

# Ex vivo human placenta models: transport of immunoglobulin G and its subclasses

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## Abstract

The comparison between fetal and maternal levels of immunoglobulins indicate that the human placenta during pregnancy develops a specific transport mechanism for IgG in the maternal to fetal direction. There are differences for the four subclasses, with preferential transfer of IgG<sub>1</sub> while the slowest transfer is seen for IgG<sub>2</sub>.

Under in vitro perfusion conditions of human term placenta IgG, when compared to other proteins, showed a significantly higher transfer rate of its subclasses from the maternal to the fetal side, indicating a specific transport mechanism. There is a preferential transfer rate, highest for IgG<sub>1</sub> and lowest for IgG<sub>2</sub>, similar to those observed under in vivo conditions. The similarity in transfer of anti-TT-IgG and tetanus antigen, which was observed under in vivo and in vitro conditions, may suggest the transport as antibody–antigen complex.

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## 1. Introduction

Intrauterine or perinatally acquired infections are important causes of perinatal mortality and morbidity. Because of the immaturity of the fetal immune system, immunoprotection of the fetus and neonate is dependent on the placental transmission of maternal antibodies, which is apparently limited to immunoglobulin class G (IgG) in an exponential profile during the last weeks of pregnancy. The transfer of maternal antibodies, which includes all IgG subclasses [1–4], providing the newborn with sufficient passive immunity against infectious pathogens until maturation of the neonatal immune system, is an important process for extrauterine survival.

A specific receptor mediated binding of the Fc $\gamma$ -portion of IgG at the maternal surface of the placenta was proposed as the first step in the transport mechanism by which IgG is transferred from the mother to the fetus [1]. The selective binding of maternal IgG at the surface of the syncytiotrophoblast is followed by transmission of IgG in coated vesicles across the trophoblastic layers [2]. The coated vesicles supposedly protect IgG against lysosomal proteolysis,

allowing intact IgG to be released into the fetal circulation. Many studies have been performed to localize IgG in placental tissue, using various techniques such as immunofluorescence, immunocytochemistry, colloidal gold complexed protein-A, and conjugated peroxidase [3–5]. The isolation of the IgG Fc $\gamma$ -receptor from detergent-solubilized membrane extracts of human placental tissue has produced bands with varying molecular weights [6,7]. Kinetic studies of human IgG binding to placental Fc-receptor have resulted in different values for binding association constants and the total number of binding sites [8–10]. While the detection of Fc $\gamma$ -receptor using immunohistochemistry has clearly indicated that all three subtypes (I, II and III) are expressed in human placental tissue [11,12], a Swedish group has suggested that placental alkaline phosphatase (PLAP), which is an enzyme with optimal activity at higher than physiological pH, acts as a receptor for IgG [13,14].

## 2. In vivo observations

Observations described in the following section are derived from an in vivo study [15,16] in which paired blood samples were collected between 17 and 41 weeks of gestation (WG) by puncture of a peripheral maternal vein and by cordocentesis (17–36 WG,  $n = 91$ ) or directly at delivery

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(37–41 WG,  $n = 16$ ) from the umbilical vein. Additional maternal samples were collected from the same individual ( $n = 16$ ) at 10, 20, 30 WG, and at term.

The mean levels of IgG and immunoglobulin class A (IgA) in maternal sera at 9–16 WG were  $13.72 \pm 2.53$  and  $3.95 \pm 1.23$  g/l, respectively. Both IgG and IgA throughout pregnancy decreased to a level of 60–70% (37–41 WG) of the initial concentration in early pregnancy. The ratio of IgG<sub>1</sub>:IgG<sub>2</sub> in the maternal circulation was 2:3 and remained constant throughout pregnancy (17–41 WG). IgG<sub>3</sub> and IgG<sub>4</sub> levels remained constant and together were less than 10% of total IgG. In the fetal circulation, a continuous rise in the level of both IgG and IgA was observed between 17 and 41 WG. Fetal levels of IgG at 17–22 WG were only 5–10% of maternal levels and at term exceeded maternal levels reaching a value of  $11.98 \pm 2.18$  g/l. IgG<sub>1</sub> at 17–22 WG was  $0.93 \pm 0.42$  g/l, which is approximately three times higher than IgG<sub>2</sub>. IgG<sub>1</sub> showed an exponential rise and at 37–41 WG its concentration was seven times higher than IgG<sub>2</sub>. IgG<sub>3</sub> and IgG<sub>4</sub> also showed an exponential rise and at term reached similar levels as in the maternal circulation. The difference from the results for IgG<sub>2</sub>, with a slow linear rise throughout gestation, was striking. Fetal IgG<sub>2</sub> levels at term remained significantly below maternal concentrations. The IgG subclasses, when characterized according to the differences in transport capacity gave the following sequence: IgG<sub>1</sub> > IgG<sub>4</sub> > IgG<sub>3</sub> > IgG<sub>2</sub>. Fetal IgA showed a slow linear rise with fetal levels at term remaining approximately 1000 times lower than concentrations in the maternal circulation.

The specificity of IgG transport across the human placenta was further confirmed by calculating fetal to maternal level ratios for anti-tetanus (TT)-IgG (specific antibody) and IgA at term. The ratio for anti-TT-IgG was 1.41 which was 1000 times higher than for IgA. In the same study, a similar ratio was found between anti-TT-IgG and its antigen (TT-AG)

with close correlation. This observation could be an indication for specific transfer of the antigen antibody complex.

The transmission of antigen across the placenta is supported by experimental studies in rats, in which the immunization of the pregnant female led to the transplacental immunization of her fetuses as indicated by the detection of fetal IgM anti-TT in the infants [17]. Similar observations were also reported from human studies. Maternal vaccination in the last trimester of pregnancy was associated with the presence of IgM anti-TT in the cord blood sera. Such antibodies might have been induced by transported TT, or through anti-idiotypic reactions [18].

Other studies have shown that maternal immunization resulted in higher infant levels of vaccine-specific IgG antibody but not in the transfer of specific T lymphocyte response(s) or production of neonatal antibody [19].

### 3. In vitro studies

Using the in vitro perfusion model (Fig. 1) of an isolated cotyledon from human term placenta [20,21], the placental transport of various proteins present in human serum, such as immunoglobulins (IgG, IgA), specific anti-tetanus-IgG (anti-TT-IgG), and tetanus toxoid-antigen (TT-AG), was investigated. In addition, the transport of IgG modified with biotin (IgG-BT) and <sup>14</sup>C-bovine serum albumin (<sup>14</sup>C-BSA, a permeability marker for macromolecules), was assessed. The perfusate was recirculated on both maternal and fetal sides. After an initial stabilization phase of 2 h (control phase), media were changed on both sides and perfusion was continued, comparing two different conditions (experimental phase). In the first group (control experiments (A,  $n = 3$ )), no test proteins were added during the experimental phase (4–6 h). In the second group (B,  $n = 5$ ),

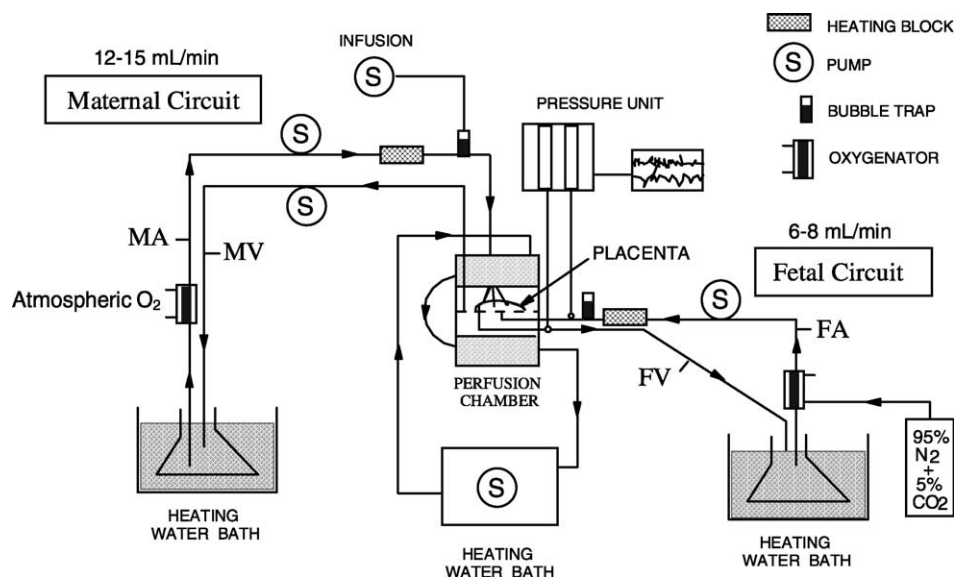


Fig. 1. Schematic diagram of the human placenta perfusion system. FA: fetal artery; FV: fetal vein; MA: maternal artery; MV: maternal vein.

during the experimental phase (6 h), the maternal perfusion medium contained IgG (Sandoglobuline<sup>®</sup>, 6–10 g/l), anti-TT-IgG (21–25 mg/l), TT-AG (0.19–0.24 mg/l), and IgA (0.13–0.19 g/l). IgG-BT (2 g/l) and <sup>14</sup>C-BSA (30–40 nCi/ml) were added to the medium on the maternal side. IgGs and TT-AG were determined in the perfusates by specific enzyme-linked immunosorbent assay.

Both groups showed stable metabolic conditions with constant rates of glucose consumption, lactate production, and hormone (human chorionic gonadotropin, human placental lactogen) release observed throughout the experiment. Washout levels of endogenous IgG and IgA observed in the maternal circuit at the end of the control period were 5 and 1000 times higher than in the fetal circuit. In the experimental phase these levels remained constant at 50–80% of control levels with no change in the last 4 h of perfusion (group A).

In group B, with the addition of the proteins described, trace amounts of IgG-BT, IgA, and <sup>14</sup>C-BSA were detectable in the fetal circuit within 1 h, with no significant further increase in circulating levels in the following 4 h of the perfusion. In contrast, the detection of IgGs in the fetal circuit was delayed by 2 h. Thereafter, a continuous linear increase was observed for all IgG subclasses. However, the ratio of immunoglobulin G<sub>1</sub> to immunoglobulin G<sub>2</sub> in the fetal perfusate was significantly higher than in the maternal perfusate (3.8 versus 1.8), suggesting preferential transfer of immunoglobulin G<sub>1</sub>. TT-AG in fetal perfusate was below the detection limit. TT-AG was found on the fetal side only after concentration of the samples obtained at the end of the experiment by ultrafiltration. For permeability comparison, the ratio between concentrations on the fetal and maternal side multiplied by 100 ((F:M) × 100), as detected after 6 h of perfusion, was assessed ( $n = 5$ , mean ± S.D.). Labeling of IgG with biotin IgG-BT reduced its placental transfer by a factor of 10 ( $0.04 \pm 0.01$ ) when compared with the natural IgG ( $0.49 \pm 0.08$ ) or the specific antibody (anti-TT-IgG). The relative fetal-to-maternal ratio found for TT-AG ( $0.48 \pm 0.12$ ) was similar to anti-TT-IgG ( $0.46 \pm 0.11$ ), and approximately 4 and 50 times that of <sup>14</sup>C-BSA ( $0.12 \pm 0.03$ ) and IgA ( $0.01 \pm 0.01$ ), respectively. Considering that the molecular weights of TT-AG and anti-TT-IgG were at least twice that of BSA and similar to IgA, the difference in transfer suggests a specific mechanism of transport.

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