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REVIEW ARTICLE

Kynurenine metabolism in health and disease

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Abstract Kynurenine is a small molecule derived from tryptophan when this amino acid is metabolised via the kynurenine pathway. The biological activity of kynurenine and its metabolites (kynurenines) is well recognised. Therefore, understanding the regulation of the subsequent biochemical reactions is essential for the design of therapeutic strategies which aim to interfere with the kynurenine pathway. However, kynurenine concentration in the body may not only be determined by the efficiency of kynurenine synthesis but also by the rate of kynurenine clearance. In this review, current knowledge about the mechanisms of kynurenine production and routes of its clearance is presented. In addition, the involvement of kynurenine and its metabolites in the biology of different T cell subsets (including Th17 cells and regulatory T cells) and neuronal cells is discussed.

Keywords Brain · Indoleamine 2,3 dioxygenase · Immunity · Kynurenine · Th17 · Treg

Introduction

Serotonin and kynurenines are metabolites of the essential amino acid tryptophan and influence a wide range of physiological processes, including mood (Russo et al. 2003; Kanai et al. 2009), blood pressure (Wang et al. 2010), and immunity (Moffett and Namboodiri 2003; Prendergast 2008). However, the biochemical pathways which are responsible for the production of serotonin and kynurenines are different. Serotonin is produced from tryptophan in two steps mediated by two distinct enzymes: tryptophan hydroxylase (EC1.14.16.4) and aromatic L-amino acid decarboxylase (EC4.1.1.28), respectively (Russo et al. 2003). In contrast, kynurenines are produced from tryptophan in a series enzymatic reactions collectively referred to as the kynurenine pathway (Fig. 1). The name of the pathway is derived from kynurenine, the most abundant molecule amongst kynurenines, which are by-products of tryptophan metabolism of this pathway (Beadle et al. 1947). Functionally, the kynurenine pathway can be grouped into three separate branches (Moffett and Namboodiri 2003). The first one is involved in initiation of tryptophan metabolism and kynurenine production. Once kynurenine is produced, it can enter two other branches of the kynurenine pathway. Hence, kynurenine can be metabolised into xanthurenic acid (XaA) and/or kynurenic acid (KA) (Amori et al. 2009). The formation of KA in particular was shown to play an important role in the central nerve system (CNS) (Schwarcz and Pellicciari 2002). KA is a neuro-protective molecule, which can reduce activation of *N*-methyl-D-aspartate receptors (NMDAR) (Nemeth et al. 2005). Thus, KA can protect neuronal cells from excitatory cell death evoked by over-activation of NMDA receptors (Nemeth et al. 2005) and (Schwarcz and Pellicciari 2002). In addition, it was shown that upon physiological flow conditions, KA might be involved in early stages of neutrophil and monocyte adhesion to surfaces coated with fibrinogen (Barth et al. 2009). This process was shown to be dependent on G-protein coupled receptor 35 (GPR35) (Barth et al. 2009), which is activated by KA (Wang et al. 2006). In addition, pre-treatment of mononuclear cells with KA effectively reduced tumour necrosis factor alpha (TNF-α) secretion

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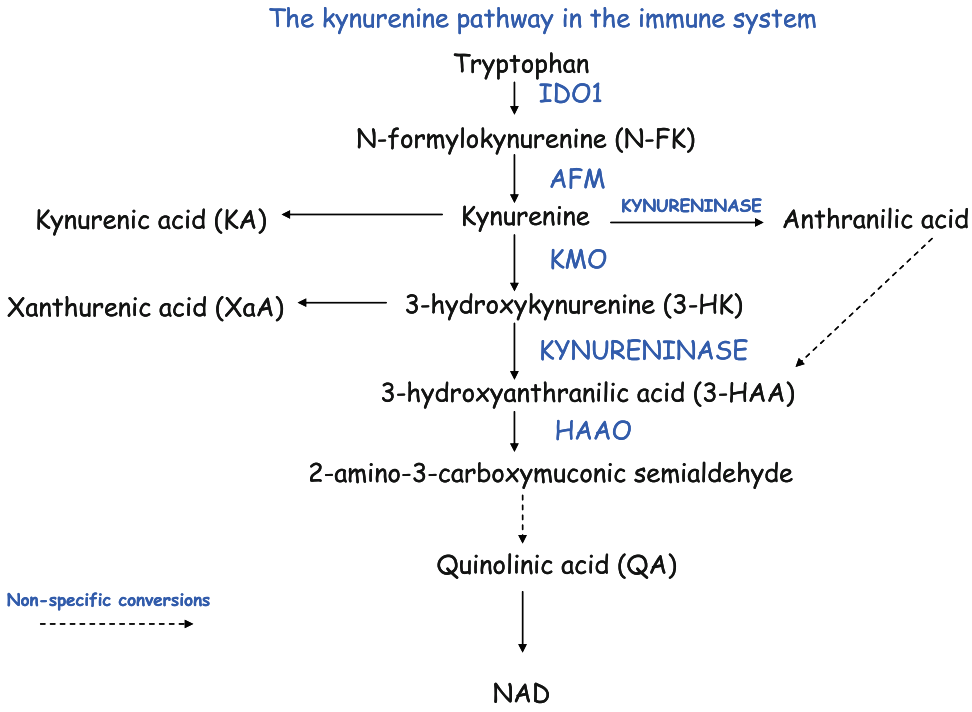

Fig. 1 Schematic presentation of the kynurenine pathway

from these cells upon LPS stimulation (Wang et al. 2006). Hence, KA may play an important but still not fully appreciated role in health and disease. Nonetheless, kynurenine catabolism to QA is a major route of kynurenine clearance (Saito et al. 1993). In addition, the biological activity of 3-hydroxykynurenine (3-HK) and 3-hydroxyanthranilic acid (3-HAA) is relatively well recognised (Stone and Darlington 2002). Therefore, the aim of this article is to summarise current knowledge about the biological activity of kynurenine and its metabolites via this branch of the pathway. Also, the regulatory mechanisms which dictate the efficiency of kynurenine metabolism are discussed.

Systemic metabolism of kynurenine

It is estimated that only around 1% of dietary delivered tryptophan can be converted into serotonin (Russo et al. 2003). The remaining 99% is metabolised via the kynurenine pathway. Thus, increased kynurenine production may result in inadequate serotonin synthesis and predispose for certain psychiatric diseases like, e.g. depression (Russo et al. 2003). The causative relationship between increased tryptophan metabolism via the kynurenine pathway, depressive-like behaviour and the immune response is well established in experimental animals. Mice infected with Bacillus Calmatte-Guérin (O'Connor et al. 2009) or injected with lipopolysaccharide (LPS) (O'Connor et al. 2009) develop depressive-like behaviour which is associated with decreased tryptophan and increased kynurenine concentrations in the serum. A similar observation was made when gerbils were injected with pokeweed mitogen (PWM) (Saito et al. 1993). In these animals, as in mice infected with Bacillus Calmatte-Guérin or injected with LPS, decreased tryptophan concentration resulted from increased gene expression and enzymatic activity of indoleamine 2,3 dioxygenase (IDO1). This is the first and rate limiting enzyme on the pathway which is activated by pro-inflammatory cytokines such as TNF-α and IFN-gamma (IFN-γ) (Robinson et al. 2005). However, in gerbils the enzymatic activity of IDO1 was restricted to specific tissues, including: lungs, cecum, colon, and epididymis (Saito et al. 1993). In addition, Takikwawa and colleagues have found that LPS could induce IDO1 enzyme activity only in specific tissues. However, this local induction of tryptophan metabolism resulted in a threefold increase in kynurenine concentration in sera taken from mice 24 h after LPS injection (Takikawa et al. 1986). Thus, it has been suggested that tryptophan metabolism can be initiated locally; whereas kynurenine may diffuse into the bloodstream. Therefore, increased kynurenine concentration in the serum can indicate increased tryptophan metabolism taking place in a specific tissue.

Upon normal conditions, kynurenine is readily cleared from blood in the liver and kidneys (Takikawa et al. 1986; Saito et al. 1993). This has been demonstrated by the intravenous administration of radioactively labelled [U-^{14}C]kynurenine into naïve mice (Takikawa et al. 1986). In this study, it was shown that 4 h after initial injection,

approximately 80% of the radioactivity was incorporated into XaA and excreted in urine. About 5–10% of ^{14}C was expired as CO_2. LPS stimulation did not change these proportions but the level of XaA production in urine was increased approximately threefold. Nonetheless, upon LPS treatment, kynurenine turnover did not change substantially, from 5.3 h^{-1} in naive mice to 6.1 h^{-1} in the LPS challenged mice (Takikawa et al. 1986). Thus, increased kynurenine concentration could result as a consequence of its higher production and inadequate to demand clearance from blood (Takikawa et al. 1986). Interestingly, increased excretion of kynurenine and XaA was reported in urine from rats (Takeuchi and Shibata 1984) and humans (Yeh and Brown 1977) loaded with tryptophan, but depleted of pyridoxal phosphate (PLP). This molecule is a cofactor for kynureninase (Jakoby 1954). Without PLP, kynureninase is inactive and kynurenine cannot be further converted into downstream metabolites (Yeh and Brown 1977). Thus, experimental depletion of PLP can abolish kynureninase activity and mimics the situation of insufficient metabolism of kynurenine. However, increased extrication of kynurenine and XaA in urine of PLP-depleted animals and humans could be a physiological route of kynurenine clearance upon its insufficient metabolism. In addition, Takikawa et al. have found that in the liver and kidneys, upon immune challenge, enzymatic activities of the following enzymes on the kynurenine pathway: kynureninase, kynurenine 3-monooxygenase (KMO) and 3-hydroxyanthranilate 3,4 dioxygenase (HAAO) have not been changed (Takikawa et al. 1986). Similar observation was made in rabbits injected with LPS (Bertazzo et al. 2001). In addition, in vitro experiments with various cell lines have indicated that the enzymatic activity of kynureninase was not affected by IFN-γ in these cells (Heyes et al. 1997). Taken together, these findings suggest that inadequate activities of the downstream enzymes on the kynurenine pathway could be a limiting factor for kynurenine metabolism upon increased kynurenine production evoked by the immune challenge. This may explain why, in sera from humans, accumulation of kynurenine is positively correlated with the level of neopterin, which is a biochemical marker of inflammation (Ploder et al. 2009; Iwagaki et al. 1995). Neopterin is a metabolite of guanosine triphosphate (GTP) produced excessively in activated macrophages. Thus, increased neopterin indicates an inflammatory reaction (Widner et al. 2002), whereas increased kynurenine indicates activation of tryptophan metabolism via the kynurenine pathway.

Increased kynurenine concentration has been observed in many diseases, including rheumatoid arthritis (Schroecksnadel et al. 2003), systemic lupus erythematous (SLE) (Widner et al. 2000), sepsis (Pellegrin et al. 2005) and Huntington disease (Leblhuber et al. 1998). Moreover, kynurenine and its downstream metabolites are biologically active. For example, in the immune system, kynurenine and its metabolites are involved in immunosuppression (Moffett and Namboodiri 2003). Therefore, reduced kynurenine metabolism could dampen physiological role of the anti-inflammatory metabolites of kynurenine like, e.g. 3-HK and 3-HAA.

Enzymes involved in kynurenine metabolism

Kynurenine anabolism

As mentioned in the introduction, kynurenine and its metabolites are biologically active. Therefore, their synthesis and further metabolism must be tightly controlled. Kynurenine production and its metabolism into the downstream metabolites is an example of such process in which subsequent biochemical reactions are controlled in a coordinated manner.

Kynurenine is not directly produced from tryptophan. Instead, kynurenine is produced from *N*-formylkynurenine (N-FK) as a result of arylformamidase (AFM) enzyme activity (Mehler and Knox 1950). Thus, the formation of N-FK may be equally important for kynurenine synthesis as initiation of tryptophan metabolism. N-FK is produced as result of tryptophan oxidation mediated by three distinct enzymes: IDO1, indoleamine 2,3 dioxygenase 2 (IDO2) and tryptophan 2,3 dioxygenase (TDO). When purified, N-FK is unstable and tends to degrade (Mehler and Knox 1950). Moreover, it has been demonstrated that N-FK is soluble in water and far less in organic solvents like ethanol or acetone. The crystalline form of N-FK decomposes at +170°C without melting. In addition, N-FK exhibits maximum absorbance at 315 nm whereas maximal absorbance of kynurenine is observed at 360 nm (Mehler and Knox 1950). As a result of acidic hydrolysis at 100°C, N-FK can be converted into kynurenine. In living organisms, the conversion of N-FK to kynurenine is catalysed by AFM (Mehler and Knox 1950).

AFM enzyme (EC 3.5.1.9) is a 305 amino acid protein with an ancient evolutionary origin (Schuettengruber et al. 2003). In mammals, AFM is expressed predominantly in the kidneys and liver (Schuettengruber et al. 2003; Dobrovolsky et al. 2005). Also, AFM enzymatic activity is higher in the kidneys and liver than in other organs and tissues in which AFM is expressed such as brain, submandibular gland, heart, trachea, lung, thymus, oesophagus, stomach, intestine, cecum, colon, urinary bladder, testis, epididymis, seminal vesicle, and spleen (Takikawa et al. 1986; Dobrovolsky et al. 2005). At the intracellular level, AFM is predominantly expressed in the cytoplasm (Schuettengruber et al. 2003).

At the genomic level, the murine *Afm* gene is localised on chromosome 11 and consists of 10 exons (Schuettengruber et al. 2003). The *Afm* gene shares the same gene promoter with the *Thymidine kinase* (*Tk*) gene. Translation initiation of the *Tk* and *Afm* genes is separated by 172-bp-long nucleotide sequence (Schuettengruber et al. 2003). Reduced acetylation of the histone H4 on this bidirectional gene promoter drives expression of *Afm*, whereas its hyperacetylation results in *Tk* gene expression (Schuettengruber et al. 2003). Two separate transcription initiation sites drive expression of the *Afm* gene. The first one is located in the bidirectional promoter and gives a 2.6 kb transcript. A 2.9 kb transcript is produced when transcription of *Afm* gene starts in the second exon of *Tk* gene. However, both transcripts, 2.6 and 2.9 kb, encode the same open reading frame of the AFM enzyme (Schuettengruber et al. 2003). In addition, transcription of the longer and shorter transcripts is regulated in an SP1-dependent manner. SP1 transcription factor can bind into the GC-rich sequences like: CACCC and GC boxes and induce expression of various genes involved in cell growth and metabolism (Black et al. 2001).

What can animal studies teach us about arylformamidase?

Gene expression of AFM enzyme was reported to be restricted to resting cells (Schuettengruber et al. 2003). In contrast, the *Tk* gene was highly expressed in proliferating cells (Schuettengruber et al. 2003). However, in vivo, functional studies focused on the physiological role of AFM enzyme provide rather puzzling results (Dobrovolsky et al. 2003, 2005). Nonetheless, an interesting comparison could be drawn between *Ido1* and *Afm* KO mice. In wild-type (WT) animals, pharmacological inhibition of either IDO1 or AFM has a minor effect in experimental animals. Thus, it could be predicted that genetic deletion of *Ido1* or *Afm* gene could have a similar, rather mild effect (Dobrovolsky et al. 2003; Baban et al. 2004). As expected, the phenotype of naive *Ido1* KO mice is indistinguishable from WT mice (Baban et al. 2004). However, *Ido1* KO mice develop exacerbated immune response when challenged with pathogens (Harrington et al. 2008) or immunised with antigens (Criado et al. 2009). However, in contrast with naive *Ido1* KO mice, naive mice with *Afm* gene deletion exhibit relatively severe abnormalities in their immune system and physiology (Dobrovolsky et al. 2003). *Afm* KO mice usually die at age of 6 months, develop (IgG but not IgM) spontaneous immune-complex mediated kidney failure, and exhibit reduced motor coordination. In the spleens isolated from *Afm* KO mice lymphoid atrophy was observed. Infiltration of lymphoid cells into the liver was noticed (Dobrovolsky et al. 2003). Also, inflammation of arteries and myocardial arteriosclerosis was reported in these KO mice. Nonetheless, final conclusions about the functional role of AFM enzyme in the immune system and in physiology of mammals should be made very carefully (Dobrovolsky et al. 2003). It has been shown that the shared promoter region and the first exons of *Afm* and *Tk* genes were affected by the transgenesis procedure. Thus, effectively double knock out mice were produced. Therefore, it might be very difficult to correlate observed abnormalities in *Afm/Tk* KO mice with a particular function of AFM and TK enzymes. It is known that inactivation of one gene may be compensated by other genes with similar functions. Also, changes in thymidine metabolism may affect tryptophan metabolism via the kynurenine pathway and vice versa. Nevertheless, as far as the kynurenine pathway is concerned in *Afm/Tk* KO mice, the lack of functional *Afm* and *Tk* genes cause the accumulation of N-FK, kynurenine and kynurenic acid (KA) in the plasma. This effect was especially pronounced in 20-week-old mice in comparison with 9-week-old mice. However, in the liver and kidneys, the concentration of nicotinamide adenine dinucleotide (NAD), the final product of tryptophan metabolism via the kynurenine pathway, was not affected in either of experimental groups. This observation may be explained by the fact that in the kidneys from *Afm/Tk* KO mice 13% of AFM activity is preserved (Dobrovolsky et al. 2005). This suggests that there might be an undiscovered gene which encodes an enzyme capable of N-FK conversion into kynurenine.

Kynurenine catabolism

However, irrespective of the enzyme which converts N-FK into kynurenine, once this molecule is produced it can be further metabolised (Oxenkrug 2007). This process may occur in the liver, kidneys or in tissues which cells express enzymes involved in kynurenine metabolism. Belladonna et al. (2006) have elegantly showed that dendritic cells express functional enzymes involved in tryptophan and kynurenine metabolism. Upon IFN-γ stimulation, functional enzymes involved in kynurenine conversion into (QA) are expressed in both tolerogenic ($CD8^+$) and immunogenic ($CD8^-$) splenic DC (Belladonna et al. 2006). However, these two cell types are different in their ability to produce kynurenine. Although IFN-γ could initiate increase *Ido1* gene expression in both $CD8^+$ and $CD8^-$ DC in a comparable manner, $CD8^-$ DC cells were unable to produce kynurenine (Belladonna et al. 2006). Nonetheless, $CD8^-$ could be converted into tolerogenic cells by exogenous administration of kynurenine. However, this process was dependent on the enzymatic activity of KMO enzyme (IC1.14.13.9) (Belladonna et al. 2006).

Kynurenine 3-monoxygenase (KMO)

KMO can catalyse kynurenine conversion into 3-hydroxykynurenine (3-HK) (Nishimoto et al. 1979). This could explain the KMO-dependency of the $CD8^+$ DC's for their ability to metabolise kynurenine. Human KMO is a protein of approximately 55.8 kDa protein with its monooxygenase activity localised on the outer mitochondrial membrane (Breton et al. 2000; Nishimoto et al. 1979). KMO requires nicotinamide adenine dinucleotide phosphate (NADPH) for its catalytic action. As a consequence of the reaction between KMO, NADPH, and kynurenine, 3-HK and water is produced. In mice, the activity of KMO is specifically high in the kidneys 901.50 ± 73.50 nmol/(min g) tissue in comparison with the liver 656.55 ± 18.99 nmol/(min g) tissue (Allegri et al. 2003). Nonetheless, pharmacological inhibition of KMO by (*m*-nitrobenzoyl)-alanine (mNBA) (Chiarugi and Moroni 1999) and UPF 648 (Amori et al. 2009) results in accumulation of kynurenine in the serum isolated from experimental animals. In addition, inhibition of KMO was found to reduce locomotor activity in rats and protect from audiogenic convulsions in DBA/2 mice (Carpenedo et al. 1994). It has been also shown that inhibition of KMO could be beneficial in Huntington disease (Giorgini et al. 2005) and (Sapko et al. 2006). In addition, ischemic brain damage in rats could be reduced by KMO enzyme inhibition (Cozzi et al. 1999). Interestingly, it has been observed in rats that the specific activity of the KMO was reduced in the liver during aging (Comai et al. 2005). Stroke, which is a primary source of ischemic brain damage, is more likely to occur in older people. Thus, reduced KMO activity could be protective in patients affected by stroke. However, KMO activity in humans has not been assessed yet.

Although inhibition of KMO could be a therapeutically important target not much is known how expression of the *Kmo* gene could be regulated. Nevertheless, it has been shown that 4 h after LPS injection, the expression of *Kmo* gene was statistically significantly reduced in the cortex but not in the hippocampus of mice injected with LPS (Connor et al. 2008). In contrast, 24 h after LPS injection, *Kmo* gene expression was markedly increased in both brain structures: the cortex and hippocampus (Connor et al. 2008). Interestingly, gene expression for the pro-inflammatory cytokines like: *Tnf-α, Ifn-γ,* and *Interleukin 6* (*Il-6*) was significantly increased in the cortex 4 h after LPS stimulation, suggesting that these cytokines could be important for *Kmo* gene expression. However, in the hippocampus gene expression for *Ifn-γ* was neither changed 4 nor 24 h after immune stimulation suggesting that IFN-γ may not be involved in the initiation of the *Kmo* gene transcription in this brain structure. Moreover, it has been demonstrated that LPS can induce *Kmo* gene expression in the microglia in vitro (Connor et al. 2008). Taken together, these results suggest that in the brain, *Kmo* gene expression is tightly regulated in the tissue and in a cell-type specific manner. It has been also shown that in human monocytes, taken from atopic individuals, *Kmo* gene expression was initiated 24 h but not 4 h after activation of the high-affinity receptor for IgE (FCεRI) receptor (von Bubnoff et al. 2002). In addition, in non-atopic donors, *Kmo* gene was constitutively expressed in human monocytes. Moreover, in healthy donors, FCεRI stimulation did not result in increased transcription of the *Kmo* gene. Therefore, increased expression of the *Kmo* gene might be restricted to the pathological situation. Nonetheless, further studies are needed to fully understand FCεRI-induce *Kmo* gene expression.

Kynureninase

As KMO produces 3-HK, kynureninase can convert 3-HK into 3-HAA. However, kynureninase can also directly convert kynurenine into 3-HAA (Gal and Sherman 1978; Tanizawa and Soda 1979a). In this case, AA and L-alanine are produced (Tanizawa and Soda 1979b). In addition, Ueda et al. (1978) have reported that AA can undergo a non-specific hydroxylation on the microsomes in the liver, resulting in 3-HAA production (Fig. 1). Moreover, in vivo data indicates that KMO-dependant kynurenine metabolism might be the preferred route of kynurenine disposal (Guidetti et al. 1995; Bertazzo et al. 2001). Nonetheless, significant differences may exist between species (Fujigaki et al. 1998).

Human kynureninase is a 52-kDa, cytoplasmic enzyme. In mammals, the enzymatic activity of kynureninase is low in comparison with than other kynurenine pathway enzymes (Allegri et al. 2003). In murine kidneys the enzymatic activity of kynureninase is: 1.43 ± 0.04 nmol/(min g) tissue whereas in the liver: 10.69 ± 0.26 nmol/(min g) tissue (Allegri et al. 2003). Thus, kynureninase may be considered as the rate limiting enzyme involved in kynurenine metabolism (Salter et al. 1986). Comai et al. have also reported that in rats, specific kynureninase activity decreased in the liver with aging. In contrast, in the kidneys, kynureninase activity was increased in 18-month-old rats in comparison with 1-week-old animals (Comai et al. 2005). Thus, in old animals, kynurenine could be preferentially metabolised in the kidneys rather than in the liver. In addition, it has been reported that 3-HAA can inhibit kynureninase in porcine liver (Tanizawa and Soda 1979a). This observation suggests that kynureninase enzyme activity may be regulated by the auto-inhibitory loop. However, this has to be validated with more contemporary methods and in other tissues and species.

Although pro-inflammatory signals were not found to increase kynureninase activity in organs isolated from experimental animals (Takikawa et al. 1986) and (Saito et al. 1993), another study showed that TNF-α and a TLR3 ligand (PolyI:C), induced *Kynureninase* gene expression in mature DC's in vitro (McIlroy et al. 2005). However, following this stimulus, *Ido1* and *Kmo* gene expression was highest 2 h after stimulation; whereas *Kynureninase* gene expression peaked 12 h after initial stimulus (McIlroy et al. 2005). In addition, Belladonna et al. (2006) have found that, in splenic $CD8^+$ DCs, kynurenine could be detected 5 h after initiation of tryptophan metabolism induced by IFN-γ. In contrast, QA was detected 18 h after IFN-γ stimulation. However, delayed *Kynureninase* gene expression and late kynurenine metabolism were observed in different, in vitro, experimental settings. Nonetheless, it shows that specific kinetic of *Kynureninase* gene expression may account for an increased kynurenine concentration in the immune system. In addition, described earlier in vivo observations of delayed kynurenine clearance from sera of immune challenge animals indicate that accumulation of kynurenine may play some physiological role. For example, immune cells could be exposed to kynurenine for longer time. Thus, from the resolution of inflammation point of view, it might be beneficial to delay kynurenine metabolism in order to produce toxic metabolites some time after initial stimuli.

Kynurenine and its metabolites

Cellular and molecular mechanisms of kynurenines' action

In vitro, the longer T cells were kept under stimulatory conditions (anti-CD3) the smaller doses of tryptophan metabolites (equimolar mixture of kynurenine, AA, 3-HAA, and QA) were required to induce cell death (Terness et al. 2002). It has been also reported that kynurenine was able to reduce proliferation of human peripheral blood lymphocytes (PBL) in vitro (Frumento et al. 2002). However, the effectiveness of kynurenine treatment was dependent on time. When kynurenine was present in the cell culture in the first 36 h after phytohaemagglutinin (PHA) stimulation, the treatment resulted in decreased cell proliferation (Frumento et al. 2002). However, when exogenous kynurenine was added to the cell culture 36 h after initial stimulation, the treatment did not reduce PBL proliferation (Frumento et al. 2002). Moreover, only activated cells were sensitive to the cytotoxic action of kynurenine, whereas naive T and NK cells were not affected by kynurenine (Frumento et al. 2002; Fallarino et al. 2002). Nevertheless, accumulated kynurenine in the blood could immediately affect newly stimulated cells and limit immune response. However, the interpretation of in vitro results for the in vivo situation should be made carefully.

In addition, kynurenine (and possibly *N*-formyl kynurenine (N-FK) and 3-hydroxykynurenine (3-HK) could be also involved in exacerbation of tryptophan starvation in T cells. Kaper et al. (2007) have proposed the existence of a positive feedback between IDO1-mediated tryptophan metabolism in DCs and kynurenine-induced tryptophan starvation in CD98 expressing T cells. The CD98 complex is a Na^+-independent large neutral amino acid transporter able to transport neutral branched amino acids (valine, leucine and isoleucine) as well as aromatic amino acid like tryptophan (Speciale et al. 1989; Wagner et al. 2001). CD98 is expressed on astrocytes (Speciale et al. 1989) and activated T cells (Wagner et al. 2001). It has been demonstrated that extracellular kynurenine can be exchanged for intracellular tryptophan in a LAT1-dependent manner (Kaper et al. 2007). LAT1 protein is a component of CD98 complex (del Amo et al. 2008). Thus, extracellular kynurenine could potentiate intracellular tryptophan starvation in cells expressing active CD98 complex by increasing tryptophan efflux (Kaper et al. 2007). As a consequence, local and/or systemic kynurenine concentration could be also regulated by the efficiency of kynurenine import into cells. On the other hand, extracellular kynurenine could potentiate intracellular tryptophan starvation in cells expressing active CD98 complex by increasing tryptophan efflux. As a result of tryptophan starvation, the pool of uncharged tRNA (free tRNA, without attached amino acid) is increased (Zaborske et al. 2009; Munn et al. 2005). Such tRNA can bind into the histidyl-tRNA-synthetase (HisRS)-related region located on the C-terminus of GCN2 kinase (Qiu et al. 1998). As a result of tRNA binding, GCN2 kinase undergoes conformational changes and becomes active (Qiu et al. 1998). Activated GCN2 can phosphorylate eukaryotic translation initiation factor 2A (eIF2α) and inactivate it (Qiu et al. 1998; Wek et al. 2006). As a consequence, translation of the vast majority of transcripts become inhibited and cell cycle is arrested (Wek et al. 2006), and (Munn et al. 2005). Thus, this mechanism could account for the observation that kynurenine-dependent reduction in human T cell proliferation was potentiated by medium containing reduced tryptophan concentration (Terness et al. 2002). In this experimental model, kynurenine could have actively depleted T cells of tryptophan, which might have been exchanged for exogenous kynurenine. Also, kynurenine imported via the CD98 complex could have been converted into 3-HAA and further into QA. However, to the best of our knowledge, it has not been tested yet if the expression of CD98 overlaps with the expression of kynureninase, KMO and HAAO enzymes in cells. Nevertheless, Moffett et al. (1998) have found that, in

rats, intraperitoneal injection of kynurenine resulted in increased concentration of QA in lymphoid organs, including thymus and spleen. In addition, QA accumulation was observed in macrophages. Thus, these results suggest that kynurenine could be readily taken up and metabolised in various cells. Interestingly, kynurenine injection did not result in QA accumulation in hepatocytes (Moffett et al. 1998), suggesting that either kynurenine is not transported into these cells, or cannot be converted into QA. Moreover, intraperitoneal injection of tryptophan resulted in an increase in QA in hepatocytes but with mild effect on the QA in macrophages (Moffett et al. 1998). Thus, kynurenine is unlikely to be taken up and metabolised by hepatocytes. Moreover, the mechanism of kynurenine transportation into the immune cells described here may explain some of the therapeutic effects of exogenously administrated kynurenine. Treatment with kynurenine was found to ameliorate allergic airway inflammation (Taher et al. 2008), prolong transplanted skin graft survival in rats (Bauer et al. 2005) and reduce severity of experimental arthritis (Criado et al. 2009).

In addition, Wang et al. (2010) have demonstrated that kynurenine is also be involved in blood pressure regulation upon immune challenge. It has been shown that endothelial cells are primary sites of kynurenine production in an IDO1-dependant manner and IFN-γ can induce *Ido1* gene and protein expression in these cells (Wang et al. 2010). In addition, intravenous injection of kynurenine into rats with spontaneous hypertension resulted in a transient decrease in the mean arterial blood pressure. It has been also demonstrated that kynurenine is able to induce relaxation in pre-constricted porcine coronary arteries in a dose-dependant manner. This pharmacological effect of kynurenine was mediated by soluble guanidine cyclase (sGC), cyclic guanosine monophosphate (cGMP) and the protein kinase G (PKG) pathway. Thus, increased kynurenine could account for hypotension observed in immunologically challenged mice, e.g. mice with sepsis or infected with *Plasmodium berghei* ANKA (PbA) (Wang et al. 2010).

Although downstream kynurenine metabolites like 3-HK, 3-HAA, and QA may not be involved in blood pressure regulation (Wang et al. 2010), they play a pivotal role in immune regulation (Stone and Darlington 2002). It has been demonstrated that T cell proliferation can be inhibited with micromolar concentrations of 3-HK (IC_{50} 187 μM) (Terness et al. 2002). The cytotoxic action of 3-HK can be attributed to the production of hydrogen peroxidise which results in the damaging action of free hydroxyl radical (Okuda et al. 1996, 1998). Also, as with kynurenine, exogenous administration of 3-HK effectively reduced symptoms in allergic airway inflammation (Taher et al. 2008).

In contrast to 3-HK, the toxic action of 3-HAA is more complex. Although the final effect of 3-HAA results in cell death of neurons, T cells (Fallarino et al. 2002), monocyte-derived macrophages (Morita et al. 2001) and thymocytes (Fallarino et al. 2002), the mechanisms involved in cell death may be cell-type specific. The formation of cytotoxic free hydroxyl radical might be involved in 3-HAA induced cell death in monocyte-derived macrophages (Morita et al. 2001). In contrast, Lee et al. (2010) have shown that, in human T cells, 3-HAA could deplete cells of glutathione (GSH) resulting in apoptosis in these cells in a caspase 3-dependent manner. In addition, treatment of mice with 3-HAA increased the survival rate from in acute graft-versus-host disease (Lee et al. 2010). However, in addition to GSH-dependant mechanism, 3-HAA can trigger apoptosis in T cells in another way. For example, in $CD4^+$ T cells and Jurkat cells stimulated with anti-CD3/anti-CD8, 3-HAA was shown to inhibit autophosphorylation of the serine 241 in PDK1 kinase (Hayashi et al. 2007). As an explanation of this observation, it has been suggested that 3-HAA forms hydrogen bounds with the following amino acids of PDK1: serine 160, alanine 162 and threonine 222. Such molecular interaction results in inhibition of PDK1 kinase and in an inadequate NFκB activation in response to anti-CD3/anti-CD8 stimulation. As a consequence, Th1 and Th2 cells undergo apoptotic cell death upon stimulation (Hayashi et al. 2007). However, in addition to the free hydroxyl radical formation (Morita et al. 2001) and inhibition of autophosphorylation, 3-HAA can also induce apoptotic cell death in a caspase 8-dependent manner (Fallarino et al. 2002). This was observed in Th1 but not in Th2 cells and this may account for the prolonged graft survival in skin graft transplantation (Bauer et al. 2005) and reduced symptoms of allergic airway inflammation upon treatment with exogenous 3-HAA (Hayashi et al. 2007; Taher et al. 2008).

In addition, it has been shown that QA, like 3-HAA, can also induce cell death via caspase 8 and cytochrome c release in Th1 cells and thymocytes (Fallarino et al. 2002). Interestingly, the involvement of QA in immune regulation was predicted before IDO1-dependant tryptophan depletion was shown to be immunosuppressive (Moffett and Namboodiri 2003). This prediction was based on the observation that of all the tissues tested, the concentration of QA was the highest in the spleen amongst other analysed tissues (Saito et al. 1993; Moffett et al. 1998). Concentration of QA was increased in lymph nodes from mice injected with LPS (Espey et al. 1995). In fact, this was very first report showing accumulation of tryptophan metabolites in the secondary lymphoid organs upon immune challenge.

QA, in addition to its immunomodulatory role, is also a natural agonist of the *N*-methyl-D-aspartate receptor (NMDA) which is primarily expressed in neuronal cells (Kincses et al. 2010). Thus, overproduction of QA can cause excitotoxic cell death in these cells (Stone et al. 2001).

Cumulative effects of kynurenines on the immune cells

Kynurenines can also exhibit a cumulative (additive) effect on T, B, and NK cells (Terness et al. 2002). Desvignes and Ernst (2009) have demonstrated that an equimolar mixture of kynurenines (L-kynurenine, 3-HK, 3-HAA, anthranilic acid and QA) can inhibit IL-17 production in a dose-dependent manner with an IC_{50} value of 11.7 μM. However, when tested separately, 3-HAA was the most potent tryptophan metabolite with an IC_{50} value of 27.7 μM. Anthranilic acid and quinolinic acid on their own had no effect (Desvignes and Ernst 2009). Also, the differentiation of Th17 cells from naive $CD4^+$ T cells was reduced by the equimolar mixture of kynurenine. Interestingly, kynurenines were able to abrogate the Th17- promoting capacity of IL-23 in Th17 cells. However, the mechanisms responsible for this effect remain unknown (Desvignes and Ernst 2009).

In addition to the above findings, Fallarino et al. (2006) have shown that in long-term cell culture (7 days), low tryptophan concentration (35 μM) and an equimolar (10 μM) mixture of kynurenine, AA, 3-HK, 3-HAA, and QA promote conversion of naive $CD4^+$ T cells into $CD25^+$ $Foxp3^+$ regulatory T cells (Treg). Tregs are a subset of T cells which are able to reduce immune response by promoting tolerogenic signals in the immune system (Fallarino et al. 2006). When tested in vivo, these cells were able to protect NOD-SCID mice from diabetes transfer in a CTLA-4 and IL-10 dependent manner (Fallarino et al. 2006). Therefore, in the long-term, decreased tryptophan concentration and increased concentration of kynurenines could promote tolerance in the immune system. Similarly, $CD8^+$ T cells, exposed to medium containing a suboptimal concentration of tryptophan and increased concentrations of kynurenines, were characterised by the decreased expression of receptor ζ, which is a part of the T cell receptor complex (TCR) (Fallarino et al. 2006). As a result, $CD8^+$ T cells exhibited compromised lytic activity and reduced production of IFN-γ and interleukin 2 (IL-2). Also, proliferation of $CD8^+$ but not $CD4^+$ T cells was affected by decreased tryptophan concentration and increased kynurenines upon anti-CD3 or concanavalin (Con A) stimulation. However, when $CD8^+$ and $CD4^+$ T cells were stimulated with PMA and calcium ionophore, using the same culture conditions as for anti-CD3 stimulation, neither cell type was affected (Fallarino et al. 2006).

HAAO enzyme and 3-HAA metabolism

Although HAAO enzyme is not directly involved in kynurenine metabolism it regulates metabolism of 3-HAA and QA. As described in the previous section, these molecules play a pivotal role in the immune system. Therefore, it is worth to summarise some knowledge about HAAO enzyme and 3-HAA metabolism.

Direct product of 3HAA metabolism by HAAO is 2-amino-3-carboxymuconic semialdehyde. This molecule is further converted into QA as a result of non-specific cyclisation (Fig. 1). Thus, the rate of QA synthesis is primarily dependant on HAAO enzyme activity. It has been estimated that in rats, only 10% of injected 3-HAA was converted into QA and excreted in urine (Hankes and Henderson 1957). The remaining 3-HAA expired as carbon dioxide (CO_2) within 3 h after of the initial injection (Hankes and Henderson 1957). Thus, 3-HAA might be metabolised very efficiently. Therefore, not surprisingly, HAAO was found to exhibit the highest enzymatic activity amongst kynurenine pathway enzymes (Allegri et al. 2003). In murine liver, the activity HAAO was reported to reach 4,803 ± 256 nmol/(min g) tissue whereas in the kidneys this value was 1,108 ± 59 nmol/(min g) tissue (Allegri et al. 2003). Comai et al. (2005) have demonstrated that, in the liver and kidneys of aging rats, the specific activity of HAAO enzyme was increased. Thus, efficiency of 3-HAA metabolism may be higher in old animals in comparison with younger ones. This may have physiological implications since 3-HAA is biologically active. It is also possible to reversely inhibit HAAO enzyme activity by administration of a synthetic HAAO inhibitor which is NCR-631 molecule (Walsh et al. 1991). It has been shown that NCR-631 effectively protected organotypic hippocampal cultures from LPS and interleukin 1 beta (IL-1β)-induced cell death (Luthman et al. 1998). In addition, pre-treatment of mice and rats with NCR-631 reduced severity of pentylenetetrazol (PTZ)-induced seizures (Luthman 2000). Similar observation was made in DBA/2J mice. In these animals, pre-treatment with NCR-631 30 or 15 min before sound-induced seizures, exhibited an anti-convulsive effect. However, therapeutic effects of NCR-631 warn off quickly. 60 min after drug administration seizures were comparable with vehicle-treated animals (Luthman 2000). Thus, in the long-term pharmacological intervention into 3-HAA metabolism may be challenging. Therefore, modulation of *Haao* gene expression could be more effective strategy.

However, not much is known about the regulation of *Haao* gene expression. Nonetheless, Huang et al. (2010) have demonstrated that the *Haao* gene promoter is hypermethylated in endometrioid endometrial carcinoma (Huang et al. 2010). As a result of this epigenetic modification, *Haao* gene expression is lost in carcinous tissues (Huang et al. 2010). Also, hypermethylated *Haao* gene has been proposed as a biomarker of ovarian cancer (Huang et al. 2009). Epigenetic modifications of the *Haao* gene were also found to result in loss of function of this gene (Huang et al. 2010). Thus, pharmacological intervention into

epigenetic mechanisms regulating *Haao* gene expression may be effective for regulation of 3-HAA concentration.

Concluding remarks

Tryptophan metabolism via the kynurenine pathway is an example of how metabolism of small molecules can impact on the immune system. Based on the cytotoxic mechanisms utilised by the individual tryptophan metabolites, new therapeutic strategies could be designed. For example: tranilast (*N*-3′4′-dimethoxycinnamoyl) anthranilic acid, commercially known as Rizaben, is an anti-allergic drug derived from AA. In addition to its anti-asthmatic and allergic properties, tranilast effectively ameliorated clinical symptoms of experimental autoimmune encephalomyelitis (EAE) (Platten et al. 2005) and collagen induced arthritis (CIA) (Inglis et al. 2007). EAE is an animal model of multiple sclerosis, whereas CIA is an animal model of rheumatoid arthritis. Thus, chemical derivates of AA could be useful as therapeutic agents. Therefore, other tryptophan metabolites like 3-HK and 3-HAA may serve as leading compounds for synthesis of new drugs. In addition, understanding the subsequent steps on the kynurenine pathway and physiological mechanisms responsible for regulation of kynurenine and its metabolites concentration in biological fluids may be important for future drug development.

Conflict of interest The authors declare that they have no conflict of interest.

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