

Dose-Dependent and Preterm-Accentuated Diaplacental Transport of Nutritive Allergens in vitro

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Key Words

Placenta · Allergen transport · Prenatal · Allergy prevention

Abstract

Objective: The mechanisms by which nutritive allergens are transported from mother to fetus and the ensuing immunological response are incompletely understood. We investigated the role of different allergen concentrations in influencing the diaplacental allergen transport in preterm and term placentas. **Method:** Twenty-seven human term placentas and 12 preterm placentas were dually perfused in vitro for up to 4 h by adding alternately two different nutritive allergens, β -lactoglobulin (BLG) or ovalbumin (OVA), at four different allergen concentrations (0.02, 0.2, 2 and 20 mg/ml) to the maternal perfusate medium. Allergen concentrations in fetal venous outflow samples collected during perfusion were measured by using specific ELISAs. **Results:** Perfusion of increasing allergen concentrations via the maternal circulation resulted in a concentration-dependent increase of fetal allergen uptake in all term and preterm placentas. A mean maternal-to-fetal ratio of 20,000/1 and 3,000/1 for BLG, and 40,000/1 and 5,000/1 for OVA was found in term and preterm placentas, respectively. Preterm placentas

(27–36 weeks of gestation) were found to favor the diaplacental passage of nutritive allergens compared with placentas at term (>36 weeks of gestation). **Conclusion:** Maternal-to-fetal allergen transport occurs in a dose-dependent and molecular weight-dependent manner with clear accentuation in preterm placentas.

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Introduction

Given the increasing number of infants developing atopic disease, the investigation of factors, such as time point, route and level of exposure to an allergen, which play a decisive role in triggering sensitization or tolerance, is of great importance. Several studies showed cord blood reactivity to nutritive or inhalant allergens [1–4]. These antigen-specific immune responses show (1) that the immune system can be primed with allergens before birth, and (2) that fetal exposure occurs to nutritive as well as inhalant allergens. Two pathways of allergen exposure in the fetus have been identified, the diaplacental transport of allergens [5] and the transamniotic transfer [6]. The underlying mechanisms, whereby allergen-specific responses are initiated and modified before and after birth, are not completely understood. It is still an open question,

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as to which maternally derived factors may influence the development of allergen-specific Th cell memory. Given the fact that pregnancy is a Th2 phenomenon [7, 8], the environment in which the fetus encounters antigen is likely to favor Th2 reactivity. In part, hyperreactivity of control mechanisms operating in utero to protect the fetoplacental unit against toxic effects of Th1 cytokines is suggested to be involved in genetic susceptibility to allergy [9].

However, beside the influence of the cytokine environment in determining the fate of naive CD4⁺ T cells, the priming antigen dose is one critical parameter leading to a differential activation of naive CD4⁺ T cells [10, 11]. Nutrients, antibodies, and infectious agents, passing from the maternal to the fetal circulation within the villous placenta, must first cross the fetally derived syncytiotrophoblast cell layer, which is in direct contact with maternal blood and forms the initial barrier limiting or supporting transplacental transfer processes [12].

Perfusion of placental tissues provides an accurate *ex vivo* model to study substance movement from the maternal to the fetal side of the placenta [5, 13–16]. Using a previously established placental perfusion model [5], our aim was to determine the role of increasing maternal levels of nutritive allergens [ovalbumin (OVA) and β -lactoglobulin (BLG)] in influencing the transfer and uptake of these allergens by the human placenta.

In this study, we show that there is a consistent correlation between the allergen dose in the maternal circulation and the fetal allergen uptake during placental perfusion. Furthermore, the capacity of preterm placentas to transport allergens exceeds clearly that of term placentas.

Material and Methods

Placental Perfusion

The technique used to prepare and perfuse the isolated human placenta has been described by several groups [5, 13–15]. Briefly, human term (>36 weeks of gestation) and preterm placentas (27–36 weeks of gestation) were obtained immediately after spontaneous vaginal delivery or cesarean section. After inspection, an intact lobe suitable for dual perfusion was selected, and a small artery and vein of the chorionic plate were cannulated for perfusion. The whole placenta was placed in a thermostable (37°C) chamber with the apical side on top. Five catheters were pierced through the basal plate into the intervillous space. In this way cotyledons were perfused from the maternal side. Perfusate and placenta were maintained at 37°C throughout perfusion. Maternal and fetal flow rates were changed stepwise between 1.2 and 3.2 ml/min. Evans blue, a dye not capable of crossing the placental barrier, was added to the maternal circuit to ensure the integrity of the membranes of all preterm and term placentas. The use of human placentas was approved by the National Ethical Review Board.

Medium

Perfusion medium containing 11 g/l technetium-Medium 199 (Biochrom, Berlin, Germany), 3 g/l bovine albumin (Behringwerke, Marburg, Germany), and 20 g/l dextran T40 (Pharmacia, Uppsala, Sweden) is saturated with 95% oxygen and 5% carbon dioxide. 10–20 mmol sodium bicarbonate are added to adjust the pH to 7.38–7.42.

Allergens

Two nutritive allergens, OVA (molecular weight 44.4 kD) and BLG (molecular weight 18.5 kD), were infused alternately via the maternal circulation for up to 4 h at four concentrations (0.02, 0.2, 2 and 20 mg/ml). Fetal venous outflow samples were collected at 1-min intervals for the first 10 min, followed by 5-min intervals. The samples were stored at –70°C for future allergen concentration determination.

Detection of Allergens by Specific ELISA

The concentrations of BLG and OVA in fetal samples were determined by using specific ELISA. For the detection of BLG plates were coated with rabbit anti-bovine BLG (affinity-purified, Bethyl, Montgomery, Tex., USA) for 2 h at 37°C. After washing with Na-phosphate buffer, nonspecific binding was blocked with 1% human serum albumin for 30 min. The plates were washed with Na-phosphate buffer, and samples and standards were applied. After incubation overnight and washing of the plates with Na-phosphate buffer rabbit anti-bovine BLG (alkaline phosphatase-conjugated, Bethyl) was applied for 2 h. The plates were washed again, and the substrate pNPP (Sigma, 100 μ l/well) was applied, and the color developed for 25 min. Optical density at 405 nm was determined, and the concentration of BLG in the samples was determined by extrapolation from a standard curve.

For the determination of OVA concentrations goat anti-chicken egg albumin (Biometricals, Eschwege, Germany), rabbit anti-chicken egg albumin (Dako, Glostrup, Denmark) and alkaline phosphatase-conjugated swine anti-rabbit Ig (Dako) were used.

Statistical Analysis

Statistical comparison was made, using the Mann-Whitney U test and the Kruskal-Wallis test. A p value of less than 0.05 was accepted as indicating significance. Nonparametric statistical analysis was performed with the computer program SAS. Results are expressed as means $\pm$ SD.

Results

Ex vivo Placental Transfer

In all 39 experiments performed in this study, a marked influence of the allergen dose, present in the maternal circulation, on the diaplacental transfer rate could be observed.

Effect of Different Maternal Allergen Concentrations on Diaplacental Transport

The addition of increasing allergen doses to the maternal circulation (0.02, 0.2, 2 and 20 mg/ml) resulted in

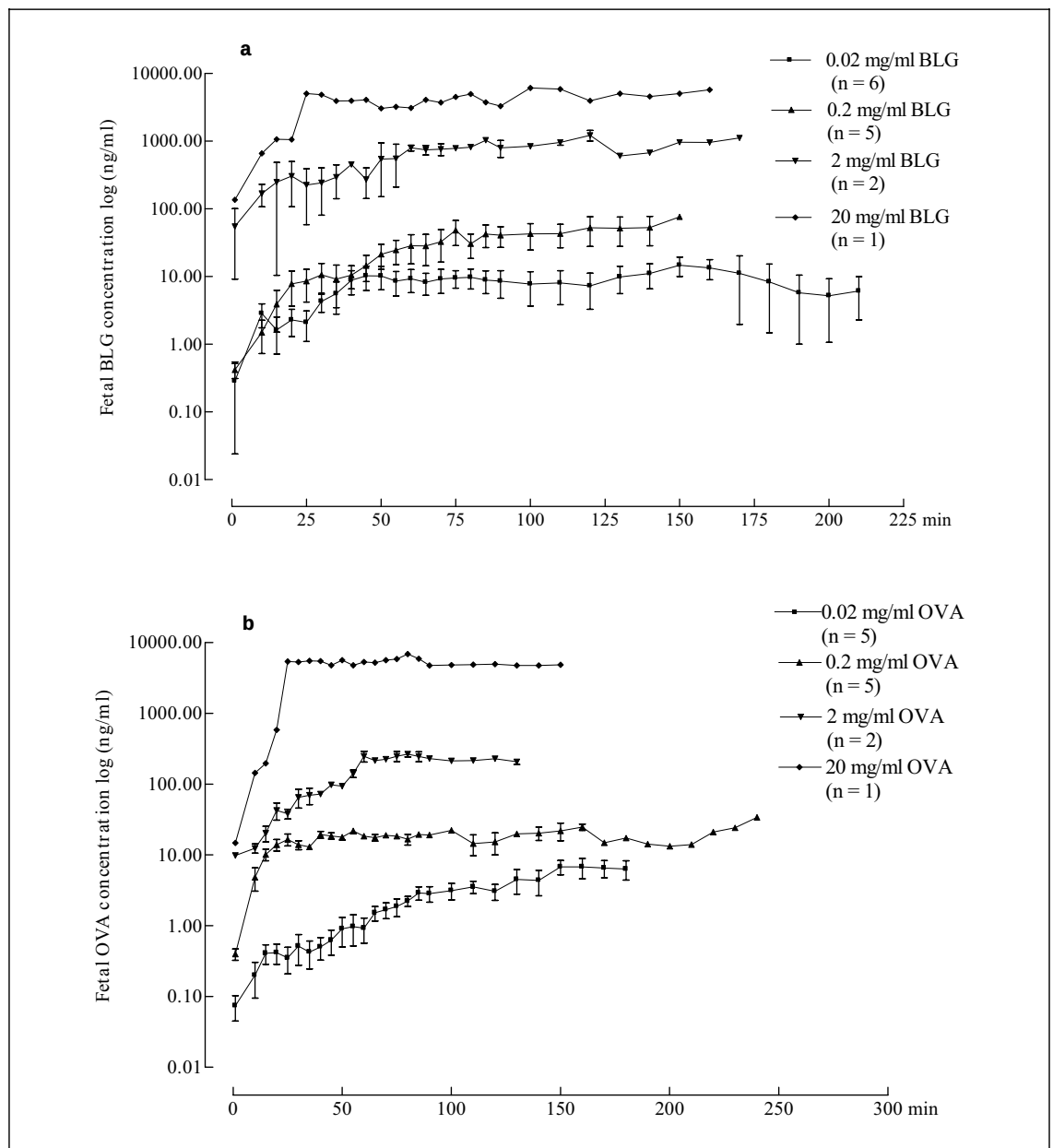


Fig. 1. Concentration dependence of allergen uptake by perfused human term placentas. a Fetal allergen concentrations, obtained during in vitro perfusion of human term placentas ($n = 14$) with increasing concentrations of BLG (0.02, 0.2, 2 and 20 mg/ml), were plotted against time from their addition to the maternal perfusate. The symbols represent the allergen levels in fetal perfusate in relationship to the maternal allergen concentration of 0.02, 0.2, 2 and 20 mg/ml. b Concentrations of OVA, detected in fetal perfusate samples, which were collected during placental perfusion with increasing levels of OVA (0.02, 0.2, 2 and 20 mg/ml) infused via the maternal circulation ($n = 13$).

increasing allergen concentrations in the fetal circulation. The allergen transmission ratio was nearly constant for all concentrations.

In preterm placentas, perfused with 0.02 mg/ml BLG maternally, mean fetal allergen uptake during perfusion

was 17 ± 3 ng/ml. The mean fetal BLG uptake level in preterm placentas was 81 ± 14 , 815 ± 40 and $8,170 \pm 210$ ng/ml, according to maternal allergen doses of 0.2, 2 and 20 mg/ml, respectively. The mean fetal OVA concentrations obtained during the perfusion of preterm placen-

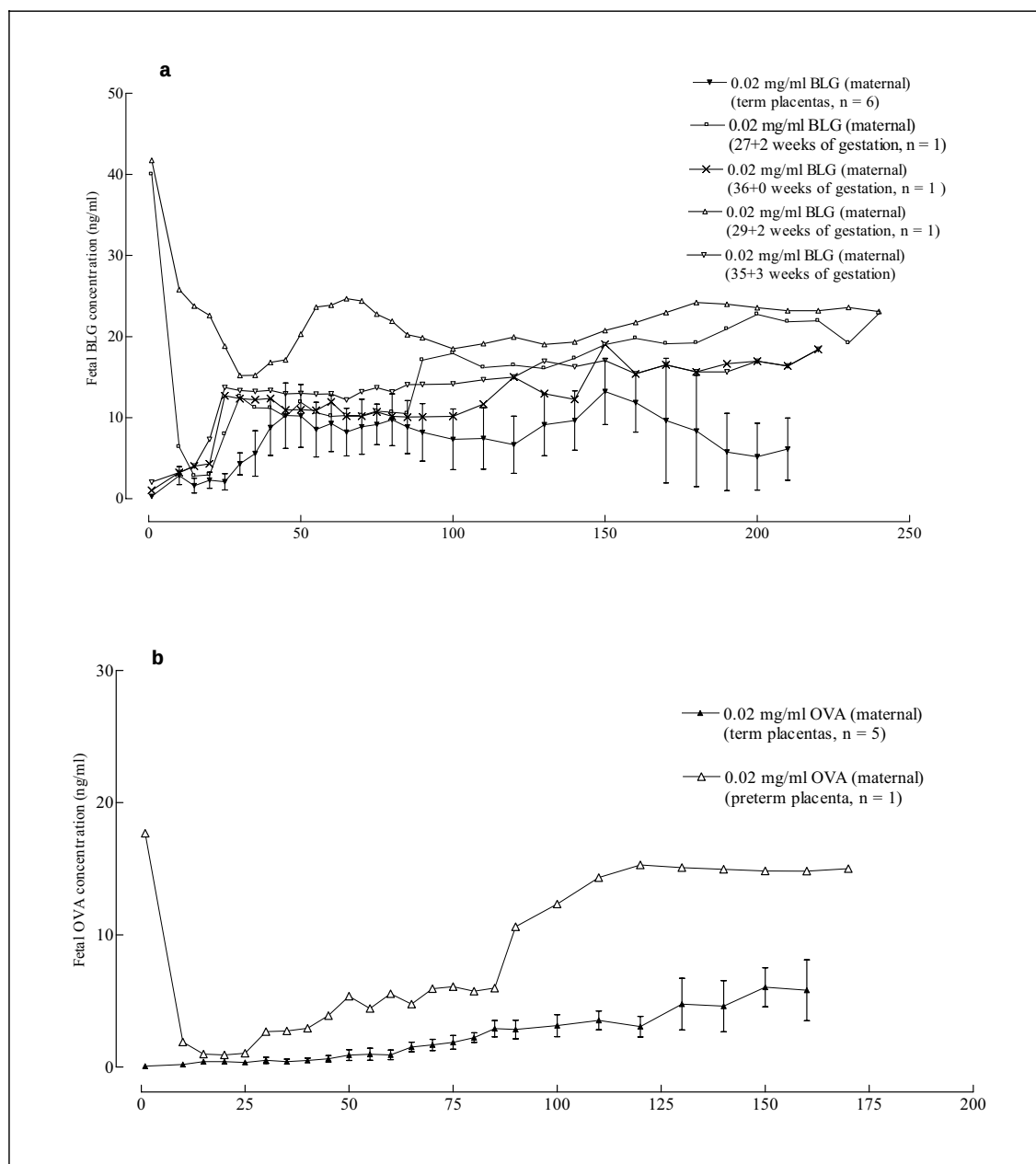
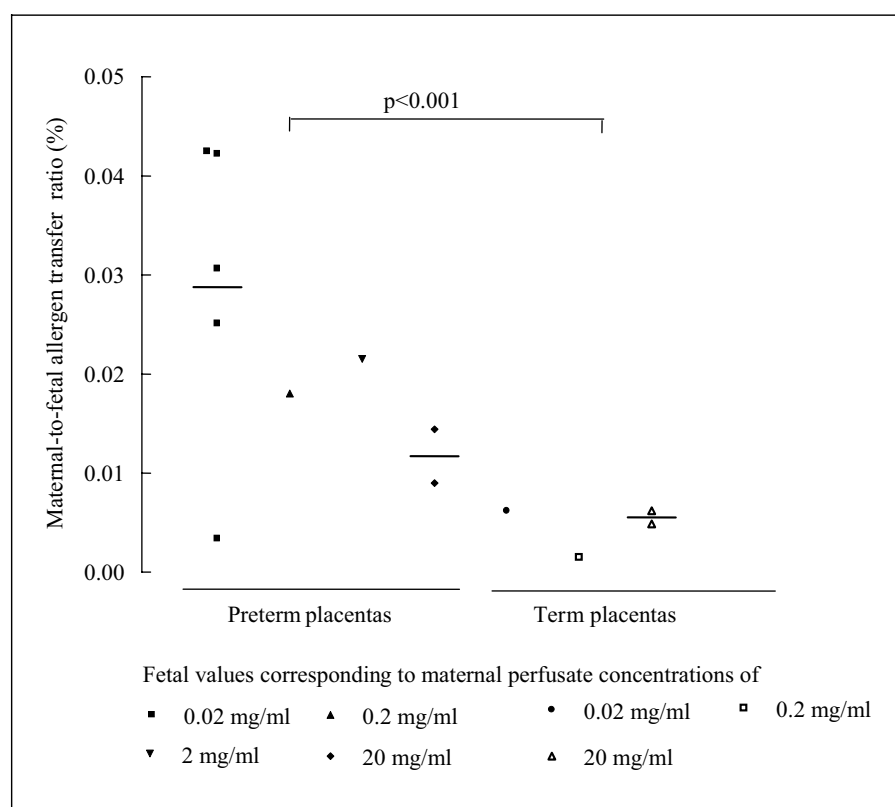


Fig. 2. Differences in allergen transfer according to molecular weight and gestational age of placentas. Fetal BLG (a) and OVA levels (b), detected during dual placenta perfusion with 0.02 mg/ml BLG and 0.02 mg/ml OVA, are shown. Preterm placentas (27–36 weeks of gestation) compared to placentas at term (>36 weeks of gestation) were plotted against time of the perfusion period.

tas with 0.02, 0.2, 2 and 20 mg/ml were 10 ± 2 , 32 ± 3 , 475 ± 23 and $5,774 \pm 486$ ng/ml, respectively. Kinetics of allergen transfer was comparable for all 12 preterm placentas, with an initial rapid increase of the fetal allergen concentration.

Mean fetal BLG transfer values in samples from term placentas were 7 ± 1.6 , 53 ± 5 , 630 ± 53 and $5,569 \pm 478$ ng/ml (fig. 1). In respect to OVA, mean fetal values were 4 ± 1.2 , 21 ± 4 , 260 ± 25 and $4,738 \pm 380$ ng/ml, corresponding to the maternal allergen levels of 0.02, 0.2, 2 and 20 mg/ml, respectively (fig. 1).

Fig. 3. Maternal-to-fetal transfer ratio of allergens in preterm and term placentas. The ratios were calculated, by relating the total allergen concentration that was added to the maternal perfusate (0.02, 0.2, 2 and 20 mg/ml) to the total allergen level that was detected in all fetal perfusate samples during the whole perfusion period. Maternofetal transfer ratios of BLG and OVA in preterm placentas were compared to placentas at term.



Influence of Gestational Age of the Placenta and Molecular Weight of the Allergen on Diaplacental Transfer

In preterm placentas (27–36 weeks of gestation), the maternal-to-fetal transfer of BLG and OVA was found to be consistently higher than in term placentas, suggesting that the mechanisms involved in allergen transfer are less powerful in term compared to preterm placentas. The diaplacental transport of allergens seems to be most effective around the 30th week of gestation and declines with increasing maturity. The transfer rates of the two allergens used, OVA and BLG, differed, indicating that the molecular weight may influence the transport mechanism of these allergens. Lower transfer rates were observed for the allergen with the higher molecular weight (OVA). Figure 2 illustrates the difference of diaplacental allergen transport between preterm and term placentas, and of the transfer of BLG and OVA, showing fetal allergen concentrations, corresponding to the lowest maternal allergen dose used in this study (0.02 mg/ml). Endogenous BLG and OVA could be detected in preterm placentas during the initial perfusion period (fig. 2), reflecting the potential influence of the maternal diet on these antigen levels.

Allergen Concentration in Maternal Samples

During the perfusion, samples were also collected on the maternal outflow corresponding to the draining medium. Allergen concentrations were determined at various time points. A constant allergen concentration was measured throughout the perfusion, in the range of 10 times the fetal levels, indicating that placental tissues may take up most of the perfused allergen.

Maternofetal Ratio of Placental Allergen Transport

In attempting to define the total fetal allergen uptake during the whole perfusion period of 4 h, the amount of allergen, detected in all fetal samples that were collected during perfusion, was correlated with the total allergen amount added to the maternal perfusate (maternofetal transfer ratio). The mean maternofetal transfer ratio in preterm placentas was 3,275:1 for BLG and 4,750:1 for OVA. In term placentas, the mean maternal-to-fetal transfer ratio was 16,200:1 for BLG and 44,100:1 for OVA. To illustrate the significant difference of diaplacental allergen transport between preterm (27–36 weeks of gestation) and term placentas (>36 weeks of gestation), we compared the maternofetal transfer ratio of preterm and term placentas (fig. 3).

Influence of Mean Pressure and Flow Rate on the Allergen Transfer

In order to investigate the nature of the transport mechanism, we attempted to define whether a paracellular transfer is involved in diaplacental allergen transport. The flow rates of placental cotyledons perfused varied between 1.2 and 3.2 ml/min without leading to a change of fetal allergen transport fraction, indicating that the paracellular route may be rather irrelevant for the diaplacental allergen transport.

Discussion

We examined the mechanism of diaplacental allergen transport with particular focus on the effect of the allergen dose, by using the placental perfusion model. There are three key findings in our study: (1) increasing levels of allergen in the maternal circulation lead to a dose-dependent rise of allergen concentration detectable in fetal venous outflow, as shown in all experiments performed; (2) the maternal-to-fetal transfer rate in preterm placentas was found to be greater than in term placentas, and (3) increasing molecular weight of the allergen was associated with reduced diaplacental allergen transfer.

The addition of increasing levels of allergen to the maternal perfusate did not alter the maternofetal transfer ratio. Thus, BLG and OVA may cross the placenta by passive diffusion, as suggested for IgA, human chorionic gonadotropin and human placental lactogen [17]. The asymptotical transfer curves, which reach a maximum far under the maternal allergen levels in every case, suggest that in addition to a possible diffusion a more complex mechanism must drive the diaplacental allergen transfer, such as transcytosis or transport of allergen-IgG immune complexes.

The anatomical correlate for a paracellular route of diffusion in the human placental villus is controversial, since the syncytiotrophoblast is a true syncytium [18–20]. Discontinuities of the syncytiotrophoblast, representing fibrioid deposits, have been discussed as one possible paracellular route for the diffusion of hydrophilic molecules from the maternal to the fetal circulation [19]. The change of maternal mean pressure and flow rate did not alter the maternofetal transfer rate of allergens. According to this, the paracellular pathway may not be relevant for the allergen transfer across the human placenta.

The maternal-to-fetal transfer ratio was found to be approximately 5 and 9 times greater in preterm than in term placentas, for BLG and OVA, respectively. The

mechanism, involved in diaplacental transport of allergens, showed a tendency to be most effective around the 30th week of gestation and to decline with increasing maturity. Thus, preterm placentas favor the diaplacental allergen transport. Assuming that the diaplacental allergen transfer is based on an Na⁺-dependent uptake by human placental microvillous membrane vesicles, this observation is consistent with the notion that due to the smaller electrochemical gradient in term placentas, transport mechanisms are less powerful in term than in preterm placentas [21]. The observation of a difference in diaplacental transfer between BLG and OVA is consistent with the finding of an inverse relationship between placental transport rates and molecular weight of oral hypoglycemic agents [22].

In previous studies BLG and OVA were detected in the serum of healthy adults at levels ranging from 0.1 to >1,000 ng/ml [23, 24]. The highest fetal allergen concentration (10,430 ng/ml), obtained during the perfusion of preterm placentas with 20 mg/ml allergen, is similar to fetal concentrations found for endogenous IgG during placental perfusion [25]. Maternofetal transfer of IgG across the placenta provides passive immunity for the fetus and neonate. The level of maternal allergen-specific IgG, however, does not significantly influence the cellular immune responses to specific allergens in late infancy [26]. Binding of maternal IgG to the MHC class I-related neonatal Fc receptor expressed in human syncytiotrophoblast has been shown to be a prerequisite for the diaplacental transport of maternal IgG [27]. Neonatal Fc receptor has been observed to transport also antigen-antibody immune complexes across the intestine of suckling rats and rodents [28], and has been predicted to transport IgG across human intestinal epithelium [29]. Therefore, it is also conceivable that the mechanism mediating the transport of free allergens as well as IgG/allergen immune complexes across the human placenta is transcytosis.

The implication of these findings for the developing fetal immune system and their contribution to the induction of tolerance versus sensitization remain to be determined.

Regarding the studies showing an initiation of antigen-specific responses in utero [1–4], fetal life may be critical in influencing the subsequent development of atopic disease. The capacity of fetal T cells to proliferate in response to dietary antigens and the results of this study indicate that antigenic stimulation occurs in the fetal circulation through the diaplacental passage of dietary antigens.

In animals, exposure to dietary antigen during pregnancy has been observed to be associated with the devel-

opment of profound oral tolerance [30], supported by experiments in rats confirming that macromolecules, derived from dietary protein, cross the placenta [31]. In addition, antigen exposure in neonatal life has been suggested to result in clonal inactivation, deletion or suppressor cell induction, as well as immune deviation from Th1 to Th2 [32]. However, tolerance is not an intrinsic property of the neonatal immune system. The type of antigen-presenting cell and the antigen dose determine tolerance or immunization upon antigenic challenge in the neonate [33].

Adaptive immune responses are largely dependent on the induction of effector CD4⁺ T cell function during the initial exposure to antigen [10]. Dominance of one or the other effector cell type has largely been attributed to cytokines present in the priming environment. However, the priming antigen dose is another critical parameter in determining the differential activation of naive CD4⁺ T cells, encountering antigen for the first time [10, 11]. Midrange peptide doses direct the development of Th0/Th1-like cells, while very low and very high doses of antigenic peptide favor the development of Th2-like cells

[11]. These observations support the hypothesis that different doses of nutritive allergens, transported across the human placenta, may exert different effects on the fetal immune system.

Here, we demonstrate the capacity of human placentas to upregulate the maternal-to-fetal allergen transport for up to 1,000-fold in a dose-dependent and molecular weight-dependent manner with clear accentuation in pre-term placentas. Pinocytotic transcytosis of the allergen and Fc receptor-mediated transcytosis for IgG/allergen immune complexes may be involved in the diaplacental allergen transport. How any of these factors affect the fetal immune response to allergens is likewise uncertain and remains to be elucidated.

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