



Editorial

Tryptophan Metabolism- Indoleamine 2,3-Dioxygenase- Friend and Foe

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Indoleamine 2,3-dioxygenase (IDO) (EC 1.13.11.42) is a cytoplasmic, heme-containing enzyme that mediates the initial and rate-limiting step in the oxidative catabolism of the essential amino acid L-tryptophan (L-Trp) [1]. Recently, an additional IDO molecule, termed IDO2 has been identified [2]. The gene encoding IDO2 is adjacent to the IDO gene. The IDO2 protein has a different expression profile to IDO and while it is able to metabolize L-Trp, IDO2 has a much higher Km for this substrate. In addition both enzymes differ in their selectivity for some inhibitors. Degradation of L-Trp via IDO leads to the production of several metabolites, including N-formyl-kynurenine and Kynurenine (Kyn). The main physiological inducer of IDO is the pro-inflammatory cytokine, IFN γ , although other cytokines such as interleukin-1 (IL-1) [3] and TNF- α [4] have also been shown to enhance IFN γ -induced IDO activity, as has the bacterial cell wall component, lipopolysaccharide [3]. IDO is hypothesized to suppress the immune response by two mechanisms. Firstly, by exhausting the local environment of L-Trp, a process referred to as immunosuppression by starvation [5]. Secondly, Trp catabolites, such as Kyn and 3-hydroxyanthranilic acid also inhibit by inducing apoptosis of activated T cells [6,7] and inducing production of regulatory T cells (Tregs) [8]. In terms of its biological actions, IDO has traditionally been recognized for its immunomodulatory role in infection, pregnancy, autoimmunity and neoplasia.

Infection Control

The biological importance of IDO was first identified in studies examining its role in bacterial, viral and parasitic infections. Such pathogens elicit a strong IFN γ cytokine response that in turn leads to an elevation in local IDO activity. IDO expression and activity was found to increase in antigen presenting cells, such as dendritic cells and macrophages. The increased expression in these cells led to a decrease in the local environment of free Trp, aiding in the control of intracellular pathogens [9]. Further evidence demonstrating the importance of IDO in controlling infections has been the demonstration that inhibition of IDO activity is an evasion mechanism used by herpes simplex virus [10]. Thus the local metabolism and production of Kyn metabolites aids in the localization of both intra and extracellular pathogens.

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Pregnancy

Historically, a major question in reproductive biology centered on why the maternal immune system does not reject the allogeneic fetus. The seminal studies of Munn et al. [5], determined that IDO expression at the maternal-fetal interface is important in maintaining fetal tolerance. Using the IDO inhibitor, 1-methyl Trp (1-MT), reduced IDO activity, and hence reduced local immunosuppressive activity, was found to lead to rejection of semi-allogeneic, but not syngeneic, fetuses in mice. Thus at the fetal interface, immunosuppression of the maternal immune response is beneficial to the host and provides a means of inducing immune tolerance.

Transplantation

Elevated levels of IDO have also been demonstrated to be beneficial in the setting of transplant acceptance. In a similar situation as maternal-fetal differences, transplantation of allogeneic tissue is rejected via a normal cellular and humoral immune response. Immunosuppression in the local graft environment assists long term maintenance of the transplanted tissue. In a model of lung transplantation, increased IDO expression or one of its metabolites, 3-hydroxyanthranilic acid (3HAA), resulted in near normal lung function and little acute rejection [11]. The effects of IDO and 3HAA were due to modulation of T-cell receptor mediated T-cell activation, and a decrease of intracellular calcium and phospholipase C- γ 1 phosphorylation. Elevated levels of IDO are also associated with increased levels of Tregs which in turn mediate local immunosuppressive effects. Sucher et al. [12] demonstrated that CTLA4Ig treatment leads to IDO induction and Tregs generation, which were essential for murine cardiac allograft survival. IDO and its metabolites are also essential in the control of Graft v/s Host Disease (GvHD). Inhibition of IDO activity through second hand smoke has recently been demonstrated to prevent long-term allograft survival [13]. However there is still some controversy as to the role of IDO, as Landfried et al. demonstrated that elevated levels of Kyn were associated with a worsening of GvHD in human allogeneic stem cell transplantation [14].

Autoimmunity

Given its immunosuppressive activity, it is not surprising that up-regulation of IDO facilitates peripheral tolerance in autoimmune diseases, such as non-obese diabetes [15] and systemic lupus erythematosus. As mentioned above, IDO has been linked to the generation of Tregs *in vivo*, and this has now been identified to be due to the action of Kyn, which has been identified as an endogenous ligand of the aryl hydrocarbon receptor [8]. Kyn production leads to the activation of the AhR transcription factor, which in turn leads to the generation of Tregs. These cells display potent inhibitory activity, modulating effector T cell activation, differentiation and survival leading to an amelioration of autoimmune symptoms [8,15].

Allergy

Recently, IDO has been implicated in the control of allergic inflammation. Crosslinking of the high-affinity IgE receptor (Fc ϵ RI)

by allergens on monocytes from atopic individuals has been shown to induce Trp degradation [16]. In turn, these cells inhibit T cell proliferation due to the increased levels of IDO and decreased levels of Trp [17]. Elevated levels of IDO lead to a local anti-inflammatory response minimising allergic inflammatory responses. Elevated levels of IDO in immature dendritic cells led to a reduction in T_H2 cytokine production and reduced allergic airway inflammation to ovalbumin [18].

Cancer

To this point, positive consequences of IDO activity have been discussed. However, it is now appreciated that expression of IDO can also be accompanied with negative consequences to the host. These effects are most clearly seen in cancer. A large proportion of primary cancer cells express elevated levels of IDO [19]. In addition, the level of IDO activity is a prognostic marker in endometrial [20], colorectal [21], esophageal squamous cell [22] and vulvar squamous cell carcinoma's [23]. Increased expression of IDO is one effective evasion mechanism used by cancer cells. Increased IDO leads to depleted levels of free Trp in the local micro-environment and a corresponding increase in its toxic metabolites which lead to T cell apoptosis and T- cell cycle arrest and increase in Tregs. For example, 1-MT can delay Lewis lung carcinoma tumour growth in syngeneic mice [24]. Recently, the hepatic enzyme Tryptophan Dioxygenase (TDO) has been shown to play a role in human brain tumours [25]. TDO was previously thought to play a role in maintaining homeostatic levels of L-Trp. However this study clearly establishes that TDO can also play an immunosuppressive role in the host, enhancing tumour evasion.

Prostaglandins and the Control of IDO Activity

Antigen presenting cells such as dendritic cells express high levels of IDO following activation or maturational signals. In terms of maturational signals, the arachidonic acid (AA) derived prostaglandin, E₂ (PGE₂) is commonly used *in vitro* for the maturation of these cells. Interestingly PGE₂ has been found to increase IDO mRNA expression, yet enzyme activity is not detectable until cells are stimulated with TNFα. Recently, we investigated the ability of the parent fatty acid, AA to modulate IDO activity in the human monocytic cell line THP-1 and primary human monocytes [26]. Our results demonstrated that cyclooxygenase derived metabolites of AA were able to inhibit the IFNγ mediated upregulation of IDO activity at both the transcriptional and translational level in both THP-1 and monocytes. Interestingly, PGE₂ was excluded as the inhibitory molecule, suggesting that different metabolites of AA vary in their ability to modulate IDO activity. In addition we have also examined the ability of other fatty acids, in particular the two major n-3 fatty acids, eicosapentaenoic and docosahexaenoic acids to inhibit IDO activity. Both of these fatty acids were significantly less inhibitory than AA. It is generally appreciated that AA gives rise to pro-inflammatory metabolites while both EPA and DHA produce anti-inflammatory metabolites [27]. These opposing biological functions and activity towards IDO have prompted us to postulate that the AA-metabolite derived inhibition of IDO is a novel pro-inflammatory mechanism of action of AA. We suggest that the inhibition of IDO activity leads to decreased anti-inflammatory effects, leading to a net pro-inflammatory effect, as is seen in the body. Consistent with this hypothesis, the n-3 fatty acids do not significantly inhibit IDO activity and have immunosuppressive activity, consistent with their lack of effect on IDO.

Summary

In summary, it is clear that IDO is an enzyme with clear immunomodulatory properties with major benefits in health and tolerance. However it is also now clear that dysregulation or hijacked use of IDO activity as is seen in cancerous states is another mechanism by which cancer cells evade the immune response. Recent studies examining the role of PG derived metabolites may allow selective targeting of IDO activity in cancer cells.

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