Exploitation of the IDO Pathway in the Therapy of Rheumatoid Arthritis

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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

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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. Activation of IDO causes localized depletion of tryptophan, resulting in activation of the stress kinase, GCN2, and the accumulation of pro-apoptotic kynurenines. 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. 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. 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. 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. 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. 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%, 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. More recently, genome-wide association studies have identified a number of 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. 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 pre-clinical 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 the basis of its pathological similarities to RA. 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-A<sup>d</sup> and I-A<sup>q</sup>. Most gene knockout strains of mice are on a C57Bl/6 background (H-2<sup>b</sup>), 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, 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.

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.\textsuperscript{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 whereas levels of kynurenine in patients did not differ significantly from controls.\textsuperscript{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.\textsuperscript{19} Similarly, tryptophan concentrations were found to be lower in 22 patients with RA compared to healthy controls.\textsuperscript{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.\textsuperscript{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.\textsuperscript{21} These findings are in agreement with those of Szanto et al\textsuperscript{22} who showed that inhibition of IDO using 1-MT increased the severity of CIA and enhanced humoral and cellular immune responses.\textsuperscript{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\textsuperscript{−/−}) mice versus wild-type mice.\textsuperscript{23} The first finding to emerge was an earlier onset of arthritis in Indo\textsuperscript{−/−} mice compared to wild-type C57Bl/6 mice. Clinical severity showed a similar progression in Indo\textsuperscript{−/−} and wild-type mice in early arthritis but reached a plateau in wild-type mice and continued to increase in Indo\textsuperscript{−/−} mice.\textsuperscript{21} Similarly, histological analysis showed increased erosion and cellular infiltration in Indo\textsuperscript{−/−} mice. Analysis of cytokine production in collagen-immunized mice revealed higher production of IL-17 and IFN\textgamma in the lymph nodes of Indo\textsuperscript{−/−} mice compared to wild-type controls and FACS analysis showed that the frequency of Th1 and Th17 cells in the inflamed paws of Indo\textsuperscript{−/−} mice also increased.\textsuperscript{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.\textsuperscript{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\textsuperscript{+} T cells, CD68\textsuperscript{+} macrophages. There was also a reduction in Th17 cell activity, as evidenced by decreased IL-17 ROR\textgamma expression within the joints and draining lymph nodes of CIA rats.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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 kynurenine pathway enzymes.\textsuperscript{29} It was known from previous studies that changes in IDO expression during arthritis were largely confined to the draining (inguinal) lymph nodes\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{29} In contrast, expression levels of genes involved in the catabolism of kynurenines (kynurenine 3-monooxygenase, kynureninate and 3-hydroxyanthranilate 3,4 dioxygenase) increased during the induction phase of arthritis but decreased during the resolution phase of arthritis.\textsuperscript{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\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{3} In contrast, the addition of kynurenine promoted the differentiation of regulatory T cells and suppressed the differentiation of pathogenic Th17 cells.\textsuperscript{3} AHRs are also expressed on naïve CD4\textsuperscript{+} 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\textsuperscript{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-\(\gamma\) 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.\textsuperscript{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.\textsuperscript{35} In contrast, the interaction of kynurenic acid with GPR35 inhibits lipolysaccharide-driven TNF production.\textsuperscript{36}
Another downstream tryptophan metabolite, 3-hydroxyanthranilic acid, was shown to enhance the percentage of regulatory T cells, inhibit Th1 and Th17 cells, and ameliorate EAE. 24 3-hydroxyanthranilic acid has also been shown to inhibit the generation of nitric oxide synthase in macrophages 37 and to promote the production of TGFβ. 3-hydroxyanthranilic 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-hydroxyanthranilic 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-hydroxyanthranilic 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 over-expressed 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.

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**Competing Interests**

Author(s) disclose no potential conflicts of interest.

**Disclosures and Ethics**

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