Arginase: a key enzyme in the pathophysiology of allergic asthma opening novel therapeutic perspectives

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Allergic asthma is a chronic inflammatory airways’ disease, characterized by allergen-induced early and late bronchial obstructive reactions, airway hyperresponsiveness (AHR), airway inflammation and airway remodelling. Recent ex vivo and in vivo studies in animal models and asthmatic patients have indicated that arginase may play a central role in all these features. Thus, increased arginase activity in the airways induces reduced bioavailability of L-arginine to constitutive (cNOS) and inducible (iNOS) nitric oxide synthases, causing a deficiency of bronchodilating and anti-inflammatory NO, as well as increased formation of peroxynitrite, which may be involved in allergen-induced airways obstruction, AHR and inflammation. In addition, both via reduced NO production and enhanced synthesis of L-ornithine, increased arginase activity may be involved in airway remodelling by promoting cell proliferation and collagen deposition in the airway wall. Therefore, arginase inhibitors may have therapeutic potential in the treatment of acute and chronic asthma. This review focuses on the pathophysiological role of arginase in allergic asthma and the emerging effectiveness of arginase inhibitors in the treatment of this disease.


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Abbreviations: ABH, 2(S)-amino-6-boronohexanoic acid; ADMA, asymmetric dimethylarginine; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; BEC, S-(2-boronoethyl)-L-cysteine; CAT, cationic amino acid transporter; EAR, early asthmatic reaction; eIF2α, eukaryotic initiation factor-α; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; HAT, histone acetyltransferase; Hsp47, heat shock protein-47; IkB, inhibitory factor-κB; IL-, interleukin; iNANC, inhibitory nonadrenergic noncholinergic; L-NAME, Nω-nitro-L-arginine methyl ester; NF-κB, nuclear factor-κB; NO, nitric oxide; NOHA, Nω-hydroxy-L-arginine; nNOS, neuronal NOS; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; SNPs, single nucleotide polymorphisms; STAT6, signal transducer and activator of transcription 6; TGF-β, transforming growth factor-β; Vmax, maximal enzymatic rate

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 synthesized from L-citrulline by the subsequent actions of argininosuccinate synthase and argininosuccinate lyase, is converted into L-ornithine and urea by the action of arginase. Besides in liver, arginase is also expressed in cells and tissues that lack a complete urea cycle, including the airways and the lung (Jenkinson et al., 1996; Que et al., 1998; Wu and Morris, 1998; Meurs et al., 2000). Two isoenzymes of arginase have been identified: arginase I and arginase II, which are encoded by different genes and differ in cellular location. Arginase I is a cytosolic enzyme, and the gene for human arginase I is assigned to chromosome 6q23, whereas arginase II is a mitochondrial enzyme, with its gene located on chromosome 14q24 in humans (Sparkes et al., 1986; Jenkinson et al., 1996; Gotoh et al., 1997; Wu and Morris, 1998). Arginase I is the
predominant isoform in the liver, but is expressed extrahepatically as well. Although low levels have been detected in the liver (Klasen et al., 2001), arginase II is mainly expressed in extrahepatic tissues (Jenkinson et al., 1996; Wu and Morris, 1998). Since L-arginine is also the substrate for nitric oxide synthase (NOS), yielding nitric oxide (NO) and L-citrulline (Moncada et al., 1989), one of the biological functions of extrahepatic arginase may be the regulation of NO levels via competition with NOS for their common substrate (Modolell et al., 1995; Wang et al., 1995; Hey et al., 1997; Figure 1). Although the affinity for L-arginine is much (~1000-fold) higher for NOS than for arginase, arginase can still compete with NO synthesis for L-arginine, since the V_max (maximal enzymatic rate) of arginase is approximately 1000-fold greater than that of NOS (Reczkowski and Ash, 1994; Griffith and Stuehr, 1995; Wu and Morris, 1998). On the other hand, NOS can inhibit the activity of arginase via the production of N^3-hydroxy-L-arginine (NOHA), an intermediate in the NO synthesis (Boucher et al., 1994; Daghigh et al., 1994; Hecker et al., 1995). In addition, the arginase product L-ornithine competitively inhibits arginase activity (Reczkowski and Ash, 1994; Cox et al., 2001; Maarsingh et al., 2007b), as well as L-arginine transport into NO-producing cells (Deves and Boyd, 1998; Schapira et al., 1998; Messeri Dreissig et al., 2000). These findings indicate a delicate balance between arginase and NOS in L-arginine homeostasis and in the control of NO synthesis (Figure 1).

Arginase may also regulate a number of cellular functions by NO-independent mechanisms (Figure 1). Thus, arginase may be involved in tissue repair processes by the synthesis of L-ornithine, which is the precursor of polyamines and L-proline that are involved in cell proliferation and collagen synthesis, respectively (Shearer et al., 1997; Wu and Morris, 1998; Satriano, 2004).

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Both arginase I and II are constitutively expressed in the airways, particularly in epithelial cells, (myo)fibroblasts, endothelial cells and macrophages (Que et al., 1998; Klasen et al., 2001; Lindemann and Racke, 2003), while expression of arginase II has also been observed in alveolar epithelial cells (Que et al., 1998). Moreover, arginase I and II may be conditionally expressed in airway smooth muscle cells (Zuyderduyn et al., 2006; Bergeron et al., 2007). Since arginase regulates a variety of processes involved in the regulation of airway func-

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**Figure 1** Interactive role of arginase and NOS in regulating airway function. L-arginine is the substrate for both arginase, yielding L-ornithine and urea, and NOS, yielding NO and L-citrulline. Arginase attenuates the production of NO by competing with NOS for their common substrate. On the other hand, arginase activity is inhibited by NOHA, an intermediate in the NO synthesis by NOS. In addition, L-ornithine causes feedback inhibition of arginase and inhibits cationic amino acid transporters involved in cellular uptake of L-arginine. L-Ornithine is also a precursor of the polyamines putrescine, spermidine and spermine, which are involved in cell proliferation and differentiation, and of L-proline, which is required for collagen synthesis. In the respiratory tract, NO induces airway smooth muscle relaxation and inhibits inflammation and airway smooth muscle cell proliferation. Increased arginase activity in allergic asthma compromises these processes by inducing reduced NO production, as well as increased formation of ONOO^−, as a consequence of reduced bioavailability of L-arginine to constitutive and inducible NOS isoforms, respectively. Moreover, increased production of L-ornithine by arginase may contribute to airway remodelling in this disease, by enhanced synthesis of polyamines and L-proline. ASM, airway smooth muscle; CAT, cationic amino acid transporter; NO, nitric oxide; NOHA, N^3-hydroxy-L-arginine; NOS, nitric oxide synthase; O_2^−, superoxide anion; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; ONOO^−, peroxynitrite; PSC, L-pyrroline-5-carboxylate.
tion, a key role for arginase in the pathophysiology of various airways diseases, including asthma, is emerging (Meurs et al., 2003; Maarsingh et al., 2008a).

Interactive role of arginase and NOS in regulating airway function

NO is generated from L-arginine by a family of NOS isoforms, utilizing oxygen and NADPH as substrates and yielding L-citrulline as a coproduct (Moncada et al., 1989; Boucher et al., 1999). Three isoforms of NOS have been identified: neuronal NOS (nNOS or NOS I) and endothelial NOS (eNOS or NOS III), which are constitutively expressed, and inducible NOS (iNOS or NOS II). All three NOS isoforms are (conditionally) expressed in the airways. The constitutive NOS (cNOS) isoforms are mainly expressed in the airway epithelium (nNOS and eNOS), inhibitory nonadrenergic noncholinergic (iNANC) neurons (nNOS) and endothelial cells from the airway vasculature (eNOS). cNOS isoforms produce relatively low levels of NO in response to increased intracellular calcium concentrations evoked by membrane depolarization or by the action of (contractile) agonists (Ricciardolo et al., 2004). The activity of iNOS, producing large amounts of NO, is regulated at the expression level, particularly by proinflammatory cytokines during airway inflammation. During airway inflammation, iNOS is predominantly expressed in macrophages and epithelial cells (Nijkamp et al., 1993; De Boer et al., 1996; Ricciardolo et al., 2004).

NO is an important endogenous bronchodilator by inducing airway smooth muscle relaxation (Tucker et al., 1990; Belvisi et al., 1991; Li and Rand, 1991; Belvisi et al., 1992; Ellis and Undem, 1992; Ricciardolo et al., 2004). NO-induced relaxation involves production of cGMP by soluble guanylyl cyclase, as well as activation of calcium-activated potassium channels, collectively leading to decreased intracellular Ca2+ concentrations, decreased Ca2+ sensitivity and membrane hyperpolarization of the airway smooth muscle cell (Ricciardolo et al., 2004).

In the airways, the availability of L-arginine to NOS isoforms is a limiting factor in NO production, as illustrated by the observation that levels of exhaled NO in healthy subjects are increased after oral or inhalational treatment with L-arginine, without affecting (normal) lung function (Khartonov et al., 1995; Sapienza et al., 1998). Using guinea pig airway preparations in vitro, it has been demonstrated that L-arginine inhibits the airway responsiveness to methacholine (De Boer et al., 1999) and increases iNANC nerve-mediated airway smooth muscle relaxation (Maarsingh et al., 2005), both through increased production of cNOS-derived NO.

That the substrate availability to cNOS in the airways may be attenuated by endogenous arginase activity was first demonstrated in isolated perfused guinea pig tracheal preparations (Meurs et al., 2000) using the specific arginase inhibitor Nω-hydroxy-nor-L-arginine (nor-NOHA), which – in contrast to NOHA – is not a substrate for NOS (Tenu et al., 1999). In these preparations, incubation with nor-NOHA decreased the airway responsiveness to methacholine, which was reversed by the nonselective NOS inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) (Meurs et al., 2000). Moreover, endogenous arginase activity attenuated iNANC nerve-mediated airway smooth muscle relaxation by inhibition of NOS-derived NO production (Maarsingh et al., 2005). That arginase regulates airway responsiveness by limiting the substrate availability to cNOS was supported by the observation that the effects of nor-NOHA were quantitatively similar to those of L-arginine (De Boer et al., 1999; Meurs et al., 2000; Maarsingh et al., 2005).

cNOS-derived NO and AHR in asthma

Allergic asthma is a chronic inflammatory disorder of the airways, characterized by allergen-induced, IgE-mediated early and late bronchial obstructive reactions, development of acute and transient airway hyperresponsiveness (AHR) after these reactions, and infiltration of inflammatory cells into the airways, particularly eosinophils and Th2 lymphocytes (Bousquet et al., 2000). Chronic inflammation may induce airway remodelling, characterized by structural changes in the airway wall, including thickening of the basement membrane, subepithelial fibrosis and increased airway smooth muscle mass (Bousquet et al., 2000). These structural changes may cause a progressive decline in lung function, as well as persistent AHR (Cockcroft and Davis, 2006; Meurs et al., 2008). Recent evidence suggests that changes in L-arginine homeostasis may importantly contribute to the development of both acute and chronic allergen-induced AHR via altered NO production and synthesis of polyamines and L-proline, respectively (Maarsingh et al., 2008c; Meurs et al., 2008).

The beneficial effect of cNOS-derived, bronchodilator nitric oxide on AHR is illustrated by the observation that the allergen-induced AHR to methacholine is completely abolished in eNOS-overexpressing mice (Ten Broeke et al., 2006), whereas the AHR is markedly increased in allergen-challenged eNOS knockout mice (Feletou et al., 2001). Loss of bronchoprotective NO after allergen challenge could thus underlie the AHR in allergic asthma. Indeed, various studies in animal models of allergic asthma and in asthmatic patients have indicated that the development of acute allergen-induced AHR may result from a deficiency of cNOS-derived NO in the airways (De Boer et al., 1996; Mehta et al., 1997; Ricciardolo et al., 1997; Schuiling et al., 1998b; Ricciardolo et al., 2001; Samb et al., 2001; Maarsingh et al., 2006). Thus, in a guinea pig model of allergic asthma, in which iNOS is not induced before the late asthmatic reaction (LAR; Yan et al., 1995), L-NAME did not affect the AHR immediately after the early asthmatic reaction (EAR) ex vivo (De Boer et al., 1996; Maarsingh et al., 2006) and in vivo (Schuiling et al., 1998b). Involvement of attenuated production of cNOS-derived NO in acute allergen-induced AHR was also found in mild asthmatic patients (Ricciardolo et al., 2001). Animal model studies further indicated that reduced substrate bioavailability to cNOS importantly accounts for the NO deficiency after the EAR, as treatment with L-arginine reduced the AHR in perfused tracheal preparations from allergen-challenged guinea pigs ex vivo (De Boer et al., 1999) and increased iNANC-mediated airway smooth muscle relaxation in tracheal strip preparations from these animals (Maarsingh et al., 2006).
Interestingly, treatment with inhaled L-arginine also markedly reduced the allergen-induced AHR to histamine after the EAR in guinea pigs in vivo (Maarsingh et al., 2008d). In human asthmatics, oral treatment with L-arginine did not significantly affect the AHR to histamine, although the slope of the dose-response curve was slightly reduced (De Gouw et al., 1999). However, since there was not an effect on exhaled NO either, the administered dose of L-arginine could have been too low.

Besides contributing to the development of acute, allergen-induced AHR after the EAR, the deficiency of eNOS-derived NO could also be involved in the induction of the inflammatory response during the late asthmatic reaction (LAR). Thus, it has been shown that eNOS-derived NO inhibits airway inflammation by constitutively suppressing NF-κB activity, thereby inhibiting the expression of inflammatory cytokines, as well as of iNOS (Cirino et al., 2003; Ckless et al., 2007; Thomassen et al., 1997; Marshall and Stamler, 2001; Cook et al., 2003; Ten Broeke et al., 2006). Fully in line with these findings, allergen-induced airway inflammation was markedly reduced in eNOS overexpressing mice as compared with wild type mice (Kobayashi et al., 2006; Ten Broeke et al., 2006).

**iNOS-derived NO and AHR in asthma**

In inflamed asthmatic airways, particularly during the LAR, the expression of iNOS is markedly increased by proinflammatory cytokines, such as TNF-α and IFN-γ, predominantly in inflammatory cells and in the airway epithelium (Hamid et al., 1993; Asano et al., 1994; Barnes, 1998). Since the NO production by iNOS is more than 1000-fold higher than the NO produced by eNOS, levels of NO in the exhaled air are increased in patients with active and persistent asthma (Kharitonov et al., 1994; Ricciardolo et al., 2004). iNOS-derived NO is generally considered to be detrimental in allergic asthma, as it has been shown to be associated with epithelial damage, infiltration of inflammatory cells, amplification and perpetuation of the Th2-mediated inflammatory response, mucus hypersecretion and vascular hyperpermeability (Kuo et al., 1992; Flak and Goldman, 1996; Schuiling et al., 1998a; Ricciardolo et al., 2004). However, most – if not all – of these detrimental effects in the airways may not be caused by iNOS-derived NO itself, but rather by peroxynitrite, the reaction product of NO and superoxide anions (Sadeghi-Hashjin et al., 1998; Saleh et al., 1998). This highly reactive nitrogen species promotes airway smooth muscle contraction (De Boer et al., 2001) and has proinflammatory actions, including the induction of epithelial damage, eosinophil activation, MUC5AC expression and vascular hyperpermeability (Sadeghi-Hashjin et al., 1996; Sugiera et al., 1999; Fischer and Voynow, 2002; Muijsers et al., 2002). Increased formation of peroxynitrite may importantly contribute to the development of AHR after the LAR in acute and chronic asthma (De Boer et al., 2001; Sadeghi-Hashjin et al., 1996; Sadeghi-Hashjin et al., 1998; Muijsers et al., 2001; Maarsingh et al., 2007a). In support, a marked increase in immunoreactivity for nitrotyrosine – a marker of protein nitration by peroxynitrite – has been observed in airway biopsies of asthmatic patients, particularly in epithelial and inflammatory cells, which correlated with iNOS expression, airway inflammation and AHR (Saleh et al., 1998).

Remarkably, in transgenic mice overexpressing iNOS in the airways, increased levels of exhaled NO were found, while airway resistance and airway responsiveness to methacholine were decreased, and no changes in airway inflammation, mucus secretion, airway fibrosis or nitrotyrosine staining were observed (Hjoberg et al., 2004). In addition, it has been shown that iNOS-derived NO may have a beneficial bronchodilating action in asthmatic airways (De Gouw et al., 1998; Schuiling et al., 1998a). Moreover, ovalbumin-induced AHR and airway inflammation, as well as expression of arginase I and II, were more pronounced in iNOS knockout mice than in wild-type animals (Bratt et al., 2009). However, in other studies, it was shown that the allergen-induced AHR was similar in iNOS knockout compared with wild-type mice (Xiong et al., 1999), and that the inflammation was also unchanged (De Sanctis et al., 1999; Koarai et al., 2002) or even decreased (Xiong et al., 1999; Duguet et al., 2001) in the iNOS knockout mice. For a comprehensive review on the role of the different NOS isoforms in allergic asthma – as studied by using NOS isoform selective inhibitors and transgenic mice – see Mathrani et al. (2007).

Taken together, these findings indicate that iNOS-derived NO by itself does not induce, but may even prevent, asthma-like pathology and support the hypothesis that it is increased formation of peroxynitrite from iNOS-derived NO that contributes to AHR and airway inflammation in asthma. Remarkably, increased formation of peroxynitrite may be the result of a decreased bioavailability of L-arginine to NOS. Studies have indicated that at low L-arginine concentrations, 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 macrophages stimulated NO production and inhibited the formation of superoxide and hence peroxynitrite (Xia and Zweier, 1997).

Interestingly, the *ex vivo* AHR after the LAR in perfused tracheal preparations obtained from guinea pigs after a single (Maarsingh et al., 2009) or repeated allergen challenges (Maarsingh et al., 2007a) was significantly reversed by treatment with exogenous L-arginine. In addition, inhalation of L-arginine also reduced the AHR to histamine after the allergen-induced LAR in guinea pigs in vivo (Maarsingh et al., 2008d). These findings indicate that a reduced bioavailability of L-arginine also contributes to the AHR after the late asthmatic response, presumably by inducing an increased formation of peroxynitrite by iNOS.

**Arginase and asthma**

Since increased L-arginine consumption by arginase could explain the NO deficiency and AHR in allergic asthma, a number of studies have focused on the expression and activity of this enzyme in this disease. In guinea pig tracheal homogenates obtained after the allergen-induced EAR, arginase activity was almost fourfold increased compared with tracheae from unchallenged control animals (Meurs et al.,
Increased arginase activity and/or expression has also been demonstrated in other animal models using different antigens in various species and strains (Maarsingh et al., 2003). Microarray analysis of gene expression revealed that both arginase I and II isoforms are induced by these allergens, which was confirmed by Northern blot analysis. Interestingly, arginase I and II belonged to the most predominantly overexpressed genes among the 291 common genes that were induced by these antigens (Zimmermann et al., 2003). Protein expression of arginase I, and, to a lesser extent, of arginase II, was also increased after repeated ovalbumin challenges of BALB/c mice for 2 weeks (Kenyon et al., 2008). Although the induction was lower than in animals challenged with ovalbumin, lung arginase I mRNA expression and arginase activity were also increased in BALB/c mice challenged with trimellitic anhydride (Greene et al., 2005). Marked up-regulation of arginase I after repeated ovalbumin challenge has also been detected by proteomics of lung tissue from C57BL/6 mice (Fajardo et al., 2004). In addition, lung arginase activity, as well as mRNA and protein expression of both arginase I and II, were increased in NC/Nga mice after a single challenge with Dermatophagoides farinae (Takemoto et al., 2007). In the allergen-challenged mice, high expression of arginase I has been observed in the airway epithelium and in perivascular and peribronchial pockets of inflammation in the asthmatic lung, particularly in alveolar macrophages and infiltrating cells around the bronchioles (Zimmermann et al., 2003; Takemoto et al., 2007). Increased expression of arginase II has been reported in the distal airways and peripheral lung, including the conducting airways from allergen-challenged mice (Kenyon et al., 2008). Besides in guinea pigs and mice, increased arginase activity in lung homogenates has also been observed in a rat model of allergic asthma after challenge with ovalbumin for three consecutive days (Abe et al., 2006).

Th2 cytokines, like IL-4 and IL-13, have been shown to increase arginase activity in mouse macrophages (Corraliza et al., 1995; Modolell et al., 1995). Although arginase activity in human alveolar macrophages was not induced by IL-4, it greatly enhanced the induction of arginase activity in response to CAMP-elevating agents (Erdey et al., 2005). Since allergic asthma is a Th2 cytokine-mediated disease, increased arginase expression in allergic asthma may be the result of increased release of these cytokines. Interestingly, both arginase isoforms were significantly increased in the lungs of IL-4 overexpressing mice (Zimmermann et al., 2003), animals that display several features of asthma (Rankin et al., 1996). In addition, intratracheal instillation with IL-13 markedly increased arginase I expression in mouse lungs, while the arginase II expression was only slightly or not induced (Zimmermann et al., 2003; Yang et al., 2006). IL-25, a novel member of the IL-17 family, which induces Th2-like airway...
inflammation and AHR, also increased arginase I mRNA expression in mouse lung (Sharkhuu et al., 2006). IL-4 also increased the expression of arginase II, but not arginase I, in isolated human airway smooth muscle cells (Zuyderduyn et al., 2006).

Several studies have indicated that Th2 cytokine-induced up-regulation of arginase I expression is regulated by the transcription factors CCAAT-enhancer binding protein (Gray et al., 2005) and STAT6 (Wei et al., 2000; Rutschman et al., 2001; Zimmermann et al., 2003; Gray et al., 2005; Yang et al., 2006). By contrast, arginase II expression is largely STAT6 independent, since IL-4-induced arginase II expression in the lung was hardly affected in STAT6−/− mice (Zimmermann et al., 2003).

The importance of the Th2-response in the induction of arginase expression in allergic asthma was supported by microarray analysis of gene expression in the lung of five different mouse models of Th2 cytokine-mediated inflammation in animals challenged with ovalbumin (BALB/c and C57BL/6 mice), the parasite Nippostrongylus brasiliensis (BALB/c) or the fungus Aspergillus fumigatus (C57BL/6), and in IL-13 overexpressing mice. Thus, among the 26 characteristic transcript that were commonly expressed in the lungs from these different models, arginase I was strongly increased in all (Lewis et al., 2007). Moreover, in mice sensitized to and challenged with Schistosoma mansoni eggs, increased arginase I gene expression was observed in Th2 polarized, but not in Th1 polarized, animals (Sandler et al., 2003). In conclusion, arginase activity and expression are markedly increased in different animal models of asthma, which may involve induction of the enzyme by Th2-cytokines.

**Functional consequences of increased arginase activity in asthma**

The contribution of increased arginase activity to acute allergen-induced AHR was first studied in perfused tracheal preparations obtained from allergen-challenged guinea pigs after the EAR. Incubation with the arginase inhibitor nor-NOHA completely reversed the allergen-induced AHR by restoring NO production, as illustrated by the reversal of this effect by coincubation with L-NAME (Meurs et al., 2002; Figure 2B). Using the same inhibitor, it was also shown that increased arginase activity after the EAR impairs iNANC nerve-mediated airway smooth muscle relaxation by inducing a deficiency of nNOS-derived NO (Maarsingh et al., 2004; 2008c; 2009). The effects of nor-NOHA were quantitatively similar to the effects of exogenous L-arginine (De Boer et al., 1999; Meurs et al., 2002; Maarsingh et al., 2006), suggesting that increased L-arginine consumption by arginase induces deficiency of both neuronal and non-neuronal NO and AHR. This hypothesis was further supported by a recent study in a mouse model of allergic asthma demonstrating that increased arginase activity in the airways of ovalbumin-challenged mice is associated with reduced levels of L-arginine and L-citrulline in these airways (Kenyon et al., 2008).

As described above, the AHR after the allergen-induced LAR may involve increased synthesis of peroxynitrite due to limitation of L-arginine availability to iNOS (De Boer et al., 2001; Maarsingh et al., 2009). Accordingly, in perfused guinea pig tracheal preparations obtained after the LAR, inhibition of arginase activity by nor-NOHA fully reversed the AHR to methacholine (Maarsingh et al., 2009). Coincubation with L-NAME prevented the effect of nor-NOHA, clearly showing that this normalising effect was due to increased production of bronchodilating NO (Maarsingh et al., 2009). Similar observations were made in a guinea pig model of chronic asthma (Maarsingh et al., 2007a). Since L-arginine administration similarly reduced the AHR after the LAR (Maarsingh et al., 2007a; 2009), the conclusion seems inevitable that increased arginase causes reduced L-arginine availability to iNOS, subsequently leading to increased formation of peroxynitrite and AHR. In support of this hypothesis, increased nitrotyrosine staining and increased arginase and iNOS expression were observed in Dermatophagoides farinae-challenged mice (Takemoto et al., 2007).

In addition to direct substrate competition with NO isoforms, increased arginase could also contribute to decreased L-arginine bioavailability to these enzymes through the production of L-ornithine. Thus, it has been demonstrated that L-ornithine competes with L-arginine for cellular uptake via cationic amino acid transporters (CATs) of the y+ system (Deves and Boyd, 1998; Schapira et al., 1998; Messeri Dreissig et al., 2000). Accordingly, incubation with L-ornithine increased airway responsiveness to methacholine in perfused guinea pig tracheal preparations by inducing a deficiency of cNOS-derived NO (Maarsingh et al., 2007b). Of note, reduced transport of L-arginine into NO-producing cells by inhibition of CATs can also be induced by (eosinophil-derived) polycations (Hammermann et al., 1999), which may also contribute to the NO-deficiency and AHR in asthma (Meurs et al., 1999; Maarsingh et al., 2004; 2008c; 2009).

Taken together, increased arginase activity in the airways contributes to the development of allergen-induced AHR by limiting the L-arginine bioavailability to cNOS and iNOS isoforms, leading to a deficiency of bronchodilating NO and increased formation of procontractile and proinflammatory peroxynitrite.

**Effects of arginase inhibition in vivo**

Thus far, only a few studies have addressed the effectiveness of arginase inhibition on features of allergic asthma in vivo. In BALB/c mice, Yang et al. (2006) demonstrated that treatment with IL-13 increased lung arginase activity and expression of arginase I, but not of arginase II, whereas NO synthesis was decreased. The increased arginase I expression temporally correlated with the development, persistence, and resolution of IL-13-induced AHR to methacholine. Interestingly, treatment with arginase I RNA interference prevented the IL-13-induced up-regulation of arginase I, as well as the cytokine-induced AHR, supporting the major role of increased arginase activity in the development of AHR in asthma (Yang et al., 2006).

In a guinea pig model of allergic asthma, it was recently demonstrated that inhalation of the specific, isoenzyme-nonspecific arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH) acutely reversed the allergen-induced AHR after...
the EAR and LAR at 6 and 24 h after single allergen challenge, respectively (Maarsingh et al., 2008d). This effect was mimicked by inhalation of L-arginine, but not D-arginine, indicating that arginase-induced deficiency of substrate for NOS isoenzymes in the airways is involved. Surprisingly, pre-treatment with ABH 30 min before allergen challenge considerably reduced the sensitivity of the airways to the inhaled allergen, as indicated by a more than 30-fold higher allergen dose needed to induce bronchial obstruction as compared with saline-treated animals (Maarsingh et al., 2008d). Moreover, in these animals, pre-treatment with ABH followed by an additional treatment at 8 h after allergen challenge reduced the AHR after the EAR and LAR, indicating that pre-treatment with the arginase inhibitor protects against the development of AHR irrespective of its acute anti-allergic effect. Interestingly, in ABH-treated animals challenged with the same allergen dose that induced airway obstruction in salinetreated guinea pigs, the AHR after the EAR was even further reduced, whereas the development of AHR after the LAR was fully prevented (Maarsingh et al., 2008d; Figure 3). Moreover, the ABH treatment in these animals caused a considerable reduction of the initial allergen-induced bronchial obstruction (Figure 4A,B) and of the magnitude of both the EAR and LAR (Maarsingh et al., 2008d; Figure 4A). Similarly, intraperitoneal treatment with nor-NOHA significantly decreased allergen-induced AHR in C57BL/6 mice after 2 weeks of ovalbumin challenge, confirming the therapeutic potential of arginase inhibitors in the treatment of allergic asthma (Bratt et al., 2009). However, oropharyngeal aspiration of the arginase inhibitor S-(2-boronoethyl)-L-cysteine (BEC) 2 h after the last of three ovalbumin challenges did not affect allergen-induced AHR of the central airways (Ckless et al., 2008). Although treatment with BEC appeared to reduce the allergen-induced increase in peripheral airway responsiveness at 40 s after methacholine inhalation, BEC did not inhibit – but rather accelerated – the peak-response to methacholine (Ckless et al., 2008). An explanation for this unexpected discrepancy is presently not at hand. However, a recent study of acetylcholine-induced vasorelaxation suggested that BEC may also have cellular targets other than arginase, including guanylyl cyclase (Huynh et al., 2009).
Arginase and airway inflammation

Increased arginase may also be involved in airway inflammation in asthmatics by limiting the production of cNOS-derived NO. As mentioned, cNOS-derived NO has been shown to inhibit inflammation by suppressing the activation of NF-κB, thereby inhibiting the production of inflammatory cytokines, as well as the expression of iNOS (Cirino et al., 2003; Thomassen et al., 1997; Cook et al., 2003; Ten Broeke et al., 2006; Ckless et al., 2007). NO may inhibit the activity of NF-κB in at least two ways: via S-nitrosylation of the DNA-interacting p50 subunit (Marshall and Stamler, 2001) and via suppression of the phosphorylation and degradation of IkB (Chang et al., 2004). Overexpression of arginase I in lung epithelial cells reduced NO production and increased cytokine-induced NF-κB activity, which was associated with decreased S-nitrosylation of p50 (Ckless et al., 2007). Conversely, inhibition of arginase decreased NF-κB activity, whereas the NO synthesis and S-nitrosylation of p50 were increased. These effects of arginase inhibition were prevented by the NOS inhibitor L-NAME (Ckless et al., 2007). Recently, the effect of arginase inhibition on allergen-induced airway inflammation in vivo has also been studied. A first report in ovalbumin-challenged guinea pigs indicated that pretreatment with inhaled ABH significantly reduced total inflammatory cell number, eosinophils and macrophages in the bronchoalveolar lavage (BAL) by approximately 50%, indicating that increased arginase activity in allergic asthma also contributes to airway inflammation (Maarsingh et al., 2008d). This was confirmed in a C57BL/6 mouse model of allergic asthma using intraperitoneally applied nor-NOHA (Bratt et al., 2009). The inhibitory effects of the arginase inhibitors on airway inflammation could indeed involve increased production of NO, as allergen-induced cellular infiltration was higher in iNOS knockout mice than in wild-type animals (Bratt et al., 2009). By contrast, in vivo treatment with BEC in BALB/c mice did not reduce allergen-induced inflammatory cell numbers, nor levels of cytokines in the BAL, and even slightly enhanced peribronchiolar and perivascular inflammation in the lung of these animals (Ckless et al., 2008). Remarkably, in the same study, arginase inhibition by BEC also caused further increase in allergen-induced NF-κB activity in the lung, which seems to be at variance with previous findings in cultured epithelial cells (Ckless et al., 2007). Clearly, the role of arginase in allergen-induced airway inflammation in asthma warrants further investigation.

Arginase and airway remodelling in chronic asthma

It has been postulated that increased arginase activity in chronic asthma may contribute to airway remodelling via the synthesis of polyamines and L-proline downstream from L-ornithine, which induce cell proliferation and collagen synthesis, respectively (Meurs et al., 2003; Ricciardolo et al., 2005; Figure 1). The synthesis of polyamines from L-ornithine is initiated by the action of ornithine decarboxylase (ODC), which converts L-ornithine into putrescine. Putrescine is countered into spermidine and subsequently into spermine by spermidine synthase and spermine synthase, respectively (Wu and Morris, 1998). L-Ornithine may also be converted to L-proline, which is a precursor of collagen, in a two-step reaction involving ornithine aminotransferase (OAT) and pyrrole-5-carboxylate reductase (Wu and Morris, 1998). The expression of arginase, ODC as well as OAT, can be increased by growth factors, resulting in increased synthesis of polyamines, L-proline and collagen (Thyberg and Fredholm, 1987a,b; Durante et al., 1998; 2001; Nelin et al., 2005). Growth factor-induced induction of ODC expression and activation has recently also been shown in bovine tracheal smooth muscle (Maarsingh et al., 2008b). Since growth factors are elevated in asthmatics, induction of these enzymes could contribute to airway remodelling.

Polyamines are known to stimulate the expression of genes involved in cell proliferation by promoting histone acetyltransferase (HAT) activity and chromatin hyperacylation (Hobbs and Gilmour, 2000), and may thus contribute to proliferation of structural cells in the airways (Wu and Morris, 1998; Hoet and Nemery, 2000; Meurs et al., 2003; Ricciardolo et al., 2005). The potential significance of this mechanism in asthma is supported by the observation that HAT activity is increased in bronchial biopsies of asthmatics (Ito et al., 2002). Importantly, elevated levels of putrescine have been observed in mouse lung after allergen challenge (Zimmermann et al., 2003), whereas elevated levels of putrescine, spermidine and spermine were detected in serum of asthmatic patients (Kuroswa et al., 1992).

In support of a potential role for arginase in airway smooth muscle cell proliferation, it has been demonstrated that transfection of vascular smooth muscle cells with arginase I increased polyamine levels and enhanced proliferation of these cells (Wei et al., 2001). Increased arginase activity may also contribute to airway remodelling by reducing NO synthesis. NO-mediated inhibition of ODC has been observed via S-nitrosylation of the enzyme (Bauer et al., 1999). In addition, inhibitory effects of NO on airway smooth muscle proliferation have been demonstrated (Hamad and Knox, 2001).

Subepithelial fibrosis in asthmatic airways may be caused by increased formation of L-proline, and subsequently collagen, from L-ornithine due to increased arginase activity. Interestingly, arginase expression and activity in vascular smooth muscle cells was increased by transforming growth factor-β (TGF-β) (Durante et al., 2001), a mediator that is also important in the development of airway fibrosis in asthma (Howell and McAnulty, 2006). Moreover, the non-specific arginase inhibitor NOHA inhibited TGF-β-induced increase in collagen content in primary mouse fibroblasts in a post-transcriptional manner (Kitowska et al., 2007). In further support of a potential role of arginase in airway fibrosis in asthma, the Th2 cytokines IL-4 and IL-13 increased arginase I and II expression in cultured rat fibroblasts (Lindemann and Racke, 2003).

Increased expression of arginase I and II, as well as of collagen I, have also been reported in a mouse model of bleomycin-induced lung fibrosis (Endo et al., 2003). Recently, it was shown that chronic infusion of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) increased
arginase activity, as well as collagen deposition in mouse lung (Wells et al., 2009). Interestingly, ADMA-induced arginase activity and collagen I expression in cultured primary mouse lung fibroblasts were fully reduced by treatment with NOHA, strongly suggesting that increased arginase activity underlies elevated collagen synthesis by these cells (Wells et al., 2009).

In conclusion, evidence for a role of arginase in tissue remodelling is accumulating. However, the involvement of this enzyme in airway remodelling in asthma remains to be established.

Arginase and human asthma

Already in the early eighties, arginase activity was shown to be increased in expectorated sputum of patients with asthma (Kochanski et al., 1980). However, it took more than 20 years to appreciate the potential significance of this finding for the disease (Meurs et al., 2003; Zimmermann et al., 2003). Increased expression of arginase I in the airways of asthmatic patients was first described by Zimmermann et al. (2003). Thus, increased numbers of cells expressing arginase I, presumably macrophages, were found in the BAL from these patients. In addition, increased mRNA expression of the enzyme was observed in inflammatory cells, as well as in the airway epithelium in bronchial biopsies obtained from asthmatics (Zimmermann et al., 2003). That increased consumption of L-arginine by arginase may also be involved in the pathophysiology of human asthma, was indicated by Morris et al. (2004). Thus, in asthmatics experiencing an exacerbation, a marked increase in arginase activity was found in serum, whereas plasma L-arginine levels were strikingly reduced. Moreover, improvement of asthma symptoms in some of these patients was associated with a decline in arginase activity and an increase in L-arginine concentrations (Morris et al., 2004). In severe asthmas, it was recently shown that serum arginase activity is inversely correlated to forced expiratory volume in 1 second (FEV1) and FEV1/forced vital capacity (FVC), whereas a positive correlation was found between L-arginine bioavailability and lung function (Lara et al., 2008).

Several single nucleotide polymorphisms (SNPs) have been reported for both the arginase I and arginase II gene. A significant association between SNPs in arginase II and increased risk of childhood asthma has been observed (Li et al., 2006). In the same study, evidence was also found for association between arginase I and arginase II SNPs and atopy (Li et al., 2006). Remarkably, screening 844 SNPs from 111 candidate genes in association with inhaled β2-agonist-induced bronchodilation recently identified arginase I as a potential β2-agonist response gene (Litonjua et al., 2008).

Arginase as a novel therapeutic target in allergic asthma

In conclusion, research during the last few years has revealed a remarkable causal role for L-arginine homeostasis by arginase in the pathophysiology of allergic asthma. Evidence obtained from animal studies indicates that arginase, particularly arginase I, belongs to the most prominently up-regulated genes in asthma, which may involve Th2 cytokines, as well as growth factors. Ex vivo studies in animal models of allergic asthma have shown that a deficiency of bronchodilating NO as well as an increased production of peroxynitrite, induced by reduced L-arginine availability to cNOS and iNOS, may be important involved in the development of allergen-induced AHR in this disease. In addition, a potential role for arginase in airway inflammation and airway remodelling by NO-dependent as well as NO-independent mechanisms has also been indicated, suggesting a central role of the enzyme in both acute and chronic asthma.

The functional relevance of arginase in asthmatic patients remains to be established. However, both pulmonary and systemic expression and activity of the enzyme are remarkably increased in these patients, which correlates with symptoms. In addition, genetic studies have indicated associations of gene polymorphisms in both arginase I and II, with increased risk of asthma, atopy and bronchodilator effectiveness in these patients.

Initial animal model studies in vivo have indicated the effectiveness of some specific arginase inhibitors in the protection and/or reversal of allergen-induced bronchial obstructive reactions and AHR, which, in addition to acute bronchoprotective effects, may involve both anti-allergic and anti-inflammatory actions. If the animal model studies can be translated to human disease, arginase inhibitors may have great therapeutic potential in the treatment of asthma. Topical application of arginase inhibitors via inhalation would be the preferred route in order to minimize possible unwanted (systemic) side effects, such as inhibition of the urea cycle in the liver, which may lead to hyperammonemia. Administration of L-arginine could also restore the bioavailability of this amino acid to NOS isoforms, and hence increase the production of bronchoprotective NO and reduce the detrimental formation of superoxide and peroxynitrite. However, chronic administration of L-arginine would similarly increase the activity of arginase, leading to enhanced formation of downstream products of L-ornithine that are involved in airway remodelling. Therefore, prolonged treatment of asthmatics with L-arginine may be contraindicated.

Collectively, it can be concluded that arginase represents a drug target that opens exciting novel therapeutic perspectives for the treatment of asthma.

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Statement of conflict of interest

The authors state no conflict of interest.

References


