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Review

Indoleamine 2,3 dioxygenase and regulation of T cell immunity

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Abstract

Regulation of adaptive immune responses is critically important to allow the adaptive immune system to eradicate infections while causing minimal collateral damage to infected tissues, as well as preventing autoimmune disease mediated by self-reactive lymphocytes. Tumors and pathogens that cause persistent infections can subvert immunoregulatory processes to protect themselves from destruction by T cells, to the detriment of patients. A growing body of evidence supports the hypothesis that specialized subsets of dendritic cells expressing indoleamine 2,3 dioxygenase (IDO), which catalyzes oxidative catabolism of tryptophan, play critical roles in regulation of T cell-mediated immune responses. IDO-dependent T cell suppression by dendritic cells suggests that biochemical changes due to tryptophan catabolism have profound effects on T cell proliferation, differentiation, effector functions, and viability. This has critical implications for immunotherapeutic manipulations designed for patients with cancer and chronic infectious diseases. In this review, I focus on dendritic cells that can express IDO, and which acquire potent T cell regulatory functions as a consequence.

Keywords: Indoleamine 2,3 dioxygenase; Dendritic cells; T cells; Immunosuppression

Tryptophan is an essential amino acid for mammals. Even bacteria that can synthesize tryptophan obtain it from their environment when available, presumably because tryptophan is the most energy consuming amino acid to synthesize. Hence, it appears counter-productive that mammals possess two oxygenases that catabolize the first and rate-limiting step in oxidative degradation of tryptophan, leading to the production of a number of downstream metabolites collectively known as kynurenines (Fig. 1). One enzyme, tryptophan dioxygenase (TDO, EC 1.13.11.11), is expressed primarily in liver where it catabolizes excess dietary tryptophan to maintain serum tryptophan concentrations below threshold levels. The second oxygenase, indoleamine 2,3 dioxygenase (IDO, EC 1.13.11.42), is expressed in a wider range of tissues, though a limited range of cell types can express IDO in these tissues.

IDO expression increases when inflammation occurs as a consequence of normal tissue functions, or when inflammation is induced by wounding, infection or tumor growth. Striking correlations between inflammation, and enhanced IDO expression imply that biochemical changes due to IDO activity affect molecular and cellular functions during inflammation. Local inflammation is critically important for activating innate and adaptive immune responses. Onset of inflammation is followed closely by induction of counter (anti) inflammatory mechanisms that protect tissues from collateral damage and facilitate tissue healing. Correlations between enhanced IDO expression and inflammation do not address whether localized IDO activity is beneficial or detrimental to tissues undergoing inflammation. Moreover, they do not reveal if critical biological effects are brought about by depletion of tryptophan and reactive oxygen species (ROS), or by production of toxic metabolites (kynurenines). Beneficial effects might accrue from local removal of toxic ROS, or free tryptophan, which is an essential nutrient for T cells that can acquire effector functions capable of causing tissue damage. Detrimental effects of IDO expression may accrue from production of toxic metabolites, as when HIV-induced IDO expression and production of quinolinate damages neurologic functions.

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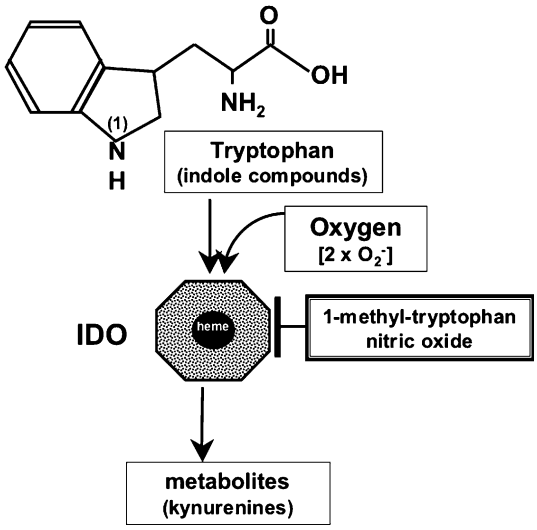

Fig. 1. IDO biochemistry.

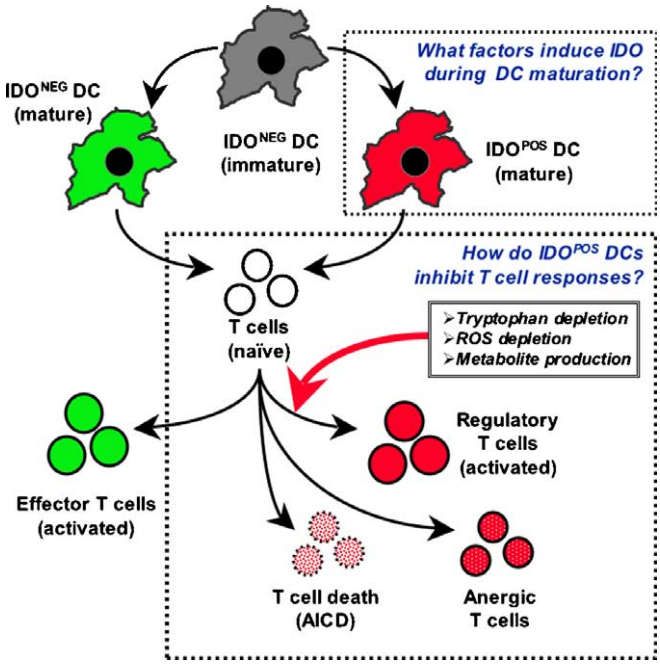

Fig. 2. Effect of IDO expression in DCs on T cell responses.

In this review, I will focus on evidence that IDO expression in certain antigen presenting cells (APCs), specifically some dendritic cell (DC) subsets, causes them to acquire potent and dominant regulatory functions that actively suppress T cell responses and promote T cell tolerance to further antigenic challenges. Mechanistic connections between oxidative tryptophan catabolism by cells expressing IDO, tissue inflammation, and immunosuppression have considerable implications for understanding cellular, molecular, and biochemical processes in chronic infectious and autoimmune diseases, and cancer [1,2]. In addition, correlations between IDO and immunoregulation provide opportunities for developing innovative therapeutic approaches to treat these chronic disease states, which affect millions of humans.

IDO expression and dendritic cells

IDO is expressed in tissues with large areas of mucosal surface (lungs, gut, and fetal-maternal unit during pregnancy), as well as in male epididymis and thymus [3]. Healthy tissues that experience "constitutive" inflammation, such as mucosal surfaces of the gut, lung, and uterus during pregnancy, express IDO during their normal function. IDO is induced by tumor growth and by pathogens that promote inflammatory responses in cancerous and infected host tissues. Parasitic intracellular pathogenic organisms that promote chronic infections, such as *Toxoplasma* and *Leishmania*, induce macrophages to express IDO. This response may be a host defense mechanism that slows the spread of microbial infections by limiting access to tryptophan, an essential nutrient for proliferating cells. A limited range of cell types express IDO constitutively, or following tissue wounding, infection, and inflammation. Some fibroblasts, epithelial and tumor cells, tumor-associated cells, and immune system cell types express IDO. Myeloid lineage cells, including macrophages, DCs, and microglial cells in the CNS are of particular interest to immunologists since these cells function as APCs that stimulate T cell activation [2].

DCs are specialized to induce or to suppress adaptive immune responses by acquiring, processing, and presenting antigens to T cells (Fig. 2). Thus, DCs present antigens from normal, cancerous, and infected cells, transplanted tissues, and developing fetal tissues during mammalian pregnancy to elicit T cell responses that have the potential to destroy (immunity) or protect (tolerance) infected or healthy cells expressing the same antigens. Inflammatory processes may influence the priming (afferent) and effector stages of immune responses. Hence, increased IDO activity in DCs may be a factor that regulates the type of T cell response elicited under specific circumstances. Hence, effective immune outcomes depend on appropriate regulation of T cell responses as T cell responses that are either too potent, or too weak might result in autoimmunity, or persistence of tumors and infectious pathogens, respectively.

IDO and cancer

Tumors may rely on local inflammatory processes to evade host T cell immunity, explaining why tumor cells transfected to express foreign antigens (alloantigens) resist T cell immunity that provokes rejection of normal tissues expressing the same antigens. This is analogous to the situation of fetuses implanted in maternal uterus during pregnancy, since paternally inherited fetal genes encode histocompatibility antigens foreign to the mother. IDO activity is essential for survival of allogeneic fetus' in mice [4,5]. Similarly, IDO expression by tumor cells, or by

tumor-associated APCs might inhibit T cell responses to tumor antigens by suppressing T cell priming in tumor-draining lymph nodes (LNs), or by rendering activated effector T cells ineffective at the site of tumor growth [6–9]. Other immunoregulatory mechanisms can compensate for genetic ablation of IDO since pregnant IDO-deficient mice bear normal numbers of healthy offspring [10]. Hence, compensatory mechanisms, combined with the innate plasticity of tumor cells, may explain why tumors grow normally in IDO-deficient mice [6,8]. Since most (if not all) humans are not IDO-deficient, it may be possible to enhance anti-tumor immunity in cancer patients using pharmacologic IDO inhibitors to block IDO-mediated T cell suppression [2].

The goal of cancer immunotherapy is to provoke effective immune responses against tumor antigens to exploit the exquisite specificity of the adaptive immune system to attack tumors. Regulatory T cells suppress natural T cell immunity to tumor antigens [11,12] and may inhibit attempts to boost anti-tumor immunity through immunotherapeutic interventions. Likewise, immunosuppression by T cell regulatory IDO+ DCs may suppress natural and induced immunity to tumor antigens. In addition, IDO+ DCs may promote regulatory T cell generation by biasing $CD4^+$ T cell responses against Th1 effectors and towards regulatory T cells. Several reports implicate regulatory DCs as generators of regulatory T cells during tolerogenic processes [13–18]. Hence, IDO+ DCs may both suppress effector T cell responses and enhance regulatory T cell generation. If so, IDO expression by tumor cells and/or tumor-associated DCs may be detrimental to cancer patients by reducing the chances of successful immunotherapeutic intervention to eradicate tumors.

IDO and regulation of T cell responses

Evidence that IDO expression regulates T cell responses came initially from studies on human macrophages that suppressed T cell proliferation [4]. These studies prompted further studies that revealed a critical role for IDO in preventing fetal rejection by maternal T cells during pregnancy [5,19]. A considerable body of evidence now supports the hypothesis that cells expressing IDO regulate T cell-mediated immune responses in inflammatory diseases, transplant rejection, pregnancy, and cancer [2]. One mechanistic hypothesis advanced to explain the correlation between IDO expression and regulation of T cell responses posits that reduced access to free tryptophan inhibits T cell activation and/or decreases T cell viability following activation (the tryptophan depletion hypothesis). A recent report [20] showed that the GCN2-kinase-dependent integrated stress response (ISR) was induced specifically in T cells activated in the presence of DCs expressing IDO, leading to induction of T cell anergy (unresponsiveness to subsequent antigenic stimulation). This finding provides experimental support for the tryptophan depletion hypothesis because GCN2-kinase activation is a specific response to increased levels of uncharged tRNA molecules in cytoplasm. An alternative hypothesis is that downstream metabolites produced by IDO+ APCs inhibit T cell proliferation, promote T cell death, and exert differential effects on helper T cell responses by altering the Th1/Th2 balance [21–23]. The biological significance of IDO-mediated tryptophan depletion and production of kynurenines in tissues are not known with certainty as biochemical changes due to IDO expression are difficult to measure in tissues.

Regulation of IDO expression in DCs by interferons

Several mammalian IDO genes have been cloned and sequenced revealing transcriptional promoter elements (GAS and ISRE motifs) that confer responsiveness to interferons via the JAK-STAT signaling pathway [3,24]. Other cytokines and growth/differentiation factors also affect IDO expression synergistically with interferons, though molecular regulatory mechanisms are not completely understood. Inducible IDO expression in some DC subsets occurs in response to ligation of immune receptor molecules leading to induction of potent T cell regulatory functions mediated by IDO+ DCs [25–29]. This has important implications for therapeutic manipulation of adaptive immunity through administration of immunodulatory ligands that bind to specific receptor molecules on DCs.

Grohmann et al. [25] reported that IFNγ was essential for IDO induction in splenic DCs following B7 ligation in vitro. This response to B7 ligation protected allografts and tissues from destruction by allogeneic effector T cells [25,26]. However, IFN type I (IFNαβ), but not IFNγ, was essential for selective IDO up-regulation in a minor subset of DCs expressing CD19, a marker often used to remove B cells from DC preparations [27,29]. STAT-1 activation also correlated with subsequent IDO induction in $CD19^+$ DCs following B7 ligation [29]. Distinct requirements for IFNγ or IFNα signaling might have emerged for technical reasons, as different methods were used to isolate DCs, assess signaling requirements, and measure T cell suppression. Recently, CD200R ligation was reported to induce IDO in plasmacytoid DCs (pDCs) a subset of splenic DCs that can produce IFNα [28]. Moreover, immunostimulatory DNA sequences containing unmethylated CpG motifs (CpG-ODNs) that bind to TLR-9-induced lung epithelial cells and DCs to express IDO leading to IDO-mediated suppression of experimentally induced asthma in mice [30]. Though IDO induction was IFNγ-dependent in lung epithelial cells, IDO induction in lung DCs was IFNγ-independent, suggesting that lung DCs may have specific requirements for IFN type I signaling with respect to IDO induction. Taniguchi and co-workers [31–34] have reported that IRF-7 is the master regulator of IFNα production in pDCs following TLR-9 ligation. These data show that a number of factors can signal IFN-dependent IDO expression in DCs, which has potent effects on the ability of DCs to stimulate T cell responses that contribute to disease processes and tissue pathology.

Summary and conclusions

As depicted in Fig. 2, biochemical changes due to induced IDO expression in some DC subsets might profoundly affect immune outcomes by altering the course of T cell responses following T cell activation. Key unanswered questions arising from this concept relate to the precise nature of critical biochemical changes in IDO substrate levels and metabolites produced by DCs expressing IDO, the regional extent of their influence on T cells activated in localized tissue microenvironments, and the molecular mechanisms that affect subsequent changes in T cell (and DC) functions.

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