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REVIEW

Arginase: an emerging key player in the mammalian immune system

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The enzyme arginase metabolizes L-arginine to L-ornithine and urea. Besides its fundamental role in the hepatic urea cycle, arginase is also expressed the immune system of mice and man. While significant interspecies differences exist regarding expression, subcellular localization and regulation of immune cell arginase, associated pathways of immunopathology are comparable between species. Arginase is induced in murine myeloid cells mainly by Th2 cytokines and inflammatory agents and participates in a variety of inflammatory diseases by down-regulation of nitric oxide synthesis, induction of fibrosis and tissue regeneration. In humans, arginase I is constitutively expressed in polymorphonuclear neutrophils and is liberated during inflammation. Myeloid cell arginase-mediated L-arginine depletion profoundly suppresses T cell immune responses and this has emerged as a fundamental mechanism of inflammation-associated immunosuppression. Pharmacological interference with L-arginine metabolism is a novel promising strategy in the treatment of cancer, autoimmunity or unwanted immune deviation.
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Keywords: arginase; L-arginine; inflammation; tumour immunology; myeloid-derived suppressor cells

Abbreviations: ABH, 2(S)-amino-6-boronohexanoic acid; ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; BEC, S-(2-boronoethyl)-L-cysteine; CAT, cationic amino acid transporter; CD, cluster of differentiation; C/EBPβ, CCAAT/enhancer binding protein β; eIF, eukaryotic Initiation Factor; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MDSC, myeloid-derived suppressor cell; MLR, mixed lymphocyte reaction; NO, nitric oxide; NOHA, N^G-hydroxy-L-arginine; nor-NOHA, N^ω-hydroxy-nor-L-arginine; OAT, ornithine aminotransferase; ODC, ornithine decarboxylase; PBMC, peripheral blood mononuclear cells; PEG-ADI, pegylated arginine deiminase; PMN, polymorphonuclear leukocytes; rhArg-PEG, pegylated recombinant human arginase; ROS, reactive oxygen species; STAT, signal transducer and activator of transcription; TCR, T cell receptor; Th, T helper

In the last couple of years literature on arginase in the immune system has increased enormously. This is due to the fact that the enzyme is crucially involved in various aspects of inflammation. Arginase has been shown to be either responsible for or to participate in, for example, inflammation-triggered immune dysfunction, tumour immune escape, fibrosis, immunosuppression and immunopathology of infectious diseases (Bronte and Zanovello 2005b). This review therefore aims to summarize key aspects of arginase in the immune system. After looking back briefly on important historical findings regarding immune cell arginase, the expression and regulation of arginase in the murine and human immune system and its involvement in a variety of immunologically mediated diseases will be discussed. Novel findings on the role of myeloid cell-associated arginase in immunosuppression in general and specifically in the context of tumour immune escape will be analysed in more detail. Finally, pharmacological ways to manipulate arginase and arginine metabolism will be summarized as they hold great promise for the treatment of cancer, autoimmunity and unwanted immunosuppression.

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Arginase: from urea cycle enzyme to immune cell component

The enzyme arginase hydrolyzes L-arginine to the products L-ornithine and urea. In mammals, two arginase isoenzymes

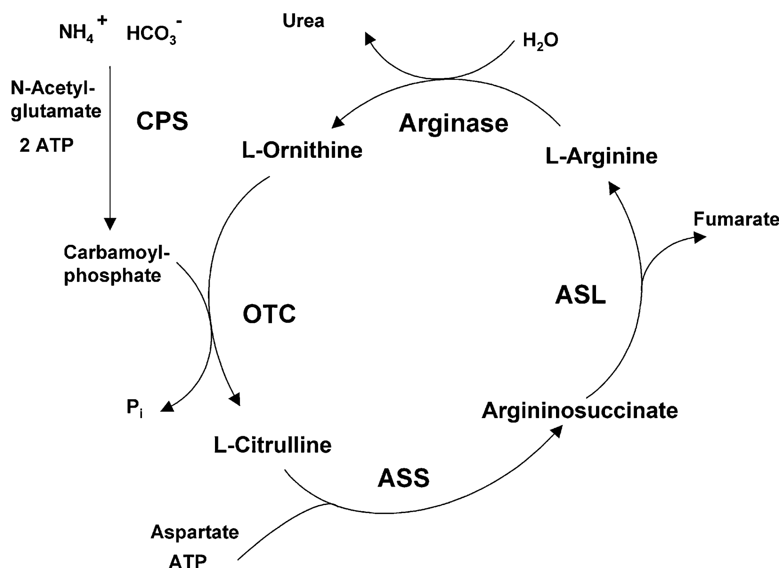

Figure 1 Arginase as part of the hepatic urea cycle. ASL, argininosuccinate lyase; ASS, argininosuccinate synthetase; CPS, carbamoyl phosphate synthetase; OTC, ornithine transcarbamoylase.

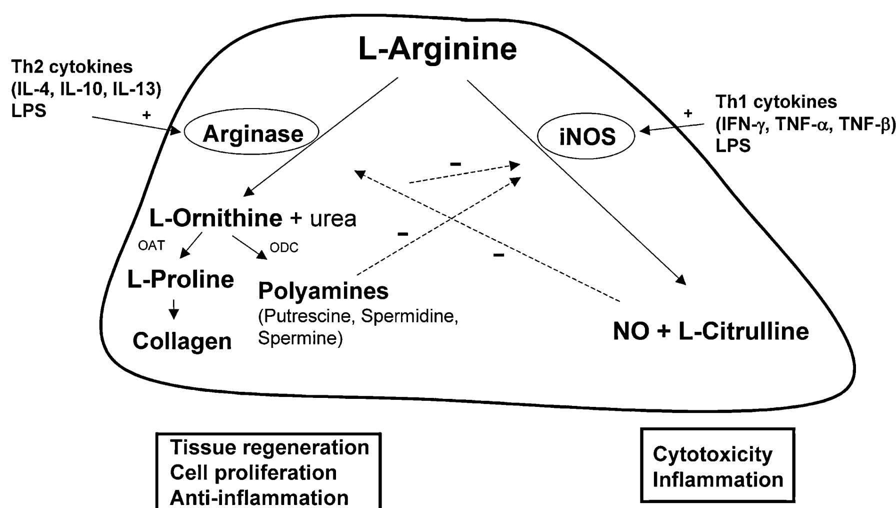

Figure 2 Reciprocal regulation of arginase and inducible nitric oxide synthase (iNOS) in murine myeloid cells. Downstream metabolic products of arginase and their association with components of inflammatory responses. OAT, ornithine aminotransferase; ODC, ornithine decarboxylase.

(designated arginase I and II) exist. They catalyse the same biochemical reaction but differ in cellular expression, regulation and subcellular localization (Jenkinson *et al.*, 1996). The isoform arginase I (L-arginine ureahydrolase, AI, EC 3.5.3.1) is expressed in the liver as one of the enzymes of the urea cycle which detoxifies ammonia in mammals (Figure 1). The cycle is distributed over two cellular compartments (mitochondrion/cytosol) with arginase acting as a cytosolic protein (Jenkinson *et al.*, 1996). Rat liver arginase was cristallized as a trimeric enzyme with a binuclear manganese cluster as part of the catalytic site cleft (Kanyo *et al.*, 1996). Human arginase I is a 322-amino acid protein and demonstrates 58% sequence identity to human arginase II. It was cloned more than 20 years ago (Dizikes *et al.*, 1986; Haraguchi *et al.*, 1987) and the gene for human arginase I was localized on chromosome 6q23 (Sparkes *et al.*, 1986). Human arginase II was cloned in 1996 (Gotoh *et al.*, 1996; Vockley *et al.*, 1996) and the gene was mapped to chromosome 14q24.1–24.3 (Gotoh *et al.*, 1997a). Arginase II protein is expressed as a mitochondrial protein in a variety of peripheral mammalian tissues, most prominently in kidney, prostate, small intestine and the lactating mammary gland. Due to its generation of L-ornithine, arginase is involved in several important downstream metabolic pathways (Figure 2). L-ornithine can be further metabolized to polyamines (putrescine, spermidine, spermine) via ornithine decarboxylase (ODC). Polyamines are small cationic molecules which participate in a variety of fundamental cellular functions (e.g. proliferation, cell membrane transport). The metabolism of L-ornithine via ornithine aminotransferase (OAT) generates L-proline, which is an essential component of collagen. Alternatively, L-arginine serves as a substrate for nitric oxide synthase (NOS) leading to

NO and other reactive nitrogen intermediates (e.g. peroxynitrite). L-arginine can be recycled from L-citrulline via argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL) (Morris, 2002).

Myeloid cell-associated arginase was described as an immunosuppressive principle already 30 years ago. The addition of peritoneal cells to murine mixed lymphocyte reaction (MLR) assays suppressed splenocyte cytotoxicity which correlated with (i) inducible expression of arginase within the peritoneal cells/macrophages and (ii) complete depletion of arginine in cell culture medium of suppressed cultures (Kung *et al.*, 1977). In fact, a complete urea cycle was demonstrated in murine bone marrow and rat peritoneal macrophages (Hofmann *et al.*, 1978). The proliferation of mouse lymphoma cells and immune functions of murine splenocytes were inhibited by the release of arginase from activated murine macrophages into the cell culture medium with subsequent degradation of L-arginine (Chen and Broome, 1980). Murine macrophage arginase was characterized as a high molecular weight protein (110 kDa) with alkaline pH optimum and a preference for manganese as cofactor (Chen and Broome, 1980). A little later, a lymphokine with arginase-inducing potential during murine secondary MLR reactions was partially characterized (Dy *et al.*, 1983). Several reports also demonstrated that the replication of malignant cells (Currie, 1978), parasites (Olds *et al.*, 1980) and viruses (Wildy *et al.*, 1982) is inhibited by the depletion of L-arginine (Schneider and Dy, 1985). Summarizing all these data, a first review on arginase in the immune response was published in 1985, concluding that macrophages can be activated to express arginase leading to L-arginine depletion in the microenvironment (Schneider and Dy, 1985). In this review it was already argued that L-arginine deficiency might lead to suppression of T lymphocyte proliferation (immunosuppression) on the one hand and to enhanced killing of malignant cells, parasites and viruses on the other hand.

Expression and regulation of arginase in the immune system

The view on the role of L-arginine in the immune response shifted completely when murine macrophages were found to express the inducible isoform of nitric oxide synthase (iNOS) upon stimulation by cytokines or microbial products (Nathan and Xie, 1994; MacMicking *et al.*, 1997). Numerous reports demonstrated that activated macrophages consume L-arginine, convert it to L-citrulline, NO and reactive nitrogen species and that this is the primary mechanism of cytostatic or cytotoxic activity of macrophages against viruses, bacteria, fungi, protozoa, helminths and tumour cells (MacMicking *et al.*, 1997). Interest in arginase slowly increased again when it was reported that the expression of both L-arginine-degrading enzymes in macrophages is regulated by T cell cytokines. While macrophage iNOS is induced by the prototypical T helper 1 (Th1) cytokine Interferon (IFN)-γ (Nathan and Xie, 1994), arginase expression in murine macrophages is inducible by activation of the cells with the Th2 cytokines Interleukin (IL)-4, IL-10 and IL-13 (Corraliza *et al.*, 1995; Modolell *et al.*, 1995; Munder *et al.*, 1998; Louis *et al.*, 1999; Munder *et al.*, 1999; Chang *et al.*, 2000). Synergism among Th1 (Nathan and Xie, 1994) and Th2 cytokines (Munder *et al.*, 1998) regarding their induction of iNOS and arginase, respectively, was demonstrated and reciprocal suppression of the alternative L-arginine-metabolizing enzyme was shown. Specifically, the NOS intermediate N^G-hydroxy-L-arginine (NOHA) was shown to inhibit co-expressed macrophage arginase activity, thereby increasing L-arginine availability for NO production (Hecker *et al.*, 1995). Th2-mediated induction of arginase I is a general feature of murine myeloid cell types and was also found in dendritic cells (Munder *et al.*, 1999; Mayer *et al.*, 2008) and granulocytes (Munder *et al.*, 2005). Also, the cytokine IL-21 acts as an amplifier of Th2-mediated arginase induction by increasing the expression of the IL-4Rα and IL-13Rα1 chains in murine macrophages (Pesce *et al.*, 2006). The dichotomous regulation of iNOS versus arginase is likely an old evolutionary program as it can already be detected in leukocytes of fish (Joerink *et al.*, 2006). The role of macrophage arginase in inflammation is also clearly demonstrated by the fact that the murine enzyme is inducible by pathogen-derived macromolecules like lipopolysaccharide (LPS) or lipoproteins (Corraliza *et al.*, 1995), by a broad range of inflammatory stimuli (Hrabak *et al.*, 2006) and in rat alveolar macrophages by the reactive oxygen product hydrogen peroxide (H_2O_2) (Matthiesen *et al.*, 2008). Recently, the induction of arginase I in murine macrophages by oxidized and acetylated lipoproteins was demonstrated (Gallardo-Soler *et al.*, 2008) connecting macrophage alternative activation and an inflammatory phenotype with lipid metabolism and vascular atherosclerosis. Arginase expression is regulated by glucocorticoids in a cell type and species-specific way. While rat liver arginase is induced by glucocorticoids via the binding of the transcription factor CCAAT/enhancer binding protein β (C/EBPβ) to the arginase I enhancer (Gotoh *et al.*, 1997b), both the LPS-induced up-regulation of rat alveolar macrophage arginase (Klasen *et al.*, 2001) as well as the IL-4/IL-13-mediated up-regulation of rat airway fibroblast arginase (Lindemann and Racke, 2003) are inhibited by dexamethasone. The constitutive expression of arginase I in human polymorphonuclear granulocytes (PMN) is not modulated *in vitro* by a variety of pro- and anti-inflammatory stimuli including glucocorticoids (Munder *et al.*, 2005).

Several groups have addressed the intracellular signalling mechanisms responsible for arginase induction in murine cells. Th2-mediated induction of arginase I is regulated by the coordinated action of the transcription factors PU.1, signal transducer and activator of transcription (STAT)-6 and C/EBPβ (Gray *et al.*, 2005) at an enhancer 3 kilobases (kb) upstream of the basal promotor (Pauleau *et al.*, 2004). The cAMP-mediated induction of murine arginase I (Corraliza *et al.*, 1997; Morris *et al.*, 1998) is mediated by Protein Kinase A Type I (PKAI) (and not PKA-II or EPAC) involving histone deacetylation (Haffner *et al.*, 2008), while arginase I induction by modified lipoproteins is mediated via Peroxisome Proliferator-Activated Receptor (PPARγ and PPARδ) (Gallardo-Soler *et al.*, 2008). The Src-homology 2 (SH2)-containing inositol-5′-phosphatase SHIP1 restrains the inducibility of murine macrophage arginase I as macrophages are skewed towards an M2 phenotype with high levels of arginase I in $SHIP1^{-/-}$ mice (Rauh *et al.*, 2004).

The role of myeloid cell arginase in disease models

Based on the regulatory principles (see above) and the different metabolic pathways associated with up-regulated immune cell arginase, it was hypothesized that the enzyme might causally be involved in disease pathogenesis because of (i) suppression of NO-mediated cytotoxicity via L-arginine consumption, (ii) enhanced collagen synthesis and fibrosis via proline generation and (iii) enhancement of cellular proliferation via polyamine generation. Consecutively, arginase expression was analysed in various disease models. *In vivo*, the enzyme was demonstrated in murine inflammatory cell infiltrates in experimental glomerulonephritis (Waddington *et al.*, 1998), schistosomiasis (Hesse *et al.*, 2001), trypanosomiasis (Gobert *et al.*, 2000; Duleu *et al.*, 2004), leishmaniasis (Iniesta *et al.*, 2001; Kropf *et al.*, 2005), autoimmune encephalomyelitis (Xu *et al.*, 2003), asthma (Meurs *et al.*, 2002; Zimmermann *et al.*, 2003; Maarsingh *et al.*, 2006), several viral (Mistry *et al.*, 2001; Wang *et al.*, 2005) and bacterial (Gobert *et al.*, 2002; Bussiere *et al.*, 2005) infections, lung fibrosis (Liu *et al.*, 2005), sepsis (Carraway *et al.*, 1998), trauma (Makarenkova *et al.*, 2006) and tumours (Chang *et al.*, 2001; Bronte *et al.*, 2003; Liu *et al.*, 2003; Kusmartsev *et al.*, 2004; Rodriguez *et al.*, 2004; Sinha *et al.*, 2005). The reciprocal regulation of iNOS and arginase that was found *in vitro* is replicated in various inflammatory pathologies *in vivo*. In murine disease models that follow the Th1/Th2 paradigm with regard to disease susceptibility or resistance iNOS is induced in the context of a Th1-dominated resistant phenotype while macrophage arginase is up-regulated during Th2-mediated disease progression (e.g. Leishmaniasis; Iniesta *et al.*, 2001; Kropf *et al.* 2005). In chronic murine schistosomiasis, arginase is similarly induced in Th2 granuloma-associated macrophages and is critical for enhanced granuloma size and fibrosis (Hesse *et al.*, 2001). On the other hand, arginase I expressing alternatively activated macrophages protect the host during acute schistosomiasis by reducing massive Th1-mediated immunopathology and iNOS activity, as demonstrated by 100% mortality of mice with a macrophage/neutrophil – targeted deletion of the IL4Rα chain (i.e. absence of alternatively activated macrophages) during acute infection with *Schistosoma mansoni* (Herbert *et al.*, 2004). Tumour growth or rejection can also be dictated by the reciprocal expression of iNOS or arginase (Sinha *et al.*, 2005). Genes of arginine metabolism like cationic amino acid transporter (CAT)-1, arginase I and arginase II are highly up-regulated in the lung in different murine asthma models (Zimmermann *et al.*, 2003) and arginase has been linked pathogenetically with bronchial hyperreactivity by the depletion of L-arginine and consecutively reduced synthesis of the bronchodilatory agent NO (Meurs *et al.*, 2002; Maarsingh *et al.*, 2006). A pathogenetically oriented summary of arginase in different disease models is presented in Table 1.

In contrast to its involvement in host-detrimental immunopathology, myeloid cell arginase can also serve a crucial host-protective function by down-regulating excessive Th1-induced inflammation (Table 1). Finally, data have emerged recently which demonstrate a host-protective effect of macrophage arginase I by itself during infections. Infection with different nematodes leads to induction of Th2 immunity, intestinal smooth muscle hypercontractility, increased luminal fluid secretion and consecutive worm expulsion. Infection of mice with the nematodes *Heligmosomoides polygyrus* (Anthony *et al.*, 2006) or *Nippostrongylus brasiliensis* (Reese *et al.*, 2007; Zhao *et al.*, 2008) leads to the IL-4/IL-13/STAT6-mediated infiltration of alternatively activated (CD206+, arginase 1+, FIZZ1+, Ym1+) macrophages into the intestinal wall. Depletion of these alternatively activated macrophages with clodronate-liposomes or pharmacological inhibition of macrophage arginase with S-(2-boronoethyl)-l-cysteine (BEC) both inhibit helminth expulsion (Anthony *et al.*, 2006; Zhao *et al.*, 2008). The impaired nematode clearance from the intestinal lumen due to arginase inhibition is associated with abrogated smooth muscle hypercontractility during *N. brasiliensis* infection (Zhao *et al.*, 2008). Although the exact mechanism of control of intestinal smooth muscle contractility by macrophage-associated arginase is still unclear, it is reminiscent of the arginase-mediated airway hyperresponsiveness during asthmatic inflammation (Maarsingh *et al.*, 2006). Additionally, metabolic products downstream of arginase-induced ornithine might participate in the anti-helminth immune response.

A mouse model for arginase I deficiency was published (Iyer *et al.*, 2002), but as the animals die between postnatal days 10–14 it cannot be used to verify hypotheses on the role of arginase I in murine immunology. It is therefore of great interest that a model of macrophage/neutrophil-specific deletion of arginase I was published recently (El Kasmi *et al.*, 2008). Infection of this mouse with *Toxoplasma gondii or Mycobacterium tuberculosis* demonstrated decreased bacterial load, correlating with increased NO production, upon ablation of myeloid cell-specific arginase I (El Kasmi *et al.*, 2008). These data are compatible with the notion of an anti-inflammatory pathway utilized by invading pathogens to suppress anti-microbial, NO-based effector pathways. In contrast, murine viability in the context of sepsis models (by LPS challenge or *Streptococcus pneumoniae* inoculation) was unaltered in the context of macrophage arginase-deficiency (El Kasmi *et al.*, 2008).

While myeloid cell arginase I is induced in a variety of infectious diseases via a Th2 driven adaptive immune response (see above) it became clear that arginase I can also be directly up-regulated in macrophages via Pathogen-Associated Molecular Patterns (PAMP) so that the nature of the pathogen dictates the type of the evolving innate immune response. Chitin, a widespread polymer of N-acetyl-β-D-glucosamine and part of, for example, fungal cell walls and helminths, leads to arginase I expression and the acquisition of an alternative activation phenotype of resident murine macrophages during infection with *Nippostrongylus brasiliensis* (Reese *et al.*, 2007). The ensuing, leukotriene B4-mediated tissue infiltration of IL-4 expressing innate immune cells (eosinophils/basophils) then leads to the Th2-mediated, STAT6-dependent induction of chitinase-like proteins acidic mammalian chitinase (AMCase), Ym1 and Ym2, which are characteristic of alternatively activated macrophages. Interestingly, chitin degradation by AMCase is able to abrogate Th2 inflammatory cell infiltration so that a possible feedback inhibition of chitin-induced allergic or helminth-induced innate immune response by AMCase is likely (Reese *et al.*, 2007). Another pathogen with direct arginase-inducing potential is

Table 1 Examples of pathogenetic association of arginase and downstream metabolic consequences with different diseases

Disease model	*Mechanism*	*Species*	*Reference*
Depletion of arginine/reduced synthesis of nitric oxide			
Allergen-induced hyperresponsiveness	Deficiency of cNOS/nNOS-derived NO	gp	Meurs *et al.*, 2002; Maarsingh *et al.*, 2006
Trypanosoma brucei brucei infection	Reduced synthesis of NO due to high arginase activity in macrophages	m	Gobert *et al.*, 2000; Duleu *et al.*, 2004
Leishmania mexicana infection	Parasite-encoded arginase: Suppression of macrophage microbicidal activity by reducing NO production via arginine depletion)	m	Gaur *et al.*, 2007
Psoriasis	Arginase overexpression in psoriatic epidermis – reduction of anti-proliferative NO	hu	Bruch-Gerharz *et al.*, 2003
Apoptosis	Induction of arginase II in macrophages upon phagocytosis of apoptotic cells	m	Johann *et al.*, 2007
Cystic fibrosis	Sputum arginase activity inversely correlated with exhaled NO	hu	Grasemann *et al.*, 2005
Toxoplasma gondii infection	Stat6-dependent macrophage arginase induction: depletion of L-arginine	m	Rutschman *et al.*, 2001; El Kasmi *et al.*, 2008
Mycobacterium tuberculosis infection	TLR-mediated induction of macrophage arginase I: L-arginine substrate competition	m	El Kasmi *et al.*, 2008
Synthesis of collagen via proline			
Schistosomiasis	Th2-mediated induction of arginase I	m	Hesse *et al.*, 2001
Lung allograft fibrosis	TGF-β-mediated induction of arginase; inhibition of arginase = reduction of fibrosis	r	Liu *et al.*, 2005
Synthesis of polyamines			
Helicobacter pylori (H.p.) infection	Inhibition of iNOS translation = NO production via spermine (generated via arginase-ODC pathway) – persistence of H.p.	m	Bussiere *et al.*, 2005
Helicobacter pylori infection	Induction of macrophage apoptosis via arginase-ODC-pathway: spermidine/spermine synthesis	m	Gobert *et al.*, 2002
Leishmania infantum/major infection	N$^{\omega}$-hydroxy-L-arginine: inhibition of arginase = infection control	m	Iniesta *et al.*, 2001; Kropf *et al.*, 2005; Muller *et al.*, 2008
Colitis	Protective role of arginase within the intestinal inflammatory milieu	m	Gobert *et al.*, 2004
Breast adenocarcinoma	Macrophage arginase: providing polyamines for growth of tumour cells	hu	Chang *et al.*, 2001
Inhibition of protein synthesis due to arginine depletion			
Pseudorabies infection	Reduced synthesis of viral structural proteins and folding	m	Wang *et al.*, 2005
Inhibition of T cell activation via arginine depletion			
Tumours	Tumour progress: correlation with TCRζ down-regulation	m/hu	Zea *et al.*, 2005; Rodriguez *et al.*, 2004; Sinha *et al.*, 2005
Colon carcinoma/fibrosarcoma	L-arginine limitation → ROS production via NOS reductase domain → Suppression of T cell activation	m	Bronte *et al.*, 2003; Kusmartsev *et al.*, 2004
Pregnancy	correlation with TCRζ down-regulation	hu	Kropf *et al.*, 2007
Helicobacter pylori infection	*H. pylori* arginase: down-regulation of T cell TCRζ	hu	Zabaleta *et al.*, 2004
Hepatitis B	Inhibition of CD8 T cell proliferation and IL-2 production	hu	Das *et al.*, 2008
Trauma	correlation with TCRζ down-regulation	m	Makarenkova *et al.*, 2006
Host protection in infectious disease			
Acute *Schistosoma mansoni* infection	Down-regulation of detrimental Th1 inflammatory response	m	Herbert *et al.*, 2004
Heligmosomoides polygyrus infection	Intestinal Worm expulsion	m	Anthony *et al.*, 2006
Nippostrongylus brasiliensis infection	Intestinal smooth muscle hypercontractility: worm expulsion	m	Zhao *et al.*, 2008

CD, cluster of differentiation; gp, guinea pig; hu, human; IL, interleukin; iNOS, inducible nitric oxide synthase; m, murine; NO, nitric oxide; ODC, ornithine decarboxylase; r, rat; ROS, reactive oxygen species; TCR, T cell receptor; TGF, transforming growth factor; Th, T helper; TLR, toll like receptor.

Mycobacterium tuberculosis, which induces arginase I in murine macrophages through the toll like receptor (TLR) – MyD88 pathway. This induction is independent of STAT6 but involves the up-regulation of C/EBPβ and binding of this transcription factor to the C/EBPβ site within the upstream enhancer of the murine arginase I gene (Pauleau *et al.*, 2004).

The analysis of the role of myeloid cell-associated arginase during infection is further complicated by the fact that various pathogens express an enzymatically active arginase themselves (McGee *et al.*, 2004; Viator *et al.*, 2008). In parallel to the myeloid cell-expressed arginase of the host, the pathogen-encoded arginase might contribute, for example, to the down-regulation of T lymphocyte functions in the context of *Helicobacter pylori* infection (Zabaleta *et al.*, 2004) or to the down-regulation of macrophage microbicidal activity by reducing NO production via L-arginine depletion in *Leishmania mexicana* infection (Gaur *et al.*, 2007). Another critical component in the system of arginase-mediated L-arginine metabolism clearly is the capacity and regulation of L-arginine transport via the cell membrane. The transport system y+ is selective for the transport of cationic amino acids like L-arginine (Closs *et al.*, 2004). Murine macrophages express CAT-1 constitutively and up-regulate CAT-2 upon classical (IFN-γ) or alternative (IL-4, IL-10) activation (Louis *et al.*, 1999; Rodriguez *et al.*, 2003; Yeramian *et al.*, 2006) so that enhanced catabolism via induced iNOS or arginase,

respectively, is met by coordinated increased cellular uptake capacity. On the other hand, murine infections with the helminth parasite *Schistosoma mansoni* or the protozoan pathogen *Toxoplasma gondii* are exacerbated in the absence of CAT-2, demonstrating a crucial regulatory role of L-arginine membrane transport for immune responses (Thompson *et al.*, 2008). The expression and regulation of L-arginine transport systems in cells of the human immune system is largely unresolved so far.

Arginase in the human immune system

In humans, arginase was detected in the peripheral blood mononuclear cell (PBMC) fraction after injury (Ochoa *et al.*, 2001), inflammatory synovial fluid macrophages (due to arginase II) of patients with arthritis (Corraliza and Moncada, 2002), inflammatory cells of bronchoalveolar lavage fluid of asthmatic patients (Zimmermann *et al.*, 2003), psoriatic lesions (Bruch-Gerharz *et al.*, 2003), in activated monocytes of patients with autoimmune diseases (Rouzaut *et al.*, 1999) and in the PBMC fraction of patients with active pulmonary tuberculosis (Zea *et al.*, 2006). We have shown that among peripheral circulating human leukocytes of normal blood donors only PMN express arginase (Munder *et al.*, 2005). By biochemical fractionation and immunoelectron microscopy we demonstrated that the enzyme is constitutively present in azurophil granules of human PMN, where it constitutes a novel oxygen-independent anti-microbial defense mechanism (Munder *et al.*, 2005). After fusion of azurophil granules with a phagosome, arginase is present in the phagosome and likely depletes the intraphagosomal microenvironment of L-arginine during phagocytosis of pathogenic microorganisms which enhances the fungicidal activity of human PMN. Interestingly, *Saccharomyces cerevisiae* and *Candida albicans* up-regulate genes of their endogenous L-arginine biosynthetic pathways upon phagocytosis by human neutrophils (Rubin-Bejerano *et al.*, 2003). This transcriptional response likely reflects the L-arginine-deprived intraphagosomal micromilieu of PMN and is not detectable upon phagocytosis by human monocytes (Rubin-Bejerano *et al.*, 2003) which do not express arginase (Munder *et al.*, 2005). Another study confirmed the expression of arginase I in human PMN but localized the enzyme to the gelatinase granules (Jacobsen *et al.*, 2007). The discrepancy in results is still unclear at the moment. *In vitro*, constitutive human PMN arginase activity was not modulated by a variety of pro- and anti-inflammatory stimuli, including cytokines that typically lead to arginase induction in murine myeloid cells (Munder *et al.*, 2005). In contrast, arginase is inducible in a variety of other human cell types like, for example, endothelium, epithelial cells and smooth muscle. Arginase I shares this feature with other important constitutive PMN proteins or peptides involved in inflammation and microbial defense like human cationic anti-microbial protein of 18 kDa (hCAP18), neutrophil gelatinase-associated lipocalin (NGAL), bactericidal/permeability-increasing protein (BPI) and the defensins (Borregaard *et al.*, 2007). The fundamental disrepancies of arginase expression and regulation between murine and human immune cells fit into a growing list of differences in the immune systems of both species (Mestas and Hughes, 2004). This must be kept in mind when data from animal models are extrapolated to the human situation. Also, data on arginase expression in the human PBMC fraction without further purification need to be interpreted with caution. It remains to be analysed if arginase protein and activity is really induced in monocytes within the PBMC fraction. Alternatively, activated PMN are known to aberrantly co-purify within the PBMC fraction of patients with tumours or inflammation (Schmielau and Finn, 2001), so that *de novo* arginase activity in the PBMC population under conditions of inflammation might actually be confined to the neutrophil subset (M. Munder, unpublished).

Arginase: an endogenous immunosuppressive pathway

Inflammation is often associated with immunosupression locally within the inflammatory micromilieu as well as systemically (Nathan, 2002). While inflammation-induced immunosuppression has likely evolved as a homeostatic mechanism to prevent excessive tissue destruction during inflammation (Baniyash, 2004) it might be detrimental in situations of infection- and tumour-associated inflammation by impeding the clearance of the relevant microorganism or inhibiting tumour cytotoxicity. During the last couple of years, arginase expression and L-arginine depletion have emerged as a powerful immunosuppressive pathway of the mammalian immune system (Bronte and Zanovello 2005b). Several years ago, Ochoa *et al.* analysed the influence of L-arginine deficiency on the function of human T lymphocytes and found a down-regulation of the T cell receptor (TCR) ζ chain, a critical signaling element of the TCR, as a possible mechanism for the impaired T cell function under conditions of L-arginine depletion (Rodriguez *et al.*, 2002). Also, an arrest of T cells in the G0-G1 phase of the cell cycle, associated with the absence of up-regulated cyclin D3 and cyclin-dependent kinase 4 (cdk4) was seen upon L-arginine depletion (Rodriguez *et al.*, 2007). Murine macrophages express arginase after Th2 stimulation (Munder *et al.*, 1998) and this also leads to depletion of extracellular L-arginine and consecutive down-regulation of the TCRζ chain in activated T cells (Rodriguez *et al.*, 2003). T cell hyporesponsiveness associated with down-regulated TCRζ chain can be the consequence of various circumstances (Baniyash, 2004) and transcriptional (Tsokos *et al.*, 2003), posttranscriptional (Rodriguez *et al.*, 2002) or posttranslational (Bronstein-Sitton *et al.*, 2003) mechanisms of TCRζ down-regulation have been described. In humans, immunosuppression in association with T cells that have partially down-regulated their TCRζ chain is a recurrent finding in patients with cancer, autoimmunity or chronic infections (Baniyash, 2004). The mechanism(s) and cells that induce the observed T cell phenotype and/or the associated immunosuppression *in vivo* are largely unknown.

As intracellular constituents are liberated from dying PMN and accumulate in the micromilieu we hypothesized that human T cell activation should be blunted in the case of

arginase liberation from human PMN. In fact, of all naturally occuring amino acids only L-arginine is depleted (and L-ornithine and urea synthesized) within the extracellular micromilieu of dying PMN (Munder *et al.*, 2006). Within such an L-arginine-depleted milieu human T lymphocytes remained viable, but stopped proliferation and secretion of cytokines, while TCR activation-induced transcription of cytokine genes remained intact. Interestingly, human purulent exudate contains extraordinarily high arginase activities and liberated PMN arginase I fully accounts for the profound T cell suppressive properties of human pus by L-arginine depletion (Munder *et al.*, 2006). Arginase-mediated T cell hyporesponsiveness is also involved in the suppression of the maternal anti-fetal immune reponse. A successful pregnancy depends on largely unknown mechanisms by which the immune system of the mother is made tolerant to the semi-allogeneic fetus. Myometrial arginase activity in the vicinity of the placental implantation site is >25 higher than in myometrium from non-pregnant guinea pigs (Weiner *et al.*, 1996). In humans, arginase activity is also highly up-regulated in term placenta and increased in the peripheral blood of pregnant women (Kropf *et al.*, 2007). While placental arginase might be important in the supply of polyamines, the local depletion of L-arginine via PMN-expressed arginase clearly dampens invading T lymphocytes (Kropf *et al.*, 2007), possibly by down-regulating their TCRζ chain. Arginase I is also expressed constitutively in human erythrocytes (Kim *et al.*, 2002) and is liberated into the extracellular milieu upon hemolysis. It was recently shown that the immune suppression associated with transfusion of packed red blood cells might be due to the liberation of arginase from erythrocytes during storage and consecutive systemic L-arginine hydrolysis upon transfusion (Bernard *et al.*, 2008).

How does L-arginine depletion translate into T cell suppression? The regulation of gene expression by amino acid availability is a fundamental regulatory mechanism in lower eukaryotes. In yeast, amino acid deprivation induces the accumulation of uncharged tRNA which leads to the phosphorylation of the α-subunit of eukaryotic Initiation Factor 2 (eIF2α). As a consequence, the synthesis of the 43S pre-initiation complex (Met-tRNA, GTP, eIF2) is impaired and protein translation inhibited (Hinnebusch, 1994). In contrast, translation of the transcription factor GCN4 is augmented which results in the induction of more than 30 genes in multiple biosynthetic pathways. Amino acid deprivation therefore does not globally repress protein translation but specifically interferes with selected cellular activation programs via a general control response (Fafournoux *et al.*, 2000). Mammalian cells express multiple eIF2α kinases (e.g. GCN2 kinase) which are activated upon different types of cellular stress. Furthermore, various transcription factors (ATF-2, ATF-4, CHOP) are up-regulated and the mTOR-p70S6Kinase pathway is blunted in different mammalian cell types upon amino acid withdrawal (Rohde *et al.*, 2001; Averous *et al.*, 2004). This amino acid depletion finally inhibits the progression of various mammalian cell types through the cell cycle. The specificity of the response to amino acid withdrawal is at least partially mediated via amino acid response elements (AARE) in the promotor region of certain transcription factors (Averous *et al.*, 2004). In murine macrophages the induction of arginase I via IL-13 leads to intra- and extracellular depletion of L-arginine and this inhibits the translation of inducible nitric oxide synthase (iNOS). This inhibitory effect seems to be specific as total protein synthesis of the cells is unaltered and, for example, the synthesis of the proinflammatory cytokine TNF-α is unimpaired (El-Gayar *et al.*, 2003). Translation of iNOS is also inhibited in astrocytes by L-arginine depletion via activation of GCN2K and increased eIF2α-phosphorylation (Lee *et al.*, 2003). The induction of amino acid transport proteins (e.g. CAT-1, -2) upon intracellular amino acid depletion is a likely compensatory mechanism (Aulak *et al.*, 1999). In murine T lymphocytes it was demonstrated that the suppression of T cell proliferation upon depletion of L-tryptophan via indoleamine dioxygenase (IDO) is mediated through the activation of GCN2 Kinase with consecutive induction of the transcription factor CHOP (Munn *et al.*, 2005). It is unclear so far, if human T lymphocytes also use this ancient nutrient sensing system to regulate their activation program. No published data are available on L-arginine depletion and the GCN2K-eIF2α-system in human T cells in particular and on the protein expression profile of human T cells upon L-arginine depletion in general. Alternatively, mammalian cellular responses to amino acid deficiency might be regulated by micro-RNA binding. Human hepatocarcinoma cells up-regulate CAT-1 upon extracellular L-arginine depletion. This is mediated by replacement of CAT-1 mRNA-associated microRNA miR-122 and consecutive relief from translational inhibition (Bhattacharyya *et al.*, 2006).

Arginase and cancer

Research over the last couple of years has convincingly demonstrated a crucial role for arginase in tumour immunobiology (Sica and Bronte, 2007; Rodriguez and Ochoa, 2008). Earlier reports focused on the expression of arginase in murine or human primary cancer tissue as well as malignant cell lines (Wu *et al.*, 1996; Mumenthaler *et al.*, 2008) and emphasized its potential role in the promotion of tumour growth via polyamine synthesis or down-regulation of NO-mediated tumour cytotoxicity. It also became clear that malignant tumours have evolved strategies to evade an effective tumour-cytotoxic immune response by inducing pathways of inflammation-associated immunosuppression (Smyth *et al.*, 2001; Rabinovich *et al.* 2007; Sica and Bronte, 2007). The fate of a developing tumour is dictated not only by the properties of the malignant cells but also by the phenotype of tumour-infiltrating and tumour-interacting myeloid cells (Lewis and Pollard, 2006). Leukocyte-tumour interaction can result in tumour destruction as well as promotion of tumour growth, tissue invasion or metastasis (Lewis and Pollard, 2006). A key mechanism of tumour evasion from immune-mediated destruction is the induced impairment of T cell functions (Kershaw *et al.*, 2005). Alternatively, tumour progression can be enhanced by infiltrating CD4+ T cells and a reduced carcinogenesis interestingly correlated with decreased infiltration of neutrophils (Daniel *et al.*, 2003) recapitulating an earlier report on the tumour growth-promoting potential of PMN (Pekarek *et al.*, 1995).

In tumour-bearing mice a heterogenous mixture of myeloid cells expands at various stages of development. This population is characterized by expression of CD11b and Gr-1 and efficiently suppresses T cell immune functions (Serafini *et al.*, 2006a; Rodriguez and Ochoa, 2008). Murine MDSC clearly comprise monocyte-like cells as well as PMN-like cells at different stages of maturation (Movahedi *et al.*, 2008). MDSC can suppress T cell immune functions by constitutive expression of arginase with consecutive L-arginine depletion (Bronte *et al.*, 2003; Liu *et al.*, 2003; Kusmartsev *et al.*, 2004; Rodriguez *et al.*, 2004; Sinha *et al.*, 2005; Gallina *et al.*, 2006; Serafini *et al.*, 2006b). The immunosuppressive function of murine MDSC is enhanced further by IL-4-mediated increased expression of arginase (Bronte *et al.*, 2003). When arginase of tumour-associated MDSC (Rodriguez *et al.*, 2004; De Santo *et al.*, 2005; Sinha *et al.*, 2005) or tumour-infiltrating Gr-1^{-} mature myeloid cells (Rodriguez *et al.*, 2004) is inhibited in various murine tumour models, T cell functions are restored and tumour growth is inhibited. Additionally, murine MDSC can suppress T cells also via production of reactive nitrogen (Bronte *et al.*, 2003) or oxygen (Bronte *et al.*, 2003; Kusmartsev *et al.*, 2004) intermediates or by cooperation of the different pathways, for example, by producing peroxynitrite from O_2^{-} and NO under conditions of L-arginine limitation (Bronte *et al.*, 2003; Gallina *et al.* 2006; Sica and Bronte, 2007).

Human arginase-expressing myeloid suppressor cells are CD13^{+}, CD33^{+}, HLA-DR$^{low/-}$ cells, that variably express monocyte (CD14) or granulocyte markers (CD15). Cells with this phenotype were described in patients with renal cell carcinoma (Zea *et al.*, 2005) and the suppressive cells resemble PMN morphologically and by surface marker profile (CD11b^{+}, CD14^{-}, CD15^{+}) although they appear in the PBMC fraction within the monocyte gate. They express arginase and down-regulate the TCRζ chain in tumour-infiltrating T cells via L-arginine depletion. Depletion of the myeloid suppressor cells re-establishes T cell receptor- and costimulation-induced T cell activation (proliferation, IFN-γ secretion and TCRζ chain expression) in cell culture experiments (Zea *et al.*, 2005). Arginase was also shown to participate in the suppression of tumour-infiltrating lymphocytes in patients with prostate carcinoma (Bronte *et al.*, 2005a), non small cell lung carcinoma (Rodriguez *et al.*, 2004) and multiple myeloma (Serafini *et al.*, 2006b).

Pharmacological modulation of L-arginine metabolism

Pharmacological strategies that interfere with L-arginine metabolism hold great promise for the treatment of inflammatory diseases and cancer. Two opposing treatment options seem reasonable depending on the type of inflammation and tumour:

(A) Arginase-mediated L-arginine depletion can lead to direct tumour cell death (Cavanaugh and Nicolson, 2000; Philip *et al.*, 2003) so that recombinant L-arginine degrading enzymes (arginase, arginine deiminase) have been suggested as anti-tumour agents (Philip *et al.*, 2003; Cheng *et al.*, 2007), analogous to the clinically employed anti-leukemia agent L-asparaginase (Figure 3). Pegylated arginine deiminase (PEG-ADI) depleted L-arginine (by producing L-citrulline) and demonstrated clinical activity

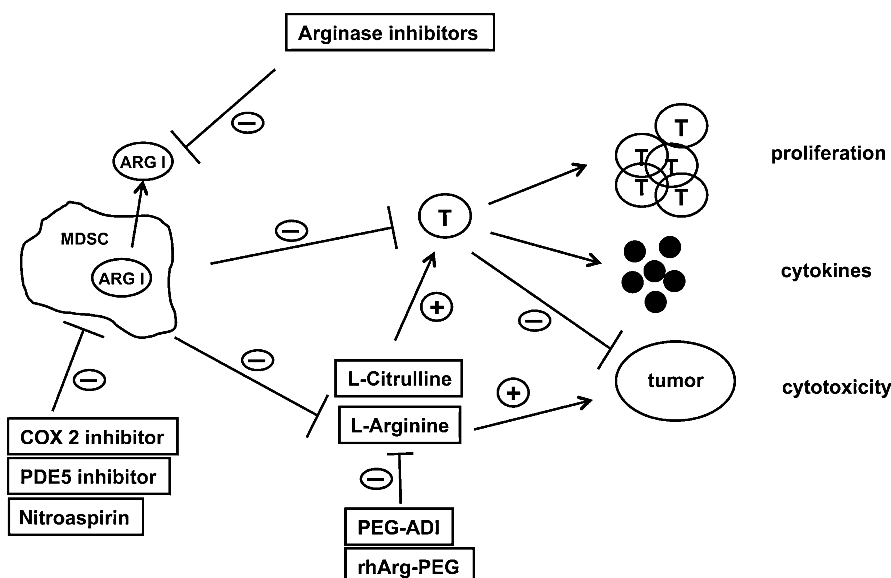

Figure 3 Opposing roles of L-arginine in tumour biology. L-arginine can directly support tumour growth and myeloidcell arginase-mediated or pharmacological depletion (PEG-ADI/rhArg-PEG) of the amino acid inhibits tumour growth. L-arginine deficiency, in contrast, can also suppress anti-tumour immune responses by down-regulating key functions of T lymphocytes. Pharmacological interference with arginase-mediated L-arginine depletion is demonstrated here in the context of myeloid-derived suppressor cell (MDSC)-associated immune suppression. T cell functions are restored by direct inhibition of cell-bound or liberated arginase I by arginase inhibitors, by supplementation of L-arginine or L-citrulline (for regeneration of L-arginine via the L-citrulline-L-arginine cycle) and by inhibition of various arginase-inducing mechanisms in MDSC. ARG I, arginase I; COX 2, cyclooxygenase 2; PDE5, phosphodiesterase 5; PEG-ADI, pegylated arginine deiminase; rhArg-PEG, recombinant human pegylated arginase; T, T lymphocyte.

in patients with malignant melanoma (Ascierto *et al.*, 2005). PEG-ADI-mediated L-arginine depletion induces autophagy and caspase-independent apoptosis in human prostate cancer cells (Kim *et al.*, 2009) and inhibits growth of human pancreatic cancer xenografts in mice (Bowles *et al.*, 2008). Interestingly, tumour susceptibility to L-arginine deprivation was associated with lack of tumour-expressed ASS as a possible L-citrulline-L-arginine recycling pathway for the tumour cells (Bowles *et al.*, 2008). Pegylated recombinant human arginase (rhArg-PEG) demonstrated anti-tumour activity in ASS-expressing hepatocellular carcinoma (Cheng *et al.*, 2007) as, in contrast to the L-citrulline generating enzyme ADI, arginase hydrolyses L-arginine to L-ornithine. Autoimmunity and unwanted inflammatory reactions might also be treated with approaches to lower local or systemic L-arginine concentrations. Clearly, problems of L-citrulline-to-L-arginine recycling and tolerability of low L-arginine concentrations over extended periods of time need to be addressed before such treatment options can be successfully implemented into clinical practice.

(B) While L-arginine depletion is a potential direct anti-tumour mechanism against certain malignant entities, the reverse scenario holds true for other tumour types due to the tumour-induced arginase-mediated immune dysfunction (as outlined above in section 'Arginase and Cancer'). It clearly depends on the tumour type, biochemical signature of the tumour cells (e.g. ASS expression) and the inflammatory context within the tumour micromilieu if L-arginine inhibits or suppresses tumour growth within the complex situation of cancer *in vivo*. Arginase-mediated L-arginine depletion might be adressed pharmacologically by either L-arginine supplementation or by inhibition of the enzyme. While pronounced local depletion of L-arginine in the inflammatory microenvironment of different immunopathologies is evident, L-arginine concentration in the systemic circulation can also be reduced due to liberated arginase. The normal concentrations of L-arginine in human plasma are 60–140 μM (Schwedhelm *et al.*, 2008). Reduced plasma levels of L-arginine and elevated plasma arginase were reproducibly described in patients after surgery, trauma or during sepsis (Luiking *et al.*, 2004). Various animal studies or small clinical trials and several randomized, placebo-controlled patient trials have analysed the influence of oral or parenteral L-arginine supplementation on different parameters of the immune system. In summary, no clear-cut reproducible advantage regarding immune functions or clinical outcome emerged so far (Nieves and Langkamp-Henken, 2002). More promising results were seen in trials to improve vascular physiology that was impaired in various diseases associated with L-arginine deficiency and impaired NO bioavailability, like, for example, in patients with pulmonary hypertension due to hemolysis and liberation of erythrocyte arginase in sickle cell disease (Morris *et al.*, 2003) or thalassemia (Morris *et al.*, 2005). In a recently published clinical trial, L-arginine supplementation could increase L-arginine plasma concentrations and improve endothelial NO bioavailability in patients with falciparum malaria and endothelial dysfunction due to hypoargininemia secondary to hemolysis-associated erythrocyte arginase release (Yeo *et al.*, 2007). While L-arginine is extensively metabolized by arginases in gut and liver (Nieves and Langkamp-Henken, 2002) and also possibly by liberated inflammation-associated arginase (Munder *et al.*, 2005), no significant intestinal degradation of L-citrulline takes place and its supplementation was already tested as alternative to L-arginine (Curis *et al.*, 2005). Physiologically, L-citrulline is derived in the intestine from L-glutamine through the L-glutamate-L-ornithine pathway. Normally, the kidneys take up ca. 80% of intestinally released L-citrulline, which contributes about 10–15% of whole body L-arginine synthesis. L-arginine can be recycled from L-citrulline via the enzymes ASS and ASL (Morris, 2002), both enzymes colocalize in various tissues and are inducible by a variety of inflammatory stimuli. L-Citrulline can be taken up and partially recycled to L-arginine in J774 murine macrophages upon activation with IFN-γ and LPS, but this mechanism is unable to sustain maximal nitric oxide production (Baydoun *et al.*, 1994). In LPS-stimulated rat alveolar macrophages L-arginine is metabolized to L-citrulline (presumably via nitric oxide synthase) and L-ornithine (presumably via arginase), whereas no metabolism of exogenously applied L-citrulline to L-arginine takes place despite induction of ASS (Hammermann *et al.*, 1998). ASS is also expressed constitutively in murine bone marrow-derived macrophages (Hofmann *et al.*, 1978) and inducibly in the RAW264.7 macrophage cell line (Nussler *et al.*, 1994). The induction of ASS was also demonstrated in the immortalized human Jurkat T lymphocyte line (Bansal *et al.*, 2004) suggesting a potential metabolic bypass for T cells under conditions of L-arginine limitation. The oral administration of L-citrulline significantly increased L-arginine plasma concentrations without relevant side effects in a double-blind, placebo-controlled study (Schwedhelm *et al.*, 2008).

The development of arginase inhibitors for clinical use is of prime importance in light of all the accumulated data on the role of arginase in tumour-associated MDSC and its pathogenetic role in inflammation-induced immunosuppression. Several specific arginase inhibitors have already been developed and tested *in vitro*. The intermediate of NO-synthesis, N^G-hydroxy-L-arginine (NOHA) is a well-known arginase inhibitor (Hecker *et al.*, 1995). It was successfully employed, for example, in human prostate carcinoma organ cultures, inhibited arginase activity and restored reactivity of tumour-infiltrating lymphocytes in cooperation with a NOS inhibitor (Bronte *et al.*, 2005a). The L-arginine derivative N^ω-hydroxy-nor-L-arginine (nor-NOHA) was, for example, able to completely reverse PMN-mediated T cell suppression in purulent inflammation (Munder *et al.*, 2006) and to restore airway responsiveness in an arginase-mediated asthma animal model (Maarsingh *et al.*, 2006). The boronic acids 2(S)-amino-6-boronohexanoic acid (ABH) and BEC are potent inhibitors of both arginase isoforms at physiologic pH, binding with much higher affinity than the natural substrate (K_M L-arginine 80.000 nM; K_d ABH 5 nM; K_d BEC 270 nM) (Ash, 2004; Chris-

tianson, 2005). Human arginase I was cristallized in association with both inhibitors and new insights into the catalytic mechanism have been gained (Di Costanzo *et al.*, 2005). Arginase inhibitors can potentially interfere with the urea cycle in the liver causing hyperammonemia and associated clinical problems. Another approach is therefore to inhibit the induction of iNOS and arginase in tumour-promoting MDSC. The NO-liberating compound nitroaspirin (NCX 4016) inhibits iNOS via NO feedback and arginase most likely indirectly by interfering with the arginase inducing pathways (De Santo *et al.*, 2005). Nitroaspirin corrected the MDSC-mediated T cell immune dysfunction and thereby restrained tumour growth of a murine colon carcinoma (De Santo *et al.*, 2005). Alternatively, inhibition of cyclooxygenase-2 (expressed by murine 3LL lung carcinoma) and prevention of Prostaglandin E_2-mediated arginase induction in MDSC led to effective tumour control (Rodriguez *et al.*, 2005). In a similar approach, phosphodiesterase-5 inhibitors were shown to down-regulate arginase I in murine MDSC and this led to an increased spontaneous anti-tumour response as well as to a more efficient adoptive T cell therapy (Serafini *et al.*, 2006b). Finally, molecular interference by inhibition of nuclear factor kappaB (NF-κB) signaling in arginase I-expressing tumour-associated macrophages transformed them into tumour-cytotoxic effector cells (Hagemann *et al.*, 2008).

In summary, arginase has emerged as a key player in the mammalian immune system and the enzyme is involved in various aspects of inflammation. Pharmacological interference with arginase specifically and L-arginine metabolism in general (Figure 3) therefore holds great promise for the treatment of cancer, autoimmunity and unwanted immunosuppression in clinical medicine.

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Conflict of interest

The author states no conflict of interest.

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