Adoptively Transferred Allergen-Specific T Cells Cause Maternal Transmission of Asthma Risk

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In addition to genetics and environment, maternal asthma is an identified risk factor for developing the disease during childhood. The mechanisms of this maternal effect remain poorly understood. We tested the role of allergen-specific T cells in the maternal transmission of asthma risk by modifying a model where offspring of asthmatic mothers are more prone to develop asthma after an intentionally suboptimal asthma induction. Normal BALB/c females were injected with allergen-specific T cells from ovalbumin-specific T cell receptor (TCR) transgenic DO11.10 donors before mating. Using the protocol of suboptimal asthma induction, offspring of normal and recipient mothers were tested for their susceptibility to develop asthma. Only pups of recipient mothers showed increased airway responsiveness (Penh), allergic airway inflammation with eosinophilia, and local Th2-skewed cytokine production. Although recipient mothers did not develop asthma, serum levels of interferon- γ , interleukin (IL)-4, IL-10, and IL-13 were significantly increased during pregnancy. Consistent with this finding, a subset of DO11.10 T cells persisted in the spleen and placenta of expectant recipient mothers. We conclude that allergen-specific T cells are sufficient to orchestrate the maternal transmission of asthma risk. Because overt maternal asthma was not required, our results suggest that similar maternal-fetal interactions may occur in other allergic disorders. (*Am J Pathol* 2006, 168:1931–1939; DOI: 10.2353/ajpath.2006.051231)

Allergic asthma is a respiratory disorder with origins in early life.^{1,2} Its complex etiology includes genetic susceptibility and exposure to environmental factors.^{3,4} An additional element, revealed by epidemiological studies, is that maternal asthma significantly increases the risk for

children to develop the disease.^{5–7} This maternal effect suggests that prenatal events dramatically influence the early susceptibility to allergic airway disease.⁸

To investigate the immunological mechanisms involved in the maternal transmission of asthma risk, we have developed a murine model that allows focused investigations on mothers and neonates without the confounding influence of genetics⁹ or exogenous stimuli (eg, infections).¹⁰ Similar to epidemiological data, pups of asthmatic BALB/c mother mice are more susceptible to develop asthma, even in response to an allergen (casein) unrelated to the ovalbumin (OVA) used to sensitize their mothers. This model thus excludes a critical role for transplacental passage of the allergen itself or allergen-specific immunoglobulins produced by the mother.^{11,12} In fact, because treatment of asthmatic females with anti-IL-4 monoclonal antibodies before mating significantly ameliorated the offspring susceptibility to asthma,⁹ our results point to a prominent role for maternal Th2 cytokines. Because T lymphocytes can provide the Th2 cytokines that both induce allergic inflammation and alter the lung function of asthmatics,¹³ we postulated that allergen-specific T cells are sufficient, in our model, to mediate the maternal transfer of asthma risk.

We addressed this hypothesis by replacing the asthmatic mothers used in our previous studies^{9,14,15} with normal BALB/c females injected with OVA-specific T cells from the DO11.10 strain.^{16,17} The females were then challenged with OVA aerosols before mating with normal BALB/c males. This protocol allowed us to distinguish immunological changes exclusively due to pro-allergic T cells during the pregnancy of otherwise normal mother mice.

Materials and Methods

Mice

Adult 6- to 7-week-old BALB/c mice and 4-day-old litters were obtained from Charles River Laboratories (Wilmington,

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Figure 1. Model of adoptive T-cell transfer and maternal transfer of asthma risk using recipient BALB/c females injected with DO11.10 T cells.

ton, MA). DO11.10 mice were originally purchased from the Jackson Laboratory (Bar Harbor, ME) and maintained in our facility by in-house breeding. All of the mice were housed in a pathogen-free barrier facility, and experiments were conducted under a protocol approved by our Institutional Animal Care and Use Committee.

Adoptive Transfer of T Cells from DO11.10 Donor Mice

T lymphocytes were collected from the spleens of age-matched DO11.10 female mice. Spleens were placed in chilled RPMI 1640 (Bio-Whittaker, Walkersville, MD) and cut into four to five pieces. Cells were expelled from the splenic capsule through a 70- μ m nylon mesh filter. After red blood cell lysis, splenocytes were resuspended in separation buffer (phosphate-buffered saline, pH 7.2, supplemented with 0.5% bovine serum albumin and 2 mmol/L ethylenediamine tetraacetic acid). T cells were collected by means of magnetic beads, MidiMACS separation columns, and pan-T-cell isolation kits from Miltenyi Biotec (Auburn, CA). The kits consisted of a mixture of biotin-conjugated monoclonal antibodies (mAbs) against CD11b (Mac-1, rat IgG2b), CD45R (B220, rat IgG2a), DX5 (rat IgM), and Ter-119 (rat IgG2b), in addition to anti-biotin (Bio3-18E7.2; mouse IgG1)-conjugated "superparamagnetic" microbeads. After this purification by negative selection, the unmanipulated T cells were injected (intraperitoneally) into recipient BALB/c female mice. Pilot studies evaluating the susceptibility of mouse pups used the suboptimal protocol for asthma induction (see below; Figure 1) to identify as "optimal" the injection of 5×10^6 DO11.10 T cells per recipient BALB/c female mouse. This was based on increased variability with a lower inoculum (2×10^6 T cells), results similar to that seen in pups from asthmatic mothers after transfer of 5×10^6 cells, and lack of any further change with a higher inoculum (10^7 T cells).

Allergen Sensitization and Aerosol Exposure

The model of maternal asthma using OVA-sensitized and aerosol-exposed mice is detailed elsewhere.⁹ The present protocol is described in Figure 1. Briefly, normal BALB/c female mice were injected (intraperitoneally) with 5×10^6 T cells from DO11.10 spleens (day 0). Recipient females were allowed to mate with normal BALB/c males (day 3) after exposure to 3% OVA aerosols (days 1 to 3; 10 minutes per day). Offspring of recipient mothers and untreated BALB/c controls were all submitted to an intentionally suboptimal asthma induction protocol that fea-

tures a single (rather than two) intraperitoneal injection of 5 μ g of OVA with 0.5 mg of Al(OH)₃ (day 4 of life), followed by 3% OVA aerosols (days 12 to 14; 10 minutes per day). When indicated, OVA was replaced by casein (CAS), with the only difference that 1% CAS aerosols were performed for 20 minutes. Final physiological and pathological analyses were performed on days 15 and 16, as previously described.⁹

Pulmonary Function Testing and Pathological Analysis

Airway responsiveness (Penh) to increasing concentrations (0 to 100 mg/ml) of aerosolized methacholine (MCh) was evaluated 24 hours after the last allergen challenge in mothers and offspring. The use of unrestrained plethysmography, in particular enhanced pause (Penh), in assessing mouse lung function is relatively controversial.¹⁸ However, Penh and compliance are highly correlated with lung resistance in the BALB/c strain that was exclusively used in the present experiments.¹⁹ Moreover, it is worth noting that our model uses mouse pups that are too small to be tested through more invasive techniques. Bronchoalveolar lavage (BAL) was performed on euthanized animals 48 hours after the last allergen challenge (day 5 of the protocol for mothers; day 16 of life for offspring). Total cell counts were quantified by hemocytometer. BAL differential cell counts were performed on cytocentrifuge slides stained with Diff-Quick (VWR, Boston, MA).

Multiplex Cytokine Assay

BAL and serum cytokines were quantitated using multiplex biomarker immunoassay kits from Linco Research (St. Charles, MI) and xMAP technology (Luminex, Austin, TX), according to the manufacturer's instructions. Color-coded microspheres were used for the detection of mouse interferon- γ (IFN- γ), interleukin (IL)-4, IL-5, IL-10, and IL-13. Standards and internal controls were added for each cytokine. Separate assays were performed for BAL and serum samples, using appropriate dilution buffers. Raw data were analyzed using Masterplex QT 2.0 software (Luminex). Graphs are representative of data averaged from three to six animals per group, with each sample assayed in duplicate. Final concentrations were expressed in picograms/milliliter $\pm$ SEM. Minimal detectable concentrations for each cytokine ranged from 0.3 pg/ml (IL-4) to 10.3 pg/ml (IL-10).

Flow Cytometric Analysis of DO11.10 T Cells in Recipient Animals

Cells were collected from the spleen and placenta of recipient mothers in the 3rd week of mouse pregnancy. After red blood cell lysis, cells were resuspended in staining buffer (Hanks' balanced salt solution containing 0.1% NaN₃ and 1% bovine serum albumin) and labeled with a combination of fluorescent mAbs. Fluorescein iso-

thiocyanate-conjugated mouse anti-mouse DO11.10 TCR mAbs (clone KJ1-26, IgG2a) were purchased from Caltag Laboratories (Burlingame, CA). Other mAbs were purchased from Pharmingen (San Diego, CA): peridinin chlorophyll protein (PerCP)-conjugated rat anti-mouse CD4 (clone RM4-5, IgG2a), allophycocyanin (APC)-conjugated rat anti-mouse CD8a (clone 53-6.7 IgG2a), and R-phycoerythrin (R-PE)-conjugated rat anti-mouse natural killer (NK)/natural killer T (NKT) cell antigen (clone U5A2-13, IgG2a). U5A2-13 mAb allowed identification of cell populations with a profile and function similar to T cells expressing NK1.1 (CD161c).^{20,21} Four-color flow cytometric analysis was performed with a FACScalibur (Becton-Dickinson Immunocytometry Systems, San Jose, CA). Cells were gated on the lymphocyte population based on forward and side light-scatter properties. T-cell subsets were analyzed according to fluorescent labeling, whereas nonlabeled cells and cells incubated with control isotypes were used to determine the staining background. Cells labeled with a single fluorescent mAbs were used to adjust the separation threshold of the cytometer channels. A minimum of 100,000 events were acquired, and analysis was performed on a semilogarithmic scale using FlowJo for Macintosh (version 6.0; Tree Star Inc., Ashland, OR).

Statistical Analysis

All data were presented as means $\pm$ SEM. Differences between groups were compared using one-way analysis of variance with protected least significant differences Fisher's test. StatView software program (Abacus Concepts, Berkeley, CA) was used for statistical analysis, and a $P < 0.05$ was considered significant.

Results

Increased Asthma Susceptibility in Offspring of Recipient Mothers

Our first goal was to determine whether allergen-specific T cells can recreate the maternal effect. Therefore we compared offspring of recipient and normal mothers after all of the pups were subjected to the intentionally suboptimal protocol of asthma induction described in Figure 1.

Figure 2A shows that inflammatory infiltration comprised of mononuclear cells and eosinophils was observed only in the airways of pups from recipient mothers. Airway inflammation was confirmed in BAL samples (Figure 2B) where total cell counts were significantly higher in pups of recipient mothers ($P = 0.001$). Moreover, a prominent eosinophilia was present in BAL samples from pups of recipient mothers (Figure 2C; $P = 0.017$). Airway responsiveness was tested by means of unrestrained plethysmography. During a MCh aerosol challenge (Figure 3A), pups of recipient mothers produced higher Penh values compared with normal control pups ($P = 0.035$ at 12 mg/ml MCh, and $P < 0.001$ from 25 to 100 mg/ml MCh). Finally, multiplexed cytokine assay (Luminex) with BAL

Figure 2. Offspring of recipient mothers show increased allergic airway inflammation after an intentionally suboptimal asthma induction. **A:** Pathological examination showed that only offspring of recipient mothers develop inflammatory infiltrates comprised of eosinophils and mononuclear cells surrounding the bronchi and vessels. **B:** BAL sample analysis showed increased total cell count in pups of recipient mothers (** $P = 0.001$). **C:** This inflammatory influx was characterized by eosinophilia in pups of recipient mothers (** $P = 0.017$) ($n = 14$ – 27 pups per group).

samples showed a local Th2 cytokine imbalance (Figure 3B), as illustrated by increased levels of IL-5 ($P = 0.027$) and IL-13 ($P = 0.02$), whereas the increase in IL-4 levels remained just below significance ($P = 0.059$). A trend toward decreased IFN- γ and IL-10 production was also observed, yet not statistically significant. These results show that recipient mothers injected with OVA-specific T cells from DO11.10 donors

Figure 3. Offspring of recipient mothers show increased airway responsiveness and Th2-skewed cytokine production after an intentionally suboptimal asthma induction. **A:** During a MCh aerosol challenge, offspring of recipient mothers showed increased Penh values from 12 ($*P = 0.035$) to 100 ($***P < 0.001$) mg/ml MCh ($n = 36$ pups per group). **B:** Multiplex cytokine assay on BAL samples showed Th2-skewed cytokine production in pups of recipient mothers, with IL-5 ($*P = 0.027$) and IL-13 ($*P = 0.02$) levels significantly increased ($n = 3$ –6 samples per group, duplicate).

can transfer asthma risk to their pups, as do asthmatic mothers.⁹

Maternal Responses Are Allergen Specific, but the Maternal Effect Is Allergen Nonspecific

The use of recipient females injected with DO11.10 T cells raises a number of questions requiring experimental controls. For example, harvesting and adoptive transfer could nonspecifically activate T cells in a manner unrelated to maternal asthma or allergy. Because DO11.10 donors are otherwise normal, BALB/c mice expressing an OVA-specific transgenic TCR, we tested the role of their OVA specificity by performing the adoptive transfer of T cells from normal, unsensitized BALB/c donors in lieu of DO11.10 mice. We then tested the asthma susceptibility of offspring from these recipient mothers (BALB/c T cells). Conversely, we tested the specificity of the maternal effect by submitting the offspring of recipient mothers (DO11.10 T cells) to the intentionally suboptimal asthma induction using CAS, an antigen unrelated to the OVA recognized by DO11.10 T cells. Figure 4 summarizes these control experiments.

Recipient mothers injected with BALB/c T cells did not transfer asthma risk to their offspring, because these

Figure 4. Maternal responses are allergen specific but the maternal effect is allergen nonspecific. Pups of control and recipient mothers were submitted to an intentionally suboptimal asthma induction using casein (CAS model) in lieu of OVA. Adoptive transfer of plain BALB/c T cells into recipient females and OVA challenge before mating did not induce maternal transmission of asthma risk. Pups of recipient mothers (BALB/c T cells) showed airway responsiveness (**A**), BAL total cell counts (**B**), and BAL differential cell count (**C**) similar to those of pups from normal mothers. Conversely, pups of recipient mothers injected with DO11.10 T cells and OVA-challenged before mating were susceptible to suboptimal asthma induction using CAS in lieu of OVA. Pups of recipient mothers (DO11.10 T cells) developed higher Penh values in response to a MCh aerosol challenge ($*P = 0.05$ and $***P < 0.001$) (**A**), increased BAL total cell counts ($***P < 0.001$) (**B**), substantial eosinophilia ($***P < 0.001$) (**C**), and increased lymphocyte numbers ($**P = 0.01$) ($n = 11$ –19 pups per group).

pups showed a minimal response to MCh aerosol challenge (Figure 4A), normal BAL cell counts (Figure 4B), and low eosinophil numbers (Figure 4C), all of which were similar to those found in normal offspring. Conversely, pups of recipient mothers injected with DO11.10 T cells showed higher susceptibility to asthma after an intentionally suboptimal CAS sensitization and CAS-aerosol exposure. When compared with normal offspring, pups of recipient mothers (DO11.10 T cells) showed increased Penh values in response to MCh aerosol challenge (Figure 4A; $P = 0.05$ at 12 to 25 mg/ml MCh, and $P < 0.001$ at 50 to 100 mg/ml MCh), as well as increased BAL cell counts (Figure 4B; $P < 0.001$) with substantial eosinophilia and increased lymphocyte numbers (Figure 4C; $P < 0.001$ and $P = 0.001$, respectively). These results indicate that only OVA-specific T cells can transfer asthma risk to offspring. However, pups of recipient mothers (DO11.10 T cells) are just as susceptible to developing asthma syndrome in response to CAS as they are in response to OVA.

Recipient Females Do Not Develop Allergic Airway Disease

Because recipient mother mice transferred asthma risk to their offspring similarly to asthmatic mothers (OVA sensitized and challenged), we sought to determine whether this effect was associated with allergic airway disease in the recipient females. We compared recipient females with controls 24 hours after the last OVA aerosol challenge. This time point corresponds to a strong allergic response in asthmatic mice,⁹ and it is the first day of mating for future mothers (see protocol in Figure 1).

The airway response of recipient females was similar to that of normal mice, whereas asthmatic females showed a strong asthma-like response (Figure 5). During MCh aerosol challenge, only asthmatic females developed increased Penh values ($P < 0.03$ from 6 to 25 mg/ml MCh, and $P < 0.001$ for 50 to 100 mg/ml MCh), whereas recipient females remained indistinguishable from normal mice (Figure 5A). In analysis of allergic airway inflammation, histopathology confirmed that recipient females were similar to normal females, whereas the airways of asthmatic mice were infiltrated by mononuclear cells and eosinophils (Figure 5B). Finally, no significant difference was found between recipient females and normal controls in terms of BAL cell counts (Figure 5C) and minimal eosinophilia (Figure 5D), whereas asthmatic females showed increased cell counts ($P < 0.001$) with substantial eosinophilia ($P < 0.001$). These studies show that allergen-specific T cells recreate the maternal effect in the absence of overt maternal asthma.

Systemic Pro-Inflammatory Imbalance in Pregnant Recipient Mothers

Based on these data and previous findings,⁹ cytokines were likely candidates for mediators of the maternal effect. The hypothesis was that IL-4 and potentially other

Figure 5. Recipient mothers do not show allergic airway disease. Recipient females were compared with normal females at the time of mating with normal BALB/c males. **A:** During MCh aerosol challenge, recipient females responded similarly to normal controls, whereas only asthmatic females (ie, OVA sensitized and challenged) showed increased Penh values from 6 to 100 mg/ml MCh ($*P < 0.03$ and $***P < 0.001$). **B:** Pathological examination showed similar airway morphology between normal and recipient females, whereas asthmatic females showed typical inflammatory infiltrates surrounding the bronchi and vessels. **C:** Recipient females showed BAL total cell counts identical to those of normal females, whereas asthmatic females showed increased BAL total cell counts ($***P < 0.001$) with eosinophilia (**D**) ($***P < 0.001$) ($n = 7-14$ females per group).

Th2 cytokines influence the fetal development toward higher asthma susceptibility in early life.⁴ Cytokine levels were therefore assayed in serum samples collected from recipient mothers during the 3 weeks of mouse gestation (also corresponding to weeks 1, 2, and 3 after OVA aerosol challenge for recipient mice). Because pregnancy may modulate the asthma status of expectant mothers,²² we included virgin controls (normal and recipient females) to distinguish the effects of adoptive T-cell transfer from those of pregnancy.

No statistically significant differences were found in the first 2 weeks of pregnancy (data not shown). In the 3rd week of mouse gestation, however, serum levels of IFN- γ (Figure 6A; $P = 0.0015$), IL-10 (Figure 6B; $P = 0.025$), IL-4 (Figure 6C; $P = 0.001$), and IL-13 (Figure 6D; $P < 0.001$) were all higher in recipient compared with normal pregnant mothers. A pregnancy effect was also found, with expectant recipient mothers showing significantly higher levels of IL-4 (Figure 6C; $P = 0.007$) and lower levels of both IL-10 (Figure 6B; $P < 0.001$) and IL-13

Figure 6. Pro-inflammatory systemic imbalance in pregnant recipient mothers. A systemic pro-inflammatory imbalance appeared during the pregnancy in recipient mothers (closed bars) compared with control groups (virgin recipient females [dark gray bars] and pregnant normal mothers [light gray bars]). Levels of circulating cytokines found in virgin normal females (open bars) are presented in reference. At week 3 of recipient mothers' gestation, serum levels of IFN- γ ($P = 0.015$), IL-4 ($P = 0.001$), IL-10 ($P = 0.025$), and IL-13 ($P < 0.001$) were higher than those of normal pregnant mothers. A "pregnancy effect" was also observed when comparing recipient females, because IL-10 and IL-13 serum levels were higher in virgin recipient mice ($P < 0.001$ and $P = 0.004$, respectively), whereas IL-4 serum levels only peaked in pregnant recipient mothers ($P = 0.007$).

(Figure 6D; $P = 0.004$) compared with virgin recipient females.

Hence, these cytokine assays show that the adoptive transfer of allergen-specific T cells is associated with systemic cytokine imbalance during the pregnancy of recipient mothers. Pregnancy appears to favor a particular pattern of cytokine production, with increased serum levels of pro-inflammatory cytokines (IFN- γ , IL-4, and IL-13) and decreased serum levels of IL-10.

Adoptively Transferred T Cells Persist during the Pregnancy of Recipient Mothers

Because adoptive T-cell transfer was found to dramatically impact systemic levels of cytokines during pregnancy, we sought to determine the fate of DO11.10 T cells in expectant recipient mothers. The fact that DO11.10 T cells remained undetectable (immunocytochemistry using KJ1-26 mAb; data not shown) in cryostat section from the lungs of recipient mothers was consistent with the absence of allergic airway disease in these mice. We then performed flow cytometric analysis of splenic and placental samples harvested from expectant recipient mothers in the 3rd week of gestation. Preliminary experiments using T cells collected from the spleens of DO11.10 mice confirmed that 80% of CD4⁺ T cells bore the DO11.10 transgenic TCR (OVA-specific), although more than 10% of DO11.10⁺ T cells were in fact

Figure 7. Adoptively transferred DO11.10 T cells persist during the pregnancy of recipient mothers. **A:** Flow cytometric analysis of splenic cell suspensions harvested from recipient mothers (week 3 of pregnancy) showed that 0.07% of all lymphocytes were CD4⁺/DO11.10⁺, 0.014% were CD8⁺/DO11.10⁺, and 0.066% were U5A2-13⁺/DO11.10⁺. Similar analysis on placental cells showed that 0.006% of all lymphocytes were CD4⁺/DO11.10⁺, 0.001% were CD8⁺/DO11.10⁺, and 0.011% were U5A2-13⁺/DO11.10⁺. **B:** U5A2-13⁺ T cells thus represented 80% (0.52 versus 0.13%) and 96% (0.24 versus 0.01%) of the DO11.10 T cells found in the spleen and placenta (respectively) of recipient mothers. Analysis was based on a minimum of 100,000 acquired events (Dot plots are representative of two separate experiments).

CD4⁻ (data not shown). Cytometric analysis thus included mAbs against the DO11.10 TCR, CD4, CD8, and U5A2-13, a marker that identifies memory/previously activated T lymphocytes with a profile and function similar to that of NK1.1⁺ T cells.^{20,21}

Figure 7A shows that, in the spleen of pregnant recipient mothers, 0.07% of all lymphocytes were CD4⁺/DO11.10⁺ and 0.014% were CD8⁺/DO11.10⁺. In addition, 0.066% of splenic lymphocytes were U5A2-13⁺/DO11.10⁺. Notably, U5A2-13⁺/DO11.10⁺ T cells constituted 80% of all DO11.10⁺ T cells that persisted in the spleen of pregnant recipient mothers (Figure 7B; 0.52 versus 0.13%). We also examined the placenta of recipient mothers, a critical location where allergen-specific T

cells could potentially influence fetal development. Placental samples contained fewer immune cells than the spleen did. However, DO11.10 T cells were detectable in this organ and could be analyzed. In expectant recipient mothers 0.006% of placental lymphocytes were CD4⁺/DO11.10⁺, 0.001% were CD8⁺/DO11.10⁺, and 0.011% were U5A2-13⁺/DO11.10⁺. Most interestingly and similar to the data from spleen samples, a high proportion (96%) of placental DO11.10⁺ T cells found in recipient mothers were U5A2-13⁺ (Figure 7B; 0.24 versus 0.01%). These experiments indicate that allergen-specific T cells persist during the pregnancy of recipient mothers, including at the maternal-fetal interface, and they display a memory/previously activated phenotype.

Discussion

The etiology of allergic asthma is multifactorial and involves genetic factors as well as individual history of exposure to airborne allergens and pathogens.^{1,3,10} Epidemiological studies have highlighted maternal asthma as an additional risk factor to developing the disease during childhood.⁵⁻⁷ The apparently greater influence of maternal versus paternal asthma⁶ supports the idea that, besides genes, maternal allergic disease may be more critical to transferring asthma risk. This assumption is also consistent with the fact that maternal cigarette smoking, which skews the immunity toward a Th2 profile,^{23,24} is associated with higher asthma risk in children.^{5,25} We previously developed a murine model of maternal transfer of asthma risk.⁹ We used a variation on this model in the present studies that were aimed at directly testing the contribution of T cells.

Our previous finding that IL-4 blockade significantly decreased susceptibility to asthma in offspring pointed to the importance of Th2 cytokines in the maternal transmission of asthma risk. Nonlymphoid gestational tissues²⁶ and indirect effects of hormones such as progesterone²⁷ are potential sources of Th2 cytokines during pregnancy. However, the best known Th2 cytokine producers are T lymphocytes themselves. We thus postulated that T lymphocytes can orchestrate the maternal effect, especially because they are prominent effectors of both acute and chronic asthma,²⁸ and adoptive transfer of T-cell subsets is sufficient to stimulate asthma in recipient animals.¹

In the present studies, we injected otherwise normal BALB/c females with OVA-specific T cells from the DO11.10 mouse strain.¹⁶ Recipient females were OVA-challenged before mating with normal BALB/c males. Importantly, recipient females received no further stimulus that could interfere with conception or fetal development. Other studies of maternal influence on asthma risk have made significant progress using pregnant female mice injected with exogenous cytokines,²⁹ sensitized to allergens,¹¹ or allergen-challenged during pregnancy.¹² However, in these experiments the exogenous stimuli may theoretically reach the fetus and directly induce responses attributed to the modulation of maternal immunity, which confounds interpretation.³⁰ Hence, our protocol sought to examine the role of T-cell responses in the

maternal transmission of asthma risk and offered the advantage of avoiding the direct exposure of fetuses to exogenous stimuli.

The results presented here confirmed that allergen-specific T cells are sufficient to recreate the maternal transmission of asthma risk. Offspring of recipient mothers were more prone than normal pups to develop asthma after an intentionally suboptimal induction protocol using either OVA or CAS antigens. Interestingly, the recipient mothers did not develop allergic airway disease despite the injection of OVA-specific T cells and OVA aerosol challenges, an important difference with asthmatic mothers. This result, however, is in agreement with Aronica et al,³¹ who reported that only Th2-differentiated (*in vitro*) memory-type DO11.10 T cells can stimulate asthma syndrome in recipient BALB/c mice, whereas the injection of naïve DO11.10 does not alter the lung function of recipient mice compared with untreated controls. In our model, the absence of allergic airway disease in recipient females was also consistent with the absence of detectable DO11.10 T cells in lung samples from these mice (data not shown).

Additional aspects of the model merit discussion here. Mediators transferred during nursing may contribute to the maternal effect, as we recently reported.¹⁵ However, offspring of asthmatic mothers adoptively nursed by normal mothers still show increased asthma susceptibility, indicating a critical intrauterine effect (the focus of the present investigation). Also, the allergen independence of the maternal effect in previous studies⁹ and in the data presented here indicates that maternal transfer of allergen-specific antibody does not contribute mechanistically. A final mechanism that we have considered is the transplacental passage of maternal cells, eg, adoptively transferred DO11.10 T cells. However, we could not detect any DO11.10 T cells in either fetal or neonatal tissues by immunohistochemistry using KJ1-26 mAb (data not shown).

Our protocol used injections of the whole splenic T-cell population collected from DO11.10 mice, based in large part on the fact that multiple subsets of T cells, including CD4⁺ T cells, CD8⁺ T cells,³² and iNKT cells,³³ are critically involved in modulating allergic asthma. The pan-T-cell "strategy" was even more relevant after we found that splenic T cells in DO11.10 donor mice were CD4⁺, CD8⁺, or double negative. In recipient mothers, cytometric analysis of spleen and placenta samples showed the persistence of these adoptively transferred DO11.10 T cells more than 2 weeks after injection. Importantly, most cells displayed a memory/previously activated phenotype (expression of U5A2-13), a finding of particular interest for several reasons. First, this indicates the activation of DO11.10 T cells after injection, their homing to both lymphoid and nonlymphoid tissues, and their persistence as memory cells in expectant recipient mothers. Second, U5A2-13 was initially described as a marker defining T-cell subsets similarly to NK1.1, yet suitable for use with non-NK1.1 mice such as the BALB/c strain.²¹ Thus, the expression of U5A2-13 makes the DO11.10 T cells found in recipient mothers reminiscent of "splenic NKT cells" that more recently have been called "CD1d-

unrestricted NKT-like cells."³⁴ Despite their potential heterogeneity,³⁴ splenic NKT-like cells are thought to constitute a primary source of IFN- γ (transient) and IL-4 (sustained), which explains that they promote the long-term differentiation of naïve T cells into Th2 effectors.³⁵ This function of NKT-like cells supports our postulation that injected DO11.10 T cells may have stimulated endogenous T cells to participate in the systemic cytokine imbalance found during the pregnancy of recipient mothers. The systemic cytokine release suggests an endocrine, hormone-like effect in recipient mothers, consistent with their effects on fetal development.⁴ This concept is further supported by the fact that injection of IFN- γ at high doses during pregnancy prevents the offspring susceptibility to allergy.²⁹

Increased levels of IL-13 were found both in pregnant and virgin recipient mice, suggesting a direct link between IL-13 overproduction and the adoptive transfer of allergen-specific T cells. Moreover, IL-13 levels were higher than any other cytokine assayed in these studies. Also, IL-4 was detected exclusively in pregnant recipient mothers. A dual role for IL-4 and IL-13 in our model may explain why anti-IL-4 mAb pretreatment of asthmatic mothers only partly abrogated the maternal effect in a prior study.⁹ The striking elevation of IL-4 and IL-13 in expectant recipient mothers suggests that the maternal effect takes place through the influence of Th2 cytokines on the fetal development. Increased levels of IFN- γ , a Th1 cytokine, in expectant recipient mothers might theoretically counteract a Th2 cytokine imbalance. However, considering that the presence of IFN- γ neither prevented peaks of IL-4 and IL-13 nor abrogated the maternal transmission of asthma risk, it is more likely that this cytokine potentiates the undergoing Th2 imbalance in recipient mothers, because Th1 and Th2 responses may cooperate to enhance allergic inflammation *in vivo*.^{36,37} Whether a similar cytokine imbalance also occurs at the maternal-fetal interface certainly merits further investigations, because the placenta itself is thought to release various cytokines.³⁸

The influence of maternal cytokines on fetal development suggests their transplacental passage, which is thought to occur during pregnancy.^{4,30,39} Cytokines such as granulocyte-macrophage-colony-stimulating-factor (GM-CSF) and IFN- α have been reported to cross the placental barrier at amounts that can have a biological effect on the fetus.^{40,41} Our results suggest that pregnancy and allergic responses interact to modulate the systemic release of cytokines. Pregnancy is thought to produce a Th2 milieu that promotes tolerance to the fetus.³⁹ Conversely, allergic asthma is a disorder already biased toward Th2-dominated responses, and asthma exacerbations requiring medical intervention are increased in expectant patients.⁴² Because this phenomenon is more common in the third trimester of pregnancy,²² the systemic cytokine imbalance found in the 3rd week of mouse pregnancy in our model appears roughly in agreement with clinical studies.

In conclusion, we report that allergen-specific DO11.10 T cells are sufficient to recreate the maternal effect and elicit an IL-4/IL-13-dominated, pro-inflamma-

tory cytokine imbalance during the pregnancy of recipient mothers. Because the maternal transmission of asthma risk can take place in the absence of overt allergic airway disease in recipient mothers, this effect may be distinguished from that of asthma per se. We have also observed higher asthma risk in pups of mother mice with chemically induced skin allergy (C. Hubeau, M. S. Arredovani, and L. Kobzik, unpublished data). Therefore we propose that not only asthma but, more broadly, allergic inflammatory diseases can increase asthma risk in offspring through changes in the gestational cytokine milieu.

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