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Alcohol reduces airway hyperresponsiveness (AHR) and allergic airway inflammation in mice

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Oldenburg PJ, Poole JA, Sisson JH. Alcohol reduces airway hyperresponsiveness (AHR) and allergic airway inflammation in mice. *Am J Physiol Lung Cell Mol Physiol* 302: L308–L315, 2012. First published November 23, 2011; doi:10.1152/ajplung.00077.2011.—There is very limited knowledge about the effects of alcohol on airway hyperresponsiveness and inflammation in asthma. Historical accounts of alcohol administration to patients with breathing problems suggest that alcohol may have bronchodilating properties. We hypothesized that alcohol exposure will alter airway hyperresponsiveness (AHR) and pulmonary inflammation in a mouse model of allergic asthma. To test this hypothesis, BALB/c mice were fed either 18% alcohol or water and then sensitized and challenged with ovalbumin (OVA). AHR was assessed by means of ventilation or barometric plethysmography and reported as either total lung resistance or enhanced pause, respectively. Airway inflammation was assessed by total and differential cell counts in bronchoalveolar lavage fluid (BALF), cytokine levels in BALF, lung histology, and serum immunoglobulin E (IgE) levels. Alcohol feeding significantly blocked methacholine-induced increases in AHR compared with water-fed controls. Alcohol feeding significantly reduced total cell numbers (64%) as well as the number of eosinophils (84%) recruited to the lungs of these mice. Modest changes in lung pathology were also observed. Alcohol exposure led to a reduction of IgE in the serum of the EtOH OVA mice. These data demonstrate that alcohol exposure blunts AHR and dampens allergic airway inflammation indices in allergic mice and suggest that there may be an important role for alcohol in the modulation of asthma. These data provide an *in vivo* basis for previous clinical observations in humans substantiating the bronchodilator properties of alcohol and for the first time demonstrates an alcohol-induced reduction of allergic inflammatory cells in a mouse model of allergic asthma.

asthma; enhanced pause; ovalbumin; BALB/c mice

THE LUNG, LIKE THE LIVER AND THE BRAIN, undergoes detrimental effects in response to alcohol exposure. Alcohol abuse increases the risk of pneumonia (34), upper respiratory infections, and acute respiratory distress syndrome (21). The exposure of the airways through the volatility of alcohol likely accounts for many of the biological effects of alcohol on lung airway functions (10, 11). Alcohol, at modest levels of consumption, has been shown to have protective effects in the cardiovascular system (24). It may also have positive effects in lung diseases such as asthma (1, 5) and chronic obstructive pulmonary disease. Others have demonstrated that alcohol exposure in the lung can cause inhibition of clearance (2), impaired ciliary motility (2, 10, 46), inflammation (41), suppression of pulmonary neutrophil recruitment (7), and lung chemokine production (3).

Asthma is an airway disease occurring when airflow is reversibly obstructed leading to shortness of breath, wheezing and cough. The predominant pathophysiological findings of asthma are airway hyperresponsiveness (AHR) and airway inflammation (6). Asthmatic airway inflammation is classically marked by influx of eosinophils (4) as well as increases in CD4⁺ T cells infiltrating the lungs polarized toward a T-helper type 2 (Th2) cell response. This response is mediated by increased immunoglobulin E (IgE) and altered levels of Th2 cytokines such as IL-4, IL-5, and IL-13, resulting in the pulmonary inflammation, AHR, goblet cell metaplasia, and mucus production that characterizes asthma (9, 10). The effects of alcohol on asthma pathophysiology are not known, and therefore the objective of this study is to gain greater insight into these effects.

Because alcohol has historically been used as an experimental treatment for asthma, we explored the effects of alcohol on airway hyperresponsiveness and pulmonary inflammation in an allergic mouse model of asthma. Previous studies from our laboratory in nonasthmatic mice demonstrate that alcohol feeding decreases airway responsiveness (28) and airway smooth muscle contraction through a PKG-mediated pathway (29). In this study, we hypothesize that brief alcohol exposure modulates AHR and pulmonary inflammation in a mouse model of allergic asthma. We describe the ability of alcohol to attenuate allergic asthma parameters, blunt bronchoconstriction, and significantly reduce the total cell numbers and influx of eosinophils into the lung.

MATERIALS AND METHODS

Mice. Male BALB/c mice 6–8 wk old were obtained from Charles River Laboratories (Frederick, MD). The mice were kept in community cages with 12-h periods of light and dark cycles and maintained on standard rodent chow with access to water *ad libitum*. All animal care and experimentation was approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center in accordance with the principles and guidelines of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Ethanol administration. Mice were either fed alcohol *ad libitum* directly in their drinking water using a ramping method where the alcohol groups drank 10% wt/vol alcohol for 2 days, 15% wt/vol alcohol for 5 days, and 18% wt/vol alcohol for 6 to 10 wk, while the control groups drank water only; see Fig. 1 (10, 11, 38). As previously reported (11), no significant weight loss or nutritional disturbances were observed in mice fed this *ad libitum* diet. All alcohol administration was sufficient to achieve a blood alcohol content (BAC) level of at least 45 mg/dl, which is equivalent to a human consuming 1–2 alcoholic beverages within 1 h.

Sensitization. Mice were sensitized with chicken egg ovalbumin (OVA; Sigma, St. Louis, MO) via a 29-day protocol adapted from Klein et al. (23) (see Fig. 1). Briefly, after prior sensitization by

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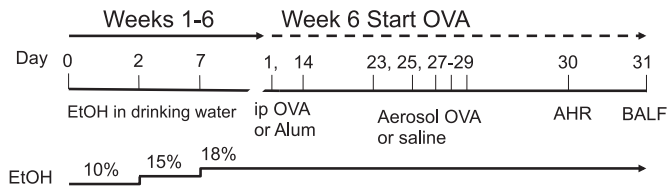


Fig. 1. Protocol for the administration of EtOH and ovalbumin (OVA). EtOH was ramped up to 18% beginning on day 0. The OVA sensitization protocol was started at week 6 of EtOH drinking, during which time the mice continued to drink EtOH for the remainder of the experiment. AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; ip, intraperitoneal.

intraperitoneal (ip) injection of OVA ($2 \times 20 \mu\text{g}/0.1 \text{ ml}$, 2 wk apart), mice were exposed to an OVA aerosol. Exposure to OVA (1%) aerosols was performed using a Plexiglas chamber and ultrasonic nebulizer as described elsewhere (27). Age-matched control animals were injected ip with alum adjuvant and exposed to saline aerosols only. Preliminary experiments demonstrated significantly increased plasma IgE concentrations in sensitized mice exposed to OVA aerosols.

IgE determination. Total IgE concentrations were monitored throughout the sensitization protocol in all mouse groups to verify that the mice had become sensitized. Blood samples were drawn by retroorbital bleeds 24 h before the first ip OVA injection, 24 h before the first OVA aerosol challenge, and 24 h after the last aerosol challenge. The serum was isolated and stored at -80°C until assayed. A mouse IgE ELISA set from BD Biosciences (San Diego, CA) was used to assay for total IgE concentrations following the manufacturer's instructions.

Invasive pulmonary function measurement. In these studies, mice were anesthetized with a ketamine (166 mg/kg) and xylazine (8 mg/kg) cocktail, tracheotomized with a steel 18-gauge cannula, and mechanically ventilated at a rate of 160 breaths/min, and tidal volume of 0.15 ml, using a computerized small animal ventilator (Finepoint; Buxco Electronics, Wilmington, NC) as previously described (19, 28, 32, 35). Mice were allowed to stabilize on the ventilator for 5 min before measurements commenced. Once the mice were stabilized, dose responsiveness to aerosolized methacholine ($1.5\text{--}48.0 \text{ mg/ml}$) was obtained and reported as total lung resistance (R_L).

Bronchoalveolar lavage. Twenty-four hours after exposure to MCh (48 h after final aerosol challenge), the mice were euthanized by ip injection of 75 mg/kg pentobarbital sodium, and bronchoalveolar lavage was performed according to established protocols (13, 39). Briefly, the trachea was exposed and cannulated. Three washings, each consisting of 1.0 ml of phosphate-buffered saline, were introduced into the lungs via the cannula and withdrawn to collect the cells. Bronchoalveolar lavage fluid (BALF) from each wash was placed in polypropylene tubes on ice. The collected BALF was then centrifuged at 1,600 revolution/min for 7 min at 4°C . The supernatant from the first wash was collected and stored at -80°C for cytokine determination. After supernatant removal, cells were resuspended in 1.0 ml of phosphate-buffered saline (pH 7.4). Total cell numbers were counted on a hemocytometer, and $1\text{--}5 \times 10^3$ cells were spun onto glass microscope slides (cytospin 3; Shandon Scientific, Cheshire, UK). The cell slides were air dried for 24–36 h, fixed, and stained with a Diff-Quik stain set (Dade Behring, Newark, DE). Differential cell counts of at least 300 cells per slide were made according to morphological criteria. The number of cells recovered was calculated and expressed as absolute cell numbers.

Lung cytokine analyses. Cytokine concentrations in whole lung lavage fluid were determined using a commercial Quantikine ELISA kits specific for mouse IL-4, IL-5, and IL-13 (R&D Systems, Minneapolis, MN). Briefly, assay diluent and samples were added and incubated for 2 h and then washed. Conjugate was added and further incubated for 2 h and then washed. Substrate was added next, and,

following a 30-min incubation, stop solution was added. Absorbance was measured at 450 nm with a 540-nm wavelength correction and concentrations calculated per the manufacturer's instructions. The sensitivity of the ELISA assay was 7 pg/ml .

Lung fixation and histological analysis. Lungs were removed from the mouse and inflated with 1.0 ml of a 10% neutral-buffered formalin solution (Sigma Chemical) following an established protocol (30). After inflation, the lungs were suspended under 18 cm H_2O pressure for 24 h in 10% formalin. Lungs were then removed and placed in 10% formalin for a further 48 h before being processed for histological sectioning. After paraffin embedding, $5\text{-}\mu\text{m}$ sections were cut and stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and calcitonin gene-related peptide (CGRP) stains for qualitative measures of cellular infiltration, goblet cell metaplasia, and neuropeptide identification, respectively.

Quantification of PAS-stained images. The positive areas for PAS cells were obtained using a Zeiss Primo Star microscope combined with a Canon EOS Rebel XS digital camera and analyzed using Image Pro image analysis software version 7.0 (Media Cybernetics, Bethesda, MD) for windows. On the basis of methods by Cho et al. (8) and Takagi et al. (40), an average of 10–15 photos of large and small airways were taken at random from mice from each group in a blinded fashion, and the total percentage of positive area for PAS per image was calculated. Color segmentation was used to identify total PAS-positive stained area per airway. This area was then divided by the total area of the image to quantitate the percentage of PAS-positive areas for the large and small airways and averaged per group of mice.

Cell culture. Beas-2B cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in bronchial epithelial cell growth medium (BEGM kit; Lonza/Clonetics, Walkersville, MD) at 37°C in a humidified atmosphere with 5% $\text{CO}_2\text{--}95\%$ air. Cells were seeded at $\sim 50\%$ confluence and used when 90–95% confluent. Cells were treated with either culture media (control) or 100 mM EtOH for the indicated amounts of time.

Assessment of PPAR- γ activity. Activation of peroxisome proliferator-activated receptor- γ (PPAR- γ) was determined with a commercially available ELISA kit (TransAM PPAR γ Transcription Factor Assay Kit; Active Motif, Carlsbad, CA) according to the manufacturer's instructions. The Nuclear Extraction Kit from Active Motif was used to isolate nuclear extracts by following the manufacturer's instructions. Results were normalized to protein concentrations as determined by a Bradford assay.

BAC. The BAC was monitored closely to verify that the mice had elevated levels of alcohol following each experiment. Upon euthanasia, 0.8–1.0 ml of whole blood was collected into serum separator tubes (BD Scientific, Franklin Lakes, NJ). The tubes were placed on ice for 30 min and then centrifuged at 8,000 revolution/min for 10 min. Serum was transferred to microcentrifuge tubes containing a rubber gasket and frozen at -80°C until assayed. The serum was assayed using an alcohol reagent set and alcohol control (Pointe Scientific, Canton, MI). Briefly, samples and controls were added to reconstituted reagent at 30°C , mixed, and incubated in a shaking water bath for 5 min. Samples were then transferred to a 96-well flat-bottom plate, and the absorbance was read at 340 nm. The ethanol concentration in mg/dl was calculated according to the manufacturer's defined procedure.

Chemicals. Ethyl alcohol dehydrated (200 proof) was obtained from PHARMCO-AAPER (Louisville, KY). Acetyl- β -methylcholine chloride and pentobarbital sodium were obtained from Sigma Chemical.

Statistics. All experimental data are expressed as means $\pm$ SE. Data were plotted using GraphPad Prism 4.0c (GraphPad Software, San Diego, CA), and the average airway responses between different groups were analyzed by analysis of variance followed by Bonferroni's post hoc analysis for multiple comparisons, or by Student's *t*-test where applicable. These tests were used to determine the level of

significance between all treatment groups. Probability levels <0.05 were considered statistically significant.

RESULTS

Ethanol feeding attenuates methacholine-induced AHR in OVA-sensitized mice. R_L was directly measured to determine AHR following inhalation of the bronchoprovocant, MCh. Six weeks of EtOH feeding and OVA sensitization significantly decreased ($P < 0.001$) the bronchoconstrictive response following administration of the 12.0-, 24.0-, and 48.0-mg MCh doses compared with the water-OVA group (Fig. 2). AHR to MCh was significantly increased in the EtOH-OVA groups compared with the EtOH-only group at the 24- and 48-mg doses (Fig. 2). The EtOH-only group exhibited no response to the MCh challenge, as observed in previous studies from our laboratory (28). Airway responses to alcohol using a noninvasive whole body plethysmography method complemented the direct measures of lung resistance (data not shown). Monitoring the BAC confirmed that EtOH-OVA and EtOH-only mice had elevated blood alcohol levels (>45 mg/dl) and that water-only control mice had no detectable alcohol in the blood (data not shown). We also observed that the sensitization process had no effect on BAC levels in these mice. Individual BAC changes did not directly correspond to individual changes in R_L (data not shown). These data demonstrate that 6 wk of ethanol consumption results in a significant attenuation of airway responsiveness in an allergic mouse model.

EtOH consumption reduces BALF total cell counts and BALF eosinophils. We observed a significant ($P < 0.05$) reduction in the total cells present in the BALF of the EtOH-OVA mice compared with the water-OVA mice (Fig. 3). There was no difference in lavage cellularity between the nonsensitized water-only and EtOH-only mice. Cell differentials of the water-only, water-OVA, EtOH-only, and EtOH-OVA groups of mice are reported in Fig. 4. We observed a significant ($P < 0.01$) decrease in the absolute numbers of eosinophils in the EtOH-OVA group compared with the water-OVA group. There was a significant reduction in BALF eosinophils in the EtOH-OVA group compared with the water-OVA group (38% eosinophilia vs. 70%, $P < 0.01$). The EtOH-OVA group had a significant ($P < 0.01$) increase in eosinophil recruitment to the

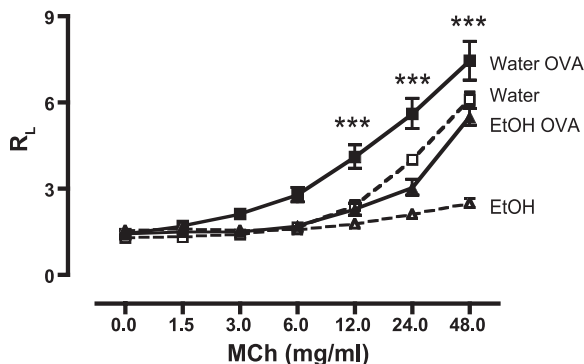


Fig. 2. Ethanol feeding blocks methacholine-induced airway responsiveness. EtOH-drinking OVA-sensitized mice demonstrated a highly significant attenuation in total lung resistance to MCh compared with water-drinking sensitized mice $***P < 0.001$. The EtOH-OVA group also had increased responsiveness compared with EtOH-only-consuming mice. No differences were observed between the EtOH-OVA and the water-only groups. $n = 8-10$ mice per group. Data are presented as means $\pm$ SE. R_L , lung resistance.

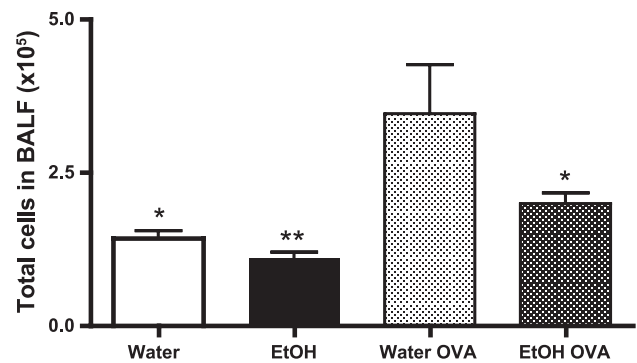


Fig. 3. Ethanol attenuates total cell counts in BALF. EtOH consumption resulted in a significant attenuation in total cells ($*P < 0.05$, $**P < 0.01$) compared with the water-OVA mice. No differences were observed between the nonsensitized groups. $n = 8-12$ mice per group.

lungs compared with the EtOH-only group. The water-OVA group also showed a significant ($P < 0.01$) increase in eosinophil recruitment to the lungs compared with the water only group. Eosinophilia was absent in the nonsensitized groups of mice, where macrophages were the primary observed cell type. There were no differences in absolute macrophage numbers among any groups of mice. There was a small but statistically significant decrease in neutrophil numbers in the BALF of the EtOH-OVA compared with water-OVA groups with no changes in the nonsensitized control groups ($43,867 \pm 19,192$ vs. $10,092 \pm 6,667$; $P < 0.05$). BALF lymphocytes were not different among any of the four groups. We also observed that the individual BAC changes did not directly correspond to individual changes in inflammation (data not shown). From these data, we conclude that EtOH impairs recruitment of inflammatory cells into the lung in this murine model of allergic asthma.

Effect of EtOH on Th2 cytokine levels. Because eosinophils and bronchial hyperreactivity were significantly diminished with EtOH consumption in the allergic OVA-challenged group, we next investigated whether Th2 cytokines in the BALF were reduced in EtOH-OVA group compared with the

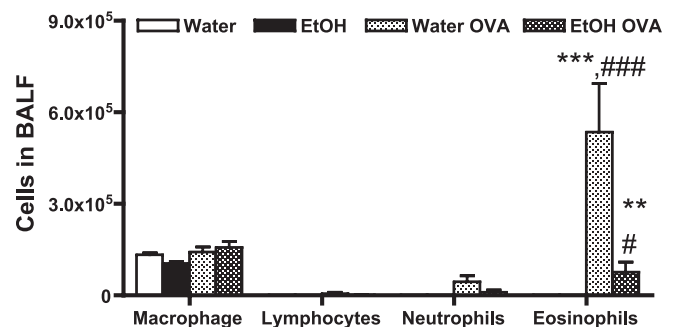


Fig. 4. EtOH consumption decreases lung eosinophilia in sensitized mice. EtOH-OVA mice demonstrated a significant attenuation in the total number of eosinophils ($**P < 0.01$) in the BALF compared with water-OVA mice and a significant potentiation in eosinophils compared with the EtOH only mice ($\#P < 0.05$). The water-only group contained significantly more eosinophils than the water-only and EtOH-only groups of mice ($***, ###P < 0.001$). No eosinophils were detected in the water-only and EtOH-only groups of mice. No observable differences were observed in the total number of macrophage, lymphocyte, or neutrophil cells present between any groups in the BALF. Both control groups contained primarily all macrophage cells. $n = 8-12$ mice per group.

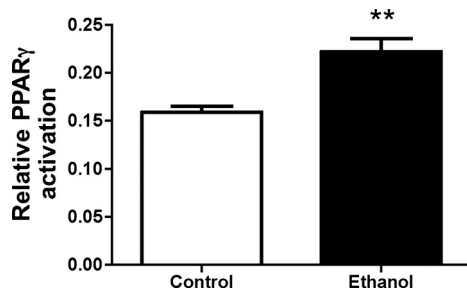


Fig. 5. EtOH activates peroxisome proliferator-activated receptor (PPAR)- γ in Beas-2B cells. Pretreatment of Beas-2B cells with 100 mM EtOH for 6 h results in a significant activation of PPAR- γ (** $P < 0.006$) compared with media only-treated control cells. No statistical differences were observed with 3-h treatments. Data represent pooled results from at least 3 separate experiments and are reported as means $\pm$ SE.

water-OVA group. Although trends for decreased IL-5 levels were observed in the EtOH-OVA group compared with the water-OVA group, these did not reach statistical significance (8.98 ± 4.97 vs. 20.66 ± 9.69 , $P = 0.6$). Samples from the nonsensitized mice had undetectable levels of IL-5. Levels of other Th2 cytokines (IL-4, IL-13, granulocyte/macrophage colony-stimulating factor) were not different among the four groups (data not shown).

EtOH increases PPAR- γ activity in Beas-2B cells. Recently, PPAR- γ activity has been shown to modulate inflammation (20, 31), and in these studies we sought to determine whether EtOH modulated PPAR- γ activity in bronchial epithelial cells. Beas-2B cells grown to $\sim 90\%$ confluence were treated with either 100 mM EtOH or media (control) to determine the activity of PPAR- γ . Following 6 h of exposure, EtOH induced increased PPAR- γ activity ($P < 0.006$, Fig. 5). These data demonstrate that EtOH can induce the anti-inflammatory effects of PPAR- γ . Increased PPAR- γ activity may explain, at least in part, why there was diminished airway inflammation in the lungs of the mice that consumed EtOH.

EtOH consumption reduces IgE levels in allergic asthmatic mice. EtOH consumption resulted in a significant ($P < 0.01$) decrease in serum IgE levels following OVA aerosol challenges (Fig. 6). IgE levels increased subsequent to the ip sensitization injections and were further increased ~ 10 -fold following the aerosol OVA challenges. No increases in IgE levels were observed in nonsensitized mice. These data suggest that EtOH consumption inhibits the development of the allergic phenotype given that serum IgE levels and BALF eosinophils were lower in the EtOH-OVA group compared with the water-OVA group.

EtOH exposure reduces inflammation, goblet cell metaplasia, and CGRP expression in the airways. H&E-stained sections from the EtOH-OVA group demonstrate less peribronchial and perivascular inflammation surrounding the large airways compared with the water-OVA mice (Fig. 7, A–D). No cellular infiltrates around the airways or vasculature of nonsensitized mice were observed. PAS-stained sections of EtOH-OVA mouse lungs also showed significantly ($P < 0.05$) less PAS-positive mucus staining in the large airways compared with the water-OVA group (Fig. 7, E–I). Nonsensitized control mice did not have any positive PAS staining. Because CGRP is associated with airway responsiveness and inflammation, we performed immunohistochemistry on lung sections to deter-

mine whether EtOH or allergen sensitization was altering its expression. We observed decreased positive staining for CGRP in airways of the EtOH-OVA mouse lungs compared with water-OVA mouse lungs (Fig. 8, A–D). Nonsensitized control mice did not demonstrate any differences in staining for CGRP, suggesting that levels of this neuropeptide are increased in response to allergen sensitization and can be attenuated with exposure to EtOH. Collectively, these data demonstrate that consumption of EtOH diminishes lung inflammation, goblet cell metaplasia, and CGRP in this allergic mouse model.

DISCUSSION

Alcohol has been historically used as a treatment for airway diseases (33, 36), as small clinical studies in humans have demonstrated that EtOH can act as a bronchodilator and improve the vital capacity in asthmatics (5, 17, 33). Although it is increasingly recognized that alcohol consumption alters many lung responses (12, 22, 43), the effect of alcohol in allergic asthma is poorly understood and may have important clinical implications.

Alcohol consumption has been previously found to cause inhibition of pulmonary clearance (2), impaired ciliary motility (2, 10, 46), inflammation (41), suppression of lung neutrophil recruitment (7), and chemokine production (3). Our laboratory has shown that alcohol can modify ciliary beat frequency through a nitric oxide (NO) (37) and dual kinase activation pathways (45).

Our laboratory has previously demonstrated that EtOH in nonsensitized mice significantly dampens AHR to MCh when administered via the drinking water (28). Our work, as well as that of others, has also previously reported that ethanol directly reduces smooth muscle contraction to methacholine in vitro, and, moreover, methacholine-induced AHR in mice is diminished following either ip or alcohol-feeding strategies (28). It is possible that the effects observed here could be secondary to the effect of ethanol on dampening allergen-induced inflammation. This was evident by the observed decrease in the

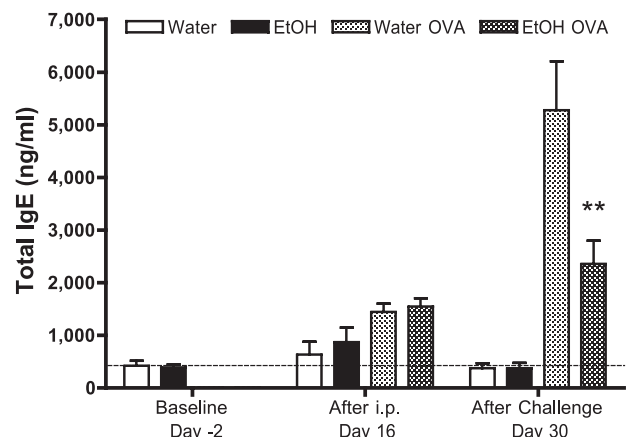


Fig. 6. EtOH exposure attenuates immunoglobulin E (IgE) levels in OVA-sensitized mouse serum. Effect of EtOH drinking and OVA sensitization on IgE levels in mouse serum. Following the initial serum collection, mice were separated into 4 groups. IgE levels were increased 24 h after the second ip OVA sensitization injection. Following the 1% OVA aerosol challenges, the EtOH-OVA group demonstrated significantly (** $P < 0.01$) lower IgE levels compared with the water-OVA group. $n = 8$ –12 mice per group.

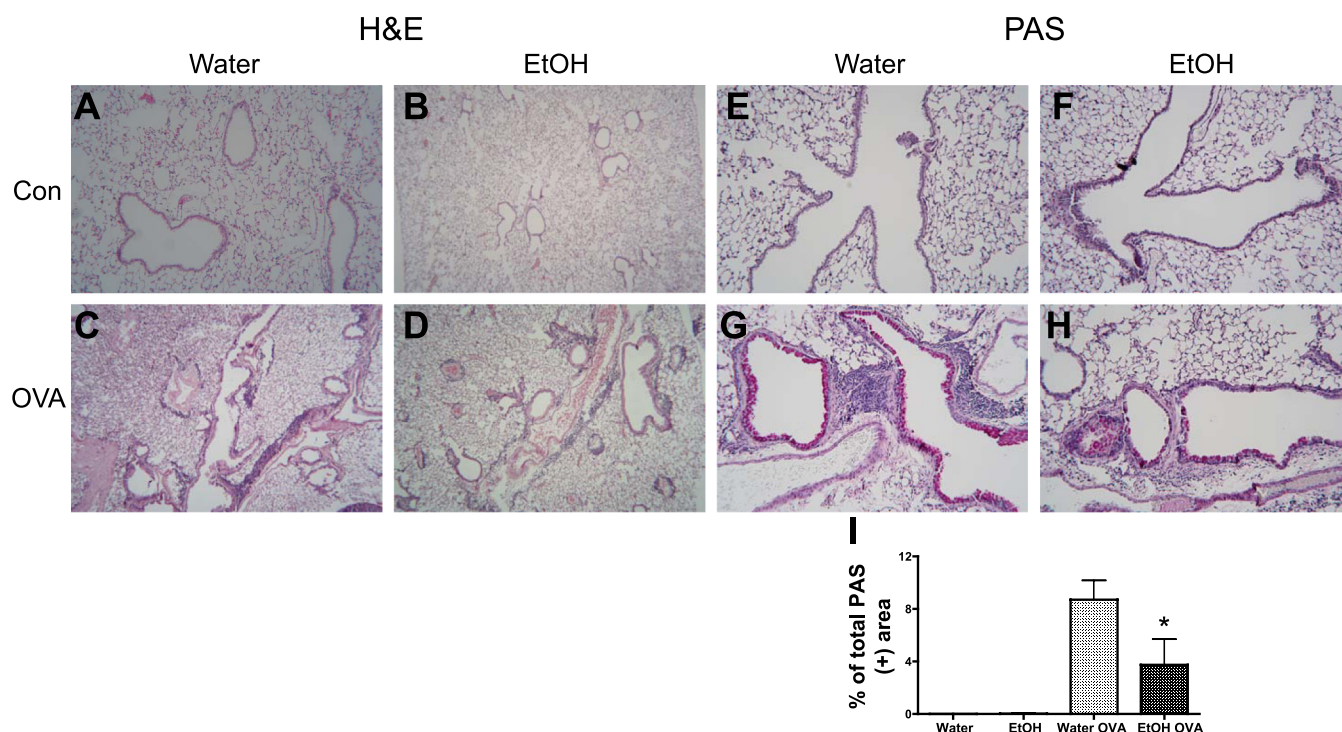


Fig. 7. Effect of EtOH on peribronchial and perivascular infiltrates and goblet cell metaplasia following OVA sensitization. Representative hematoxylin and eosin (H&E)-stained (A–D) and periodic acid Schiff (PAS)-stained (E–H) sections of the lungs. EtOH consumption decreases the amount of peribronchial and perivascular inflammation and goblet cell metaplasia in the sensitized mice (D and H, respectively) compared with water-drinking control mice (C and G, respectively). No increased inflammation or goblet cell metaplasia was observed in the control groups of mice (A, B, E, and F). $n = 8–10$ mice per group, images A–D were taken at $\times 4$, images E–H were taken at $\times 10$. Quantification of PAS staining of mouse lung tissue (I). $n = 8–12$ mice in each group. $*P < 0.05$. Results shown here are representative of 3 separate experiments.

recruitment of allergen effector cells, eosinophils, dampening of lung parenchymal inflammatory indices, and mild reduction in Th2 cytokine expression.

Allergen challenge in sensitized mice generates many of the same characteristic features observed in human asthma, namely increased AHR, airway eosinophilia, and damaged

lung tissue. In the present study, we observed that EtOH consumption significantly attenuates OVA-induced AHR compared with OVA-induced AHR in water-fed mice. These data are consistent whether direct (R_L) or indirect (enhanced pause) measures of airway reactivity are assessed and correlate well with results from older clinical evidence of alcohol having

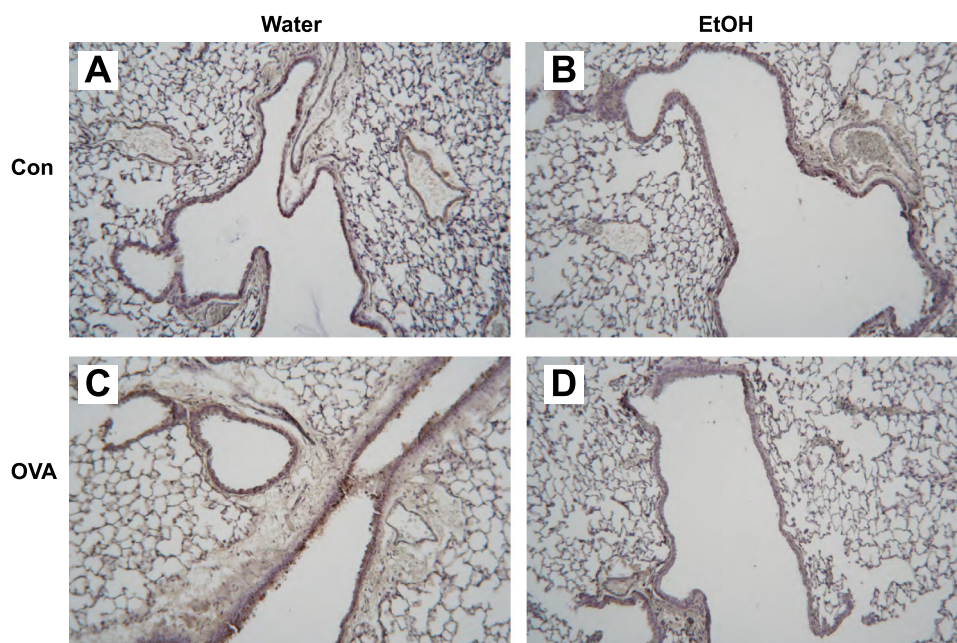


Fig. 8. Effect of EtOH consumption and OVA challenges on calcitonin gene-related peptide (CGRP) in mouse lungs. EtOH consumption decreases the amount of positive stained areas for CGRP in OVA-sensitized mice. Ubiquitous staining was observed in the water-OVA mice. Nonsensitized control mice did not demonstrate any differences in staining. $n = 8–10$ mice per group, images A–D were taken at $\times 10$. Images are representative of 3 separate experiments. All samples were stained at the same time to avoid experimental artifacts.

bronchodilatory properties (1, 17). In isolated rat airway smooth muscle cells exposed to EtOH, we observed a highly significant inhibition of contraction, which is reversible, demonstrating that EtOH is not lethal to the cells (29). From these studies we established that EtOH is likely modulating contraction through a NO/cGMP-dependent protein kinase (PKG)-dependent pathway. Collectively, these data strongly establish a role for EtOH attenuating AHR.

We also found evidence that EtOH consumption partially prevents the development of an allergic airway phenotype. Although our findings demonstrate a novel role for the effect of ethanol on attenuating bronchial hyperreactivity and airway inflammatory responses, future studies are warranted to investigate specific cellular responses that could also be targeted by ethanol, such as Th2 cellular responses, antigen-presenting cells, or dendritic cells. It is well established that sensitization and challenge with OVA potentiates the influx of eosinophils to the lungs of mice through a Th2-mediated pathway (4, 16). Th2 cells together with other inflammatory cells such as mast cells, B cells, and eosinophils have been shown to play critical roles in the initiation, development, and maintenance of asthma (6, 19, 25). Upon activation, these inflammatory cells produce Th2 cytokines and chemokines and mediate several regulatory and effector functions. These cytokines induce the production of allergen-specific IgE via B cells, eosinophil development and recruitment, mucus production, smooth muscle contraction, and ultimately AHR. Interestingly, we found that EtOH consumption before allergic sensitization and challenge may modulate allergic development and inflammation. Influx of airway eosinophils and serum IgE levels were significantly reduced in EtOH-fed OVA-sensitized mice compared with water-fed OVA-sensitized mice. The decrease in cellular influx paralleled our AHR data and supports current evidence that pulmonary inflammation enhances AHR (6). Decreased IgE levels influence how many eosinophils get recruited into the airways as well as the levels of cytokines present in the lung lavage fluid. Therefore, the differences we observed in the IgE levels correspond to decreases in cytokine levels, decreased eosinophilia, and decreased perivascular and peribronchial inflammation. Although there were trends for differences in IL-5 levels between the EtOH-OVA and water-OVA groups, these differences did not reach statistical significance (data not shown). IL-4 and IL-13 levels were not different between EtOH-OVA and water-OVA groups of mice (data not shown). Whereas we hypothesized that there would be significant changes in these inflammatory cytokines, as these cytokines are crucial to the development of airway inflammation and the influx of airway eosinophils, our studies did not find a significant role for EtOH modulating IL-4, IL-5, and IL-13.

It is possible that EtOH is exerting its bronchodilatory effects via noncytokine mediators, such as NO. Because ethanol has also been shown by others to reduce antigen-presenting cell responses and modulate T-cell phenotypes, future studies should investigate the role of these cellular specific responses in the present model.

EtOH affecting allergic airway inflammation is further supported by the lung histology, which demonstrates less perivascular and peribronchial infiltration in the OVA-sensitized mice that drank alcohol. EtOH consumption also significantly decreased goblet cell metaplasia in allergic mice, implying that less mucus was secreted in the alcohol-fed allergic mice. Less

mucus in the airways of allergic mice correlates with less obstruction in the airway, resulting in better airflow. We do not know whether the effects of EtOH on AHR and airway inflammation occur through a common mechanism or by separate pathways. Furthermore, it is possible that timing of either allergen or ethanol exposure is important in explaining these findings and should be further explored.

Overall, the data presented in this study support EtOH as a modulator of the Th2 allergic response in these mice although this mechanism remains undefined. Although trends for Th2 downregulation following ethanol exposure were observed, this did not reach significance. Future studies could consider investigating alternative time points or T-cell isolation techniques to phenotype the T-polarization status. From the data presented in this study, it is clear that multiple mechanisms for AHR and inflammation may be involved.

It remains unclear what distinct mechanism alcohol is acting through to cause decreased inflammation in this model. We have explored multiple possibilities in an effort to elucidate this. Data presented here strongly support a role for PPAR- γ activation in the airways, the involvement of NO, and also a role for the neuropeptide CGRP. One anti-inflammatory mechanism gaining attention in asthma is PPAR- γ activation, which can alter Th2 allergic responses. Luvizotto et al. (26) has recently reported that hepatic expression of PPAR- γ is increased in the liver of rats chronically fed EtOH (26). Recent studies have demonstrated that PPAR- γ activation reduces AHR and eosinophil activation (42, 44), decreased IgE production, and decreased recruitment of eosinophils in BALF (15). The results of these studies parallel the results we observe when mice are fed EtOH; therefore a role for PPAR- γ as a potential mechanistic target in this EtOH model of allergic asthma is an attractive hypothesis that needs further exploration. In an effort to further delineate the mechanistic effects of ethanol on inflammation, we observed a roughly 30% increase in PPAR- γ activity in bronchial epithelial cells following EtOH exposure for 6 h. Not only do these data corroborate the findings of Luvizotto et al. (26) in the livers of rats exposed to EtOH, it also can be related to data from others demonstrating decreased IgE, cytokine, and eosinophilia, which in turn helps strengthen our hypothesis that EtOH attenuates AHR and allergic inflammation in the lungs. Additionally, PPARs are upstream triggers of NO synthase activation and NO production, and clinically NO has been shown to be a weak bronchodilator in asthmatics but not in normal subjects (18).

Because NO can act as a weak bronchodilator, and increased NO production with alcohol stimulation in cultured bronchial epithelial cells has been reported (45), the cGMP/NO pathway also seems a strong alternative mechanism. We have previously established that airway smooth muscle cell contraction is significantly blocked by EtOH, which is inhibited by pharmacological blockade of the NO/PKG pathway in rat primary airway smooth muscle cells (29). NO is also involved in airway inflammation by the recruitment of eosinophils to the lungs from the circulation. Feder et al. (14) has demonstrated that treatment with nitro-L-arginine methyl ester in allergic mice results in a decrease eosinophilia, which could be restored through L-arginine treatment (14). Interestingly, in the present study, EtOH exposure decreases eosinophilia in the EtOH-OVA mice, possibly through a similar pathway.

The neuropeptide CGRP could also be a possible modulator of constriction and inflammation that may be modulated by alcohol. Preliminary studies from our laboratory utilizing immunohistochemistry in lung have identified that in this model CGRP levels in EtOH-consuming mice are decreased compared with their water-drinking littermates. This decrease was evident by the amounts of positive stained CGRP in the sensory nerves and neural epithelial bodies of the airways.

In summary, we have demonstrated the new finding that EtOH attenuates AHR in OVA-sensitized mice and for the first time have shown that EtOH consumption decreased airway total cellular infiltration and eosinophilia in the lung of allergic mice. These data demonstrate a novel role for the effect of ethanol on attenuating bronchial hyperreactivity and airway inflammatory responses. Future studies are warranted to investigate specific cellular responses that could also be targeted by ethanol such as Th2 cellular responses or dendritic cells.

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DISCLOSURES

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

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