Inflammation and remodelling in experimental models of COPD - Mechanisms and therapeutic perspectives

Pera, Tonio

Chapter 6

ARGINASE AND PULMONARY DISEASES

Harm Maarsingh, Tonio Pera, Herman Meurs

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Abstract

Recent studies have indicated that arginase, which converts L-arginine into L-ornithine and urea, may play an important role in the pathogenesis of various pulmonary disorders. In asthma, COPD and cystic fibrosis, increased arginase activity in the airways may contribute to airways obstruction and hyperresponsiveness by inducing reduced production of bronchodilatory nitric oxide (NO) due to competition with constitutive (cNOS) and inducible (iNOS) NO synthases for their common substrate. In addition, reduced L-arginine availability to iNOS induced by arginase may result in the synthesis of both NO and superoxide anion by this enzyme, thereby enhancing the production of peroxynitrite, which has procontractile and pro-inflammatory actions. Moreover, increased synthesis of L-ornithine by arginase might contribute to airway remodelling in these diseases. L-Ornithine is a precursor of polyamines and L-proline, which may promote cell proliferation and collagen production, respectively. Increased arginase activity may also be involved in other fibrotic disorders of the lung, including idiopathic pulmonary fibrosis. Finally, by inducing reduced levels of vasodilating NO, increased arginase activity has been associated with primary and secondary forms of pulmonary hypertension. Drugs targeting the arginase pathway could have therapeutic potential in these diseases.

Introduction

Arginase is the final enzyme of the urea cycle in the liver and is the key enzyme for the removal of highly toxic ammonium ions from the body. To this aim, L-arginine, which is produced by argininosuccinate lyase, is converted into L-ornithine and urea by the action of arginase. Besides in the liver, arginase is also expressed in cells and tissues that lack a complete urea cycle, including the airways and the lung.

Arginase exists in two distinct isoenzymes: arginase I and II, which are encoded by different genes. Arginase I is a cytosolic enzyme and is the predominant isoform in the liver, where it is highly expressed (1, 2). Although low levels of arginase II have been detected in liver as well (3), this mitochondrial enzyme is mainly expressed in extrahepatic tissue (1, 2). In the airways, both arginase I and II are constitutively expressed in bronchial epithelial cells, endothelial cells, (myo)fibroblasts and alveolar macrophages (3-5), while arginase II is also expressed in parenchymal epithelial cells (4). Although arginase expression in airway smooth muscle was below detection limit in some studies (4), other

studies have indicated that either isoform may be (conditionally) expressed in these cells (6, 7).

One of the biological functions of extrahepatic arginase may be regulation of the synthesis of nitric oxide (NO), via competition with NO synthase (NOS) for the common substrate, L-arginine (Fig. 1). In activated macrophages, for example, arginase activity limits the utilization of L-arginine by inducible NOS (iNOS) and suppress the cytotoxic response by these cells (8-10). The activity of arginase, on the other hand, is inhibited by N^{ω}-hydroxy-L-arginine (NOHA), an intermediate in the NO synthesis, showing a delicate balance between NOS and arginase activity in the control of NO production (11, 12). In addition, by the synthesis of L-ornithine extrahepatic arginase may be involved in tissue repair processes. Thus, L-ornithine is a precursor of polyamines and proline, which are involved in cell proliferation and collagen synthesis respectively (2, 13, 14).

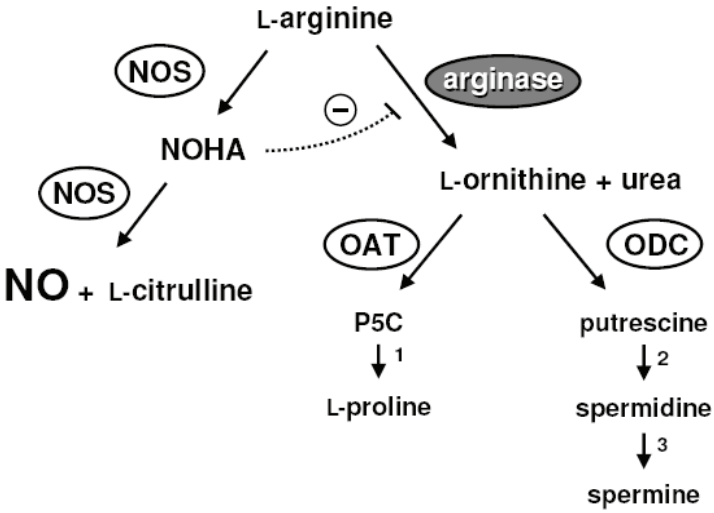

Figure 1: L-Arginine metabolism by arginase and NOS. L-Arginine is a substrate for NOS, yielding NO and L-citrulline, as well as for arginase, which produces L-ornithine and urea. Arginase regulates the production of NO via competition with NOS for their common substrate. On the other hand, NOHA, an intermediate in the NO synthesis by NOS, inhibits arginase activity. In addition, the arginase product L-ornithine is the precursor of the polyamines putrescine, spermidine and spermine, as well as of L-proline. Abbreviations: NO, nitric oxide; NOHA, N^{ω}-hydroxy-L-arginine; NOS, nitric oxide synthase; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; P5C, L-pyrroline-5-carboxylate; 1, pyrroline-5-carboxylate reductase; 2, spermidine synthase; 3, spermine synthase.

A functional role for constitutively expressed arginase in the airways has been established in guinea pig tracheal preparations, by using the specific arginase inhibitor N^{ω}-hydroxy-nor-L-arginine (nor-NOHA). Thus, nor-NOHA decreased methacholine-induced airway constriction by increasing the production of non-neural, presumably epithelium-derived, bronchodilating NO (15). Moreover, the arginase inhibitor increased NO-mediated airway smooth muscle relaxation induced by inhibitory nonadrenergic noncholinergic (iNANC) nerve stimulation (16). In both studies, the effects of nor-NOHA were quantitatively similar to the effects of exogenously applied L-arginine, supporting that arginase is involved in the control of airway responsiveness by attenuation of substrate availability to NOS (15-17).

Aberrant NO homeostasis as well as exaggerated tissue repair are involved in various inflammatory airway diseases associated with reduced lung function, airway hyperresponsiveness (AHR) and/or airway remodelling, such as allergic asthma (18, 19), chronic obstructive pulmonary disease (COPD; (20-22) and cystic fibrosis (23-25). In addition, reduced levels of NO have also been observed in lungs of patients with pulmonary arterial hypertension (PAH; (26), while dysregulated tissue repair and excessive fibrosis in the lung interstitium is observed in patients with idiopathic pulmonary fibrosis (27). This review will address the potential role of arginase in the pathophysiology of these diseases.

Allergic asthma

Role of NO in allergic asthma

Allergic asthma is a chronic inflammatory airways disease, characterized by allergen-induced early and late bronchial obstructive reactions and AHR to a variety of stimuli, including allergens, chemical irritants, cold air and pharmacological agents like histamine and methacholine (28). The development of bronchial obstructive reactions as well as of AHR is associated with infiltration and activation of inflammatory cells, particularly Th2 lymphocytes and eosinophils, in the airways (28). The cause of AHR may be multi-factorial, involving changes in the neurogenic and non-neurogenic control of airway smooth muscle function as well as structural changes in the airways such as epithelial damage, mucosal swelling and airway remodelling that is characterized by increased airway smooth muscle mass, subepithelial fibrosis, hyperplasia of mucous cells and angiogenesis (28). All these changes can be induced by a cascade of inflammatory reactions involving various mediators, including NO (19, 28, 29).

NO is produced by a family of NOS isoforms, which convert L-arginine into NO and L-citrulline, using oxygen and NADPH as cosubstrates (30). Three NOS isozymes are known: neuronal NOS (nNOS or NOS I), inducible NOS (iNOS or NOS II) and endothelial NOS (eNOS or NOS III). In the respiratory tract, nNOS and/or eNOS are constitutively expressed in iNANC neurons (nNOS), and in epithelial (nNOS and eNOS) and endothelial (eNOS) cells. These constitutive NOS (cNOS) isoenzymes are primarily involved in the neural and non-neural regulation of airway and vascular smooth muscle tone via both cGMP-dependent and -independent mechanisms (19). In addition, eNOS-derived NO has been shown to inhibit airway inflammation by suppressing the activation of NF-κB, thereby inhibiting the expression of iNOS as well as the production of inflammatory cytokines (31-35). It has been demonstrated that an impaired production of cNOS-derived bronchodilating NO contributes to the development of AHR in allergic asthma, both in animal models and in human asthma (36-42).

iNOS is induced in the airways by pro-inflammatory cytokines, particularly in inflammatory and epithelial cells (43-45). iNOS-derived NO may be involved in infiltration of inflammatory cells (46), mucosal swelling (47) and epithelial damage (46, 48), but may also have a beneficial bronchodilating action (46, 49), indicating a dualistic role in the airways. In contrast to cNOS, iNOS produces large amounts of NO, which causes increased concentrations of NO in the exhaled air of asthmatics (50). In experimental asthma, iNOS is induced in the airways during the allergen-induced late asthmatic reaction, similarly leading to increased levels of NO in the exhaled air (51, 52). In asthmatics, increased nitrotyrosine staining in the airways correlates well with iNOS expression, AHR and airway inflammation, suggesting that not iNOS-derived NO itself, but rather its reaction with superoxide to the highly reactive oxidant peroxynitrite may importantly account for the detrimental effects of iNOS in the airways (53). Indeed, peroxynitrite has procontractile and proinflammatory actions and is involved in the development of AHR after the late asthmatic reaction or after repeated allergen challenge (54-57).

Arginase in animal models of allergic asthma

In a guinea pig model of allergic asthma using ovalbumin-sensitized animals, it was demonstrated that a deficiency of NO underlies the development of AHR after the allergen-induced early asthmatic reaction (36, 39) and that this NO deficiency is caused by a decreased availability of L-arginine to cNOS (17, 37, 58). Using airway preparations from the same animal model, it was demonstrated that increased arginase activity may be involved in the reduced L-arginine bioavailability and AHR. Thus, after the early asthmatic reaction arginase activity

in the airways of allergen-challenged guinea pigs was 3.5-fold increased as compared to unchallenged animals, while incubation with the arginase inhibitor nor-NOHA completely reversed the allergen-induced AHR of perfused tracheal preparations from these animals by restoring NO production (58). L-Arginine limitation induced by increased arginase activity appeared also to underlie an impaired iNANC nerve-mediated airway smooth muscle relaxation after the early asthmatic reaction by inducing a deficiency of nNOS-derived NO (37). Collectively, these findings indicate a key role for arginase in the development AHR after the allergen-induced early asthmatic reaction by inducing a deficiency of both neuronal and non-neuronal NO (Fig. 2).

A second mechanism by which increased arginase activity may contribute to AHR in allergic asthma is via stimulation of peroxynitrite formation. Thus, studies in macrophages have indicated that under conditions of low L-arginine availability iNOS not only produces NO by its oxygenase moiety, but also synthesizes superoxide anions by its reductase moiety, which leads to an efficient formation of peroxynitrite (59). Increasing the L-arginine concentration in these cells stimulates NO production, while the formation of superoxide – and hence peroxynitrite – is reduced (60). In perfused guinea pig tracheal preparations obtained after the allergen-induced late asthmatic reaction, the AHR to methacholine was reduced by both the NOS inhibitor L-NAME and the superoxide anion scavenger superoxide dismutase (SOD), indicating the involvement of peroxynitrite in this process (54). Remarkably, the AHR was similarly diminished by the arginase inhibitor nor-NOHA and by exogenous L-arginine, highly suggesting that reduced L-arginine availability caused by increased arginase activity is involved in iNOS-induced production of peroxynitrite and AHR (61). This was underscored by the observation that the effect of nor-NOHA was fully reversed by L-NAME, indicating that arginase inhibition restores the production of bronchodilating NO. Moreover, the arginase activity in tracheal tissue as well as in bronchoalveolar lavage cells of the challenged animals was increased after the late asthmatic reaction (61).

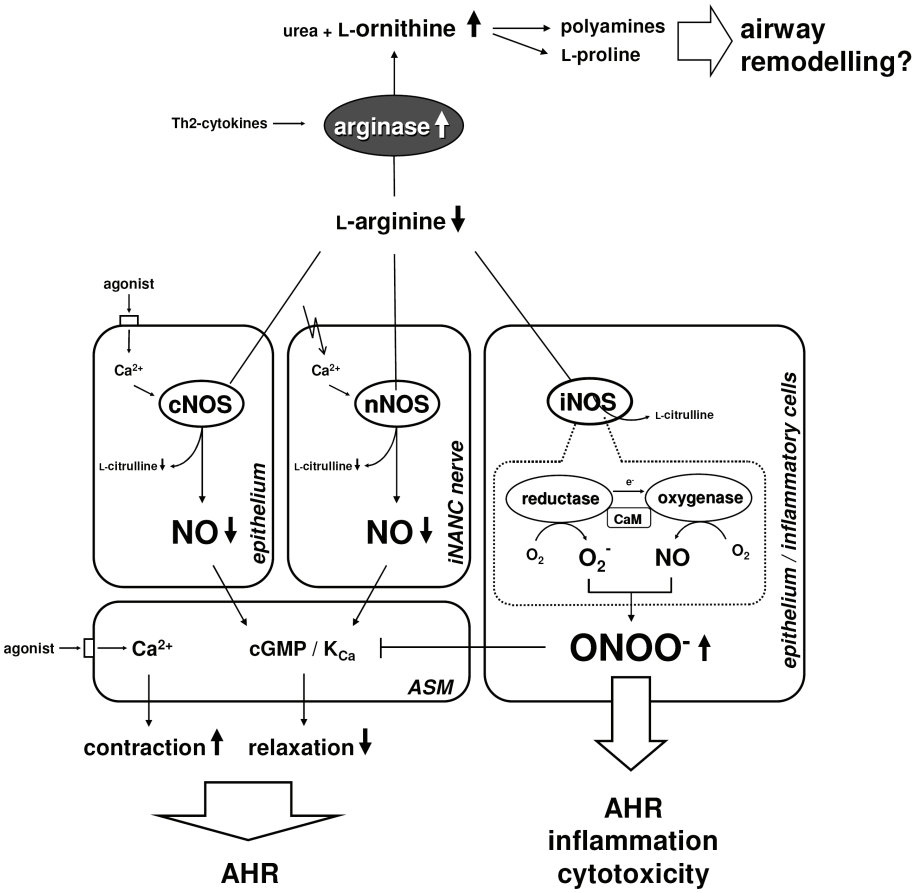

Figure 2: Role of increased arginase activity in the pathophysiology of allergic asthma. In allergic asthma, arginase expression and activity is increased by Th2-cytokines. Increased arginase activity limits the bioavailability of L-arginine to cNOS, leading to a reduced production of agonist-induced NO by the airway epithelium as well as of neuronal NO by iNANC nerves. Under basal conditions, NO induces airway smooth muscle relaxation by increasing the production of cGMP and/or by opening of calcium-activated potassium channels, thereby attenuating the airway responsiveness to contractile stimuli. Therefore, arginase-induced deficiency of cNOS-derived NO in allergic asthma contributes to AHR in this disease. Increased arginase activity also attenuates the availability of L-arginine to iNOS, which is induced during the late asthmatic reaction or in chronic asthma. The reduced L-arginine availability to iNOS results in the simultaneous production of NO and O_2^- by the oxygenase and reductase moieties of the enzyme, respectively, This leads to rapid formation of the highly reactive nitrogen species $ONOO^-$, which has procontractile, proinflammatory and cytotoxic actions in the airways. In addition, increased production of polyamines and L-proline downstream of L-ornithine may contribute to the process of

airway remodelling. Abbreviations: AHR, airway hyperresponsiveness; ASM, airway smooth muscle; CaM, calmodulin; e^-, electron; cGMP, cyclic 3',5'guanosine monophosphate; cNOS, constitutive nitric oxide synthase; iNANC nerve, inhibitory nonadrenergic noncholinergic nerve; iNOS, inducible nitric oxide synthase; K_{Ca}, calcium-activated potassium channel; nNOS, neuronal nitric oxide synthase; NO, nitric oxide, O_2^-, superoxide anion; $ONOO^-$, peroxynitrite.

The importance of increased arginase activity in the pathophysiology of asthma was confirmed in various animal models of allergic asthma, using different antigens. In BALB/c mice sensitized to ovalbumin and to *Aspergillus fumigatus* increased arginase activity in the lung was measured after allergen challenge (62). Notably, by microarray analysis of gene expression it was shown that among the 291 common genes that were induced by these allergens, enzymes involved in L-arginine metabolism, particularly arginase I and II, belonged to the most predominantly overexpressed genes. Northern blot analysis confirmed the increase in arginase I and II gene expression. In contrast to arginase I, arginase II was constitutively present, but induced to a much lesser extent. In situ hybridization and immunohistochemistry of ovalbumin-challenged animals demonstrated that high levels of arginase I were observed in the perivascular and peribronchial pockets of inflammation in asthmatic lung (62). In line with the microarray study mentioned above, proteomics of lung tissue from repeatedly ovalbumin-challenged C57BL/6 mice indicated considerable upregulation of arginase I (63).

According to previous studies in mouse macrophages (9, 64), lung arginase activity and mRNA expression of both arginase I and arginase II were strongly induced by the Th2 cytokines IL-4 and IL-13, which are abundant in allergic airway inflammation (62). Moreover, arginase I mRNA expression in mouse lung was also increased by IL-25, a novel member of the IL-17 family which induces Th2-like airway inflammation and AHR (65). Furthermore, increased arginase I gene expression was observed in Th2 polarized, but not in Th1 polarized, mice sensitized to and challenged with *Schistosoma mansoni* eggs (66), supporting the importance of Th2 cytokines in inducing arginase in asthma. Although IL-4 alone did not induce arginase activity in human alveolar macrophages, it greatly enhanced the response to cAMP-elevating agents (67). Recently, it was demonstrated that isolated human airway smooth muscle cells have low expression of particularly arginase II (6). Remarkably, the expression of arginase II, but not of arginase I, was increased after stimulation with IL-4 (6). Of note, in rat vascular smooth muscle cells the expression of arginase I was induced by IL-4 as well as by IL-13, while arginase II was not induced by these cytokines (68).

Several studies have indicated that cytokine-induced expression of arginase I is under important control of the transcription factors STAT6 (62, 68-71) and CCAAT-enhancer binding protein (69). However, IL-4-induced arginase II expression in the lung was hardly affected in STAT6$^{-/-}$ mice, indicating that the induction of arginase II is largely STAT6 independent (62).

Lung arginase activity and arginase I mRNA expression were also increased in BALB/c mice challenged trimellitic anhydride, although the induction was lower than in animals challenged with ovalbumin (72). In addition, lung arginase activity as well as mRNA and protein expression of both arginase I and II were shown to be increased in NC/Nga mice challenged with *Dermatophagoides farinae* (73). While arginase I was not detected in the lung of control animals, increased arginase I expression was observed in alveolar macrophages and infiltrating cells around the bronchioles in challenged mice (73). In different mouse models of Th2 cytokine-mediated inflammation, using ovalbumin (BALB/c and C57BL/6 mice), the parasite *Nippostrongylus brasiliensis* (BALB/c) or the fungus *Aspergillus fumigatus* (C57BL/6), as well as in IL-13 overexpressing mice, gene expression in the lung was studied to identify the genes that are commonly expressed in lung inflammation. Among the 26 characteristic transcripts of these 5 different models, arginase I was strongly increased in all (74). In another study in mice, instillation of IL-13 in the airways increased arginase activity and the expression of arginase I, but not arginase II, while NO synthesis was decreased (71). IL-13-induced arginase expression was also temporally correlated with the development, persistence, and resolution of IL-13-induced AHR to methacholine. *In vivo* treatment with RNA interference against arginase I abrogated the development of IL-13-induced AHR, supporting the importance of increased arginase activity for AHR in asthma (71). Further studies demonstrating the effects of specific arginase inhibitors and/or RNA interference against arginase I and II on allergen-induced AHR are clearly indicated.

Besides in guinea pigs and mice, increased arginase activity has also been observed in a rat model of allergic asthma. Thus, arginase activity was increased in lung homogenates of rats challenged with ovalbumin for three consecutive days (75).

Taken together, increased arginase induction has been observed in a wide variety of animal models of asthma, using different species and allergens (Table 1). The increased arginase activity may contribute to AHR by reducing the production of bronchodilating NO as well as by stimulation of the formation of procontractile

and proinflammatory peroxynitrite (Fig. 2). Interestingly, in lung epithelial cells it was recently demonstrated that overexpression of arginase may increase NF-κB activation by decreased production of NO, suggesting that increased arginase activity in allergic asthma could also promote airway inflammation and AHR by increased production of inflammatory cytokines (76).

Arginase in human asthma

The significance of increased arginase expression and activity may for the pathophysiology of human asthma was first demonstrated by Zimmermann et al. (2003), who demonstrated that arginase I protein expression is increased in BAL cells of asthmatic patients. Moreover, in bronchial biopsies of these patients, enhanced mRNA expression of arginase I was observed in inflammatory cells as well as in the airway epithelium. Surprisingly, arginase activity was increased in serum of asthmatic patients experiencing an exacerbation, which was associated with reduced plasma L-arginine levels, indicating that changes in arginase expression in asthma are not confined to the airways and that reduced levels of circulating L-arginine could contribute to NO deficiency and hyperresponsiveness of the airways (77). Moreover, in some of these patients arginase activity declined and L-arginine concentrations increased after improvement of symptoms (77). Quite remarkably, enhanced arginase activity was already found in expectorated sputum of asthmatic patients over two decades ago (78).

The effect of smoking on arginase expression in human airways has recently also been studied (7). Endobronchial biopsy specimens from steroid-naïve mild asthmatics were investigated for changes in immunoreactivity for arginase I, ornithine decarboxylase (ODC, the rate limiting enzyme in polyamine synthesis, Fig. 1) and iNOS in smoking versus non-smoking patients. Interestingly, increased immunoreactivity for arginase I and ODC was observed in both the epithelium and smooth muscle layers of the smokers, while iNOS-immunoreactivity was similar in both groups. In addition, arginase I mRNA expression was increased in the epithelium and smooth muscle bundles of smoking asthmatics as compared to the non-smoking patients. To investigate which component of cigarette smoke may attribute for the increase in arginase expression, the effect of nicotine on arginase and ODC expression in cultured airway epithelial and smooth muscle cells and fibroblasts was studied. Nicotine significantly increased arginase I mRNA in the epithelial cells, while a trend towards increased arginase I mRNA expression was observed in airway smooth muscle cells and fibroblast after nicotine treatment. In addition, nicotine significantly increased ODC mRNA expression in fibroblasts and epithelial cells,

but not in the airway smooth muscle cells (7). These observations might also be of relevance for the pathogenesis of COPD (see below).

Interestingly, single nucleotide polymorphisms (SNPs) in arginase I and arginase II have recently found to be associated with atopy and risk of childhood asthma, respectively (79).

COPD

COPD is an inflammatory disease characterized by a progressive, irreversible decline in lung function. The leading cause of COPD is cigarette smoke (80). Smoking initiates pulmonary inflammation characterized by prominent infiltration of neutrophils, macrophages and T-lymphocytes, particularly of the $CD8^+$ subset (81, 82). Major features of COPD are airway hyperresponsiveness and progressive decline in lung function associated with structural changes of the peripheral lung, including small airway remodelling - characterized by mucus cell hyperplasia, airway fibrosis and increased airway smooth muscle mass - and alveolar wall destruction (20-22, 81, 82).

Using the single expiratory flow technique measuring NO derived from the (predominantly larger) airways, COPD has been associated with increased levels of exhaled NO (eNO) in patients with severe stage disease and during exacerbations (83-85). However, using this method, eNO is often low or in the normal range in patients with stable COPD (86). This has been attributed to the effect of tobacco smoking, which down-regulates eNOS (87) and iNOS (88). Indeed, smokers exhale lower amounts of NO compared to nonsmokers (89). Reduced levels of NO may also result from smoking-induced increased oxidative stress, by formation of peroxynitrite from NO and superoxide anions. Indeed, peroxynitrite generation is considerably increased in sputum macrophages of COPD patients, which is negatively correlated with FEV_1 in these patients (90). Recently, methods for measuring eNO at multiple respiratory flows have indicated that, while airway NO is relatively low in COPD, there is an increase in alveolar NO that is related to disease severity and not affected by smoking (91).

Table 1: Changes in arginase activity and the expression of arginase I and II in animal models and human pathology of pulmonary diseases

	Species	Condition/ stimulus	Localization	Arg I	Arg II	Activity	References
Asthma							
	Human		Sputum	n.d.	n.d.	+	Kochanski, 1978
			Serum	n.d.	n.d.	+	Morris, 2004
			BAL cells, epithelium, lung, MΦ	+	n.d.	n.d.	Zimmermann, 2003
		Cigarette smoke	Smooth muscle, epithelium	+	n.d.	n.d.	Bergeron, 2007
	Guinea pig	Ovalbumin	BAL cells, trachea	n.d	n.d.	+	Maarsingh, 2004; Meurs, 2002
	Mouse	*A. fumigatus*	Lung	+	+	+	Lewis, 2007; Zimmermann, 2003
		D. farinae	Lung, serum, MΦ	+	+	+	Takemoto, 2007
		IL-4	Lung	+	+	n.d.	Zimmermann, 2003
		IL-13	Lung	+	=	+	Lewis, 2007; Yang, 2006; Zimmermann, 2003
		N. brasiliensis	Lung	+	=	n.d.	Lewis, 2007
		Ovalbumin	Lung, MΦ	+	+	+	Fajardo, 2004; Greene, 2005; Lewis, 2007; Zimmermann, 2003
		S. mansoni eggs	Lung	+	n.d.	n.d.	Sandler, 2003
		TMA	Lung	+	+	+	Greene, 2005
	Rat	Ovalbumin	Lung	n.d.	n.d.	+	Abe, 2006

COPD							
	Human		Sputum	n.d.	n.d.	+	Chachaj, 1978; Kochanski, 1980
		GOLD 0, I, II-A	Lung	=	=	n.d.[1]	Tadie, 2007
	Rat	Cigarette smoke	Lung	+	n.d.	n.d.	Gebel, 2006
CF							
	Human		Plasma, sputum,	n.d.	n.d.	+	Grasemann, 2005b; 2006b
Fibrosis							
	Human	IPF	Lung	-	=	=	Kitowska, 2007
		IPF	Epithelium, fibroblasts, lung, MΦ	+	n.d.	n.d.	Mora, 2006
	Mouse	Bleomycin	Epithelium, fibroblasts, lung, MΦ	+	+	n.d.	Endo, 2003; Kitowska, 2007
		Herpes virus	Lung, MΦ	+	n.d.	+	Mora, 2006
		Silica	BAL cells, lung, MΦ	+	n.d.	+	Misson, 2004
	Rat	Silica	BAL cells, lung	+	=	+	Nelin, 2002; Poljakovic, 2007; Schapira, 1998
PH							
	Human	Primary PH	PAEC, serum	=	+	+	Xu, 2004
		Secondary PH in sickle cell disease	Erythrocytes, plasma, serum	n.d.	n.d.	+	Morris, 2003; 2005
	Mouse	Hemoglobin$^{-/-}$	Lung	n.d.	n.d.	+	Hsu, 2007
	Rat	Monocrotaline	PAEC	n.d.	n.d.	+	Sasaki, 2007

-, decreased; +, increased; =, unchanged, n.d., not determined; [1]arginase inhibition decreases the sensitivity to acetylcholine in COPD patients compared to control subjects.
Arg I, arginase I; Arg II, arginase II; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; IPF, idiopathic lung fibrosis; MΦ, macrophages; PAEC, pulmonary arterial endothelial cells; PH, pulmonary hypertension; TMA, trimilletic anhydride

Quite interestingly, microarray analysis of gene expression, followed by reverse transcription real-time quantitative PCR in lungs from smoking rats revealed a marked time- and dose-dependent up-regulation of arginase I expression during 2-13 weeks of smoke exposure (2 times 1 h/day, 5 days/week; (92). Smoke-induced expression of arginase I might be involved in the relatively low NO production in the airways from COPD patients as well as in peroxynitrite production and AHR found in these patients. The latter was supported by the recent observation that increased arginase activity may be involved in the enhanced sensitivity to methacholine of bronchial preparations from patients with mild COPD (93). Interestingly, subcutaneous injection (5 weeks, once daily) of cigarette smoke extract in rabbits increased expression of arginase I and an increase in arginase activity in cavernous tissue, while NOS activity and nNOS expression were significantly decreased (94). Moreover, electric field stimulation-induced neurogenic and NO mediated cavernous smooth muscle relaxation was attenuated by cigarette smoke extract administration (94). Whether cigarette smoke-induced increase in arginase activity in the airways also leads to reduced iNANC-mediated NO production and airway smooth muscle relaxation has not been studied yet.

Cystic fibrosis

Cystic fibrosis (CF) is a progressive disease, characterized by pulmonary inflammation and bacterial infection, chronic airway obstruction, airway remodelling and AHR (23-25). Despite the inflammatory nature of CF, levels of eNO are decreased (98, 99). The reduced NO levels in the airways of CF patients might contribute to microbial infection and colonization as well as to functional changes of the airways. Several mechanisms may contribute to low pulmonary levels of NO in CF, including reduced expression of iNOS (100), polymorphisms of nNOS (101, 102) and eNOS (103), mechanical retention of NO in airway secretions (104), increased metabolism to peroxynitrite (105), consumption of NO by denitrifying bacteria (106) and increased arginase activity in the airways (107, 108).

In a mouse model of cystic fibrosis, an impaired electrical field stimulation-induced airway smooth muscle relaxation was found, which was reversed by L-arginine and NO (109). This indicates that a deficiency of NO due to substrate limitation to nNOS compromises airway relaxation and contributes to airway obstruction. Indeed in CF patients a positive correlation was found between pulmonary function and exhaled NO and NO metabolite concentrations in sputum (98, 104). Moreover, inhalation of L-arginine increased exhaled NO

levels and improved lung function in these patients (110). However, oral L-arginine treatment increased L-arginine levels in sputum and plasma as well as levels of exhaled NO, but failed to improve pulmonary function (111). Increased consumption by arginase may account for the L-arginine limitation in CF, since sputum arginase activity in CF is markedly increased as compared to controls and is even further increased during pulmonary exacerbation (108). Interestingly, increased arginase activity negatively correlated with lung function (FEV_1), while increased eNO and improvement of lung function by antibiotic treatment were associated with a decrease in arginase activity (108). At least some portion of the increased arginase activity in the above-mentioned study could have been derived from microorganisms in the sputum. However, increased arginase activity was also observed in plasma of CF patients during pulmonary exacerbation, while plasma L-arginine levels were decreased (107). Treatment with antibiotics decreased the arginase activity and restored the L-arginine levels in these patients, confirming the close relationship between increased arginase activity and decreased levels of L-arginine (107). Taken together, these findings indicate that increased arginase activity in CF contributes to the NO deficiency and pulmonary obstruction in CF by limiting the availability of L-arginine to NOS.

Arginase in airway remodelling and fibrotic pulmonary disorders

Increased arginase activity in asthma, COPD and cystic fibrosis might also contribute to the airway remodelling observed in these diseases by increased production of the L-proline and the polyamines putrescine, spermidine and spermine from L-ornithine (Fig. 1, 2). L-Proline, the precursor of collagen, is synthesized from L-ornithine in a two step reaction involving ornithine aminotransferase and pyrroline-5-carboxylate reductase, while ODC initiates the synthesis of polyamines that could be involved in proliferation of structural cells in the airways (2, 18, 112, 113) Fig. 1). In support of this concept, transfection of rat vascular smooth muscle cells with arginase I induced increased polyamine levels as well as enhanced proliferation of these cells (114). The involvement of arginase in airway remodelling remains to be established, however. In support of a potential role of arginase in airway fibrosis in asthma, IL-4 and IL-13 increased arginase I and II expression and arginase activity in cultured rat fibroblasts (5). Moreover, IL-4 induced increased arginase II expression in human airway smooth muscle cells (6). In addition, increased levels of polyamines have been observed in mouse lung after allergen challenge (62) and in serum of asthmatic patients (115), respectively. Notably, growth factors like EGF and PDGF, known to be enhanced in asthma, may be involved in the induction of arginase and of

enzymes of the polyamine synthetic pathway (116-118). Polyamines can stimulate the expression of genes implicated in cell proliferation by promoting histone acetyltransferase activity resulting in chromatin hyperacetylation (119). Interestingly, in bronchial biopsies of asthmatic patients, the activity of histone acetyltransferase is increased (120).

As mentioned above, exposure to cigarette smoke and/or nicotine may induce increased expression of arginase I and ODC in human airway structural cells (7). In support, long-term exposure to mainstream smoke increased ODC activity in rat trachea and lung (121). That nicotine could account for the effect of cigarette smoke was shown by the observation that a single subcutaneous injection of nicotine also induces a transient increase in ODC activity in rat trachea (122). These findings suggest that smoking may contribute to airway remodelling in asthma as well as in COPD.

Expression of collagen I mRNA as well as arginase I and II mRNA and protein was increased in bleomycin-induced lung fibrosis in mice (123). Arginase II expression colocalized with the collagen-specific chaperone Hsp47, indicating a prominent role for arginase in collagen synthesis in lung fibrosis (123). In another study, bleomycin induced a time-dependent increase in arginase I and II expression in mouse lung, which was accompanied by decreased levels of L-arginine (124). Arginase was localized to macrophages and epithelial cells as well as in interstitial fibroblasts, especially in fibrotic lesions (124). Increased arginase expression in fibrotic areas was also observed in herpes virus-induced lung fibrosis in mice (125). Interestingly, arginase I and II expression was induced by TGF-β in primary mouse fibroblasts and treatment with the non-specific arginase inhibitor NOHA prevented TGF-β-induced increase in collagen collagen content in a post transcriptional manner (124). In several animal models of silicosis, another inflammatory lung disease characterized by fibrosis, arginase activity and arginase I expression were also enhanced in lung and alveolar macrophages (126-129). However, a direct relationship between increased arginase activity and expression and fibrosis was not always found (127). Thus, arginase I expression and activity in lung of silica-exposed mice were increased at 3 days, but not 30 or 60 days, after silica exposure, while levels of hydroxyproline – a marker of fibrosis – were increased at all three time points (127). Treatment with an arginase inhibitor could clarify whether (increased) arginase activity is indeed involved in silica-induced fibrosis.

In line with the above findings in mouse models of lung fibrosis, arginase I expression was also increased in patients with idiopathic pulmonary fibrosis

(IPF), especially in alveolar macrophages, epithelial cells and areas with pleura thickening and interstitial fibrosis (125). However, in another study lung arginase I expression in IPF patients was decreased, while no differences were observed in arginase II expression and arginase activity in lung, nor in arginase I and II expression in cultured fibroblasts from these patients (124). Therefore, more investigation is needed to establish the role of arginase in human pulmonary fibrotic disease.

Arginase and pulmonary hypertension

Reduced levels of NO have been observed in lungs of patients with pulmonary hypertension (PH; (26). These reduced NO levels may be caused by changes in L-arginine metabolism, since low levels of plasma L-arginine have been found in patients with primary and secondary forms of hypertension (130-132). Inhalation of NO as well as L-arginine supplementation have shown to be of potential benefit in the treatment of PH (131-133). The decreased L-arginine levels may result from increased arginase activity. Thus, increased arginase activity in serum has been detected in patients with primary PH (PPH), which was associated with reduced L-arginine to L-ornithine levels in these patients (130). Moreover, increased arginase II expression has been observed in PPH patients, especially in the endothelium of arteries and arterioles, as well as in cultured pulmonary arterial endothelial cells from these patients (130). Increased arginase activity was also observed in a mouse model of PH. Thus, in mice with monocrotaline-induced PH arginase activity was increased, while NOS activity and cGMP production were reduced (134). Of note, reduced eNOS expression and increased accumulation of endogenous NOS inhibitors, such as monomethylarginine and asymmetric dimethylarginine, did also contribute to reduced NO production in this model (134).

Decreased plasma levels of L-arginine and NO metabolites are also observed in infants with persistent PH (135); however, whether this is caused by increased arginase activity is presently unknown. Interestingly, a recent study indicated that arginase II expression in pulmonary arteries is regulated developmentally, with maximal expression and activity during fetal life (136). It is therefore tempting to speculate that developmentally high levels of arginase activity may be involved in the pathogenesis of PPH of the newborn.

Reduced plasma L-arginine levels and increased serum and plasma arginase activity have also been observed in patients with sickle cell anemia and associated secondary PH (131, 132). Oral treatment with L-arginine increased

plasma levels of both L-arginine and L-ornithine and reduced pulmonary artery systolic pressure (131). The increased plasma arginase activity in patients with sickle cell anemia and PH may be primarily caused by release of the enzyme from erythrocytes during intravascular hemolysis. In addition, hemolysis may contribute to reduced NO bioavailability and endothelial dysfunction via release of erythrocyte hemoglobulin, which scavenges NO (132). Increased arginase activity in the lung and in plasma as well as reduced eNOS activity in the lung were observed in a mouse model of sickle cell disease and PH (137). Similar pathobiology was observed in a nonsickle mouse model of acute alloimmune hemolysis, indicating that hemolysis is sufficient to cause these changes (137).

In conclusion, increased arginase activity appears to be importantly involved in the pathophysiology of both primary and secondary PH by limiting the bioavailability of L-arginine to eNOS in the pulmonary vasculature.

Conclusions

It has been well established that changes in L-arginine metabolism by NO synthases are involved in a variety of diseases, including diseases of the respiratory system. During the last few years there is growing interest in the potential role of arginases as a key regulators of the synthesis of NO, as well as of polyamines and L-proline, in these diseases. Animal model studies have indicated that arginase is importantly involved in the regulation of airway responsiveness, and pharmacological studies using specific arginase inhibitors have revealed a pathophysiological role of the enzyme in the pathogenesis of allergic asthma. Moreover, remarkable increases in pulmonary arginase activity and/or expression associated with altered NO and L-arginine homeostasis have been observed in animal models and human pathology of asthma, COPD, cystic fibrosis, idiopathic pulmonary fibrosis and pulmonary hypertension. Although the functional role of arginase overexpression in most of these diseases has not yet fully been established, there is accumulating evidence that arginase may be an important novel target for drug therapy of these diseases.

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