Airway responsiveness in CD38-deficient mice in allergic airway disease: studies with bone marrow chimeras

Alonso G. P. Guedes,1 Joseph A. Jude,2 Jaime Paulin,6 Laura Rivero-Nava,3 Hirohito Kita,4 Frances E. Lund,3,5 and Mathur S. Kannan6

1Department of Surgical and Radiological Sciences, University of California, Davis, California; 2Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; 3Trudeau Institute, Saranac Lake, New York; 4Departments of Immunology and Medicine, Mayo Clinic, Rochester, Minnesota; 5Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama; 6Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, Minnesota

Submitted 15 August 2014; accepted in final form 4 January 2015

Guedes AG, Jude JA, Paulin J, Rivero-Nava L, Kita H, Lund FE, Kannan MS. Airway responsiveness in CD38-deficient mice in allergic airway disease: studies with bone marrow chimeras. Am J Physiol Lung Cell Mol Physiol 308: L485–L493, 2015. First published January 9, 2015; doi:10.1152/ajplung.00227.2014.—CD38 is an ectoenzyme that catalyzes the conversion of β-nicotinamide adenine dinucleotide (β-NAD) to cyclic adenosine diphosphoribose (cADPR) and ADPR (18, 26). Recent investigations have provided evidence that CD38 also catalyzes the conversion of NAD(P)+ to nicotinic acid adenine dinucleotide phosphate (NAADP+) and ADPR (14, 25). The metabolites of β-NAD and NAD(P)+ are known to play important roles in calcium signaling in a variety of cell types (24). In airway smooth muscle (ASM), cADPR mediates intracellular calcium release from the sarcoplasmic reticulum through ryanoide receptor channels (9, 33). CD38 also regulates leukocyte function through enzyme-dependent and enzyme-independent (receptor-mediated) mechanisms and has a role in regulating inflammation by modulating leukocyte responses (32). Results of other investigations indicate that CD38 regulates the migration of neutrophils and the in vivo and in vitro migration of dendritic cells and monocytes to sites of inflammation (30, 31). There is also evidence that T cell-dependent immune responses to allergens are significantly attenuated in the CD38-deficient (Cd38−/−) mice, possibly attributable to defective leukocyte trafficking (27).

Airway inflammation and airway hyperresponsiveness (AHR) are hallmark features of asthma. Evidence from mouse models shows that CD38 is involved in AHR and airway inflammation (13, 15, 16). Our laboratory reported evidence that Cd38−/− mice exhibit attenuated contractile response to inhaled methacholine, suggesting an airway phenotype of these mice (10, 15). Furthermore, these mice also are hyporesponsive to inhaled methacholine following intranasal challenge with the Th2 cytokine IL-13 or following intranasal challenge with TNF-α (15, 16). ASM cells obtained from Cd38−/− mice have attenuated intracellular calcium responses and contractility to airway spasmogens, such as endothelin-1 and acetylcholine (10). In addition to contractility, there is substantial evidence supporting an immunomodulatory role for ASM. ASM cells secrete a variety of chemokines in response to inflammatory cytokines (1, 17, 19, 29), and ASM of asthmatic patients express chemokines and chemokine receptors (2, 3, 20, 34). Therefore, the contribution of ASM to the pathophysiology of asthma probably stems from a multitude of mechanisms, from contractile responses to local modulation of inflammation.

Allergic asthma in humans is a complex disease that is mediated by CD4+ T cells polarized to a Th2 phenotype (6, 35, 36). Th2 cytokines such as IL-4, IL-5, and IL-13 contribute to the pathophysiology of asthma that is characterized by airway eosinophilia, antigen-dependent IgE response, AHR, mucus production, and airway wall remodeling (6, 12). There is a strong association between airway exposure to environmental fungi such as Alternaria and asthma in humans (4, 7). The mechanisms by which such exposure results in a Th2-skewed immune response are complex but appear to require dendritic cell-derived factors and activation of protease-activated receptor 2 (PAR-2) receptors in the airways (21, 28).

In this study, we evaluated the contribution of CD38 to AHR and airway inflammation in the well-known ovalbumin model and following exposure to Alternaria, a clinically relevant
environmental allergen. In the ovalbumin model, we also evaluated the contribution of CD38 expressed in inflammatory cells and airway structural cells to AHR. We asked whether CD38+ inflammatory cells recruited into the lungs of CD38−/− hosts following ovalbumin challenge would recapitulate the airway phenotype of the wild-type (WT) mice.

MATERIALS AND METHODS

Animals. Pathogen-free, 8–12-wk-old C57BL/6 WT and CD38−/− mice, obtained by in-house breeding, were used in the studies. Mice were maintained in a 12-h:12-h light/dark schedule with food and water available ad libitum. The institutional animal care and use committees of the University of Minnesota and of the Trudeau Institute approved the experimental protocols.

Development of bone marrow chimeras. Bone marrow chimeras were produced at Trudeau Institute, Saranac Lake, NY. The recipient mice (WT and CD38−/−) were irradiated and subsequently reconstituted with WT or CD38−/− bone marrow–derived cells. Bone marrow was obtained from the femurs and tibias of WT and CD38−/− donor mice. Recipient mice were irradiated with 1,000 rad, in a split dose administered 4–5 h apart, from an x-ray source operated at 200 kV delivering 85 rad/min. A total of 5 × 10^6 bone marrow cells from each source (WT and CD38−/−) was injected retroorbitally into irradiated recipients (WT and CD38−/−). After irradiation and reconstitution, the recipient animals were maintained on antibiotic-containing water (polyminix B sulfate 10^6 UI and neomycin sulfate 1.1 g/l) for at least 6 wk. Reconstituted mice were analyzed at 12 wk by fluorescence-activated cell sorting staining of peripheral blood for CD38.

Ovalbumin sensitization and intranasal challenge protocol. WT and CD38−/− mice (n = 10/group) were primed with an intraperitoneal injection of 10 µg of ovalbumin (Sigma, St. Louis, MO) adsorbed in aluminum sulfate (ALUM; 2 mg; Injec alum; Pierce, Rockford, IL) on days 0 and 14, intranasally challenged with ovalbumin (10 µg/mouse) diluted in nonpyrogenic saline (50 µl) on days 21, 23, and 25 under isoflurane anesthesia. Control mice were primed with ALUM and intranasally challenged with nonpyrogenic sterile saline. On day 26, 24 h following the last intranasal ovalbumin challenge, airway responsiveness, inflammatory cell numbers, and cytokine/chemokine levels in the lung parenchyma were evaluated, as described below. Bone marrow chimeras (n = 5/group) were subjected to the same allergen exposure protocol.

Intranasal challenge with Alternaria extract. Under isoflurane anesthesia, WT and CD38−/− mice (n = 6–10/group) were intranasally challenged with 10, 50, or 100 mg/ml of Alternaria extract (Greer Laboratories, Lenoir, NC) diluted in 50 µl of nonpyrogenic sterile water or vehicle control on days 0, 3, and 6. On day 8, airway responsiveness, BALF inflammatory cell numbers, cytokine/chemokine levels, and inflammation of the lung parenchyma were evaluated, as described below. The Alternaria extract contained minimal endotoxin activity (11.7 EU/mg extract) by a limulus lysate assay (Wako, Osaka, Japan). The endotoxin in Alternaria extract at the highest dosage used (100 mg/ml) was equivalent to ~0.038 ng of LPS, which should not affect airway responses to allergens (31).

Measurement of airway responsiveness to inhaled methacholine. The procedure for the measurement of airway responsiveness to inhaled methacholine has been described in previous publications (10, 15, 16). Briefly, mice were anesthetized (sodium pentobarbital 100 mg/kg, ip), paralyzed (pancuronium bromide 6 µg/g, ip), intubated via tracheostomy, and subsequently transferred to a plethysmograph chamber (Buxco Electronics, Sharon, CT), where they were mechanically ventilated (Harvard Apparatus, March-Hugstetten, Germany) with a tidal volume of 150 µl at 160 breaths/min. Respiratory flow signal was obtained through a flow transducer (Sen Sym SCXL004, Buxco Electronics) connected to the plethysmograph chamber, and integration of the flow signal was used to calculate lung volume.

Intraesophageal and airway pressures were measured with a dedicated pressure transducer (Validyne DP45, Buxco Electronics). The signals generated were preamplified (MaxII, Buxco Electronics) and captured with a computer software (Biosystem XA, Buxco Electronics). Data were digitized and stored on a desktop computer for later analysis. Body temperature was maintained at physiological limits throughout the experiment.

Respiratory resistance (Rr) was measured in response to increasing doses of inhaled methacholine (0.00, 6.25, 12.50, 25.00, 50.00, and 100.00 mg/ml of saline). A volume of 2 µl of each dose of methacholine was delivered through an ultrasonic nebulizer during 10 consecutive breaths. Tidal volume was increased to 350 µl, and respiratory rate was reduced to 100 breaths/min during each nebulization and then returned to prenebulization values. Rr to airflow was determined continuously, and the peak responses following each dose of methacholine were obtained and analyzed offline.

Assessment of airway inflammation. The lungs were washed with three aliquots of 1 ml of cold Hank’s balanced salt solution and with EDTA (3 mM) such that peak inflation pressure was ~25 cmH2O. The total number of cells in the BALF was counted immediately using a hemocytometer. Subsequently, the BALF was centrifuged at 2,000 revolution/min for 5 min, and the supernatant was collected and frozen at ~80°C for later measurement of cytokine concentration (as described below). Differential cell counts of inflammatory cells (macrophages, neutrophils, lymphocytes, and eosinophils) were performed by counting 200 cells from cytospin preparations stained with Diff-Quick stain.

Cytokine and chemokine levels in the BALF. IL-5, IL-13, and eotaxin-2 levels were measured in the BALF supernatants using a commercial ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. The minimum detectable levels for IL-5, eotaxin-2, and IL-13 are respectively 7.0 pg/ml, 3.0 pg/ml, and 1.5 pg/ml.

Histopathological analysis of lung sections in Alternaria-exposed mice. Inflammatory changes in the lung parenchyma were evaluated as described previously (15, 16). After assessment of lung function, the lungs were collected and fixed in 10% phosphate-buffered formalin (n = 6 mice/group). The tissues were subsequently embedded in paraffin and cut at 10-µm thickness at regions representing central and peripheral airways. A random code was assigned to each specimen to blind the examiner as to the identity of each specimen. Sections were stained with hematoxylin and eosin for evaluation of inflammatory infiltrate and with Alcin blue/periodic acid–Schiff for evaluation of mucous-secreting cells (e.g., goblet cells). Lungs were first evaluated for the general nature of the inflammatory infiltrate. Scoring was then performed at a magnification of ×250 by examining 40 consecutive fields to evaluate peribronchiorial, perivascular, and alveolar inflammation separately. For peribronchiorial and perivascular inflammation, the numerical scores for each view field were determined as follows: 0, normal; 1, few cells; 2, a ring of inflammatory cells one cell layer deep; 3, a ring of inflammatory cells 2–4 cell layers deep; 4, a ring of inflammatory cells of >4 cell layers deep. For alveolar inflammation, the numerical scores were determined as follows: 0, normal; 1, alveolar walls normal, few macrophages in alveoli; 2, mild thickening of alveolar walls and increased alveolar macrophages and eosinophils; 3, marked thickening of alveolar walls and alveolar multinucleated giant cells and eosinophils in 30–50% of the field; 4, same as 3 but with inflammatory cells in >50% of the field; 5, complete consolidation. Mucous-secreting cells/high-power field were evaluated in the central airway by using the following scoring system: 0, <5% goblet cells; 1, 5–25%; 2, 25–50%; 3, 50–75%; 4, >75%. An experienced pathologist who was blinded as to the identity of the specimens carried out the scoring.

Sample size and statistical analysis. Sample sizes were calculated using data from preliminary and previous studies considering a power of 0.8 and a 95% confidence interval. Data were analyzed with one-way ANOVA or two-way repeated-measures ANOVA using
controls (Fig. 1, B and C, respectively). In ovalbumin-sensitized and -challenged mice, airway responsiveness to methacholine was significantly higher in the WT compared with Cd38−/− mice (Fig. 1D).

The airway responsiveness to methacholine was also assessed following intranasal challenge with three doses of an extract of the common environmental fungus Alternaria. The airway responsiveness to methacholine was significantly higher in the WT compared with Cd38−/− mice at the three doses of the extract studied (Fig. 2).

Airway inflammation following challenge with ovalbumin or Alternaria extract. Cd38 is known to regulate trafficking and migration of leukocytes to the lungs (27). Therefore, we assessed whether the attenuated methacholine responsiveness in the Cd38−/− mice following ovalbumin sensitization and challenge or following intranasal Alternaria extract was attributable to decreased airway inflammation. In naïve groups and in ovalbumin-sensitized-only groups of Cd38−/− and WT mice, total cell counts in the BALF were comparable. Following ovalbumin sensitization and challenge, there were significant and comparable elevations in the total cells in the BALF of Cd38−/− mice and WT mice (Fig. 3A). Total cells in the BALF following challenge with Alternaria (50 and 100 mg/ml) also showed significant and comparable elevations in Cd38−/− mice and WT mice (Fig. 3B). In these mice, differential cell counts in the BALF revealed significant and comparable ele-
Methacholine responsiveness and airway inflammation in ovalbumin-challenged mice following bone marrow transfer. Although the magnitude of airway inflammation was comparable in C3d8−/− mice and WT mice, it is possible that the absence of AHR in the C3d8−/− mice following allergen challenge may be related to the fact that the cells recruited into the lungs lack expression of CD38. We generated bone marrow chimeras to address whether C3d8−/− inflammatory cells would restore AHR in the C3d8−/− mice following allergen challenge.

Initially, we assessed the effect of irradiation and bone marrow transfer on the phenotype of naïve mice. The methacholine responsiveness of the WT mice did not change following irradiation and WT bone marrow transfer but was attenuated following transfer of C3d8−/− bone marrow (Fig. 5A). Methacholine responsiveness of the C3d8−/− mice decreased following irradiation and bone marrow transfer from either WT or C3d8−/− mice (Fig. 5B). As demonstrated previously (10, 15, 16) and in the present study, airway responsiveness to inhaled methacholine under naïve conditions is significantly greater in WT than in C3d8−/− mice both in the intact mice and in the chimeras (Fig. 5C). These studies indicate that radiation and reconstitution do not exacerbate the airway resistance of either WT or C3d8−/− naïve mice in response to inhaled methacholine. If anything, lack of C3d8−/− cells of inflammatory or parenchymal origin appears to promote attenuation of responsiveness to methacholine compared with the intact mice (both WT and C3d8−/−) following irradiation and bone marrow cell transfer under naïve conditions.
Following bone marrow transfer and recovery, mice were sensitized and intranasally challenged with ovalbumin, and their methacholine responsiveness was assessed. The airway responsiveness is decreased in ovalbumin-sensitized and -challenged intact CD38−/− mice compared with intact sensitized and challenged WT mice (Fig. 1 and Fig. 6A). Airway responsiveness to methacholine is also lower in the chimeric CD38−/− mice compared with the chimeric WT mice following ovalbumin sensitization and challenge (Fig. 6B). Methacholine responsiveness of the chimeric WT mice following ovalbumin sensitization and challenge was similar regardless of whether they received bone marrow from CD38−/− or WT mice (Fig. 6C). Interestingly, ovalbumin sensitization and challenge caused significantly higher methacholine responsiveness in chimeric CD38−/− mice transferred with CD38−/− or WT bone marrow compared with the intact mice (Fig. 6D). In the bone marrow chimeras, BALF cell numbers were the same under naïve conditions and increased to similar magnitude following ovalbumin sensitization and challenge (Fig. 6E). These results indicate that, upon ovalbumin sensitization and challenge, the CD38−/− chimeras behave differently than the intact CD38−/− mice and that the WT chimeras look like their intact WT counterparts. The change in methacholine responsiveness in the CD38−/− chimeras cannot be attributed to differences in cell numbers in the airways.

**DISCUSSION**

In this study, we investigated the contribution of CD38, a cell-surface protein that has calcium-signaling properties and is involved in innate immunity, to allergen-induced AHR in a mouse model. We evaluated the contribution of CD38 to AHR...
in mice following intraperitoneal sensitization with ovalbumin followed by intranasal challenge with the same allergen. We also used the extract of the environmental fungus Alternaria that induces a Th2-skewed airway inflammation when administered directly into the airways by activating PAR-2 receptors in the airway epithelium (28). Our results demonstrate that WT mice develop significantly higher airway responsiveness to inhaled methacholine compared with naïve intact WT mice with naïve intact Cd38−/− mice following intranasal Alternaria extract or following ovalbumin sensitization and challenge. There were comparable airway inflammation and elevations in levels of BALF cytokines/chemokines following allergen challenge in both groups of mice. We previously showed that airway responsiveness to inhaled methacholine is higher in naïve intact WT mice compared with naïve intact Cd38−/− mice (10, 15, 16). Naïve irradiated and bone marrow-reconstituted Cd38−/− hosts also exhibited decreased responsiveness to methacholine compared with naïve irradiated and bone marrow-reconstituted WT hosts. This was true regardless of whether the Cd38−/− host was reconstituted with WT or Cd38−/− bone marrow. These data suggest that airway resistance is decreased in naïve Cd38−/− hosts, regardless of which bone marrow-derived cells are present, indicating that, in the naïve state, Cd38−/− lung parenchymal cells exhibit less airway hyperreactivity.

Following irradiation, the phenotype observed in Cd38−/− hosts sensitized and challenged with ovalbumin is partially reversed, regardless of which bone marrow cells are present. These results suggest that the mechanism by which deleting CD38 protects the lung cells (or nonhematopoietic cells) from methacholine challenge is largely overcome if the lung cells have been exposed to radiation. Furthermore, Cd38−/− bone marrow cells only confer a very modest “protection” to the naïve WT hosts but not to the sensitized and challenged WT hosts, suggesting that the loss of CD38 on hematopoietic cells is not sufficient to prevent airway hyperreactivity in mice.

This finding is somewhat different from that reported earlier by other investigators, who observed a more significant effect of the Cd38−/− bone marrow (13). However, in our study, we did not shield the lungs before radiation, which may have contributed to some of the observed differences. One conclu-

![Fig. 4. Cytokine/chemokine concentrations in the BALF in WT and Cd38−/− mice. A: IL-5, IL-13, and eotaxin-2 levels in the BALF obtained from naïve mice and from mice following ovalbumin sensitization and challenge (WT P+Ch and Cd38−/− P+Ch). Note significant elevations in the levels of IL-13 and eotaxin-2 in WT and Cd38−/− mice following ovalbumin challenge (n = 10/group). B: IL-5, IL-13, and eotaxin-2 levels in the BALF obtained from naïve mice and from mice following challenge with 50 mg/ml Alternaria extract (n = 6/group). Values are shown as means ± SE. Within each cytokine/chemokine, statistical significance is denoted with superscripted letters (a, b, c) above group values such that treatment groups without common letters are significantly different (P < 0.05).](image1)

![Fig. 5. Effect of irradiation and bone marrow transfer on airway responsiveness to inhaled MCh in naïve WT and Cd38−/− mice. A: changes in Rl in response to increasing doses of inhaled MCh in naïve intact WT mice or in naïve chimeric WT mice transferred with WT bone marrow (WT BM) or Cd38−/− bone marrow (Cd38−/− BM). Note that MCh responsiveness in the WT mice is unchanged following irradiation and transfer of WT bone marrow but is slightly decreased following irradiation and transfer of Cd38−/− bone marrow. B: changes in Rl in response to increasing doses of inhaled MCh in naïve intact Cd38−/− mice or in naïve chimeric Cd38−/− mice following transfer of Cd38−/− bone marrow or WT bone marrow. Note significant attenuation of MCh responsiveness following transfer of both types of bone marrow. C: MCh responsiveness in naïve WT chimeras and Cd38−/− chimeras. Note that MCh responsiveness is greater in the WT chimeras compared with Cd38−/− chimeras. *P < 0.05. Values are shown as means ± SE, n = 5–10/group.](image2)
CD38 AND ALLERGIC AIRWAY DISEASE

Fig. 6. Changes in $R_L$ in response to different doses of MCh in WT and Cd38$^{-/-}$ mice following bone marrow transfer and ovalbumin sensitization and challenge. A: changes in $R_L$ measured in ovalbumin-sensitized and -challenged WT (WT P+Ch) and Cd38$^{-/-}$ (Cd38$^{-/-}$ P+Ch) mice. B: MCh responsiveness in chimeric mice sensitized and challenged with ovalbumin. Note lower $R_L$ in response to the highest dose of MCh in the chimeric Cd38$^{-/-}$ mice compared with the chimeric WT mice. C: MCh responsiveness in intact and chimeric WT mice following ovalbumin sensitization and challenge. Note similar $R_L$ regardless of whether the WT mice received bone marrow from Cd38$^{-/-}$ or WT mice. D: MCh responsiveness in intact and chimeric Cd38$^{-/-}$ mice following ovalbumin sensitization and challenge. Note the significantly higher MCh responsiveness in chimeric Cd38$^{-/-}$ mice transferred with Cd38$^{-/-}$ or WT bone marrow compared with the intact mice. E: total cell numbers (individual mice and groups mean ± SE) in BALF obtained from naïve WT and Cd38$^{-/-}$ mice following radiation and bone marrow transfer. Note significant elevations in cell numbers in ovalbumin-sensitized and -challenged mice regardless of the source of bone marrow. Data are shown as means ± SE (n = 5/group). *P < 0.05.

The contribution of CD38 to AHR, a hallmark feature of asthma, can result from a multitude of mechanisms. CD38 is a signaling receptor on lymphocytes, and its activation by anti-CD38 antibodies in the presence or absence of IL-4, IL-5, or anti-IgM results in lymphocyte proliferation (27). The Cd38$^{-/-}$ mice develop poor humoral immune response to antigens (5, 13), and Cd38$^{-/-}$ neutrophils migrate less efficiently to chemoattractants or bacterial peptides (30). Thus CD38 regulates the humoral immune response by regulating B cell activation, leukocyte and dendritic cell trafficking, and inflammatory response. There is evidence that T cells are inefficiently primed in Cd38$^{-/-}$ mice attributable to an inability of Cd38$^{-/-}$ dendritic cells to mobilize Ca$^{2+}$ in response to CC chemokine receptor 7 and CX chemokine receptor 4.
ligation although the cells appear to possess the normal complement of the chemokine receptors (27, 30). CD38 expression on CD4+ T cells appears to be necessary for their expansion, differentiation to Th2-type effector cells, and migration to the lungs upon antigen challenge (27, 31). By controlling the migration of dendritic cells in response to antigen challenge, CD38 facilitates humoral immune responses. Thus it is not surprising that, in the Cd38−/− mice, there is a defective antibody response to allergens and decreased Th2-type inflammation.

Dendritic cells exposed to Alternaria also exhibit distinct inflammatory responses and Th2-skewed humoral and cellular immune responses when they are transferred into the airways of naive animals (21). Thus it is possible that dendritic cell-mediated polarization of the Th2 responses to Alternaria may be defective in the Cd38−/− mice and may contribute to the observed airway phenotype following intranasal challenge with Alternaria extract. However, the levels of IL-13, a major Th2-derived cytokine that mediates AHR at least partly via CD38 (8, 16), were similar in the BALF from WT and Cd38−/− mice following Alternaria or ovalbumin challenge. Thus it seems unlikely that defective Th2 polarization following Alternaria exposure is sufficient to explain the attenuated airway responses observed in the Cd38−/− mice. Interestingly, a previous study (13) reported significantly lower Th2 cytokine release from ovalbumin-stimulated Cd38−/− cells. Although we do not know why the two studies appear to contradict one another, we examined the response in the effector site (lung airway), and the prior study examined the cytokine release in cultures of peribronchial-draining lymph node cells that were exposed to ovalbumin in vivo and then in vitro (13). Given the differences in the CD4 T cell subsets that are present in these two tissues, it is possible that CD38 differentially affects the cytokine-producing capacity of effector Th2 cells compared with the lymphoid tissue T follicular helper cells.

In this study, we evaluated the role of Alternaria allergens in the development of airway inflammation and AHR in the Cd38−/− mice because the mechanisms by which these allergens induce airway inflammation appear to be unique. Prior studies have provided evidence for protease activities in Alternaria in the development of Th2-type airway inflammation (21). The cysteine and aspartate protease activities are known to induce the secretion of several cytokines and chemokines from airway epithelium by activating PAR-2 receptors (28). Furthermore, the cysteine protease activity of Alternaria induces the production of thymic stromal lymphopoietin through PAR-2 receptors in the airway epithelium, which is sufficient for the development of Th2-type inflammation (23). In addition, exposure of airway epithelial cells to Alternaria allergens causes IL-33 secretion into the airways with the production of Th2 cytokines (22). More recent reports provide evidence that the aspartate protease activity of Alternaria, through PAR-2 receptors in the airway epithelium, is capable of inducing the secretion of a variety of cytokines, such as granulocyte-macrophage colony-stimulating factor, IL-6, and IL-8 (28). The fact that Cd38−/− mice failed to develop AHR to a magnitude comparable to WT mice strongly suggests that one or more of these mechanisms may be defective. The decreased AHR in the Cd38−/− mice following allergen challenge may also be related to defective response of other lung structural cells, such as ASM cells to Th2-type inflammation. This conclusion is further supported by our previously reported results that show attenuated methacholine responsivity of Cd38−/− mice following intranasal challenge with the Th2 cytokine IL-13 (16).

In summary, we have demonstrated that Cd38−/− mice develop significantly attenuated AHR following ovalbumin sensitization and challenge. Reconstitution of bone marrow from WT or Cd38−/− mice restored part of the hyperresponsive phenotype of the WT mice in the Cd38−/− mice following ovalbumin challenge although the naive phenotypic differences between these two mice did not change. The difference in the magnitude of AHR between WT and Cd38−/− mice was also seen following exposure to Alternaria extract, whose protease activities are known to activate PAR-2 receptors in the airway epithelium to cause release of cytokines and a Th2-type inflammation. Airway inflammation characterized by total cells or the numbers of different inflammatory cells in the BALF or levels of cytokines/chemokines following allergen challenge was similar in the WT and Cd38−/− mice. These results indicate that the magnitude of airway inflammation is not the predominant underlying determinant of AHR. Structural cells such as ASM cells in the lungs may contribute to the observed difference in airway responsiveness to methacholine challenge through mechanisms such as augmented calcium signaling and contractility.

GRANTS
This study was supported by National Institutes of Health Grants HL-057498 (to M. Kannan) and AI-057996 (to F. Lund) and Grants from Academic Health Center, University of Minnesota and Comparative Medicine Signature Program, College of Veterinary Medicine, University of Minnesota (to M. Kannan).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


