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Involvement of Mast Cells in Adenosine-Mediated Bronchoconstriction and Inflammation in an Allergic Mouse Model.

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d) Nonstandard abbreviations:

C48/80, compound 48/80; NECA, 5'-N-ethylcarboxamidoadenosine; A_1AR, adenosine A_1 receptor; A_{2A}AR, adenosine A_{2A} receptor; A_{2B}AR, adenosine A_{2B} receptor; A_3AR, adenosine A_3 receptor; ANOVA, analysis of variance; SEM, standard error measurements; vs., versus; BAL, Bronchoalveolar Lavage

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ABSTRACT

In allergen-induced asthma, activation of lung mast cells leads to bronchial constriction, increased mucus secretion, as well as an increase in the localization of inflammatory cells to the airways. The purpose of this study was to explore the role of mast cells in adenosine-mediated airway reactivity and inflammation using the mast cell degranulating agent, compound 48/80 (C48/80). Mice were sensitized and challenged with ragweed (or 0.9% saline) followed by C48/80 administration twice a day in increasing doses for five days. Dose responsiveness to the nonspecific adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA) was established and lung lavage was performed 24 hours later for cell differential analysis to evaluate inflammation. At a dose of 375 μg/ml (aerosolized NECA), C48/80 pretreatment resulted in a significant attenuation in airway reactivity when compared to sensitized control mice (330.07% vs. 581.57%, respectively). Lung lavage from the C48/80 treated mice showed a decrease in eosinophils (17.7% vs. 60.9%, respectively), and an increase in macrophages when compared to the sensitized control group (76.4% vs. 30.8%, respectively). These results support the conclusion that mast cell degranulation plays an important role in adenosine receptor mediated airway hyperresponsiveness and inflammation.

INTRODUCTION

Allergic airway diseases such as asthma are characterized by a dual phase response, specifically airway constriction and lung inflammation. Many studies support the findings that airway challenges in sensitized animals will cause an increase in airway constriction. Previous studies from this laboratory have demonstrated that inhalation of adenosine causes a dose-related bronchoconstriction and the recruitment of inflammatory cells to the lung (Fan and Mustafa, 2002). Many other studies have also demonstrated that the acute inflammatory component of asthma can be characterized by the recruitment of eosinophils to the lungs (Spruntulis and Broadley, 2001). This recruitment of eosinophils to the lungs has been postulated to be the result of mast cell degranulation.

Adenosine, an endogenous purine nucleoside, acts as a mediator of asthma and can cause bronchoconstriction in asthmatics but not in normal subjects (Cushley et al., 1983). Adenosine acts on four extracellular G-protein coupled adenosine receptors: A_1, A_{2A}, A_{2B}, and A_3. Through their ability to promote or enhance mediator release from mast cells, these receptors have been shown to have a contributory role in inflammation and are important in the etiology of asthma and chronic obstructive pulmonary disease (COPD). Blackburn *et al*. have demonstrated that knocking out the gene for adenosine deaminase results in adenosine accumulation in the lungs. The deleterious effects of adenosine accumulation in the lungs include extensive mast cell degranulation, eosinophilia, increased airway responsiveness, and mucus secretion (Blackburn et al., 2000; Zhong et al., 2001; Zhong et al., 2003).

Current research supports that activation of adenosine receptors potentiates mast cell mediator release after being induced by allergen challenge (Church et al., 1983; Fozard

and Hannon, 2000; Meade et al., 2001). Activation of the A_1 adenosine receptor has been shown to cause bronchoconstriction in rabbit airway smooth muscle (Ali et al., 1994). The A_{2B} and A_3 receptors have been shown to be responsible for initiating mast cell degranulation (Feoktistov and Biaggioni, 1995; Zhong et al., 2003) and airway reactivity (Fan et al., 2003). Little is known about the effects of A_{2A} adenosine receptor activation in the development of asthma and inflammation, but it has been postulated that it is not involved in adenosine-induced bronchoconstriction in rats (Pauwels and Joos, 1995). Ohta and Sitkovsky recently reported that activation of the A_{2A} adenosine receptor is a critical part of the negative feedback mechanism for limitation and termination of both tissue-specific and systemic inflammatory responses (Ohta and Sitkovsky, 2001).

Allergen induced activation of lung mast cells leads to the release of histamine (Polosa et al., 1995; Forsythe et al., 1999), adenosine (Forsythe et al., 1999), 5-HT (Fozard and Hannon, 2000) as well as a myriad of other mediators. Bronchial constriction (Polosa et al., 1995), increased mucus secretion (Sommerhoff et al., 1989), as well as increases in the localization of inflammatory cells to the airways (Forsythe and Ennis, 1999) are hallmark signs of allergic asthma. Our laboratory has shown that allergen challenge in allergic mice leads to increased airway reactivity and inflammation (Fan and Mustafa, 2002; Fan et al., 2003). Adenosine induced bronchoconstriction is also associated with increased levels of histamine, PGD_2, and tryptase (Fozard and Hannon, 2000). It has been shown that pretreatment with disodium cromoglycate and nedocromil sodium (Phillips et al., 1989) will attenuate adenosine-induced bronchoconstriction by inhibiting mast cell mediator release through the inhibition of degranulation (Eady and Norris, 1997).

Therefore, in order to study the role of the mast cell in airway constriction and inflammation, the mast cell degranulating agent, compound 48/80 (C48/80) was administered to ragweed sensitized and control mice. We hypothesized that the chronic degranulation of mast cells would result in attenuation of adenosine-mediated airway constriction and a decreased recruitment of eosinophils to the lungs. We sought to test this hypothesis through the use of the nonspecific adenosine receptor agonist NECA to obtain dose-responsiveness for bronchoconstriction, and by performing cell differentials on the cells obtained from the bronchoalveolar lavage fluid (BALF) from each group of mice.

MATERIALS AND METHODS

Mice and Sensitization

Male BALB/c mice 6-8 weeks old were obtained from Harlan laboratories (Indianapolis, IN). The mice were kept in community cages with 12-hour periods of light and dark cycles and were maintained on a ragweed-free diet with access to water *ad libitum*. All animal care and experimentation was approved and carried out in accordance with the East Carolina University institutional animal care and use committee, and in accordance with the principles and guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The sensitization was performed according to previously described methods including the one from this laboratory (Sommerhoff et al., 1989; Fan and Mustafa, 2002). In short, mice were sensitized by i.p. injections of short ragweed 200 μg (Greer Laboratories, Lenoir, NC) adsorbed to 200 μl Imject alum (Pierce Laboratories, Rockford, IL) per dose on days 1 and 6. On days 11, 12, and 13 the mice were placed in a plexiglass chamber and challenged with an aerosolized 1% ragweed solution via an ultrasonic nebulizer (DeVilbiss, Somerset, PA) for 20 minutes in the morning and afternoon. The control animals received i.p. injections of alum only (same volume as sensitized mice) on days 1 and 6, and were subjected to a 0.9% saline aerosolization on days 11, 12, and 13 in the morning and afternoon.

The following groups of mice were used in this study: Sensitized; mice sensitized to ragweed and treated with MCh or NECA (0.9% saline was used in place of NECA as a sensitized control). Sensitized with C48/80 pretreatment (Sens + C48/80); mice sensitized to ragweed and treated with MCh or NECA. Sensitized + saline (Sens + S);

mice sensitized to ragweed but treated with saline in place of MCh or NECA. Control; mice with no ragweed exposure and treated with MCh or NECA. Control with C48/80 pretreatment (Con + C48/80); mice with no ragweed exposure and treated with MCh or NECA.

Experimental Protocol:

Compound 48/80 treatments. BALB/c mice were injected chronically to deplete mast cells of their granular contents. This protocol is modified from previous work by Karadag *et al* (Karadag et al., 2000). C48/80 was injected i.p. twice daily as follows: 0.5 mg/kg on day 9, 1.0 mg/kg on day 10, 2.0 mg/kg on day 11, 3.0 mg/kg on day 12, and 4.0 mg/kg on day 13. Groups of mice not receiving C48/80 were given i.p. injections of 0.9% saline twice daily in the same manner.

Airway responsiveness to NECA and Methacholine. The airway responsiveness was assessed by unrestrained whole body plethysmography (Max II; Buxco, Troy, NY). The Buxco system uses a dimensionless parameter known as enhanced pause (Penh) to estimate the total pulmonary airflow. This parameter has been shown to correlate with direct invasive measures of airway obstruction, primarily airway resistance and dynamic compliance (Justice et al., 2001). Twelve hours after the last i.p. injection of C48/80, the mice were placed in separate plexiglass chambers and allowed 10 minutes to accommodate to their surroundings. After the accommodation period, the mice were exposed to either methacholine (MCh) or the nonselective adenosine receptor agonist (NECA) via the Buxco Aerosol delivery system (version 1.5; Buxco, Sharon, CT). Drug was aerosolized for 2.0 minutes with increasing concentrations (1.5-48.0 mg/ml MCh; 23.44 – 375.0 μg/ml NECA) to establish a dose-response relationship. Readings were

taken for five minutes immediately following drug aerosolization. Each consecutive dose was not administered until the mice returned to baseline Penh levels. Airway responsiveness (Penh values) were normalized to the vehicle values and then expressed as a percentage increase in Penh compared to vehicle.

Bronchoalveolar Lavage (BAL). Twenty-four hours after exposure to NECA the mice were euthanized by i.p. injection of 100 mg/kg sodium pentobarbital and the BAL was performed according to established protocols (Sur et al., 1996; Fan and Mustafa, 2002). In brief, the trachea was exposed and cannulated. Three washings, each consisting of 1.0 ml phosphate buffered saline (PBS), were introduced into the lungs via the cannula and the fluid was withdrawn in order to collect the cells. The pooled lavage fluid was placed in tubes on ice. The collected BALF was then centrifuged at 1600 rpm for 7 min at 4°C (Beckman, Model TJ-6 centrifuge). After removing the supernatant, the cells were resuspended in 0.5 ml PBS. Total cell numbers were counted on a hemocytometer (Fisher) and 1-5 $x10^3$ cells were spun onto glass microscope slides (cytospin 3, Shandon, UK). The cell slides were air dried for 24-36 hours then fixed and stained with a Diff-Quik stain set (Dade Behring, Newark, DE). A differential cell count of at least 300 cells per slide was made according to morphologic criteria. The number of cells recovered was calculated and expressed as a percentage of total cells.

Chemicals

Acetyl-β-methylcholine chloride (MCh), NECA, compound 48/80, and sodium pentobarbital were purchased from Sigma Chemical (St. Louis, MO). Diff-Quik stain set was purchased from Dade Behring Inc. (Newark, DE).

Statistical Analysis

All experimental data are expressed as means ± SEM. Data were plotted using Graphpad Prism 3.0 and the average Penh between different groups was analyzed by analysis of variance (ANOVA) followed by Bonferroni's post hoc analysis for multiple comparisons. This test was used to determine the level of significance between all treatment groups. Probability levels less than 0.05 was considered to be statistically significant.

RESULTS

Airway Responsiveness to methacholine (MCh):

We examined baseline lung function and assessed airway responsiveness to the inhaled bronchoprovocant MCh. Baseline Penh values between control, control + C48/80, sensitized, and sensitized + C48/80 groups of mice are comparable and no significant differences were found (0.6636 ± 0.0355, 0.7812 ± 0.0469, 0.735 ± 0.0268, 0.7087 ± 0.0269, respectively). Administration of MCh caused a dose-dependant increase in bronchoconstrictor responsiveness in all groups of mice with the sensitized group being significantly higher when compared to control mice. Figure 1 shows the dose-responsiveness of sensitized and control BALB/c mice after exposure to aerosolized MCh. At the 12.0, 24.0 and 48.0 mg/ml dose of MCh, a significant difference ($p < 0.05$) was observed between the sensitized and control groups. No significant differences were observed between the sensitized + C48/80, control, and control + C48/80 groups. Further, no significant differences could be found between the sensitized and sensitized + C48/80 and control + C48/80 groups. ANOVA analysis of the differences between the sensitized and sensitized + C48/80 groups of mice did not reveal significant differences; a type two error due to the relatively small number of animals used per group cannot be completely excluded. These data show that C48/80 pretreatment does not cause a significant dose-dependent attenuation in Penh when mice are challenged with a non-specific receptor agonist, MCh. These data, therefore, support that the observed attenuation in Penh is not the result of a bronchodilator action of C48/80 pretreatment.

Airway Responsiveness to NECA:

We examined baseline lung function and assessed airway responsiveness to the inhaled nonspecific adenosine receptor agonist, NECA. Baseline Penh values between control, control + C48/80, sensitized, and sensitized + C48/80 groups of mice are comparable and no significant differences were found (0.6073 ± 0.0219, 0.6862 ± 0.0299, 0.7079 ± 0.0330, 0.7461 ± 0.0497, respectively). These values were similar to the baseline values with MCh administration. Administration of NECA caused a dose-dependant increase in bronchoconstrictor responsiveness in all groups of mice with the sensitized group being significantly higher. Figure 2 shows the dose-responsiveness of sensitized and control BALB/c mice after exposure to aerosolized NECA. The sensitized group was significantly different ($p < 0.05$) from all other groups at the 187.5 μg/ml and 375.0 μg/ml dose. No significant differences were observed between the sensitized + C48/80, control, and control + C48/80 groups. These data show that C48/80 treatment caused a dose-dependent attenuation in Penh when compared to sensitized control mice but did not cause a further attenuation in Penh in control mice. These data illustrate that degranulation of mast cells results in a dose-dependent attenuation in Penh in sensitized mice with NECA.

Bronchoalveolar cellularity:

BALF was collected to determine if chronic pretreatment with C48/80 had effects on cell recruitment. The sensitized mice showed significant ($p < 0.05$) increases in total cell number when compared to the other groups of mice (Figure 3). These data demonstrate that chronic depletion of the granular contents of mast cells result in attenuation of inflammatory cell recruitment to the lungs mediated by the activation of adenosine receptors by NECA.

The sensitization and challenge protocol with ragweed had an effect on the composition of inflammatory cells collected in the BALF. The cell differentials of the sensitized, sensitized + C48/80, control, and control + C48/80 groups of mice are shown in Figure 4. Macrophage cells were the primary observed cell type in the control, and control + C48/80 groups and very little inflammation was evident in these groups. The sensitized group showed a significant ($p < 0.05$) increase in eosinophil recruitment to the lungs comprising roughly 65% of the cells in that group. This increased eosinophil recruitment is a key hallmark of asthma and correlates with the increase in airway responsiveness observed in these mice. The proportion of macrophages decreased ($p < 0.05$) in the sensitized mice when compared to the other groups. Neutrophils were also increased in the BALF of the sensitized mice. The lymphocyte component of BALF was not different between groups. Sensitized mice aerosolized with saline showed a significant ($p < 0.05$) decrease in the proportion of macrophages and a significant ($p < 0.05$) increase in eosinophil recruitment to the lung when compared to the control and control + C48/80 groups of mice. The saline aerosolized group showed significantly ($p < 0.05$) more macrophage and significantly ($p < 0.05$) less eosinophils than the sensitized group treated with NECA. Based on these data, it is evident that NECA is involved in the recruitment of inflammatory cells to the lung.

The BALF from sensitized + C48/80 group showed significantly ($p < 0.05$) fewer macrophages than the control and control + C48/80 groups but showed significantly ($p < 0.05$) more macrophage cells than the sensitized only group (Figure 4). The sensitized + C48/80 group had ($p < 0.05$) more neutrophils than the control groups but had less neutrophils ($p < 0.05$) than the sensitized only group. Eosinophil numbers for the

sensitized + C48/80 group were increased significantly when compared to the control and control + C48/80 groups ($p < 0.05$); but when compared to the sensitized only group, the sensitized + C48/80 group contained significantly ($p < 0.05$) fewer eosinophils. Chronic treatment with C48/80 had anti-inflammatory effects as observed by an approximate 66% decrease in eosinophils when compared to the sensitized group. From these data, it can be concluded that mast cells indeed have a definitive role in this murine model of lung inflammation due to NECA activation as is observed in allergic asthma.

DISCUSSION

In the past few decades adenosine has been shown to play an important role in the development and worsening of asthma (Jacobson and Bai, 1997; Forsythe and Ennis, 1999; Polosa, 2002). Adenosine has many inflammatory effects such as modulating histamine release from mast cells, influencing eosinophil function, and stimulating mucus secretion in the airways (Blackburn et al., 2000; Polosa et al., 2002). The data presented in this paper show that a non-selective adenosine analog, NECA, can cause the degranulation of mast cells, which in turn results in increased airway responsiveness and inflammation. Our data show that the adenosine agonist NECA has a significant effect on airway reactivity as demonstrated by the increased Penh response to NECA in the sensitized mice. Also, the data show that chronic treatment with C48/80 results in a significant attenuation of Penh in sensitized mice. Moreover, our data demonstrate that inflammatory cells including mast cells have an important role in the bronchoactive response to adenosine agonist, NECA.

Chronic treatment with C48/80 has been shown to deplete mast cells of their granular contents (Jaffery et al., 1994; Karadag et al., 2000). Adenosine is involved in asthma through its ability to promote or enhance mediator release from mast cells (Peachell et al., 1988; Jacobson and Bai, 1997). The depletion of mast cells leads to the release of mediators such as histamine, tryptase, chemokines, and interleukins all of which are important in the etiology of asthma (Forsythe et al., 1999; Polosa et al., 2002).

In vivo studies by various groups (Tilley et al., 2000; Zhong et al., 2003) have shown that adenosine is a potent activator of mast cell degranulation. In contrast to this, *in vitro* studies of mast cells have shown adenosine to elicit only a minimal level of mast cell

degranulation (Ramkumar et al., 1993). This suggests that the mast cell response and the participation of adenosine receptors may be different when grown in culture.

Our data illustrate that Penh measurements in conscious unrestrained mice confirm previous observations in anesthetized animals that allergen challenge enhances airway responsiveness (Fozard and Hannon, 2000; Fan and Mustafa, 2002). Previous data from this laboratory demonstrated that ragweed sensitization results in increased IgE levels (Oldenburg and Mustafa, 2003). These increased levels of IgE may perhaps allow mast cells to become more susceptible to activation by adenosine and its analogs as confirmed by the increased airway responsiveness of the sensitized mice to NECA observed in this study. Treatment with C48/80 resulted in the activation of mast cells, which may predispose them to become less susceptible to the effect of adenosine analog, which is made evident by the attenuation in the Penh response to NECA (Figure 2). We feel that this phenomenon was most likely the result of the mast cell activation. Control mice that were not sensitized to ragweed still showed a modest response to NECA. This we believe is due to a direct activation of adenosine receptors located on airway smooth muscle. Data from this laboratory (Fan and Mustafa 2002) have also shown that treatment with theophylline, a nonspecific adenosine receptor antagonist will not block the hyperreactivity effects of MCh. Our laboratory has also reported that the airway hyperreactivity to NECA can be attenuated with specific adenosine receptor antagonists (Fan M, Qin W and Mustafa SJ (2003)). This, therefore, shows that NECA is acting through specific adenosine receptors. Previous data from this laboratory have shown that adenosine when acting directly on its receptors on airway smooth muscle will lead to

bronchoconstriction in a rabbit model of adenosine-mediated bronchoconstriction (Ali et al., 1994).

Inflammation is a key hallmark of asthma. Fan *et al*. have shown that aerosolized adenosine potentiates the infiltration of inflammatory cells induced by allergen challenge into the lungs (Fan et al., 2003). The data presented in this paper support these findings as significant potentiation of neutrophils and eosinophils were observed in the BALF of both sensitized and the sensitized + C48/80 groups of mice (Figure 4). We observed nearly a 70% increase in eosinophils in the sensitized mice over the control mice, and an approximate 20% eosinophil increase in the sensitized + C48/80 group when compared to control groups (Figure 4). This leads us to speculate that degranulating mast cells through treatment with C48/80 may result in an anti-inflammatory effect of decreasing eosinophil, and neutrophil recruitment to the lungs after exposure to NECA. It may also be feasible that the inhibition of release of mast cell mediators may in turn be leading to an inhibition of inflammatory cell recruitment with C48/80 pretreatment. NECA challenge may, in addition, cause recruitment of inflammatory cells independent of mast cell into the lungs.

We also observed a decrease in the proportion of macrophage cells upon NECA challenge (Figure 4), which was not expected, although clinical studies have found that macrophage numbers in BALF of humans are also decreased in asthma (Ndukwu et al., 1999). The mechanism for this decrease in alveolar macrophages needs further investigation. No change was observed in the cellularity of the control mice BALF possibly due to the absence of allergen to potentiate IgE levels and increase the adenosine

response. The results support a role for mast cell activation (or the release of mast cell mediators) in inflammation after exposure to an adenosine receptor agonist.

The recruitment of eosinophils to the lungs is the direct result of mast cell activation caused by adenosine receptor stimulation. We speculate that this recruitment is caused by the collective action of multiple mediators released from mast cells including cytokines and interleukins. Recent studies have shown a role for IL-13 in airway inflammation (Wills-Karp et al., 1998; Zhu et al., 1999). These studies have demonstrated that inflammation can be blocked through concurrent treatment with IL-13 inhibitors. It is likely that the potent eosinophil chemoattractant IL-13 is involved in inflammation.

With all that is known about the role of adenosine and its effects in asthma including increased production during periods of hypoxia, its ability to induce bronchoconstriction in asthmatics but not normal individuals, and the elevation of adenosine in the BALF of asthmatics (Driver et al., 1993; Fozard, 2003), the specific adenosine receptor responsible for degranulation of mast cells in humans remains elusive. This may be due to the fact that each species is different and has a different adenosine receptor expression profile. Feoktistov *et al*. have recently shown in an HMC-1 mast cell line the presence of A_{2A}, A_{2B}, and A_3 receptors with only the A_{2B} receptor being functionally coupled to stimulation of phospholipase Cβ (Feoktistov and Biaggioni, 1995; Forsythe and Ennis, 1999). Ramkumar *et al*. have shown in the RBL-2H3 mast cell line that the A_3 receptor is the primary receptor involved in mast cell activation (Ramkumar et al., 1993) whereas, in canine BR mastocytoma cells, Marquardt *et al*. have shown that the A_{2B} receptor is the primary activator of mast cells (Marquardt et al., 1994). Murine mast cells express the

A_{2A}, A_{2B}, and A_3 adenosine receptors (Marquardt et al., 1994; Forsythe and Ennis, 1999; Salvatore et al., 2000; Zhong et al., 2003) and lack the A_1 adenosine receptor (Marquardt, 1997). Zhong *et al*. have recently shown large transcript levels of the A_3 adenosine receptor through the use of RT-PCR techniques in murine primary lung mast cells (Zhong et al., 2003). It can therefore be speculated that mast cell degranulation in the murine model of asthma is most probably due to activation of the A_3 adenosine receptor (Salvatore et al., 2000; Tilley et al., 2000; Zhong et al., 2003).

The involvement of the A_3 adenosine receptor may be critical to the degranulation of murine mast cells. A recent report by Tilley *et al*. using mice genetically deficient in A_3 adenosine receptors showed a significant attenuation in the degranulation of mast cells *in vivo* (Tilley et al., 2003). In their study, airway responses were also measured by whole body plethysmography and the A_3 receptor knockout mice had decreased Penh levels consistent with the attenuation caused by C48/80 treatment reported in the present study. Activation of adenosine A_3 receptors by selective A_3 receptor agonists has recently been shown to activate cultured murine mast cells resulting in the release of histamine and other mast cell mediators (Zhong et al., 2003). These findings suggest an important role for the A_3 adenosine receptor and mast cells in adenosine induced bronchoconstriction and inflammation.

In conclusion, the data presented here strongly implicate a role for adenosine in initiating the degranulation of mast cells and their involvement in bronchoconstriction and inflammation, which occurs in the pathophysiology of asthma. However, the identification of the adenosine receptor subtype responsible for this effect in our model is not known and further studies are needed.

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Figure Legends

Figure 1: Dose-responsiveness to MCh in sensitized, sensitized + C48/80 (Sens + C), control, control + C48/80 (Con + C) groups of mice Sensitized mice showed significantly higher levels of airway responsiveness when compared to other groups. Data are represented as means ± SEM, n = 8 animals per group. $*p < 0.05$ for sensitized vs. control mice.

Figure 2: Dose-responsiveness to NECA in sensitized, sensitized + C48/80 (Sens + C), control, control + C48/80 (Con + C) groups of mice. Sensitized mice showed significantly higher levels of airway responsiveness than did any other group. Data are represented as means ± SEM, n = 9-12 animals per group. $*p < 0.05$.

Figure 3: Total cell counts for BALB/c control, control + C48/80 (Con + C), sensitized, sensitized + C48/80 (Sens + C), sensitized + saline (Sens + S) groups of mice 24 hours post allergen challenge. n = 8-10 for each group. Values are means ± SEM. $*p < 0.05$ for sensitized vs. all other groups.

Figure 4: Macrophage, lymphocytes, neutrophils, and eosinophils recovered from BALF of BALB/c mice 24 hours post allergen challenge. Control, control + C48/80 (Con + C), sensitized, sensitized + C48/80 (Sens + C), sensitized + saline (Sens + S), groups. n = 8-10 for each group. Values are means ± SEM. $^{*}p < 0.05$ for Sensitized vs. all other groups (macrophage, neutrophils, and eosinophils). $^{+}p < 0.05$ for Sensitized + C48/80 vs.

control groups (macrophage, neutrophils, and eosinophils). $^{\#}p < 0.05$ for Sensitized + saline vs. control groups (Macrophage and eosinophils).

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Figure 1

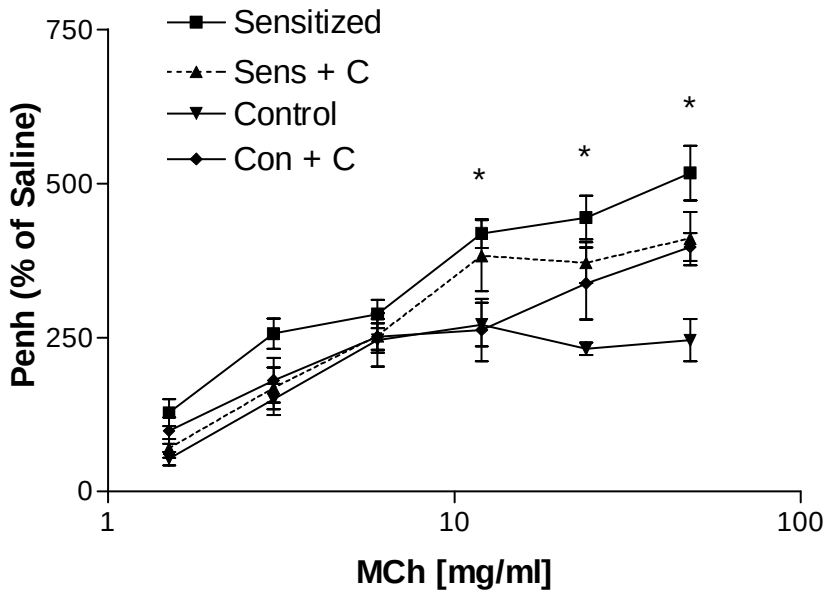

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Figure 2

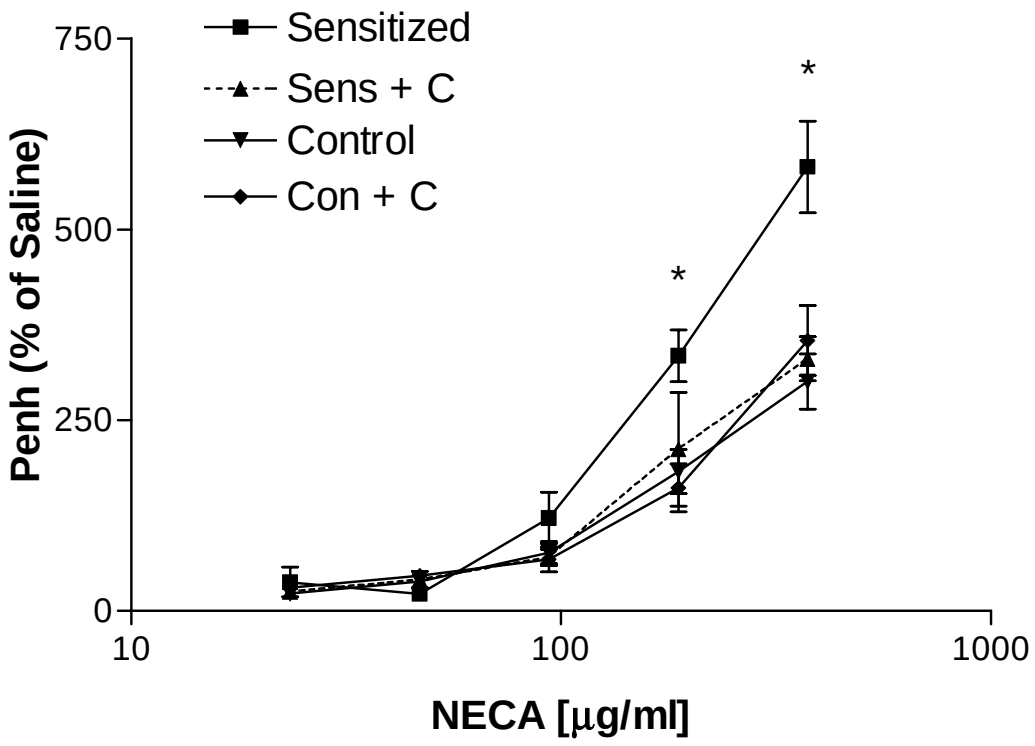

Figure 3

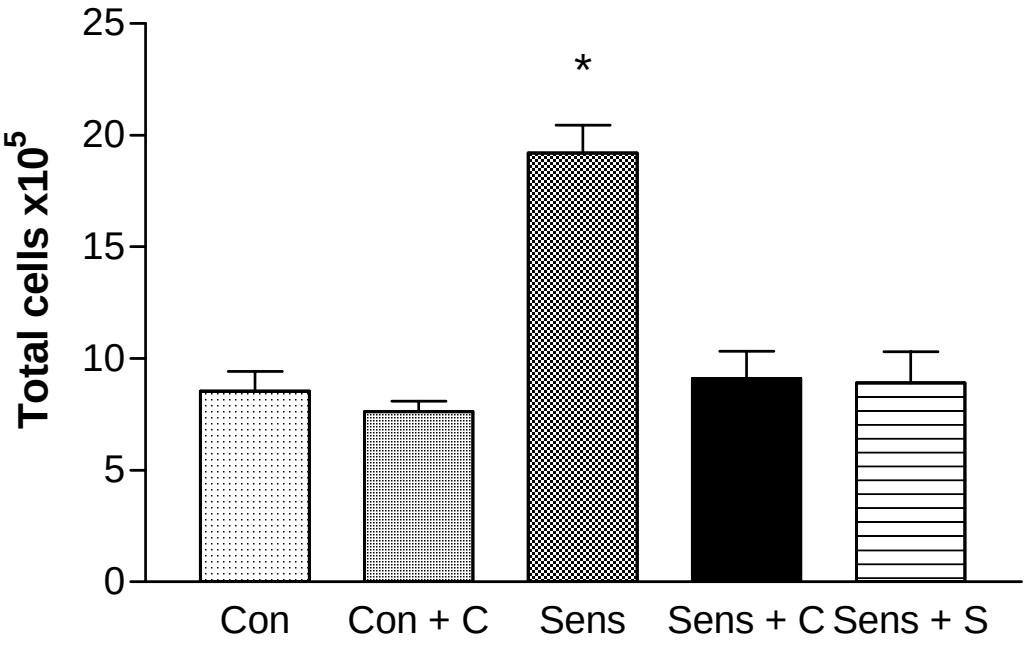

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Figure 4