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REVIEW

The role of indoleamine 2,3-dioxygenase in transplantation

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Summary

Indoleamine 2,3-dioxygenase (IDO), by enzymatic tryptophan degradation, has recently been proposed to have profound immunoregulatory activity. By most recent findings IDO induction follows reverse signaling of cytotoxic T-lymphocyte antigen-4 (CTLA-4) to its ligands CD80/86 and acts as a counter-regulatory mechanism to T-cell stimulation. With regard to transplantation, experimental evidence suggests that IDO has the potential to down-regulate allo-responses of T cells *in vitro* and to promote tolerance in murine models of pancreatic islet transplantation and of allogeneic T-cell transfer *in vivo*. However, the physiologic role of IDO in human organ transplantation still is to be elucidated. Experiments that clearly identify a significance of IDO in tolerance induction to vascularized organ allografts or in effecting costimulation blockade are required. In this review we provide a conceptual view of the current knowledge of IDO in the context of transplantation and, in light of its particular biological features, speculate about its potential application in novel therapeutic approaches for tolerance induction.

Introduction

The immune system comprises a nonlinear network of pathways that orchestrate the balance of survival of an individual in its environment. By evolution mammal organisms, in order to generate broadly founded immune competence, have developed primary and secondary lymphoid organs with specific immune activity but also have adopted other biological systems. One outstanding example of a single compound to serve diverse biological functions, including immunologic activity, is the amino-acid tryptophan. Tryptophan plays an important role for protein synthesis and for the generation of the neurotransmitter serotonin. The focus of this review is the role of tryptophan and its metabolism in immunology, particularly to give a comprehensive view on the current understanding and controversies of tryptophan metabolism in transplantation immunology.

In the immune system tryptophan metabolism has been originally recognized as a host defense mechanism but recently has attracted additional scientific interest for its proposed role in being involved in tolerance induction. Tryptophan metabolism is intimately linked to the enzymatic activity of indoleamine 2,3-dioxygenase (IDO), which has been identified as the key enzyme to initiate the metabolic breakdown of tryptophan. Tryptophan itself was found to be essential to many cellular organisms, to survive and divide such as *Toxoplasma gondii* and *Chlamydia* [1–4]. IDO activity, as present in cells of the monocyte/macrophage lineage, would deprive these pathogens from their access to tryptophan and also expose them to toxic tryptophan metabolites and thus help to terminate infection. It was in the late 1990s, when Munn *et al.* [5] showed that IDO activity was critically involved in the immunologic acceptance of semi-allogeneic fetuses in a murine model. This study has profoundly changed the view of IDO and has fuelled a new understanding of its activity as a central pathway for down-regulating potentially dangerous immune reactions [6]. Comprehensively, sustained IDO activity would represent an immunosuppressive pathway. Recent studies have addressed the immunomodulatory effects of IDO in many aspects, including allergy, tumor immunology, autoimmunity and HIV infection [7–11]. In the appropriate context, IDO might contribute to limiting immune effector mechanisms and prevent exaggerated immune activity, or in circum-

stances of its sustained activity, generate a state of immunosuppression. Thus, IDO activity is considered to have an ambivalent potential as it may act for the benefit or detriment of the host.

The IDO immunologic activity, above all, should be viewed as a feedback mechanism that counter-regulates immune activation [12]. IDO expression and activity, as described in detail below, will be induced by the same mechanisms and molecules that initiate immune activation.

Biological background

IDO, the rate limiting enzyme for tryptophan metabolism

Tryptophan taken up from diet, generally, is the least abundant of the essential amino acids in mammal organisms [13]. Metabolization of tryptophan may occur along several pathways (Fig. 1). A small proportion of ingested tryptophan (1%) is converted to serotonin in the nervous system and gut or used for the synthesis of melatonin in the pineal gland. The main proportion (>95%) is metabolized along the kynurenine pathway [13], ultimately leading to the biosynthesis of nicotinamide adenine dinucleotide (NAD; Fig. 1). The initial and thus the rate limiting step of the kynurenine metabolic pathway is the oxidative cleavage of the pyrrole ring that converts tryptophan to kynurenine. This step is catalyzed by two enzymes, tryptophan 2,3-dioxygenase (TDO), which is exclusively expressed in the liver [14], and IDO, which is inducible in many tissues and cell types, including tumor cells [15], bone marrow stromal cells [16], eosinophilic granulocytes [17], astrocytes [18], endothelial cells [19], but most prominently, in antigen-presenting cells (APCs). In steady-state conditions tryptophan metabolism mainly takes place in the liver through TDO activity [20,21]. In an inflammatory environment tryptophan metabolism will increase by enhanced IDO activity. *In vivo*, reduced levels of serum tryptophan and simultaneously increased levels of tryptophan metabolites, e.g. kynurenines, likely may result from systemic IDO activity and, in fact, are frequent findings in states of sustained immune activation, e.g. autoimmune diseases or pregnancies or transplantation [22–25]. On the contrary, low serum tryptophan levels without elevated levels of tryptophan metabolites and in the absence of immune activation markers, such as neopterin, rather reflect reduced dietary uptake [26].

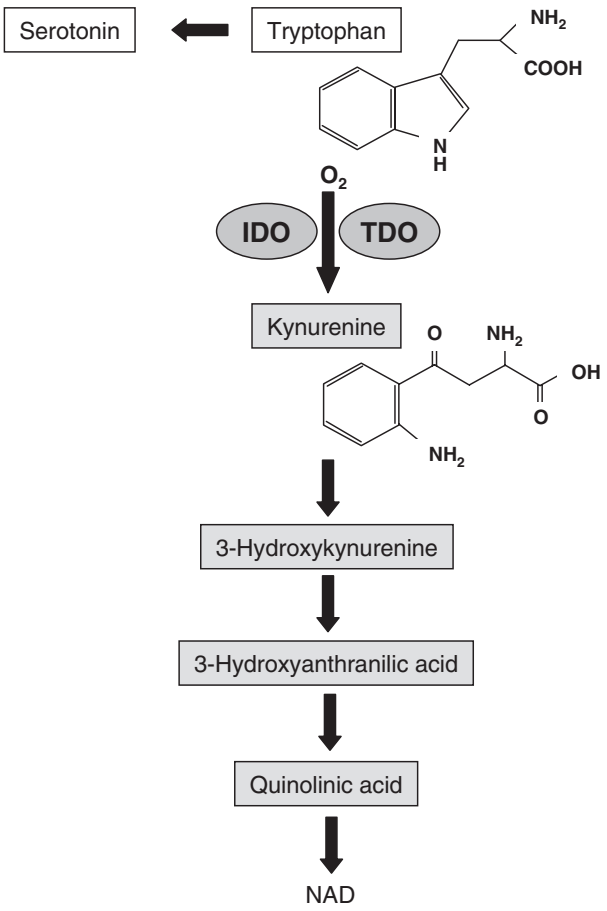

Figure 1 IDO initiates tryptophan metabolism. TDO and IDO are the two rate limiting enzymes that initiate tryptophan metabolism by oxidative cleavage of the pyrrole ring of tryptophan. The enzymatic activity generates N-formylkynurenine that rapidly converts into kynurenine. The downstream products of kynurenine shown in this graph (grey) have been described to possess immunosuppressive activity.

Regulation of enzymatic activity

Genetically, IDO in mice and humans is encoded by a single gene, termed *Indo*, located on the short arm of chromosome 8 (8p12–8p11) [27] and, as shown in murine and human dendritic cells (DCs), was found to be co-regulated by a limited number of genes [28]. Gene transcription, in general, occurs in response to inflammatory mediators, most prominently interferon-γ (IFN-γ), or toll-like receptor (TLR) ligation (e.g. through lipopolysaccharide) [29]. Intracellular signaling following ligation of IDO inducers occurs along the JAK-STAT pathway and nuclear factor κB (NFκB) [29,30] to finally result in expression of the monomeric, cytosolic, 45 kDa IDO glycoprotein [31].

To comprehensively understand the role of IDO in immunoregulation it is of central importance to bear in mind that cellular expression of IDO protein does not necessarily mean IDO activity [32]. IDO protein may be expressed constitutively by some subgroups of DCs but

needs further stimulatory signals to be activated [33]. The IDO glycoprotein contains a heme-prosthetic group with iron in its ferric (Fe^{3+}) form. Ferric IDO is inactive and requires redox active compounds (e.g. superoxide) to generate the active (Fe^{2+}) form and allow for tight binding of L-tryptophan and O_2 [34–36] such that tryptophan metabolism can proceed [30]. Thus, IDO activity is ultimately dependent on an environment enriched for redox active components. One such environment is inflamed tissue. This explains the apparent association of inflammation (immune activation) and IDO enzymatic activity [6].

Mechanisms of immunosuppression

Two theories have been proposed to explain how tryptophan catabolism regulates immune responses, tryptophan depletion and the accumulation of tryptophan metabolic compounds in the microenvironment.

The *tryptophan depletion theory* is founded on the concept that tryptophan is essential for protein synthesis and thus supposed to be necessary for T-cell proliferation. *In vitro*-activated human and mouse T cells when deprived of the access to tryptophan, e.g. by use of tryptophan depleted cell culture medium, have been shown to undergo initial steps of activation (e.g. acquire cell surface expression of CD69) but subsequently exhibit cell-cycle arrest [37] and become sensitive to apoptosis [38]. IDO-expressing cells themselves are thought to be protected from tryptophan self-starvation by the IFN-γ inducible enzyme tryptophanyl-tRNA-synthetase [39], which induces the formation of a tryptophan-tRNA complex. This complex is protected from IDO-mediated degradation and provides a reservoir of tryptophan in a form that is directly available for protein synthesis [40].

Several recent observations demonstrating IDO mediated modulation of T-cell function, despite tryptophan being readily available, contradict the concept that tryptophan depletion is the prime mechanism by which IDO mediates T-cell inhibition [6]. In *in vitro* T-cell stimulation assays, metabolic products of tryptophan themselves (*tryptophan utilization theory*) [6] were observed to have a direct immunosuppressive effect. 3-hydroxyanthranilic acid (3-HAA) and quinolinic acid induced selective apoptosis of murine thymocytes, and of Th1 but not Th2 cells [41]. Pro apoptotic activity was suggested to be Fas-dependent in conditions of tryptophan depletion [38] and Fas-independent in case of the activity of tryptophan metabolic compounds [41]. Kynurenine and 3-hydroxykynurenine also were shown to be cytotoxic for human T cells, B and NK cells [42,43].

Yet, since in general the activation of IDO will cause both, tryptophan depletion and accumulation of tryptophan metabolic compounds, it is conceivable to speculate that *in vivo* both these mechanisms will contribute to the immunoregulatory effect of IDO.

IDO, part of the immunoregulatory circuit

In general, enhanced tryptophan degradation is observed in conditions of sustained cellular immune activation [26,44]. In the acute phase of an inflammatory response pro-inflammatory mediators, e.g. IFN-γ, tumor necrosis factor α (TNF-α), or TLR ligation [45,46] initiate immune effector mechanisms. However at the same time negative feedback mechanisms are also initiated, such as the expression of the transcription factor FOXP3 and cytotoxic T lymphocyte antigen-4 (CTLA-4) in T cells or IDO activity in DCs and cells of the monocyte/macrophage lineage. A recent report suggested that in fact the initiation of IDO activity is a mechanism by which regulatory T cells exert their immunosuppressive function [47].

In a balanced state, immune activation followed by immunosuppression will rapidly restore immune homeostasis (Fig. 2, upper panel). However, in a state of persistent immune activation (e.g. prolonged activity of IFN-γ), immune effector mechanisms, although ongoing, will be down-regulated and immunosuppressive feedback mechanisms (e.g. prolonged IDO activity) may prevail [48] (Fig. 2, lower panel). Thus, enhanced IDO activity may represent an effector mechanism for the paradoxically appearing immunoregulatory activity of otherwise proinflammatory cytokines, e.g. IFN-γ [45,49]. As a state of chronic inflammation is a key feature of transplantation, IDO immunoregulatory activity may play a central role in the modulation of post-transplant immunity and the generation of tolerance [50].

Under homeostatic conditions, IDO mediated immunoregulation does not appear to be essential for maintenance of central or peripheral tolerance to self-antigens, as IDO-deficient mice show a normally developed immune system and do not display spontaneous autoimmunity [51]. However, IDO deficient mice failed to regulate potentially lethal T-cell responses upon the transfer of allogeneic CD8+ cells, even after treatment with the tolerance inducing agent CTLA-4-Ig. This was in contrast to wild type animals, which up-regulated IDO after treatment with CTLA-4-Ig and efficiently suppressed allogeneic T-cell expansion. This observation was interpreted such that immunoregulation by IDO affects tolerance to neoantigens, including allo-antigens [51].

The role of IDO in transplantation tolerance

Transplantation tolerance essentially involves two critical processes, (i) deletion of allo-reactive T cells as a first step and (ii) the development of anergy and regulatory activity

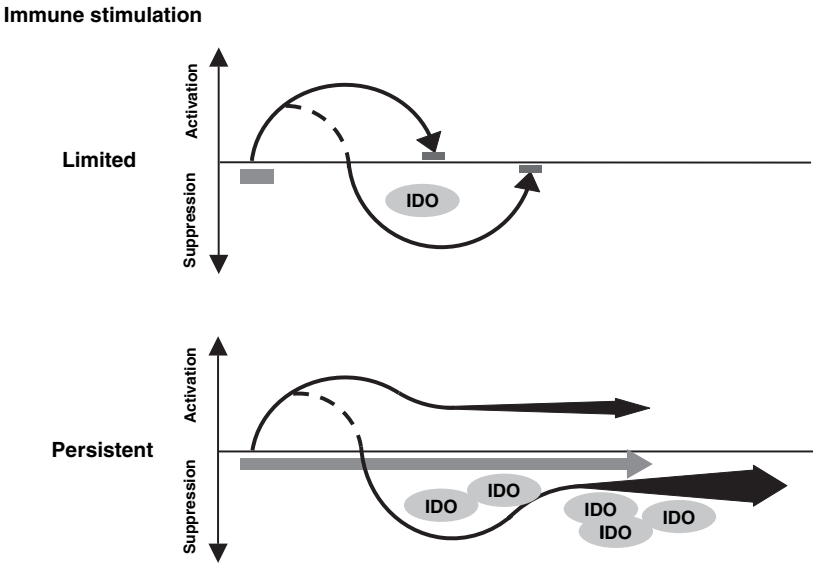

Figure 2 A dominant immunosuppressive effect of IDO activity is associated with persistent immune activation. Immune stimulation (grey bar) initiates immune activation (e.g. IFN-γ secretion) which, in turn, initiates counter-regulatory immunosuppression (e.g. IDO activity) (dashed line). When immune stimulation is limited (upper panel) counter-regulatory immunosuppression will terminate the immune reaction and rapidly restore immune homeostasis. In contrast, when immune stimulation is persistent (lower panel) ongoing immune activation will continuously activate counter-regulatory immunosuppression. By time, immunosuppression, such as mediated by IDO activity, will predominate over immune activation leading to an immunosuppressed state as frequently observed in chronic immune activation [73].

of allo-reactive T cells [52]. After allogeneic cell transplantation a state of immune activation, driven by recognition of major or minor histocompatibility antigens, invariably will emerge in the recipient, even in HLA-matched donors. In addition, some immune activation will result from tissue damage in the recipient caused by surgery or, in hematopoietic stem cell transplantation (HSCT), by the conditioning regimens. This state of immune activation will include the secretion of pro-inflammatory cytokines including IFN-γ by APCs or activated T cells. Because of the intimate association of IFN-γ and induction of IDO, it appears sound to assume that IDO by its immunoregulatory effects may actively participate in down-regulating allogeneic immune responses in transplantation.

In their seminal work that initiated broader interest in IDO mediated modulation of immune responses and tolerance, Munn *et al.* [5] employed a murine model of semi-allogeneic pregnancy and showed that female CBA mice mated to male C57BL/6 mice accepted the semi-allogeneic fetuses only when tryptophan metabolism was intact. Upon blockade of tryptophan metabolism *in vivo*, by implantation of pellets that constantly released a specific IDO inhibitor, 1-methyl tryptophan (1-MT), maternal T cells effectively mounted an allogeneic response against the fetus leading to fatal abortion. This was interpreted as evidence that tryptophan metabolism through IDO activity was involved in transplantation tolerance in a dominant fashion. Further investigations extending this finding to humans showed that, indeed, IDO is expressed in the human placenta and tryptophan metabolites are detectable in the sera of pregnant women [53], all together supporting that IDO-mediated tryptophan metabolism contributes to materno-fetal tolerance in humans.

Subsequent experiments, mostly carried out *in vitro*, corroborated the evidence that IDO activity possesses the potential to down-regulate allo-responses [42,43,54]. Tryptophan metabolites, 3-hydroxykynurenine, quinolinic acid and 3-HAA, which act as apoptosis inducing compounds and particularly affect activated T cells, were shown to suppress allogeneic T-cell responses in an *in vitro* mixed leukocyte reaction (MLR). In an *in vivo* rat model, a systemic application of tryptophan metabolites resulted in a delayed rejection of allogeneic skin grafts [55]. Moreover, in a murine model of experimental autoimmune encephalomyelitis natural and synthetic tryptophan metabolites were shown to down-regulate activity of disease [56]. Albeit less effective than CyA the findings basically confirmed a suppressive effect of an enhanced tryptophan metabolism upon allogeneic responses. Furthermore, IDO transfected cell lines and transgenic mice overexpressing IDO both were shown to suppress allogeneic T cell-responses *in vitro* and *in vivo* [57].

Important insights into the pathway by which IDO activity is induced to participate in immunoregulation

came from a study of Grohmann *et al.* [58], in a model of pancreatic islet transplantation. These authors showed that a rejection of Balb/c pancreatic islets, transplanted into diabetic C57BL/6 mice, was prevented by a treatment of the recipients with CTLA-4-Ig. Strikingly, tolerance induction by CTLA-4-Ig was overcome by the administration of 1-MT releasing pellets. Subsequent experiments demonstrated that DCs prepared from C57BL/6 recipient mice and exposed to CTLA-4-Ig showed increased kynurenine release. The induction of IDO activity required the interaction of CTLA-4-Ig with the costimulatory molecule B7 (CD80/86) on the recipient DCs. This finding led the authors to propose a model in which a reverse signaling of CTLA-4, to molecules of the CD80/86 family, induces IDO and thus renders DCs tolerogenic rather than immunogenic [59]. This process was critically dependent on IFN-γ and the STAT-1 pathway of DCs [58].

Support for the understanding that the exposure of DCs to CTLA-4-Ig induces IDO activity came from a further murine model that directly tested a potential role of IDO in modulating allo-reactivity [51]. Splenic DCs of F1 (CBA × C57BL/6) mice that expressed the CD11c cell surface molecule and the CD8α homodimer were found to express IDO upon exposure to CTLA-4-Ig. When these mice were injected with allogeneic TCR transgenic CD8+ T cells, recipients having received CTLA-4-Ig treatment and being able to up-regulate IDO expression and activity (i.e. IDO competent) maintained their normal splenic structure. In contrast, in animals in which the IDO gene was disrupted the transfer of TCR transgenic allo-reactive CD8+ T cells resulted in a massive splenic infiltration of allogeneic lymphocytes, even when having been treated with CTLA-4-Ig. The reduced clonal expansion of allogeneic CD8+ T cells in IDO competent animals was partially overcome by exposing the recipients to pellets releasing 1-MT. These observations were interpreted such that the tolerogenic effect of CTLA-4-Ig was IDO dependent.

Subsequent studies indicated that the regulatory murine CD4+CD25+ T cells, as they constitutively express high levels of cell surface CTLA-4, exert their immunosuppressive activity through IDO induction that follows the interaction of CTLA-4 with B7 expressed by DCs [60,61].

The concept of an association of costimulation blockade and IDO activity was also investigated in a rat kidney transplantation model [62]. In this model tolerance was induced *in vivo* by administration of anti-CD28 antibodies in an antigen-specific fashion. The expression of B7 in an otherwise poorly defined recipient non-T cell population was proposed to mediate T-cell suppression. The *in vivo* tolerance could be broken when the animals were fed with 1-MT and the exposure to 1-MT restored the *in vitro* proliferative capacity of recipient cells to stimulation with donor cells. Unfortunately, the authors did not provide further evidence for enhanced IDO activity in tolerized animals, a fact that precludes to unequivocally concluding on the role for IDO in this model and weakens this otherwise interesting observation. In another study DCs transfected with the extracellular part of the CTLA-4 gene conferred tolerance in an IDO-independent fashion [63]. Notably, in the transfected DCs cell surface expression of CD80 molecules was nearly absent and it was not addressed by the authors whether the disrupted signaling via CD80 was responsible for the absence of IDO induction.

In human immunology a potential relevance for IDO induction by reverse signaling from T cells to DCs was then provided by Munn *et al.* [33] studying human monocyte-derived DCs *in vitro*. The authors described a CD11c+ CCR6+ CD123+ DC subset as particularly IDO competent DCs, i.e. they expressed IDO and efficiently metabolized tryptophan. Cross-linking of CD80 and CD86 molecules stabilized IDO expression in DCs in the absence of T cells and up-regulated IDO activity in an MLR. These DCs were observed to be able to suppress the proliferation of allogeneic T cells and suppression was reversed by addition of 1-MT.

In their composite, these data (i) suggest a potentially dominant role of IDO governing allo-reactivity and (ii) propose a mechanistic pathway, in which IDO is induced by reverse signaling through costimulatory receptors. This concept is compatible with viewing IDO as a negative feedback mechanism in which activated T cells that express CTLA-4 interact with CD80/86 expressed by DCs. This interaction then induces IDO and finally results in suppression of T-cell effector responses.

As a matter of fact, evidence for IDO activity being involved in human transplantation immunology came from our own study [25] in which we observed that the state of immunosuppression after HSCT involved an enhanced tryptophan metabolism mediated by activated monocytes that were able to suppress T-cell proliferation. A striking finding was that after HSCT monocytes were highly sensitive to up-regulate IDO activity upon exposure to even low doses of IFN-γ. The study has two important implications. It first showed that IDO competent cells, in this case post-HSCT monocytes, might become sensitive to up-regulate IDO activity upon exposure to inflammatory cytokines such that the conversion into suppressor cells rather than stimulatory cells is facilitated. Further, it indicates that systemic IDO activity is a double edged sword, as the enhanced tryptophan catabolism (i) may be associated with general immunosuppression after transplantation but (ii) at the same time may contribute to tolerance induction and prevention of graft-

versus-host disease (GvHD) or rejection. To this point a previous study, although small scaled, showed that lack of IDO activity in recipients of HSCT was associated with high-grade GvHD [64].

IDO in therapeutic tolerance induction

By the concept outlined above, IDO mediated tryptophan metabolism appears to have a considerable potential to accomplish the requirements of induction of specific transplantation tolerance (Fig. 3): local tryptophan depletion and the accumulation of tryptophan metabolites will arrest allo-responsive T cells in cell cycling and render them susceptible to apoptosis, respectively [38,41]. Even in case of some residual T-cell activation, signaling from activated T cells to DCs via CTLA-4/CD80/86 interaction may render DCs tolerogenic and thus enhance the regulatory circuit. These effects are concentrated in the immediate DC/T cell microenvironment and should, by enlarge, neither affect T cells that do not interact with the allo-antigen presenting DC nor cause overt systemic immunosuppression. In other words, ideally, potent IDO-mediated immunoregulation acts locally at the site of inflammation in an antigen-specific fashion.

Therefore, IDO mediated immune regulation appears attractive for being used in a therapeutic setting. Employing IDO activity in an intelligent manner may be en route to achieve the magic goal of transplantation immunology which is to tolerize recipients against allo-antigens while preserving immunity against pathogens. As to date, evidence to support a therapeutic role of IDO for tolerance induction in clinical organ transplantation is limited. In the reverse approach, a blockade of IDO has been suggested to break tolerance in tumor bearing hosts and to improve anti-tumor immunity [8,65].

Considering the therapeutic adoption of IDO-mediated immunoregulation in transplantation one has to take into account the particular features of IDO. A systemic enhancement of IDO activity *in vivo* e.g. by pharmacologic IDO induction or the adoptive transfer of IDO competent cells, certainly carries the risk of inducing general immunosuppression and probably would have, if any, little advances over general immunosuppressive treatment approaches, e.g. use of CyA or

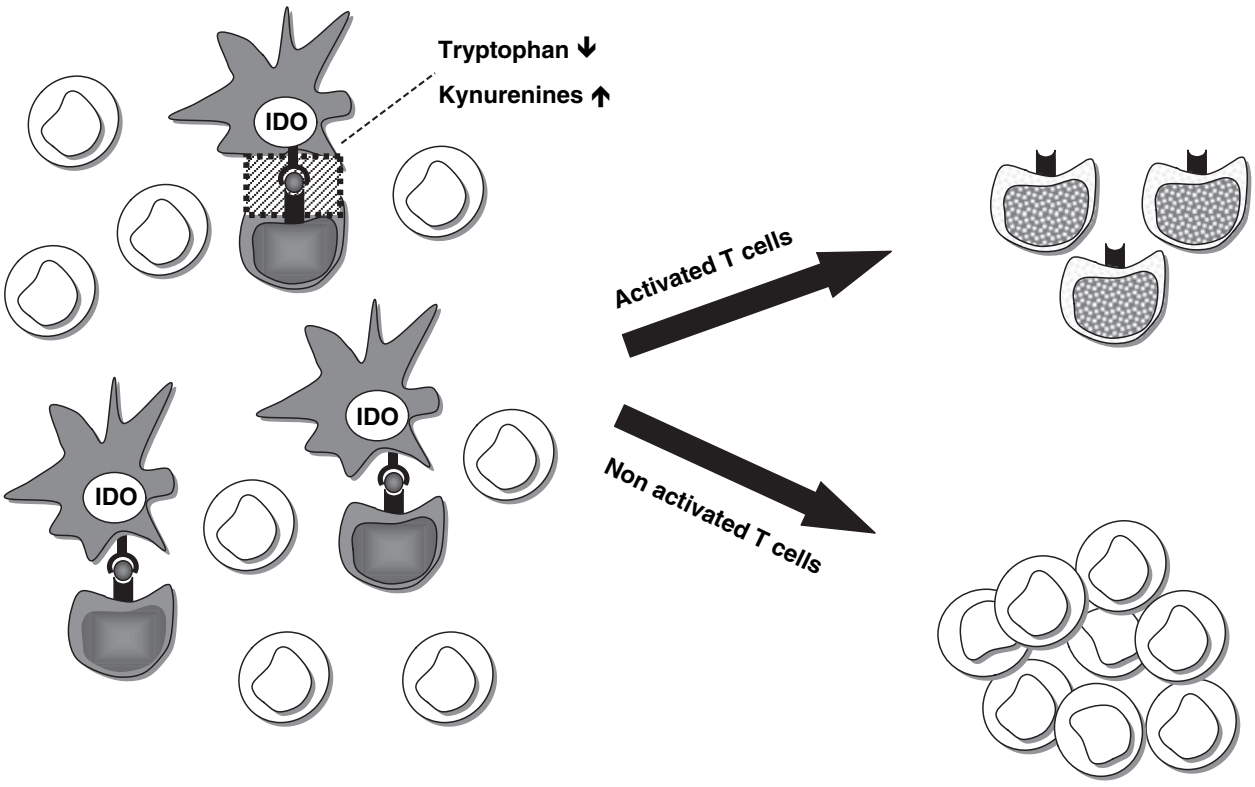

Figure 3 Antigen-specific activity of IDO. By concept, IDO competent antigen-presenting DCs exert their T-cell suppressive effect particularly within the immunological synapse (dashed area), which is formed by a DC derived MHC/antigen complex and a T-cell receptor complex recognizing the antigen. The effects of IDO activity, namely low tryptophan concentration and high levels of tryptophan breakdown products (kynurenines) are focussed in this microenvironment and therefore specifically affect antigen-specific T cells. These are arrested in cell cycle progression and are rendered susceptible to apoptosis. T cells that are not antigen-specific remain unaffected.

CTLA-4-Ig. Furthermore, Bauer *et al.* [55] (see above) observed in their *in vitro* studies that exogenously added tryptophan metabolites induced extensive T-cell apoptosis, which also affected nonactivated T cells. As IDO activity itself is modulated to a significant extent by the microenvironment and factors that cannot be controlled for *in vivo*, e.g. redox molecules, the effect of a systemic enhancement of IDO will be hard to predict. These considerations argue against a therapeutic approach of systemic IDO induction as a means of facilitating the generation of tolerance.

Nevertheless, as IDO activity is assumed to act locally rather than systemically, one approach of taking advantage of the tolerogenic potential of IDO would be to enrich IDO activity at targeted sites. IDO competent DCs might be generated *ex vivo* and be enriched in organ grafts. Some evidence exists that IDO transfected cells in fact are able to mediate tolerance *in vivo* [57] although the approaches to optimize stability of IDO expression and the survival capacity of transfected cells still have to be developed [66]. Another approach could be to exploit the tolerogenic potential of IDO for the generation of specifically allo-antigen tolerized T-cell populations *in vitro*.

Concluding remarks

While experimental systems including murine and human *in vitro* studies and some *in vivo* rodent models support a role for IDO in mediating transplantation tolerance, its true relevance to human transplantation immunology remains to be defined. Recently, some criticism has emerged questioning whether the experimental evidence as summarized above does not overemphasize the physiologic role of IDO in human immunology [32]. Terness *et al.* [67] stated that, given the interspecies differences in tryptophan metabolism, an extrapolation from mouse studies to human immunology was not justified. In addition, it was criticized that in a majority of the experiments a role for IDO was supposed when by use of the IDO inhibitor 1-MT T-cell responses increased. However, 1-MT *per se* would also inhibit the tryptophan transporter and therefore have profound effects on protein synthesis in general and thus influence T-cell responses in an IDO independent fashion. Finally, Terness *et al.* [67] found that in their experiments the human CD123+ CCR6+ DC subset, that was formerly described by Munn *et al.* [33] as being highly IDO competent, neither expressed IDO nor was able to suppress allogeneic T-cell responses. In a public debate it was suggested that whether or not human DCs would have IDO-mediated immunoregulatory activity depended on minute experimental conditions *in vitro* [68].

In the context of human organ transplantation, it is up to future studies to clearly elaborate the clinical significance of IDO induction as a mechanistic pathway of the systemic immunosuppressive activity of CTLA-4-Ig. In a recent review, Bluestone *et al.* [69] pointed out that, still, the major effect of CTLA-4-Ig is the blockade of the engagement of CD28 with its ligands CD80 and CD86. A considerable body of evidence including human therapeutic trials demonstrates that pharmacologic preparations of CTLA-4-Ig, such as the CTLA-4-Ig fusion protein abatacept [70] and the CTLA-4-Ig mutant LEA29Y, betalacept, [71] promote the acceptance of vascularized organ grafts [72], while the current evidence for a role of IDO in mediating tolerogenic effects of CTLA-4-Ig *in vivo* is based on experimental islet transplantation or adoptive transfer of allo-reactive T cells. Thus, studying a potential involvement of IDO-mediated tryptophan metabolism associated with CTLA-4-Ig treatment in organ transplantation using renal or cardiac allografts is urgently warranted to clearly learn about the biological relevance of IDO in human transplantation immunology.

Given, IDO does have a physiologic role in transplantation the ultimate understanding of its role and effects will be challenging. Because of the multiple microenvironmental factors regulating its activity, one has to be aware that more of IDO (e.g. when measured by protein expression or IDO mRNA levels) does not necessarily mean more immunoregulatory activity in the direction of tolerance. Furthermore, any level of IDO expression or activity is not necessarily purely beneficial to the host. Endogenous IDO expression and activity may, similarly as in infectious diseases, have an ambivalent value in transplantation. It may protect the graft from potentially deleterious allo-reactivity but at the same time leave the host organism susceptible to infection. This complexity has to be taken into account to truly estimate the significance for IDO in humans and human transplantation [32].

On the other hand it is this complexity that makes IDO a fascinating field of research. An ultimately better understanding of its complex role in regulating allogeneic immune responses will probably contribute to better understand the principle mechanisms of ups and downs in immunoregulation. The elaboration of conditions in which IDO-mediated immunoregulation is optimized towards the induction of antigen-specific tolerance will potentially open new windows of therapeutic opportunities.

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