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Indoleamine 2,3-dioxygenase as a Modifier of Pathogenic Inflammation in Cancer and other Inflammation-Associated Diseases

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Abstract

Chronic inflammation underlies the basis for development and progression of cancers and a variety of other disorders, but what specifically defines its pathogenic nature remains largely undefined. Recent genetic and pharmacological studies in the mouse suggest that the immune modulatory enzyme indoleamine 2,3-dioxygenase (IDO), identified as an important mediator of immune escape in cancer, can also contribute to the development of pathology in the context of chronic inflammatory models of arthritis and allergic airway disease. IDO-deficient mice do not display spontaneous disorders of classical inflammation and small molecule inhibitors of IDO do not elicit generalized inflammatory reactions. Rather, in the context of a classical model of skin cancer that is promoted by chronic inflammation, or in models of inflammation-associated arthritis and allergic airway disease, IDO impairment can alleviate disease severity. Here we offer a survey of preclinical literature suggesting that IDO functions as a modifier of inflammatory states rather than simply as a suppressor of immune function. We propose that IDO induction in a chronically inflamed tissue may shape the inflammatory state to support, or in some cases retard, pathogenesis and disease severity.

Keywords

Immunosuppression; immunoediting; immune escape; IDO; IDO2

CHRONIC INFLAMMATION IN DISEASE

Inflammation is an important contributory factor in the development and progression of many diseases, such as cancer, asthma and arthritis. Moreover, the generalized increase in inflammation that occurs with aging is thought to be an important contributory factor in age-

associated diseases like cancer. Although chronic inflammation is not invariably associated with increased disease risk or progression, in many individuals the connection is clear. What specific factors dictate the manifestation of pathogenic inflammation in some individuals but not others? Diverse studies of the mediators of inflammation and adaptive or innate immunity have suggested the existence of genetic and possibly epigenetic predispositions to disease, for example, on the basis of associations between disease incidence, progression, severity, or outcome with the presence of single nucleotide polymorphisms or other genetic variants in an individual that impact immune-based inflammatory responses (e.g. IL-1 β in lung cancer susceptibility [1]). In this review, we survey preclinical studies of indoleamine 2,3-dioxygenase in the literature that suggest a role in determining the pathogenicity of chronic inflammatory states which impacts the incidence or severity of cancer and other inflammation-associated diseases.

IDO IN IMMUNE MODULATION

The tryptophan catabolic enzyme indoleamine 2,3-dioxygenase (IDO or IDO1) mediates immune tolerance in a variety of physiological and pathological settings (reviewed in ref. [2]). This single chain oxidoreductase initiates tryptophan degradation to produce downstream products referred to in aggregate as kynurenines, constituting the first step in the *de novo* biosynthesis of nicotinamide adenine dinucleotide (NAD). IDO1 does not handle dietary tryptophan catabolism and NAD levels in mammals are maintained by salvage rather than synthesis. Thus, the role of IDO1 in mammalian cells was generally unclear before seminal studies of Munn, Mellor and colleagues who correlated IDO activity with T cell suppression in the context of pregnancy [3]. IDO1 is expressed in antigen-presenting cells in lymph nodes, where an immunosuppressive function has been suggested. T cells exhibit sensitivity to tryptophan deprivation and to inhibitory effects of kynurenines, both of which blunt T cell activation. Through local tryptophan starvation, IDO1 can stimulate a Gcn2-induced stress signaling pathway in T cells, blocking their activation and upregulating translation of the NF-IL6/CEBP β transcription factor isoform LIP, thereby altering expression of the immune modulatory factors IL-6, TGF- β , and IL-10 [4, 5]. In addition to directly suppressing T cell activation, IDO+ pDCs also inform the organization of a population of T regulatory cells (Tregs) by inducing naïve CD4+ T cells to differentiate to FoxP3+ Tregs, as well as by activating resting natural Tregs to become potentially suppressive [6, 7]. Notably, IDO is sufficient to convert pDCs from a T cell-activating to a T cell-suppressing phenotype [8]. Interestingly, Tregs have been shown to induce IDO in pDCs through CTLA-4-mediated reverse signaling [9–11], suggesting that communication between IDO+ pDCs and Tregs may comprise a positive feedback loop for immunosuppression. In an initial dramatic illustration of how IDO can mediate local immunosuppression to neoantigens, administration of the bioactive IDO inhibitor 1-methyl-tryptophan (1MT) was shown to trigger MHC-restricted T cell-mediated rejection of allogeneic pregnancies by revealing ‘foreign’ paternal antigens to the maternal immune system [3].

IDO1 is expressed ubiquitously but at relatively higher levels in lung, gut, epididymis, and thymus. It is inducible by virus, lipopolysaccharide, and interferons [12]. In addition to catabolizing tryptophan, IDO1 can cleave other indole-containing compounds such as

serotonin, tryptamine, and 5-hydroxytryptophan. The IDO1 enzyme is composed of a two-domain structure of alpha-helical domains with a centrally located heme group [13], which is required for catalytic activity along with an electron donor such as a flavin or cytochrome [14]. The human *IDO1* gene comprises 10 exons spanning ~15 kb at chromosome 8p12 that encodes the 403 amino acid IDO1 polypeptide [15, 16]. Genetic variability may contribute to differences in IDO activity between individuals, based on the identification of IDO1 variants including non-synonymous single nucleotide polymorphisms in coding exons 1 and 3, at least one of which compromises IDO activity [17].

Recently IDO2 was discovered as the product of a novel IDO gene termed *IDO2* or *INDOL1* [4, 18]. The human *IDO2* gene is located immediately downstream of *IDO1* on chromosome 8p12. Both the human and mouse *IDO2* genes span 11 exons in an ~74 kb genomic region, encoding IDO2 proteins of 420 and 405 amino acids, respectively. Expression of *IDO2* is much narrower than *IDO1*, confined mainly to liver, placenta, and antigen-presenting immune cells [4]. Although the IDO1 and IDO2 enzymes do not share a high degree of homology (43% identity), they share amino acids determined by crystallographic and mutagenesis studies to be critical for tryptophan catalytic activity that has been documented for IDO2 [19]. Interestingly, structural considerations of the *IDO2* genes conserved across evolution in different species suggest that *IDO2* is ancestral to *IDO1* [20]. Even more than is the case for *IDO1*, genetic variability contributes to differences in IDO2 activity between individuals based on the presence of non-synonymous single nucleotide polymorphisms in *IDO2* that ablate its activity yet occur commonly in human populations [19]. Indeed, as many as 50% of individuals of European or Asian descent and 25% of individuals of African descent appear to lack functional *IDO2* alleles [19]. The frequent occurrence of inactive genetic variants suggests that there may be some evolutionary benefit in human populations to attenuating IDO2 activity, perhaps reflecting competing selective pressures to maintain immunological responsiveness and tolerance in an optimal balance, in complex environments composed of pathogenic and symbiotic microorganisms.

IDO IN IMMUNE ESCAPE AND INFLAMMATION IN CANCER

IDO1 has gained broad attention as an immunosuppressive enzyme in cancer, based on its frequent upregulation in human tumors and tumor-draining lymph nodes (TDLNs) where it facilitates immune escape [21]. Mouse genetic studies have linked IDO to a tumor suppressor pathway controlled by Bin1, a multifunctional adapter protein that normally inhibits IDO1 expression but is often attenuated in human cancers [22]. Bin1 has also been shown to repress interferon- γ -mediated expression of IDO in myeloid cells [22]. 1MT and other structural classes of IDO inhibitors that have been identified can overcome IDO1-mediated immune escape, stanch tumor growth and synergize with chemotherapy to regress established tumors in diverse mouse models of cancer [22–26]. Other work has established that IDO1 must be present for 1MT and other IDO inhibitors to manifest their antitumor activity [25, 27, 28]. In TDLNs, IDO1 supports the function of regulatory pDCs that drive development of FoxP3⁺ Tregs and tumor growth [7, 29–31]. T cell suppression by TDLN-derived IDO⁺ pDCs relies upon Type I and II interferons and on MyD88 and Gcn2 signaling [28]. In supporting development of Tregs, IDO may also blunt their conversion to Th17 cells that may exert antitumor activity [29, 30].

In contrast to the situation for IDO1, very little is presently known about the possible role of IDO2 in cancer. IDO2 upregulation has been documented in pancreatic carcinomas and TDLNs [32]. In contrast to IDO1, it seems that IDO2 is not commonly overexpressed in cancer, based on *in silico* analyses (G.C.P., unpublished observations). However, this does not rule out the notion that IDO2 may be supportive to cancer. Indeed, some recent studies of the D isomer of 1MT have suggested that its antitumor activities are based on inhibition of IDO2 rather than IDO1 [4, 27].

IDO upregulation in cancer may exert several pathophysiological benefits. Cancer is initiated by cumulative genetic and epigenetic changes to a normal cell, but beyond the cancer cell it is clear that the tissue microenvironment exerts powerful effects in determining progression versus dormancy or destruction of an oncogenically initiated cell. Indeed, loss of microenvironmental controls is probably essential to unleash the invasive and metastatic behaviors which pose the major clinical challenges. Many types of immune cells contribute to tumor suppression. Yet it is also evident that tumor cells can evolve tactics to 'tilt' the immune balance in the tumor microenvironment from antagonistic to supportive for tumor growth. Although an appropriately activated immune system can eradicate cancer in an animal, it is clear that tumors erect barricades to thwart immunity, and there is now widespread recognition that improvement in immunotherapy outcomes will require defeating immune escape mechanisms [33]. Emerging evidence suggests that IDO activity in tumor cells and the tumor microenvironment is one key factor affecting the balance of immunosuppressive and immunostimulatory signals that determine tumor eradication, dormancy, or outgrowth.

Recent work suggests that IDO1 could be a key defining feature of 'cancer-associated' inflammation. Chronic inflammation is a key component in the genesis and progression of many solid tumors. Indeed, the earliest descriptions of cancer histopathology in the 1800s by Virchow and others noted striking hallmarks of inflammation [34]. However, despite the strong evidence linking inflammation and cancer, there remains a gap in knowledge concerning the specific factors that define 'cancer-associated' inflammation as it is distinguished from chronic inflammations that are not associated with cancer [35]. The impact of such a factor would be strengthened by its ability to shed light on how 'cancer-associated' inflammation is integrated with the process of immunoediting, as enunciated by Schreiber and colleagues [36]. IDO1 offers such a candidate, because its activation may permit tumor cells to move toward immune escape as a result of 'tilting' the inflammatory microenvironment toward a more supportive stance.

In support of this concept, other studies have linked IDO1 and cancer-associated inflammation [28, 37]. One study focused on the role of IDO1 in inflammatory carcinogenesis in the skin [28]. Dermal application of the pro-inflammatory phorbol ester PMA was found to induce formation of regulatory IDO+ pDCs in skin-draining lymph nodes. In this setting, where IDO1 connects inflammation to the production of immune suppressive pDCs, it was hypothesized that IDO1 would also connect inflammation to cancer progression where immune escape is pivotal [21, 38, 39]. If IDO1 had a generalized immunosuppressive role in inflammation, its deficiency in mice might alter the course of normal wound healing or the epidermal thickening that occurs after application of pro-

inflammatory agents such as phorbol ester PMA. Alternately, if IDO1 had a more subtle role in contextualizing the inflammatory microenvironment, then IDO1 deficiency in mice would not be expected to affect general processes of inflammation. In fact, there were no apparent differences in the response of IDO-deficient mice to abrasion wounds or topical treatment with PMA. In striking contrast, however, IDO1-deficient mice were resistant to inflammatory skin carcinogenesis involving chronic exposure to PMA [28]. Since IDO1-deficient mice can support the growth of engrafted tumors, IDO1 is not required for tumor growth *per se*. Moreover, IDO-deficient mice are not resistant to carcinogenesis protocols that lack a pro-inflammatory stimulus (A.J.M and G.C.P., unpublished observations). Thus, the link between IDO1 and carcinogenesis was inferred to be derivative of a more proximal link between IDO1 and the generation of a pathogenic inflammation that can support cancer.

IDO1 and ‘cancer-associated’ inflammation have also been linked in mechanistic studies of two anti-inflammatory agents, the simple agent ethyl pyruvate and the COX-2 inhibitor celecoxib. Studies of celecoxib in two murine models of cancer determined that IDO1 inhibition was a critical correlate of this well-documented anticancer compound [40, 41]. More recently, the anti-inflammatory properties of ethyl pyruvate were found to be effective in blocking the outgrowth of skin tumors through a mechanism based on inhibition of IDO1 expression [37]. These studies are notable in that they each link IDO1 more proximally to an inflammatory state(s) that is known to support cancer (on the basis of drug antagonism). In summary, ongoing studies tend to support the concept that elevated IDO activity can alter chronic inflammation to a pathogenic state of ‘cancer-associated’ inflammation that supports malignant development.

IDO IN RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease of unknown etiology. Tryptophan catabolism is elevated in RA patients suggesting the possible involvement of IDO [42]. Two murine models have been used to evaluate the role of IDO in RA. In one model, collagen type II is injected into DBA/1 mice to induce chronic inflammation of the joints that mimics human RA [43]. In this model, IDO restricts the RA phenotype on the basis of its susceptibility to 1MT [43–49]. Results in the collagen model align with the common perspective that IDO plays a suppressive role in inflammatory immune responses. In a second model, K/BxN transgenic mice spontaneously develop a joint inflammatory disease similar to human RA, exhibiting cellular infiltrates, proinflammatory cytokines, autoantibodies, and cartilage and bone destruction [50]. The K/BxN model differs from the collagen type II model in that arthritis occurs spontaneously, without the need for immunization with adjuvants. In this model, IDO supports the RA phenotype, on the basis of its amelioration by 1MT, which decreased autoantibody titers, reduced inflammatory cytokine levels, and attenuated disease course [51]. The alleviation of arthritis was not due to an altered T cell response, but rather a diminished autoreactive B cell response, revealing a novel role for IDO in stimulating B cell responses. The finding that an enzyme that is considered to be immunosuppressive paradoxically exacerbates autoimmunity in the K/BxN model suggested that IDO is not simply immunosuppressive, but instead plays a more complex role in modulating the inflammatory milieu that ultimately leads to the expansion of autoreactive B cells that drive pathology in this model.

Other data on the role of IDO in arthritis conveys a similarly complex picture. Synovial fluid (SF) from RA patients contains large numbers of autoreactive T cells that, upon adoptive transfer, can induce inflammatory arthritis in SCID mice. Partial elimination or inhibition of T cells by immunosuppressive drugs or T cell-specific antibodies ameliorated the disease. Counterintuitively, DCs derived from RA patients expressed higher levels of IDO1 than DCs derived from healthy donors; however, they did not suppress the proliferation of autoreactive T cells derived from RA patients [52]. IFN- γ , TNF and other cytokines in the synovial fluid of RA patients increased the activity of tryptophanyl-tRNA-synthetase (TTS) in synovial T cells. Thus, increased TTS activity may promote resistance to IDO-mediated tryptophan deprivation in T cells from RA patient-derived synovial fluid, making them difficult to restrain. Importantly, IDO-expressing RA dendritic cells can stimulate allogenic T cell proliferation, but the proliferation is greatly enhanced by 1MT [52]. This finding suggested that RA synovial DCs express functional IDO that can inhibit T cell proliferation, however, the RA synovial T cells can resist the effect of IDO due to the increased TTS activity, which enhances the reservoir of tryptophan-tRNA available for protein synthesis. Although IFN- γ induces IDO in DCs, it also upregulates TTS in T cells in the RA synovial fluid microenvironment. Thus, IDO may not suppress the autoreactive T cells in RA because TTS counters the immunosuppressive effect of IDO. In summary, IDO may drive autoreactivity or immunosuppression in RA, depending on contributions of the local microenvironment.

IDO IN INFLAMMATORY BOWEL DISEASES

In inflammatory bowel disease, IDO1 is highly expressed by epithelia and monocytes infiltrating inflamed lesions [53]. Similarly, IDO1 is strongly expressed in celiac disease associated with elevated kynurenine levels [54]. Crohn's disease also exhibits elevated IDO1 expression in mononuclear cells infiltrating submucosal areas of inflamed lesions with associated kynurenine elevation. In Crohn's disease (CD), IDO1 is also strongly expressed in perifollicular regions of lymphoid follicles. Treatment of Crohn's disease patients with TNF-blocking antibody reduces IDO1 expression in CD patients in conjunction with good clinical response [55]. Studies of intestinal inflammation models in IDO1-deficient mice are needed to evaluate whether IDO may support or antagonize these inflammatory states.

IDO IN ALLERGIC AIRWAY DISEASE AND ASTHMA

Allergic responses are characterized by a polarization of T cells to a Th2 phenotype. The role of IDO1 has been evaluated in the well-characterized Th2-mediated antigen-induced allergic airway inflammation. IDO1-deficient mice do not exhibit an impairment of airway tolerance, but rather an attenuation in establishment of allergic airway disease, as defined by reduced Th2 cytokine production, airway inflammation, mucus secretion, airway hyperresponsiveness, and serum ovalbumin-specific IgE production [56]. In IDO1-deficient mice, myeloid DCs from lung-draining lymph nodes of mice immunized to elicit either a Th1 or Th2 response included fewer mature DCs, but the impact of IDO1 deficiency on antigen-induced responses was more exaggerated in the Th2 model. In summary, IDO1 was dispensable to induce airway tolerance, but it promoted Th2-mediated allergic airway inflammation *via* effects on lung DCs [56].

Other studies employing 1MT have argued that IDO1 can contribute to airway tolerance, with the caveat that IDO2 or other targets might also be impacted with this pharmacological probe. In one study, the mouse model used involved intranasal inoculation of lipopolysaccharide-free ovalbumin with subsequent tests for tolerance by intraperitoneal sensitization and intranasal re-challenge [57]. In the same model, another study reported that 1MT could partly reverse the suppressive effects of immunotherapy on airway eosinophilia and Th2 cytokine levels. Additionally, administration of the IDO-generated catabolite kynurenine could potentiate reduction of eosinophilia and Th2 cytokine levels in bronchoalveolar lavage fluid [58]. In a different model of allergic bronchopulmonary aspergillosis, it was found that IDO1 induction was critical for mediating the anti-inflammatory action of the glucocorticoid dexamethasone, which activates IDO1 *via* a symmetric induction of the B7 receptor family member GITR in CD4+ T cells and the B7 ligand GITRL in pDCs to exert protection [59]. In addition to supporting the concept that IDO limits allergy by promoting immune tolerance, these latter findings were quite interesting in implying that IDO is an essential part of the mechanism for anti-inflammatory action of corticosteroids, which are used widely in medicine to limit immunity and inflammation. The Th1 versus Th2 orientation of the different models used to assess the role of IDO in allergic airway disease offers a possible explanation of differing effects of IDO on pathological inflammation.

Two studies have evaluated the role of IDO in experimental models of ovalbumin-induced asthma. In a Th2-driven model, administering a TLR9 ligand induced high levels of IDO activity in lung and other organs that was associated with reduced measures of asthma. It appeared that IDO1 activated in resident lung cells rather than pulmonary DCs suppressed lung inflammation and airway hyperreactivity [60]. Studies of the mechanism by which splenic CD8 γ + DCs can tolerize the Th2 responses of cells in asthma identified a role for IDO based on 1MT treatment, the reversal of which was associated with diminished airway hyperreactivity, eosinophilic airway responses, and pulmonary Th2 cytokine expression [61].

IDO AS A 'MOLECULAR FLAVOR' OF PATHOLOGICAL INFLAMMATION?

Inflammation can be triggered by infection, tissue damage, or both. With infection, inflammation activates innate immune functions and enhances immune cell infiltration into affected tissues and proximal lymph nodes. After tissue damage, 'alarmins' or other 'danger-associated' signals released by dying cells in the absence of infection can also provoke inflammation. In chronic infections or chronic tissue damage, inflammatory responses that stimulate effective T cell immunity and memory appear to be blunted through multiple mechanisms of T cell suppression. By analogy, chronic inflammatory responses associated with the development and progression of various inflammatory pathologies may be similar to those associated with chronic infection, where functions like IDO that can suppress T cell immunity and memory are invoked.

In cancer, there are several implications of connecting IDO and inflammation that are briefly noted below and discussed in detail elsewhere [62]. One implication is that mechanisms underpinning immune escape and 'cancer-associated' inflammation in cancer may be

overlapping rather than distinct. Models suggesting how the inflammatory microenvironment contributes to cancer tend to be based on the notion that the inflammatory conditions precede and engender the subsequent evolution of immune escape mechanisms. However, studies in the IDO-deficient mouse argue that this concept may be invalid, since a single gene that supports immune escape is also needed at early times to support the generation of a ‘cancer-associated’ inflammatory state needed for tumorigenesis [28]. If immune escape pathways are understood merely as pathogenic elements of inflammation needed to support common cancers – a ‘molecular flavor’ to inflammation – then ‘cancer-associated’ inflammation might be defined as a generalized chronic inflammation plus a genetic or epigenetic signature of immune escape. A second implication is that therapeutic benefits may accrue from altering the ‘molecular flavor’ of inflammation instead of simply inhibiting it. Thus, the concept of IDO as a modifier of inflammation suggests that it may be relevant to cancer only in settings where its ‘flavoring’ is relevant. If this perspective is valid, further studies of the role of IDO in cancer would be expected to reveal that IDO may exert no effect or even antagonize some types of cancer, rather than invariably supporting it as broad models of immune suppression would predict [62].

In arthritis and allergic airway disease or asthma, the implications of connecting IDO and inflammation are similar to those in cancer. In different models, IDO has been reported to support or to antagonize pathological inflammation. Putting aside the possibility that the models may differ in the extent of their clinical relevance, the concept of IDO as an inflammatory ‘flavor’ that determines pathologic outcomes may offer a novel basis to consider therapeutic approaches, by altering the inflammatory milieu (e.g. by IDO inhibition) rather than by generally suppressing inflammation or inhibiting immunity. Work in the K/BxN model of rheumatoid arthritis has unexpectedly revealed that IDO can support B cell-driven autoimmunity [51]. These findings would be consistent with evidence from allergic airway/asthma studies that IDO can support a Th2-polarized model of this disease [56]. The possibility that IDO confers a ‘molecular flavor’ that intensifies disease severity in a Th2-polarized environment is interesting in light of the support IDO can provide in eliciting the development of cancer in the context of chronic inflammation, in which Th2 polarization is generally thought to be pro-tumorigenic. If the impact of IDO on a Th2-polarized environment does indeed provide a common link with arthritis and asthma, then clinical exploration of IDO inhibitors may be rationalized for these diseases as well.

In the context of inflammatory intestinal disease, the picture appears to be more consistent with IDO1 in its traditionally viewed role as a suppressor of T cell immunity. Mechanistic studies in IDO1-deficient mice or with IDO inhibitors are needed to more firmly corroborate this prediction as it relates to intestinal cancer as well as inflammatory intestinal disease, where the ‘molecular flavor’ that may be conferred by IDO to inflammation in other settings may not be as relevant. In the small intestine, IDO1 levels are normally high for reasons that have yet to be investigated. In the large intestine, probiotics are abundant and vital but must be balanced by high levels of immune suppression where IDO may be important. Consistent with this perspective, IDO1-deficient mice have been reported recently to display a shift in intestinal secretion of IgA and colonization by commensal bacteria which impacts the response to intestinal pathogens [63]. It will be important to learn whether IDO1-deficient mice have increased susceptibility to stimuli that cause intestinal inflammation or cancers.

In summary, emerging evidence suggests that IDO may function not simply as a suppressor of T cell activation, but more broadly as a modifier of inflammatory states. While the functional complexity of the IDO genes are only just beginning to be unraveled, it is already clear from the growing number of IDO1 studies that it can support or antagonize inflammation in a context- or tissue-dependent manner. We propose that in a chronically inflamed tissue, IDO activity may define a state that can support, or in some cases retard, disease development and progression. Considering the practical application of IDO inhibitors in medicine, which is now receiving increasing attention at the clinical level, it will be important to understand how IDO informs inflammatory states that can direct heightened or attenuated immune responses.

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