Neurokinins and inflammatory cell iNOS expression in guinea pigs with chronic allergic airway inflammation


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Prado, Carla M., Edna A. Leick-Maldonado, Vanessa Arata, David I. Kasahara, Milton A. Martins, and Iolanda F. L. C. Tibério. Neurokinins and inflammatory cell iNOS expression in guinea pigs with chronic allergic airway inflammation. Am J Physiol Lung Cell Mol Physiol 288: L741–L748, 2005. First published December 3, 2004; doi:10.1152/ajplung.00208.2004.—In the present study we evaluated the role of neurokinins in the modulation of inducible nitric oxide synthase (iNOS) inflammatory cell expression in guinea pigs with chronic allergic airway inflammation. In addition, we studied the acute effects of nitric oxide inhibition on this response. Animals were anesthetized and pretreated with capsaicin (50 mg/kg sc) or vehicle 10 days before receiving aerosolized ovalbumin or normal saline twice weekly for 4 wk. Animals were then anesthetized, mechanically ventilated, given normal saline or NO-nitro-L-arginine methyl ester (L-NAME, 50 mg/kg ic), and challenged with ovalbumin. Prechallenge exhaled NO increased in ovalbumin-exposed guinea pigs (P < 0.05 compared with controls), and capsaicin reduced this response (P < 0.001). Compared with animals inhaled with normal saline, ovalbumin-exposed animals presented increases in respiratory system resistance and elastance and numbers of total mononuclear cells and eosinophils, including those expressing iNOS (P < 0.001). Capsaicin reduced all these responses (P < 0.05) except for iNOS expression in eosinophils. Treatment with L-NAME increased postantigen challenge elastance and reduced both resistance and elastance previously attenuated by capsaicin treatment. Isolated L-NAME administration also reduced total eosinophils and mononuclear cells, as well as those cells expressing iNOS (P < 0.05 compared with ovalbumin alone). Because L-NAME treatment restored lung mechanical alterations previously attenuated by capsaicin, NO and neurokinins may interact in controlling airway tone. In this experimental model, NO and neurokinins modulate eosinophil and lymphocyte infiltration in the airways.

Nitric oxide; inducible nitric oxide synthase; asthma; lymphocytes; eosinophils

Neurokinins such as substance P and neurokinin A are involved in the modulation of several aspects of inflammatory responses in the lungs. These peptides induce airway smooth muscle contraction, peribronchial edema, and airway mucous secretion and are considered mediators of the excitatory nonadrenergic-noncholinergic response (43). It has been previously shown that substance P and neurokinin A play significant roles in priming eosinophils and in lymphocyte recruitment in allergic lung inflammation (33, 43, 44). In guinea pigs and other mammals, pretreatment with capsaicin has been used as a research tool to elucidate the physiological effects of neurokinins (18, 29). It has been previously shown that administration of high doses of capsaicin reduces tissue concentrations of substance P and other neurokinins (28, 29).

Nitric oxide (NO), a gaseous molecule that is generated during the conversion of the amino acid L-arginine to L-citrulline by NO synthase (NOS), is considered a mediator of the inhibitory nonadrenergic-noncholinergic response (3, 26, 32). The role of NO in the modulation of allergic inflammation and bronchial hyperresponsiveness is currently unclear. There is some evidence that NO has both inflammatory and anti-inflammatory effects in experimental models of airway inflammation (38). The NO derived from constitutive NOS can act as a vasodilator and bronchodilator. However, some proinflammatory cytokines provoke inducible NOS (iNOS) to release large quantities of NO in pathological situations that contribute to maintaining the inflammatory process (2). Various mammalian cells, such as endothelial cells (34), neurons (32), epithelial cells (16), and immune cells (24), can produce NO. A variety of immunocompetent cells express iNOS, including macrophages (17, 49), neutrophils (6, 30, 49), and eosinophils (9, 49). There is evidence that NO is involved in eosinophil recruitment in various models of lung inflammation (13, 14). In contrast, some authors have observed that NO derived through iNOS activation contributes to neutrophil recruitment rather than to eosinophil recruitment (11).

Because both neurokinins and NO have been considered neurotransmitters of nonadrenergic-noncholinergic fibers and are involved in the regulation of some inflammatory responses in the lungs, we reasoned that neurokinins could modulate iNOS expression in inflammatory cells. Thus the purpose of the present study was to evaluate the effects of neurokinin depletion on iNOS expression in eosinophils and mononuclear cell, recruited in the airways of guinea pigs with chronic allergic airway inflammation. In addition, we studied the acute effects of NO inhibition on this response.

Materials and methods

All guinea pigs received humane care in compliance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health publication 85-23, revised 1985), and the study was approved by the institutional review board.

Antigen sensitization. Male Hartley guinea pigs, weighing 300–400 g, were placed in a Plexiglas box (30 × 15 × 20 cm) coupled to an ultrasonic nebulizer (Soniclean, São Paulo, Brazil). A solution of ovalbumin (Sigma Chemical, St. Louis, MO) diluted in 0.9% NaCl (normal saline) was prepared. This solution was continuously aerosolized into the environment until respiratory distress (sneezing, coryza, cough, or retraction of the thoracic wall) occurred or until 15
min had elapsed. The observer who made the decision to withdraw the guinea pig from the inhalation box was blinded as to the treatment status of the animal. This protocol was repeated twice a week for 4 wk (23, 44) with increasing concentrations of ovalbumin (from 1 to 5 mg/ml) to counteract tolerance. Control animals received aerosolized normal saline.

**Capsaicin treatment.** Neurokinin was depleted with a single dose (50 mg/kg sc) of capsaicin (Sigma Chemical, Gardena, CA), as previously described (28, 29, 44). Each guinea pig received amnophylline (10 mg/kg ip) and terbutaline (0.1 mg/kg sc) and was anesthetized with ketamine (50 mg/kg im) and xylazine (0.1 mg/kg im). Capsaicin was suspended in a 50 mg/ml solution consisting of 80% normal saline, 10% ethanol, and 10% Tween 80 (Sigma Chemical). Guinea pigs received supplemental oxygen during anesthesia and recovery.

**Experimental groups and pulmonary mechanics evaluation.** Eight groups of guinea pigs were used in the experimental protocol. The first group was submitted to inhalation of aerosolized normal saline only (NS group, n = 9). The second group was exposed to aerosolized ovalbumin (OVA group, n = 10). The third group was treated with capsaicin and exposed to aerosolized normal saline (CAP-NS group, n = 9). The fourth group was treated with capsaicin and exposed to aerosolized ovalbumin (CAP-OVA group, n = 9). The fifth group was exposed to aerosolized normal saline and received N\textsuperscript{\textdegree}G-nitro-L-arginine methyl ester (L-NAME; NS-L group, n = 10; see below). The sixth group was exposed to aerosolized ovalbumin and received L-NAME (OVA-L group, n = 10). The seventh group was treated with capsaicin, submitted to inhalation of aerosolized normal saline, and given L-NAME (CAP-NS-L group, n = 9). The eighth and final group was treated with capsaicin, exposed to aerosolized ovalbumin, and given L-NAME (CAP-OVA-L group, n = 9).

Seventy-two hours after the last inhalation, the guinea pigs were anesthetized with pentobarbital sodium (50 mg/kg ip). They were then tracheostomized and mechanically ventilated at 60 breaths/min with a tidal volume of 8 ml/kg using a Harvard 683 ventilator (Harvard Apparatus, South Natick, MA). Tracheal pressure (Ptr) was measured with a 142PC05D differential pressure transducer (Honeywell, Freeport, IL) connected to a side tap in the tracheal cannula. Airflow (V\textsuperscript{\prime}) was determined using a pneumotachograph (Fleisch 4–0, Richmond, VA) connected to the tracheal cannula and to a Honeywell 163PC01D36 differential pressure transducer. Changes in lung volume were determined by digital integration of the airflow signal. Nine to ten respiratory cycles were averaged to provide one data point (15, 36, 44). After baseline measurements of Ptr and V\textsuperscript{\prime}, guinea pigs received intracardiac injection of either L-NAME (50 mg/kg, Sigma Chemical) (50) diluted in normal saline (1 ml) or normal saline in the same volume. Ten minutes after administration of either L-NAME or vehicle, we performed two 1-min challenges with either aerosolized ovalbumin (30 mg/ml) or normal saline delivered into the breathing circuit through the air inlet of the ventilator. Measurements of Ptr and V\textsuperscript{\prime} were taken 1, 3, and 5 min after the beginning of the first challenge (Fig. 1). Respiratory system elastance and resistance were obtained using the equation of motion of the respiratory system: Pt(t) = Ers\cdot V(t) + Rrs\cdot V\textsuperscript{\prime}(t) where t is time, V is lung volume, Ers is respiratory system elastance, and Rrs is respiratory system resistance.

**Concentration of exhaled NO.** Concentrations of exhaled NO (ENO) were measured by chemiluminescence using a fast-responding analyzer (NOA 280; Sievers Instruments, Boulder, CO). Before each measurement, the analyzer was calibrated with a certified 47 parts per billion NO source (White Martins, São Paulo, Brazil) and a zero NO filter (Sievers Instruments). To avoid environment contamination, we attached the NO filter to the breathing circuit. We measured ENO before and 15 min after L-NAME administration (Fig. 1). We measured ENO after L-NAME or vehicle administration and antigen or normal saline challenge to observe the effect of L-NAME in reducing endogenous NO production.

**With the guinea pigs under anesthesia and after measurement of pulmonary mechanics and ENO, we opened the anterior chest wall and washed the lungs with heparinized saline solution (1:40). Immediately after that, guinea pigs were exsanguinated, a positive end-expiratory pressure of 5 cmH\textsubscript{2}O was applied to the respiratory system, and the airways were occluded at the end of expiration. The lungs were removed en bloc for J) morphometric studies, 2) histochemical analysis of eosinophil peroxidase (EPO) activity, and 3) immunohistochemical analysis with monoclonal antibodies against iNOS.

**Morphometric studies.** One lung was removed, fixed with 4% buffered paraformaldehyde for 24 h, and then transferred to 70% ethanol. Sections representing peripheral areas of the lung were cut and processed for paraffin embedding. Histological sections (5 μm in thickness) were cut and stained with hematoxylin and eosin and were evaluated by researchers blinded to the protocol design. We evaluated mononuclear cells and eosinophils around the airway (between the bronchial epithelium and the adventitia) through an integrating eyepiece (10\textsuperscript{\textdegree} μm\textsuperscript{2} of total area). We analyzed 10–20 fields per lung at a magnification of ×1,000 and expressed as cells/unit area (10\textsuperscript{\textdegree} μm\textsuperscript{2}).

**EPO activity.** The other lung was inflated via the trachea with 5 ml of optimum cutting temperature (OCT) compound (Reichert-Jung, Heidelberg, Germany), covered with OCT, and cooled in liquid nitrogen. Sections were cut on a cryostat, mounted on glass slides precoated with aminopropyltriethoxysilane (Sigma Chemical), and fixed in chloroform-acetone (Merck, Rio de Janeiro, Brazil) vol/vol for 10 min at room temperature. A histochemical method for cytidine-resistant EPO activity employing diaminobenzidine (Sigma Chemical), H\textsubscript{2}O\textsubscript{2}, and potassium cyanide (Sigma Chemical) was used to stain eosinophils, as previously described (25, 44, 52). Counterstaining with hematoxylin was employed to reveal cellular nuclei, and counts of positive cells in the airway walls were performed as previously described for morphometric studies (44). We analyzed 10–20 fields per lung at a magnification of ×1,000 and expressed as cell/unit area (10\textsuperscript{\textdegree} μm\textsuperscript{2}).
Evaluation of iNOS. For iNOS detection, we used the same sections employed for histochemical evaluation of cells containing EPO (EPO+ cells). Immunohistochemistry was performed as previously described (7). Subsequently, the sections were incubated for 30 min at room temperature with a blocking solution containing normal mouse serum (Dako, Carpinteria, CA). Monoclonal antisera raised in mouse against iNOS (IgG2a - iNOS/NOS Type II - N32020; BD Transduction Laboratories, San Diego, CA) were used as primary antisera (incubation overnight at room temperature, 1:5 dilution in Tris buffer). After three 5-min washes in Tris-buffered saline (TBS), sections were incubated with a secondary antibody (LSAB+AP Link Universal, Dako) for 30 min at 37°C in a humid chamber. Slides were given three more 5-min washes in TBS and were coverslipped with prediluted (for 30 min) alkaline phosphatase (LSAB+AP-Streptavidin AP, Dako). This was followed by incubation with substrate Fast Red TR (Sigma Chemical) for 6 min and light hematoxylin counterstaining for 1 min. We analyzed 10–20 fields per lung at a magnification of ×1,000 and expressed as cells/unit area (10^4 μm²).

Statistical analysis. Values are expressed as means ± SE. Statistical analysis was performed with SigmaStat software (Jandel Scientific, San Rafael, CA). Data were evaluated by ANOVA. Multiple comparisons were made using Tukey test or Bonferroni test. A P value of <0.05 was considered significant (51).

RESULTS

Pulmonary mechanics evaluation. There were no significant differences among the study groups in respiratory system elastance or resistance values measured before antigen challenge/vehicle administration (data not shown). In addition, l-NAME administration did not affect respiratory system elastance and resistance values. Figure 2, A and B, shows the maximal increase in respiratory system elastance and resistance (percentage of baseline) obtained after ovalbumin challenge or normal saline administration. The OVA group showed increased elastance and resistance of respiratory system after antigen challenge of (means ± SE) 452 ± 46% and 230 ± 29%, respectively (P < 0.001). If one considers maximal responses of both elastance and resistance of respiratory system, the increases in both parameters were more attenuated in the CAP-OVA group than in the OVA group (P < 0.05).

After l-NAME treatment, there was an increase in maximal values of respiratory system elastance after antigen challenge in ovalbumin-exposed guinea pigs. In fact, maximal increase in respiratory system elastance was greater in the OVA group than in the OVA group (P < 0.001). In addition, in the presence of l-NAME, maximal values of respiratory system resistance and elastance induced by ovalbumin challenge in sensitized guinea pigs were similar those found for capsaiacin-treated and noncapsaicin-treated guinea pigs (CAP-OVA-L and OVA-L groups, respectively).

Concentration of ENO. Figure 3 shows levels of ENO measured before and 15 min after l-NAME administration. Baseline values of ENO were higher in the OVA group than in the saline-only (NS) group (P < 0.05). Pretreatment with capsaiacin reduced ENO in sensitized guinea pigs (P < 0.05). We observed a significant reduction in ENO after 15 min of l-NAME treatment in all experimental groups, thereby confirming that l-NAME has an acute effect of inhibiting NO production.

Morphometric analysis and histochemistry for eosinophils. Figure 4A shows the numbers of mononuclear cells, eosinophils, and EPO+ cells in the distal airways of the eight experimental groups studied. There were no significant differences among the four control groups (NS, NS-L, CAP-NS, and CAP-NS-L). The number of mononuclear cells, eosinophils, and EPO+ cells in ovalbumin-exposed guinea pigs was greater than that found in controls (P < 0.001). In ovalbumin-sensitized animals, capsaiacin pretreatment and l-NAME treatment (P < 0.001 and P < 0.01, respectively) reduced recruitment of mononuclear cells, eosinophils, and EPO+ cells. In addition, the combination of the two treatments in ovalbumin-exposed guinea pigs had no additional effects over those observed in response to isolated treatment with one agent alone.

Immunohistochemistry for iNOS detection. Figure 4B shows the numbers of mononuclear cells and eosinophils showing iNOS expression and infiltrating the airways of the experimental groups studied. There were few of these inflammatory cells in control guinea pigs, and no differences were found among the control groups. Ovalbumin-treated animals presented increases in the total numbers of mononuclear cells and eosinophils showing iNOS expression in the airways compared with
controls ($P < 0.001$). We noticed that the eosinophil populations were divided between iNOS-positive and iNOS-negative cells (45.7 and 54.3%, respectively, in the OVA group). Inhibition of NO production by L-NAME treatment reduced the total number of eosinophils, but the reduction was primarily within the iNOS-positive subgroup (Table 1). In contrast, most (84.3%) of the mononuclear cells were iNOS negative. Although the total number of mononuclear cells was reduced by L-NAME treatment, we did not observe any preferential effect in iNOS-positive or iNOS-negative mononuclear cells, with similar reductions in both subgroups (Table 1).

Although neurokinin depletion by capsaicin treatment reduced the total number of eosinophils, we observed no reduction in iNOS-positive eosinophils, showing a preferential effect in the iNOS-negative subgroup (Table 1). Neurokinin depletion by capsaicin pretreatment reduced the total numbers of both iNOS-positive and iNOS-negative mononuclear cells (Table 1). The combination of neurokinin depletion and L-NAME treatment did not modify this response.

Figure 5 shows photomicrographs of the distal airway walls of guinea pigs exposed to aerosolized ovalbumin, highlighting a large amount of EPO+ cells and iNOS-positive inflammatory cells. In normal saline-exposed animals, we observed low numbers of inflammatory cells stained for EPO and iNOS around distal airway walls.

DISCUSSION

In a previous investigation, we evaluated airway inflammation induced by repeated exposure to aerosolized ovalbumin in guinea pigs and the effects of neurokinin depletion (by capsaicin pretreatment) on that inflammation in guinea pigs (44). In that experimental model, we observed peribronchial edema, an increase in lymphocytes and eosinophils (both in bronchoalveolar lavage fluid and in distal airways), and an increase in pulmonary responsiveness to methacholine. Immunohistochemistry with monoclonal antibodies revealed that most mononuclear cells present in the airway walls are CD4+ T cells. Neurokinin depletion resulted in lower maximal values of respiratory system elastance and resistance after antigen challenge, less intense bronchoconstriction and airway edema formation, and a decrease in the number of CD4+ T cells in the airway wall (44).

In the present study, using the same experimental model of chronic allergic airway inflammation in guinea pigs, we evaluated the effects of neurokinin on iNOS inflammatory cell expression. In ovalbumin-exposed guinea pigs, in contrast to the effects of neurokinin depletion, L-NAME administration induced a decrease in NO production through inhibition of NOS, resulting in a significant increase in respiratory system elastance response to allergen challenge. In addition, the pulmonary mechanical response to allergen challenge in the CAP-OVA-L group was greater than that of those in the CAP-OVA group and comparable to that of those in the OVA group. These results suggest in vivo correlations of the effects of NO and neurokinins in modulation of proximal and distal airway tone since the attenuation of the acute effects of antigen challenge in animals depleted of neurokinins was negated by...
the inhibition of NO. The inhibitory effect of L-NAME on NO production was confirmed by the observation that all animals receiving L-NAME presented a significant decrease in the concentration of ENO (Fig. 3).

Our findings are in accordance with previous studies that demonstrated the bronchoprotective effect of NO. Dupuy et al. (10) showed that inhaled gaseous NO is a potent bronchodilator in guinea pigs, acting in the distal airways only in high doses. However, many authors have suggested a more pronounced effect of NO in the proximal airways (8, 35, 48) and have proposed that this may be due to decreased nitrergic innervation density in the distal airways (48). Nevertheless, in the present study, we observed that the increased respiratory system mechanical response to ovalbumin challenge in L-NAME-treated animals was more evident in respiratory system elastance than in respiratory system resistance. This suggests that NO inhibition affects distal airways and lung parenchyma more than it does proximal airways. One possible explanation is that changes in respiratory system elastance may also be related to vascular effects in the alveolar wall. We noticed that sensitized L-NAME-treated guinea pigs presented extensive blood extravasation in the alveolar spaces (data not shown) probably resulting from NO modulation of vascular tone (12, 27, 46).

Endogenously produced NO has been measured in the expired gases of animals and humans. It has been reported that ENO is a useful marker of airway inflammation in asthma (1, 21, 22). Asthmatic patients have higher ENO levels than normal subjects do, and there is a decline in ENO levels when asthmatic patients receive treatment with corticosteroids. Increased ENO after antigen challenge in experimental models of allergic airway inflammation has also been shown (11, 31). However, the precise source of the increased NO measured in expired air in asthma and experimental models of allergic airway inflammation remains to be determined. In our study, we also noticed increased ENO in guinea pigs with chronic airway inflammation induced by repeated exposure to aerosolized ovalbumin. We observed that neurokinin depletion in guinea pigs chronically exposed to antigen reduced ENO levels to values similar to those obtained for guinea pigs not exposed to ovalbumin (Fig. 3). A possible explanation is that in animals sensitized and pretreated with capsaicin, the reduction in total inflammatory cells could induce some changes in NO metabolism, as suggested by Hunt et al. (19).

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<th>Mononucleated Cells</th>
<th>Eosinophils</th>
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<tr>
<td></td>
<td>Total</td>
<td>iNOS – (%)</td>
</tr>
<tr>
<td>OVA</td>
<td>34.3</td>
<td>28.9 (84.3%)</td>
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<tr>
<td>CAP-OVA</td>
<td>7.0</td>
<td>4.3 (62.0%)</td>
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<tr>
<td>OVA-L</td>
<td>19.5</td>
<td>16.6 (85.1%)</td>
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<tr>
<td>CAP-OVA-L</td>
<td>4.1</td>
<td>2.5 (61.3%)</td>
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The cells were counted at a magnification of ×1,000 and expressed as cells/unit area (10^4 μm²). OVA, ovalbumin exposure; CAP, capsaicin pretreatment; L, N^G-nitro-L-arginine methyl ester treatment; iNOS, inducible nitric oxide synthase.

Table 1. Number and percentage of iNOS-positive and iNOS-negative mononucleated cells and eosinophils in guinea pigs submitted to the protocol of multiple exposures to aerosols of ovalbumin, pretreated with capsaicin, and treated with L-NAME.

Fig. 5. Photomicrograph of distal wall airway submitted to histochemical and immunohistochemical techniques. A: distal airway of an OVA-sensitized guinea pig demonstrating a great number of EPO+ cells (arrows) around and in epithelium (×400). B: distal airway of control guinea pig (×400). C: airway wall of an OVA-sensitized guinea pig demonstrating iNOS+ inflammatory cells (×1,000). The arrows identify the positive cells. D: airway wall of normal saline group showing iNOS-negative cells (×1,000).
As a counterpoint to the increased mechanical response to allergen challenge, we observed that \( l\)-NAME treatment reduced numbers of both lymphocytes and eosinophils in the airway wall. This implies that NO plays an important role in inflammatory cell recruitment in this animal model of asthma. This role became clear, even despite the fact that only one dose of NO inhibitor was given to sensitized animals and the lungs were not excised until after 20 min of antigen challenge. By that time, in sensitized-only guinea pigs, the inflammatory cells had aggregated to blood vessels, resulting in acute bronchial wall infiltration. As we had expected, the blockage of NO production in these animals resulted in diminished cell recruitment. In accordance with these results, some other authors have also observed the acute effect that administration of NO inhibitors has on inflammatory cell recruitment (13, 38).

The lung represents an important target organ for NO generation, and NO is a crucial mediator of the inflammatory response. It has been previously suggested that NO is involved in the development of pulmonary eosinophilia (13) and that treatment with \( l\)-NAME may reduce eosinophilic and/or neutrophilic influx, both in vitro and in models of antigen-induced airway inflammation (5, 11, 14, 20, 38, 39, 42). In contrast, some authors have found no participation of NO in inflammatory cell recruitment (45).

This \( l\)-NAME-induced anti-inflammatory effect was similar to that observed in capsaicin-treated guinea pigs, in which there was also a decrease in numbers of both lymphocytes and eosinophils in the airway wall. However, we did not notice any additional effects when capsaicin and \( l\)-NAME treatments were combined.

Some mechanisms could explain the effects of capsaicin pretreatment and \( l\)-NAME administration in reducing inflammatory cells around airways of sensitized animals. One possibility is that neurokinins and NO act in apoptosis of these inflammatory cells. Some evidence suggested that substance \( P\) and neurokinin A can delay the apoptosis process in some types of cells (4, 37). As for NO, its effect in cell death is unclear. Taylor et al. (40) suggested that NO could have different effects on inflammatory cell apoptosis (anti- and proapoptotic properties), depending on the concentration and flux of NO and the source from which NO is derived. Another possibility could be related to the effects of neurokinins and/or NO in other mediators that can act directly in the inflammatory cells, delaying or anticipating the apoptosis process.

We also performed immunohistochemical evaluation of the expression of iNOS in inflammatory cells surrounding the airway wall. We detected increases in the total numbers of mononuclear cells and eosinophils showing iNOS expression in the airways of repeated ovalbumin-exposed animals. Of greater interest, we demonstrated that there were both iNOS-positive and iNOS-negative populations of eosinophils (45.7 and 54.3%, respectively, in the OVA group). Treatment with \( l\)-NAME primarily reduced the numbers of the iNOS-positive eosinophils, whereas neurokinin depletion had a preferential effect in the iNOS-negative eosinophil subgroup. In light of these various findings, one possible explanation is that nonadrenergic-noncholinergic actions in eosinophilic recruitment may be related to autocrine or paracrine effects of NO and neurokinins.

Both neurokinins and NO may contribute to lymphocyte cell recruitment with no preferential effect in the iNOS-positive or iNOS-negative cell subgroups. The combination of neurokinin depletion and reduction of NO production did not modify this response.

Most mononuclear cells infiltrating the airway wall in chronically ovalbumin-exposed guinea pigs were iNOS negative (84.3%). Although total numbers of mononuclear cells were reduced by \( l\)-NAME treatment, we did not observe any preferential effect in the iNOS-positive or iNOS-negative mononuclear cells, with similar reductions in both subgroups (Table 1). The same behavior was observed with neurokinin depletion by capsaicin pretreatment, which reduced total numbers of both iNOS-positive and iNOS-negative mononuclear cells (Table 1). The combination of neurokinin depletion and reduction of NO production did not modify this response.

Some studies in isolated cells have also shown iNOS expression in inflammatory cells such as macrophages (17, 47, 49), neutrophils (6, 30, 49), and eosinophils (9, 49). In the present study, a model of chronic allergic inflammation was used for the first time to show to describe iNOS expression in various subgroups of eosinophils and lymphocytes. Taylor-Robinson et al. (41) found that cloned murine Th1 cells activated by antigens or mitogens expressed iNOS and produced substantial amounts of NO. The authors proposed that both proliferation of Th1 cells and production of IL-2 and IFN-\( \gamma\) may be inhibited by high concentration of NO, although Th2 cells neither produced nor were affected by NO. In this animal model, the majority of lymphocytes in the distal airways are T lymphocytes, mainly of the CD4+ subset (44). We therefore speculate that the decrease in inflammatory cells induced by either capsaicin pretreatment or \( l\)-NAME administration in our experimental asthma model may be attributed to a reduction in the Th2 lymphocyte population.

In conclusion, even in the case of neurokinin depletion, NO modulates airway tone. Using a model of chronic allergic airway inflammation, we were able to identify subgroups within the eosinophils and lymphocytes that showed expression of iNOS. Neurokinins and NO amplify recruitment of both iNOS-positive and iNOS-negative lymphocytes. Neurokinins are involved in iNOS-negative eosinophil recruitment, and NO affects recruitment within the iNOS-positive eosinophil subgroup. In the guinea pig model, NO may have both beneficial and detrimental effects, acting as a bronchodilator and promoting eosinophil and mononuclear cell response in the distal airways.

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