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μ-Chain–Deficient Mice Possess B-1 Cells and Produce IgG and IgE, but Not IgA, following Systemic Sensitization and Inhalational Challenge in a Fungal Asthma Model

Sumit Ghosh, Scott A. Hoselton, and Jane M. Schuh

Allergic bronchopulmonary aspergillosis is often difficult to treat and results in morbidity associated with chronic airway changes. This study assessed the requirement for B cells and their products in the allergic pulmonary phenotype in a murine model of fungal allergic asthma that mimics allergic bronchopulmonary aspergillosis. C57BL/6 and μMT mice (assumed to lack peripheral B cells) were sensitized with Aspergillus fumigatus extract and challenged with two inhalation exposures of live conidia to induce airway disease. Airway hyperresponsiveness after methacholine challenge, peribronchovascular inflammation, goblet cell metaplasia, and fibrotic remodeling of the airways was similar between μMT mice and their wild-type counterparts (C57BL/6). Surprisingly, even in the absence of the μ-chain, these μMT mice produced IgE and IgG Abs, although the Abs induced did not have specificity for A. fumigatus Ags. In contrast, IgA was not detected in either the lavage fluid or serum of μMT mice that had been exposed to A. fumigatus. Our findings also reveal the existence of CD19+CD9+B-1 cells in the lungs of the μMT animals. These data show the μMT mice to have a developmental pathway independent of the canonical μ-chain route that allows for their survival upon antigenic challenge with A. fumigatus conidia, although this pathway does not seem to allow for the normal development of Ag-specific repositories. Additionally, this study shows that IgA is not required for either clearance or containment of A. fumigatus in the murine lung, as fungal outgrowth was not observed in the μMT animals after multiple inhalation exposures to live conidia. The Journal of Immunology, 2012, 189: 1322–1329.
repeated *A. fumigatus* inhalation in C57BL/6 wild-type (WT) controls and μMT animals. We found that the absence of the μ-chain did not alter the pulmonary pathology that results from inhalation of *A. fumigatus* in allergic animals: airway hyper-responsiveness (AHR), peribronchial inflammation, epithelial changes, and collagen deposition were equivalent to WT controls. Surprisingly, we found that repeated *A. fumigatus* conidia exposure resulted in elevated IgE, IgG1 (in bronchoalveolar lavage [BAL]), and IgG2a production in sensitized μMT mice, although IgA was undetectable in the μMT animals. This has implications both for the role of the B cell in the allergic lung and for IgA in the response to fungal allergens. To our knowledge, we report for the first time that, even in the absence of the Ig μ-chain, IgG1 (only in BAL), IgG2a, and IgE isotypes were produced in animals after exposure to fungal Ags, but IgA was not made. The Abs produced after fungal exposure showed no Ag specificity for *A. fumigatus*. Our findings also reveal the previously unreported presence of B-1 cells (CD19^+CD9^+IgD^+) in the lungs of μMT mice, even in the complete absence of B-2 cells. Taken together, our data demonstrate that μMT mice have B-1 cells in the lungs and that these animals produce selected isotypes through a μ-independent pathway in the context of the fungal allergen-exposed lung.

**Materials and Methods**

**Experimental animals**

C57BL/6 and μMT mice (5–9 wk of age) were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were housed on Alpha drip paper bedding (Shepherd Specialty Papers, Watertown, TN) in microfilter-topped cages (Acare, Belmont, NY) in a specific pathogen-free facility with ad libitum access to food and water. The study described was performed in accordance with the Office of Laboratory Animal Welfare guidelines and was approved by the North Dakota State University Institutional Animal Care and Use Committee.

**Ag preparation and *A. fumigatus* culture**

Soluble *A. fumigatus* Ag extract was purchased from Greer Laboratories (Lenoir, NC), and fungal culture stock (strain NIH 5233) was purchased from the American Type Culture Collection (Manassas, VA). The *A. fumigatus* culture was reconstituted in 5 ml PBS, and 60-μl aliquots were stored at 4°C until use. All experiments that used *A. fumigatus* were conducted with prior approval of the Institutional Biological Safety Committee of North Dakota State University.

**Allergen sensitization and challenge by airborne delivery system**

Animals were sensitized per Hogaboam et al.’s (30) published protocol, with the exception that aluminum hydroxide was used as the adjuvant. Mice were sensitized globally with 10 μg *A. fumigatus* Ag (Greer Laboratories) in 0.1 ml normal saline mixed with 0.1 ml Imject Alum (Pierce, Rockford, IL), which was injected s.c. (0.1 ml) and i.p. (0.1 ml). After 2 wk, 20 μg *A. fumigatus* Ag was administered to naïve mice, and 20 μg *A. fumigatus* Ag in 20 μl normal saline. Mice were challenged as previously described with a 10-min nose-only aerosol exposure to live *A. fumigatus* conidia (29). Each anesthetized mouse was placed supine with its nose in an inoculation port inhaling the live fungal conidia for 10 min. Two weeks after the first allergen challenge, mice were subjected to a second 10-min aerosol fungal challenge. Naïve animals from both groups were further sensitized nor challenged. After the second allergen exposure, the mice were separated into groups of five for analysis at day 0 (sensitized, but not challenged) or days 7 or 28 after the second aerosol challenge. Day 7 after challenge had been previously determined to be the peak of B cell recruitment into the allergic lungs, and leukocyte inflammation was assessed at this time point. Airway wall remodeling can be seen as early as 7 d after the second aerosol challenge in this model, and the changes to the lung architecture continue to accrue through at least day 28 after the second inhalation of fungal conidia. The day 28 time point was chosen to assess epithelial changes as well as peribronchial fibrosis. The experimental protocol is depicted in Fig. 1.

**AHR measurement**

Mice were anesthetized using sodium pentobarbital (Butler, Columbus, OH; 0.1 mg/0.01 kg of mouse body weight), intubated, and ventilated with a Harvard pump ventilator (Harvard Apparatus, Reno, NV) to assess allergic airway responses. Restraint plethysmography (Buxco, Troy, NY) was used to assess AHR. Before performing readings, the system was first calibrated and the stroke volume set at 225 with the strokes per minute set at 150. The value for baseline airway resistance was measured for each animal before an i.v. injection of acetyl-b-methacholine (420 μg/kg) was administered to determine AHR at each time point.

**Sample collection**

Approximately 500 μl blood was removed from each mouse via ocular bleed and centrifuged at 13,000 × g for 10 min to yield serum. Serum was stored at −20°C until use. BAL was performed on each mouse with 1.0 ml sterile normal saline. The BAL contents were centrifuged at 2000 × g for 10 min to separate cells from fluid. The BAL fluid was stored at −20°C until use, and cells were used immediately for morphometric analysis. Left lungs were harvested and fixed in 10% neutral buffered formalin for histological analysis.

**Morphometric and histological analyses**

BAL cells were cytospun (Shandon Scientific, Runcorn, U.K.) onto microscope slides and differently stained (Quick-Dip stain; Mercedes Medical, Sarasota, FL). Cells from five random high-powered fields were counted to determine the mean number of each cell type per high-powered field in the airway lumen of each mouse.

Formalin-fixed, paraffin-embedded lungs were cut longitudinally across the coronal plane in 5-μm sections and stained with H&E to assess inflammation and with periodic acid-Schiff stain (Richard-Allan Scientific, Kalamazoo, MI) for the analysis of goblet cells.

**Evaluation of collagen thickness**

Gomori trichrome (Richard-Allan Scientific) was used to stain histological sections to assess collagen deposition in naive and allergic mice as described previously by Hoselton et al. (29). For each sample, at least 50 discrete points were measured at 50-μm intervals along the largest lateral bronchial branch visible on the histological section (the second or third lateral branch). A perpendicular line was drawn from the point on the basement membrane through the full thickness of the collagen immediately below. The mean collagen thickness was reported for each sample, and the mean of the means was reported for each group.

**Quantification of serum and BAL IgE, IgG1, IgG2a, and IgA**

The total IgE (BD OptEIA; BD Biosciences, San Diego, CA), IgG1 (Immunology Consultants Laboratory, Portland, OR), IgG2a (BD OptEIA), and IgA (Bethyl Laboratories, Montgomery, TX) in serum and BAL were quantified via specific ELISA according to manufacturers’ guidelines. Serum samples were diluted in PBS at 1:100 for IgE, at 1:500 and 1:5000 for IgG1 and IgG2a, and at 1:500 for IgA. BAL samples were pooled and diluted at 1:5 for IgE and IgG2a, at 1:2 for IgG1, or undiluted for IgA. The detection limits for the kits were 1.6 ng/ml for IgE, 6.25 ng/ml for IgG1, 3.1 ng/ml for IgG2a, and 15.625 ng/ml for IgA.
ELISA plates were coated with 100 μl/well of a 20-μg/ml sample of A. fumigatus Ag (Greer Laboratories) diluted in coating buffer (pH 9.6; 15 mM Na2CO3, 35 mM NaHCO3) and incubated overnight at 4°C. The next day, the plates were washed three times with PBS containing 0.05% Tween 20 and 200 μl blocking (3%) BSA in coating buffer) was added to each well. Plates were incubated in the dark for 2 h at room temperature and washed three times with PBS-Tween 20. After blocking, 100 μl/well serially diluted serum or BAL from C57BL/6 and μMT mice diluted in PBS-Tween 20/1% BSA (10−1 to 10−8 for serum and 1:2 to 1:64 for BAL fluid) was added to each well and incubated for 1 h. Plates were washed five times with PBS-Tween 20, and 100 μl/well diluted goat anti-mouse Ig-GHRP (SouthernBiotech, Birmingham, AL) secondary Ab was added. Following a 1-h incubation, the plates were again washed five times, and 100 μl/well tetramethylbenzidine substrate (BD Biosciences) was added. The absorbance was read at 650 nm using a Synergy HT microplate reader (BioTek, Winooski, VT). Additionally, serum and BAL samples were tested to check the specificity of individual subclasses of Ab (IgG1 and IgG2a) for this, rat anti-mouse IgG1-AKP (1:5000 dilution; BD Pharmingen, San Jose, CA) and goat anti-mouse IgG-HRP (1:500 dilution; Thermo Scientific, Rochester, NY) secondary Abs were used in place of IgG-HRP. The absorbance was read at 650 nm when tetramethylbenzidine was used as a substrate and at 405 nm when p-nitrophenyl phosphate (disodium salt, hexahydrate) (Sigma-Aldrich, St. Louis, MO) was used as a substrate using a Synergy HT microplate reader.

Flow cytometry

Minced lungs from naive animals and at days 0 (sensitized, but not challenged) and 7 were subjected to collagenase IV (Sigma-Aldrich) digestion and RBC lysis. For collagenase digestion, minced lung sections were treated with 0.04% collagenase IV in DMEM and incubated at 37°C for 1 h with gentle agitation. For flow cytometry analysis, the cells were suspended in PBS with 1% BSA (Sigma Aldrich) to a final concentration of 1 × 10^7 cells/ml. Fe receptors were blocked with anti-mouse CD16/32 (BD Pharmingen, San Diego, CA) and 100 μl/well tetramethylbenzidine substrate (BD Biosciences) was added. The samples were preincubated with combinations of directly labeled Abs for 30 min in the dark and then washed with PBS/1% BSA twice before the samples were analyzed on an Accuri C6 flow cytometer (Accuri Cytometers, Ann Arbor, MI) or a FACSCalibur flow cytometer (BD Biosciences). A minimum of 50,000 events were acquired and the data were analyzed using FlowJo software (Tree Star, Ashland, OR).

Statistical analysis

Allergic C57BL/6 WT and μMT animals were compared with each other and to their respective naive controls at each time point. An unpaired Student two-tailed t test with a Welch correction was used to determine statistical significance with GraphPad Prism software (GraphPad Software, San Diego, CA). For each of the mouse strains compared with its naive control, statistical significance is indicated as follows: *p = 0.01–0.05, **p = 0.001–0.01, ***p < 0.001. Where appropriate, # indicates statistical difference between the C57BL6 and μMT mice (*p < 0.05). All results are expressed as the means ± SEM.

Results

Airborne fungal challenge results in AHR in μMT mice after sensitization to A. fumigatus

In the present study, airway physiology of both murine groups (i.e., C57BL/6 and μMT animals) was monitored before allergen challenge at day 0 and at days 7 and 28 after the second conidia inhalation (Fig. 2). Airway response measurements from all study animals were used to determine the baseline mean for AHR prior to methacholine challenge (Fig. 2, dotted line). Peak increases in airway resistance were recorded after i.v. methacholine injection (420 μg/kg). AHR was increased following allergen challenge, and the trend was quite similar in both the murine groups throughout the course of the study. Data were analyzed using an unpaired Student two-tailed t test with a Welch correction. All values are expressed as the means ± SEM (n = 3–5 mice/group). *p < 0.05 when compared with the respective naive controls.

Leukocytes are recruited to the allergic airways after fungal conidia challenge in μMT mice

Leukocyte recruitment to the lungs of allergen-sensitized animals that had inhaled conidia was evaluated using H&E-stained lung sections and morphometric analysis of BAL cells. Naive animals from both groups exhibited no pulmonary inflammation (Fig. 3A, 3B). Similarly, sensitized C57BL/6 and μMT animals that did not inhale spores (day 0) showed no evidence of inflammation (Fig. 3C and 3D, respectively). However, upon allergen challenge, both C57BL/6 and μMT animals actively recruited inflammatory cells to the lungs. Allergic animals exhibited prominent perivascular and peribronchial leukocyte inflammation 7 d after the second spore challenge (Fig. 3E, 3F). The pattern of perivascular and peribronchial inflammation was similar in the C57BL/6 and μMT animals at day 7, and inflammation was largely resolved in both strains by day 28 after challenge (Fig. 3G, 3H).

Morphometric analysis of monocyte/macrophage lineage cells, neutrophils, eosinophils, and lymphocytes was performed to estimate the relative makeup of the cellular inflammation and to monitor leukocyte egress into the airway lumen (Fig. 4). In naive and sensitized animals that were not challenged (day 0), alveolar macrophages were the dominant cell type (Fig. 4A). Neutrophils, lymphocytes, and particularly eosinophils were prominent cell types identified in the BAL 7 d after the second conidia challenge (Fig. 4B–D). Eosinophils were the most numerous cell type counted (Fig. 4C) in the BAL of both C57BL6 and μMT mice at day 7 after the second conidia exposure, emphasizing the polarization of the immune response in favor of allergy after multiple inhalations of conidia. At day 28 after challenge, macrophages were again the major cellular component of the BAL compartment with very few neutrophils (Fig. 4A, 4B). The inflammation pattern was similar between C57BL6/6 and μMT animals, with eosinophils dominating at day 7 in both murine groups when they were compared with their naive controls.

Inhalation of fungal conidia changes the airway architecture in allergic C57BL/6 mice and allergic μMT mice

In the present study, goblet cells were assessed by counting periodic acid-Schiff–stained cells and representing them as a percentage of total epithelial cells lining the second or third lateral airways in each histological section. Goblet cell metaplasia was not observed...
in the day 0 (sensitized, but not challenged) animals of either the C57BL/6 or μMT groups (Fig. 5C). Challenge with *A. fumigatus* conidia resulted in a marked increase in the percentage of goblet cells lining the airways (Fig. 5C). As compared with sensitized animals that did not receive inhaled conidia, the number of goblet cells was increased dramatically (~65% of total) but equally in both groups 7 d after challenge (Fig. 5). By day 28 after the second conidia challenge, fewer goblet cells were noted in the allergic lungs of both the C57BL/6 and μMT group as compared with the day 7 time point, although there was no difference in the number of goblet cells between the WT and the μMT groups (Fig. 5C; ~27% of the total epithelial cells for each).

Collagen accumulation in the peribronchial space of allergic C57BL/6 or μMT animals was significantly increased at both day 7 and day 28 after the second conidia inhalation as compared with sensitized animals that had not been exposed to inhaled conidia (Fig. 6). In contrast to the pattern seen in goblet cell metaplasia, collagen accumulation did not diminish over the time course of this study. This phenomenon has been seen and extended in other studies by our laboratory in both BALB/c and C57BL/6 mice (29, 31–33).

**FIGURE 3.** Inhalation of *A. fumigatus* conidia increases pulmonary inflammation in C57BL/6 and μMT mice. Representative photomicrographs of H&E-stained lung sections of allergen-challenged C57BL/6 (left column) and μMT (right column) mice. Naive and day 0 mice in both groups did not show inflammation (A–D). Peribronchovascular inflammation was prominent at day 7 after the second conidia challenge in both groups (E, F) and subsided well into day 28 (G, H). Scale bars, 100 μm.

**FIGURE 4.** Effect of *A. fumigatus* conidia inhalation on inflammatory leukocytes in the allergic lung. Airway inflammation was marked by the presence of (A) macrophages, (B) neutrophils, (C) eosinophils, and (D) lymphocytes in naive, allergic C57BL/6, and μMT mice. The inflammation pattern was similar in both C57BL/6 and μMT mice throughout the course of the study. Data were analyzed using an unpaired Student two-tailed *t* test with a Welch correction. All values are expressed as the means ± SEM (n = 4–5 mice/group). *p < 0.05, **p < 0.01, ***p < 0.001 when compared with the respective naive controls. *p < 0.05 when μMT animals were compared with C57BL/6 animals. HPF, High-powered field.

**FIGURE 5.** Inhalation of *A. fumigatus* conidia increases goblet cell metaplasia in C57BL/6 and μMT mice. Representative photomicrographs of periodic acid-Schiff–stained whole lung sections of C57BL/6 and μMT mice show that goblet cells and mucus were evident in the airways at day 7 after the second conidia challenge (A, B). Goblet cell numbers were reported as the percentage of total epithelial cells along segments of airway epithelium lining the large lateral branches of the bronchi (C). Data were analyzed using an unpaired Student two-tailed *t* test with a Welch correction. All values are expressed as the means ± SEM (n = 3–5 mice/group). *p < 0.01, **p < 0.001 when compared with the respective naive controls.

Fungal inhalation resulted in increased serum IgA, IgG1, IgG2a, and IgE levels in allergic C57BL/6 mice, whereas μMT mice exhibited elevated IgG1 in BAL and IgG2a and IgE in serum.

In the present study, inhalation of *A. fumigatus* conidia resulted in an increase in the BAL IgA from C57BL/6 mice at day 7 after two conidia inhalations. IgA Abs were not detected in either serum or the BAL fluid of μMT mice (Fig. 7A, 7B). IgG1 was detected in the BAL fluid of allergic μMT mice 7 d after two exposures to conidia, but it was not found in the serum (Fig. 7C, 7D). Although serum IgG2a levels in naive μMT animals were significantly lower than in WT animals, sensitization with fungal Ags stimulated its
production to levels equivalent to those of WT animals, and the μ-deficient animals matched IgG2a levels throughout the rest of the study (Fig. 7E). IgG2a was also detected in the BAL fluid of the μMT mice (Fig. 7F). IgE was elevated in A. fumigatus-sensitized and challenged C57BL/6 and μMT animals (Fig. 7G, 7H). Although there was a significant difference in the IgE levels of C57BL/6 and μMT mice, the production of IgE was significantly higher at day 7 after the second conidia challenge in both the murine groups when they were compared with their respective naive controls, suggesting that isotype switching to an allergic phenotype was possible even in the μMT mice. However, IgE production in C57BL/6 mice was 4-fold higher than μMT levels at day 7 after the second inhalation.

To investigate the extent to which the Abs produced as a result of fungal sensitization and inhalation exposure were specific to A. fumigatus, serial dilutions of serum and BAL samples from C57BL/6 and μMT mice were collected at day 7 after the second conidia exposure and analyzed against the sensitizing Ag. The specificity of Abs to A. fumigatus at day 7 after the second conidia challenge (when the Ab levels are higher in serum and BAL) of both C57BL/6 and μMT mice are shown in Fig. 8. The serum and BAL Abs produced in the C57BL/6 mice were specific to A. fumigatus (filled square), whereas the ones produced in μMT mice (filled circle) appeared to be nonspecific and the values were comparable to those of naive control animals (Fig. 8). When the specificity of individual subclasses of Abs (IgE and IgG1) for A. fumigatus was tested, we observed similar results (data not shown).

CD19+CD9+ IgD+ B-1 cells are present in the lungs of μMT mice despite a lack of the Igμ-chain

It has previously been shown that μMT mice on the BALB/c, but not C57BL/6, background display an incomplete block in B cell development and harbor mature B cells in secondary lymphoid organs (34, 35). Although all μMT animals used in this study were

FIGURE 6. Effect of A. fumigatus conidia inhalation on peribronchial collagen thickness. Gomori trichome stain was used to visualize subepithelial collagen deposition in histological sections. Peribronchial collagen thickness was similar in both C57BL/6 and μMT mice throughout the course of the study. Approximately 50 discrete points were measured at 50-μm intervals along the largest lateral bronchiolar branch visible on the histological section (L2 or L3). A perpendicular line was drawn from the point on the basement membrane through the full thickness of the collagen immediately below. The mean collagen thickness was reported for each sample. Data were analyzed using an unpaired Student two-tailed t test with a Welch correction. All values are expressed as the means ± SEM (n = 3–5 mice/group). *p < 0.05, **p < 0.01 when compared with their respective naive controls.

FIGURE 7. Inhalation of A. fumigatus conidia induces μMT mice to produce IgG1 (only in BAL), IgE, and IgG2a in serum and BAL fluid (BALF). (A and B) IgA Ab levels in serum and BAL fluid of C57BL/6 and μMT mice. (C–F) IgG1 and IgG2a Ab levels in serum and BAL of naive and allergic C57BL/6 and μMT mice. (G and H) IgE Ab levels in serum and BAL of C57BL/6 and μMT mice. The Ab levels of C57BL/6 and μMT mice were compared with naive animals and to each other at each time point. ELISAs indicated that the μMT mice produced Abs in response to A. fumigatus allergen challenge. Data were analyzed using an unpaired Student two-tailed t test with a Welch correction. All values are expressed as the means ± SEM (n = 4–5 mice/group). *p < 0.05, **p < 0.01, ***p < 0.001 when compared with naive controls. #p < 0.05 when μMT animals were compared with C57BL/6 animals. No statistics are shown for Ig ELISAs for BAL fluid, as the samples from each time point were pooled and run as a single sample.

Days post second conidia challenge
on the C57BL/6 background, we considered the possibility that A. fumigatus exposure might overcome the B cell developmental block (28, 35). In the present study, after inhalation with A. fumigatus conidia, CD19+ B cells were detected in the lungs of μMT mice at day 7 after the second conidia inhalation and their numbers were fewer than those in WT controls (Fig. 9C–F). When evaluated, CD19+ B cells were also detected in the lungs of naive μMT mice (data not shown).

Given the fact that the Abs produced in the μMT mice were not specific for A. fumigatus, we looked for the presence of B-1 lymphocytes in the lungs, as these cells are known to produce Abs in a nonspecific manner and they predominate in the pleural and peritoneal cavities (36). In addition to B-1 cells, we also looked for B-2 cells in the lungs of μMT mice, as these conventional B-2 cells form a major population of lymphocytes that is present in the body (37, 38). The CD19+ B cell population in the lungs of C57BL/6 WT mice expressed either CD9 (as B-1 cells are CD9+) (36, 39) or CD23 (as B-2 cells are CD23+ and have low to no expression of CD9) (38, 40), showing the presence of both B-1 and B-2 cells (Fig. 9C, 9E). Conversely, CD19+ B cells that were present in the lungs of μMT mice did not express CD23, indicating the absence of B-2 lymphocytes (Fig. 9F). Similar to the B-1 population in C57BL/6 WT mice, the μMT CD19+ B cells expressed CD9, illustrating the presence of B-1 lymphocytes (36) (Fig. 9D).

It has been shown that IgD can substitute for IgM when it is expressed early in the B cell development process (41). As such, we looked for the expression of IgD on the CD19+CD9+ cells that were present in the lungs of C57BL/6 WT and μMT mice using flow cytometry. IgD was expressed on the CD19+CD9+ cells present in the lungs of C57BL/6 and μMT mice at day 7 after the second conidia challenge (Fig. 9G, 9H). As expected, IgM+ cells were not detected in either naive or A. fumigatus-challenged μMT mice (data not shown). These data demonstrate that in the μMT mice, IgD can substitute for IgM early in B-1 cell development.

**Discussion**

In the present study, we show that the localized production of IgG1, IgG2a, and IgE is elicited in μMT mice in response to systemic fungal sensitization and inhalational challenge in an experimental allergic asthma model. In addition to the localized production, our work demonstrates that μ-deficient mice produced systemic IgG2a and IgE Abs after exposure to A. fumigatus extract Ags followed by inhalation of A. fumigatus conidia. However, when tested in binding assays with the Aspergillus Ags that were used to sensitize the animals, the Ab isotypes from the μMT animals proved to be...
The canonical pathway of B cell ontogeny requires surface expression of the μ Ig chain at an early pre-B cell stage (48). Indeed, until recently only B cells that express IgM were thought to migrate from the bone marrow to the peripheral lymphoid organs (35), and membrane-bound IgM expression was thought to be essential for B cell maturation and differentiation to Ab-producing cells. However, recent research using μMT mice has shown that the expression of the μ Ig chain is not an absolute requirement for B cell survival (35, 45, 46, 49). These genetically altered animals have been useful tools in understanding the complex biological processes associated with different diseases.

Although Ab-binding ELISAs were not attempted in the helmint infection study, the IgE was functional in that it elicited IL-4 production by basophils (46). In that study, the Ab was produced at a low concentration and Ab-producing B cells could not be detected in the central or peripheral lymph organs. In the present study, whereas μMT mice were able to produce Abs after sensitization to and challenge with fungal Ags, our results show that the Abs produced by the μ-deficient animals had no affinity for Astacus astacus Ags as compared with those from WT animals. Taken together, these results suggest a tissue-centric Ab production, which mandated the assessment of B cell populations in the lung.

In investigating the potential source of Abs in μMT mice, to our knowledge, no other study has examined the presence of CD19+ IgD+ cells in the lungs. Because we did not find CD19+IgD+ or CD19+IgM+ cells in the bone marrow or spleens of naïve mice (data not shown), we hypothesized that tissue-resident B-1 cells act as the source of Abs in μMT mice. We were able to detect IgD-expressing CD19+CD9+ B-1 cells in the lungs of μMT mice using flow cytometry, supporting a tissue-resident B-1 cell as a source for localized Ab production. These observations are consistent with the notion that B cells can receive switching signals in peripheral sites (45, 46, 50, 51), a process that may occur in the allergic lung.

In summary, we provide conclusive evidence that B-1 cells can impact asthma pathophysiology in the absence of conventional B-2 lymphocytes. From these studies, we report two significant conclusions. First, the route of the pathogenic/allergenic challenge as well as the type of Ag has a significant impact on the generation of Ab responses in μMT mice lacking the normal pathway for B-2 cell maturation, as different types of Ag yield very different outcomes. The second major finding is that as a B-2 cell knockout mouse, μMT animals may be very useful to determine the role of B-1 cells in response to various pulmonary insults. Future studies may include elucidating the mechanism for B-1 isotype switching in the lung and the contribution of B-1 cells to protective responses, which would have important implications for experimental analysis and for understanding normal B-1 and B-2 cell activation in health and disease.

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Disclosures
The authors have no financial conflicts of interest.

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