

EDITORIAL

The placenta: a portal of fetal allergen exposure

Introduction

The observation of allergen-dependent proliferation by cord blood mononuclear cells has stimulated many areas of research. Although the immunological processes that underlie this response are characterized poorly, there has been much interest in elucidating the route of fetal allergen exposure and how this might be manipulated as a prevention strategy for IgE-mediated allergic disease. However, an increasing number of studies indicate an inconsistent relationship between maternal allergen exposure and either the cord blood response to allergen or the subsequent development of disease [1–3]. Nevertheless, such observations do not preclude further study of possible routes of exposure of the fetus to allergen and their role in the developing immune response and the offspring's atopic outcome, as these might identify alternative therapeutic strategies. In this issue, the passage of allergen across not only the term (> 37 weeks gestation) but also the preterm placenta in an *ex vivo* perfusion model is shown [4]. This offers an opportunity to review the features of the biology of the materno-fetal interface that might help us understand the significance of fetal allergen exposure.

Development of the placenta and placental membranes

The implanting blastocyst has an outer covering of two cell layers – an inner cytotrophoblast layer and an outer syncytiotrophoblast layer – surrounding the inner cell mass of the embryo proper. Invasion of the uterine epithelium by the blastocyst causes erosion of maternal endometrial blood vessels such that maternal blood bathes the syncytiotrophoblast. Chorionic villi develop from the trophoblast cell layers as outgrowths of cytotrophoblast covered with syncytiotrophoblast. Trophoblast cells at the tips of the villi proliferate and infiltrate the uterine stroma, thereby securing implantation and increasing blood flow within the intervillous space. The chorionic villi are invaded by embryonic mesodermal cells, which differentiate into fetal capillaries and permit the circulation of fetal blood through the villi by 3 weeks post-fertilization.

Until the 8th week of gestation, chorionic villi surround the developing conceptus. However, as the embryo enlarges, the villi at the abembryonic pole begin to atrophy, becoming part of the amniochorionic membrane that lines the amniotic fluid-filled cavity in which the fetus develops. The remaining villi continue to increase in number and branch profusely, projecting in all directions within the intervillous space [5]. These chorionic villi and the overlying endometrium (decidua basalis) constitute the placenta.

Fetal allergen exposure

Two possible routes of intrauterine allergen transfer have been postulated: passage of maternally-derived allergen from the maternal vasculature of the decidua across the amniochorionic membranes into the amniotic fluid, and across the placenta from the maternal to the fetal circulation [6]. Of these, trans-placental transport offers the greater reservoir of antigen, reflecting the placenta's primary function in enabling the exchange of nutrients and waste materials between the mother and the fetus. However, from the overview provided above it is clear that any substances that pass from the maternal circulation to the fetal blood via the placenta must traverse a histological barrier comprised of trophoblast, fetal endothelial cells and the intervening stroma.

Mechanisms of trans-placental antigen transport

A wide range of substances can be transferred to the fetus via the placenta. The trans-placental passage of the common dietary allergens, cow's milk β -lactoglobulin and hen's egg ovalbumin is described to occur from 26 weeks of gestation using an *ex vivo* model of transfer, while transfer of Bet v 1 across the term placenta is also described [4]. Furthermore, allergen passage was enhanced in the preterm placenta, and with increasing dose and decreasing molecular weight (MW) of the allergens. The question then arises as to how these proteins cross the placental membranes. As noted by the authors [4], the ability of a molecule to traverse the placenta is associated with its molecular weight, lipid solubility, polarity and ionization, as well as the existence of specific transport mechanisms. Most low molecular weight substances (< 500 Da) simply diffuse through placental tissue. However, many other substances, including amino acids [7], require specific transport mechanisms. Of particular relevance to the field of allergy is the specific transport of IgG.

Maternofetal IgG transport begins at about 16 weeks of gestation. From 22 weeks of gestation fetal IgG levels increase rapidly, reaching maternal serum concentrations by 26 weeks of gestation, and exceeding those of the mother by birth [8]. However, this relationship is isotype-specific with fetal IgG1 exceeding, IgG3 and IgG4 equivalent to, and IgG2 levels less than maternal levels, reflecting the transfer preference of IgG1 > IgG3 > IgG4 > IgG2. The transfer of maternal IgG is essential for survival of the newborn whose endogenous production of IgG begins slowly and only attains adult levels by 3 years of age [8]. It is mediated by IgG receptors on cell types within the placenta.

IgG must first cross the syncytiotrophoblast that is bathed in IgG-containing maternal blood. As a true multinucleated syncytium, formed by cell fusion of the underlying cytotrophoblast, the syncytiotrophoblast lacks lateral membranes and IgG passage must occur via an apical-basal route. Syncytiotrophoblast express IgG-binding proteins such as annexin II and placental alkaline phosphatase but these neither bind monomeric IgG nor possess the transmembrane/intracellular domains required for IgG uptake [9]. The neonatal Fc receptor (FcRn), an MHC Class I-like Fc receptor expressed by syncytiotrophoblast [10], can bind all subtypes of IgG with high affinity but is unable to bind IgG at the neutral pH of the intervillous space. Therefore, the proposed model is the uptake of IgG in the fluid phase by endocytosis within coated pits, enabling binding to FcRn within vesicles that have the appropriate pH of 5–6.5. In this way, IgG is delivered to the basal membrane where it dissociates from the receptor in response to the neutral pH of the interstitial fluid of the stroma [10–12].

The underlying cytotrophoblast do not express IgG receptors and it is the intact cytotrophoblast layer that is postulated to limit the passage of IgG across the placenta in the first trimester. The degeneration of this layer as pregnancy progresses combined with the expression of IgG receptors by other cell populations facilitates the transfer of IgG [9, 11]. Transfer across the villous stroma is postulated to occur by bulk phase flow, although placental macrophages (Hofbauer cells) within the stroma express Fc γ RI (CD64), Fc γ RII (CD32) and Fc γ RIII (CD16) and have a scavenging role in the clearance of immune complexes [11]. Fetal endothelial cells represent the next barrier to the passage of IgG to the fetal circulation. A candidate transport molecule expressed by these cells is Fc γ RIIb [13]. This receptor can bind immune complexes but has not been described to bind monomeric IgG. However, expression of Fc γ RIIb transcripts increases markedly at 20 weeks of gestation which is around the time that IgG transfer begins, further implicating a role for this receptor [14].

Trans-placental allergen transfer

The active transport of maternal IgG offers an ideal vehicle for allergen carriage to the fetus. The inhalant cat allergen, Fel d 1, has been detected in IgG complex in up to 40% of infant umbilical cord sera [15], and, previously, Szepfalusi et al. demonstrated that *ex vivo* trans-placental transport of cow's milk β -lactoglobulin was enhanced by the addition of human immunoglobulin, and was essential for the transfer of the inhalant birch pollen allergen, Bet v 1 [16]. These authors now report trans-placental transfer of free allergen as early as 26 weeks of gestation (although no data for Bet v 1 transfer across the preterm placenta are presented) and the rapid elution of endogenous IgG from the perfused placental cotyledon [4]. Thus, an IgG-independent route of transfer seems to be in operation. A number of alternative routes are possible and a role for placental fibrinoids is suggested. Placental fibrinoids occur at sites of syncytiotrophoblast degeneration, which can be found in all placentas at all stages of pregnancy, are postulated to play an important role in materno-fetal interactions and are permeable to macromolecules [17]. This is the pathway proposed by

Loibichler et al. to operate in their perfusion model but this mechanism would not account for the differences in transfer rate of allergens of different molecular weights.

Further work is needed to clarify whether a paracellular porous route (as described for *ex vivo* experiments with horseradish peroxidase [18]) or an endocytic mechanism (as demonstrated for HIV-1 infection [19]) underlies the passage of free antigen. As allergen-specific IgG will also cross the placenta as part of the bulk IgG transferred, the impact of this should also be considered. It is also necessary to reconcile the apparent universal transfer of Bet v 1 (as well as β LG and OVA) with the low proportion of responders in cord blood mononuclear cells proliferation assays at term (5% to Bet v 1 as reported by this group [20]). Furthermore, allergen exposure does not seem to be essential for proliferation responses to allergen, as reactivity occurs when the pregnancy does not overlap with the relevant pollen season [20], and, as already noted maternal allergen exposure, at least for house dust mite, does not appear to show any association with responses/disease in the first year of life [1–3].

Despite these shortcomings it seems that allergen can indeed cross the placenta, although the details of the mechanism(s) remain to be elucidated. Moreover, altered placental function has been observed in asthmatic women [21], and although it remains unclear if this is disease or treatment associated, it is feasible that transfer rates (both IgG-dependent and -independent) across the placenta might differ in women of differing clinical histories. Also, the relationship between the level of allergen in the environment and that in the circulation of pregnant women may differ to that in non-pregnant individuals; high levels of circulating progesterone, and other endocrine changes, are associated with an increased respiratory rate [22], and there are profound changes in maternal haemodynamics [23]. These might combine to increase the allergen load in the pregnant compared to the non-pregnant state.

Finally, for immunological priming to occur it is insufficient for allergen to be merely present in the circulation, it must access tissue sites where it can be taken up by dendritic cells for processing and presentation to naïve T cells. This does not occur in the circulation but in the secondary lymphoid organs (lymph nodes and spleen) where naïve T cells normally reside. It is however worthy of note that animal studies have revealed that there is less restricted trafficking of naïve T cells during fetal and neonatal life [24, 25], and, indeed, cord blood T cells show a different pattern of chemokine receptor expression compared to the equivalent adult population [26]. The trafficking of naïve T cells to the peripheral tissues is postulated to facilitate the interaction of tissue-resident immature dendritic cells bearing tissue antigens with naïve T cells for the initiation of peripheral self-tolerance [24, 25]. In the absence of inflammatory and other signals that induce maturation of dendritic cells it is probable that allergen encounter in the fetal period leads to tolerance rather than immunological priming to allergen, presumed to have some association with the development of clinical disease. However, much more information is required about the journey of allergen not only across but once it has crossed the placenta, the functional status of the cell types encountered (especially dendritic and T cells), and the consequences of the transition from the germ-free to the germ-laden environment at birth on any response generated during fetal life.

References

- 1 Chan-Yeung M, Ferguson A, Chan H et al. Umbilical cord blood mononuclear cell proliferative response to house dust mite does not predict the development of allergic rhinitis and asthma. *J Allergy Clin Immunol* 1999; 104:317–21.
- 2 Marks GB, Zhou J, Yang HS, Joshi PA, Bishop GA, Britton WJ. Cord blood mononuclear cell cytokine responses in relation to maternal house dust mite allergen exposure. *Clin Exp Allergy* 2002; 32:355–60.
- 3 Smillie FI, Elderfield AJ, Patel F et al. Lymphoproliferative responses in cord blood and at one year. no evidence for the effect of in utero exposure to dust mite allergens. *Clin Exp Allergy* 2001; 31:194–204.
- 4 Liobichler C, Pichler J, Gerstmayr M et al. Materno-fetal passage of nutritive and inhalant allergens across placentas of term and preterm deliveries perfused *in vitro*. *Clin Exp. Allergy* 2002; 32: 1546–51.
- 5 Kingdom J, Huppertz B, Seaward G, Kaufmann P. Development of the placental villous tree and its consequences for fetal growth. *Eur J Obstet Gynecol Reprod Biol* 2000; 92:35–43.
- 6 Holloway JA, Warner JO, Vance GH, Diaper ND, Warner JA, Jones CA. Detection of house-dust-mite allergen in amniotic fluid and umbilical-cord blood. *Lancet* 2000; 356:1900–2.
- 7 Jansson T. Amino acid transporters in the human placenta. *Pediatr Res* 2001; 49:141–7.
- 8 Malek A, Sager R, Kuhn P, Nicolaides KH, Schneider H. Evolution of maternofetal transport of immunoglobulins during human pregnancy. *Am J Reprod Immunol* 1996; 36:248–55.
- 9 Saji F, Samejima Y, Kamiura S, Koyama M. Dynamics of immunoglobulins at the feto-maternal interface. *Rev Reprod* 1999; 4:81–9.
- 10 Kristoffersen EK, Matre R. Co-localization of the neonatal Fc gamma receptor and IgG in human placental term syncytiotrophoblasts. *Eur J Immunol* 1996; 26:1668–71.
- 11 Simister NE, Story CM. Human placental Fc receptors and the transmission of antibodies from mother to fetus. *J Reprod Immunol* 1997; 37:1–23.
- 12 Kristoffersen EK. Human placental Fc gamma-binding proteins in the maternofetal transfer of IgG. *APMIS Suppl* 1996; 64:5–36.
- 13 Lyden TW, Robinson JM, Tridandapani S et al. The Fc receptor for IgG expressed in the villus endothelium of human placenta is Fc gamma RIIB2. *J Immunol* 2001; 166:3882–9.
- 14 Koyama M, Saji F, Kameda T et al. Differential mRNA expression of three distinct classes of Fc gamma receptor at the feto-maternal interface. *J Reprod Immunol* 1991; 20:103–13.
- 15 Casas R, Bjorksten B. Detection of Fel d 1-immunoglobulin G immune complexes in cord blood and sera from allergic and non-allergic mothers. *Pediatr Allergy Immunol* 2001; 12:59–64.
- 16 Szepefalusi Z, Loibichler C, Pichler J, Reisenberger K, Ebner C, Urbanek R. Direct evidence for transplacental allergen transfer. *Pediatr Res* 2000; 48:404–7.
- 17 Kaufmann P, Huppertz B, Frank HG. The fibrinoids of the human placenta: origin, composition and functional relevance. *Anat Anz* 1996; 178:485–501.
- 18 Edwards D, Jones CJ, Sibley CP, Nelson DM. Paracellular permeability pathways in the human placenta: a quantitative and morphological study of maternal-fetal transfer of horseradish peroxidase. *Placenta* 1993; 14:63–73.
- 19 Phillips DM, Tan X. HIV-1 infection of the trophoblast cell line BeWo: a study of virus uptake. *AIDS Res Hum Retroviruses* 2002; 8:1683–91.
- 20 Szepefalusi Z, Pichler J, Elsasser S et al. Transplacental priming of the human immune system with environmental allergens can occur early in gestation. *J Allergy Clin Immunol* 2000; 106:530–6.
- 21 Clifton VL, Giles WB, Smith R et al. Alterations of placental vascular function in asthmatic pregnancies. *Am J Respir Crit Care Med* 2001; 164:546–53.
- 22 Bayliss DA, Millhorn DE. Central neural mechanisms of progesterone action: application to the respiratory system. *J Appl Physiol* 1992; 73:393–404.
- 23 Ekholm EM, Erkkola RU. Autonomic cardiovascular control in pregnancy. *Eur J Obstet Gynecol Reprod Biol* 1996; 64:29–36.
- 24 Alferink J, Tafuri A, Vestweber D, Hallmann R, Hammerling GJ, Arnold B. Control of neonatal tolerance to tissue antigens by peripheral T cell trafficking. *Science* 1998; 282:1338–41.
- 25 Cahill RN, Kimpton WG, Washington EA, Holder JE, Cunningham CP. The ontogeny of T cell recirculation during foetal life. *Semin Immunol* 1999; 11:105–14.
- 26 Sato K, Kawasaki H, Nagayama H et al. Chemokine receptor expressions and responsiveness of cord blood T cells. *J Immunol* 2001; 166:1659–66.

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