Arginase and pulmonary diseases

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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, chronic obstructive pulmonary disease (COPD) and cystic fibrosis, increased arginase activity in the airways may contribute to obstruction and hyperresponsiveness of the airways by inducing a reduction in the production of bronchodilatory nitric oxide (NO) that results from its 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 the superoxide anion by this enzyme, thereby enhancing the production of peroxynitrite, which has procontractile and pro-inflammatory actions. Increased synthesis of L-ornithine by arginase may also contribute to airway remodelling in these diseases. L-Ornithine is a precursor of polyamines and L-proline, and these metabolic products 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, through its action of 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.

Keywords Arginase · Asthma · Chronic obstructive pulmonary disease · Cystic fibrosis · Nitric oxide · Pulmonary hypertension

Introduction

Arginase, which converts L-arginine into L-ornithine and urea, is a key enzyme of the urea cycle in the liver, but it is also expressed in cells and tissues that lack a complete urea cycle, including the lung. Arginase exists as 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 (Jenkinson et al. 1996; Wu and Morris 1998). Although low levels of arginase II have been detected in liver as well (Klasen et al. 2001), this mitochondrial enzyme is mainly expressed in extrahepatic tissue (Jenkinson et al. 1996; Wu and Morris 1998). In the airways, both arginase I and II are constitutively expressed in bronchial epithelial cells, endothelial cells, and alveolar macrophages. Klasen et al. 2001; Lindemann and Racke 2003; Que et al. 1998), while arginase II is also expressed in parenchymal epithelial cells (Que et al. 1998). A number of studies have reported that arginase expression in airway smooth muscle was below detection limit (e.g. Que et al. 1998), while other studies have indicated that either isofrom may be (conditionally) expressed in these cells (Bergeron et al. 2007; Zuyderduyn et al. 2006).

One of the biological functions of extrhepatic arginase may be regulation of the synthesis of nitric oxide (NO) by means of 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 suppresses the
cytotoxic response by these cells (Hey et al. 1997; Modolell et al. 1995; Wang et al. 1995). The activity of arginase, however, is inhibited by Nω-hydroxy-L-arginine (NOHA), an intermediate in the NO synthesis catalysed by NOS, inhibits arginase activity. In addition, the arginase product L-ornithine is the precursor of the polyamines putrescine, spermidine and spermine and of L-proline. 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 using the specific arginase inhibitor Nω-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 (Meurs et al. 2000). Moreover, the arginase inhibitor increased NO-mediated airway smooth muscle relaxation induced by inhibitory nonadrenergic noncholinergic (iNANC) nerve stimulation (Maarsingh et al. 2005). In both studies, the effects of nor-NOHA were quantitatively similar to the effects of exogenously applied L-arginine, thereby providing experimental evidence that arginase is involved in the control of airway responsiveness through attenuation of substrate availability to NOS (De Boer et al. 1999; Maarsingh et al. 2005; Meurs et al. 2000).

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 (Meurs et al. 2003; Ricciardolo et al. 2004), chronic obstructive pulmonary disease (COPD; Barnes et al. 2003; Hogg 2004; Postma and Kerstjens 1998) and cystic fibrosis (Hays et al. 2005; Hilliard et al. 2007; Tiddens and De Jong 2007). In addition, reduced levels of NO have also been observed in lungs of patients with pulmonary arterial hypertension (PAH; Kaneko et al. 1998), while dysregulated tissue repair and excessive fibrosis in the lung interstitium are observed in patients with idiopathic pulmonary fibrosis (Gross and Hunninghake 2001). 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, such as histamine and methacholine (Bousquet et al. 2000). The development of bronchial obstructive reactions as well as of AHR is associated with an infiltration and activation of inflammatory cells, particularly Th2 lymphocytes and eosinophils, in the airways (Bousquet et al. 2000). The cause of AHR may be multifactorial, involving changes in both 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 (Bousquet et al. 2000). All of these changes can be induced by a cascade of inflammatory reactions involving various mediators, including NO (Bousquet et al. 2000; Ricciardolo et al. 2004; Ricciardolo 2003).

Nitric oxide is produced by a family of NOS isoforms that convert L-arginine into NO and L-citrulline using oxygen and NADPH as cosubstrates (Moncada et al. 1989). Three NOS isoforms have been identified to date: 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 (Ricciardolo et al. 2004). In addition, eNOS-derived NO has been shown to inhibit airway inflammation by suppressing the activation of NF-κB, thereby inhibiting both the expression of iNOS and the production of inflammatory cytokines (Cirino et al. 2003; Cook et al. 2003; Marshall and Stamler 2001; Ten Broeke et al. 2006; Thomassen et al. 1997). A number of studies have 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 (De Boer et al. 1996; Maarsingh et al. 2006; Mehta et al. 1997; Ricciardolo et al. 1997, 2001; Samb et al. 2001; Schuling et al. 1998b).

Inducible NOS is induced in the airways through the action of pro-inflammatory cytokines, particularly those found in inflammatory and epithelial cells (Asano et al. 1994; Barnes 1998; Hamid et al. 1993). Inducible NOS-derived NO may be involved in the infiltration of inflammatory cells (Schuling et al. 1998a), mucosal swelling (Kuo et al. 1992) and epithelial damage (Flak and Goldman 1996; Schuling et al. 1998a), but it may also have a beneficial bronchodilating action (De Gouw et al. 1998; Schuling et al. 1998a), 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 (Khaitov et al. 1994). In experimentally induced 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 (Khaitov et al. 1995a; Yan et al. 1995). 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 be an important factor accounting for the detrimental effects of iNOS in the airways (Saleh et al. 1998). 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 (De Boer et al. 2001; Muijsers et al. 2001; Sadeghi-Hashjin et al. 1996, 1998).

Arginase in animal models of allergic asthma

A study using a guinea pig model of allergic asthma and ovalbumin-sensitized animals demonstrated that a deficiency of NO underlies the development of AHR following the allergen-induced early asthmatic reaction (De Boer et al. 1996; Schuling et al. 1998b) and that this NO deficiency is caused by a decreased availability of L-arginine to eNOS (De Boer et al. 1999; Maarsingh et al. 2006; Meurs et al. 2002). 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, arginase activity in the airways of allergen-challenged guinea pigs was 3.5-fold increased after the early asthmatic reaction 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 (Meurs et al. 2002). L-Arginine limitation induced by increased arginase activity also appeared to underlie an impaired iNANC nerve-mediated airway smooth muscle relaxation after the early asthmatic reaction by inducing a deficiency of nNOS-derived NO (Maarsingh et al. 2006). Collectively, these findings indicate a key role for arginase in the development AHR following 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 through the stimulation of peroxynitrite formation. 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, leading to an efficient formation of peroxynitrite (Xia et al. 1998). Increasing the L-arginine concentration in these cells stimulates NO production, while the formation of superoxide — and hence peroxynitrite — is reduced (Xia and Zweier 1997). 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 (De Boer et al. 2001). Remarkably, the AHR was similarly diminished by the arginase inhibitor nor-NOHA and by exogenous L-arginine, which is highly suggestive of the reduced L-arginine availability caused by increased arginase activity being involved in the iNOS-induced production of peroxynitrite and AHR (Maarsingh et al. 2004). This mechanism 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 the tracheal tissue and bronchoalveolar lavage cells of the challenged animals was increased after the late asthmatic reaction (Maarsingh et al. 2004).
animal models of allergic asthma and using different antigens. Increased arginase activity in the lung was measured after allergen challenge in BALB/c mice sensitized to ovalbumin and to *Aspergillus fumigatus* (Zimmermann et al. 2003). Notably, microarray analysis of gene expression revealed that among the 291 common genes that are induced by these allergens, enzymes involved in L-arginine metabolism, particularly arginase I and II, belong to the most predominantly overexpressed genes. Northern blot analysis confirmed the increase in arginase I and II gene expression; however, 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 the presence of high levels of arginase I in the perivascular and peribronchial pockets of inflammation in the asthmatic lung (Zimmermann et al. 2003). In line with the results of the microarray study mentioned above, proteomics of lung tissue from repeatedly ovalbumin-challenged C57BL/6 mice showed a considerable upregulation of arginase I (Fajardo et al. 2004).

Based on results from earlier studies in mouse macrophages (Corraliza et al. 1995; Modolell et al. 1995), lung arginase activity and mRNA expression of both arginase I and arginase II are strongly induced by the Th2 cytokines interleukin (IL)-4 and IL-13, which are abundant in allergic airway inflammation (Zimmermann et al. 2003). More recently, IL-25, a novel member of the IL-17 family which induces Th2-like airway inflammation and AHR, has also been shown to increase arginase I mRNA expression in mouse lung (Sharkhuu et al. 2006). Increased arginase I gene expression was also observed in Th2-polarized—but
arginase II expression in the lung was hardly affected in induced expression of arginase I. However, IL-4-induced (2005) are key factors regulating the control of cytokine-
2003) and CCAAT-enhancer binding protein (Gray et al. 
W e i et al. 2000; Y a n ge t a l . 2006; Z i m m e r m a n n e t al. 
We are observed to increase in lung homogenates of rats challenged with ovalbumin for 3 consecutive days (Abe et al. 
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 stimulating the formation of procontractile and proinflammatory peroxynitrite (Fig. 2). Interestingly, it was recently demonstrated in lung epithelial cells that over-expression of arginase may increase NF-κB activation through a decreased production of NO, suggesting that increased arginase activity in allergic asthma could also promote airway inflammation and AHR by increasing the production of inflammatory cytokines (Ckless et al. 2007).

Arginase in human asthma

The significance of increased arginase expression and activity on the pathophysiology of human asthma was first reported by Zimmermann et al. (2003), who demonstrated that arginase I protein expression increases in bronchialveolar lavage (BAL) cells of asthmatic patients. Moreover, bronchial biopsies of these patients revealed enhanced mRNA expression of arginase I in both the inflammatory cells and in the airway epithelium. Surprisingly, elevated levels of arginase activity were found in the 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 (Morris et al. 2004). These researchers also observed that arginase activity declined and L-arginine concentrations increased in some of these patients following an improvement of symptoms (Morris et al. 2004). It is worth noting that enhanced arginase activity in expectorated sputum of asthmatic patients had been reported over two decades ago (Kochanski et al. 1980).

Bergeron et al. (2007) recently published their findings on the effect of smoking on arginase expression in human airways. Endobronchial biopsy specimens from steroid-naïve mild asthmatics were investigated for changes in
| Clinical condition | Species | Condition/stimulus | Localization | Arginase I | Arginase II | Activity | References |
|--------------------|---------|--------------------|--------------|------------|-------------|----------|------------|
| Asthma             | Human   | Sputum             | n.d.         | n.d.       | +           |          | Kochanski et al. 1980 |
|                    |         | Serum              | n.d.         | n.d.       | +           |          | Morris et al. 2004   |
|                    |         | BAL cells, epithelium, lung, MΦ | +           | n.d.       | n.d.       |          | Zimmermann et al. 2003 |
|                    |         | Smooth muscle, epithelium | +           | n.d.       | n.d.       |          | Bergeron et al. 2007 |
|                    | Cigarette smoke | Smooth muscle, epithelium | +           | n.d.       | n.d.       |          | Maarsingh et al. 2004; Meurs et al. 2002 |
| Guinea pig         | Ovalbumin | BAL cells, trachea | n.d.         | n.d.       | +           |          | Lewis et al. 2007; Zimmermann et al. 2003 |
| Mouse              | Aspergillus fumigatus | Lung | +           | +           | +           |          | Takemoto et al. 2007 |
|                    | Dermatophagoides farinae | Lung, serum, MΦ | +           | +           | +           |          | Lewis et al. 2007 |
|                    | IL-4    | Lung               | +           | +           | n.d.       |          | Zimmermann et al. 2003 |
|                    | IL-13   | Lung               | +           | +           | =           |          | Lewis et al. 2007; Yang et al. 2006; Zimmermann et al. 2003 |
|                    | Nippostrongylus brasiliensis | Lung | +           | =           | n.d.       |          | Fajardo et al. 2004; Greene et al. 2005; Lewis et al. 2007; Zimmermann et al. 2003 |
|                    | Schistosoma mansoni eggs | Lung | +           | n.d.       | n.d.       |          | Sandler et al. 2003 |
| Chronic obstructive pulmonary disease (COPD) | Rat | Ovalbumin | Lung | +           | +           | +         | Greene et al. 2005 |
|                    | Human   | Sputum             | n.d.         | n.d.       | +           |          | Chachaj et al. 1978; Kochanski et al. 1980 |
|                    | GOL D 0, I, II-A | Lung | =           | =           | n.d.       |          | Tadie et al. 2008 |
|                    | Rat | Cigarette smoke | Lung | +           | n.d.       | n.d.       | Gebel et al. 2006 |
| Cystic fibrosis (CF) | Human | Plasma, sputum | n.d.         | n.d.       | +           |          | Grasemann et al. 2005b; 2006b |
| Fibrosis           | Human   | IPF                | –            | =           | =           |          | Kitowska et al. 2008 |
|                    |         | IPF                | +           | n.d.       | n.d.       |          | Mora et al. 2006 |
| Mouse              | Bleomycin | Epithelium, fibroblasts, lung, MΦ | +           | +           | n.d.       |          | Endo et al. 2003; Kitowska et al. 2008 |
| Herpes virus       | Silica | BAL cells, lung    | +           | n.d.       | +           |          | Mora et al. 2006 |
|                    |         |                  | +           | n.d.       | +           |          | Misson et al. 2004 |
| Rat                | Silica | BAL cells, lung    | +           | =           | +           |          | Nelin et al. 2002; Po lj kovic et al. 2007; Schapira et al. 1998 |
| Pulmonary hypertension (PH) | Human | Primary PH | PAEC, serum | =           | +           | +         | Xu et al. 2004 |
|                    | Secondary PH in sickle cell disease | Erythrocytes, plasma, serum | n.d.       | n.d.       | +           |          | Morris et al. 2003; 2005 |
|                    | Mouse   | Haemoglobin<sup>−/−</sup> | Lung | n.d.       | n.d.       | +           |          | Hsu et al. 2007 |
|                    | Rat     | Monocrotaline      | PAEC | n.d.       | n.d.       | +           |          | Sasaki et al. 2007 |

<sup>−</sup>, Decreased; <sup>+</sup>, increased; <sup>−/−</sup>, unchanged, n.d., not determined

<sup>a</sup> IPF, Idiopathic pulmonary fibrosis; TMA, trimilletic anhydride; IL, interleukin

<sup>b</sup> MΦ, Macrophages; PAEC, pulmonary arterial endothelial cells; BAL cells, bronchoalveolar lavage cells

<sup>c</sup> Arginase inhibition decreases the sensitivity to acetylcholine in COPD patients compared to control subjects
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, these researchers studied the effect of nicotine on arginase and ODC expression in cultured airway epithelial and smooth muscle cells and fibroblasts. 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 fibroblasts after nicotine treatment. In addition, nicotine significantly increased ODC mRNA expression in the fibroblasts and epithelial cells, but not in the airway smooth muscle cells (Bergeron et al. 2007). These observations may also be of relevance for the pathogenesis of COPD (see below).

Interestingly, single nucleotide polymorphisms (SNPs) in arginase I and arginase II have recently been found to be associated with atopy and the risk of childhood asthma, respectively (Li et al. 2006).

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease is an inflammatory disease characterized by a progressive, irreversible decline in lung function. The leading cause of COPD is cigarette smoke (Pauwels et al. 2001). Smoking initiates pulmonary inflammation characterized by the prominent infiltration of neutrophils, macrophages, and T-lymphocytes, particularly those of the CD8+ subset (Jeffery 2000; Sutherland and Martin 2003). The main features of COPD are airway hyperresponsiveness and progressive decline in lung function associated with structural changes in the peripheral lung, including small airway remodelling that is characterized by mucus cell hyperplasia, airway fibrosis and increased airway smooth muscle mass- and alveolar wall destruction (Barnes et al. 2003; Hogg 2004; Jeffery 2000; Postma and Kerstjens 1998; Sutherland and Martin 2003).

Based on results obtained using the single expiratory flow technique to measure 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 (Agusti et al. 1999; Bhowmik et al. 2005; Maziak et al. 1998). However, this method has also shown that eNO is often low or in the normal range in patients with stable COPD (Kharitonov and Barnes 2001), a state that has been attributed to the effect of tobacco smoking, which downregulates eNOS (Hutchison et al. 2001) and iNOS (Hoyt et al. 2003). Indeed, smokers exhale lower amounts of NO than nonsmokers (Kharitonov et al. 1995b). Reduced levels of NO may also result from smoking-induced increased oxidative stress through the formation of peroxynitrite from NO and superoxide anions. In support of this hypothesis, peroxynitrite generation has been found to be considerably increased in sputum macrophages of COPD patients, which is negatively correlated with forced expiratory volume in the first second (FEV1) in these patients (Achenes et al. 2000). Measurements of eNO taken 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 (Brindicci et al. 2005).

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 upregulation of arginase I expression during 2–13 weeks of smoke exposure (2 × 1 h/day, 5 days/week; Gebel et al. 2006). Smoke-induced expression of arginase I may be involved in the relatively low NO production in the airways of COPD patients as well as in the peroxynitrite production and AHR found in these patients. The latter development is supported by the recent observation that increased arginase activity may be involved in the enhanced sensitivity to methacholine shown by bronchial preparations from patients with mild COPD (Tadie et al. 2008). Interestingly, subcutaneous injection (5 weeks, once daily) of cigarette smoke extract in rabbits increased the expression of arginase I and increased arginase activity in cavernous tissue, while NOS activity and nNOS expression were significantly decreased (Imamura et al. 2007). Moreover, electric field stimulation-induced neurogenic and NO-mediated cavernous smooth muscle relaxation was attenuated by cigarette smoke extract administration (Imamura et al. 2007). Whether cigarette smoke-induced increase in arginase activity in the airways also leads to reduced sNANC-mediated NO production and airway smooth muscle relaxation has yet to be studied.

As already mentioned, nicotine, a major constituent of tobacco smoke, may upregulate arginase I in airway structural cells (Bergeron et al. 2007). Interestingly, recent evidence suggests that human neutrophils constitutively express high levels of arginase I in azurophilic granules (Munder et al. 2005) which, like other constituents of these granules, including neutrophil elastase, may be liberated in patients with COPD (Renkema et al. 1991). Since neutrophil abundance is also demonstrated in patients with severe asthma as well as in those with cystic fibrosis, neutrophil arginase could also be involved in the pathogenesis of these
diseases; however, the role of neutrophil arginase in airway diseases remains to be established. Remarkably, as with asthma, increased arginase activity in the sputum of COPD patients had already been found as early as the late 1970s (Chachaj et al. 1978; Kochanski et al. 1980).

Cystic fibrosis

Cystic fibrosis is a progressive disease, characterized by pulmonary inflammation and bacterial infection, chronic airway obstruction, airway remodelling and AHR (Hays et al. 2005; Hilliard et al. 2007; Tiddens and De Jong 2007). Despite the inflammatory nature of CF, this disease is characterized by decreased levels of eNO (Elphick et al. 2001; Grasemann et al. 1997), and reduced NO levels in the airways of CF patients may contribute to microbial infection and colonization as well as to functional changes of the airways. Several mechanisms may contribute to the low pulmonary levels of NO in CF, including a reduced expression of iNOS (Downey and Elborn 2000), polymorphisms of nNOS (Grasemann et al. 2000, 2002) and eNOS (Grasemann et al. 2003), mechanical retention of NO in airway secretions (Grasemann et al. 1998), increased metabolism to peroxynitrite (Robbins et al. 2000), consumption of NO by denitrifying bacteria (Gaston et al. 2002) and increased arginase activity in the airways (Grasemann et al. 2005b, 2006b).

In a mouse model of CF, an impaired electrical field stimulation-induced airway smooth muscle relaxation was found, which was reversed by L-arginine and NO (Mhanna et al. 2001). This result indicates that a deficiency of NO due to substrate limitation to nNOS compromises airway relaxation and contributes to airway obstruction. In support of this mechanism, a positive correlation has been found between pulmonary function and exhaled NO and NO metabolite concentrations in the sputum of CF patients (Grasemann et al. 1997, 1998). Moreover, inhalation of L-arginine increased exhaled NO levels and improved lung function in these patients (Grasemann et al. 2006a). 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 (Grasemann et al. 2005a). Increased consumption by arginase may account for the L-arginine limitation in CF, since sputum arginase activity in CF is markedly increased in comparison to controls and is even further increased during pulmonary exacerbation (Grasemann et al. 2005b). Interestingly, increased arginase activity was found to be negatively correlated with lung function (FEW$_1$), while increased eNOS and improved lung function due to antibiotic treatment were associated with a decrease in arginase activity (Grasemann et al. 2005b). 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 (Grasemann et al. 2006b). Treatment with antibiotics decreased the arginase activity and restored the L-arginine levels in these patients, thereby confirming the close relationship between increased arginase activity and decreased levels of L-arginine (Grasemann et al. 2006b). 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 may also contribute to the airway remodelling observed in these diseases through the increased production of 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 the proliferation of structural cells in the airways (Hoet and Nemery 2000; Meurs et al. 2003; Ricciardolo et al. 2005; Wu and Morris 1998; Fig. 1). In support of this concept, transfection of rat vascular smooth muscle cells with arginase I induced increased polyamine levels and enhanced proliferation of these cells (Wei et al. 2001). 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 (Lindemann and Racke 2003), and IL-4 induced increased arginase II expression in human airway smooth muscle cells (Zuyderduyn et al. 2006). In addition, increased levels of polyamines have been observed in the mouse lung after allergen challenge (Zimmermann et al. 2003) and in the serum of asthmatic patients (Kurosawa et al. 1992), respectively. Notably, growth factors, such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), which are known to be enhanced in asthma, may be involved in the induction of arginase and of enzymes participating in the polyamine synthetic pathway (Nelin et al. 2005; Thyberg and Fredholm 1987a, b). Polyamines can stimulate the
expression of genes implicated in cell proliferation by promoting histone acetyltransferase activity, resulting in chromatin hyperacetylation (Hobbs and Gilmour 2000). The activity of histone acetyltransferase was found to be increased in bronchial biopsies of asthmatic patients (Ito et al. 2002).

As mentioned above, exposure to cigarette smoke and/or nicotine may induce increased expression of arginase I and ODC in human airway structural cells (Bergeron et al. 2007). The results of a study showing that long-term exposure to mainstream smoke increased ODC activity in rat trachea and lung provides experimental support for this possibility (Olson 1985). That nicotine could account for the effect of cigarette smoke was shown by the observation that a single subcutaneous injection of nicotine also induced a transient increase in ODC activity in rat trachea (Olson and Crooks 1985). These findings suggest that smoking may contribute to airway remodelling in asthma as well as in COPD.

Expression of collagen I mRNA and arginase I and II mRNA and protein was increased in bleomycin-induced lung fibrosis in mice (Endo et al. 2003). Arginase II expression co-localized with the collagen-specific chaperone Hsp47, indicating a prominent role for arginase in collagen synthesis in lung fibrosis (Endo et al. 2003). In another study, Kitowska et al. (2008) observed a bleomycin-induced time-dependent increase in arginase I and II expression in mouse lung that was accompanied by decreased levels of L-arginine; arginase was localized to macrophages and epithelial cells as well as in interstitial fibroblasts, especially in fibrotic lesions. Increased arginase expression in fibrotic areas was also observed in herpes virus-induced lung fibrosis in mice (Mora et al. 2006). Interestingly, arginase I and II expression was induced by transforming growth factor (TGF)-β in primary mouse fibroblasts, and treatment with the non-specific arginase inhibitor NOHA prevented the TGF-β-induced increase in collagen content in a post-transcriptional manner (Kitowska et al. 2008). 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 (Misson et al. 2004; Nelin et al. 2002; Poljakovic et al. 2007; Schapira et al. 1998). However, a direct relationship between increased arginase activity and expression and fibrosis has not always been found: Misson et al. (2004) reported that arginase I expression and activity in lung of silica-exposed mice were increased at 3 days, but not 30 or 60 days, following silica exposure, while levels of hydroxyproline—a marker of fibrosis—were increased at all three time points. A study involving 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 found to be increased in patients with idiopathic pulmonary fibrosis (IPF), especially in the alveolar macrophages, epithelial cells and areas with pleura thickening and interstitial fibrosis (Mora et al. 2006). However, in another study on IPF patients, lung arginase I expression was decreased, while no differences were observed in arginase II expression and arginase activity in the lung, nor in arginase I and II expression in cultured fibroblasts from these patients (Kitowska et al. 2008). Therefore, further 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; Kaneko et al. 1998). 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 (Morris et al. 2003, 2005; Xu et al. 2004). Inhalation of NO as well as L-arginine supplementation have shown to be of potential benefit in the treatment of PH (Atz and Wessel 1997; Morris et al. 2003, 2005). The decreased L-arginine levels may result from increased arginase activity, and 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 (Xu et al. 2004). 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 (Xu et al. 2004). Increased arginase activity was also observed in a mouse model of PH: in mice with monocrotaline-induced PH, arginase activity was increased, while NOS activity and cGMP production were reduced (Sasaki et al. 2007). Of note, reduced eNOS expression and increased accumulation of endogenous NOS inhibitors, such as monomethylarginine and asymmetric dimethylarginine, also contributed to reduced NO production in this model (Sasaki et al. 2007).

Decreased plasma levels of L-arginine and NO metabolites have also been observed in infants with persistent PH (Pearson et al. 2001); 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 (Belik et al. 2008). 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 (Morris et al. 2003, 2005). Oral treatment with L-arginine was found to increase the plasma levels of both L-arginine and L-ornithine and to reduce pulmonary artery systolic pressure (Morris et al. 2003). The primary cause of the increased plasma arginase activity in patients with sickle cell anemia and PH may be the release of the enzyme from erythrocytes during intravascular haemolysis. Haemolysis may also contribute to reduced NO bioavailability and endothelial dysfunction via the release of erythrocyte haemoglobin, which scavenges NO (Morris et al. 2005). Increased arginase activity in the lung and in plasma and reduced eNOS activity in the lung were observed in a mouse model of sickle cell disease and PH (Hsu et al. 2007). Similar pathobiology was observed in a nonsickle mouse model of acute alloimmune haemolysis, indicating that haemolysis is sufficient to cause these changes (Hsu et al. 2007).

In conclusion, increased arginase activity appears to be a major factor in the pathophysiology of both primary and secondary PH through its action of limiting the bioavailability of L-arginine to eNOS in the pulmonary vasculature.

Conclusions

It is a well-established fact that changes in L-arginine metabolism mediated by NO syntheses are involved in a variety of diseases, including diseases of the respiratory system. During the last few years, the potential role of arginases in these diseases as key regulators of the synthesis of NO and of polyamines and L-proline has been attracting increasing interest. Results from animal model studies have indicated that arginase is a key factor in the regulation of airway responsiveness, and pharmacological studies using specific arginase inhibitors have revealed that this enzyme has a pathophysiological role 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.

Acknowledgements The authors wish to thank the Netherlands Asthma Foundation (grant 00.24), the Ubbo Emmius Foundation and N.V. Organon, a part of Schering Plough corporation, Oss, the Netherlands, for financial support.

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