

Mother to Child Transfer of IgG and IgA Antibodies Against *Dermatophagoides pteronyssinus*

P. Macchiaverni*, C. Arslanian*, J. B. Frazão*, P. Palmeira†, M. Russo*, V. Verhasselt‡¹ & A. Condino-Neto*¹

*Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, SP, Brazil; †Laboratory of Medical Investigation (LIM-36), Department of Pediatrics, Hospital das Clínicas, University of São Paulo Medical School, São Paulo, SP, Brazil; and ‡Institut National de la Santé et de la Recherche Médicale (INSERM), U924 Université de Nice-Sophia Antipolis, Valbonne, France

Received 6 June 2011; Accepted in revised form 16 August 2011

Correspondence to: A. Condino-Neto, MD, PhD, Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, 1730, Lineu Prestes Avenue, São Paulo, SP 05508-000, Brazil. E-mail: condino@icb.usp.br

¹These authors contributed equally to this work.

Abstract

There is strong evidence from animal models that placental and/or breast milk-mediated transfer of maternal allergen-specific IgG prevents allergic immune responses in the progeny. Both human and animal data also point to IgA as having an important regulatory role. In contrast, little is known about maternal transfer of IgG and IgA specific for respiratory allergens in humans. *Dermatophagoides pteronyssinus* (*Der p*) is an indoor allergen that is a major cause of asthma worldwide. We analysed maternal to child *Der p*-specific IgG and IgA transfer in a cohort of 77 paired maternal and child samples. We found *Der p*-specific IgG and its IgG1, IgG2 and IgG4 subclasses in all cord blood samples. Except for IgG1, cord levels were higher in newborns from atopic mothers ($n = 29$) compared to non-atopic mothers ($n = 48$). *Der p*-specific IgA was found in all colostrum samples and levels were independent of maternal atopic status. Notably, anti-*Der p* IgG was also found in colostrum and levels were higher in atopic mothers. We believe that our work is a critical first step in the identification of early factors that may impact asthma development and should guide the development of clinical studies that assess whether *Der p*-specific IgG and IgA protect children from allergy as demonstrated in animal models.

Introduction

Atopic asthma affects millions of children worldwide [1]. Pathogenesis of allergic disease results from complex interactions between genetic and environmental factors such as pollution, tobacco and microbial exposure including microbiota of the gastrointestinal tract. In most cases, symptoms of allergic asthma manifest in childhood, and the immunological changes leading to atopy can occur very early in life and even during gestation [2]. Thus, identifying early factors that predispose to asthma development may help to improve primary prevention.

During pregnancy, mothers transfer to the foetus immunoglobulins (Ig) that recognize antigens to which she has been exposed [3]. IgG is the main Ig isotype transferred across the placental barrier [3–5], and its subclasses are ordered according to their relative serum levels: IgG1 > IgG2 > IgG3 > IgG4. IgG1 and IgG3 are generally induced in response to protein antigens,

IgG2 is associated with polysaccharide antigens, and IgG4 class switching is stimulated by TH2-type cytokines [6].

After delivery, Ig can be transferred by breastfeeding as it is the most abundant Ig found in human milk [7]. Most studies in humans have focused on placental transfer of IgG or milk transfer of IgA molecules specific for microbial antigens and have demonstrated their role in infectious disease prevention [7, 8]. There is also some evidence from animal models that transferred maternal Ig could exert a regulatory role in their progeny. Experimental data in rodents indicate that maternal allergen-specific IgG transferred by placenta and/or breastfeeding prevents allergic sensitization in the progeny [2, 9–16], and animal and human studies indicate that IgA can exert an immunoregulatory role [17–20]. In humans, only a few studies have demonstrated the presence of IgG [21, 22] or IgA [23–26] specific for food and respiratory antigens in cord blood or breast

milk, respectively. To date, no study has demonstrated the transfer of IgG specific for respiratory allergens by breast milk.

In this study, we investigated whether mothers can provide to their children antibodies specific for *Dermatophagoides pteronyssinus* (Der p), a major allergen in house dust and one of the most frequently implicated respiratory allergens in allergic asthma [27–30]. In particular, we assessed whether anti-Der p antibodies were detected in cord blood and/or colostrum and whether maternal atopic status had any influence on the amount of antibody.

Methods

Study design. A total of 77 healthy mothers and their newborns were selected at *Maternidade de Campinas* Hospital in Campinas, São Paulo, Brazil, between February and July 2006. The selection criteria included mothers giving birth to healthy, full-term and adequate-for-gestational-age-weight infants. Demographic data and details about the antenatal care of the mothers were obtained from their medical records and a directed questionnaire. The information included maternal age, parity, medications during pregnancy and atopic status (e.g. atopic rhinitis or asthma) established by a typical clinical history. Total and Der p-specific IgE were assayed in blood samples from all mothers. Inclusion criteria for atopic mothers were clinical manifestations of rhinitis, asthma or atopic dermatitis and anti-Der p IgE concentration ≥ 3.5 KU/l ($n = 29$). A group of non-atopic healthy mothers (anti-Der p IgE concentration ≤ 0.3 KU/l and absence of atopic symptoms) was included in the study as a control group ($n = 48$). Exclusion criteria for enrolment of all mothers were hypertension, diabetes, infections, immunodeficiency, and those who had received corticosteroids, transfusion of blood-derived products or other drugs related to chronic diseases during pregnancy. The study was approved by the University of São Paulo Institute of Biomedical Sciences Ethics Committee in accordance with the Brazilian Ministry of Health Resolution 96/1996 and the Helsinki Declaration.

Serum and colostrum samples. After mothers signed the informed consent, cord blood was collected from large veins on the foetal side of the placenta immediately after delivery. Maternal peripheral venous blood and colostrum samples were collected within 48 h after delivery. Approximately 5 ml of colostrum was collected manually and, on the same day, centrifuged for 30 min at 160 g at 4 °C. The top layer of fat and the pellet were discarded, and the intermediate fluid fraction was aliquoted and stored at –80 °C until analysed. Serum was separated from maternal and cord blood and stored at –80 °C until assayed.

Total and Der p-specific IgE quantification. Total and anti-Der p IgE antibodies from maternal serum samples

were analysed by chemiluminescent immunoassay (ADVIA Centaur® and Cap System Pharmacia®, respectively), according to manufacturer's recommendations [31].

In the Cap System Pharmacia® assay, the specific IgE concentration is expressed in KU/l; values ≥ 3.5 KU/l were considered positive for specific IgE. In the ADVIA Centaur® assay, total IgE concentration is expressed in IU/ml, with a detection level of 1.5 IU/ml.

Total IgA quantification. Total IgA was measured in colostrum samples by enzyme-linked immunosorbent assays (ELISA), as described [32] with modifications. Briefly, colostrum samples were diluted 1:10,000 in duplicate and incubated for 2 h in anti-human IgA (I-0884; Sigma, St. Louis, MO, USA) coated plates. As a standard, we used IgA purified from human colostrums (I-2636; Sigma), and as secondary antibody, peroxidase-conjugated anti-human IgA (A0295; Sigma) diluted 1:6000 (1 h 30 min) was used. *Ortho*-phenylenediamine (OPD) was used as the chromogenic substrate, and IgA concentration was expressed as mg/ml.

Anti-Der p IgG and IgA quantification. Microplates (Costar, Cambridge, MA, USA) were coated overnight at 4 °C with 5 µg/ml of Der p extract from IPI-ASAAC, São Paulo, BR, or with Der p extract from Greer Laboratories, Lenoir, NC, in phosphate-buffered saline (PBS). Both Der p preparations gave similar results. Plates were then saturated with 5% non-fat dry milk in PBS–Tween 0.1% for 1 h at room temperature. Samples and secondary antibodies were added as described below and bound antibodies were revealed by the addition of a solution containing 0.4 mg/ml OPD and 0.01% H₂O₂ in 0.1 M phosphate–citrate buffer (pH 5.0). After 30 min of incubation, the reaction was stopped with 50 µl of 2.5 N H₂SO₄. Plates were washed with PBS–Tween 0.1% between each step. Optical absorbance at 492 nm was measured by a microplate reader (Labsystems Multiskan MS, Farnborough, Hampshire, UK).

For Ig detection, sample dilution and secondary antibodies were prepared as follows.

Serum anti-Der p IgG: Maternal and cord serum were added in duplicate at a dilution of 1:100 followed by two-fold serial dilutions and incubated at 37 °C for 2 h. HRP-conjugated anti-human IgG (A8419; Sigma) at a dilution of 1:400 was used as secondary antibody and incubated at 37 °C for 2 h. Results were expressed as arbitrary units (AU)/ml obtained by comparison of optical density (OD) values of a serum pool (collected from 24 mothers with anti-Der p IgE concentration ≥ 17.5 KU/l) and defined to contain 1000 AU/ml of anti-Der p IgG.

Serum anti-Der p IgG subclasses: Paired maternal and cord serum samples were added in duplicate at dilutions of 1:5 (IgG1), 1:2 (IgG2) and 1:2 (IgG4), followed by twofold serial dilutions, and incubated for 1.5 h on Der p-coated plates. As secondary antibody, biotinylated anti-human IgG1 (555869; BD Pharmingen, San Diego, CA, USA),

IgG2 (555874; BD Pharmingen) and IgG4 (555882; BD Pharmingen) were used at dilutions of 1:500, 1:1000 and 1:100, respectively, and incubated for 1.5 h. This step was followed by incubation with streptavidin-HRP (554066; BD Pharmingen) diluted 1:500, 1:1000 and 1:500, respectively, for 1.5 h. Concentrations were expressed as arbitrary units (AU/ml) as described previously.

Colostrum anti-Der p IgA: Colostrum samples in duplicate were diluted 1:100 followed by two steps of twofold serial dilutions and incubated at 37 °C for 2 h on purified Der p-coated plates. As secondary antibody, we used peroxidase-conjugated anti-human IgA (A0295; Sigma) diluted 1:6000 and incubated 1.5 h at 37 °C. The results were expressed as arbitrary units (AU/ml) obtained by comparison with a colostrum pool (collected from 24 mothers with anti-Der p IgE concentration ≥ 17.5 KU/l) and defined to contain 1000 AU/ml of colostrum anti-Der p IgA.

Colostrum anti-Der p IgG: Colostrum anti-Der p IgG quantification was performed as described for colostrum anti-Der p IgA with some modifications: colostrum samples were diluted 1:2 and incubated at 37 °C for 2 h on purified Der p-coated plates. As secondary antibody, we used anti-human biotinylated IgG (555785; BD Pharmingen) followed by streptavidin-HRP (554066; BD Pharmingen), both diluted 1:500 and incubated for 1.5 h at 37 °C. OPD was used as the chromogenic substrate, and concentrations were expressed as arbitrary units (AU/ml) obtained by comparison with a colostrum pool as described previously.

Statistical analyses. Statistical analyses were performed using GRAPHPAD PRISM version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). Dots represent individual data points, and horizontal lines, the medians of each group. Mann–Whitney test was used to determine statistical differences because the D'Agostino–Pearson normality test was not passed. Kruskal–Wallis test was performed to compare more than two groups. When significant differences were found, a Mann–Whitney test was performed to determine which groups differed. Correlation coefficients of antibody levels in maternal serum versus colostrum or cord blood were determined using Spearman's tests. Two-tailed *P*-values < 0.05 were considered statistically significant and graphically represented as **P* < 0.05 ; ***P* < 0.01 ; ****P* < 0.001 ; *****P* < 0.0001 .

Results

Characteristics of the study population

The study population was divided into two groups according to maternal atopic status defined by the presence of anti-Der p IgE ≥ 3.5 KU/l and associated allergic symptoms. The characteristics of the two groups are sum-

marized in Table 1. Total and anti-Der p IgE concentrations in blood samples from atopic mothers were significantly higher than non-atopic mothers (Table 1). Maternal age, infant weight and height, and male-to-female ratios were similar between the two groups (Table 1).

Der p-specific IgG in cord blood and maternal serum

Anti-Der p IgG was detected in cord blood of all neonates. Anti-Der p IgG concentrations were significantly higher in cord blood of neonates from atopic mothers compared to neonates from non-atopic mothers (Fig. 1A and Table 2). In addition, neonatal anti-Der p IgG correlated with anti-Der p IgE levels in maternal blood (data not shown; Spearman *r* = 0.2, *P* = 0.006). Similarly to their children, atopic mothers showed higher concentration of anti-Der p IgG compared to non-atopic mothers (Fig. 1A and Table 2), and Der p-specific IgG in maternal blood correlated with anti-Der p IgE levels (Spearman *r* = 0.2, *P* = 0.009). Anti-Der p IgG levels in cord blood correlated strongly with the maternal concentration for both atopic and non-atopic groups (Fig. 1B). The ratio of cord blood to maternal blood anti-Der p IgG levels was not affected by maternal antibody concentration (Fig. 1C).

Der p-specific IgG1, IgG2 and IgG4 in cord blood and maternal serum

Anti-Der p IgG2 and IgG4 concentrations were significantly higher in cord blood of neonates of atopic mothers compared to non-atopic mothers (Fig. 2B,C and Table 2),

Table 1 Characteristics of the study population.

	Atopic (<i>n</i> = 28)	Non-atopic (<i>n</i> = 48)
Mother		
Total IgE (UI/ml)	263.2 (17.3–3910)****	16.45 (1.5–1543)
Der p-specific IgE (KU/ml)	20.7 (3.4–100)****	<0.35 (<0.35–<0.35)
Age (years)	26.5 (16–40)	26.5 (15–37)
Gestation, % (number)		
1	55	35
2	31	38
3	14	17
≥ 4	0	10
Newborn		
Weight (kg)	3.2 (2.3–4.2)	3.3 (2.4–4.5)
Height (cm)	48.6 (45–53)	49.1 (44.5–52.8)
Sex (%)		
Male	52	42
Female	48	58

Data represent median values and range (shown in brackets). Results obtained in atopic and non-atopic groups were compared, and statistical difference was calculated by Mann–Whitney test.

****Statistically significant values.

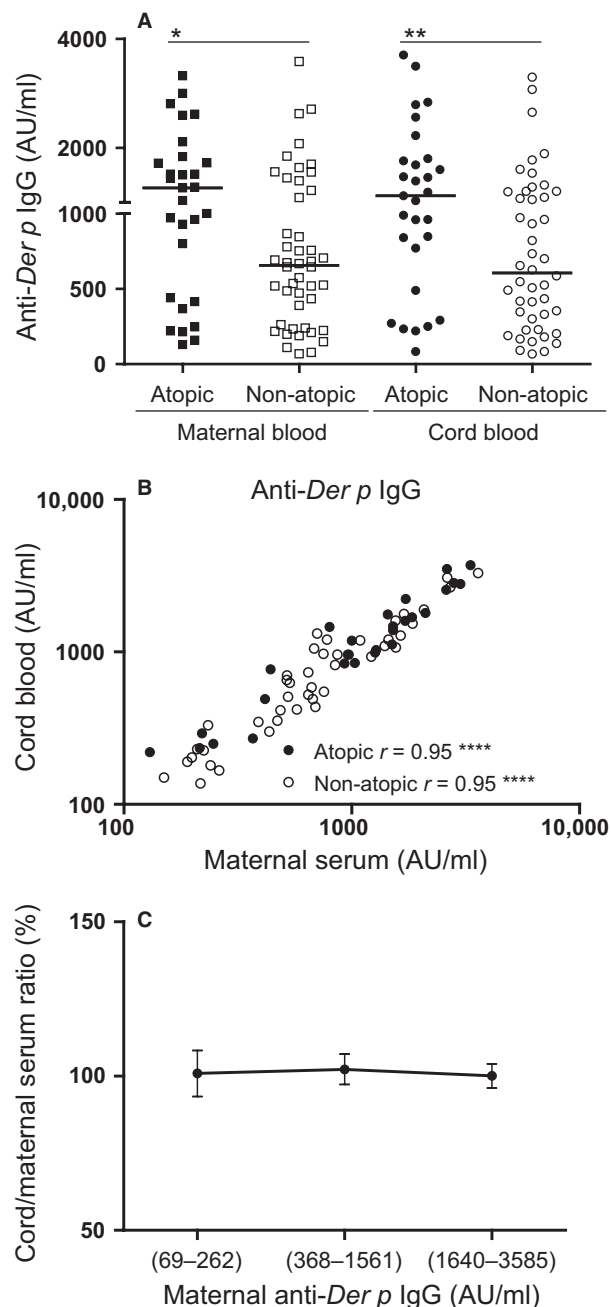


Figure 1 *Der p*-specific IgG concentration in maternal and cord blood. *Der p*-specific IgG levels were determined in paired maternal and cord blood samples. Data are compared according to maternal atopic status by Mann–Whitney test (A). *Der p*-specific IgG concentrations obtained in cord blood are correlated with concentrations in respective maternal blood by Spearman test (B). Cord/maternal serum ratios of *Der p*-specific IgG obtained at low, intermediate and high levels of respective maternal anti-*Der p* IgG levels (C).

while the anti-*Der p* IgG1 concentration was equivalent in both groups (Fig. 2A and Table 2). Further, cord blood anti-*Der p* IgG2 and IgG4, but not IgG1, correlated with maternal anti-*Der p* IgE concentrations (Spearman $r = 0.2$, $P = 0.03$ and $r = 0.5$, $P < 0.0001$ for

IgG2 and IgG4, respectively). As observed in the neonates, maternal blood IgG2 and IgG4 levels were higher in the serum of atopic mothers compared to non-atopic, while IgG1 levels were similar in both groups (Fig. 2A–C), and *Der p*-specific IgG subclasses in maternal blood correlated with anti-*Der p* IgE levels with the exception of IgG1 (data not shown; Spearman $r = 0.2$, $P = 0.03$ and $r = 0.5$, $P < 0.0001$ for IgG2 and IgG4, respectively).

Cord blood anti-*Der p* IgG1, IgG2 and IgG4 correlated strongly with respective maternal levels in both groups (Fig. 2D–F), and the ratio of cord blood to maternal blood antibody levels decreased at high maternal antibody concentration (Fig. 2G–I). We also found that the ratio of cord blood to maternal serum anti-*Der p* IgG1 was higher than for the other IgG subclasses in both groups (Table 2).

Der p-specific IgA and IgG in colostrum

Total and anti-*Der p* IgA were detected in all colostrum samples without significant differences between atopic and non-atopic mothers (Fig. 3A). For both groups, a positive correlation was found between total and anti-*Der p* IgA concentrations in colostrum (Fig. 3B). We did not find any correlation between anti-*Der p* IgA levels in colostrum and anti-*Der p* IgG or IgE levels in maternal blood, as expected, because most of the IgA found in colostrum is produced peripherally, in mammary tissue.

In addition to anti-*Der p* IgA, we found anti-*Der p* IgG in all colostrum samples (Fig. 4 and Table 2). Anti-*Der p* IgG concentrations in colostrum were higher in atopic mothers (Fig. 4A) and correlated with maternal anti-*Der p* IgE concentrations (Spearman $r = 0.3$; $P = 0.002$). Colostrum anti-*Der p* IgG concentrations correlated with maternal blood anti-*Der p* IgG in the non-atopic group but not in the atopic group (Fig. 4B).

Discussion

This study demonstrates the presence of *Der p*-specific IgG in all cord blood samples as well as *Der p*-specific IgA and IgG in all colostrum samples. Others have previously shown the presence of IgG antibodies specific for respiratory antigens from birch pollen (*Bet v* 1), cat (*Fel d* 1) and *Dermatophagoides farinae* (*Der f* 1) in cord blood samples [22, 33, 34]. In those studies, not all samples were positive, which probably reflects differences in immunogenicity of the allergen tested and in the maternal exposure to the allergens. In this case, *Der p* is an indoor allergen that is widely distributed in the humid regions of the world [27–30].

The analysis of IgG subclass concentrations in maternal and cord blood demonstrates that cord blood concentrations of anti-*Der p* IgG, IgG1, IgG2 and IgG4

Table 2 *Der p*-specific IgG levels in maternal serum and colostrum and neonate umbilical cord serum.

	Maternal serum	Umbilical cord serum	Colostrum	Cord/maternal serum ratio (%)
Atopic (<i>n</i> = 29)				
Anti-Der p IgG	1266 (130–3321)	1121 (84–3704)	352 (17.8–10807)	98.72 (52.5–182.1)
Anti-Der p IgG1	133.5 (10.9–1205)	213.1 (36.2–1902)	–	177.7 (78.1–462.9)

Anti-Der p IgG2	96 (4.8–1344)	65.8 (1.5–1162)	–	89.9 (31.2–189.1)

Anti-Der p IgG4	44.6 (0.5–5357)	29.4 (0.0–4498)	–	88.7 (0.0–222)
Non-atopic (<i>n</i> = 48)				
Anti-Der p IgG	656.4 (69–3585)	606.1 (67–3296)	115.6 (3.5–1061.2)	94.35 (62.86–187)
Anti-Der p IgG1	96.2 (0–405)	144.7 (17.6–1366)	–	170.5 (66.5–1398)

Anti-Der p IgG2	37.6 (0.8–1219)	33 (0.6–1393)	–	82.1 (17.39–8441)

Anti-Der p IgG4	4.9 (0.0–42.3)	3.1 (0.0–48.5)	–	87.8 (5.4–1317)

–, not performed.

Anti-Der p IgG levels are expressed in AU/ml. Data represent median values and ranges (shown in bracket). Cord/maternal serum ratios represent the median of the ratio calculated for each pair of cord/maternal samples. For both atopic and non-atopic group, we compared IgG1, IgG2 and IgG4 cord/maternal serum ratio.

***Statistically significant values.

correlated strongly with respective maternal values. We also found that both maternal serum and cord blood anti-Der p IgG, IgG2 and IgG4 correlated with maternal IgE levels, and we found higher levels of IgG, IgG2 and IgG4 in cord blood of neonates from atopic mothers as compared to non-atopic mothers. Such correlation was not found for anti-Der p IgG1, and concentrations of IgG1 were equivalent in both groups. In addition, as previously described by others [33], we detected anti-Der p IgG and subclasses in maternal serum and cord blood in the absence of maternal *Der p*-specific IgE. In addition to the presence or absence of atopy, differences in maternal exposure to *Der p* could also be responsible for differences in IgG levels in maternal blood, colostrum and cord blood. Although we did not measure *Der p* levels in subjects' homes, we did not favour this hypothesis because all subjects live in a region where *Der p* is found uniformly in very high concentration [35].

The source of the *Der p*-specific IgG found in cord blood might be of foetal origin as a result of *in utero* sensitization or might be of maternal origin as a result of maternal transfer across the placenta. Many studies have reported that allergen-specific IgE detected in cord blood is synthesized *in utero* and can be a marker of risk of atopic disease development in children [36–38]. However, this concept was recently challenged by Bonnelykke *et al.* [4, 5]. Comparison of allergen-specific IgE in maternal and cord blood indicated that specific IgE in cord blood completely matched specific IgE in maternal blood with respect to allergen specificity, level of specific IgE and ratio of total IgE to specific IgE. In addition, correspond-

ing specific IgE was not found in infant blood at 6 months of age. These observations let the authors conclude that the presence of cord blood IgE was, in the majority of cases, a result of maternal transfer. Our results showed a strong correlation between cord blood anti-Der p IgG, IgG1, IgG2 and IgG4 and respective maternal levels. Although we do not have data on IgG levels in children at 6 months of age, our data suggest a maternal transfer of anti-Der p IgG subclasses across placenta.

In addition, the decreased ratio of cord blood to maternal levels of these antibodies at high maternal concentrations suggests a saturable receptor-mediated transfer. Notably, the syncytiotrophoblast expresses a neonatal Fc receptor (FcRn) that is essential for IgG transfer [3, 39] and is saturable [40]. This receptor has a higher affinity for IgG1, compared to other IgG subclasses, which may explain the more efficient *in utero* transfer of *Der p*-specific IgG1, compared to other subclasses [3], as also shown here.

Several studies in rodents have reported that maternal allergen-specific IgG inhibits allergic responses in the offspring [2, 9–16]. Proposed mechanisms of protection by maternal IgG include the following: (1) IgG binding to allergen, leading to allergenic determinant masking and clearance of the immune complexes by phagocytosis, (2) IgG blockade of IgE binding to allergen and hence inhibition of mast cell degranulation and (3) interactions with inhibitory receptor FcγRIIb on neonatal B lymphocytes or dendritic cells [2, 41]. More recently, protection from allergic airway disease by antigen transfer through breast milk was shown to be more stronger and of longer

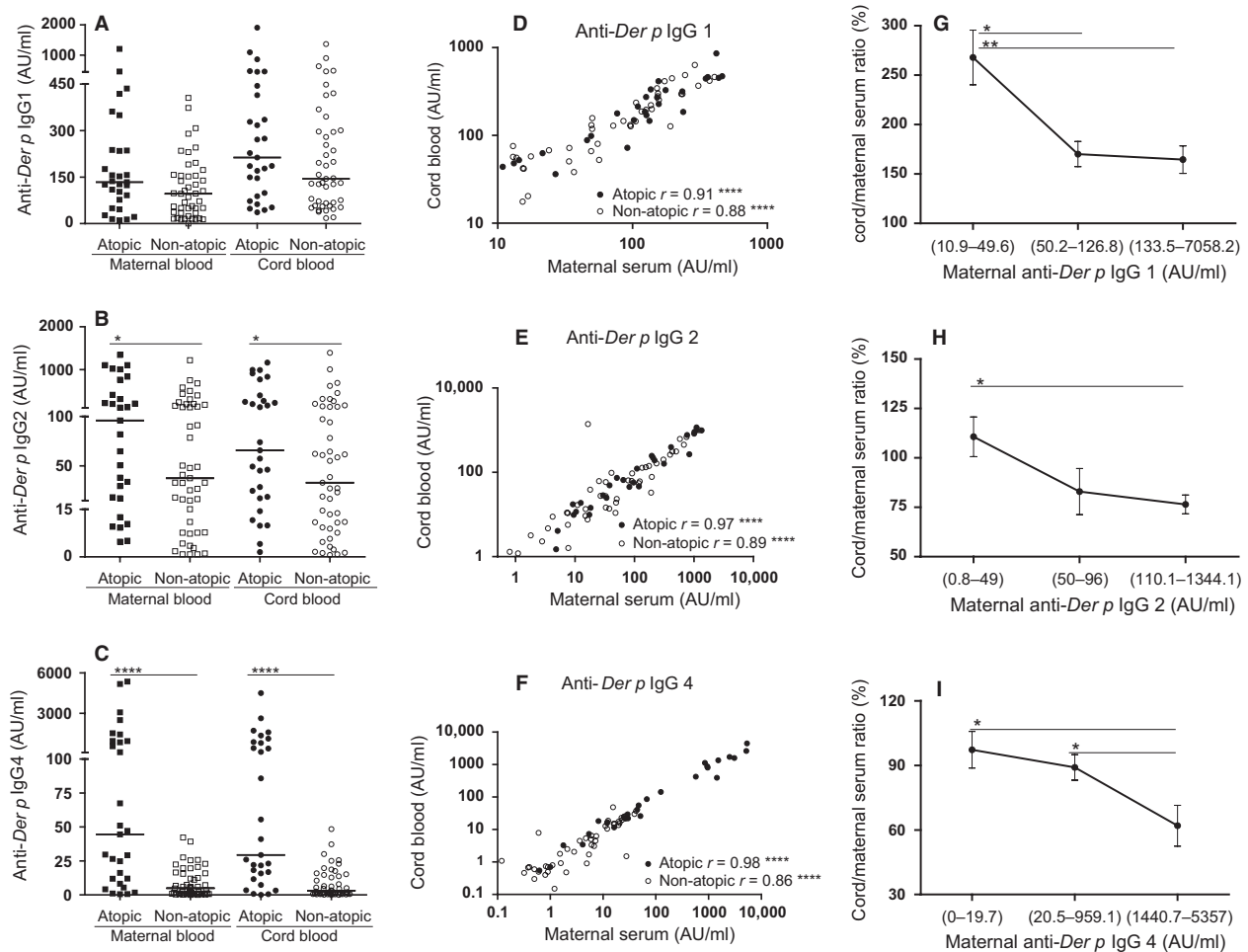


Figure 2 Der p-specific IgG1, IgG2 and IgG4 concentrations in maternal and cord blood. Specific IgG1, IgG2 and IgG4 levels were determined in paired maternal and cord blood samples. Data are compared according to maternal atopic status by Mann–Whitney test (A–C). Der p-specific IgG1, IgG2 and IgG4 concentrations obtained in cord blood are correlated with concentrations in respective maternal blood by Spearman test (D–F). Cord/maternal serum ratios of Der p-specific IgG1, IgG2 and IgG4 obtained at low, intermediate and high levels of respective maternal anti-Der p IgG subclasses (G–I).

duration when maternal allergen-specific IgG is present in breast milk. The authors attributed the increased protection to the formation of allergen–IgG immune complexes that are easily transferred across the neonatal gut barrier compared to uncomplexed antigen and display tolerogenic properties [42].

Human studies also suggest an immunoregulatory role for *in utero* transfer of maternal IgG. A study by Glovsky *et al.* [43] analysed the effect of specific immunotherapy during pregnancy on allergic sensitization in children. Their data suggested that blocking antibodies induced by immunotherapy were transferred across the placenta and were responsible for decreased allergic sensitization in their children. Jenmalm and Björkstén [21] found that high concentration of IgG directed to inhaled allergens in cord blood was associated with reduced atopy in children. Another study showed a transient protective effect of placental transfer of maternal antibodies on allergic

immune response [22]. The current study demonstrated a higher concentration of specific IgG4 and, to a lesser extent, of IgG2 in cord blood of neonates from atopic mothers compared to non-atopic mothers. Although we cannot conclude that these IgG subclasses exert an immunoregulatory role, a protective effect has previously been reported for IgG4 [44–46].

After delivery, breastfeeding maintains a strong interaction between the mother and her infant and in particular allows the continued transfer of maternal Ig. Secretory IgA is the predominant class of Ig found in human breast milk. This class of non-inflammatory Ig inhibits microbial colonization through decreased adherence of bacteria and viruses to mucosal surfaces and thereby protects against gut and respiratory infections in breastfed children [7]. IgA can also trap food antigens, leading to immune exclusion of dietary antigens by favouring degradation by pancreatic enzymes [47]. In addition to

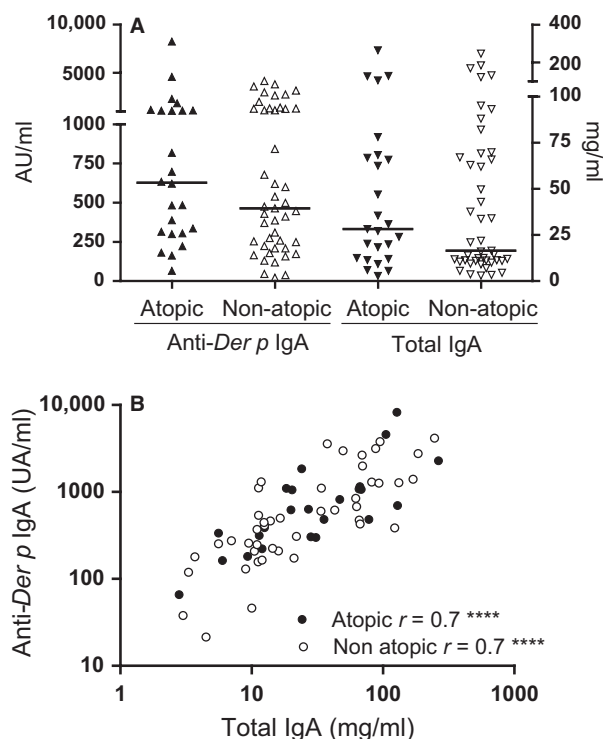


Figure 3 Total and *Der p*-specific IgA concentration in colostrum of atopic and non-atopic mothers. Total and *Der p*-specific IgA levels were determined in colostrum samples. Data are compared according to maternal atopic status by Mann–Whitney test. Total IgA concentration is expressed in mg/ml (right Y-axis), and anti-*Der p* IgA concentration is expressed in AU/ml (left Y-axis) (A). *Der p*-specific IgA concentrations are correlated with total IgA concentrations by Spearman test (B).

immune exclusion, IgA can exert immunoregulatory effects [17–20]. The epidemiological evidence of food allergy prevention by IgA [48–51] might be explained by these two mechanisms. As the majority of inhaled antigens reach the gut [52], the presence of milk-borne *Der p*-specific IgA may then protect the newborn from respiratory allergens as proposed for food allergens. Notably, we found anti-*Der p* IgA in all colostrum samples tested. The range of values was broad, and we did not observe significant differences in antibody concentrations between atopic and non-atopic mothers. One previous study assessed the presence of IgA to cat allergen in human breast milk from atopic and non-atopic mothers. This study also found a similar concentration of IgA in both groups [26]. The absence of an effect of atopy on IgA levels could be explained by the fact that IgA class switching depends mainly on the presence of TGF- β [53]. In fact, we found similar levels of TGF- β in colostrum of atopic and non-atopic mothers, and we observed that both total IgA and *Der p*-specific IgA levels correlated with TGF- β levels in colostrum (Figure S1).

In addition to IgA specific for respiratory allergen, our study demonstrated, for the first time, the presence of *Der p*-specific IgG in colostrum. *Der p*-specific IgG con-

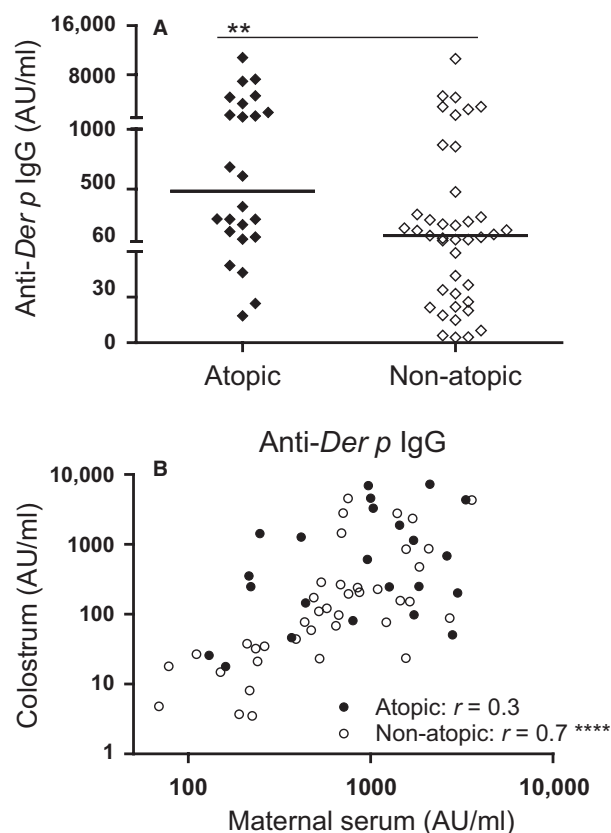


Figure 4 *Der p*-specific IgG concentration in colostrum. *Der p*-specific IgG levels were determined in colostrum samples. Data are compared according to maternal atopic status by Mann–Whitney test (A). Concentrations obtained in colostrum are correlated with concentrations in respective maternal serum by Spearman test (B).

centrations were higher in colostrum from atopic mothers compared to non-atopic mothers, and colostrum levels correlated with maternal IgE serum levels. It is worth noting that colostrum *Der p*-specific IgG concentration correlated with maternal serum IgG levels in the non-atopic but not in the atopic group. IgG in colostrum could come from maternal serum, as supported by the observation that intravenous administration of Ig to immunodeficient mothers results in the presence of Ig in breast milk [54]. In addition, IgG may be synthesized locally in the mammary gland. The latter mechanism may operate in the atopic group because there was no correlation between maternal serum and colostrum *Der p*-specific IgG levels in that group. Studies in rodents suggest that, as in the placenta, FcRn can be involved in IgG transfer across mammary gland epithelium [55]. Notably, in contrast to IgA that stays in the gut lumen, anti-*Der p* IgG can then be transferred to the neonate by FcRn expressed in the human proximal intestine [39, 56]. This continuous supply of IgG after delivery may be sufficient to maintain systemic levels in the neonate and allow protection from asthma when *in utero*-transferred

antibodies have been degraded. In support of this hypothesis, a meta-analysis of prospective studies and a multidisciplinary review of studies performed between 1966 and 2000 concluded that breastfeeding protection from asthma was higher in the subgroup of children with a positive family history of asthma or atopy compared with children with no parental history of atopy [57, 58]. In the light of experimental data obtained in animal models, our work suggests that the higher concentration of Der p-specific IgG in colostrum from atopic mothers may contribute to the better protection afforded upon breastfeeding by atopic mothers.

Conclusions

Our study indicates that Der p-specific IgG can be found in both cord blood and colostrum and identifies maternal atopy as a critical factor for increased levels of allergen-specific IgG in these compartments. In addition, Der p-specific IgA is present in colostrum. Clinical studies will be necessary to assess whether Der p-specific IgG and IgA protect the child from allergy as demonstrated in animal studies. In view of the increasing evidence from animal models and importance of neonatal prevention of allergy, this study would be a timely and necessary way to elucidate the role of allergen-specific Ig in early life and its effect on allergy development.

Acknowledgment

The authors thank Maternidade de Campinas Hospital, Prof. Maria Notomi Sato (Laboratory of Clinical and Experimental Allergy and Immunology, School of Medicine, University of São Paulo) for supplying us with anti-human IgG antibodies, Dr José Carlos Mori (IPI-ASAC, Brasil) for Der p purified extract, nurse Silvana S. Dalgé for her excellent assistance in the colostrum collection, Dr Meri Tulic and Dr Peter Newburger for critical reading of the manuscript, as well as the mothers who kindly agreed to participate in this study. We also acknowledge the State of São Paulo Research Foundation (FAPESP) for financial support: Grant 08/58825-7 to Antonio Condino-Neto, Grants 05/57593-7 and 08/51535-3 to Patricia Macchiaverni.

References

- Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 2004;59:469–78.
- Boyle RJ, Tang ML. Can allergic diseases be prevented prenatally? *Allergy* 2006;61:1423–31.
- Simister NE. Placental transport of immunoglobulin G. *Vaccine* 2003;21:3365–9.
- Bonnelykke K, Pipper CB, Bisgaard H. Sensitization does not develop in utero. *J Allergy Clin Immunol* 2008;121:646–51.
- Bonnelykke K, Pipper CB, Bisgaard H. Transfer of maternal IgE can be a common cause of increased IgE levels in cord blood. *J Allergy Clin Immunol* 2010;126:657–63.
- Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. *J Allergy Clin Immunol* 2010;125:S41–52.
- Brandtzaeg P. Mucosal immunity: integration between mother and the breast-fed infant. *Vaccine* 2003;21:3382–8.
- Hanson LA, Korotkova M, Haversen L et al. Breast-feeding, a complex support system for the offspring. *Pediatr Int* 2002;44:347–52.
- Jarrett EE, Hall E. IgE suppression by maternal IgG. *Immunology* 1983;48:49–58.
- Seeger M, Thierse HJ, Lange H, Shaw L, Hansen H, Lemke H. Antigen-independent suppression of the IgE immune response to bee venom phospholipase A2 by maternally derived monoclonal IgG antibodies. *Eur J Immunol* 1998;28:2124–30.
- Fusaro AE, Maciel M, Victor JR, Oliveira CR, Duarte AJ, Sato MN. Influence of maternal murine immunization with *Dermatophagoides pteronyssinus* extract on the type I hypersensitivity response in offspring. *Int Arch Allergy Immunol* 2002;127:208–16.
- Melkild I, Groeng EC, Leikvold RB, Granum B, Lovik M. Maternal allergen immunization during pregnancy in a mouse model reduces adult allergy-related antibody responses in the offspring. *Clin Exp Allergy* 2002;32:1370–6.
- Uthoff H, Spenner A, Reckelkamm W et al. Critical role of pre-conceptional immunization for protective and nonpathological specific immunity in murine neonates. *J Immunol* 2003;171:3485–92.
- Fusaro AE, Brito CA, Victor JR et al. Maternal-fetal interaction: preconception immunization in mice prevents neonatal sensitization induced by allergen exposure during pregnancy and breastfeeding. *Immunology* 2007;122:107–15.
- Victor JR, Muniz BP, Fusaro AE et al. Maternal immunization with ovalbumin prevents neonatal allergy development and up-regulates inhibitory receptor Fc gamma RIIB expression on B cells. *BMC Immunol* 2010;11:11.
- Polte T, Hennig C, Hansen G. Allergy prevention starts before conception: maternofetal transfer of tolerance protects against the development of asthma. *J Allergy Clin Immunol* 2008;122:1022–30 e5.
- Smits HH, Gloudemans AK, van Nimwegen M et al. Cholera toxin B suppresses allergic inflammation through induction of secretory IgA. *Mucosal Immunol* 2009;2:331–9.
- Favre L, Spertini F, Cortes B. Secretory IgA possesses intrinsic modulatory properties stimulating mucosal and systemic immune responses. *J Immunol* 2005;175:2793–800.
- Sletten GB, Halvorsen R, Egaas E, Halstensen TS. Casein-specific immunoglobulins in cow's milk allergic patient subgroups reveal a shift to IgA dominance in tolerant patients. *Pediatr Allergy Immunol* 2007;18:71–80.
- Pilette C, Durham SR, Vaerman JP, Sibille Y. Mucosal immunity in asthma and chronic obstructive pulmonary disease: a role for immunoglobulin A? *Proc Am Thorac Soc* 2004;1:125–35.
- Jenmalm MC, Bjorksten B. Cord blood levels of immunoglobulin G subclass antibodies to food and inhalant allergens in relation to maternal atopy and the development of atopic disease during the first 8 years of life. *Clin Exp Allergy* 2000;30:34–40.
- Prescott SL, Holt PG, Jenmalm M, Bjorksten B. Effects of maternal allergen-specific IgG in cord blood on early postnatal development of allergen-specific T-cell immunity. *Allergy* 2000;55:470–5.
- Juto P, Holm S. Gliadin-specific and cow's milk protein-specific IgA in human milk. *J Pediatr Gastroenterol Nutr* 1992;15:159–62.
- Chirido FG, Rumbo M, Anon MC, Fossati CA. Presence of high levels of non-degraded gliadin in breast milk from healthy mothers. *Scand J Gastroenterol* 1998;33:1186–92.
- Rumbo M, Chirido FG, Anon MC, Fossati CA. Detection and characterization of antibodies specific to food antigens (gliadin, ovalbu-

- min and beta-lactoglobulin) in human serum, saliva, colostrum and milk. *Clin Exp Immunol* 1998;112:453–8.
- 26 Casas R, Bottcher MF, Duchon K, Bjorksten B. Detection of IgA antibodies to cat, beta-lactoglobulin, and ovalbumin allergens in human milk. *J Allergy Clin Immunol* 2000;105:1236–40.
 - 27 Arlian LG, Platts-Mills TA. The biology of dust mites and the remediation of mite allergens in allergic disease. *J Allergy Clin Immunol* 2001;107:S406–13.
 - 28 Arlian LG, Morgan MS. Biology, ecology, and prevalence of dust mites. *Immunol Allergy Clin North Am* 2003;23:443–68.
 - 29 Arbes SJ Jr, Cohn RD, Yin M *et al.* House dust mite allergen in US beds: results from the First National Survey of Lead and Allergens in Housing. *J Allergy Clin Immunol* 2003;111:408–14.
 - 30 Zock JP, Heinrich J, Jarvis D *et al.* Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. *J Allergy Clin Immunol* 2006;118:682–90.
 - 31 Johansson SGO, ed. *IgE Antibodies and the Pharmacia CAP System in Allergy Diagnosis*. Lidköping: Landströms: Ed. Clinical Workshop, 1988. ISBN: 91-973440-0-1.
 - 32 Nagao AT, Friedlander-Del Nero D, Arslanian C, Carneiro-Sampaio MM. Elevated levels and different repertoire profile of colostral anti-LPS antibodies may have a significant role in compensating newborn immunity. *Scand J Immunol* 2001;53:602–9.
 - 33 Platts-Mills TA, Erwin EA, Allison AB *et al.* The relevance of maternal immune responses to inhalant allergens to maternal symptoms, passive transfer to the infant, and development of antibodies in the first 2 years of life. *J Allergy Clin Immunol* 2003;111:123–30.
 - 34 Jenmalm MC, Holt PG, Bjorksten B. Maternal influence on IgG subclass antibodies to Bet v 1 during the first 18 months of life as detected with a sensitive ELISA. *Int Arch Allergy Immunol* 1997;114:175–84.
 - 35 de Oliveira CH, Binotti RS, Muniz JR, dos Santos JC, do Prado AP, de Pinho AJ Jr. Comparison of house dust mites found on different mattress surfaces. *Ann Allergy Asthma Immunol* 2003;91:559–62.
 - 36 Hansen LG, Halken S, Host A, Moller K, Osterballe O. Prediction of allergy from family history and cord blood IgE levels. A follow-up at the age of 5 years. Cord blood IgE. IV. *Pediatr Allergy Immunol* 1993;4:34–40.
 - 37 Odelram H, Bjorksten B, Leander E, Kjellman NI. Predictors of atopy in newborn babies. *Allergy* 1995;50:585–92.
 - 38 Edenharter G, Bergmann RL, Bergmann KE *et al.* Cord blood-IgE as risk factor and predictor for atopic diseases. *Clin Exp Allergy* 1998;28:671–8.
 - 39 Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. *Nat Rev Immunol* 2007;7:715–25.
 - 40 Benlounes N, Chedid R, Thuillier F, Desjeux JF, Rousselet F, Heyman M. Intestinal transport and processing of immunoglobulin G in the neonatal and adult rat. *Biol Neonate* 1995;67:254–63.
 - 41 Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIB cross-linking. *J Clin Invest* 2006;116:833–41.
 - 42 Mosconi E, Rekima A, Seitz-Polski B *et al.* Breast milk immune complexes are potent inducers of oral tolerance in neonates and prevent asthma development. *Mucosal Immunol* 2010;3:461–74.
 - 43 Glovsky MM, Ghekiere L, Rejzek E. Effect of maternal immunotherapy on immediate skin test reactivity, specific rye I IgG and IgE antibody, and total IgE of the children. *Ann Allergy* 1991;67:21–4.
 - 44 Aalberse RC, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. *Clin Exp Allergy* 2009;39:469–77.
 - 45 Ruiter B, Knol EF, van Neerven RJ *et al.* Maintenance of tolerance to cow's milk in atopic individuals is characterized by high levels of specific immunoglobulin G4. *Clin Exp Allergy* 2007;37:1103–10.
 - 46 Frew AJ. Allergen immunotherapy. *J Allergy Clin Immunol* 2010;125:S306–13.
 - 47 Brandtzaeg PE. Current understanding of gastrointestinal immunoregulation and its relation to food allergy. *Ann N Y Acad Sci* 2002;964:13–45.
 - 48 Janzi M, Kull I, Sjöberg R *et al.* Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. *Clin Immunol* 2009;133:78–85.
 - 49 Savilahti E, Tainio VM, Salmenpera L *et al.* Low colostral IgA associated with cow's milk allergy. *Acta Paediatr Scand* 1991;80:1207–13.
 - 50 Calbi M, Giacchetti L. Low breast milk IgA and high blood eosinophil count in breast-fed newborns determine higher risk for developing atopic eczema after an 18-month follow-up. *J Investig Allergol Clin Immunol* 1998;8:161–4.
 - 51 Savilahti E, Siltanen M, Kajosaari M, Vaarala O, Saarinen KM. IgA antibodies, TGF-beta1 and -beta2, and soluble CD14 in the colostrum and development of atopy by age 4. *Pediatr Res* 2005;58:1300–5.
 - 52 Holt PG, Batty JE, Turner KJ. Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. *Immunology* 1981;42:409–17.
 - 53 van Vlasselaer P, Punnonen J, de Vries JE. Transforming growth factor-beta directs IgA switching in human B cells. *J Immunol* 1992;148:2062–7.
 - 54 Palmeira P, Costa-Carvalho BT, Arslanian C, Pontes GN, Nagao AT, Carneiro-Sampaio MM. Transfer of antibodies across the placenta and in breast milk from mothers on intravenous immunoglobulin. *Pediatr Allergy Immunol* 2009;20:528–35.
 - 55 Cianga P, Medesan C, Richardson JA, Ghetie V, Ward ES. Identification and function of neonatal Fc receptor in mammary gland of lactating mice. *Eur J Immunol* 1999;29:2515–23.
 - 56 Israel EJ, Taylor S, Wu Z *et al.* Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. *Immunology* 1997;92:69–74.
 - 57 Gdalevich M, Mimouni D, Mimouni M. Breast-feeding and the risk of bronchial asthma in childhood: a systematic review with meta-analysis of prospective studies. *J Pediatr* 2001;139:261–6.
 - 58 van Odijk J, Kull I, Borres MP *et al.* Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966–2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. *Allergy* 2003;58:833–43.

Supporting Information

Additional supporting information may be found in the online version of this article.

Figure S1 Colostrum IgA levels are correlated to colostrums TGF- β levels in colostrum. TGF- β levels were determined in colostrum samples by ELISA according to manufacturer instruction (Promega, CAT G 7591). Data obtained in colostrum from atopic and non atopic mothers are compared by Mann–Whitney test (a). TGF- β concentrations obtained in colostrum are correlated with colostrum total IgA (b) and colostrum Der p-specific IgA (c) using Spearman test.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.