Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

Arginine in Asthma and Lung Inflammation1,2

Nina E. King, Marc E. Rothenberg, and Nives Zimmermann3

Division of Allergy and Immunology, Cincinnati Children’s Hospital Medical Center and Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45229

ABSTRACT Asthma, a complex chronic inflammatory pulmonary disorder, is on the rise despite intense ongoing research underscoring the need for new scientific inquiry. Using global microarray analysis, we recently discovered that asthmatic responses involve metabolism of arginine by arginase. We found that the cationic amino acid transporter (CAT)2, arginase I, and arginase II were particularly prominent among the allergen-induced gene transcripts. These genes are key regulators of critical processes associated with asthma, including airway tone, cell hyperplasia, and collagen deposition, respectively. Recent data suggest that arginase induction is not just a marker of allergic airway responses, but that arginase is involved in the pathogenesis of multiple aspects of disease. This review focuses on the current body of knowledge on L-arginine metabolism in asthma. J. Nutr. 134: 2830S–2836S, 2004.

KEY WORDS: • arginine • arginase • asthma • allergy • lung

Asthma is a disease characterized by eosinophil-rich inflammatory cell infiltrates, increased mucus production, airway hyperreactivity, and reversible airway obstruction (1–3). Analysis of lung tissue biopsies from patients with asthma has revealed that chronic airway inflammation is associated with lung remodeling. These chronic changes include epithelial shedding and hypertrophy, hyperplasia and metaplasia of submucosal mucus glands and smooth muscle cells, and fibrosis (4–6). Experimentation in the asthma field has largely focused on analysis of the cellular and molecular events induced by allergen exposure in sensitized animals (primarily mice) and humans (7–9). Although no animal model adequately mimics human disease, experimentation in animals has provided an experimental framework to dissect key cells and molecules involved in the pathogenesis of allergic lung disease. In these studies, mice are typically sensitized with antigen [e.g., ovalbumin (OVA)]4 in the presence of an adjuvant (e.g., alum) by intraperitoneal injection (10). Subsequently, mice are challenged by exposure to a respiratory allergen (via intratracheal, intranasal, or nebulized routes) and pathological responses are monitored. In other murine asthma models, unsensitized mice are repeatedly exposed to mucosal allergens (e.g., extracts of Aspergillus fumigatus) and the development of experimental asthma is monitored (11). In patient studies, naturally sensitized individuals are similarly challenged by exposure to allergen in the respiratory tract (e.g., segmental antigen challenge) (12). Collectively, these studies have provided important insight into the complexity of allergic lung disease by revealing the involvement of diverse cells including infiltrating leukocytes and resident cells including endothelial, epithelial, and smooth muscle cells (4,8,13). In addition, these studies have elucidated that Th2 cells induce asthma through the secretion of an array of cytokines (IL-4, -5, -9, -10, -13) that activate inflammatory and resident effector pathways both directly and indirectly (14,15). In particular, IL-4 and IL-13 are produced at elevated levels in the asthmatic lung and are thought to be central regulators of many of the hallmark features of this disease (8). Collectively, these studies have provided the rationale for the development of multiple therapeutic agents that interfere with specific inflammatory pathways (13,16). Despite this active ongoing research and the availability of an increasing number of medications to treat patients with asthma, there is currently an epidemic of this disease in the western world and the incidence continues to rise (17,18). This highlights the importance of taking new approaches in the scientific analysis of the asthma problem.

We hypothesized that DNA microarray analysis of lung tissue from mice undergoing induction of experimental antigen-induced asthma would provide novel and empiric insight.

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3 To whom correspondence should be addressed.

E-mail: zimmn0@cchmc.org.

4 Abbreviations used: AHR, airway hyperresponsiveness; CAT, cationic amino acid transporter; DFMO, difluoromethylornithine; iNOS, inducible NOS; LPS, lipopolysaccharide; mRNA, messenger RNA; NO, nitric oxide; NOS, nitric oxide synthase; ODC, L-ornithine decarboxylase; OAT, L-ornithine amino transferase; OVA, ovalbumin; STAT6, signal transducer and activator of transcription.
into the pathogenesis of experimental asthma. After identifying a subset of the mouse genome involved in experimental asthma, we were struck by the upregulation of specific genes involved in l-arginine metabolism (19,20). In particular, the genes for the l-arginine transporter [cationic amino acid transporter (CAT)2], arginase I, and arginase II were strongly induced. Involvement of l-arginine metabolism via nitric oxide synthase (NOS) in asthma pathogenesis has been extensively studied (21–23). However, the role of the arginase pathway is largely unexplored. Arginase may be a regulator of diverse pathways including production of nitric oxide, polyamines, and proline; these molecules regulate critical processes associated with asthma including airway tone, cell hyperplasia, and collagen deposition, respectively. Thus, in this review we will mainly focus on the role of arginase and l-arginine transport in asthma pathogenesis.

**L-arginine metabolism by NOS**

L-arginine metabolism via NOS results in the production of nitric oxide (NO), a free radical molecule involved in a wide range of biological processes (24). Three isoforms of NOS have been described (24). NOS1 and NOS3 are constitutively expressed and their activity is calcium dependent. NOS1 is expressed in neurons and is thought to have a role in neurotransmission, whereas NOS3, or endothelial NOS, has a role in smooth muscle relaxation and bronchodilation. NOS2, inducible NOS (iNOS), is calcium-independent, and is upregulated in response to pro-inflammatory mediators leading to the increased production of NO (25). All 3 NOS isoforms have been found in the respiratory tract and are involved in the regulation of multiple pulmonary functions. The role of NO depends on milieu and local concentration. For instance, NOS2 is markedly upregulated in asthmatic lungs and elevated levels of exhaled NO in asthma patients correlate with asthma severity (22,26). High concentrations of NO may be detrimental by promoting inflammation and by causing mucosal swelling and epithelial damage, therefore contributing to airway hyperreactivity (AHR) (21). However, in recent years it became clear that endogenous NO is involved in regulation of airway reactivity to bronchoconstrictor stimuli, including muscarinic receptor agonists, histamine, and bradykinin (21). A number of studies have shown that endogenous NO deficiency may potentiate AHR in response to various stimuli (27–30). NO deficiency in asthmatic airways may be a result of reduced availability of NOS substrate, l-arginine, via decreased cellular uptake or increased utilization via other metabolic pathways, as discussed below.

**L-arginine metabolism by arginase**

Besides the NO pathway, l-arginine is also metabolized by arginase as part of the urea cycle (31,32). The bulk of the urea cycle occurs in the liver, the main organ containing the full enzymatic machinery necessary for the urea cycle. The enzyme arginase is the only urea cycle enzyme that exists in 2 isoforms (60% amino acid homology), which are encoded by different genes on distinct chromosomes, designated type I and type II. Arginase I is a cytoplasmic protein that is primarily expressed in the liver, whereas arginase II is a mitochondrial protein expressed in a variety of tissues, especially the kidney and prostate (33). The downstream enzymes l-ornithine decarboxylase (ODC) and l-ornithine amino transferase (OAT) are specifically expressed in the cytoplasm and mitochondria, respectively (32), suggesting coordinated biochemical links for the 2 isoenzymes. Arginase I deficiency in humans results in hyperargininemia and a progressive neurological deterioration that is usually fatal (33). Whereas arginase I gene targeted mice die within 10–14 d after birth, arginase II-deficient mice are grossly normal (34,35). One of the most exciting developments in the past several years concerning l-arginine metabolism was the finding that arginase can be expressed in many tissues and cell types following exposure to a variety of cytokines and agents (32).

**Arginase in asthma**

In our effort to identify important allergen-induced genes that were not previously associated with asthma, we used microarray analysis of transcripts that were induced in 2 different murine asthma models. We were struck by the high level of arginase messenger RNA (mRNA) transcripts in allergen-treated lungs compared with saline-treated lungs (Fig. 1). Both arginase I and arginase II were strongly induced during experimental asthma. In addition, the CAT2 gene expression was significantly increased following allergen challenges (Fig. 1). These findings were confirmed by Northern blot analysis (19). In situ hybridization and immunohistochemistry indicated that arginase I was produced in the active inflammatory sites around airways and blood vessels during induction of allergic airway inflammation (Fig. 2). A subpopulation of mononuclear cells, most consistent with macrophages, appeared to be the major cellular source (19). The upregulation of arginase mRNA during induction of experimental asthma was associated with functional changes in arginase activity in the lung. Consistent with the absence of arginase mRNA in the lung of control mice, the level of arginase activity in control lung was close to background. Following induction of experimental asthma by the OVA/alum protocol, there was a marked increase in lung arginase activity (Fig. 3). Finally, immunocytochemistry and in situ hybridization analysis of samples from patients with asthma demonstrated that this pathway is also operational in human asthma (19).

The product of arginase, l-ornithine, is a precursor in the production of polyamines (e.g., putrescine, spermidine, and spermine) and proline, which control cell proliferation and collagen production, respectively (Fig. 1) (32,33,36–38). In fact, increased expression of arginase I alone is sufficient to result in increased proliferation rates of vascular smooth muscle (39) and endothelial cells (40). Interestingly, allergen-challenged mice had an ~3-fold increase in the level of putrescine compared with saline challenged mice (19). Increased production of putrescine in the lung can have several important ramifications on pulmonary responses (41). Based on their basic charge and small size, polyamines interact with macromolecules including RNA and DNA, thereby regulating diverse cellular responses including cell growth, division, and differentiation. The inhibitor of ODC and polyamine synthesis, difluoromethylornithine (DFMO), has been shown to block cell growth and differentiation, and is an effective anticancer therapeutic (37,42,43). With regard to asthma, DFMO inhibits smooth muscle cell proliferation in vitro (44), a process that is associated with the chronic airway remodeling in the asthmatic lung (4). Polyamines can be secreted from cells and indeed elevated levels of these molecules are found in the serum of patients with asthma (45). This may be particularly important in the lung because respiratory epithelial cells have a potent polyamine uptake system (41). Interestingly, with regard to allergic responses, polyamines have also been shown to directly interact with allergens and modulate signaling in mast cells (41,46–48).
In addition, L-ornithine (whose production is regulated by arginase) is a precursor of proline (following metabolism by OAT). Work from several laboratories led to the notion that arginase is a precursor of proline (following metabolism by ornithine and proline, secreted from arginase expressing cells (e.g., macrophages), are transported into fibroblasts, where they subsequently become incorporated into collagen (49,50).

**Regulation of arginase expression in lungs**

Although both arginase isoforms are inducible by various stimuli in vitro, arginase I appears to be more strongly induced by Th2 cytokines such as IL-4 and IL-13 (38,51–53). However, this has not been extensively studied in cell types other than macrophages. In our studies we observed that Th2 cytokines have a major role in regulation of lung arginase expression. For example, mice that overexpress the IL-4 transgene in pulmonary epithelium (under the control of the Clara cell 10 promoter) and exhibit several features of asthma including eosinophil-rich inflammatory cell infiltrates, mucus production, and changes in baseline airway hyperreactivity (54) also had a marked increase in the level of arginase I (Fig. 4). Interestingly, arginase II was also inducible in the IL-4 lung transgenic mice (Fig. 4). Another Th2 cytokine, IL-13, has been shown to induce arginase expression in vitro and IL-13-deficient mice have impaired induction of arginase during experimental Th2-associated granuloma/fibrosis formation (56–58). Pharmacological delivery of IL-13 to the lung of mice induced marked levels of arginase I compared with saline treated control mice (Fig. 4). Consistent with the finding that IL-4 transgenic mice had elevated levels of arginase II mRNA, IL-13 also increased arginase II mRNA levels (Fig. 4).

IL-4 and IL-13 share similar signaling requirements such as utilization of the IL-4Rα chain and the induction of janus kinase 1 and signal transducer and activation of transcription (STAT6). A subset of their responses has been shown to be STAT6-dependent (59–61). To test the role of STAT6 in the induction of arginase I in vivo, we examined IL-4 lung transgenic mice that contained wild type or gene targeted STAT6. We have previously described these mice (62). As shown in Figure 4, whereas IL-4 lung transgenic mice contained abundant arginase I mRNA, in the absence of STAT6 there was a complete loss of the IL-4 induced arginase I mRNA. This is in agreement with the report that arginase expression (induced by IL-4 and IL-13) is associated with inhibition of NO production in macrophages by a STAT6-dependent pathway (57). Interestingly, the IL-4 transgene-induced arginase II mRNA signal was not attenuated in STAT6-deficient mice, indicating that arginase II, in contrast to arginase I, was STAT6-independent. These findings are consistent with in vitro studies that demonstrated shared and distinct signaling requirements for these 2 isoenzymes (32,36).

The finding that both arginase isoforms are highly upregulated in asthmatic lung challenges the conventional view that L-arginine is primarily metabolized by NOS in asthmatic responses. It also suggests that L-arginine is an important molecule in asthma pathogenesis and modulation of L-arginine levels may affect L-arginine metabolism by both NOS and arginase pathways.
L-arginine transport

Extracellular L-arginine is required for sustained NO and L-ornithine generation from L-arginine (63), implicating an important role for L-arginine transport through the plasma membrane. Among the several transport systems that mediate L-arginine uptake, system $\gamma^+$ is widely expressed and considered to be the major L-arginine transporter in most cells and tissues (64).Encoded by cationic amino acid transporters CAT1, CAT2, and CAT3, system $\gamma^+$ is an Na$^+$-independent high affinity cationic amino acid transport system. With the exception of the liver, CAT1 is expressed virtually ubiquitously and is required for viability, whereas CAT2 is expressed in a more restricted number of tissues (64); CAT3 is primarily

FIGURE 2  Arginase I mRNA in situ hybridization. The hybridization signal of the arginase I anti-sense (AS) and sense (S) probes are shown for OVA/alum sensitized mice challenged with 2 doses of OVA (A–C,E) or saline (D). Tissue was analyzed 18 h after the second saline or allergen challenge. Bright field (B,E,F) and dark field images (A,C,D) are shown at 100x (A–D) and 400X (E–F) original magnification. The dark field signal is white/pink and the bright field signal is black. In the paired dark and bright field photomicrographs (A & B) a peribronchial staining pattern is shown. The hybridization of the AS probe to a subpopulation of isolated inflammatory cells is shown in E. Staining was also observed in isolated large mononuclear cells with abundant cytoplasm, typical for airway macrophages. Examples of such cells stained by in situ (F) and immunohistochemistry against arginase I (G) are shown. Arrows indicate representative positive signal. Representative photomicrographs of 4 separate mice are shown. Reproduced with permission from (19).
expressed in the brain (65). Due to differential splicing of 2 exons, the CAT2 mRNA exists in 2 isoforms: CAT2A, a low affinity transporter that is expressed primarily in the liver, and the high affinity CAT2 (CAT2B) (66). Interestingly, CAT2 was originally cloned from lymphoma cell line cDNA and was named Tea (T cell early activation factor), because it is induced early in the response of normal T cells to mitogens (66). The first indication that CAT2 may be involved in critically regulating substrate availability for iNOS or arginase was the finding that pro-inflammatory molecules [e.g., lipopolysaccharide (LPS)] regulate CAT2 expression. In contrast, cat-1 is a "housekeeping" gene that is not induced under conditions that induce CAT2 (67). A further interesting relationship has been established by the findings that eosinophil cationic proteins inhibit L-arginine uptake by macrophages and affect both NOS and arginase activity, albeit to a different degree (68). Recent analysis of CAT2-deficient mice has revealed that sustained NO production in macrophages requires CAT2 (69). The 95% decrease in L-arginine uptake by CAT2-deficient macrophages indicates that CAT2 is the major L-arginine transporter in macrophages. Upregulation of CAT2 expression mainly was studied in the context of iNOS induction after treatment with LPS or IFNγ (69). We have observed induction of CAT2 mRNA in lungs in a murine experimental asthma model that was originally cloned from lymphoma cell line cDNA and was named Tea (T cell early activation factor), because it is induced early in the response of normal T cells to mitogens (66). The first indication that CAT2 may be involved in critically regulating substrate availability for iNOS or arginase was the finding that pro-inflammatory molecules [e.g., lipopolysaccharide (LPS)] regulate CAT2 expression. In contrast, cat-1 is a "housekeeping" gene that is not induced under conditions that induce CAT2 (67). A further interesting relationship has been established by the findings that eosinophil cationic proteins inhibit L-arginine uptake by macrophages and affect both NOS and arginase activity, albeit to a different degree (68). Recent analysis of CAT2-deficient mice has revealed that sustained NO production in macrophages requires CAT2 (69). The 95% decrease in L-arginine uptake by CAT2-deficient macrophages indicates that CAT2 is the major L-arginine transporter in macrophages. Upregulation of CAT2 expression mainly was studied in the context of iNOS induction after treatment with LPS or IFNγ (69). We have observed induction of CAT2 mRNA in lungs in a murine experimental asthma model that is associated with marked increase in arginase activity (Fig. 1). Induction of CAT2 expression may be required for maintenance of intracellular L-arginine supply. Depletion of extracellular L-arginine is common in inflammatory conditions (70,71). Delivery of L-arginine in vivo resulted in increased mucosal antibody production following sensitization with antigen (72). In an asthma model, delivery of dietary L-arginine increased airway eosinophilia and levels of IL-5 production (73). Notably, aerosolized delivery of L-arginine in individuals with asthma induces bronchoconstriction (74). However, one report in guinea pigs infected with parainfluenza virus demonstrated that delivery of aerosolized L-arginine prevented airway hyperresponsiveness (75). Taken together, these studies provided evidence that modulation of L-arginine availability can have profound effects on a variety of inflammatory parameters in the lung.

The interplay between arginase and NOS

It is important to understand the inter-relationships between arginase and NOS, since both enzymes utilize the same substrate and both are implicated in asthma pathogenesis. There are several levels at which arginase and NOS affect each other. While the Km for L-arginine is in the 2–20 mmol/L range for arginase compared with the 20–200 µmol/L range for various NOS isoenzymes, the Vmax of arginase is 1000-fold higher than for NOS (32,33). Thus, arginase and NOS effectively compete for L-arginine, and thereby negatively coregulate the function of each other. Indeed, induction of arginase by IL-4 and IL-13 induces substrate depletion for nitric oxide production by macrophages (57). Furthermore, the NOS product hydroxylamine is an inhibitor of arginase (36). Conversely, polyamines inhibit the NOS enzymes (76). These biochemical properties are likely to have functional signifi-

FIGURE 3

Arginase activity analysis. Arginase activity in the lungs of saline (n = 4) and OVA (n = 3)-challenged mice is shown. Arginase activity was measured in lung lysates using the blood urea nitrogen reagent. As a control, arginase activity in the liver was 1522 ± 183 and 1390 ± 78 nmol min⁻¹ mg⁻¹ protein for saline and OVA-challenged mice, respectively. Modified and reproduced with permission from (19).

FIGURE 4

Regulation of arginase by IL-4, IL-13 and STAT6. Northern blot analyses of arginase I and arginase II in IL-4 lung transgenic mice (in the Balb/c background) containing either the wild type or deleted STAT6 gene (A) and lungs from Balb/c mice treated with IL-13 intranasally (C) are shown. The position of the 18S RNA is shown. Each lane represents a separate mouse. Ethidium bromide (EtBr) staining of the RNA gels is also shown. In (B), arginase activity in the lungs of saline (n = 4) and OVA (n = 3)-challenged wild type (WT) and STAT6-deficient (STAT6-KO, n = 4 each) mice is shown. Arginase activity was measured in lung lysates using the blood urea nitrogen reagent. Modified and reproduced with permission from (19).
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