International Journal of Tryptophan Research

REVIEW

Exploitation of the IDO Pathway in the Therapy of Rheumatoid Arthritis

Richard O. Williams

Kennedy Institute of Rheumatology, University of Oxford, UK.
Corresponding author email: richard.o.williams@kennedy.ox.ac.uk

Abstract: Indoleamine 2,3-dioxygenase (IDO) is the first and rate-limiting step along the kynurenine pathway and is thought to play a key role in immune homeostasis through depletion of tryptophan and accumulation of kynurenines. In this review we summarize recent research into the possibility of harnessing the IDO pathway for the therapy of rheumatoid arthritis. Inhibition of IDO activity, or knockout of the gene encoding IDO, was shown to cause an increase in the severity of collagen-induced arthritis, an animal model of rheumatoid arthritis. The increased severity of disease was associated with elevated numbers of pathogenic Th1 and Th17 cells in the joints and draining lymph nodes. In another study, analysis of the kinetics of expression of downstream kynurenine pathway enzymes during the course of arthritis revealed a potential role for tryptophan metabolites in resolution of arthritis. Furthermore, the therapeutic administration of L-kynurenine or [3,4-dimethoxycinnamonyl]-anthranilic acid (a synthetic derivative of 3-hydroxy-anthranilic acid) significantly reduced both clinical and histological progression of experimental arthritis. These findings raise the possibility of exploiting the IDO pathway for the therapy of autoimmune disease.

Keywords: rheumatoid arthritis, indoleamine 2,3-dioxygenase, animal models

International Journal of Tryptophan Research 2013:6 (Suppl. 1) 67–73

doi: 10.4137/IJTR.S11737

This article is available from http://www.la-press.com.

Introduction

The degradation of the essential amino acid, tryptophan, by indoleamine 2,3-dioxygenase (IDO) represents the first and rate-limiting step along the kynurenine pathway in extrahepatic tissues. IDO is encoded by an evolutionarily ancient gene that predates the adaptive immune system but nevertheless has acquired a key role in immune homeostasis.[1] Activation of IDO causes localized depletion of tryptophan, resulting in activation of the stress kinase, GCN2, and the accumulation of pro-apoptopic kynurenines.[2] In addition, the identification of kynurenine as a ligand for the aryl hydrocarbon receptor (AHR), which influences gene expression during immune cell differentiation, has raised interest in the immunomodulatory consequences of IDO activation.[3,4] In this review, recent research is summarized into the possibility of harnessing the IDO pathway for the therapy of autoimmune disease, with a particular focus on rheumatoid arthritis (RA).

Rheumatoid Arthritis

RA is a chronic, inflammatory disease that principally targets synovial joints. The disease is usually progressive and results in swelling of joints, pain and stiffness, with ankylosis developing in many cases.[5] Other features include fatigue, anaemia and flu-like symptoms. RA has a worldwide prevalence of approximately 1% and is one of the most common causes of disability in the western world.[6] The age of onset is typically 25–50, but it can occur at any age. The disease involves inflammation of the capsule surrounding the joints, hyperplasia of synovial cells, oedema and fibrosis.[5] The pathology of the disease process frequently causes destruction of articular cartilage and ankylosis of the joints. In addition, RA can cause subcutaneous nodular lesions and can result in inflammation in the lungs, pericardium, pleura and sclera.[7] Although the etiology of the disease is unknown, it is widely assumed that autoimmune processes play a major role in the initiation and/or perpetuation of the disease.

Genetic factors play an important role in determining susceptibility to RA and data from twin studies, in which concordance is approximately 15%,[8] suggests that the genetic contribution to disease susceptibility is around 50%. Further analysis has revealed a strong association between susceptibility to RA and MHC class II molecules. Thus, the association between RA susceptibility and specific HLA DRB1 alleles has been demonstrated in a number of different populations around the world.[9] More recently, genome-wide association studies have identified a number non-HLA RA susceptibility loci which associate with RA, including *AFF3*, *KIF5A*, *PTPN22*, *CTLA4*, *CD40*, 6q23, *TRAF1*/*C5* and *STAT4*, many of which are involved in lymphocyte signaling.[10] The importance of immune cells in RA is further emphasized by the presence of large numbers of $CD4^+$ T cells in the joints of RA patients.

Animal Models of Arthritis

Animal models of RA have been used widely for preclinical testing of novel therapies, analyses mechanisms of drug action, identification of both pro- and anti-inflammatory mediators, and the analyses of genetic susceptibility factors. Of the various models, collagen-induced arthritis (CIA) has been widely studied, largely on based on its pathological similarities to RA.[11,12] Like RA, susceptibility to CIA is strongly linked to MHC class II genes, with susceptibility being mainly restricted to mouse strains bearing MHC types $I\text{-}A^q$ and $I\text{-}A^r$. Most gene knockout strains of mice are on a C57Bl/6 background ($H\text{-}2^b$), which is generally regarded to be resistant to CIA. Hence, one way to study the impact of a gene deletion is to back-cross the knockout strain onto the DBA/1 background but this is costly and time-consuming. However, it has recently been shown that it is possible to induce arthritis in C57Bl/6 mice,[13–15] which greatly improves the opportunities to use the model for studying disease pathogenesis. Furthermore, CIA in C57Bl/6 mice is a relatively chronic form of arthritis with a more sustained T cell response than the conventional CIA model in DBA/1 mice.[16]

Activation of the Kynurenine Pathway in RA

As discussed above, there is abundant evidence of immune cell hyper-activation in RA. This raises the question of whether there is corresponding activation the IDO pathway, in an attempt to regulate uncontrolled T cell responses. In an early study, the plasma tryptophan concentration of 13 long-standing RA patients was found to be lower than that of controls whilst the urinary excretion of the tryptophan

metabolites, kynurenine, xanthurenic acid and 3-hydroxyanthranilic acid, was increased.[17] More recently, the concentrations of tryptophan, kynurenine, and neopterin were measured by HPLC in the sera of 38 patients with RA. Tryptophan concentrations were lower in patients with RA compared to healthy blood donors whilst levels of kynurenine in patients did not differ significantly from controls.[18] The kynurenine/tryptophan ratio was higher in RA patients than in controls and the levels of kynurenine, as well as the kynurenine/tryptophan ratio, correlated with levels of neopterin (a marker of immune cell activation). Further evidence of kynurenine pathway activation is provided by the finding of reduced baseline levels of tryptophan, 3-hydroxykynurenine and 3-hydroxyanthranilic acid and increased levels of kynurenine and xanthurenic acid in RA patients compared to healthy controls.[19] Similarly, tryptophan concentrations were found to be lower in 22 patients with RA compared to healthy controls.[20] These findings confirm that there is activation of the kynurenine pathway in RA.

The Role of IDO in RA: Lessons from Animal Models

Analysis of the levels of IDO mRNA transcripts by quantitative RT-PCR in the spleen, lymph nodes and paws of mice during the course of CIA revealed a significant increase after arthritis onset in lymph nodes, but not spleens or paws.[21] Further analysis revealed that IDO expression in lymph nodes was mainly confined to dendritic cells.

To further understand the role of IDO in arthritis, we evaluated the progression of CIA in DBA/1 mice treated with the IDO inhibitor, 1-methyl tryptophan (1-MT). Administration of 1-MT after disease onset increased the severity of CIA, as demonstrated by the clinical score assessment and the measurement of paw thickness.[21] These findings are in agreement with those of Szanto et al[22] who showed that inhibition of IDO using 1-MT increased the severity of CIA and enhanced humoral and cellular immune responses.[22]

A more comprehensive assessment of the role of IDO in arthritis was subsequently carried out by comparing the progression of CIA in IDO deficient (*Indo*$^{-/-}$) mice versus wild-type mice.[23] The first finding to emerge was an earlier onset of arthritis in *Indo*$^{-/-}$ mice compared to wild-type C57Bl/6 mice. Clinical severity showed a similar progression in *Indo*$^{-/-}$ and wild-type mice in early arthritis but reached a plateau in wild-type mice and continued to increase in *Indo*$^{-/-}$ mice.[21] Similarly, histological analysis showed increased erosion and cellular infiltration in *Indo*$^{-/-}$ mice. Analysis of cytokine production in collagen-immunized mice revealed higher production of IL-17 and IFNγ in the lymph nodes of *Indo*$^{-/-}$ mice compared to wild-type controls and FACS analysis showed that the frequency of Th1 and Th17 cells in the inflamed paws of *Indo*$^{-/-}$ mice also increased.[21] These findings confirm an important role for IDO in the progression of CIA and in regulating pathogenic Th1/Th17 responses. Similar to our own findings in CIA, it was shown that IDO-deficient mice develop exacerbated experimental autoimmune encephalomyelitis (EAE), associated with increased encephalitogenic Th1 and Th17 cell responses and reduced regulatory T cell responses.[24]

An alternative approach to inducing IDO activity that has been tested in CIA is the administration of adenoviral vectors encoding IDO (AdIDO). A reduction in both clinical and histological severity was noted in AdIDO-treated animals, as well as decreased numbers of infiltrating $CD4^+$ T cells, $CD68^+$ macrophages. There was also a reduction in Th17 cell activity, as evidenced by decreased IL-17 RORyt expression within the joints and draining lymph nodes of CIA rats.[25]

In contrast to studies in CIA, studies in the K/BxN model of RA showed that administration of 1-MT resulted in amelioration of arthritis due to a reduced level of autoreactive B cell activity.[26] Further analysis revealed that the principal effect of 1-MT was to inhibit the ability of autoreactive B cells to differentiate into autoantibody-secreting cells, but did not influence their initial activation or survival.[27] These findings clearly challenge the concept of a purely immunosuppressive role for IDO and merit further investigation.

Kinetics of Expression of Kynurenine Pathway Enzymes in Arthritis

CIA in DBA/1 mice is a self-remitting disease with a well-defined induction and resolution phase.[28] In order to gain a better understanding of the role played by the IDO pathway induction and resolution of arthritis, a study was carried out to measure the levels of tryptophan and its catabolites during the induction

and resolution phases of CIA, as well as the kinetics of expression of kinurenine pathway enzymes.[29] It was known from previous studies that changes in IDO expression during arthritis were largely confined to the draining (inguinal) lymph nodes[21] and on this basis it was predicted that most changes in kynurenine pathway enzymes would also take place in draining lymph nodes.

The tryptophan concentration in lymph nodes decreased progressively during the development and resolution of arthritis. This was paralleled by a corresponding increase in levels of kynurenine, indicating activation of IDO.[29] Measurement of the downstream kynurenine metabolites in lymph nodes revealed an accumulation of anthranilic acid, and 3-hydroxyanthranilic acid only during the resolution of arthritis.[29] This raises the possibility that the downstream kynurenine metabolites play an active role in disease resolution. It is known, for example, that 3-hydroxyanthranilic possesses potent inhibitory effects on T cell responses.[30]

Analysis of the expression levels of the enzymes along the kynurenine pathway also uncovered an interesting pattern which may explain the accumulation of these downstream metabolites during the resolution phase of arthritis. Thus, expression levels of the early enzymes along the pathway (IDO-1, IDO-2 and formamidase) were elevated throughout the development of arthritis, including induction and resolution phases.[29] In contrast, expression levels of genes involved in the catabolism of kynurenines (kynurenine 3-monooxygenase, kynureninase and 3-hydroxyanthranilate 3,4 dioxygenase) increased during the induction phase of arthritis but decreased during the resolution phase of arthritis.[29] The decrease in the expression of these enzymes prior to the period of disease resolution may be important for the accumulation of key metabolites involved in resolution of arthritis in this model.

Modulation of Immune and Inflammatory Responses by Kynurenines

As stated above, IDO activation results in tryptophan depletion as well as the accumulation of tryptophan metabolites, such as kynurenine (reviewed by Stone et al[31]). In order to determine whether kynurenine itself would be therapeutically effective in CIA, we evaluated the progression of CIA in mice treated with L-kynurenine (200 mg/kg) or vehicle alone after arthritis onset. Treatment with L-kynurenine significantly reduced both clinical and histological progression of arthritis,[21] confirming the potential of kynurenine to modify the course of arthritis. However, it remains to be established whether kynurenine itself, or one of its metabolites, is responsible for the therapeutic effect observed in CIA. For example, it has previously been shown that kynurenine loading leads to increased blood levels of kynurenic acid which may be neuroprotective.[32,33]

As discussed above, kynurenine has recently been identified as a ligand for the AHR, which is important in the detection of foreign substances and in the maturation of immune cells.[3,4] Specifically, ligation of the AHR appears to promote anti-inflammatory and tolerogenic cell phenotypes. For example, co-cultures of AHR deficient dendritic cells with naïve T cells inhibited the differentiation of regulatory T cells.[3] In contrast, the addition of kynurenine promoted the differentiation of regulatory T cells and suppressed the differentiation of pathogenic Th17 cells.[3] AHRs are also expressed on naïve $CD4^+$ T cells and play a direct role in the differentiation of Foxp3 expressing regulatory T cells. However, an alternative view has been presented by Stephens et al[34] who showed that Th17 cells preferentially express kynurenine 3-monooxygenase, which is involved in catabolism of kynurenine. Importantly, inhibition of kynurenine 3-monooxygenase, using a specific inhibitor or by siRNA-mediated silencing, led to increased IL-17 production in vitro without affecting IFN-γ production. Further studies revealed that inhibition of kynurenine 3-monooxygenase led to exacerbation of Th17-mediated gastritis in vivo, suggesting that kynurenine 3-monooxygenase plays a regulatory role in limiting Th17 responses by reducing levels of kynurenine.[34] Clearly, further work is needed to unravel these questions.

In addition to kynurenine itself, a number of kynurenine metabolites also have the potential to influence inflammatory responses. For example, kynurenic acid has been identified as a ligand of the orphan G-protein-coupled receptor GPR35. GPR35 is expressed in a variety of immune cells and appears to play an important role in monocyte extravasation.[35] In contrast, the interaction of kynurenic acid with GPR35 inhibits lipoloysaccharide-driven TNF production.[36]

Another downstream tryptophan metabolite, 3-hydroxyanthranillic acid, was shown to enhance the percentage of regulatory T cells, inhibit Th1 and Th17 cells, and ameliorate EAE.[24] 3-hydroxyanthranillic acid has also been shown to inhibit the generation of nitric oxide synthase in macrophages[37] and to promote the production of TGFβ. 3-hydroxyanthranillic acid also has a direct inhibitory effect on Th1 cells whilst sparing the activity of Th2 cells.[38–40] In addition, a metabolite of 3-hydroxyanthranillic acid, cinnabarinic acid, is a potent inducer of apoptosis in T cells.[41]

Tranilast (N-[3,4-dimethoxycinnamonyl]-anthranilic acid) is a clinically approved, synthetic derivative of 3-hydroxyanthranillic acid that has been known for many years as an anti-allergic drug. Tranilast had recently shown to be effective in a murine model of multiple sclerosis[42] and we therefore assessed its therapeutic potential in CIA, as a model for RA. Administration of tranilast after arthritis onset reduced clinical and histological severity of disease. Tranilast also completely abrogated thermal and mechanical hyperalgesia and reduced Th1 cell activity in lymph node cell cultures whilst raising serum levels of IL-10.[43] Tranilast suppressed IFNγ production and proliferation of both T and B lymphocytes in vitro, in a manner comparable to the endogenous tryptophan metabolite, 3-hydroxyanthranilic acid, suggesting similar mechanisms of action.

These findings were subsequently confirmed in another study of CIA in mice[44] and of adjuvant-induced arthritis and streptococcal cell wall-induced arthritis in rats.[44] It was subsequently shown that tranilast inhibits STAT1 phosphorylation in activated human T cells, leading to down-regulation of CXCL9 and CXCL10.[45] It was concluded that tranilast has both anti-inflammatory and analgesic properties, and may therefore be useful in the treatment of RA.[43]

Future Perspectives

It is clear that the IDO pathway plays an important regulatory role in the immune system and possibilities for manipulation of this pathway within the context of autoimmune disease include the development of synthetic analogues of tryptophan metabolites as the use of endogenous metabolites themselves is limited by their short half-life in vivo. Activation of the kynurenine pathway is an attractive therapeutic approach for autoimmune disease but progress towards this aim has been hampered by the absence of a viable strategy to induce IDO activity in vivo. It is well-known that IDO gene expression is promoted by IFNγ but more recently it has been shown that epigenetic events, in particular DNA methylation, inhibit IDO expression.[46] Furthermore, an elegant study by Xue et al[46] has shown that the DNA methylation inhibitor, zebularine, acts synergistically with IFNγ in inducing IDO expression.[46] As IFNγ is overexpressed at sites of disease activity in RA, it follows that administration of zebularine at an appropriate dose may allow for tissue specificity in the induction of IDO. Another possible route to drug development would be the development of inhibitors of the downstream enzymes along the kynurenine pathway, although the overall consequences of manipulating this pathway in vivo will need to be thoroughly explored.

Author Contributions

Wrote the first draft of the manuscript: ROW. Made critical revisions: ROW. The author reviewed and approved of the final manuscript.

Funding

This work was supported by The Kennedy Institute of Rheumatology Trustees and Arthritis Research UK.

Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the author has provided signed confirmation of compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

References

1. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol*. Oct 2004;4(10):762–74.
2. Munn DH, Sharma MD, Baban B, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity*. May 2005;22(5):633–42.
3. Nguyen NT, Kimura A, Nakahama T, et al. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. *Proc Natl Acad Sci U S A*. Nov 16, 2010;107(46):19961–6.
4. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J Immunol*. Sep 15, 2010;185(6): 3190–8.
5. Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. *Cell*. May 3, 1996;85(3):307–10.
6. Emery P, Salmon M. Early rheumatoid arthritis: time to aim for remission? *Ann Rheum Dis*. Dec 1995;54(12):944–7.
7. Prete M, Racanelli V, Digiglio L, Vacca A, Dammacco F, Perosa F. Extra-articular manifestations of rheumatoid arthritis: An update. *Autoimmun Rev*. Dec 2011;11(2):123–31.
8. Silman AJ, MacGregor AJ, Thomson W, et al. Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *Br J Rheumatol*. Oct 1993;32(10):903–7.
9. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res*. 2002;4 Suppl 3:S265–72.
10. Plant D, Flynn E, Mbarek H, et al. Investigation of potential non-HLA rheumatoid arthritis susceptibility loci in a European cohort increases the evidence for nine markers. *Ann Rheum Dis*. Aug 2010;69(8):1548–53.
11. Holmdahl R, Andersson ME, Goldschmidt TJ, et al. Collagen induced arthritis as an experimental model for rheumatoid arthritis. Immunogenetics, pathogenesis and autoimmunity. *APMIS*. 1989;97:575–84.
12. Trentham DE. Collagen arthritis as a relevant model for rheumatoid arthritis: evidence pro and con. *Arthritis Rheum*. 1982;25:911–6.
13. Campbell IK, Hamilton JA, Wicks IP. Collagen-induced arthritis in C57BL/6 (H-2b) mice: new insights into an important disease model of rheumatoid arthritis. *Eur J Immunol*. Jun 2000;30(6):1568–75.
14. Inglis JJ, Notley CA, Essex D, et al. Collagen-induced arthritis as a model of hyperalgesia: functional and cellular analysis of the analgesic actions of tumor necrosis factor blockade. *Arthritis Rheum*. Dec 2007;56(12):4015–23.
15. Inglis JJ, Simelyte E, McCann FE, Criado G, Williams RO. Protocol for the induction of arthritis in C57BL/6 mice. *Nat Protoc*. 2008;3:612–8.
16. Inglis JJ, Criado G, Medghalchi M, et al. Collagen-Induced Arthritis in C57BL/6 mice is associated with a robust and sustained T-cell response to type II collagen. *Arthritis Res Ther*. 2007;9:R113.
17. Labadarios D, McKenzie DY, Dickerson JW, Parke DV. Metabolic abnormalities of tryptophan and nicotinic acid in patients with rheumatoid arthritis. *Rheumatol Rehabil*. Nov 1978;17(4):227–32.
18. Schroecksnadel K, Kaser S, Ledochowski M, et al. Increased degradation of tryptophan in blood of patients with rheumatoid arthritis. *J Rheumatol*. Sep 2003;30(9):1935–9.
19. Forrest CM, Kennedy A, Stone TW, Stoy N, Darlington LG. Kynurenine and neopterin levels in patients with rheumatoid arthritis and osteoporosis during drug treatment. *Adv Exp Med Biol*. 2003;527:287–95.
20. Schroecksnadel K, Winkler C, Duftner C, Wirleitner B, Schirmer M, Fuchs D. Tryptophan degradation increases with stage in patients with rheumatoid arthritis. *Clin Rheumatol*. May 2006;25(3):334–7.
21. Criado G, Simelyte E, Inglis JJ, Essex D, Williams RO. Indoleamine 2,3 dioxygenase-mediated tryptophan catabolism regulates accumulation of Th1/Th17 cells in the joint in collagen-induced arthritis. *Arthritis Rheum*. May 2009;60(5):1342–1351.
22. Szanto S, Koreny T, Mikecz K, Glant TT, Szekanecz Z, Varga J. Inhibition of indoleamine 2,3-dioxygenase-mediated tryptophan catabolism accelerates collagen-induced arthritis in mice. *Arthritis Res Ther*. 2007;9(3):R50.
23. Baban B, Chandler P, McCool D, Marshall B, Munn DH, Mellor AL. Indoleamine 2,3-dioxygenase expression is restricted to fetal trophoblast giant cells during murine gestation and is maternal genome specific. *J Reprod Immunol*. Apr 2004;61(2):67–77.
24. Yan Y, Zhang GX, Gran B, et al. IDO upregulates regulatory T cells via tryptophan catabolite and suppresses encephalitogenic T cell responses in experimental autoimmune encephalomyelitis. *J Immunol*. Nov 15, 2010;185(10): 5953–61.
25. Chen SY, Wu CL, Lai MD, et al. Amelioration of rat collagen-induced arthritis through CD4+ T cells apoptosis and synovial interleukin-17 reduction by indoleamine 2,3-dioxygenase gene therapy. *Hum Gene Ther*. Feb 2011;22(2):145–54.
26. Scott GN, DuHadaway J, Pigott E, et al. The immunoregulatory enzyme IDO paradoxically drives B cell-mediated autoimmunity. *J Immunol*. Jun 15, 2009;182(12):7509–17.
27. Pigott E, Mandik-Nayak L. Addition of an indoleamine 2,3,-dioxygenase inhibitor to B cell-depletion therapy blocks autoreactive B cell activation and recurrence of arthritis in K/BxN mice. *Arthritis Rheum*. Jul 2012;64(7):2169–78.
28. Mauri C, Williams RO, Walmsley M, Feldmann M. Relationship between Th1/Th2 cytokine patterns and the arthritogenic response in collagen-induced arthritis. *Eur J Immunol*. 1996;26:1511–8.
29. Kolodziej L. An exploratory study of the interplay between decreased concentration of tryptophan, accumulation of kynurenines, and inflammatory arthritis. *IUBMB Life*. Dec 2012;64(12):983–7.
30. Bauer TM, Jiga LP, Chuang JJ, Randazzo M, Opelz G, Terness P. Studying the immunosuppressive role of indoleamine 2,3-dioxygenase: tryptophan metabolites suppress rat allogeneic T-cell responses in vitro and in vivo. *Transpl Int*. Jan 2005;18(1):95–100.
31. Stone TW, Stoy N, Darlington LG. An expanding range of targets for kynurenine metabolites of tryptophan. *Trends Pharmacol Sci*. Feb 2013;34(2): 136–43.
32. Nozaki K, Beal MF. Neuroprotective effects of L-kynurenine on hypoxia-ischemia and NMDA lesions in neonatal rats. *J Cereb Blood Flow Metab*. May 1992;12(3):400–7.
33. Kumar A, Babu GN. In vivo neuroprotective effects of peripheral kynurenine on acute neurotoxicity induced by glutamate in rat cerebral cortex. *Neurochem Res*. Apr 2010;35(4):636–44.
34. Stephens GL, Wang Q, Swerdlow B, Bhat G, Kolbeck R, Fung M. Kynurenine 3-monooxygenase mediates inhibition of Th17 differentiation via catabolism of endogenous aryl hydrocarbon receptor ligands. *Eur J Immunol*. Apr 9, 2013.
35. Barth MC, Ahluwalia N, Anderson TJ, et al. Kynurenic acid triggers firm arrest of leukocytes to vascular endothelium under flow conditions. *J Biol Chem*. Jul 17, 2009;284(29):19189–95.
36. Tiszlavicz Z, Nemeth B, Fulop F, et al. Different inhibitory effects of kynurenic acid and a novel kynurenic acid analogue on tumour necrosis factor-alpha (TNF-alpha) production by mononuclear cells, HMGB1 production by monocytes and HNP1-3 secretion by neutrophils. *Naunyn Schmiedebergs Arch Pharmacol*. May 2011;383(5):447–55.
37. Sekkai D, Guittet O, Lemaire G, Tenu JP, Lepoivre M. Inhibition of nitric oxide synthase expression and activity in macrophages by 3-hydroxyanthranilic acid, a tryptophan metabolite. *Arch Biochem Biophys*. Apr 1, 1997;340(1): 117–23.
38. Munn DH. Indoleamine 2,3-dioxygenase, Tregs and cancer. *Curr Med Chem*. 2011;18(15):2240–6.
39. Hill M, Tanguy-Royer S, Royer P, et al. IDO expands human CD4+CD25 high regulatory T cells by promoting maturation of LPS-treated dendritic cells. *Eur J Immunol*. Nov 2007;37(11):3054–62.
40. Fallarino F, Grohmann U, Vacca C, et al. T cell apoptosis by tryptophan catabolism. *Cell Death Differ*. Oct 2002;9(10):1069–1077.
41. Hiramatsu R, Hara T, Akimoto H, et al. Cinnabarinic acid generated from 3-hydroxyanthranilic acid strongly induces apoptosis in thymocytes through the generation of reactive oxygen species and the induction of caspase. *J Cell Biochem*. Jan 1, 2008;103(1):42–53.
42. Platten M, Ho PP, Youssef S, et al. Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. *Science*. Nov 4, 2005; 310(5749):850–5.
43. Inglis JJ, Criado G, Andrews M, Feldmann M, Williams RO, Selley ML. The anti-allergic drug, N-(3′,4′-dimethoxycinnamonyl) anthranilic acid, exhibits potent anti-inflammatory and analgesic properties in arthritis. *Rheumatology (Oxford)*. Sep 2007;46(9):1428–32.

44. Nagate T, Tamura T, Sato F, Kuroda J, Nakayama J, Shibata N. Tranilast suppresses the disease development of the adjuvant- and streptococcal cell wall-induced arthritis in rats. *J Pharmacol Sci*. Sep 2007;105(1):48–56.
45. Hertenstein A, Schumacher T, Litzenburger U, et al. Suppression of human CD4+ T cell activation by 3,4-dimethoxycinnamonyl-anthranilic acid (tranilast) is mediated by CXCL9 and CXCL10. *Biochem Pharmacol*. Sep 15, 2011;82(6):632–41.
46. Xue ZT, Sjogren HO, Salford LG, Widegren B. An epigenetic mechanism for high, synergistic expression of indoleamine 2,3-dioxygenase 1 (IDO1) by combined treatment with zebularine and IFN-gamma: potential therapeutic use in autoimmune diseases. *Mol Immunol*. Jun 2012;51(2):101–11.