possible alloantigenic activity on the TBM or trophoblasts, and the relation to lymphocyte antigens.

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