Immunology and Cell Biology (1997) **75**, 231–237

Elution and partial characterization of immunoglobulins bound to ovine placenta

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Summary Immunoglobulins were eluted from ovine placentae and characterized by immunoprecipitation, electrophoresis, western blotting and ELISA. IgG was shown to comprise the bulk of placental-bound immunoglobulins while smaller amounts of IgM and only trace amounts of IgA were demonstrated. Results suggest that ovine placental IgG eluted by surgical cannulation of the uterine blood vessels *in situ* is similar to that eluted from postpartum placentae *in vitro*, implying that there may be some transfer of antibodies across the maternal side of the placental barrier to the trophoblast. These antibodies are rich in IgG_1 and IgG_2, have a relative molecular weight of 158 kDa, and bind to an 80 kDa peptide prepared from pre-acidified ovine placental cotyledons.

We propose that the binding of placental IgG to the 80 kDa antigen may prevent immunological rejection of the foetus by competitively excluding cytotoxic cells of maternal origin such as NK cells. Also, given that a similar antigen (80 kDa) has been reported in humans and equines, and shown to be saturated with IgG in term placentae, we propose that this antigen may be conserved in several mammalian species for reproductive purposes. Consequently, we suggest that the ovine placental IgG and the 80 kDa antigen may be suitable as models for the study of maternal–foetal interactions in mammalian pregnancies.

Key words: abortions, immunoglobulins, immunotherapy, ovine placenta, pregnancy.

Introduction

The mechanism by which the pregnant mother tolerates the semi-allogeneic foetus remains a puzzle to immunologists since the foeto–placental unit in an outbred population is antigenically different from the mother by virtue of its complement of the paternal genes. Although many mechanisms have been proposed to explain the survival of the foetus in an immunologically competent mother, there is no clear indication that any of these mechanisms may play a dominant role. This survival of the foetus under potential immunological attack has been attributed to some extent to the down-regulation of the maternal immune responses during pregnancy by blocking antibodies,[1] restricted expression of class I MHC antigens[2] and the presence of the placental barrier among others.

Placental-bound immunoglobulins have been reported in mice and humans and have been shown to comprise mainly IgG although small amounts of IgM and IgA have been detected.[3,4] Also, IgG-rich fractions derived from full-term placenta are able to neutralize catalytic activities of reverse transcriptases from several retroviruses[4] and have been characterized as blocking antibodies capable of inhibiting blastogenic reactions of the maternal lymphocytes directed against MHC determinants in humans.[5] These immunoglobulins have also been shown to inhibit spontaneous IgE synthesis by lymphocytes from healthy and atopic subjects *in vitro*,[6] suggesting that they may be involved in the down-regulation of maternal immunity to allow for successful gestation. Elsewhere, it has been reported that in all human pregnancies including first pregnancies, there is IgG bound to the syncytiotrophoblast.[7] These antibodies were bound to a highly polymorphic 80 kDa antigen showing considerable analogies with the 80 kDa cell membrane protein to which a mAb capable of preventing the killing of human, mouse or parasitic cells by activated NK cells had been developed earlier.[8]

In addition, it has been reported that passive immunization of mice with IgG raised against paternal strain antigens reduces the incidence of foetal resorption, further suggesting the possible involvement of IgG in the immunology of pregnancy.[9] In humans, it has been demonstrated that these antibodies are bound both to Fc receptors and to antigenic sites on the placenta.[7] However, prenatal transfer of Fc-receptor-bound immunoglobulins into foetal circulation has frustrated the ease with which the antigen-specific subpopulation of these antibodies can be purified, especially after the placenta has been subjected to physical handling for *in vitro* analysis. Also, given that all the antibodies used in previous studies were eluted from isolated placentae *in vitro* and none has been eluted *in situ* and considering the complex processes associated with parturition in mammals, it may not suffice to assume that the antibodies found on the placenta after parturition are fully representative of those bound to the placenta *in vivo*. Thus, there is a need to identify an animal model

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Received 25 September 1996; accepted 20 November 1996.

from which these antibodies can be eluted by the *in situ* and by the conventional *in vitro* methods, so that these antibodies can be compared in order to determine whether or not the antibodies eluted from isolated placentae *in vitro* are suitable representatives of those bound to the placenta *in vivo*. Also, despite the fact that human placental-bound IgG has been successfully used in the treatment of rheumatoid arthritis,[10] it is not clear whether these antibodies may be useful in the treatment of immunologically compromised pregnancies such as recurrent spontaneous abortions. However, characterization of these antibodies and identification of the antigens to which they are bound on the placenta would be instrumental in advancing a possible treatment for immunologically compromised pregnancies. We have purified and partially characterized these antibodies following elution by the *in situ* and *in vitro* methods from the ovine placenta where prenatal transfer of antibodies from the maternal side into foetal circulation has not previously been reported.

Materials and methods

Sheep and human placentae

Sheep used in this study were kept in open grazing yards where their environment was not altered in any way. Term human placentae were obtained from the delivery suite of the John Hunter Hospital, Newcastle, Australia.

Antibodies

Mouse mAb to sheep IgG_1 and IgG_2 were obtained from Dr Ken Beh, CSIRO Laboratories, Sydney, Australia. Donkey anti-sheep gamma globulin and alkaline-phosphatase-conjugated donkey anti-sheep immunoglobulin was purchased from Silenus Laboratories (Melbourne, Vic., Australia). Rabbit anti-sheep α and μ chains were purchased from Immunodiagnostics (Campberdown, NSW, Australia).

Elution and purification of placental-bound immunoglobulins

Two methods were used to elute antibodies from the placenta. In one method, antibodies were eluted from sheep placenta *in situ* and in the other method *in vitro*. In both methods, 0.5 mol/L glycine buffer (pH 2.5) was used to dissociate the antibodies from their binding sites in the trophoblast-containing layer of sheep placental cotyledons. The antibody-rich fractions were concentrated by precipitation with ammonium sulfate solution (*in situ* eluates) and by polyethylene glycol (*in vitro* eluates). These eluates were further purified by gel filtration on Sephacryl S-300 (BioRad), and by affinity chromatography on protein G–Sepharose (AMRAD Pharmacia, Sydney, NSW, Australia).

In situ *elution of immunoglobulins* Pregnant ewes were killed at term with 20 mL of 300 mg/mL pentobarbitone sodium injected intravenously into the jugular vein. The uterine artery and vein were cannulated. A ligature was tied around the cervix proximal to the cannulated site to prevent flow of eluate into the systemic circulation. The tubing from the cannulated artery was attached to a pump to aid in washing the uterine vascular system with buffers. At first, 0.15 mol/L PBS with 10 U/mL of heparin was perfused through until the eluate ran clear and no protein could be detected by measuring optical density at 280 nm. Elution of bound antibodies was then started using 0.5 mol/L glycine–HCl buffer (pH 2.5) until the pH of the eluate stabilized at 2.55 for 5 min and no further proteins could be detected in the eluate. Fractions (50 mL) were collected and their pH was determined using a portable pH meter. Each fraction was then titrated to pH 7.2 with 3 mol/L Tris-HCl buffer (pH 10) and centrifuged at $1000 \times g$ for 10 min to clarify the eluate, then 1 mL fractions were drawn from each, dialysed and tested for the presence of IgG_1 and IgG_2 by ELISA.[11] Each of the 50 mL fractions were then concentrated by vacuum dialysis, dialysed in five changes of PBS and tested for the presence of immunoglobulins (IgG, IgM and IgA) by single radial immunodiffusion.[12] The immunoglobulin-containing fractions were stored at −20°C prior to use.

In vitro *elution of immunoglobulins* A thin section of the trophoblast-containing layer of placental cotyledons (about 10 g of wet tissue) was dissected out and used to prepare membrane microvesicles as previously described[5] and immunoglobulins subsequently eluted by treating these microvesicles with 0.5 mol/L glycine buffer (pH 2.5). The pH of the eluate was adjusted to 7.2 prior to dialysis for 20 h in PBS. The eluate was concentrated with polyethylene glycol, redialysed in PBS and purified by gel filtration chromatography on Sephacryl S-300 (BioRad) and then by affinity chromatography on protein G–Sepharose (AMRAD Pharmacia). Human placental IgG was purified as described above for ovine IgG on a Protein A–Sepharose column (AMRAD Pharmacia).

Detection of IgG_1 and IgG_2 in the in situ *placental acid eluates*

The presence of IgG_1 and IgG_2 in the *in situ* placental acid eluates was detected by ELISA.[11] Ninety-six-well flat bottom plates (Linbro; Flow Laboratories, McLean, VA, USA) were coated with 100 μL/well of a 1/500 dilution of mouse mAb to sheep IgG_1 and IgG_2 in PBS and incubated at 37°C for 1 h. Plates were washed in running water and blocked for 1 h at room temperature with 5% (w/v) fat-free skim milk. After washing, 100 μL of each acid-eluted, *in situ* fractions (dialysed in five changes of PBS) and negative control, and PBS containing Tween 20 to a final concentration of 0.05% (v/v) (PBST), respectively, were added to experimental wells and incubated for 1 h at 37°C. Plates were washed and 100 μL of alkaline-phosphatase-conjugated donkey anti-sheep immunoglobulin diluted 1/2000 in PBST was added to all wells and incubated for 1 h at room temperature. After washing, 100 μL of substrate (1 mg/mL of 2,4-dinitrophenyl phosphate in 50 mmol/L diethanolamine buffer, pH 9.8) was added to each well and allowed to stand for 30–60 min at room temperature in the dark until colour developed. The reaction was stopped by adding 50 μL of 3 mol/L NaOH to each well, and optical densities were read at 405 nm using a BioRad Model 450 microplate reader.

Estimation of total IgG bound to ovine placenta at term

Eluate immunoglobulin prepared from placental microvesicles *in vitro* was dialysed in PBS and concentrated by polyethylene glycol, redialysed and its IgG content estimated by ELISA[11] using protein G-purified ram systemic IgG as the standard. Plates were

coated with 100 μL/well of a 1/500 dilution of rabbit anti-sheep IgG heavy chain specific (Bethyl Laboratories, Akron, OH, USA) and incubated at 37°C for 1 h and blocked for one hour with 5% (w/v) fat-free milk in PBS. After washing, 100 μL of either serially diluted acid-eluted immunoglobulins from placental microvesicles, systemic IgG (standard) or negative control (PBST), respectively, were added to respective wells. Plates were incubated for 1 h at 37°C and developed as described above. Protein concentrations were determined by the bicinchoninic acid protein determination method[13] and a standard curve was constructed with the help of STATISTICA (Version 4.5, Statsoft, Tulsa, OK, USA), using protein G-purified ram systemic IgG as the standard.

Concentration of immunoglobulins with ammonium sulfate

The immunoglobulin containing *in situ* glycine fractions was pooled and precipitated in 50% saturated ammonium sulfate (v/v). The resulting pellet was washed in 45% saturated ammonium sulfate solution (v/v), pelleted and subsequently suspended in minimal amount of PBS, then dialysed for 20 h at 4°C with five changes of PBS and kept at −20°C until use.

Characterization of immunoglobulins by immunoprecipitation

The presence and relative concentrations of immunoglobulins in the crude *in situ* acid eluate was tested by single radial immunodiffusion[12] and two-dimensional immunodiffusion using donkey anti-sheep gamma globulin and rabbit anti-sheep α and μ chains, respectively. The presence of IgG in the eluates was further confirmed by immuno-electrophoresis.[14]

Analysis of immunoglobulin by SDS-PAGE

Electrophoresis on 10–20% gradient SDS polyacrylamide gels was carried out under reducing conditions on a vertical electrophoresis unit using 50 mol/L Tris-Tricine buffer (TTS) (pH 7.5). Samples (4.0 mg/mL) were diluted 1 : 1 with Laemmli sample buffer[15] containing 2% (w/v) SDS, 6% (v/v) 2-ME, 40% (w/v) sucrose, 0.02% (w/v) bromophenol blue in 0.125 mol/L Tris-HCl (pH 6.8) and were boiled for 5 min before applying 10 μL (40 μg) of protein per well. Electrophoresis was carried out at 50 V for the first 30 min and then at 75 V until the marker dye (bromophenol blue) reached the bottom of the gel. Proteins were fixed for 10 min in 10% (w/v) TCA and stained overnight in 0.2% (w/v) Coomassie Brilliant blue R (Sigma Chemicals) in 50% (v/v) methanol, 5% (v/v) glacial acetic acid. Gels were destained in 4–5 changes of 40% (v/v) methanol, 10% (v/v) glacial acetic acid to obtain a clear background.

Analysis of eluate immunoglobulin by western blotting

Eluate immunoglobulins were separated by SDS-PAGE and transferred to polyvinyl difluoride (PVDF) 0.4 mm pore size (Millipore, Sydney, NSW, Australia) membranes by electrophoresis[16] for 2 h at 2.5 mA/cm^2 of membrane using the BioRad transblot apparatus. Membranes were rinsed in 25 mmol/L Tris-buffered saline (TBS; pH 8.0), and blocked for 1 h at room temperature with blocking solution (0.1% (w/v) fat-free skim milk, and 3% (v/v) FCS in PBST) and then cut into 4 mm strips and incubated for 1 h at room temperature with alkaline-phosphatase-conjugated donkey anti-sheep immunoglobulin diluted 1/1000 in washing solution (TBS containing 0.5% (w/v) fat-free milk and 0.05% (v/v) Tween 20). Membranes were washed twice for 10 min each time with washing solution, and twice for 20 min each time with 25 mmol/L TBS. The antibody–antigen complexes were visualized by incubating the strips with BioRad nitroblue tetrazolium/5-bromo-4-chloro-3-indoyl phosphate (NBT/BCIP) colour development reagent (100 μL each in 10 mL of 25 mmol/L bicarbonate buffer, pH 9.8). Excessive colour development was stopped by immersing the strips in tap water.

Purification of placental-bound IgG on protein G–Sepharose column

Protein G–Sepharose (Pharmacia) was suspended in PBS and the slurry was slowly packed into a 10 mL polystyrene column. The column was connected to a peristaltic pump (Gilson Minipuls 2, Gilson Medical Electronics, France) and washed with at least 10 volumes of PBS at 4°C. Approximately 1 mL of eluate immunoglobulin was loaded on to the column and washed with an excess amount of chilled PBS until the unbound protein was completely removed as determined by measuring optical densities at 280 nm. Protein G-bound IgG was eluted with 0.1 mol/L glycine buffer (pH 2.5) at 12 mL/h and 3 mL fractions were collected into tubes containing 400 μL of 10 mmol/L Tris-HCl buffer, pH 8.0, to neutralize the pH of the eluate. The presence of IgG in the eluate was determined by ELISA[11] using plates coated with a 1/1000 dilution of mouse mAb to sheep IgG_1 and IgG_2. Ram systemic IgG was similarly purified on a protein G column while human placental-bound IgG was similarly purified on protein A–Sepharose.

Preparation of sheep cotyledon antigen

Ovine cotyledon antigens were prepared as outlined elsewhere[17] for lymphocyte glycoprotein antigens but with some modifications. About 10 g of trophoblast-containing layer was dissected from post-elution sheep cotyledons, homogenized and the pH adjusted to 2.7 using 10 mol/L glycine–HCl buffer (pH 2.5). The solution was stirred for 30 min at room temperature to remove traces of antibody still bound to the trophoblast then centrifuged for 15 min at 2000 × *g*. The pellet was resuspended in 10 mmol/L TBS (pH 8.0) to a total volume of 7.5 mL. The following solutions were added to this suspension: 4.5 mL of 10% Nonidet P-40 (NP-40) in 10 mmol/L TBS (pH 8.0), 3 mL of 5 mmol/L phenyl methyl sulfonyl fluoride (PMSF), a protease inhibitor, and 0.6 mL of 500 mmol/L iodoacetamide to obtain a final concentration of 3% NP-40, 1 mmol/L PMSF and 15 mmol/L iodoacetamide. The solution was stirred for 30 min at 4°C and centrifuged for 20 min at 3500 × *g*. A volume of 7.5 mL of 3% sodium deoxycholate in 10 mmol/L TBS (pH 8.0) was added to the supernatant to give a final concentration of 1% sodium deoxycholate and 2% NP-40 and the mixture was centrifuged at 70 000 × *g* for 60 min at 4°C. The pellet was resuspended in a minimal volume of 10 mmol/L TBS (pH 8.0), aliquoted into 50 μL fractions and stored at −20°C until use. Ovine liver and spleen antigens were similarly prepared.

Analysis of soluble cotyledon peptides by western blotting

The soluble extracts of trophoblast-containing membrane were electrophoresed under reducing conditions on 10–20% gradient

gels and subsequently transferred to PVDF membranes by electrophoresis.[16] Membranes were incubated with affinity-purified, placental IgG as the primary antibody and subsequently with alkaline-phosphatase-conjugated donkey anti-sheep immunoglobulin diluted 1/1000 in wash solution as the secondary antibody. Antibody–antigen complexes were visualized using NBT/BCIP colour development reagents. Systemic IgG similarly prepared from normal ram serum were used as positive control and PBST as negative control, respectively. These membranes were also blotted with protein A-purified human placental eluate IgG.

Results

This study revealed that IgG forms the bulk of immunoglobulins bound to the placenta of sheep and that small amounts of IgM and only trace amounts of IgA are present. Thus, while the presence of IgG was readily detected in the *in situ* placental acid eluates by single radial immunodiffusion following concentration of each 50 mL fraction to 1 mL by vacuum dialysis, IgM and IgA were not detected at this stage. However, we demonstrated the presence of IgM and extremely low amounts of IgA when all eluates were pooled and concentrated with ammonium sulfate, suggesting that IgG forms the bulk of immunoglobulins bound to the placenta of sheep and that IgM and IgA are present in lower amounts. The presence of IgG_1 and IgG_2 in the *in situ* and *in vitro* eluates was demonstrated by ELISA using mouse mAb to sheep IgG_1 and IgG_2. There was a wide variation in IgG content from one term placenta to the other ranging from as low as 25 μg to as high as 371 μg (Table 1). Electrophoresis of the *in situ*- and the vesicle-derived *in vitro* placental eluate immunoglobulin under reducing conditions revealed two distinct peptides of approximate molecular weight 57 and 27 kDa representing the heavy and light chains of IgG, respectively. These peptides blotted positive for IgG when probed with alkaline-phosphatase-conjugated donkey anti-sheep immunoglobulin (Fig. 1). A similar pattern of bands was obtained when these peptides were probed for the presence of IgG_1 and IgG_2 by using mouse mAb to sheep IgG_1 and IgG_2. This observation confirms that the 57 and 27 kDa peptides obtained on electrophoresis were

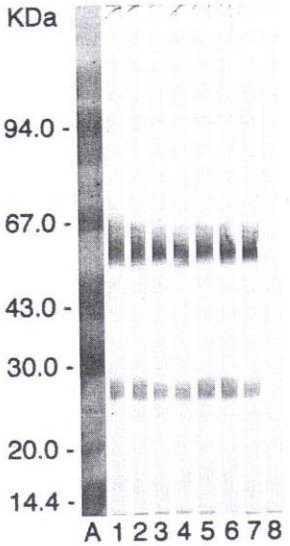

Figure 1 Analysis of eluate immunoglobulin by western blotting. Eluate immunoglobulin was separated by SDS-PAGE on 10–20% gradient gels under reducing conditions and transferred to polyvinyl difluoride membranes by electrophoresis. Membranes were probed with alkaline-phosphatase-conjugated donkey anti-sheep immunoglobulin and colour developed by NBT/BCIP colour development reagents.

representative of heavy and light chains of sheep IgG_1 and IgG_2, respectively. Following filtration of the *in situ*- and the vesicle-derived *in vitro* eluate immunoglobulins on Sephacryl S-300, IgG_1 and IgG_2 were recovered at exactly the same elution volume as Aldolase (a 158 kDa molecular weight marker protein), suggesting that sheep placental IgG has a relative molecular weight of about 158 kDa. Electrophoresis of these eluates under non-reducing conditions resulted in a relatively pure peptide with a relative molecular weight of about 152 kDa (Fig. 2) which migrated with gamma mobility on immuno-electrophoresis. Further purification of these eluates on protein G–Sepharose yielded a well-refined peak containing both IgG_1 and IgG_2 (Fig. 3). These immunoglobulins from all the third party placental eluates tested, recognized and

Table 1 Estimation of total IgG released from individual placentae

Sample number	Volume of eluate (mL)	Protein (mg/mL)	Total protein (mg)	Total IgG (μg)	IgG (% of total protein)
46	2.25	02.50	05.63	159.88	2.8
47	1.80	03.64	06.55	046.32	0.7
48	3.25	08.54	27.76	370.68	1.3
49	2.00	01.51	03.02	153.57	5.0
50	2.35	01.61	03.78	036.00	0.9
51	1.71	02.27	03.90	028.00	0.7
52	2.75	05.25	14.44	065.00	0.4
53	1.79	00.38	00.68	050.00	7.4
54	2.10	02.78	05.84	037.50	0.6
55	1.61	00.56	00.90	041.77	4.6
56	1.60	09.13	14.61	219.65	1.5
57	2.45	05.39	13.21	025.00	0.2
58	1.85	04.12	07.62	072.89	0.9

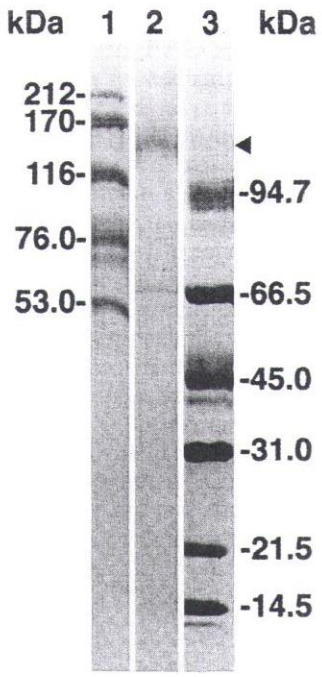

Figure 2 Analysis of Sephacryl S-300 eluate immunoglobulin by polyacrylamide gel electrophoresis under non-reducing conditions. Lane 1, high molecular weight protein standards; lane 2, Sephacryl S-300 eluate immunoglobulin; lane 3, low molecular weight protein standards.

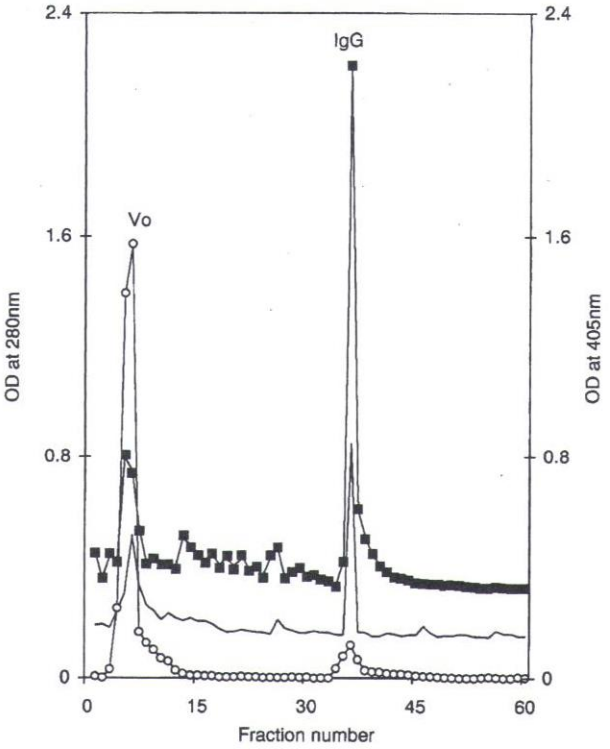

Figure 3 Elution profile of sheep placental IgG on protein G–Sepharose column. The IgG containing fractions were located by ELISA using mouse monoclonal antibodies to sheep (—) IgG_1 and (■) IgG_2. (○) OD at 280 nm.

bound to an 80 kDa peptide prepared from the 'trophoblast-containing membranes' of sheep placental cotyledons (Fig. 4). No such binding was seen between these antibodies and sheep liver and spleen antigens prepared in the same way and used at similar concentrations. Also, affinity purified ram serum IgG used in similar concentrations did not show strong binding to the 80 kDa peptide but instead showed weak binding to a number of lower molecular weight antigens. The 80 kDa sheep placental peptide was not recognized by protein A-purified human placental IgG.

Discussion

Results of this study have demonstrated that IgG forms the bulk of immunoglobulins bound to sheep placenta and that only small amounts of IgM and trace amounts of IgA are present. This observation coincides with the earlier reports that immunoglobulins eluted from the trophoblast membrane of human placenta comprised mainly IgG with only trace amounts of IgA and IgM.[3] Also, the finding in this study that an intact sheep placenta IgG is about 158 kDa suggests some degree of structural similarities with the human placental IgG reported elsewhere to be about 160 kDa.[18] However, although we estimated the molecular weight of the ovine placental IgG at 158 kDa by gel filtration chromatography, the same molecule was estimated at 152 kDa by gel electrophoresis under non-reducing conditions showing a shift of 6 kDa. This shift may have resulted partly from the retarded mobility of molecular weight marker proteins relative to placental IgG on electrophoresis due to the steep gradient of the gel used (10–20% polyacrylamide gel) under non-reducing conditions, thus leading to some degree of inaccuracy. The estimated molecular weight of the sheep placental IgG (about 158 kDa) as presented in this study is at variance with the earlier report that sheep serum IgG is about 190 kDa by gel filtration on Sephadex G-100 and 192.6 kDa by density sedimentation.[19] This variation may reflect substantial differences in the molecular structure of the sheep placental and serum IgG which may be related to differences in their respective functions.

We propose that placental IgG may down-regulate the maternal immune response to foetal antigens in order to allow successful gestation. Elsewhere, it was suggested that IgG_1 is the antibody that is predominantly produced in response to foetal antigens in pregnant mice and that it may impede the binding of cytotoxic maternal antibodies by competitive exclusion.[20] Whether or not this is the case in the sheep was not investigated in this study, but the fact that we demonstrated IgG_1, and IgG_2 and IgM in the sheep placental eluates suggests that either of these immunoglobulin subclasses as well as IgM may play some role in the immunology of pregnancy. The results presented here also show that ovine placental IgG binds to an 80 kDa peptide prepared from the 'trophoblast-containing membranes' of the sheep placental cotyledons and that ram serum IgG does not show selective binding

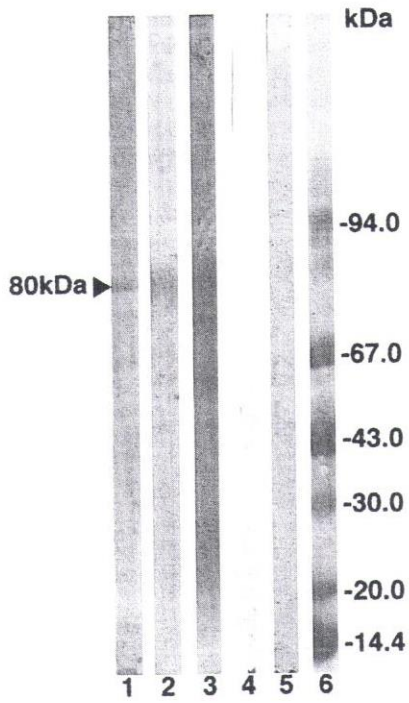

Figure 4 Analysis of soluble placental cotyledon antigens by western blotting. Cotyledon antigens from a third party sheep placenta were separated by SDS-PAGE and transferred to polyvinyl difluoride membranes by electrophoresis. Membranes 1 and 2 were incubated with affinity purified sheep placental IgG. Membrane 3 (positive control) was incubated with affinity purified ram serum IgG, while membrane 4 (negative control) was incubated with PBS containing Tween 20. These membranes (1–4) were probed with alkaline-phosphatase-conjugated donkey anti-sheep immunoglobulin. Membrane 5 was incubated with protein A-purified human placental IgG and probed with alkaline-phosphatase-conjugated sheep anti-human immunoglobulin (γ and λ chain specific). Colour was developed with NBT/BCIP reagents. Strip 6 is the molecular weight standard.

to this peptide when used at similar concentrations as the placental IgG (40–80 μg/mL) but in addition, weakly recognizes a number of lower molecular weight antigens (Fig. 4). These observations suggest that while the IgG specific for the 80 kDa placental antigen may be found in the systemic circulation to a limited extent, there is a higher concentration of this antibody in the placenta, suggesting that its distribution is related to function. In addition, the fact that ovine placental IgG did not show similar binding to sheep spleen and liver antigens prepared in the same way and used at similar concentrations further suggests specificity in the binding of sheep placental IgG to the 80 kDa placental peptide.

We proposed that the binding of placental IgG to the 80 kDa placental antigen may prevent immunological rejection of the foetus by competitively excluding the binding of cytotoxic cells of maternal origin such as the NK cells to the trophoblast. Also, given that human placental IgG has been shown to recognize an 80 kDa antigen in western blotting and that this antigen (80 kDa) has been shown to be saturated with IgG in both equine and human placentae at term,[7,21] we propose that this antigen is crucial in the immunology of pregnancy and may be conserved in several mammalian species for reproductive purposes. This thesis may be re-enforced by the recent observations that a mAb BA 11 raised to a non-polymorphic epitope of the human 80 kDa trophoblast antigen (R80K), recognizes a mouse analogue of the human R80K, inhibits human and mouse NK cell activity and reduces the incidence of foetal resorption in mouse abortion models.[22] Whether or not the 80 kDa antigen may be part of a MHC antigen was not investigated. However, the fact that a similar antigen (80 kDa) has been reported in other mammalian placentae [22] suggests that it may be crucial in immunological regulation of pregnancy in several mammalian species.

The recovery of IgG in the microvesicles prepared from the 'trophoblast-containing membranes' of the sheep placental cotyledons suggests that these antibodies may have been transported across the maternal side of the placental barrier by some unidentified mechanism. Thus, although the sheep placenta is histologically classified as an epitheliochorial placenta and has not been shown to allow transfer of immunoglobulins across the placental barrier into the foetal circulation during pregnancy, it may exhibit localized histological variations to allow the transfer of some antibodies across the maternal side of the placental barrier to the trophoblast. Thus, earlier reports have indicated that some parts of the placental membranes, particularly those at which the vascular channels of one side approach those of the other side, are much thinner than other parts. Consequently, parts of the placental membrane in epitheliosyndesmo- and endotheliochorial placentas may actually be no thicker than or involve any more layers than the barrier in many haemochorial placentas, although there may be quantitative differences between species in the amount of surface represented by such thin areas.[23] In addition, there is evidence to show that even within a single species, the placenta may change its histological class during gestation; thus the rabbit starts off with an epitheliochorial placenta, and it is not until the last 3 days of gestation that this becomes a predominantly haemoendothelial placenta.[24]

The wide variation in the amount of IgG detected in each placenta (Table 1) may reflect variations in the amount of trophoblast (and its IgG content) recovered in the wet tissue dissected from different placental cotyledons. These variations may also have arisen from excessive degradation of native IgG in some placental preparations by acid treatment during the elution process. We propose that immunological regulation of pregnancy may involve the production by the mother of antibodies directed to specific antigens on the placenta. The binding of such antibodies to placental antigens may in turn exclude potentially harmful cells of maternal origin such as NK cells and prevent immunological rejection of the foetus.

This study has provided evidence that placental IgG eluted by surgical cannulation of the uterine blood vessels *in situ* procedure is similar to that eluted from postpartum placentae *in vitro*. Thus they are rich in IgG_1 and IgG_2, have a relative molecular weight of about 158 kDa and they both bind to an 80 kDa peptide of placental

origin. Consequently, we suggest that the interaction between the ovine placental IgG and the 80 kDa antigen may be suitable as a model for the study of maternal–foetal interaction in mammalian pregnancies.

Acknowledgements

We are thankful to Drs Phil Robinson and Jun Ping of the Discipline of Reproductive Medicine of the University of Newcastle, John Hunter Hospital, for their unfailing technical support with the purification of immunoglobulins. We also thank Rhiannon Noltorp for her participation in this work. We are grateful for the financial support of the Australian Research Council and the University of Newcastle.

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