Evidence that FcRn mediates the transplacental passage of maternal IgE in the form of IgG anti-IgE/IgE immune complexes

A. Bundhoo¹², S. Paveglio², E. Rafti², A. Dhongade¹², R. S. Blumberg³ and A. P. Matson¹²⁴
¹Division of Neonatology, Connecticut Children’s Medical Center, Hartford, CT, USA, ²Department of Pediatrics, University of Connecticut School of Medicine, Farmington, CT, USA, ³Division of Gastroenterology, Brigham and Women’s Hospital, Boston, MA, USA, ⁴Department of Immunology, University of Connecticut School of Medicine, Farmington, CT, USA

Correspondence:
Adam Matson, Department of Pediatrics, MC-3213, University of Connecticut School of Medicine, 263 Farmington Avenue, Farmington, CT 06030-3510, USA. E-mail: Amatson@UCHC.edu
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Summary

Background The mechanism(s) responsible for acquisition of maternal antibody isotypes other than IgG are not fully understood. This uncertainty is a major reason underlying the continued controversy regarding whether cord blood (CB) IgE originates in the mother or fetus.

Objective To investigate the capacity of maternal IgE to be transported across the placenta in the form of IgG anti-IgE/IgE immune complexes (ICs) and to determine the role of the neonatal Fc receptor (FcRn) in mediating this process.

Methods Maternal and CB serum concentrations of IgE, IgG anti-IgE, and IgG anti-IgE/IgE ICs were determined in a cohort of allergic and non-allergic mother/infant dyads. Madin–Darby canine kidney (MDCK) cells stably transfected with human FcRn were used to study the binding and transcytosis of IgE in the form of IgG anti-IgE/IgE ICs.

Results Maternal and CB serum concentrations of IgG anti-IgE/IgE ICs were highly correlated, regardless of maternal allergic status. IgG anti-IgE/IgE ICs generated in vitro bound strongly to FcRn-expressing MDCK cells and were transcytosed in an FcRn-dependent manner. Conversely, monomeric IgE did not bind to FcRn and was not transcytosed. IgE was detected in solutions of transcytosed IgG anti-IgE/IgE ICs, even though essentially all the IgE remained in complex form. Similarly, the majority of IgE in CB sera was found to be complexed to IgG.

Conclusions and Clinical Relevance These data indicate that human FcRn facilitates the transepithelial transport of IgE in the form of IgG anti-IgE/IgE ICs. They also strongly suggest that the majority of IgE in CB sera is the result of FcRn-mediated transcytosis of maternal-derived IgG anti-IgE/IgE ICs. These findings challenge the widespread perception that maternal IgE does not cross the placenta. Measuring maternal or CB levels of IgG anti-IgE/IgE ICs may be a more accurate predictor of allergic risk.

Keywords allergy, autoantibodies, cord blood, FcRn, IgG anti-IgE, immune complexes, infant, mother, placenta

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Introduction

IgG is generally regarded as the only maternal antibody isotype capable of crossing the placental barrier [1]. The widespread belief in the selective transport of maternal IgG is a major reason underlying the continued controversy regarding whether cord blood (CB) IgE originates in the mother or fetus [2]. The receptor mediating placental transmission of maternal IgG is the β2-microglobulin-associated MHC-class-I-like molecule FcRn, which in humans localizes to the syncytiotrophoblast of the placental villi [3, 4]. In mice, FcRn also facilitates the acquisition of maternal IgG through expression in the intestinal epithelium during neonatal life [5] and yolk sac placenta [6]. It is well established that maternal passive immunity provides the neonate with protection against a wide range of infectious agents [7]; however, maternal IgG also facilitates the transplacental passage of antigens as IgG/antigen immune complexes (ICs) [8–10]. In a similar process, exogenous insulin, a protein
that by itself does not cross the placenta [11], is transported in the form of IgG anti-insulin/insulin ICs [12].

We recently demonstrated that murine FcRn facilitates the intestinal absorption of IgE in the form of IgG anti-IgE/IgE ICs [13]. In addition, IgE bound by IgG anti-IgE at the Cε4 domain remained biologically active by retaining the ability to bind FcεRI and induce rat basophil leukaemia cell degranulation [13]. Because IgG anti-IgE and IgG anti-IgE/IgE ICs are present in the sera of allergic and non-allergic individuals [14–17], we speculated that human FcRn (hFcRn) localized to the placenta would transport maternal IgE to the fetus in the form of IgG anti-IgE/IgE ICs. In this study, we sought to define the relationship between maternal and CB serum concentrations of IgG anti-IgE/IgE ICs in a cohort of allergic and non-allergic pregnant women and their infants. In addition, we aimed to investigate the capacity of hFcRn to bind and transport IgE in the form of IgG anti-IgE/IgE ICs using a well-validated in vitro model system [18, 19]. Our results strongly suggest that maternal IgE crosses the placenta predominantly in the form of IgG anti-IgE/IgE ICs, thereby providing new insight into a pathway for the fetal acquisition of maternal IgE.

Methods

**Subject recruitment, sample collection, and measurement of serum IgE levels**

The study population consisted of 152 allergic and non-allergic pregnant women and their full-term infants delivered at Hartford Hospital (Hartford, CT, USA) between January 2011 and February 2012. Details of the recruitment strategy and sample collection have been previously reported [20]. In brief, potential participants were screened for eligibility and recruited for participation upon admission to labour and delivery. Pregnant women were eligible if they were English or Spanish speaking, had not received prenatal steroids for the treatment of preterm labour, were not on high dose inhaled steroids (e.g. > 800 mcg/day beclomethasone equivalent), and were delivering an infant ≥ 37 weeks gestational age with no known major congenital anomaly. Informed written consent was obtained from all prospective mothers and on behalf of all their infants. Maternal blood was collected by venipuncture prior to delivery. Immediately following delivery, CB samples were obtained from the umbilical vein cleansed with alcohol. Pregnant women were divided into two groups based on the absence or presence of allergic disease as defined by a physician’s diagnosis of asthma, allergic rhinitis, atopic dermatitis, or food allergy and associated symptoms (e.g. cough, wheeze, skin rash) within the past 12 months. Assignment of study subjects into these groups was by chart review and personal interview. Total serum IgE concentrations were determined by Phadia (Thermo Fisher Scientific, Portage, MI, USA) using the ImmunoCAP method. The detection limit for IgE in maternal and CB specimens was 2.00 and 0.10 kU/L, respectively. As a surrogate marker for maternal blood contamination, CB samples were analysed for total IgA using a commercial ELISA kit (Mabtech Inc., Cincinnati, OH, USA), and CB samples containing ≥ 10 μg/mL IgA were excluded from the analysis [20, 21]. The study was approved by the Institutional Review Board at Hartford Hospital (IRB# MAT-003083HJU).

**Measurement of IgG anti-IgE autoantibodies in maternal and cord blood sera**

Thermo Scientific Nunc™ Immunoplates (Thermo Fisher Scientific) were coated with monoclonal human IgE (hIgE) (HE1) (Bioreclamation LLC., Westbury, NY, USA) (10 μg/mL) in 0.1 M carbonate (pH 9.5) for 16 h at 4°C. After blocking non-specific binding, maternal or CB IgG anti-IgE antibodies were captured as twofold serial dilutions of maternal or CB serum. Detection of IgG anti-IgE was with biotin-SP-conjugated mouse IgG (mlgG) anti-hFcγ (Jackson ImmunoResearch, West Grove, PA, USA), followed by avidin-horseradish peroxidase (HRP) (BD Biosciences, San Jose, CA, USA). As a reference standard, adjacent wells on the same plates were coated with ovalbumin (OVA) (grade V; Sigma Chemical Co., St. Louis, MO, USA) (10 μg/mL) in PBS, and predetermined amounts of mlgG1 anti-OVA (BioX-Cell, West Labanon, NH, USA) were added as twofold serial dilutions in duplicate. Biotin-SP-conjugated goat anti-mFcγ1 (Jackson ImmunoResearch) was used to detect bound murine IgG, followed by avidin-HRP. Development was with the TMB microwell peroxidase substrate system (Kirkgaard & Perry Laboratories, Gaithersburg, MD, USA) and A450 measured with a Bio-Rad microplate reader (Hercules, CA, USA).

**Measurement of IgG anti-IgE/IgE immune complexes in maternal and cord blood sera**

Three sandwich ELISAs were employed using different anti-IgE capture reagents with unique epitope specificities. This strategy enabled the capturing of IgE (and ICs) in which the FcεRI-binding region of IgE remained open or blocked by IgG anti-IgE. Thermo Scientific Nunc™ Immunoplates were coated under the conditions described above with mlgG anti-hIgE (m107) (Mabtech Inc.) (2 μg/mL), mlgG anti-hIgE (HP6029) (SouthernBioTech, Birmingham, AL, USA) (2 μg/mL), or recombinant FcεRI protein (rFcεRI) (NBS-C Bioscience, Vienna, Austria) (2 μg/mL). m107 competes for an epitope in
Transplacental passage of IgG anti-IgE/IgE complexes

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As a surrogate model to study the ability of hFcRn to bind and transport IgG anti-IgE/IgE complexes across the placenta, we used β2m-positive Madin-Darby canine kidney (MDCK) cells stably transfected with the heavy chain of hFcRn [18]. When grown to confluence on Transwell filters, FcRn-expressing MDCK cells are a well-established in vitro model system to study IgG transport [19, 25]. Furthermore, because hFcRn has strong affinity for human and rabbit IgG [19, 26], this system was used to evaluate the binding of humanized or rabbit IgG anti-IgE to hFcRn, prior to the generation of IgG anti-IgE/IgE ICs. Cells were incubated for 1 h at 4°C with omalizumab (Genentech, Inc.) (1 mg/ml), polyclonal rabbit IgG anti-IgE (1 mg/ml) (Dako Inc., Carpinteria, CA, USA) (130 μg/ml), or as a reference standard RhoGAM (polyclonal hIgG anti-RhD) (Ortho-Clinical Diagnostics, Rochester, NY, USA) (1 mg/ml) in PBS containing 0.2% BSA and 0.1% NaN3, buffered to pH 6.0. After washing to remove unbound antibodies, biotin-conjugated mlgG anti-hFCY (Jackson ImmunoResearch) was used to detect bound omalizumab or RhoGAM, and biotin-conjugated mlgG anti-rFCY (Sigma-Aldrich, St Louis, MO, USA) was used to detect bound rlgG anti-hlgE. Streptavidin–allophycocyanin (SA–APC; Life Technologies, Carlsbad, CA, USA) was used as a reference standard for the m107 and m107 IC assay, IgG anti-IgE/IgE ICs were generated using equal molar amounts of omalizumab (humanized IgG1 anti-hlgE(ε2ε3)) (Genentech, Inc., San Francisco, CA, USA) and hlgE (HE1) (Bioreclamation LLC). Predetermined amounts of ICs were added to adjacent wells on the same plate. Biotin-SP-conjugated mIgG anti-hFc (Sigma-Aldrich) was used to detect bound rIgG anti-hIgE/hIgE ICs. SA-APC was subsequently applied, and cell staining was examined by flow cytometry. Control conditions were cells incubated with biotinylated antibodies, followed by SA-APC. For blocking experiments, cells were pre-incubated with the hFcRn-blocking antibody DVN 24 [27] (kind gift from Dr. Derry Roopenian, Jackson Labs, Bar Harbor, ME, USA). For immunofluorescence microscopy, hlgE (U266B1) (ATCC) was labelled with Alexa Fluor 555 (AF555) (Molecular Probes Inc., Eugene, OR, USA) and used to generate rlgG anti-hlgE/hlgEAF555 ICs. Cells were incubated with mlgG anti-hFcRn (B-8) (Santa Cruz Biotechnology, Dallas, TX, USA) followed by goat anti-mlgG FITC (Santa Cruz Biotechnology) and rlgG anti-hlgE/hlgEAF555 ICs, in PBS containing 0.2% BSA and 0.1% NaN3, buffered to pH 6.0. Stained cells were adhered to glass slides via cytopsins, fixed with methanol, and counterstained with DAPI (Vectorshield, Burlingame, CA, USA). Cells were viewed using a fluorescence Olympus DP 73 microscope (Olympus, Center Valley, PA, USA), and CellSens software (Olympus) was used for image acquisition and analysis.

For transport studies, MDCK cells were plated at a density of 800 000 cells/cm² on 12 mm diameter, 0.4 μm pore, semipermeable Transwell filters (Corning Life Sciences, Corning, NY, USA). On day 3, when transepithelial electrical resistance reached 150–200 Ωhm/cm², transcytosis assays were performed as described [18, 19], with the input chamber buffered to pH 6.0 and the output chamber buffered to pH 7.4. To the input chamber, rlgG
Measurement of antibodies and immune complexes in Transwell solutions and pooled cord blood serum

Because polyclonal rabbit IgG anti-hIgE was used in hFcRn-transport experiments, different assays were required to enumerate antibodies and ICs. To determine concentrations of rIgG anti-hIgE in Transwell solutions, Thermo Scientific Nunc™ Immunoplates were coated with monoclonal hIgE (U266B1) (10 μg/mL) in 0.1 M carbonate (pH 9.5) for 16 h at 4°C. After blocking non-specific binding, rIgG anti-hIgE antibodies were captured as twofold serial dilutions of Transwell solutions. Detection of rIgG anti-hIgE was with biotin-conjugated mIgG anti-rFc (Sigma-Aldrich), followed by avidin-HRP. As a reference standard, predetermined amounts of rIgG anti-hIgE were added to the same plate and the remainder of the assay was the same. For the measurement of rIgG anti-hIgE/hIgE ICs, Thermo Scientific Nunc™ Immunoplates were coated under the conditions described above with mIgG anti-hIgE (m107) (Mabtech) (2 μg/mL). After blocking non-specific binding, rIgG anti-hIgE antibodies were captured as twofold serial dilutions of Transwell solutions. Detection of rIgG anti-hIgE was with biotin-conjugated mIgG anti-rFcγ (Sigma-Aldrich), followed by avidin-HRP. As a reference standard, predetermined amounts of rIgG anti-hIgE/hIgE ICs were applied to the remaining categorical variables between groups. All statistical analyses were performed using Prism 4 (GraphPad Software, San Diego, CA, USA).

Results

Study subjects and serum IgE levels

Of the 152 pregnant women included in this study, 62 were allergic and 90 were non-allergic. Of these, two allergic and three non-allergic mother/infant dyads were excluded because of insufficient volumes of blood
collected. One allergic and one non-allergic mother/infant dyad were excluded because of CB serum IgA concentrations > 10 μg/mL, and one non-allergic mother/infant dyad was excluded because of a CB serum IgE concentration > 7 SD from the mean. The remaining 59 allergic and 85 non-allergic infant/dyads were included in the analysis. Baseline characteristics of these mothers and infants are shown in Table S1 in the OR. Pregnant women with a history of allergic disease demonstrated higher levels of total serum IgE than pregnant women without a history of allergic disease (P = 0.03). CB serum total IgE levels were not significantly different in infants of allergic and non-allergic mothers (P = 0.31) (Table 1).

IgG anti-IgE autoantibodies in maternal and cord blood sera

IgG autoantibodies directed against IgE were detected in the serum of allergic and non-allergic pregnant women [median 82 (range 5–1854) vs. 107 (3–4238) ng/mL, P = 0.14] (Fig. 1a). In allergic pregnant women, serum concentrations of IgG correlated with serum concentrations of IgG anti-IgE (r = 0.49, P < 0.0001) (Fig. 1b). In non-allergic pregnant women, serum concentrations of IgE did not correlate with serum concentrations of IgG anti-IgE (r = 0.03, P = 0.77) (Fig. 1c). For each mother/infant dyad, levels of IgG anti-IgE were similar, regardless of maternal allergic status. A combined analysis of all mother/infant dyads demonstrated that maternal and CB serum concentrations of IgG anti-IgE were highly correlated (r = 0.90, P < 0.0001) (Fig. 1d).

IgG anti-IgE/IgE immune complexes in maternal and cord blood sera

Sandwich ELISAs utilizing antibodies directed against IgE and IgG are an established method to determine serum concentrations of IgG anti-IgE/IgE ICs [28]. Our initial studies using the rFceRIα-based assay demonstrated that IgG anti-hIgE were highly correlated (r = 0.90, P < 0.0001) (Fig. S2 in the OR). Furthermore, pre-incubation of maternal serum with omalizumab resulted in a significant reduction in the binding of IgG anti-IgE/IgE ICs to rFceRIα (Fig. S2 in the OR), indicating that IgG anti-IgE/IgE ICs were not due to endogenous IgG directed against FcεRIα [24].

IgG anti-IgE/IgE ICs were detected in the sera of allergic and non-allergic pregnant women using the m107 (median 1950 (range 186–1835) vs. 2048 (284–8732) ng/mL, P = 0.40), HP6029 (median 3472 (range 157–18486) vs. 4342 (169–15006) ng/mL, P = 0.49), and rFceRIα (median 6 (range 4–155) vs. 5 (4–171) ng/mL, P = 0.82) IC assays (Fig. 2a). In allergic pregnant women, serum concentrations of IgE correlated with serum concentrations of IgG anti-IgE/IgE ICs determined using the rFceRIα IC assay (r = 0.83, P < 0.0001) (Fig. 2b). A combined analysis of all mother/infant dyads demonstrated that maternal and CB serum concentrations of IgG anti-IgE/IgE ICs were highly correlated in each of the IC assays [m107 (r = 0.94, P < 0.0001), HP6029 (r = 0.85, P < 0.0001), and rFceRIα (r = 0.88, P < 0.0001)] (Fig. 2d–f).

To further investigate for differences in IC composition based on maternal history of allergy, serum samples from 10 allergic and 11 non-allergic pregnant women with comparable levels of total IgG anti-IgE/IgE ICs measured using the rFceRIα IC assay [median 40 (range 18–155) vs. 29 (14–171) ng/mL, P = 0.50] (data not shown) were analysed to determine the IgG subclass distribution of IgG anti-IgE/IgE ICs. In these subjects, total IgE levels were significantly higher in the allergic pregnant women as compared to the non-allergic pregnant women [median 289.00 (range 62.08–1259.00) vs. 50.90 (25.40–276.50) kU/L, P < 0.001] (Fig. 3a). Despite similar serum concentrations of total IgG anti-IgE/IgE ICs, there were significantly higher levels of IgG1-containing IgG anti-IgE/IgE ICs in the serum of allergic pregnant women as compared to non-allergic pregnant women [median 34 (range 7–70) vs. 12 (4–66) ng/mL, P = 0.026] (Fig. 3b). IgG3- and IgG4-containing IgG anti-IgE/IgE ICs were also present; however, differences in serum concentrations between allergic and non-allergic pregnant women were not statistically significant (P = 0.11 and P = 0.15, respectively) (Fig. 3b). In corresponding CB sera, total IgE concentrations were not significantly different in infants of allergic and non-allergic mothers (P = 0.31) (Table 1).

Table 1. Serum total IgE in allergic and non-allergic mother/infant dyads. * P < 0.05.

| Allergic mother/infant dyads (n = 59) | Non-allergic mother/infant dyads (n = 85) | P-value |
|--------------------------------------|------------------------------------------|---------|
| Maternal IgE (kU/L)                  |                                          |         |
| Median (range)                       | 62.89 (1.13–1258.90)                     | 34.40 (2.00–1572.52) | *0.03 |
| CB IgE (kU/L)                        |                                          |         |
| Median (range)                       | 0.26 (0.10–2.67)                        | 0.18 (0.10–3.19)    | 0.31  |

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**Fig. 1.** IgG anti-IgE autoantibodies in maternal and CB sera. Data represent results obtained from 59 allergic and 85 non-allergic mother/infant dyads. (a) Levels of IgG anti-IgE were similar between allergic and non-allergic pregnant women. Total serum IgE correlated with levels of IgG anti-IgE in (b) allergic pregnant women but not in (c) non-allergic pregnant women. (d) A combined analysis of allergic and non-allergic mother/infant dyads demonstrated that maternal and CB levels of IgG anti-IgE were highly correlated. ns, not significant. The horizontal line represents the median.

**Fig. 2.** IgG anti-IgE/IgE ICs in maternal and CB sera. Data represent results obtained from 59 allergic and 85 non-allergic mother/infant dyads. (a) Levels of IgG anti-IgE/IgE ICs were similar between allergic and non-allergic pregnant women, when determined using the m107, HP6029, or rFcRIα IC assays. Total serum IgE correlated with levels of IgG anti-IgE/IgE ICs in (b) allergic pregnant women but not in (c) non-allergic pregnant women, when ICs were measured using rFcRIα. (d–f) A combined analysis of allergic and non-allergic mother/infant dyads demonstrated that maternal and CB levels of IgG anti-IgE/IgE ICs were highly correlated when determined using the m107, HP6029, or rFcRIα IC assays. ns, not significant.
levels were similar in infants of allergic and non-allergic mothers (median 0.45 (range 0.10–1.51) vs. 0.29 (0.1–3.19) kU/L, \( P = 0.67 \) (Fig. 3c). IgG1- and IgG3-containing IgG anti-IgE/IgE ICs were the most highly represented subclasses in CB sera, however, differences in serum concentrations between infants of allergic and non-allergic mothers did not reach statistical significance (\( P = 0.18 \) and \( P = 0.30 \), respectively) (Fig. 3d). In allergic mother/infant dyads, combined levels of maternal IgG1- and IgG3-containing ICs were predictive of CB IgE levels (\( r = 0.75, P = 0.01 \)) (Fig. 3e), whereas maternal IgE levels were not (\( r = 0.35, P = 0.33 \)) (data not shown). In non-allergic mother/infant dyads, maternal IgE levels were predictive of CB IgE levels (\( r = 0.77, P = 0.006 \)), whereas combined levels of maternal IgG1- and IgG3-containing IgG anti-IgE/IgE ICs were not (\( r = 0.01, P = 0.99 \)) (data not shown).

**hFcRn binds and transports IgE in the form of IgG anti-IgE/IgE immune complexes**

Initial studies performed to compare the binding of different IgG anti-hIgE to hFcRn demonstrated that polyclonal rabbit IgG anti-hIgE bound to hFcRn-expressing cells at an equivalent level as RhoGAM, whereas omalizumab demonstrated a much lower level of binding (Fig. S3 in the OR). Thus, polyclonal rIgG anti-hIgE and hIgE were used to generate IgG anti-IgE/IgE ICs for the remaining hFcRn-binding and transport studies.

Flow cytometric analysis of hFcRn-expressing MDCK cells demonstrated no binding of the murine detection antibody biotin-conjugated mlgG anti-hlgE_{\text{Cys}} (Le27) or monomeric hIgE, whereas there was strong binding of polyclonal rlgG anti-hlgE (Fig. 4a–c). These results are consistent with published reports, which demonstrate that mouse IgG and human IgE do not bind hFcRn,
while rabbit IgG binds hFcRn strongly [26, 29]. hIgE, in the form of rIgG anti-hIgE/hIgE ICs, bound strongly to cells (Fig. 4d). The binding of rIgG anti-hIgE/hIgE ICs was pH dependent, inhibited by the hFcRn-blocking antibody DVN24, and co-localized with hFcRn (Fig. 4e–g). pH-dependent binding is a characteristic feature of FcRn which requires an acidic pH to bind IgG with high affinity [27].

In transcytosis experiments, hFcRn-mediated transport was observed for rIgG anti-hIgE and rIgG anti-hIgE/hIgE ICs, but not for monomeric hIgE (Fig. 5a). The transport of rIgG anti-hIgE/hIgE ICs occurred in basolateral to apical and apical to basolateral directions, was competitively inhibited by RhoGAM and by the hFcRn-blocking antibody DVN24 (Figs 5a,b; and data not shown). Following transcytosis, a substantial proportion of rIgG anti-hIgE/hIgE ICs retained the ability to bind rFceRIz protein (mean % antibody transport = 0.042 ± 0.005; data not shown). In addition, hIgE was detected in output solutions containing transcytosed rIgG anti-hIgE/hIgE ICs, using both the m107 and rFceRIz anti-hIgE assays (Fig. 5c, and data not shown). To determine whether the transcytosed hIgE was monomeric or complexed to rIgG anti-hIgE, output solutions from nine separate Transwells were pooled [mean IgE concentration of 7140 (range 2622–26 517) ng/mL, determined using rFceRIz anti-hIgE assay] and subjected to protein A chromatography. Following exposure to protein A, only 6% of the hIgE was recovered in the primary flow through (Fig. 5c), which coincided with removal of > 99% of the rIgG anti-hIgE/hIgE ICs (data not shown). In control solutions exposed to protein A, > 99% of rIgG anti-hIgE was removed, whereas > 95% of monomeric IgE was recovered (data not shown).

The majority of IgE in cord blood serum exists as IgG anti-IgE/IgE immune complexes

To determine whether CB IgE was monomeric or complexed to IgG anti-IgE, CB sera from 20 different infants were pooled into five separate groups of 4,
subjected to protein A chromatography, and analysed. Prior to protein A, the pooled CB serum contained a mean total IgE level of 171 (range 102–335) ng/mL, determined using the m107 anti-hIgE assay (Fig. 6). Following protein A, only 6% (range 3–10%) of the IgE was recovered \( (P = 0.021) \) (Fig. 6), which coincided with removal of >99% of the IgG anti-IgE/IgE ICs determined using the m107 IC assay (data not shown).

Total IgE levels determined using the rFcεRIa anti-hIgE assay were also reduced following protein A; however, several of the pooled specimens were at the lower limit of detection prior to exposure (data not shown).

**Binding of cord blood IgE to FcεRI in infants of allergic and non-allergic mothers**

To investigate the possibility that CB IgE (in the form of IgG anti-IgE/IgE ICs) could have differential ability to bind FcεRI based on maternal allergic status, we attempted to determine CB IgE levels using the rFcεRIa-based IgE assay. Levels of CB IgE measured using the rFcεRIa-based IgE assay were at the lower limit of detection for the majority of CB samples, precluding a comparison between infants of allergic and non-allergic mothers (data not shown). Thus, we quantified percentages of CB basophils with surface-bound IgE relative to levels of CB IgE as an indicator for the ability of CB IgE to bind FcεRI. Seven CB samples from infants of allergic mothers and 11 CB samples from infants of non-allergic mothers had adequate numbers of basophils and sufficient CB IgE (determined via the ImmunoCAP) to perform this analysis. In these samples, there was no difference in levels of CB IgE between infants of allergic and non-allergic mothers \( (r = 0.77, P = 0.04) \) (Fig. 7a). In contrast, there was no significant correlation between CB IgE levels and
percentages of CB basophils with bound IgE in infants of non-allergic mothers ($r = 0.40, P = 0.22$) (Fig. 7b).

**Discussion**

Here we report essentially equivalent maternal and CB serum concentrations of IgG anti-IgE/IgE ICs in a cohort of allergic and non-allergic mothers/infant dyads and demonstrate that hFcRn facilitates the transepithelial transport of IgE in the form of IgG anti-IgE/IgE ICs. These data, along with our discovery that the majority of IgE in CB sera is bound by IgG, strongly suggest that maternal IgE is transported across the placenta via FcRn-mediated transcytosis of IgG anti-IgE/IgE ICs. These findings challenge the widespread perception that maternal IgG is the only antibody isotype transported across the placenta and establish a new paradigm to study perinatal aspects of early allergic sensitization. CB IgE existing primarily in the form of IgG anti-IgE/IgE ICs may explain the inconsistent results observed when using CB IgE as a predictor for future allergic disease [30–32] and the variable correlations observed between maternal and CB serum IgE concentrations [33–36].

It is well established that IgG anti-IgE/IgE ICs are present in the sera of allergic and non-allergic individuals [16, 17, 37]. In the present study, we demonstrated that IgG anti-IgE/IgE ICs are also present in the sera of pregnant women and their newborn infants at essentially equivalent concentrations, which is highly suggestive of placental transmission. Because maternal IgG, but not free IgE, is transported across the human placenta via FcRn-mediated transcytosis [29, 38], we investigated the ability of hFcRn to bind and transport IgE in the form of IgG anti-IgE/IgE ICs in a well-validated in vitro model system [18, 19]. Our initial studies using omalizumab to generate IgG anti-IgE/IgE ICs demonstrated hFcRn has low affinity for this humanized IgG1 anti-hIgE$_{3.5}$. These results are consistent with the observation that hFcRn binds omalizumab less strongly than other humanized IgGs [39]. Thus, we used polyclonal rlgG anti-hlgE to form rlgG anti-hIgE/hIgE ICs, and in agreement with published literature [19, 26] found that rabbit IgG has strong affinity for hFcRn. Furthermore, using polyclonal rlgG anti-hIgE more closely recapitulates the human situation where multiple IgG anti-IgE s are present, directed against different IgE epitopes with various affinities. hIgE in the form of rlgG anti-hlgE/hIgE ICs, bound to hFcRn and was transcytosed across polarized epithelial cells in an hFcRn-dependent fashion. Taken together, these data are highly suggestive that hFcRn can bind and transfer maternal IgE across the placenta as IgG anti-IgE/IgE ICs.

Interestingly, following FcRn-mediated transcytosis of rlgG anti-hlgE/hIgE ICs, hIgE was detected in output solutions using m107 or rFc$_{eRI}$a anti-IgE as capture reagents and biotinylated antibodies directed against hlgE. Following protein A chromatography, essentially all of the hlgE was removed, indicating that the majority was in the form of rlgG anti-hlgE/hIgE ICs. The explanation for this may be that the anti-hlgEs were directed against epitopes that differed from those already occupied by rlgG anti-hlgEs. Alternatively, the anti-hlgEs could have displaced rlgG anti-hlgEs because of competing affinities. Regardless of the mechanism, these results suggest that IgE in CB serum might similarly exist as IgG anti-IgE/IgE ICs. Indeed, further supporting this notion was our finding that the bulk of IgE in pooled CB serum was removed following exposure to protein A. To our knowledge, this is the first report demonstrating that the vast majority of IgE in CB sera is complexed to IgG. The low levels of monomeric IgE recovered following exposure to protein A may represent natural IgE produced in utero in the absence of classical MHC II cognate help [40].

The ability of maternal IgG anti-IgE/IgE ICs to predispose towards allergy development in the child is of

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**Fig. 7.** CB IgE is predictive of basophil-bound IgE in infants of allergic mothers, but not in infants of non-allergic mothers. Data represent CB samples from 7 infants of allergic mothers and 11 infants of non-allergic mothers, containing $\geq 0.5$ kU/L total serum IgE (determined by ImmunoCAP) and a minimum of 200 basophils. Flow cytometry was used to calculate the percentages of CB basophils with surface-bound IgE relative to an isotype control.
interest, as the molecular basis for maternal imprinting of allergic risk remains unclear [35, 41–43]. Upon entering the fetal circulation, IgG anti-IgE/IgE ICs may bind to cells expressing FcRs (e.g. FcεRI or FcεRII), FcγRs, or co-aggregate FcRs to FcγRs [44]. Factors that influence receptor binding include the epitope specificity of the IgG anti-IgE [13], the ratio of IgG anti-IgE : IgE (unpublished results), or the IgG anti-IgE subclass which can influence binding to activating or inhibitory FcγRs [45, 46]. Our results indicate a fraction of the total pool of IgG anti-IgE/IgE ICs in maternal and CB serum retains the capacity to bind FcεRI. This is further supported by our experiments utilizing FcRn-expressing MDCK cells, which demonstrate that a significant proportion of transcytosed rIgG anti-IgE/IgE ICs bind to rFcεRIz. In mice, IgG anti-IgE/FcεRIz ICs absorbed across the intestine via FcRn retain the capacity to induce rat basophil leukemia cell degranulation [13]. Thus, it is plausible to hypothesize that maternal IgG anti-IgE/IgE ICs with the ability to bind FcRs might have yet to be identified functional effects in the fetus. In adults, it is known that allergen-specific IgE localized to the surface of FcεRI-expressing antigen-presenting cells serves as an antigen-focusing agent that optimizes the capacity to elicit T cell responses [47]. Our unpublished work has demonstrated IgG anti-IgE/IgE ICs to be present on the surface and within CB basophils and myeloid dendritic cells (DCs). It remains to be determined whether biologically active IgG anti-IgE/IgE ICs (e.g. IgG bound to IgE at Cε4) localized to FcεRI on fetal or neonatal cells could provide an adjuvant-like stimulus to promote in utero or early postnatal T cell priming. The possibility that intracellular IgE recently reported in adult myeloid DCs represents IgG anti-IgE/IgE ICs should also be considered [48].

Our analysis of IgG subclasses is relevant because it demonstrates the potential for differential regulation of neonatal immunity based on maternal history of allergic disease [35, 41, 49]. Despite total levels of IgG anti-IgE/IgE ICs being similar in allergic and non-allergic pregnant women, levels of IgG1-containing ICs with the capacity to bind FcεRI were significantly greater in the allergic group. In addition, IgG1- and IgG3-containing ICs were present at the highest concentrations in CB sera, implying that these subclasses are efficiently transported across the placenta when bound to IgE. IgG1 and IgG3 display high affinity binding to Fcγ-activating receptors [50], and IgG1 antibodies are the most common subclass found bound to IgE on the surface of adult basophils [16]. It remains to be determined whether IgG1 antibodies are the most abundant subclass bound to IgE on CB basophils [20]. Interestingly, levels of maternal IgG1- and IgG3-containing ICs that bind to FcεRI were predictive of CB IgE levels only in infants born to allergic women. Traditional IgG assays such as the ImmunoCAP do not distinguish IgE that is bound or not bound by IgG anti-IgE at Cε3 [22]. Thus, it is possible that a significant portion of CB IgE found in infants of non-allergic mothers is bound by IgG anti-IgE at Cε3, resulting in reduced binding to FcεRI. Our recent report demonstrating that IgE expression on CB basophils is greater in infants of allergic as compared to non-allergic mothers, despite similar levels of CB IgE, is supportive of this possibility [20]. In the current study, we further established that levels of CB IgE were predictive of basophil-bound IgE in infants of allergic mothers, but not in infants of non-allergic mothers. Collectively, these data suggest that the ability of CB IgE (in the form of IgG anti-IgE/IgE ICs) to bind FcεRI-expressing fetal cells can be influenced by maternal allergy. The low sensitivity of the rFcεRIz-based IgE assay precluded further characterization of CB IgE, perhaps due to natural IgG anti-IgE hindering additional IgE binding sites.

Following transport across the placental syncytiotrophoblast, maternal IgG anti-IgE/IgE ICs may encounter resident Hofbauer cells, the placental macrophages which express all FcγRs [51], and function to trap ICs [52]. This may explain the finding of IgE in Hofbauer cells regardless of maternal allergic status [53]. Our finding of essentially equivalent concentrations of IgG anti-IgE/IgE ICs in CB and maternal serum suggests the majority escape trapping by Hofbauer cells, are recycled back to the surface and released into the fetal circulation or bound to a saturable receptor with the excess being degraded. In the vascular endothelium, FcRn functions to rescue IgG from lysosomal degradation and recycles IgG back to the circulation, thereby prolonging serum half-life [54–56]. FcRn is expressed in human antigen-presenting cells [57, 58], suggesting FcRn might represent the saturable receptor hypothesized above. Although it has not been demonstrated that Hofbauer cells express FcRn, given the broad expression in antigen-presenting cells of dendritic and myeloid origin, this is likely to be the case [59].

The biologic functions of IgG anti-IgE appear diverse, with the potential to induce or suppress IgE-mediated inflammatory responses [60–62]. In our analysis of pregnant women with symptomatic allergic disease, the production of IgG anti-IgE (and generation of ICs) increased in proportion to rising serum IgE levels. Despite this positive correlation, and the finding of higher total IgE in the allergic group, levels of IgG anti-IgE were similar between allergic and non-allergic pregnant women suggesting a potential limit in the quantities of IgG anti-IgE that are produced and thus the regulatory capacity of this pathway. We speculate that as IgE production increases, IgG anti-IgE/IgE ICs are generated that retain the capacity to bind FcεRI [13, 63]. At even higher IgE production rates, the ability of IgG anti-IgE to bind the total pool of IgE becomes sur-
passed, resulting in elevated levels of monomeric IgE and increased risk for IgE-mediated pathology. Additional studies are required to confirm this possibility and to further define structural characteristics that differ between individuals with and without a history of allergy [64]. It should be noted that our IgG anti-IgE assay used adjacent wells on the same ELISA plate coated with OVA and predetermined amounts of mouse IgG1 anti-OVA as the reference standard. Similar distributions of IgG anti-IgE in maternal and CB sera may be attained using ELISA plates coated with hIgE and predetermined amounts of omalizumab as the reference standard (unpublished results).

As a method of assessing maternal blood contamination of CB samples, we used elevated CB serum IgA. While this is an established method [21], our data suggest maternal autoantibodies directed against IgA could transport maternal IgA into the fetal circulation by a similar mechanism. While we appreciate this limitation, the observed correlation between maternal and CB serum levels of IgG anti-IgE/IgE ICs argue strongly in favour of active transport as opposed to leakage of trace amounts of maternal blood into the fetal circulation. In addition, while significantly more pregnant women smoked tobacco in our allergic group, the strong correlation between maternal and infant serum levels of ICs in both allergic and non-allergic mother/infant dyads implies that smoking tobacco has a negligible effect on maternal IC transmission.

In summary, our study details a novel mechanism for the placental transport of maternal IgE and suggests the majority of CB IgE originates in the mother as IgG anti-IgE/IgE ICs. The ability of maternal-derived IgG anti-IgE/IgE ICs to modulate fetal or neonatal immune responses and influence risk for future atopy requires additional study.

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Conflict of interest

The authors declare no conflict of interest.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Fig S1.** The epitope specificity of IgG anti-hIgE impacts the binding of hIgE to rFcεRIα.

**Fig S2.** Omalizumab inhibits the binding of maternal IgG anti-IgE/IgE ICs to rFcεRIα.

**Fig S3.** Relative fluorescence intensities for the binding of rIgG anti-hIgE, RhoGAM, and omalizumab to hFcRn-expressing MDCK cells.

**Table S1.** Maternal and neonatal characteristics based on maternal history of allergic disease.