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Inhibition of Allergic Airway Inflammation in Mice Lacking Nitric Oxide Synthase 2

Yelin Xiong,* Gunasegaran Karupiah,* Simon P. Hogan, † Paul S. Foster, † and Alistair J. Ramsay2*

We have used mice rendered deficient for nitric oxide synthase 2 (NOS2) production to study the role of inducible nitric oxide (NO) in the pathogenesis of allergic airways disease. Using a model with OVA as aeroallergen, we show that the manifestations of disease, including infiltration of inflammatory cells, particularly eosinophils, loss of structural integrity of the airways walls, microvascular leakage, pulmonary edema, and airway occlusion are markedly less severe in the NOS2 mutants than in wild-type animals. Indeed, NOS2-deficiency resulted in a 55–60% reduction in both circulatory and pulmonary eosinophil numbers following aeroallergen treatment, although eosinophil maturation or efflux from the bone marrow was not suppressed. There were no obvious differences in the number of airway hyperreactivity recorded in OVA-treated wild-type and NOS2-deficient mice. Interestingly, the suppression of allergic inflammation was accompanied by marked increases in T cell production of IFN-γ but not by any obvious reduction in the secretion of either IL-4 or IL-5, nor by major changes in the IgG1 and IgE OVA-specific serum Ig profiles in the mutants. The markedly enhanced production of IFN-γ in NOS2−/− mice was apparently responsible for the suppression of both eosinophilia and disease, as in vivo depletion of this factor restored allergic pathology in these animals. Our data indicate that NOS2 promotes allergic inflammation in airways via down-regulation of IFN-γ activity and suggest that inhibitors of this molecule may represent a worthwhile therapeutic strategy for allergic diseases including asthma. The Journal of Immunology, 1999, 162: 445–452.

Nitric oxide (NO) is involved in the regulation of many physiological processes (1, 2). It is synthesized from L-arginine by the enzyme NO synthase (NOS), which exists either in constitutive or inducible forms (3). Constitutive isoforms, following their activation by increased levels of intracellular calcium ions, appear to form picomolar amounts of locally acting NO, which may act as a signaling molecule in a number of processes (3), including neural responses and vasodilation (4). Inducible NOS (now termed NOS2), which is not dependent on elevated calcium ion concentrations, is induced in a wide variety of cell types by cytokines, including IL-1β, TNF-α and IFN-γ, or microbial products such as LPSs (5), in which it catalyzes the production of much higher levels of NO mediating widespread antimicrobial effects (2). Normally, resting cells do not express NOS2 unless triggered by appropriate stimuli (6); however, cells such as macrophages, epithelial cells, vascular smooth muscle, endothelial cells, T cells, and eosinophils have the potential to express NOS2 when stimulated in this manner (7–9).

There is increasing evidence that NO also plays a role in the regulation of airways function in both health and disease. As in other tissues, both constitutive and inducible isoforms of NOS have been identified in lungs, with the latter implicated mainly in defense against infection and during airways inflammation (10). NO is produced by a variety of cell types in the respiratory tract (11, 12). It has been reported that asthmatic patients have increased expression of NOS2 in epithelial cells lining their airways (13, 14) and elevated levels of exhaled NO (15), while allergen-induced airway obstruction in a guinea pig model of allergic airway disease was also associated with high levels of exhaled NO (16). In addition, chemical inhibition of NO activity has recently been shown to suppress pulmonary eosinophilic inflammation in mice, although this was apparently not due to inhibition of NOS2 function (17).

Asthma is a chronic airways disease characterized by inflammation, airway hyperreactivity (AHR), and reversible airway obstruction (18), and it is likely that NO influences these pathophysiological events. Pulmonary inflammation is thought to play a critical role in the pathogenesis of asthma (18), with strong correlations between disease, the local expression of Th2-type cytokines (including IL-4 and IL-5) by lung T cells, and the presence of inflammatory cells, particularly eosinophils, in blood and airways (19, 20). It has recently been proposed that NO from airway epithelial cells, and possibly macrophages and Th1 cells, promotes asthmatic inflammation via down-regulation of Th1 cell-derived IFN-γ with concomitant up-regulation of Th2 cell numbers and resultant increased local expression of IL-4 and IL-5 (21). In addition, NO is a bronchodilator (22, 23) and a potent bronchial vasodilator (4), and may also mediate cytotoxic effects on airway epithelia (24). In the present study, we have used mice rendered deficient for NOS2 production in a model of allergic airway disease to more clearly define the activity of this molecule in disease pathogenesis.
Materials and Methods

**Mice**

The NOS2-deficient mice and wild-type controls used in these studies were derived as described elsewhere (25). Cells from the NOS2 mutants were deficient for NO production in response to inductive regimens, which included LPS and/or IFNs. The mice were bred in the Animal Breeding Establishment of the Australian National University and housed in an approved containment facility. Mice were treated according to Australian National University Animal Welfare guidelines, and age- and sex-matched animals were used in these studies.

**Sensitization and aerallergen treatment of mice**

A murine model of allergic airways disease was established as previously described (26). In this model, exposure to aerallergen induces airway inflammation and localized expression of Th2-type cytokines, particularly IL-4 and IL-5. Briefly, mice (8–10 wk of age) were sensitized by two i.p. injections with 50 μg OVA (Grade V; Sigma, St. Louis, MO) in 1 mg alhydrogel (Commonwealth Serum Laboratories, Parkville, Australia) given on day 0 and 12. Nonsensitized controls received 1 mg of alhydrogel in normal saline. On day 24, sensitized mice were exposed to aerosolized OVA (10 mg/ml) in normal saline) for three 30-min periods at intervals of 30 min, and the process was repeated every second day for 6 days. Nonsensitized mice received aerosolized saline only. The aerosol was generated at 6 L/min by a nebulizer producing particles of ~3.9 μm diameter into a closed chamber of 800 cm³. Mice were sacrificed on day 31, 24 h after the last aerosol. In some experiments, groups of mice were given an i.p. dose of 0.8 mg/mouse of anti-CD3 mAb against murine CD3 (anti-CD3 mAb, American Type Culture Collection, Manassas, VA) or mAb GL113 (a kind gift of Dr. J. Abrams, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA) as a rat IgG1 isotype control one day before each course of aerallergen treatment (i.e., on day 23, 25, 27, and 29). Sections were cut from paraffin-embedded lung tissues and stained with May-Grünwald-Giemsa solution for histological studies.

**Assessment of bone marrow, blood and lung inflammatory cell populations**

Twenty-four h after the final treatment with aerallergen on the day of sacrifice (day 31), bronchoalveolar lavage (BAL) was performed by tracheal cannulation and instillation of 1 ml of normal saline into the lungs. Each animal was lavaged twice, with ~0.8 ml of fluid being recovered per wash. BAL fluid (BALF) was centrifuged (1500 × g for 5 min), and total cell counts were made using trypan blue. Ten 10⁶ cells were taken for cytospin (Shandon Scientific, Cheshire, U.K.). Blood smears were made from samples drawn from the tail vein immediately before each course of aerallergen treatment and on the day of sacrifice. Numbers of eosinophilic cells in bone marrow were assessed using aspirates collected before aerallergen treatment (day 23) and on the day of sacrifice. Cytospin smears of cells from BALF, blood, and bone marrow were stained with May-Grünwald-Giemsa solution, and 200 leukocytes were counted on each slide.

**Measurement of eosinophil hemoptoisis in bone marrow**

A modification of the method of Stanley was used to assess eosinophil colony formation from bone marrow progenitor cells in vitro (27). Briefly, 24 h after the final treatment with aerallergen, bone marrow cells were collected and prepared in 1× HBSS containing 15 mM HEPES, 2% FCS, and 50 μg/ml gentamicin. Cells from mice within each group were pooled and cultured at 4 × 10⁶ cells/ml in 12-well Luc migration plates (Becto Laboratories, New South Wales, Australia) in a volume of 400 μl of 0.33% agar (Unipath, Basingstoke, U.K.) in RPMI 1640 medium (JRH Biosciences, Lenexa, KS) containing 10% FCS, 4 mM glutamine, 60 μg/ml gentamicin, 500 U/ml IL-3, and 250 U/ml IL-5 (the cytokines are a kind gift of Dr Andrew Hapel, John Curtin School of Medical Research). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 7 days. Semisolid cultures were then lifted onto microscope slides, air-dried, and stained with May-Grünwald-Giemsa solution. Numbers of colonies containing eosinophils were scored under light microscopy using morphological criteria.

**Measurement of AHR**

Mice were anesthetized with a mixture of ketamine (Troy Laboratories, Smithfield, Australia) at 60 mg/kg body mass and Rompun (Bayer Australia, Pymble, Australia) at 8 mg/kg given by i.p. injection, and their tracheae were cannulated and connected to a bronchospasm transducer (Ugo Basal 7020) coupled to a Lab Mac/8 computer analysis station (Ad-
OVA-treated NOS2-deficient mice only at background levels (Fig. 1). These results indicate that increased production of NO is not induced during allergic lung inflammation in the mutant mice.

Allergic eosinophilia in peripheral blood and BALF and airway disease are down-regulated in mice lacking NOS2

Aerosol challenge of wild-type mice sensitized with OVA normally leads to widespread eosinophilic inflammation, primarily in the peribronchial and perivascular regions as we have previously reported (26). Therefore, we initially examined blood smears taken from aerosol-treated wild-type mice and found markedly elevated numbers of eosinophils in the bloodstream of those given OVA (Fig. 2). A significant blood eosinophilia was first detected on day 28, after the second day of exposure to aeroallergen, with peak counts recorded on day 31, after the full course of treatment. Although blood eosinophil numbers had also increased in OVA-treated NOS2−/− mice by day 28, they remained relatively constant thereafter until sacrifice and were significantly lower than the rising counts recorded in wild-type mice after both the third (p < 0.05) and fourth (p < 0.01) aeroallergen treatments. In addition to blood eosinophil levels, differential counts of lung leukocyte populations were made in BALF samples taken after aeroallergen treatment (Table I). Eosinophils, whilst rarely detected in saline-treated wild-type or NOS2−/− mice, were the predominant leukocyte type in BALF from aeroallergen-treated mice. However, their numbers in NOS2−/− mice were consistently less than half of those found in wild-type animals. Other leukocyte populations, particularly lymphocytes and macrophages, were present in BALF in much smaller numbers, although these were significantly elevated in the NOS2 mutant mice. In the case of lymphocytes, a 20-fold increase over saline-treated mutants was recorded compared with a 5-fold increase in the wild-type mice,

indicating that these noneosinophilic components of the allergic inflammatory response are somewhat enhanced in the NOS2-deficient animals.

The degree of airway disease in NOS2−/− and wild-type mice was assessed following histological examination of lung tissues. A system of grading of several parameters was used, where 1 represented mild pathology and 5 represented the most severe disease. These parameters included the degree of leukocyte inflammation adjacent to the airways, particularly eosinophilic infiltration, the development of gross alterations in the structural integrity of the airway walls, microvascular leakage, mucosal edema and particulate exudation, and mucous occlusion in the lumina of the airways. Based on this system, disease severity was invariably graded at 5 in aeroallergen-treated wild-type mice but consistently only at either 2 or 3 in the NOS2 mutants.

**Eosinophil development and efflux is not suppressed in NOS2-deficient mice**

We next studied the development of eosinophils in the bone marrow and their efflux in NOS2-deficient mice in light of the marked decreases in numbers of these cells in their blood and airways following aeroallergen treatment. Analyses of bone marrow cells taken from OVA-treated NOS2−/− and wild-type mice in colony-forming assays revealed no significant differences in the percentages of colonies containing eosinophils after 7 days in culture (Table II). In addition, similar decreases in numbers of eosinophilic cells were found in the bone marrow of sensitized NOS2 mutants and wild-type mice after aerosol treatment with OVA (Table III).

**Table I. Cell populations in BALF from OVA-sensitized mice**

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Treatment</th>
<th>Macrophages (10^5/ml)</th>
<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOS2−/−</td>
<td>Saline</td>
<td>8.7 ± 1</td>
<td>&lt; 0.1</td>
<td>0.35 ± 0.3</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>NOS2+/−</td>
<td>Saline</td>
<td>10.1 ± 3.2</td>
<td>&lt; 0.1</td>
<td>0.95 ± 0.4</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>NOS2−/−</td>
<td>OVA</td>
<td>12.3 ± 1.8*</td>
<td>0.2 ± 0.1</td>
<td>7.5 ± 0.8*</td>
<td>40 ± 1.7**</td>
</tr>
<tr>
<td>NOS2+/−</td>
<td>OVA</td>
<td>5.4 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>4.8 ± 0.5</td>
<td>93 ± 3.2</td>
</tr>
</tbody>
</table>

* Mice were sensitized and treated with aeroallergen as described in Materials and Methods. BAL was performed after sacrifice on day 31 and cell types in BALF enumerated on cytospin smears stained with May-Grunwald-Giemsa solution. Results represent mean ± SEM for groups of five mice and are representative of two experiments.

* p < 0.05 and ** p < 0.001 compared to OVA-treated wild-type mice.
These findings indicate that the reduction in circulatory and pulmonary eosinophil numbers observed in NOS2-deficient mice are due neither to suppression of their maturation nor of their efflux from the bone marrow in response to aeroallergen challenge.

AHR is not inhibited in mice lacking NOS2

In our model, airway eosinophilic inflammation following aeroallergen challenge is directly associated with AHR to the spasmonogen, β-methacholine (26). Our findings of markedly decreased eosinophilic inflammation and airway disease in sensitized NOS2-deficient mice treated with aerosolized OVA prompted us to study the development of AHR in these animals. As shown in Fig. 3, AHR in allergic wild-type mice increased with dosage of β-methacholine to a maximum of 60% airway occlusion over the baseline levels seen in controls. Similar high levels of AHR were found in the OVA-treated NOS2 mutants, although these were recorded over baseline levels that were markedly higher than those found in the wild-type mice. These elevated basal readings may reflect removal of the known bronchodilatory influence of inducible NO in the mutants. Nevertheless, it appeared that the diminished airway allergic inflammatory infiltrate following OVA treatment of these mice was still sufficient to provoke substantial AHR.

Elevated IFN-γ production does not greatly modify the type 2 cytokine-driven immune response in allergic NOS2−/− mice

We next compared the cytokine profiles of CD4+ T cells and specific Ab responses in aeroallergen-treated NOS2−/− and wild-type mice. Given the correlation between allergic airway disease and expression of type 2 cytokines by lung T cells, together with the proposition that local NO production promotes airway inflammation through down-regulation of T cell-derived IFN-γ, it was of interest to determine whether the marked decrease in blood and airway eosinophilia seen in the aeroallergen-treated NOS2 mutants might reflect a shift in the characteristics of the immune response in these animals. As shown in Fig. 4, activated lung and spleen CD4+ T cells from control, nonallergic NOS2−/− and wild-type mice secreted similar levels of IFN-γ; however, neither IL-4 nor IL-5 was detected. In contrast, while CD4+ T cells from OVA-treated wild-type animals produced significantly less IFN-γ than those from saline-treated controls, there was pronounced expression of both IL-4 and IL-5, particularly among cells from the lungs. Similar patterns of type 2 cytokine expression were found in cells from OVA-treated NOS2 mutants; however, both lung and splenic T cells from these animals also secreted very high levels of IFN-γ. In addition, IL-12, TNF-α, and IL-10 were detected at similar levels only marginally above background in BALF from both wild-type and mutant mice treated with OVA (data not shown), contrasting with the elevated levels of IL-5 (55 U/ml in wild-type; 48 U/ml in NOS2 mutants) found in these fluids.

The majority of the specific Ab response in mice sensitized with OVA (measured on day 24) and treated with aeroallergen (day 31) was of the IgG1 subclass and titers recorded in NOS2−/− and wild-type animals did not differ significantly at either time point (Fig. 5). While significantly lower levels of OVA-specific IgE were found in sensitized NOS2 mutants compared with wild-type mice, titers were also similar after aerosolization. However, NOS2 deficiency increased levels of specific IgG2a, albeit at comparatively low titers, particularly after OVA aerosol challenge. Together, these observations suggest that NOS2 deficiency leads to marked increases in both local and systemic T cell-derived IFN-γ production and in levels of serum IgG2a against the allergen. However, neither the characteristic type 2 cytokine-driven specific IgG1 and IgE responses nor the production of IL-4 and IL-5 by lung T cells were modified in allergic NOS2−/− mice. Depletion of IFN-γ reverses inhibition of airway inflammation in NOS2−/− mice

These pronounced increases in IFN-γ production might account for the decreased eosinophilic inflammation and, therefore, airway disease, seen in aeroallergen-treated NOS2-deficient mice. To test this hypothesis, we treated the mutants with mAbs against IFN-γ during their course of treatment with OVA. As shown in Fig. 6, the inhibition of blood eosinophilia from day 28 (after the second treatment with aerosolized OVA) recorded in NOS2−/− mice

Table II. Eosinophilic colony formation in bone marrow cell cultures from OVA-sensitized mice

<table>
<thead>
<tr>
<th>Mouse Strain</th>
<th>Treatment</th>
<th>Percent of Colonies Containing Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOS2−/−</td>
<td>Saline</td>
<td>3.0 ± 1.6</td>
</tr>
<tr>
<td>NOS2+/+</td>
<td>Saline</td>
<td>4.4 ± 2.2</td>
</tr>
<tr>
<td>NOS2−/−</td>
<td>OVA</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td>NOS2+/+</td>
<td>OVA</td>
<td>3.8 ± 0.7</td>
</tr>
</tbody>
</table>

Table III. Eosinophilic cells in bone marrow smears

<table>
<thead>
<tr>
<th>Day</th>
<th>Eosinophilic Cells (× 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NOS2+/+</td>
</tr>
<tr>
<td>23</td>
<td>10.4 ± 0.9</td>
</tr>
<tr>
<td>31</td>
<td>10.4 ± 1.5</td>
</tr>
</tbody>
</table>

* Mice were sensitized and treated with aeroallergen as described in Materials and Methods. Bone marrow cell aspirates were collected on day 23 (prior to aeroallergen challenge in OVA-sensitized mice) and day 31, and cytospin smears were stained with May-Grünwald-Giemsa solution. A total of 200 cells per smear were scored by light microscopy using morphological criteria. Results represent mean ± SEM for six replicate cultures per group and are representative of two experiments.
given control mAb was abolished in those given mAb against IFN-γ. Similarly, the marked reduction in eosinophil numbers in BALF seen in allergic mutants was reversed in mice depleted of this factor (Fig. 7). In addition, the symptoms of severe disease as described above in wild-type mice were evident in the mAb-treated mutants (data not shown). Neutralization of IFN-γ activity did not significantly affect levels of AHR in the mutant mice (data not shown). Thus, it appears that IFN-γ was responsible for the suppression of eosinophilic inflammation and allergic pathology in NOS2−/− mice despite their capacity for continued production of IL-5.

Discussion

NO, which has been implicated in many physiological processes (1, 2), may play an important role in airways function both in health and disease (10). During asthma, the expression of inducible NOS (NOS2) by cells of the respiratory tract, particularly epithelial cells, increases markedly, while levels of exhaled NO are also elevated (10, 13–15). In the present study, we have used mice rendered deficient for the production of NOS2 in a model of allergic airway disease. Our results clearly show that the manifestations of disease, including infiltration of leukocytes, particularly eosinophils, around the airways, gross alterations in the structural integrity of the airway walls, microvascular leakage, mucosal edema and particulate exudation, and mucous occlusion of the airways lumina are markedly less severe in NOS2−/− mice than in wild-type animals. NOS2 deficiency resulted in a 55–60% reduction in both circulatory and pulmonary eosinophil numbers following allergen treatment, although no suppression of eosinophil precursor development, nor of efflux from the bone marrow following aeroallergen treatment, was apparent. Although representing a relatively small percentage of the inflammatory cell population of the lung, both macrophages and lymphocytes were recorded in significantly higher numbers in the mutant mice. These changes were accompanied by pronounced increases in the capacity of cultured T cells to secrete IFN-γ upon restimulation in vitro, but not...
by any obvious change in local production of IL-4, IL-5, IL-10, IL-12, or TNF-α, nor by major changes in the largely IgG1 and IgE OVA-specific serum Ig profiles in the mutants. The markedly enhanced production of IFN-γ in the NOS2−/− mice was apparently responsible for the suppression of both eosinophilia and disease, as in vivo depletion of this factor restored allergic inflammation and pathology in these animals.

Our findings are in partial agreement with those of Feder and colleagues, who recently showed that chemical inhibition of NO activity suppressed pulmonary eosinophilic inflammation in a murine model of allergic airway disease (17). However, these authors attributed the reduction in inflammation to NO catalyzed by enzymes other than NOS2 and speculated that localized NO production enhances extravasation of circulating eosinophils into the lung. In contrast, our study, using mice with a specific mutation, has clearly shown that NOS2 deficiency leads to a IFN-γ-mediated suppression of both pulmonary and circulating eosinophilia and allergic airways disease following aeroallergen challenge.

The severity of clinical asthma appears to correlate with the degree of airway inflammation and, therefore, the level of local Th2 cell activity (31). In murine models of allergic airway inflammation, aeroallergen challenge of sensitized mice normally leads to widespread eosinophilia, which correlates directly with disease (26, 32–34). In addition, we have shown that eosinophilia is, in turn, critically dependent on IL-5 in vivo (26) and that type 1 cytokine-mediated suppression of eosinophilia and disease is accompanied by a marked down-regulation of local IL-5 production (35). Therefore, it was somewhat surprising to find that T cells isolated from both the lungs and spleens of aeroallergen-treated NOS2−/− mutants retained the capacity to secrete high levels of both IL-4 and IL-5, as in wild-type animals, in the face of their ability to produce large amounts of IFN-γ upon restimulation in vitro, particularly given the established role of IFN-γ as a potent suppressor of Th2 cell activity (35–37). These findings lead us to suggest that the suppressive effects of IFN-γ may be counteracted by the generation of strong Th2 responses with IL-4 and IL-5 secretion, such as in our model, and that high local levels of IL-4 promote Th2 cell development despite significant local IFN-γ production. In this context, we and others have recently reported that local virus infections that normally induce IFN-γ production did not suppress eosinophilic airway inflammation in similar models of allergic airway disease (35, 38).

The enhanced T cell production of IFN-γ observed in the allergen-treated NOS2 mutants is in accordance with the finding that treatment of mice with inhibitors of NO production leads to marked increases in IFN-γ levels during staphylococcal infection (39). Similar enhancement of IFN-γ production has recently been reported in studies using NOS2−/− mutants infected with the protozoan, Leishmania major (40, 41), Mycobacterium tuberculosis (42), or viruses, including ectromelia virus and influenza virus, contributing to rapid viral clearance in the latter (G.K., unpublished observations). How NO influences IFN-γ gene expression is unclear at this stage. However, recent evidence indicates that NO may regulate the activity of NF-κB transcription factors and protein tyrosine kinases belonging to the src kinase, mitogen-activated protein (MAP) kinase, and Janus kinase (JAK) families (43–46), the latter with major implications for IFN-γ signaling (47). It has also been suggested that NO may down-regulate Th1-type immune responses and, in this way, diminish IFN-γ production (40). The delineation of mechanisms that regulate the development of different T cell subsets is of obvious importance for increased understanding of the pathogenesis of a number of diseases including allergy. In this regard, recent in vitro studies have indicated that NO may act differentially on activated murine and human Th cell populations by inhibiting Th1 cytokine production but promoting the expression of Th2-type cytokines (48, 49), although these data remain controversial (50). Nevertheless, it has been proposed that inducible NO, produced in the asthmatic airways by epithelial cells, macrophages, and possibly Th1-type cells, has an inhibitory effect on Th1-type cells and their expression of IFN-γ, facilitating the activation of Th2-type cells and the development of allergic inflammation (21). The greatly increased local expression of IFN-γ we recorded in allergen-treated NOS2−/− mutants lends partial support to this hypothesis. However, it was clear from our data that the IFN-γ-mediated protection from airway inflammation in these animals was not due to suppression of T cell production of IL-4 and IL-5 by this factor.

Our findings in NOS2−/− mutant mice indicate that inhibitors of NOS2 may be effective in suppressing allergic inflammation, particularly in the airways. They also suggest that the effects of such compounds would largely be mediated through IFN-γ activity in the face of continued production of Th2-type cytokines. These data suggest several potential mechanisms whereby allergic inflammation may be suppressed. The inhibition of eosinophilia recorded not only in BALF but also in the circulation of aeroallergen-treated NOS2 mutants and its reversal by mAbs directed against IFN-γ raised the possibility that this factor may down-regulate inflammatory cell hematopoiesis, either directly or via intermediate molecules, possibly including NOS2 itself. However, our studies using bone marrow cells indicate that eosinophil development and eflux from the bone marrow following aeroallergen challenge is unaffected by NOS2 deficiency and suggest that the inhibitory effects of IFN-γ may occur at a later stage in eosinophilic inflammation, such as the subsequent maturation of these cells in the circulation. In addition, IFN-γ may directly influence the activity of mature inflammatory cells and, in this respect, has been shown to inhibit degranulation and release of inflammatory mediators from both eosinophils (51) and mast cells (52). In the latter case at least, this suppression may be mediated by IFN-γ-induced NO (53). Selective inhibition of NO production might also protect against allergic pulmonary inflammation independently of increased IFN-γ activity, our present data notwithstanding. It has recently been shown that NO specifically disrupts Fas receptor-mediated apoptosis of eosinophils (54), while its vasodilatory properties (4) and chemotactic activity for a variety of cell types, including eosinophils (55), may enhance their recruitment into allergic airways (17).
The lack of obvious differences between AHR measured in aeroallergen-treated wild-type and NOS2−/− mice, despite suppressed pulmonary eosinophilic inflammation in the latter, probably reflects the continued production of levels of eosinophil inflammatory mediators sufficient to provoke such hyperreactivity. Our previous work in this model has clearly demonstrated a close association between local eosinophil inflammation and AHR (26). However, it should be noted that the elevated AHR readings in OVA-challenged mice were recorded over baseline levels in control (saline-treated) animals that were significantly greater in the NOS2 mutants. Deficient NO production in these mice may have been responsible for this spontaneous AHR, given the reported, albeit variable, bronchodilatory activity of this molecule (22, 23).

In conclusion, we have shown that eosinophil inflammation and related manifestations of allergic airway disease are markedly suppressed in mice deficient for NOS2 production, apparently due to pronounced IFN-γ expression in these animals. Our results support the hypothesis that NO promotes allergic inflammation and indicate that specific inhibitors of NOS2 may be worthwhile components of therapeutic strategies against allergic diseases, including asthma.

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