

IMMUNOLOGY

Fetal mast cells mediate postnatal allergic responses dependent on maternal IgE

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Mast cells (MCs) are central effector cells in allergic reactions that are often mediated by immunoglobulin E (IgE). Allergies commonly start at an early age, and both MCs and IgE are detectable in fetuses. However, the origin of fetal IgE and whether fetal MCs can degranulate in response to IgE-dependent activation are presently unknown. Here, we show that human and mouse fetal MCs phenotypically mature through pregnancy and can be sensitized by maternal IgE. IgE crossed the placenta, dependent on the fetal neonatal Fc receptor (FcRN), and sensitized fetal MCs for allergen-specific degranulation. Both passive and active prenatal sensitization conferred allergen sensitivity, resulting in postnatal skin and airway inflammation after the first allergen encounter. We report a role for MCs within the developing fetus and demonstrate that fetal MCs may contribute to antigen-specific vertical transmission of allergic disease.

Mast cells (MCs) are innate immune cells present in all vascularized tissues. They reside close to mucosal surfaces, blood vessels, and nerve fibers. Their positioning enables them to act as sentinels, constantly sampling the surrounding microenvironment, and they have the ability to respond rapidly to a wide variety of pathogens, allergens, or tissue-derived danger signals (1–4). MCs contain large numbers of secretory granules, storing an extensive array of preformed mediators such as histamine, serotonin, proteases (e.g., tryptase and chymase), proteoglycans, and some cytokines (5). Additionally, they synthesize prostaglandins, leukotrienes, and cytokines de novo (6). Therefore, they can initiate a complex program of inflammation while also regulating the development of adaptive immune responses (3, 7). MCs are required in many animal allergic disease models (8, 9) and contribute to human allergic diseases, as suggested by their activation during acute allergic

episodes and the efficacy of MC-stabilizing therapeutics in allergic conditions (10, 11).

Allergic diseases are caused by pathological immune responses to innocuous environmental substances such as plant pollen, pet dander, house dust mites, and various foods (12, 13). On the basis of the route of sensitization and exposure, they can manifest as asthma, allergic rhinitis, atopic dermatitis, food allergy, or anaphylaxis. Allergic diseases affect ~10 to 30% of the general population, and their prevalence has been increasing over time (14, 15). Allergic reactions are primarily mediated by immune mechanisms that promote Th2-polarized immunity, leading to immunoglobulin E (IgE) production that triggers MC activation upon allergen exposure (16–18). However, MC degranulation can also be triggered by alternate mechanisms, including immune complexes, anaphylatoxins, and through other G protein-coupled receptors such as MrgX2 (19–21). Sensitization of MCs to antigens occurs when IgE binds to the high-affinity receptor for the Fc region of IgE (FcεRI) present on the cell surface (2). Upon subsequent antigen exposure, the IgE-armed MCs rapidly detect antigen and degranulate, with their released products leading to inflammation (2).

During embryonic development in mice, MCs together with macrophages appear relatively early in fetal tissues, preceding other immune cell types (22, 23). MCs colonize the developing fetus in two waves, which are distinct both temporally and by the tissue of residence (22). The initial wave of yolk sac-derived progenitors with MC potential is detectable between embryonic day 7.5 (E7.5) and E10.5 (22). Fetal MCs are thought to influence the development of several organs (24–26); however, whether they are functional contributors to fetal and neonatal immune and allergic

responses through the capture of allergen-specific maternal IgE is presently unknown.

Here, we show that maternal IgE can enter the fetus, dependent on the neonatal Fc receptor for IgG (FcRN), after both passive and active maternal sensitization. IgE is able to prime fetal MCs, which are functional before birth, for allergen detection postpartum. Consequently, the offspring born from mice sensitized before pregnancy develop allergic inflammation upon the first exposure to the allergen. Furthermore, MC-bound IgE is present in human fetal skin, suggesting that a similar mechanism may occur in humans. Our results demonstrate that maternally derived IgE can predispose for allergic disease through fetal MC binding and/or activation in neonatal and early life upon the first exposure to the allergen, suggesting potential therapeutic targets to mitigate neonatal allergies.

Results

Murine fetal MCs are phenotypically mature

Although the earliest progenitors of MCs originate in primitive hematopoiesis during embryonic development (22), the time course of their maturation and the acquisition of molecules required for effector functions in allergic inflammation are not yet fully described. To address this, we used flow cytometry to analyze the expression of phenotypic markers on skin-resident MCs of fetuses and newborns (NBs) compared with adult MCs. Cells having an MC phenotype (CD45⁺F4/80⁺CD11b⁺CD90⁺MHCII⁺CD117⁺CD200R⁺) (Fig. 1A), which constituted ~20% of skin CD45⁺ cells at E17.5 (fig. S1A), were selected for further phenotypic characterization at various developmental stages. This involved the detection of other MC markers such as CD16/32, CD63, integrin-β7, the granule component heparin, and FcεRI (Fig. 1, B and C, and fig. S1B). FcεRI expression was low but confirmed to be present on fetal MCs and began increasing in NBs (Fig. 1C). Increased maturity of adult MCs compared with fetal MCs was indicated by the greater relative expression of markers that mediate allergic effector functions, such as heparin and FcεRI (Fig. 1, B and C, and fig. S2A). FcεRI expression was confirmed by immunostaining to colocalize with MCs (fig. S2B). Conversely, the MC progenitor- and migration-associated markers integrin-β7 (27) and CX3CR1 (22) were more highly expressed on fetal MCs, as was c-kit (CD117) (Fig. 1, A and B, and fig. S2A). However, fetal MCs also gradually increased the expression of the maturation marker and granule component heparin (Fig. 1B and fig. S2A), which also could be visualized by toluidine blue staining (fig. S2C), and colocalized with the MC protease MCPT1 (fig. S2D). The relative numbers of MCs that were positive for these and additional markers in E12.5, E14.5, E16.5, NB, and adult skin are provided in

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fig. S2A. Scanning electron microscopy showed that isolated MCs from multiple embryonic stages appeared granulated (Fig. 1D). By immunostaining, we confirmed the presence of MCs loaded with heparin-containing granules in the embryonic skin based on the colocalization of heparin with c-kit and CX3CR1, an early MC marker in embryos (22), beginning as early as E12.5, and their density increased with gestational age (Fig. 1E). Together, these data indicate that fetal MCs having low expression for FcεRI and containing heparin to the same or similar degree as adult MCs expand during fetal development, supporting that some fetal MCs might be functionally mature and contain the components required for allergic responses before birth.

Maternal IgE drives antigen-specific degranulation of fetal MCs

We next investigated whether fetal MCs can functionally bind IgE to become sensitized. Although allergen-specific IgE is detectable in human neonates and cord blood (28–31), intrinsic IgE production was found to be detectable only ~9 months after birth (32), suggesting that neonatal IgE is at least partially derived from the mother. Therefore, to experimentally address whether IgE transfers from mother to fetus, we passively sensitized pregnant mice with IgE and verified whether these antibodies were later detectable on fetal MCs. For this, we administered exogenous IgE to the dam at E16.5 and collected the fetal and maternal skin 24 hours later (Fig. 2A). This led to increased binding of IgE to fetal MCs, although to a lesser extent than to maternal MCs (Fig. 2, B and C). The binding of IgE to fetal MCs was also increased after injection of dams with higher doses of IgE (Fig. 2C). Moreover, IgE persisted on the fetal MCs, because it was present to an equal degree on MCs from the skin of NBs compared with E17.5 (Fig. 2C). Immunostaining of skin MCs (counterstained with toluidine blue for MC identification) after injection into dams (Fig. 2D). Fetal MCs could also be identified by toluidine blue staining (Fig. 2E) and by flow cytometry (Fig. 2F and fig. S4) of fetal lung tissue. Flow cytometry indicated the expression of FcεRI on a small proportion of skin and lung MCs, in contrast to monocytes, which did not express FcεRI (Fig. 2G and fig. S4). We also observed an increase in the granulation of fetal lung MCs after IgE injection of dams (Fig. 2H), suggesting an increase in the maturation of MCs in the presence of IgE. Together, these data show that translocation of maternal IgE to the fetus occurs and that maternal IgE can bind to fetal MCs in multiple tissues.

To address whether fetal MCs are functionally capable of degranulation mediated by maternally derived IgE, we collected E17.5

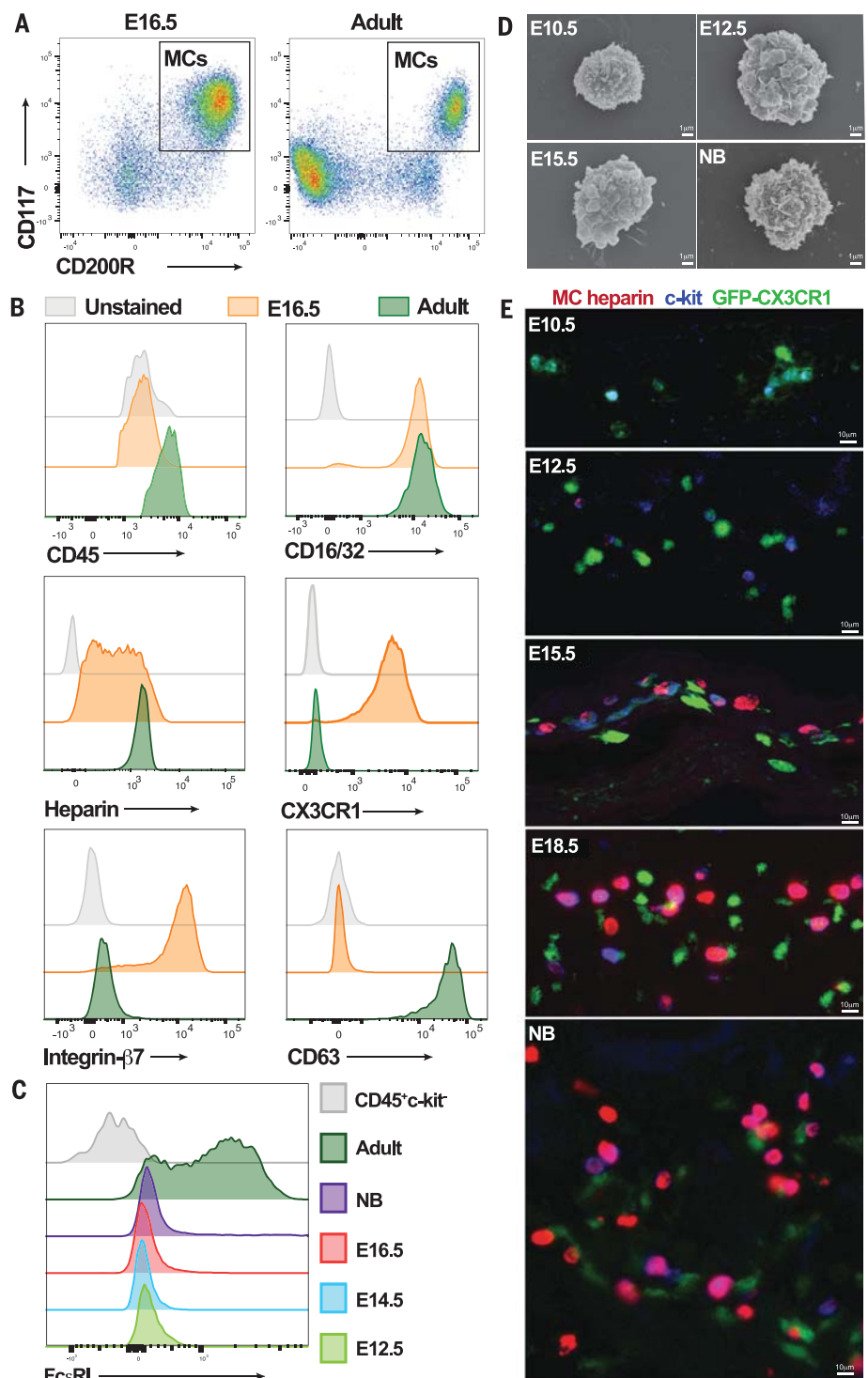


Fig. 1. Mast cells are present in the fetal skin and mature during pregnancy. (A) Flow cytometry plots of MCs identified in fetal (E16.5) and adult skin after gating on DAPI⁺CD45⁺F4/80⁺CD11b⁺CD90⁺MHCII⁺ cells. The full gating strategy is shown in fig. S1. (B) Histograms showing the expression of various MC phenotypic markers on E16.5 and adult (orange and dark green, respectively) mouse skin MCs. (C) Expression of FcεRI on fetal MCs throughout development compared with adult MCs. (D) Scanning electron microscope images of sorted mouse fetal and NB MCs. The full gating strategy is shown in fig. S3. Scale bar, 1 μm. (E) Immunofluorescence staining for MC markers (c-kit is shown in blue and heparin in red) in CX3CR1^{GFP} mouse skin sections at various time points of fetal development showing gradual increases in their density and granulation. Scale bar, 10 μm.

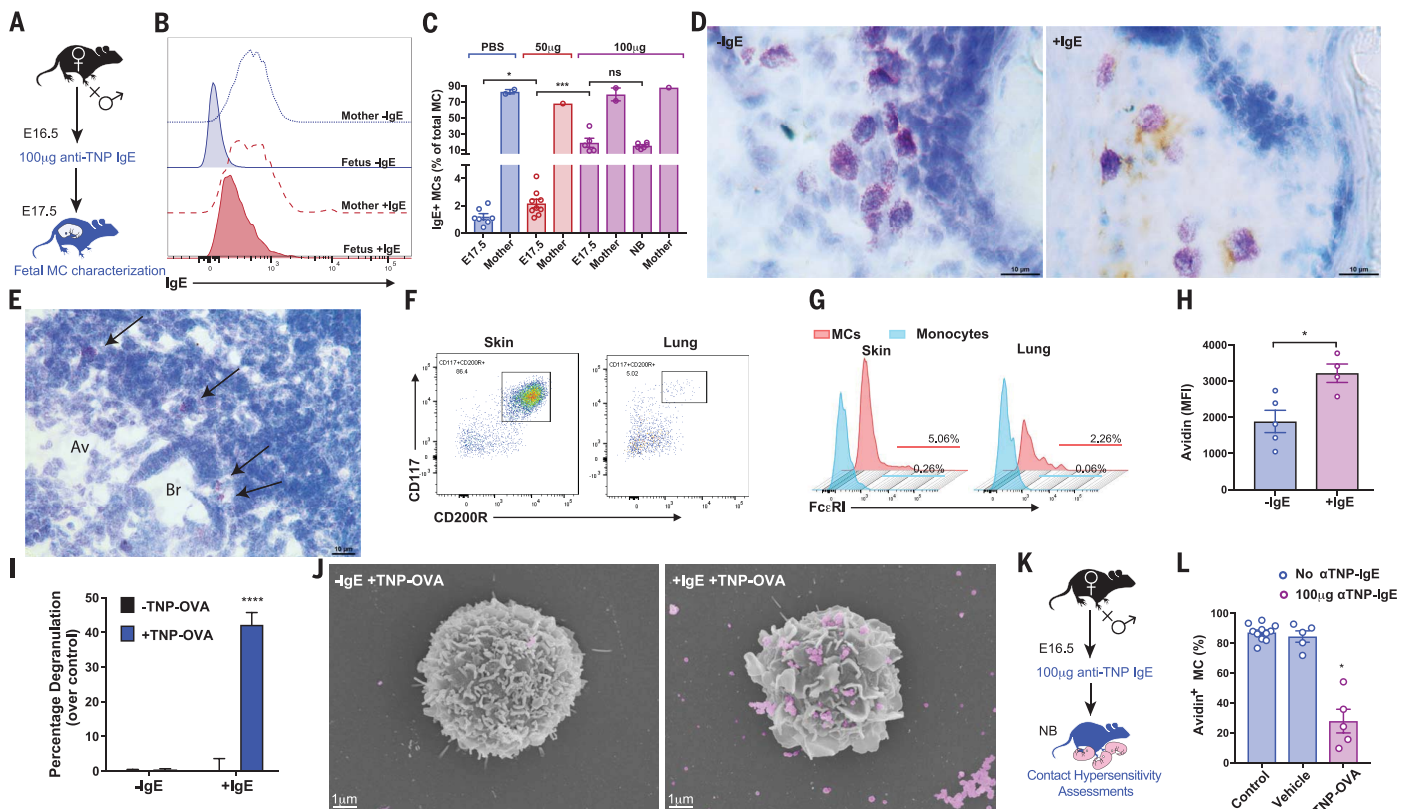


Fig. 2. Maternal IgE binds fetal MCs and induces their maturation and antigen-specific activation. (A) Schematic of the experimental design of passive maternal sensitization with TNP-specific IgE at E16.5, followed by fetal skin MC characterization. (B) IgE binding to maternal (blue) and fetal (red) MCs by flow cytometry. (C) Quantification of the percentage of IgE⁺ MCs after intravenous injection of various doses of IgE comparing the percentages in the maternal, fetal, and NB skin. Fetal groups were compared with each other by Student's unpaired *t* test. **p* < 0.05; ****p* < 0.001; ns, not significant. Data points represent individual animal responses and error bars indicate the mean ± SEM. (D) Immunohistochemistry for IgE (brown) in fetal skin sections followed by counterstaining using toluidine blue to visualize granulated MCs (purple). IgE staining could be visually observed on MCs in the fetuses of IgE-injected dams. (E) Fetal MCs could also be seen in toluidine blue-stained lung tissue sections. Arrows point to granulated MCs, as indicated by purple staining. Av, alveolar space; Br, bronchiole. (F) Identification of MCs in fetal skin and lung tissue at E17.5. The gating strategy for the identification MCs is provided in figs. S1A and S4. (G) Histograms represent staining for FcεRI on fetal skin and lung

MCs and monocytes (E17.5). (H) Mean fluorescence intensity of avidin staining (to indicate heparin content) was determined in lung MCs of NB mice (with and without IgE injection of dams at E16.5), showing increased granulation of fetal MCs in the presence of maternal IgE. Data points represent individual animal responses and error bars indicate the mean ± SEM. (I) Antigen-specific degranulation of fetal MCs after maternal sensitization was confirmed by a β-hexosaminidase assay after isolation of fetal MCs from sensitized (IgE⁺) and unsensitized (IgE⁻) groups by magnetic bead separation and challenge with antigen (TNP-OVA) or control media. *****p* < 0.001. Data indicate the mean ± SEM of technical replicates. (J) Electron micrographs of TNP-OVA-exposed MCs isolated from dams with (+IgE) and without (-IgE) IgE injection. Extracellular granules in both images are false-colored pink to aid visualization. Scale bar, 1 µm. (K) Schematic of the experimental design of the experiment to assess MC degranulation in antigen-challenged NBs. (L) Percentage of granulated MCs (Avidin⁺) of total MCs in the NB skin 6 hours after painting of TNP-OVA or vehicle. **p* < 0.05. Data points represent individual animal responses and error bars indicate the mean ± SEM.

fetuses from mothers passively sensitized with either IgE specific for the hapten 2,4,6-trinitrophenyl (TNP) or vehicle on E16.5 (Fig. 2A) and isolated fetal MCs from skin cell suspensions by immunomagnetic positive selection. When antigen-specific activation was induced ex vivo by exposing the MCs to ovalbumin (OVA)-conjugated TNP, only MCs isolated from fetuses of the maternal sensitization group, but not the control groups, showed significant degranulation (Fig. 2, I and J, and fig. S3). Next, to investigate whether the sensitization of fetal MCs with maternally derived IgE can initiate an allergic response in vivo

in NBs, we used a neonatal model of contact hypersensitivity in which anti-TNP IgE was administered to dams at E16.5 and NBs were challenged by painting a solution of TNP-OVA onto the dorsal skin (Fig. 2K). The antigen-specific allergic reaction was indicated by MC degranulation in the skin, as measured by flow cytometry. Vehicle-treated NBs developed no allergic reaction in the skin, whereas MC degranulation was detected in the skin of antigen-exposed neonates (Fig. 2L). Because allergic responses were acquired to an experimental antigen, these results indicate the ability of maternal IgE to induce antigen-

specific degranulation of fetal MCs and to mediate early postnatal allergic responses at the first encounter with the allergen.

Fetuses born to allergic mothers develop airway hyperreactivity upon their first allergen exposure

We next used an active sensitization model to determine whether maternal allergy can influence postnatal allergic responses of progeny and to investigate the mechanism of maternal IgE transfer to fetal mice. Female mice were sensitized using a protocol that is known to induce a strong type II allergic response similar to that observed in allergic humans (33, 34).

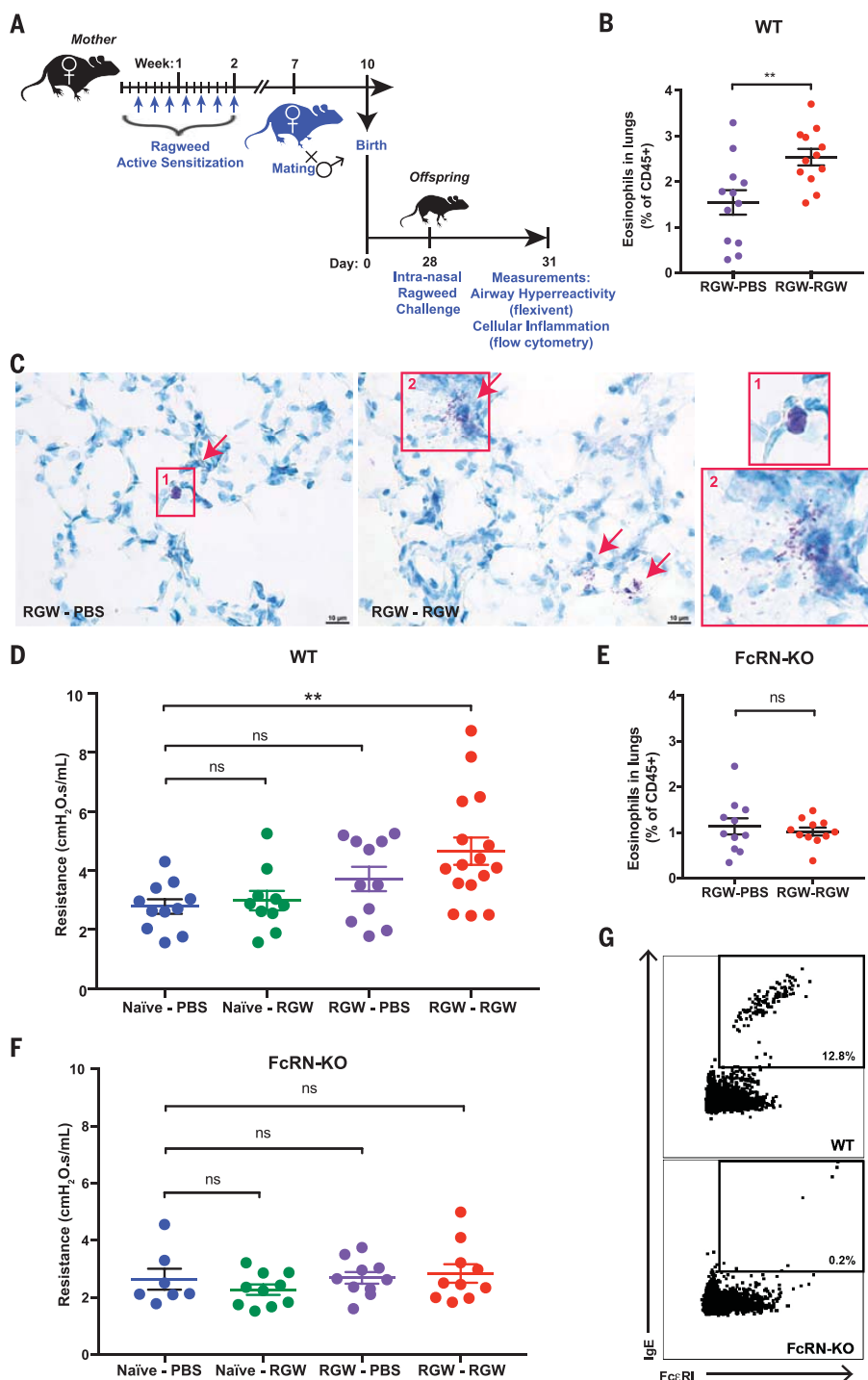


Fig. 3. Antigen-specific enhancement of allergic disease after maternal sensitization is FcRN dependent. (A) Schematic of the active sensitization protocol of mice against RGW antigen, followed by mating and measurements of allergic responses in progeny intranasally challenged with RGW or vehicle 4 weeks after birth. (B) Significant eosinophilia in the offspring of RGW-sensitized dams that were challenged with RGW (RGW-RGW) compared with littermates challenged with PBS (RGW-PBS). Eosinophils were defined as CD45⁺CD11c⁻Ly6g⁻SiglecF⁺ cells, with the gating strategy provided in fig. S5A. Data points represent individual animal responses compiled from three independent litters, with the mean $\pm$ SEM indicated. ** $P < 0.01$. (C) Visualization of intact MCs in the lungs of RGW-PBS mice and MC degranulation in the lungs of RGW-RGW mice 6 hours after challenge. Additional representative images are provided in fig. S5C. (D) Measurements of airway hyperreactivity in response to escalating doses of inhaled methacholine 72 hours after RGW or vehicle challenge. Offspring born to RGW-sensitized dams that were challenged with RGW (RGW-RGW) showed increased airway hyperreactivity compared with all other groups. The highest methacholine concentration is presented, and the full escalating dose curve is provided in fig. S6A. Data points represent individual animal responses compiled from three independent litters, with the mean $\pm$ SEM indicated. ** $P < 0.01$. (E) Eosinophilia after RGW challenge was not detected in FcRN-KO mice born to RGW-sensitized dams compared with PBS-challenged littermate controls. Data points represent individual animal responses compiled from three independent litters, with the mean $\pm$ SEM indicated. (F) In FcRN-KO mice, RGW challenge did not influence airway hyperreactivity. Data points represent individual animal responses compiled from three independent litters, with the mean $\pm$ SEM indicated. Data were compared by Student's unpaired t test for (B) and (E) and by one-way ANOVA with Holm-Sidak posttest for (D) and (F). ns, not significant. (G) IgE binding to fetal MCs is FcRN dependent, as determined by flow cytometry. Plots show MCs from the skin of E17.5 WT and FcRN-KO mice fetuses after maternal IgE injection at E16.5. Percentage of FcεRI⁺ MCs that are IgE⁺ is given.

For this, ragweed (*Ambrosia artemisiifolia*) pollen protein extract (RGW) was administered for 2 weeks for active sensitization, followed by 5 weeks without any treatments to allow for any ongoing inflammation to resolve before initiating pregnancy (Fig. 3A). Allergic reactions in the offspring were measured at 4 weeks of age by intranasal RGW allergen challenge and evaluation of their allergic inflammation after 72 hours (Fig. 3A and fig.

S5A). We observed a significant and allergen-specific increase in airway eosinophilia (Fig. 3B), which was confirmed by histology (fig. S5B). We also observed that the progeny of RGW-sensitized animals showed MC degranulation in the lungs after RGW challenge (Fig. 3C and fig. S5C). Other immune cell types, including dendritic cells and neutrophils, which are not as strongly associated with acute respiratory allergy, did not significantly differ

between the groups (fig. S5, D and E). Functionally, allergic inflammation in the lung is characterized by airway hyperreactivity (8, 33). Therefore, we measured airway hyperreactivity after RGW challenge in response to an inhaled bronchoconstricting stimulus involving intubation and exposure to escalating doses of nebulized β -methylcholine. Bronchoconstriction was reflected in the increase in airway resistance to airflow. Upon allergen

challenge, mice born from RGW-sensitized dams showed significantly higher airway hyper-reactivity compared with those born to non-sensitized dams or various controls (Fig. 3D and fig. S6A), suggesting transfer of allergy

from mother to the fetus. The control groups included mice born to sensitized or non-sensitized dams that were not challenged with an allergen (Fig. 3D and fig. S6A). Furthermore, mice born from RGW-sensitized dams

that were challenged with a nonspecific complex antigen, house dust mite, did not display allergic disease, confirming allergen specificity (fig. S6B). The effect of maternal sensitization was lost in mice aged longer when challenge was done after 6 weeks of age (fig. S6C). These data show that the offspring of allergic mothers can develop airway inflammation in an antigen-specific and time-dependent manner upon the first exposure to an allergen without prior sensitization themselves.

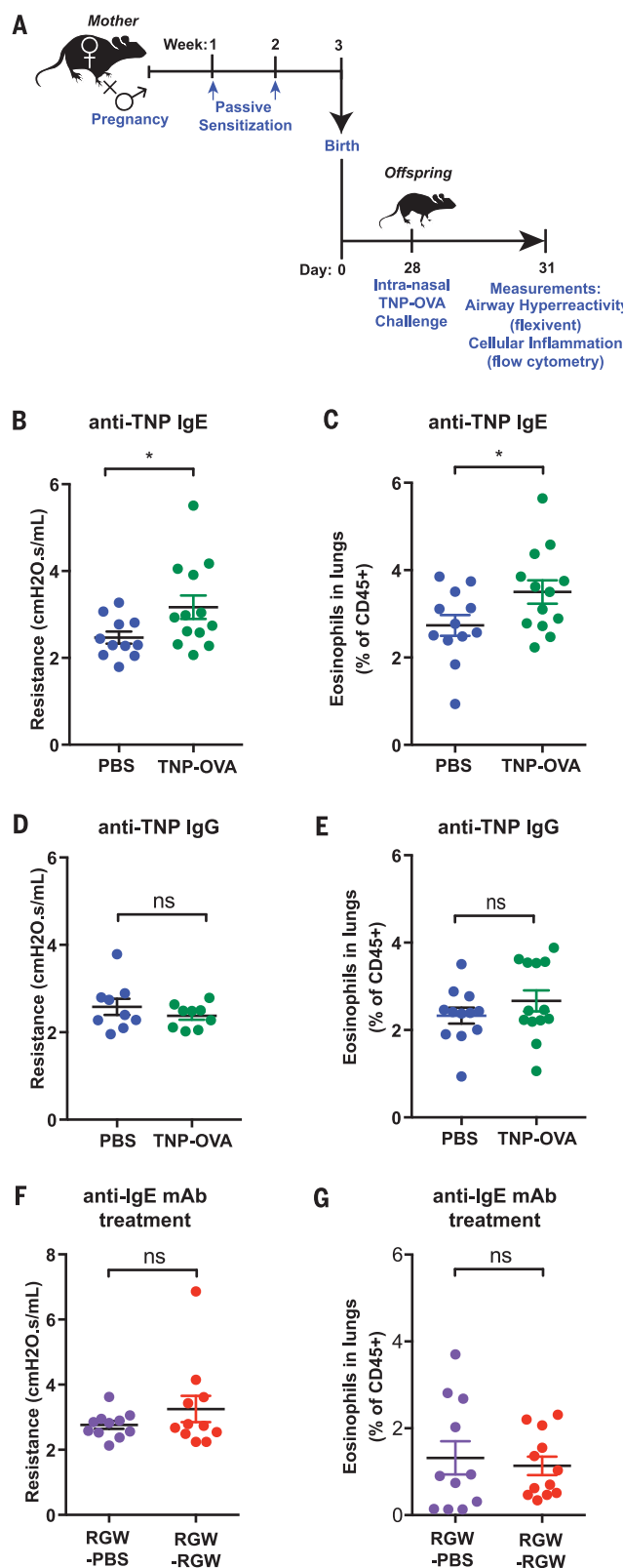
Transfer of allergic disease from mother to fetus is FcRN and IgE dependent

To gain a mechanistic understanding of how maternal allergen sensitivity is transferred to offspring in utero, we investigated the role of FcRN in this process. FcRN is expressed by the fetal endothelial cells and syncytiotrophoblast cells of the placenta, where it serves a critical role in regulating antibody transfer from mother to fetus (35, 36). Although FcRN is best characterized for its ability to transfer IgG transplacentally, we hypothesized that it was involved in IgE translocation based on recent in vitro evidence of FcRN-mediated transfer of IgG-IgE complexes (37). To test the role of FcRN in IgE translocation into the fetus and, consequently, allergy transfer, we performed the active RGW sensitization experiment in FcRN-deficient mice (Fig. 3A). In contrast to wild-type (WT) mice (Fig. 3B), there was no allergen-specific eosinophilia detected in the progeny of RGW-sensitized FcRN-deficient mice (Fig. 3E), even though adult FcRN-knockout (KO) mice had no defects in IgE production (fig. S7A) and were capable of generating intrinsic allergic responses after sensitization and challenge with antigen (fig. S7B). Furthermore, in the FcRN-deficient progeny of sensitized dams, there was no detectable increase in airway hyperreactivity to inhaled methacholine (Fig. 3F and fig. S7C). To investigate the influence of FcRN on IgE translocation into fetuses, we injected equivalent amounts of IgE into pregnant WT and FcRN-KO mice at E16.5. At E17.5, IgE could be detected by flow cytometry on the fetal skin MCs of WT but not FcRN-KO mice (Fig. 3G), supporting its ability to translocate in an FcRN-dependent manner. These results suggest that the transfer of allergen-specific sensitization from the mother to the offspring is mediated by FcRN.

Because FcRN can mediate both IgG and IgE transfer (37), we investigated whether IgE is sufficient to cause postnatal allergic inflammation. We used a passive sensitization model in which pregnant mice were injected with TNP-specific IgE (Fig. 4A). Juvenile mice were challenged 4 weeks after birth with TNP-OVA, and the allergic responses in the lungs were measured 72 hours after challenge and compared with their unchallenged control littermates. Antigen challenge induced significant

Fig. 4. Maternal IgE can mediate antigen-specific allergic disease. (A) Schematic of the passive sensitization model in which

TNP-specific IgE or IgG was injected into pregnant mice 8 and 16 days after confirmation of pregnancy, and groups of littermates from three independent litters were challenged intranasally with TNP-OVA or vehicle 4 weeks after birth. Allergic responses in terms of lung eosinophilia were measured 3 days after challenge. (B) Airway resistance was significantly elevated 3 days after challenge with the experimental antigen TNP-OVA compared with phosphate-buffered saline (PBS)-challenged littermate controls after passive sensitization of dams with TNP-specific IgE during pregnancy. (C) Eosinophilia was observed in the lungs and TNP-OVA-challenged mice compared with PBS-challenged littermate controls. (D and E) No differences in airway resistance (D) or eosinophil infiltration (E) were observed between littermates given TNP-OVA or PBS challenges after passive sensitization of their mothers with anti-TNP IgG. (F and G) No differences in airway resistance (F) or eosinophil infiltration (G) were observed between littermates of mice born to females that were actively sensitized to RGW as in Fig. 3A and treated with a monoclonal antibody against IgE at E8.5 during pregnancy. Groups were compared by Student's unpaired *t* test. **p* < 0.05; ns, not significant. For (B) to (F), data points represent individual animal responses of three litters of progeny, with the mean ± SEM indicated.



airway hyperreactivity (Fig. 4B) and eosinophil influx into the lungs (Fig. 4C), indicative of an IgE-dependent allergic response. Similar to the active sensitization model, these responses were lost in FcRN-KO mice (fig. S8, A and B). The hyperreactivity responses were also dose dependent, with a higher dose of IgE inducing a stronger effect in the progeny compared with littermate controls (fig. S8, C and D). In contrast to IgE, injection of an equivalent amount of anti-TNP IgG was insufficient to

induce detectable airway hyperreactivity or inflammation (Fig. 4, D and E). To further support that IgE mediates these allergic responses, we also used a monoclonal blocking antibody against IgE, which is a similar strategy to the anti-IgE human therapeutic omalizumab, an effective treatment for some types of allergic disease (38, 39). Injection of RGW-sensitized pregnant dams with anti-IgE on E8.5 of pregnancy (Fig. 3A) significantly reduced subsequent allergic responses in the

progeny so that RGW challenge failed to increase airway resistance (Fig. 4F) or induce eosinophilia (Fig. 4G), supporting the critical role of IgE in this model.

Because IgE transfer could occur in complex with IgG (37), we confirmed the presence of IgG-IgE immune complexes in the blood of sensitized dams (fig. S8E). We investigated whether IgE could be observed in fetal endothelial cells of the placenta after injection in pregnant dams. Staining of placental tissues

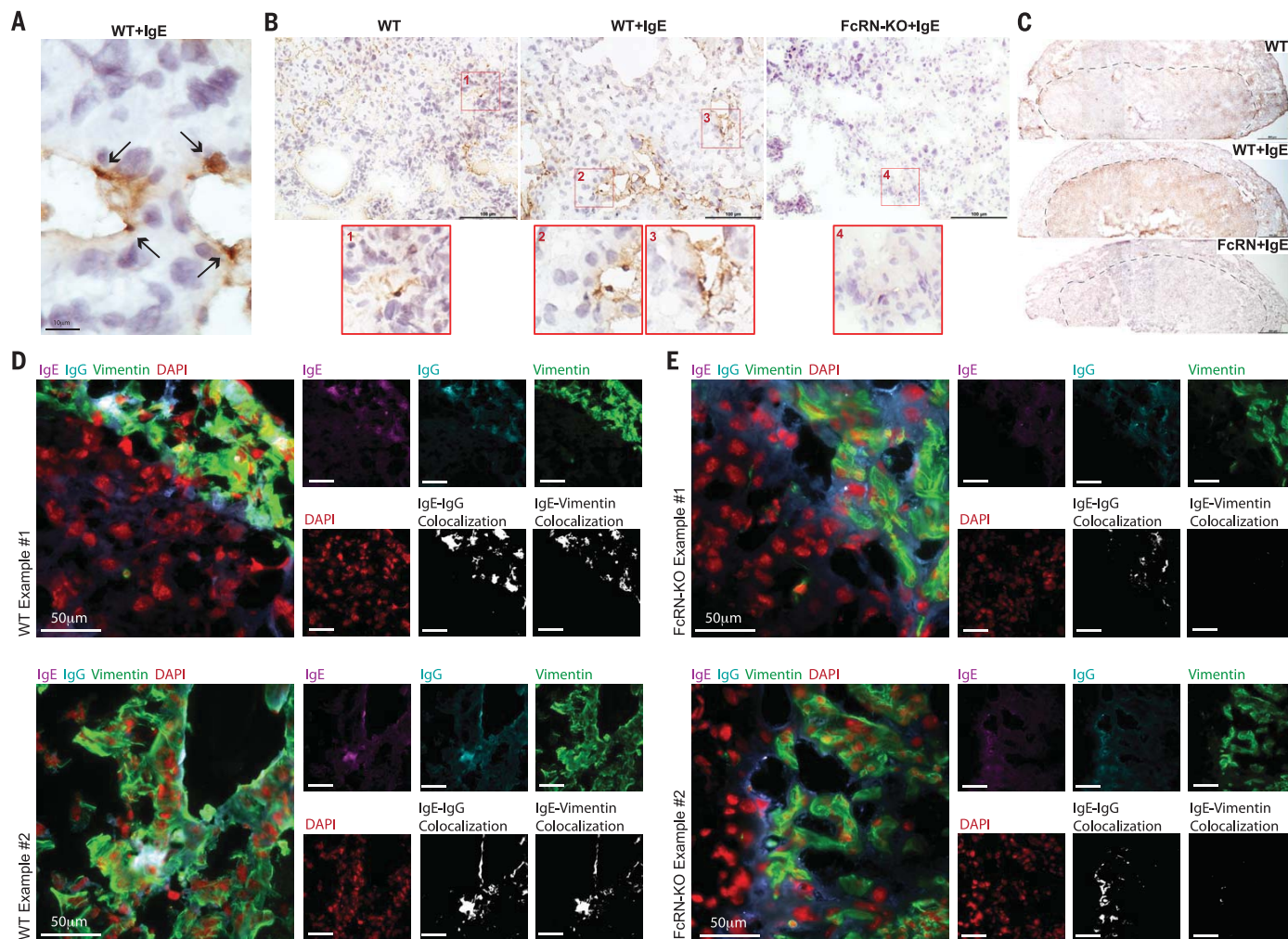


Fig. 5. IgE associates with IgG in fetal endothelial cells of the placenta.

(A) High-magnification immunohistochemistry image showing IgE-stained endothelial cells in the placenta from E17.5 of mice that received exogenous IgE. The IgE staining was noted in endothelial cells lining the blood vessels (arrow). Scale bar, 10 μ m. (B) IgE-bound endothelial cells were also observed in naive (WT) mice, albeit in small numbers. IgE injection (WT+IgE) increased areas of IgE-stained endothelial cells, which was not observed in FcRN-KO mice (FcRNKO+IgE). Scale bar, 100 μ m. The red boxed areas are shown at higher resolution below. (C) Stitched images depicting IgE staining in entire placentas from WT naive, WT+IgE, and FcRN-KO+IgE mothers. A dashed line demarcates maternal decidua (above) from the fetal labyrinth (below). IgE staining was largely observed in the fetal labyrinth, which is densely packed with fetal blood vessels. IgE staining in the labyrinth substantially increased when IgE was injected into mothers. IgE staining was not

observed in FcRN-KO mice. Scale bar, 500 μ m. For (A) to (C), immunohistochemistry was performed using anti-IgE antibody, and dark brown color shows positive signal for IgE staining. Sections were counterstained with hematoxylin to discern the tissue architecture, and the nuclei appear blue. (D and E) Multicolor immunofluorescent images showing staining of IgE (magenta), IgG (cyan), fetal endothelial vessels (green), and nucleus (red) in the placenta. (D) In WT placenta, colocalization of IgE with IgG (IgE-IgG complexes) and IgE with fetal vessels (uptake of IgE-IgG complexes by fetal endothelial cells) was noted. Scale bar, 50 μ m. Images are representative of $n = 3$ animals. (E) Although IgE-IgG complexes were formed in FcRN-KO mice, no colocalization of IgE with fetal vessels was observed. Two representative images from each group are presented as examples 1 and 2. Scale bar, 50 μ m. Images are representative of $n = 2$ animals. The data indicate that IgE binding to fetal endothelial cells is dependent on FcRN.

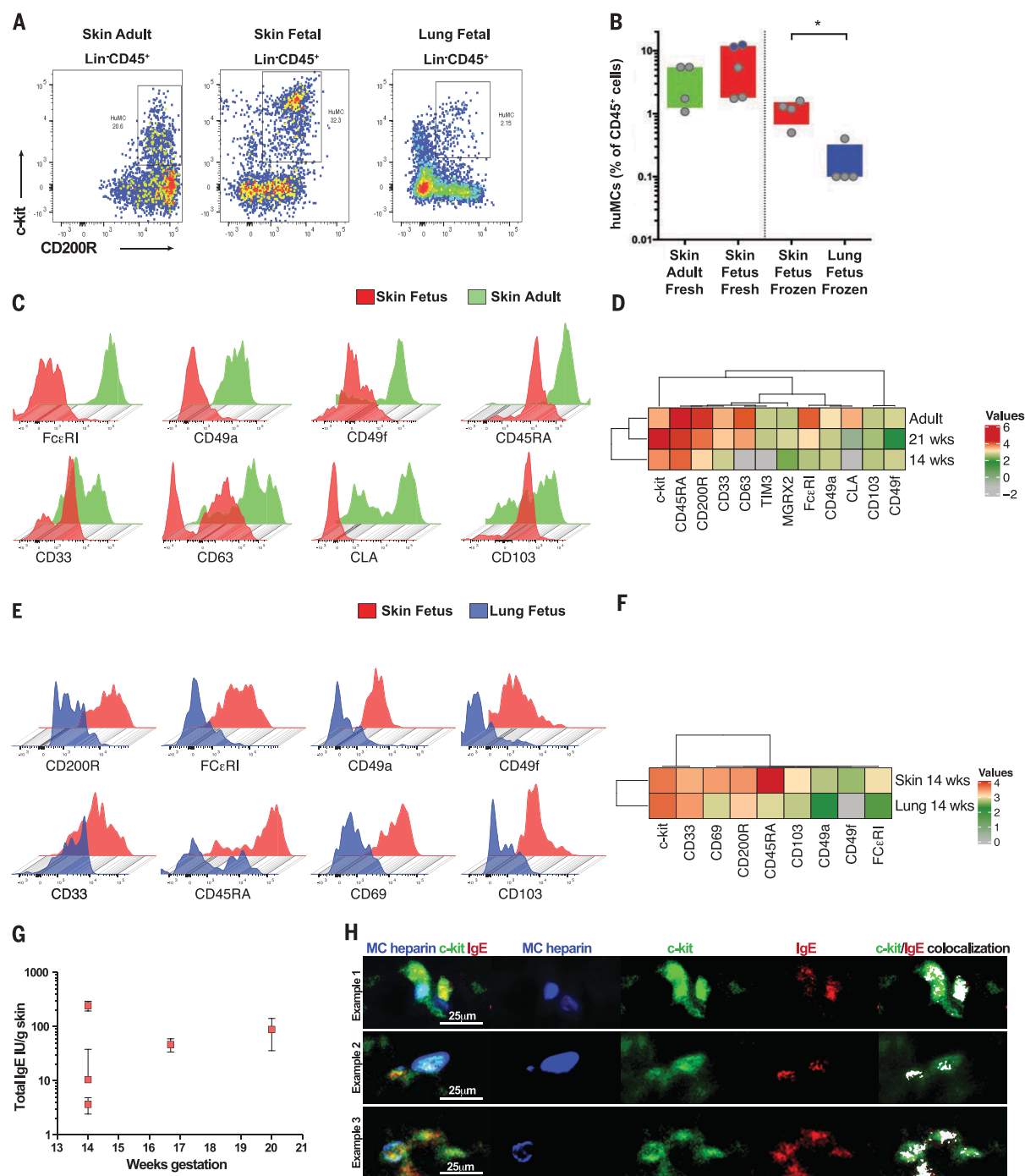


Fig. 6. Human fetal MCs mature in utero, where they also bind IgE. (A) Flow cytometry plots depicting the identification of huMCs in adult and human fetal tissues as c-kit⁺CD200R⁺ from cells gated on CD45⁺Lin⁺ (CD123⁺CD14⁺HLA-DR⁺CD56⁺CD34⁺CD127⁺, as shown in fig. S9, A and B) for skin and lung tissues at 14 weeks of gestation. (B) Quantification of huMCs in adult skin and fetal skin and lungs as a percentage of CD45⁺ cells. huMCs constituted a reduced percentage of CD45⁺ cells in the lung compared with the skin. $p < 0.05$, determined by Student's unpaired t test. Data points indicate individual donor samples. (C) Flow cytometry histograms of expression of various surface markers on huMCs (CD45⁺CD123⁺CD14⁺HLA-DR⁺CD56⁺CD34⁺CD127⁺c-kit⁺CD200R⁺ cells, as defined in fig. S9A). Control gray histograms for comparison of expression levels are adult skin T cells. (D) Heatmap of the relative expression of various markers on fetal skin huMCs compared with adult skin huMCs by multicolor flow cytometry. Heatmap presentations in the form of

flow cytometry plots are provided in fig. S9B. For (A) and (B), $n = 3$ for 14 weeks of gestation, $n = 2$ for 21 weeks of gestation, and $n = 4$ for adult samples. (E) Flow cytometry comparison of the relative expression of various markers of fetal huMCs and lung huMCs at 14 weeks of gestation. (F) Heatmap of the relative expression of various markers on fetal skin huMCs compared with paired lung huMCs from the same donors ($n = 4$ for 13 to 15 weeks of gestation) by multicolor flow cytometry, both defined as in fig. S9, A and C. (G) Quantification of human IgE in fetal skin. Graph depicts the concentrations in samples from five individual fetuses, with the error bars representing the SEM of technical replicates for each sample. (H) Immunofluorescence staining of fetal human skin showing colocalization between MC and IgE (in white), detected by overlapping signals for heparin (blue) and c-kit (green) with IgE (red). Rows are replicates showing representative MCs. Scale bar, 25 μm. An experimental repeat of this panel using an alternate antibody against human IgE is provided in fig. S10.

showed that IgE was detectable in the placenta, associated with cells having endothelial cell morphology (Fig. 5A), and the staining was brighter after maternal IgE injection (Fig. 5, B and C). By contrast, FcRN-KO mice lacked staining for IgE in the placenta (Fig. 5, B and C). We confirmed the lack of IgE association with fetal endothelial cells by confocal microscopy. IgE was detected to be colocalized with IgG within vimentin-positive fetal endothelial cells in WT mice (Fig. 5D). However, in FcRN-KO mice (Fig. 5E), IgG and IgE appeared colocalized but neither showed colocalization with the fetal endothelial cells, further supporting the FcRN-dependent mechanism of IgE transfer by vimentin-expressing placental endothelial cells.

Human fetal MCs are phenotypically mature and IgE bound

We next investigated whether fetal human MCs (huMCs) are phenotypically mature and if, similar to fetal murine MCs, they are associated with maternal IgE. To characterize the phenotype of huMCs from the early second trimester of pregnancy (14 weeks) compared with adult skin huMCs, we used a comprehensive multicolor flow cytometry panel. Skin and lung huMCs were identified in concatenated samples for each time point ($n = 3$ for 14 weeks and $n = 4$ for adult samples) by gating on lineage-negative hematopoietic cells that were positive for the MC marker c-kit, and stem cells were excluded by selecting cells positive for the myeloid lineage marker CD200R (CD45⁺CD123⁻CD14⁻HLA-DR⁻CD56⁻CD34⁻CD127⁻c-kit⁺CD200R⁺; Fig. 6A and fig. S9, A and B). The percentage of huMCs in adult and fetal skin were comparable, whereas the fetal lung had a lower percentage of huMCs than paired fetal skin from the same donors (Fig. 6B), similar to our murine results. Fetal skin samples showed heterogeneous expression of markers related to huMC functionality, such as FcεRI and CD63, but generally expressed lower levels than adult skin huMCs (Fig. 6C). Other markers were decreased in expression in fetal samples, such as cutaneous lymphocyte associated antigen (CLA) (Fig. 6C), whereas the levels of c-kit were higher in fetal compared with adult huMCs (Fig. 6A). We also used a staining panel designed to characterize the expression of a broad array of markers associated with MCs or tissue residency, including integrin and adhesion molecules. This revealed that MC maturation was also characterized by increasing expression of CD200R, CD45RA, CD33, MGRX2, and Tim-3 (T-cell immunoglobulin and mucin-domain containing-3), as well as integrin family molecules such as CD49a and CD49f (Fig. 6C and fig. S9C). Gradual increases in markers such as FcεRI, CLA, CD63, CD200R, and others from weeks 14 to 21 ($n = 2$) of pregnancy to adult huMCs suggests maturation of huMCs in the

skin throughout gestation (Fig. 6D). We also analyzed paired fetal skin and lung tissues from the same donors ($n = 4$) and found that MCs had a similar phenotype in terms of expression of cellular markers (Fig. 6, E and F), although skin MCs had higher expression levels of certain markers associated with maturation such as FcεRI and CD200R in the paired ~14-week samples (Fig. 6, E and F). Together, these results emphasize fetal MC heterogeneity and indicate that by the second trimester, there is a subset of MCs present that is phenotypically mature.

We next sought to confirm whether the other component required for allergic sensitization, IgE, is detectable in human fetal skin and bound to MCs. Quantification of IgE in human fetal skin showed that it was detectable in all samples obtained from various gestational time points (Fig. 6G). Human fetal skin was shown by confocal microscopy to contain both immature and granulated MCs (Fig. 6H). Multiple staining methods showed that IgE colocalized with c-kit staining, supporting that IgE in human fetal skin is MC bound (Fig. 6H and fig. S10). These data indicate that human fetal MCs can be sensitized with IgE in utero.

Concluding remarks

Our results demonstrate that maternal allergy predisposes progeny for allergen-specific disease upon the first antigen exposure postpartum. In humans, allergies frequently start during very early life (15, 40), and allergic symptoms can also apparently develop upon the very first exposure to a triggering antigen (29). Maternal but not paternal IgE levels are predictive of infant atopic status (41, 42). Furthermore, the sensitization profile of IgE in maternal blood highly correlates with that of fetal blood (43, 44), as does the ratio of allergen-specific and total IgE in the maternal blood and cord blood (45). These studies suggested that neonates can be exposed to factors predisposing for allergic disease even before birth and are consistent with the mechanistic role that we have defined here for maternal IgE in mediating fetal allergic responses in experimental murine models. We also observed that MCs in human fetal skin and lungs are phenotypically mature, and in the skin they appear to be bound to IgE, suggesting that similar allergic responses may occur in humans postpartum.

Increased allergic responses that were allergen nonspecific have been observed in the progeny of atopic mice and were thought to be related to active maternal Th2 inflammation during gestation (46, 47). However, those studies involved acute allergic sensitization or inflammation in the mother during pregnancy and a period of offspring sensitization to the experimental allergen after birth, which our results show may not be necessary for postnatal allergy. Thus, our study adds to a growing

literature revealing developmental origins of health and disease (48), where prenatal exposure to maternal allergen-specific antibodies can influence subsequent health and, potentially, long-term disease. Indeed, our active sensitization model indicates that allergic responses induced weeks before pregnancy in mothers can mediate antigen-specific and allergic inflammation in the skin and lungs of the progeny even several weeks after birth. Although these responses may be important during the early weeks of life, we noted that the maternally derived allergic responses waned over time and were lost by 6 weeks after birth in the airway challenge model. We also observed a dose dependency on the concentrations of IgE. Subsequent studies will be needed to determine whether this decay in maternally derived allergic disease is due to the half-life of IgE or to the turnover or reduced presence of fetal MCs or other factors, and if fetal exposure to allergen can prolong the maternal influence on allergic responses.

Until recently, the source of fetal IgE was unclear, because it was thought that FcRN mediated only IgG transfer from mother to fetus. A recent study examining IgG abundance in human fetuses showed that antibody transfer occurs earlier than previously thought (49), and in mice this FcRN-dependent transcytosis begins shortly after placentation starts (50). Our results support in vitro demonstrations that there is an FcRN-dependent mechanism for maternal IgE transcytosis (37), because colocalization of IgE with fetal endothelial cells and the effects of active maternal sensitization were both lost in mice lacking FcRN. It has also been shown that the binding of monomeric IgE to FcεRI on MCs can modify many aspects of the MC state, including expansion, survival, migration, and activation (2, 51–53). Consistent with this, we observed here that fetal MC maturation is induced by exogenous IgE. Atopic individuals frequently have high circulating levels of total IgE, which are correlated with the severity of allergic disease (54–57). Because we observed that passive sensitization of pregnant mice with IgE induced MC maturation, this suggests that fetal MC maturation in humans may be heterogeneous and influenced by the varied levels of maternal IgE available to the fetus. Our assessments of fetal huMCs supported that IgE was bound to them, but further studies are needed to assess the correlations between maternal IgE responses and fetal MC phenotypes. In adult humans, allergic disease and positive skin test reactions are correlated with serum IgE levels (58), and we also observed correlations between the amounts of IgE bound to fetal skin MCs and the concentrations injected into the dams in the mouse model.

The ratio of IgG to IgE may play a role in whether maternal antibodies are able to sensitize

offspring, because it has been shown in animal models that high levels of maternal IgG can reduce sensitization of offspring to experimental antigens (59). In our model, injection of the same concentration of IgG that induced IgE-mediated allergy did not result in detectable allergic responses. Although this does not exclude the potential of maternal IgG to participate in allergic responses in progeny, it suggests that those responses are less likely. This is consistent with the fact that 1000-fold higher concentrations of IgG compared with IgE are needed to induce MC activation (60). Although IgG-antigen complexes are able to induce MC degranulation (61, 62), IgG sensitization of MCs in the absence of antigen is also less likely to occur because the affinity of IgG for activating receptors on MCs is greatly increased in the form of immune complexes (60). Our results using the anti-IgE monoclonal antibody, which abrogated fetal allergic responses when injected into pregnant dams, further support the specific role of IgE in mediating maternally derived allergic responses. In addition, differing levels of IgE were detected in the human fetal skin samples analyzed here. Further studies are needed to determine whether these levels have any correlation with the mother's allergic status and if antibodies in maternal breast milk, which contains multiple subclasses of antibodies including IgE (63, 64), could also contribute to or modulate allergic sensitization postpartum.

Although MCs have been best described for their contributions to harmful allergic responses, they also contribute to protective immunity against multiple classes of pathogens. Conceivably, as in adults, IgE-mediated activation of fetal MCs could be protective in the context of postpartum exposure to certain pathogens, toxins, and venoms to which mothers have been exposed and against which they have subsequently mounted a protective response. Therefore, transfer of maternal IgE to the fetus may confer transient but potent immunity to various pathogens encountered during the mother's lifetime, mediating crucial protective functions in early life, aside from their implications in harmful allergic responses as shown here. However, it also remains to be understood whether maternal IgE could play a role in sensitizing progeny for intrinsic IgE production, which might either have protective functions in

some circumstances of pathogen exposure or, alternatively, exacerbate allergies later in life. Our broadened understanding that fetal MCs are mature and capable of mediating IgE-dependent responses has implications for understanding both fetal health and long-term postnatal immune homeostasis.

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Data and materials availability: All data needed to evaluate the conclusions of this study are present in the main text and/or the supplementary materials.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S10
Table S1
References (65–67)

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Fetal mast cells mediate postnatal allergic responses dependent on maternal IgE

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Maternal IgE activates fetal mast cells

Mast cells (MCs) are immune cells that participate in allergic reactions through their activation by immunoglobulin E (IgE) antibodies. MCs arise early during mammalian development, but it is unclear whether IgE-mediated activation occurs in fetal tissues and what the source of IgE stimulation is. Msallam *et al.* show that human and mouse fetal MCs can be sensitized by IgE of maternal origin, which crosses the placental barrier through the fetal neonatal Fc receptor (see the Perspective by Rothenberg). Prenatal maternal sensitization conferred transient allergen sensitivity after birth and resulted in the development of postnatal skin and airway inflammation in the offspring after their first exposure to allergen. Thus, both maternal IgE and fetal MCs may influence mother-to-child transmission of allergic disease during gestation.

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