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Am J Physiol Lung Cell Mol Physiol 291:1286-1293, 2006. First published Aug 4, 2006;
doi:10.1152/ajplung.00187.2006

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CD38-deficient mice have reduced airway hyperresponsiveness following IL-13 challenge

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Submitted 30 May 2006; accepted in final form 1 August 2006

Guedes, Alonso G. P., Jaime Paulin, Laura Rivero-Nava, Hirohito Kita, Frances E. Lund, and Mathur S. Kannan. CD38-deficient mice have reduced airway hyperresponsiveness following IL-13 challenge. *Am J Physiol Lung Cell Mol Physiol* 291: L1286–L1293, 2006. First published August 4, 2006; doi:10.1152/ajplung.00187.2006.—The transmembrane glycoprotein CD38 in airway smooth muscle is the source of cyclic-ADP ribose, an intracellular calcium-releasing molecule, and is subject to regulatory effects of cytokines such as interleukin (IL)-13, a cytokine implicated in asthma. We investigated the role of CD38 in airway hyperresponsiveness using a mouse model of IL-13-induced airway disease. Wild-type (WT) and CD38-deficient (CD38KO) mice were intranasally challenged with 5 µg of IL-13 three times on alternate days under isoflurane anesthesia. Lung resistance (R_L) in response to inhaled methacholine was measured 24 h after the last challenge in pentobarbital-anesthetized, tracheostomized, and mechanically ventilated mice. Bronchoalveolar cytokines, bronchoalveolar and parenchymal inflammation, and smooth muscle contractility and relaxation using tracheal segments were also evaluated. Changes in methacholine-induced R_L were significantly greater in the WT than in the CD38KO mice following intranasal IL-13 challenges. Airway reactivity after IL-13 exposure, as measured by the slope of the methacholine dose-response curve, was significantly higher in the WT than in the CD38KO mice. The rate of isometric force generation in tracheal segments (e.g., smooth muscle reactivity) was greater in the WT than in the CD38KO mice following incubation with IL-13. IL-13 treatment reduced isoproterenol-induced relaxations to similar magnitudes in tracheal segments obtained from WT and CD38KO mice. Both WT and CD38KO mice developed significant bronchoalveolar and parenchymal inflammation after IL-13 challenges compared with naïve controls. The results indicate that CD38 contributes to airway hyperresponsiveness in lungs exposed to IL-13 at least partly by increasing airway smooth muscle reactivity to contractile agonists.

interleukin-13; inflammation; asthma; eosinophil; airway smooth muscle

CD38 IS A MULTIFUNCTIONAL ECTOENZYME that catalyzes the conversion of β -nicotinamide adenine dinucleotide to cyclic ADP-ribose (cADPR) and ADP-ribose (16). Both CD38 and its product cADPR are present in airway smooth muscle (ASM) cells and other mammalian cell types (8, 26, 32, 39). Together with inositol 1,4,5-trisphosphate, cADPR is an important mediator of intracellular calcium ($[Ca^{2+}]_i$) release in smooth muscle cells including that of the airways (7, 20, 31). The dynamics of $[Ca^{2+}]_i$ responses to stimuli are central in the regulation of ASM contraction,

bronchomotor tone, and airway caliber (33). cADPR induces Ca^{2+} release from the sarcoplasmic reticulum through activation of ryanodine receptor channels in ASM cells (31). The $[Ca^{2+}]_i$ responses of human ASM cells exposed to acetylcholine, thrombin, and bradykinin are reduced by the competitive cADPR antagonist 8-bromo-cADPR in a magnitude that correlates positively with the level of CD38 expression (6).

CD38-deficient (CD38KO) mice have been used to elucidate the role of CD38 in the function of several organs. CD38-generated cADPR plays a critical role in Ca^{2+} -induced insulin secretion (21), osteoclast-mediated bone resorption (35), vasoconstriction (27), myometrium function (9), and immune function (28, 29). Using these mice, we have previously demonstrated that CD38 is the main source of cADPR in the lungs. We have also shown that CD38KO airway myocytes have reduced $[Ca^{2+}]_i$ responses to contractile agonists and that methacholine-induced increases in airway resistance are lower in CD38KO mice compared with wild-type (WT) controls (8). These studies demonstrated that the CD38/cADPR signaling has an important role in $[Ca^{2+}]_i$ homeostasis in ASM cells with clear implications to normal airway function.

Changes in $[Ca^{2+}]_i$ homeostasis have been proposed to be an important mechanism underlying airway hyperresponsiveness (AHR), a prominent feature of the asthmatic phenotype (3, 15, 36). Previous studies from our laboratory and from others using human ASM cells have implicated the CD38/cADPR signaling in $[Ca^{2+}]_i$ hyperresponsiveness to contractile agonists with possible relevance to inflammatory airway diseases (7, 18, 19, 37). For example, increases in CD38 expression and activity are observed in human ASM cells exposed to IL-1 β , IFN- γ , TNF- α , and IL-13. Exposure to these cytokines induced a heightened $[Ca^{2+}]_i$ response to contractile agonists that was significantly attenuated by the cADPR antagonist 8-bromo-cADPR (1, 5–7, 18, 19, 37, 38). These observations suggest that the absence of CD38 in the airways would have a protective role in AHR in vivo, a possibly important finding for airway biology in the context of bronchial asthma.

The study reported here examined whether CD38 has a role in AHR during airway inflammation induced by IL-13, a cytokine with a central role in asthma. We show that both CD38KO and WT mice develop significant airway and parenchymal inflammation and that both WT and CD38KO mice develop AHR following exposure to IL-13 compared with respective naïve controls. However, the magnitude of the AHR

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observed in the WT mice is significantly greater than that in the CD38KO mice, as was the airway reactivity to methacholine. Similarly, ASM reactivity to carbachol in vitro was significantly greater in the WT than in the CD38KO mice following IL-13 exposure. Our data indicate that CD38 contributes to ASM hyperreactivity and AHR induced by IL-13 in vivo.

METHODS

Animals. Specific pathogen-free 8- to 12-wk-old C57BL/6J WT and CD38KO mice (backcrossed 12 generations to C57BL/6J) were obtained by in-house breeding. The original breeding pairs were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed in a 12-h light-dark schedule with food and water available ad libitum. This study was approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Intranasal challenge with recombinant murine IL-13. WT and CD38KO mice were anesthetized with isoflurane and intranasally administered 5 μ g of recombinant murine IL-13 (mu rIL-13) dissolved in nonpyrogenic sterile water (provided by Wyeth Pharmaceuticals, Cambridge, MA) with $n = 10$ /group or nonpyrogenic sterile water (controls) with $n = 6$ /group in a total volume of 50 μ l. The intranasal challenges were done on days 1, 3, and 5, and the studies were conducted 24 h following the last challenge, as described below.

Airway responsiveness to methacholine. Lung resistance (R_L) was measured in anesthetized, tracheostomized, intubated, and mechanically ventilated mice as described in detail previously (8) using whole body plethysmography (Buxco Electronics, Sharon, CT). R_L was measured in response to increasing doses of inhaled methacholine (0, 6.25, 12.5, 25, 50, and 100 mg/ml of saline). As determined by preliminary experiments, each dose of methacholine was delivered in a volume of 2 μ l with the use of an ultrasonic nebulizer during 10 consecutive breaths. Tidal volume was increased to 350 μ l, and respiratory rate was reduced to 100 breaths/min during each nebulization and then returned to prenebulization values. Peak R_L to airflow was determined following each dose of methacholine and analyzed offline. The dose-response curves were further evaluated by regression analysis to obtain indices of airway reactivity (slope) and sensitivity (lowest dose to produce bronchoconstriction) as described previously (24).

Inflammatory cell numbers in the bronchoalveolar lavage fluid. In preliminary experiments, mice were intranasally challenged with sterile water or mu rIL-13 (5 μ g/mouse) up to three times, and 24 h following the last challenge, the lungs were lavaged three times with 1-ml aliquots of sterile Hanks' balanced salt solution (HBSS) containing 3 mM EDTA. The bronchoalveolar lavage (BAL) fluid was collected and analyzed for total and differential inflammatory cell counts. Highest lymphocyte and eosinophil numbers were observed following three IL-13 challenges. Therefore, in subsequent studies, the mice were subjected to three IL-13 challenges as described above. Following measurement of methacholine responsiveness, the mice were killed, and the lungs ($n = 6$ –10/group) were lavaged as described above. The retrieved BAL fluid was centrifuged at 2,000 rpm for 5 min, and the supernatant was removed and frozen at -80°C for cytokine/chemokine measurements (see below). The cell pellet was resuspended in 0.5 ml of HBSS, and the total number of inflammatory cells in the BAL was counted using a hemocytometer. Differential cell counts (macrophages, neutrophils, lymphocytes, and eosinophils) were performed by counting 200 cells from cytospin preparations stained with Diff-Quick stain.

Histopathological analysis of lung sections. Inflammatory changes in the lung parenchyma were evaluated as described previously (11). Briefly, the lungs were collected and fixed in 10% phosphate-buffered formalin ($n = 6$ –10 mice/group). Subsequently, parasagittal sections of tissue representing central and peripheral airways were embedded in paraffin, cut at 5- μ m thickness, and stained with hematoxylin and eosin for evaluation of inflammatory infiltrate and with periodic

acid-Schiff for evaluation of mucus-secreting cells (e.g., goblet cells). Lungs were first evaluated for the general nature of the inflammatory infiltrate. Scoring was then performed at $\times 200$ magnification by examining 40 consecutive fields to evaluate peribronchiolar, perivascular, and alveolar inflammation separately as described elsewhere (11). A pathologist unaware of the identity of the specimens performed all the scoring.

Cytokine and chemokine levels in the BAL fluid. IL-5, IL-13, and eotaxin-2 levels were measured in the BAL fluid supernatants ($n = 6$ –10 mice/group) using an 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 7, 3, and 1.5 pg/ml, respectively. The levels of cytokines/chemokines were normalized for BAL volume.

Isometric force measurement in the isolated trachea. WT and CD38KO mice were killed with carbon dioxide, the cervical trachea was carefully dissected, and a segment of ~ 4 ml in length was removed for the experiments. Tracheal segments were maintained in Dulbecco's minimum essential medium with no serum and supplemented with 5.7 μ g/ml insulin and 5 μ g/ml transferrin (arresting medium). The tracheas ($n = 8$ –10/group) were incubated at 37°C in 5% CO_2 for 12–14 h with the addition of 100 ng of mu rIL-13 per ml of medium. After incubation, two stainless-steel wires were passed through the lumen of the tracheal segments and fixed on the jaws of a horizontal myograph (Multi Myograph System 610M, Danish Myo Technology). The tracheal segments were submerged in a 5-ml volume bath containing HBSS under continuous oxygenation with a flow of 10 ml/min at 37°C . A steady tension of 0.5 g was applied for 45 min to each tracheal segment before baseline measurements and carbachol stimulation in all experiments. In a first set of experiments, a 1 mM carbachol solution was introduced at a constant rate of 5 μ l per min for 15 min into the 5-ml volume organ bath using a digital multiple syringe pump (WPI Instruments, Longmont, CO). With the continuous infusion, the concentration of carbachol in the bath was expected to increase continuously and linearly, reaching a maximum concentration of 15 μ M at the end of the infusion. This approach was taken to determine the minimum concentration of carbachol required to elicit contraction (e.g., ASM sensitivity) and the rate of isometric force generation (e.g., ASM reactivity). In a second set of experiments, the contractile responses of the tracheal segments to different concentrations of carbachol (1 nM to 10 μ M) were determined. In another set of experiments, tracheal rings were precontracted with carbachol to $\sim 75\%$ of maximal response, and relaxations to cumulative additions of isoproterenol (1 nM to 10 μ M) were assessed. Relaxation responses to isoproterenol are represented as % of maximal relaxation from the initial carbachol-induced contraction for the naïve and IL-13-treated conditions.

Sample size and statistical analysis. Sample size was determined using data from preliminary studies considering a power of 0.8 and a 95% confidence interval. Inflammatory cell counts in the BAL fluid were subjected to one-way ANOVA, R_L values were subjected to repeated-measures ANOVA, and inflammatory scores of lung parenchyma were subjected to Kruskal-Wallis one-way ANOVA on ranks. The Tukey-Kramer multiple comparison test was used to detect statistically significant differences between means. Statistical analysis was performed with commercial statistic software (NCSS) with $P < 0.05$ considered statistically significant. Parametric data are shown as means $\pm$ SE, and nonparametric data are shown as ranks of inflammatory scores.

RESULTS

Airway hyperresponsiveness to methacholine. We previously demonstrated that airway resistance in response to methacholine is reduced in naïve CD38KO mice relative to WT mice (8) and that the CD38/cADPR pathway plays a role in IL-13-induced calcium modulation in human ASM cells (5).

In the present study, when the naïve (sham-treated) animals were exposed to increasing amounts of methacholine, the changes in R_L were significantly higher in the WT than in the CD38KO mice (Fig. 1, A and B), confirming our previous data (8). To test the role of CD38 in AHR, we employed a well-described experimental model of IL-13-induced airway disease

(13, 41) in which mice are intranasally exposed to IL-13 three times over a 5-day period. These mice develop pulmonary eosinophilia, elevated total serum IgE levels [independent of IL-4 (10)], excessive airway mucus production, and AHR (13, 41). Using this approach, we found that IL-13 exposure resulted in larger methacholine-induced R_L in both WT and

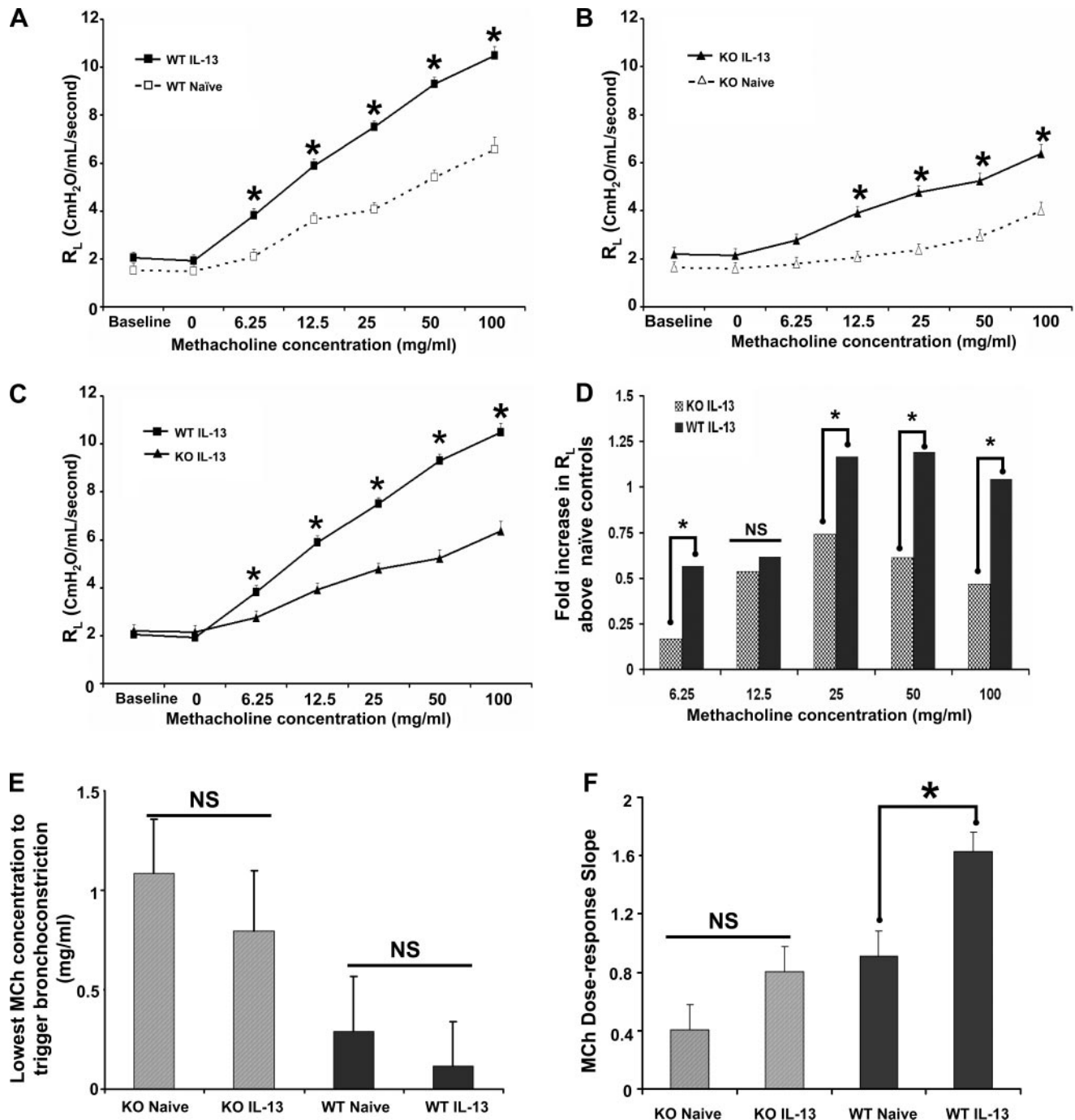


Fig. 1. The magnitude of airway hyperresponsiveness and airway reactivity is reduced in the absence of CD38. A: changes in lung resistance (R_L) in response to different doses of methacholine (MCh) in wild-type naïve (WT Naïve, $n = 6$) and IL-13-treated (WT IL-13, $n = 10$) mice. B: changes in R_L in response to different doses of MCh in CD38KO naïve (KO Naïve, $n = 6$) and IL-13-treated (KO IL-13, $n = 10$) mice. C: changes in R_L in response to different doses of MCh in the IL-13-treated (WT IL-13, $n = 10$) and CD38KO (KO IL-13, $n = 10$) mice. D: changes in MCh-induced R_L following IL-13 compared with respective naïve controls. Note significantly larger increase in R_L (responsiveness) to inhaled MCh in the WT IL-13-challenged mice than in the CD38KO mice. E: airway sensitivity to inhaled MCh does not change significantly ($P = 0.052$) following intranasal IL-13 in CD38KO or WT mice. F: airway reactivity to inhaled MCh is significantly elevated following intranasal IL-13 only in the WT mice. Values are shown as means $\pm$ SE. * $P < 0.05$. NS, not significant.



CD38KO animals compared with the respective naïve controls, characterizing AHR (Fig. 1, A and B). However, the AHR was significantly less in the CD38KO mice (Fig. 1C). Specifically, changes in R_L in response to methacholine concentrations of >6.25 mg/ml were approximately twofold lower in the CD38KO than in the WT mice. Indeed, the magnitude of the methacholine responses in the IL-13-exposed CD38KO mice was similar to that observed in naïve WT mice. The baseline values of R_L were similar in the WT and the CD38KO mice following IL-13 challenge. The changes in R_L following IL-13 treatment for each of the methacholine doses were calculated, and the results showed significantly greater increases in R_L values in the WT mice than in the CD38KO mice (Fig. 1D).

We next examined the underlying contributors to airway responsiveness to methacholine, i.e., airway sensitivity (lowest dose of methacholine that induced bronchoconstriction) and reactivity (slope of the methacholine dose-response curve) (24). Intranasal IL-13 treatment did not change airway sensitivity to methacholine in WT or CD38KO mice compared with respective naïve controls (Fig. 1E). Interestingly, airway reactivity increased significantly only in the WT mice (Fig. 1F). Since mice exhibited excessive airway secretion, bradycardia, and even death at methacholine doses >100 mg/ml, thus producing inconsistent results, the maximal dose to produce bronchoconstriction could not be determined. Nonetheless, at the highest dose of methacholine administered (100 mg/ml), the WT mice showed a 5.4-fold increase in R_L as opposed to the 2.9-fold increase observed in the CD38KO mice compared with respective R_L values following saline administration.

Isometric force measurement in the isolated trachea. Our data indicated that naïve CD38KO mice as well as CD38KO mice repeatedly exposed to IL-13 make quantitatively smaller responses to methacholine than comparably treated WT mice. In previous studies, we showed that the $[Ca^{2+}]_i$ responses to acetylcholine were lower in airway myocytes from CD38KO mice than in the WT mice (8). In naïve tracheas, concentration-response curves to carbachol were similar between the WT and CD38KO mice (data not shown). Following incubation with IL-13, carbachol-induced contractions in CD38KO and WT tracheal segments were significantly augmented compared with the respective naïve controls at concentrations between 100 nM and 10 μ M (data not shown). In addition, the tracheal segments from CD38KO and WT mice exposed to IL-13 generated similar maximal isometric force in response to carbachol (data not shown). The rate of isometric force generation (e.g., reactivity) in the IL-13-treated tracheal segments obtained from WT mice was significantly higher than that developed in the CD38KO mice (Fig. 2A). However, the minimum concentration of carbachol required to produce contraction (e.g., sensitivity) was similar between tracheal segments obtained from naïve and IL-13-treated WT and CD38KO mice (Fig. 2B). In tracheal segments obtained from WT and CD38KO mice precontracted with carbachol ($\sim 75\%$ of maximum contraction), isoproterenol-induced relaxation was significantly decreased and to a similar magnitude following IL-13 treatment compared with the respective naïve controls (Fig. 2C).

Cytokine and chemokine levels in the bronchoalveolar fluid of IL-13-treated mice. One consequence of repeated IL-13 exposure is the accumulation of inflammatory cells, particularly eosinophils and T helper type 2 lymphocytes in the airways (42). We have previously shown that CD38 regulates

leukocyte trafficking both in vitro and in vivo (29, 30). To determine whether CD38 regulates the migration of inflammatory cells to the lungs of mice repeatedly exposed to IL-13, we first measured levels of IL-13, eotaxin-2, and IL-5, which are involved in eosinophil recruitment, in the BAL fluid of IL-13-treated WT and CD38KO mice. Both the WT and CD38KO mice showed significant and comparable elevations in eotaxin-2 levels following repeated IL-13 challenge, whereas no changes were observed in IL-5 levels (Fig. 3). As expected, the IL-13 levels in the BAL fluid were significantly elevated and to similar extents in both the WT and CD38KO mice following repeated intranasal IL-13 treatment (Fig. 3).

Bronchoalveolar inflammatory cell infiltrate. Since the chemokines and cytokines associated with induction of inflammation were upregulated in the lungs of IL-13-treated WT as well as CD38KO mice, we next analyzed total and differential cell counts in the BAL fluid obtained from naïve and IL-13-treated WT and CD38KO mice. Total inflammatory cell numbers revealed significant increases following IL-13 treatment compared with the naïve for both WT and CD38KO mice (Fig. 4). There were significant and comparable increases in the numbers of neutrophils, lymphocytes, and eosinophils in the BAL of IL-13-treated WT and CD38KO mice, suggesting that IL-13 can induce airway inflammation in the absence of CD38.

Histopathological changes of the lung parenchyma. To assess whether IL-13 induced an equivalent inflammatory response in the lung parenchyma of CD38KO and WT mice, we scored histological changes in the lung of IL-13-challenged mice. IL-13 induced significant inflammatory cell infiltration into the lung parenchyma in both the CD38KO and WT mice (Fig. 5A). Alveolar, peribronchiolar, and perivascular inflammatory infiltrate were all significantly higher in the IL-13-treated mice compared with naïve animals (Fig. 5B). Goblet cell numbers in the central and peripheral bronchi significantly increased in both WT and CD38KO mice following IL-13 challenges (data not shown). Together, these data indicate that IL-13 induces a comparable inflammatory response in both CD38KO and WT animals yet induces different effects on airway reactivity.

DISCUSSION

In this study, we sought to elucidate the role of CD38 on AHR by using CD38-deficient mice in a model of IL-13-induced airway disease. We demonstrate that the absence of CD38 reduced, but did not eliminate, AHR, and the underlying mechanism appears to involve impaired ASM reactivity to muscarinic agonists. Because CD38KO mice have defective T cell priming by dendritic cells (28, 29), we decided not to use an antigen-induced allergic model but instead directly administered IL-13 to the airways. IL-13 was chosen because of its critical role in asthma, it has been studied in mouse models of the disease, and it has direct effects on airway myocytes and epithelial cells (13, 22, 40–42). We have previously shown that IL-13 induces strong CD38 expression and ADP-ribosyl cyclase activity in ASM cells. We further demonstrated that the CD38/cADPR pathway has a major role in IL-13- as well as TNF- α -induced calcium hyperresponsiveness in ASM cells (5, 6, 18).

In the present report, intranasal administration of IL-13 in WT mice produced robust AHR to inhaled methacholine, in

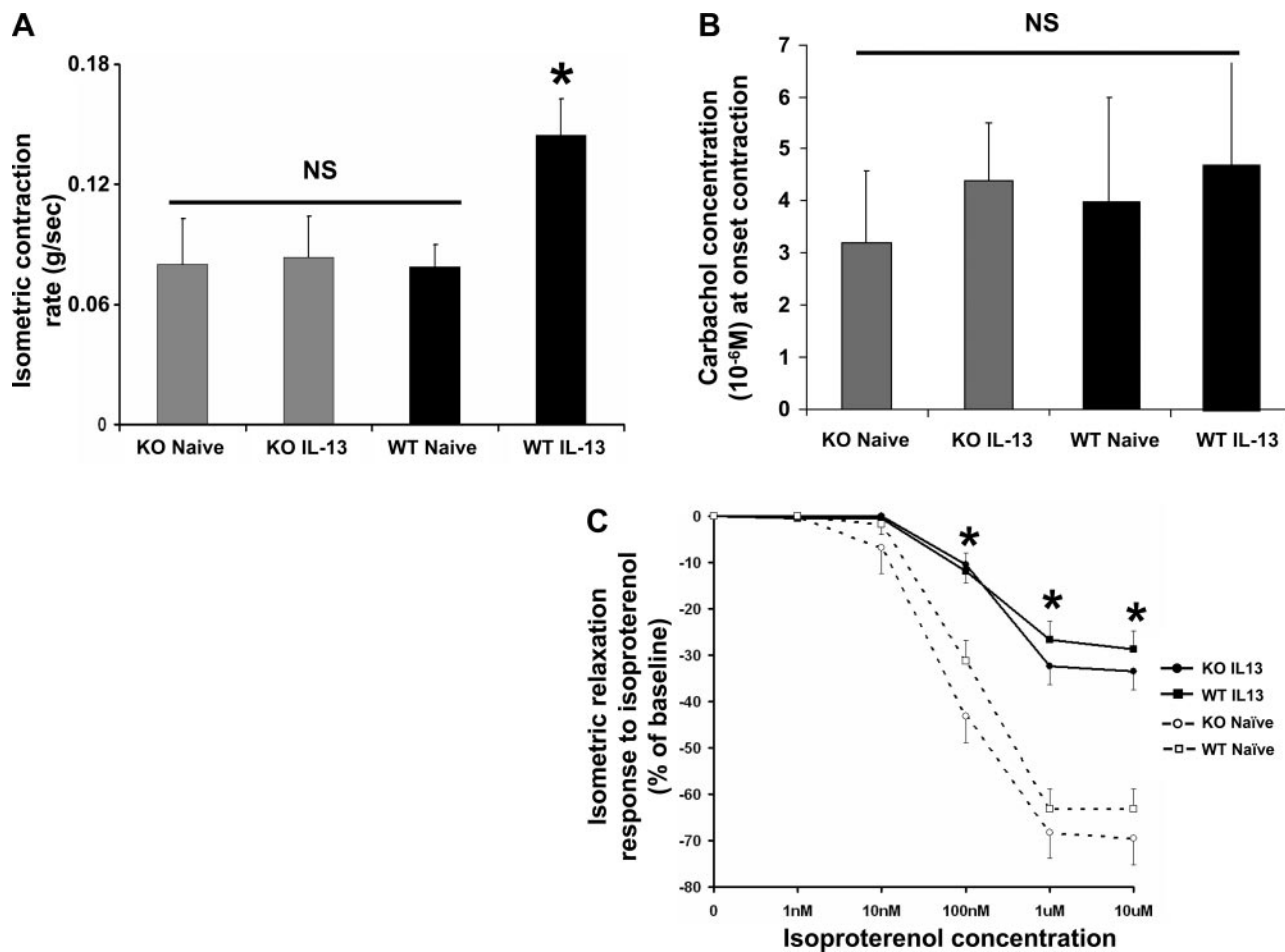


Fig. 2. Measurement of reactivity and sensitivity to carbachol stimulation and relaxation responses to isoproterenol in isolated tracheal segments of CD38KO and WT mice ($n = 8-10$ /group). **A:** rate of isometric force responses (e.g., slope or reactivity) to continuous cumulative carbachol stimulation increases significantly following incubation with IL-13 in WT but not in CD38KO tracheal segments. **B:** minimal carbachol concentration to trigger contraction (e.g., sensitivity) does not change following IL-13 exposure for both CD38KO and WT mice. **C:** relaxation responses to isoproterenol are significantly reduced by IL-13 treatment to a similar magnitude in the tracheal rings obtained from WT and CD38KO mice. All values shown are means $\pm$ SE. * $P < 0.05$.

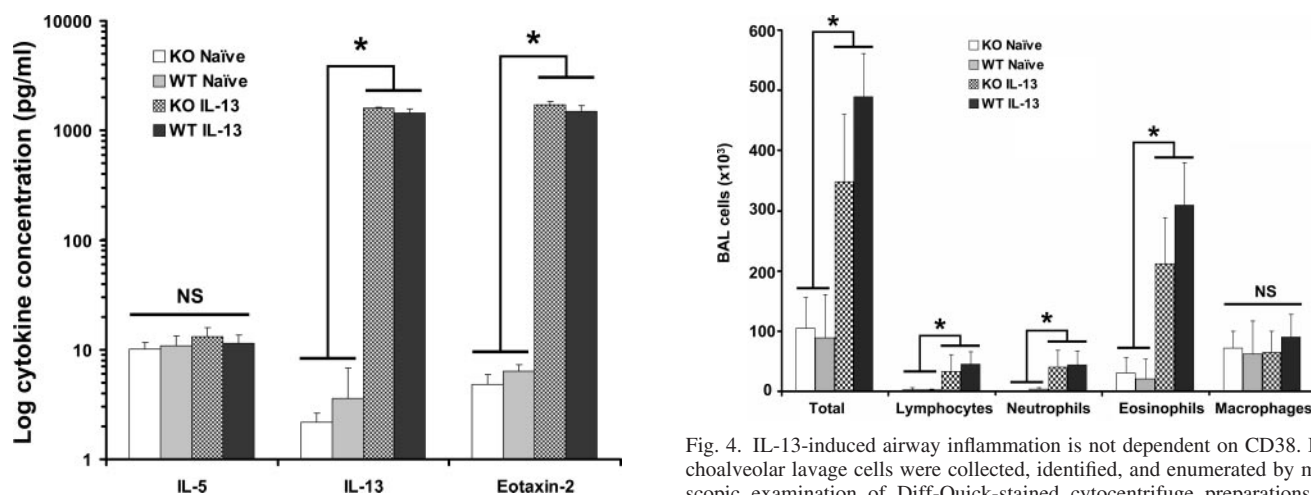


Fig. 3. CD38 is not required for IL-13-induced changes in cytokine/chemokine responses in the airways. IL-5, IL-13, and eotaxin-2 levels were measured in the bronchoalveolar lavage fluid obtained from naïve ($n = 6$ /group) and IL-13-challenged ($n = 10$ /group) mice. Note significant elevations in the levels of IL-13 and eotaxin-2 following IL-13 treatment in both the WT and the CD38KO mice. No significant changes in IL-5 levels were seen. Values are shown as means $\pm$ SE. * $P < 0.05$.

Fig. 4. IL-13-induced airway inflammation is not dependent on CD38. Bronchoalveolar lavage cells were collected, identified, and enumerated by microscopic examination of Diff-Quick-stained cytocentrifuge preparations. Repeated IL-13 challenge induced significantly greater total inflammatory cell numbers in both the WT mice (WT IL-13) and the CD38KO (KO IL-13) mice ($n = 10$ /group) compared with mice challenged with sterile water (WT Naive and KO Naive) ($n = 6$ /group). Eosinophils were the predominant inflammatory cell type following IL-13 treatment, with the numbers of lymphocytes and neutrophils elevated to a smaller magnitude. Data are shown as means $\pm$ SE. * $P < 0.05$.

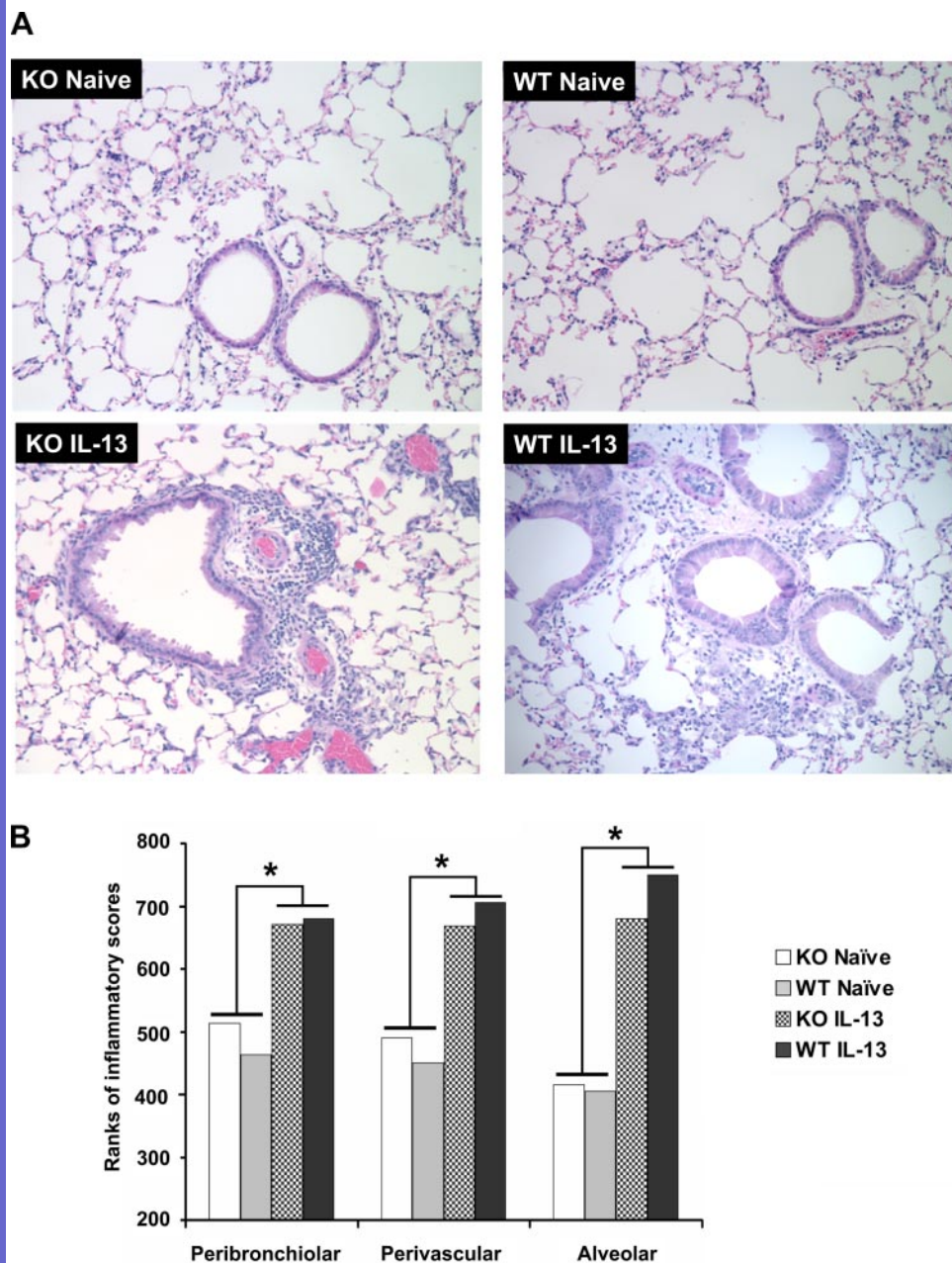


Fig. 5. Parenchymal inflammation was similarly increased in CD38KO and WT mice following IL-13 administration. Lung inflammation was assessed in tissue sections from WT and CD38KO mice that were exposed to IL-13 3 times. **A**: representative photomicrographs ($\times 200$ magnification) of hematoxylin and eosin-stained lung sections of naïve ($n = 6/\text{group}$) and IL-13-treated ($n = 10/\text{group}$) animals. **B**: ranking of inflammation scores for alveolar, peribronchiolar, and perivascular regions of the lung from naïve ($n = 6/\text{group}$) and IL-13-treated ($n = 10/\text{group}$) mice.

agreement with previous investigations (13, 22, 41, 42). IL-13 treatment also caused AHR in the CD38KO mice, although of a magnitude significantly lower than in the WT mice. Analysis of the methacholine dose-response curve showed that airway reactivity, as defined by the rate of increase in respiratory resistance for a given increase in methacholine dose (24), was significantly increased in the IL-13-treated WT mice but not in the IL-13-treated CD38KO mice. This impaired responsiveness to methacholine in the CD38KO mice is unlikely to be due to lack of an adequate inflammatory response, since these mice developed significant airway inflammation following IL-13 challenges. Eotaxin-2, a chemokine that is involved in eosinophil recruitment (2, 12), and numbers of eosinophils increased similarly in the WT and the CD38KO mice. Although we have previously shown that CD38KO mice make reduced lung

inflammatory responses to *Streptococcus pneumoniae* (28), this appears to be due to the inability of CD38KO granulocytes to migrate in response to bacteria-derived chemoattractants. The data presented here would suggest that CD38KO eosinophils and lymphocytes are competent to respond to the chemokines, such as eotaxin, that are induced by IL-13. This is not too surprising as signaling through some chemokine receptors occurs normally in the absence of CD38 and cADPR (28).

A previous investigation showed that exposure of ASM to IL-13 caused augmented contractility to acetylcholine and impaired relaxation to isoproterenol, and these effects were largely due to IL-13-mediated IL-5 expression and secretion (14). In our study, we provide similar evidence for contractility and relaxation responses in tracheal segments obtained from both the WT and the CD38KO mice following IL-13 treatment,



although IL-5 secretion by the tracheal rings was not measured. Isoproterenol concentration-relaxation responses were attenuated in tracheal rings following IL-13 treatment, but to a similar magnitude in the tracheal rings obtained from WT and the CD38KO mice. Furthermore, IL-5 levels in the BAL fluid were unchanged and comparable in the WT and CD38KO mice following IL-13 challenge. These results suggest that decreased relaxation and/or differences in IL-13-induced IL-5 secretion by immune or resident airway cells may not account for the difference in the magnitude of AHR between the WT and CD38KO mice following IL-13 challenge.

Together, our results demonstrate that AHR is attenuated in the absence of CD38, despite the presence of a robust inflammatory response in these mice. We show that in vivo airway reactivity to methacholine and in vitro ASM reactivity to carbachol increase significantly in the WT but not in the CD38KO mice upon IL-13 exposure. In previous studies in ASM cells, we found that IL-13 modulates CD38 expression and ADP-ribosyl cyclase activity. Furthermore, the CD38/cADPR pathway plays a major role in IL-13-induced calcium hyperresponsiveness, and airway myocytes from CD38KO mice have attenuated calcium responses to contractile agonists (5, 8). On the basis of our previous results, we suggest that a heightened $[Ca^{2+}]_i$ mobilization through the CD38/cADPR pathway in ASM cells upon agonist stimulation contributes to the increased ASM contraction, leading to excessive airway narrowing and AHR in vivo. This suggestion is also supported by other studies showing that enhanced calcium signaling in ASM cells is associated with enhanced ASM contractility and AHR in vivo (3, 36). This is anticipated since Ca^{2+} is an important signaling molecule regulating ASM contraction and bronchomotor tone. Another consideration is that the CD38/cADPR-calcium pathway in ASM may be just one of various signaling pathways mediating excitation-contraction coupling and AHR. For example, ASM cells from asthmatic patients have increased myosin light chain kinase mRNA expression and greater shortening velocity and capacity than cells from normal subjects (25). Increased myosin light chain phosphorylation can result in a faster rate of cross-bridge cycling and shortening velocity in the early phases of contraction (17, 33, 34). Furthermore, IL-13 has direct effects on signaling pathways in human airway myocytes inducing STAT-6 and ERK/MAP kinase phosphorylation (23). Finally, changes in calcium sensitivity of the contractile proteins through Rho kinases can also contribute to AHR (4). It is possible that the IL-13-induced changes in airway reactivity requiring the presence of CD38 may arise from one or more of the mechanisms described above.

In summary, we have demonstrated a major role of CD38 in AHR using CD38KO mice intranasally challenged with IL-13. Our data indicate that an intrinsic defect in the CD38KO myocytes leads to reduced airway reactivity to contractile agonists after IL-13 exposure both in vivo and in vitro. The mechanisms underlying the "permissive" role of CD38 in AHR may involve regulation of intracellular calcium dynamics in ASM cells.

ACKNOWLEDGMENTS

We thank Dr. Divya Chaudhary of Wyeth Pharmaceuticals, Inc., for the generous gift of mu rIL-13 used in these studies.

GRANTS

This study has been supported by National Institutes of Health Grants HL-057498 (to M. S. Kannan) and AI-43629 and AI-057996 (to F. E. Lund).

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