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Arginase AI Is Upregulated in Acute Immune Complex-Induced Inflammation

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Previous studies have shown high arginase activity at inflammatory sites. Arginase converts L-arginine to L-ornithine, sharing a common substrate with nitric oxide synthase. It exists as two isoforms, AI and AII. While the function of liver arginase (AI) in ureagenesis has been defined, the role and isoform of arginase in cells without a complete urea cycle are unknown. We therefore determined arginase isoform mRNA expression in glomerular acute immune complex inflammation, and its cultured constituent cells. AI was induced in nephritic glomeruli, and in mesangial cells stimulated with IL-4 and cAMP, and was present in elicited neutrophils and macrophages. AII was constitutively expressed. Our data strongly suggest that AI, thought to be restricted to the liver, accounts for high arginase activity at inflammatory sites where it may limit high output nitric oxide production and generate polyamines and proline essential for cell proliferation and matrix production. This identification of AI in inflamed tissue is an important step for understanding the consequences of increased arginase activity.

Key Words: **arginase; glomerulonephritis; nitric oxide; mesangial cells; neutrophils.**

The enzymes arginase and inducible nitric oxide synthase (iNOS) act on a common substrate, L-arginine. Arginase is present in the liver where its role in the urea cycle has been defined. At inflammatory sites it is highly expressed in macrophages, and possibly other cell types, which lack a complete urea cycle [1], and here its role is unknown. The persistence of arginase relative to iNOS at sites of inflammation and the fact that it is induced by anti-inflammatory molecules such as TGFβ [2] and IL-10 [3] have led to the proposal that it contributes to wound repair by inhibiting nitric oxide production [4] and increasing proline and polyamine synthesis [5].

It has recently been established that there are two arginase isoforms, AI and AII [6-10]. There is very little information regarding the regulation of these isoforms and their expression during inflammation. AI accounts for almost all hepatic arginase activity and is expressed at much lower levels elsewhere [10,11]. AII is more highly expressed outside the liver, particularly in the kidney and small intestine [9,10].

The deposition of immune complexes in tissues causes inflammation, tissue destruction, and inappropriate repair responses leading to scarring. One example of this reaction is glomerulonephritis, where acute glomerular inflammation is followed by scarring and loss of renal function.

We have previously reported that arginase activity is upregulated in inflamed glomeruli in a model of immune complex injury associated with macrophage infiltration (accelerated nephrotoxic nephritis) [12]. Candidate cell types responsible for this activity include the resident population of mesangial cells, and macrophages which infiltrate the glomerulus during inflammation, both of which express arginase activity [13]. More recently we have shown that arginase activity is modulated in glomeruli, mesangial cells, and macrophages, by endogenously-produced compounds known to modulate inflammation [14]. In this study we also reported preliminary data showing glomerular induction of AI mRNA in macrophage-associated glomerulonephritis.

Apart from this study, there is only one report of the expression of AI mRNA [15], and no studies on the differential distribution of the two isoforms, in inflamed tissue. To increase understanding of the possible roles of arginase in inflammation we have now studied the differential expression and regulation of AI and AII in immune complex glomerulonephritis and the cells involved in this reaction (mesangial cells and leu-

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kocytes). We chose to study heterologous phase nephrotoxic nephritis, for injury in this model has been previously defined as a neutrophil-dependent form of acute inflammation.

MATERIALS AND METHODS

Animals and chemicals. Male Lewis rats bred at Imperial College School of Medicine at St. Mary's were used for all experiments. All reagents were obtained from Sigma-Aldrich Company Ltd. (Dorset, UK) unless otherwise stated.

Mesangial cells. Mesangial cells were cultured and characterised as previously described [16]. Confluent cultures were incubated in 22 mm plastic wells in 0.5 ml RPMI supplemented with 1 mg/ml BSA, 100 U/ml penicillin in and 100 mg/ml streptomycin. Cells were used between passages 7 and 16 in four separate experiments. The results shown here are from one representative experiment.

Neutrophils and macrophages. To extract neutrophils, peritoneal lavage was performed with Hanks buffered salt solution without calcium or magnesium, 2 h after i.p injection of 10 ml 6% oyster glycogen. The cells were pelleted, washed, and purified by centrifugation through a Percoll gradient. The resulting cell preparation was 95% neutrophils. Thioglycollate-elicited peritoneal macrophages were obtained and cultured as previously described [17]. Cell pellets were added to RNAzol (Biogenesis, Poole, UK) and extraction performed as previously described [18].

Glomerulonephritis. Heterologous nephrotoxic nephritis was induced by rabbit anti-rat glomerular basement membrane (GBM) globulin. The preparation of this globulin has been previously described [16]. Rats were killed 2 h after i.v. 8 mg/kg anti-GBM globulin. Glomeruli were isolated from kidneys by differential sieving [16] and were snap-frozen for RNA extraction as previously described [18]. A portion of renal cortex was formalin-fixed and histology performed on paraffin-embedded 2 μm sections stained with haematoxylin and eosin. Neutrophil infiltration was quantitated in 20 glomeruli per section and expressed as the mean number per glomerulus.

Arginase activity. For arginase activity, glomeruli were incubated at 2,000/ml for 24 h at 37°C in 0.5 ml DMEM supplemented with L-[guanido-^{14}C]arginine, 300 μM L-NMMA, 4 mM L-glutamine, 1 mg/ml BSA and antibiotics. Arginase activity was measured in cultures by assaying the production of [^{14}C]urea from L-[guanido-^{14}C]arginine in culture supernatants over 24 h (extracellular arginase activity) as previously described [13]. Mesangial cells were incubated for 24 h at 37°C with L-[guanido-^{14}C]arginine as previously described [14]. Supernatant was taken for measurement of arginase activity. Data are presented as means ± SE for triplicate wells and comparisons were made using analysis of variance with Fisher's PLSD. The cell monolayer was scraped off into RNAzol for reverse transcription-polymerase chain reaction. Triplicate wells were cultured in the absence and presence of cholera toxin (1 mg/ml) and IL-4 (80 ng/ml). Recombinant rat IL-4 was produced as a cell culture supernatant from Chinese hamster ovary (CHO)-K1 cell line transfected with rat IL-4 cDNA (a gift from Dr. D. Fowell, MRC Immunology Unit, Oxford, UK) (34). The specific activity was 2000 unit per mg and the endotoxin level was 60 pg/ml as previously reported [14].

Reverse transcription–polymerase chain reaction. RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR) were performed on normal and nephritic glomeruli as previously described [18]. The PCR primers for type I arginase (AI) were those used by Buga et al. [19]. The primers for type II arginase (AII) were designed using the sequence submitted by Iyer and Grody (accession no. UY-90887 NID). AII primers, which amplify a product of predicted length 612 b.p., are 5′ GCT-GTG-TCA-CAC-TGG-GAG-GAG-ACC 3′ and 5′ CTG-CTA-GGC-TGG-CTG-TAG-CCT-TGG 3′. PCR was carried out at previously described [14]. Identity of the reaction product was confirmed by cleavage by the restriction enzyme *Dsa* I into the predicted fragment sizes. PCR reactions using internal standards of G3PDH (Clontech, Basingstoke, UK) and β-actin primers were performed with AI and AII, respectively. This technique has been previously described [20]; conditions were identical to the single PCR reactions. Ratios of arginase transcript to the respective internal control were calculated using densitometry, after staining of the gel with ethidium bromide.

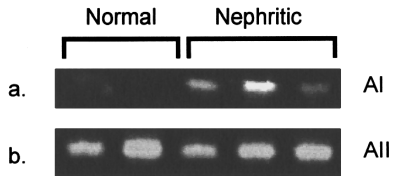

FIG. 1. Arginase isoform mRNA in glomeruli from 2 normal and 3 nephritic rats. a. AI b. AII.

RESULTS

AI and AII Expression in Nephritic Glomeruli

Administration of anti-GBM globulin induced acute neutrophil infiltration into the glomerulus (14.7± 2.2 neutrophils per glomerular cross section). AI mRNA was increased in nephritic glomeruli (n=3), but was barely detectable in normal glomeruli (n=2) (Fig. 1a). AII mRNA was present in normal glomeruli and no increase was detected in nephritic glomeruli (Fig. 1b). Arginase activity was increased in nephritic glomeruli (nephritic, 7.8 ± 0.6 nmol urea/2000 glomeruli; normal, 3.2 ± 0.6; $p<0.005$).

AI and AII Expression in Mesangial Cells

AI mRNA was just detectable in unstimulated mesangial cells and increased by IL-4 or cAMP agonists alone, and maximally by the combination of stimulants (Fig. 2a). This was confirmed by semi-quantitative analysis by co-amplification of AI and G3PDH (Fig. 3). AII mRNA was expressed in unstimulated mesangial cells but not upregulated by stimulants applied either singly or in combination (Fig. 2b). This was confirmed by co-amplification of AII and β-actin (results not shown).

Arginase activity was detectable in unstimulated mesangial cells. Activity was upregulated by cAMP agonists, maximally in combination with IL-4. IL-4 alone only variably increased arginase activity (Fig. 4).

AI and AII Expression in Leukocytes

mRNA for both arginase isoforms was detectable in elicited neutrophils and macrophages (Fig. 5)

DISCUSSION

The arginase isoform AI was increased in nephritic glomeruli in this model of acute immune complex in-

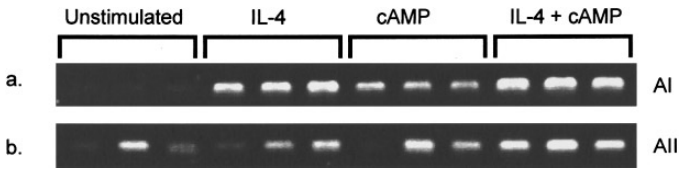

FIG. 2. Arginase isoform mRNA in mesangial cells. Triplicate extracts for each condition. a. AI b. AII.

jury; it was absent or barely detectable in the normal glomerulus. AII mRNA was highly expressed in the normal glomerulus and not increased in the nephritic glomerulus. These results are the same as macrophage-dependent accelerated NTN [14] and this strongly suggests that induction of this isoform is responsible for the substantial increase in arginase activity by nephritic glomeruli.

This isoform pattern was repeated in the mesangial cell, in that AII was expressed in unstimulated cells and did not show upregulation when the cells were stimulated with IL-4 or cAMP while in contrast, AI was barely detectable in unstimulated cells and increased by these stimulants. The mesangial cell response to these mediators, which are known to modulate inflammation [21,22] suggests that induction of AI in these cells could also provide a major source of arginase activity in nephritic glomeruli, as we have previously suggested [13]. The increase in AI mRNA accompanied by a parallel increase in arginase activity supports this, and is the first demonstration of differential transcriptional regulation of arginase isoforms by such compounds. IL-4 increased transcript but did not increase enzyme activity or increased it variably, as we have previously reported [14]. This suggests that AI is subject to post-transcriptional regulation.

The increase in arginase, and the expression of AI in this heterologous model of NTN where the predominant inflammatory cell is the neutrophil is of some interest, for there is only one report of arginase activity in neutrophils [23]. We found AI in the activated rat

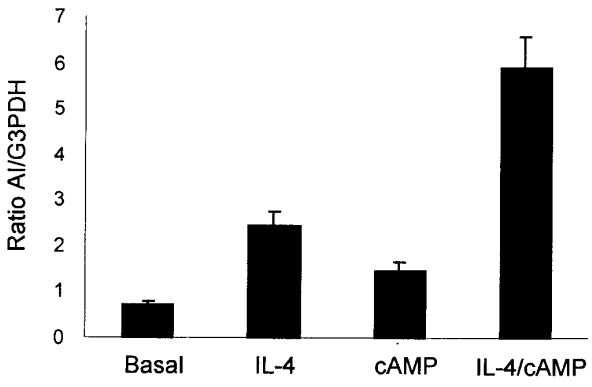

FIG. 3. AI/G3PDH mRNA ratio in mesangial cells.

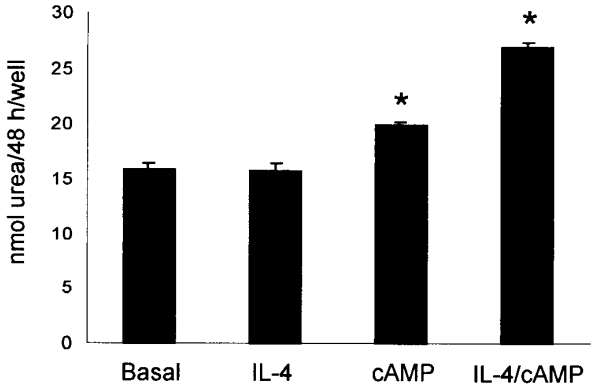

FIG. 4. Arginase activity in mesangial cells; *$p<0.05$ vs. unstimulated.

neutrophil, demonstrating that this cell type can contribute to the enhanced arginase activity in nephritic glomeruli. We now show for the first time the presence of mRNA for both arginase isoforms in rat neutrophils; this confirms a preliminary report of AI protein in human neutrophils [24]. Expression of AI, but not AII, has been shown in LPS-stimulated polypeptone-elicited rat peritoneal macrophages [15]. We, however, found expression of both isoforms in thioglycollate-elicited macrophages without further stimulation.

The role of these distinct isoforms of arginase in inflammation is unknown. The demonstration that AI, an enzyme previously thought to be almost exclusively expressed in the liver catalysing the final step of urea synthesis, is upregulated at an inflammatory site, is an important step to understanding the role of arginase in tissues that do not possess a complete urea cycle. This enzyme shares a common substrate with nitric oxide synthase; several studies support the hypothesis that one function in inflammation is to limit high output production of NO by reducing L-arginine availability [4,15,19]. This is supported by our previous demonstration of competition between the two pathways in immune complex glomerulonephritis [13]. Control of NO production may modulate tissue injury and inflammatory cell responses. Arginase also synthesises L-ornithine, a precursor for polyamines and proline. These compounds have important roles in inflammation and tissue repair. Polyamines can modulate apoptosis [25] and cell proliferation [26], and proline is

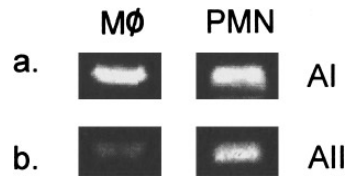

FIG. 5. Arginase isoform mRNA in macrophages (MØ) and neutrophils (PMN).

a constituent of extracellular matrix [27]. The role of AII is currently unknown; the constitutive presence of this isoform in the normal glomerulus may be to generate physiological amounts of polyamines and proline for maintenance of normal tissue structure including cell replacement and mesangial matrix and basement membrane proteins.

These results showing constitutive AII expression, and upregulation of AI in immune complex glomerulonephritis, point to differing control and functions of these two isoforms in normal and inflamed tissues. They further suggest an important role for arginase in inflammatory responses.

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