REVIEW ARTICLE
Iran J Allergy Asthma Immunol
December 2007; 6(4): 167-179

The Immunoregulatory Function of Indoleamine 2, 3 Dioxygenase and Its Application in Allotransplantation

Reza B. Jalili[1,2], Farshad Forouzandeh[1], Mohammad Ali Bahar[3], and Aziz Ghahary[1]

[1] Department of Surgery, University of British Columbia, Vancouver, British Columbia V6H 3Z6, Canada
[2] Endocrinology and Metabolism Research Center, Medical Sciences/ University of Tehran, Tehran, Iran
[3] Department of Immunology, Iran University of Medical Sciences, Tehran, Iran

Received: 22 May 2007; Received in revised form: 29 September 2007; Accepted: 30 September 2007

ABSTRACT

Indolemine 2, 3-dioxygenase (IDO) is a cytosolic monomeric hemoprotein enzyme that catalyses tryptophan, the least available essential amino acid in the human body, to N-formylkynurenine, which in turn rapidly degrades to give kynurenine. IDO is expressed in different tissues, especially and prominently in some subsets of antigen presenting cells (APCs) of lymphoid organs and also in the placenta of human and other mammals. Expression of IDO by certain dendritic cells, monocytes and macrophages has a regulatory effect on T cells probably by providing a tryptophan-deficient microenvironment and/or accumulation of toxic metabolites of tryptophan. This immunomodulatory function of IDO plays an essential role in different physiological and pathological states. IDO was shown to prevent rejection of the fetus during pregnancy, possibly by inhibiting alloreactive T cells. Moreover, IDO expression in APCs was suggested to control autoreactive immune responses. In this review we discuss the molecular and biological characteristics of IDO and its function in immune system as well as the potential application of this enzyme in improving the outcome of allogeneic transplantation as a local immunosuppressive factor.

Key words: Allogeneic transplantation; Immunosuppression; Indoleamine 2, 3 dioxygenase; T- lymphocytes

INTRODUCTION

The immune system continuously modulates the balance between responsiveness to pathogens and tolerance to non-harmful antigens.

Corresponding Author: Aziz Ghahary, PhD;
Director, Burn and Wound Healing Research Lab, Rm 351, Jack Bell Research Centre,2660 Oak Street, Vancouver, BC, Canada, V6H 3Z6. Tel: (604) 875 4185, Fax: (604) 875 4212,
Email: aghahary@interchange.ubc.ca

The mechanisms that mediate tolerance are not well understood, but recent findings have implicated tryptophan catabolism through the kynurenine metabolic pathway as one of many mechanisms involved. Tryptophan is the least abundant of all essential amino acids in the human body and is required by all forms of life for protein synthesis and other important metabolic functions. In addition to being essential for protein synthesis in mammals, tryptophan is also the only source of substrate for the

production of several very important molecules. The major catabolic route of tryptophan in mammals is the kynurenine pathway that can ultimately lead to the biosynthesis of nicotinamide adenine dinucleotide (NAD).[1] The initial and rate-limiting reaction of the kynurenine pathway is the oxidation of tryptophan to *N*-formyl-l-kynurenine, catalysed by hepatic tryptophan 2,3-dioxygenase (TDO, EC 1.13.11.11) or the ubiquitous, extra-hepatic, indoleamine 2,3-dioxygenase (IDO or INDO, EC 1.13.11.17)[1]. Of the dietary tryptophan that is not used in protein synthesis, 99% is metabolized by IDO.[2] IDO has recently been proposed to have profound immunoregulatory activity and the concept that cells expressing IDO can suppress T-cell responses and promote tolerance is a relatively new paradigm in immunology. Considerable evidence now supports this hypothesis, including studies of mammalian pregnancy, tumor resistance, chronic infections and autoimmune diseases. In this review we explain the molecular and biological characteristics of IDO and discuss its function in immune system as well as the potential use of this enzyme to improve the outcome of allogeneic transplantation as a local immunosuppressive factor.

IDO STRUCTURE, EXPRESSION AND REGULATION

IDO Structure and Biochemical Properties

Mature IDO is a 42-45 kDa monomeric protein containing heme as its sole prosthetic group. The tertiary structure of recombinant human IDO was recently defined using X-ray crystallography.[3] Overall, IDO is folded into two distinct alpha-helical domains, one small and one large, with the heme prosthetic group positioned between them. Once synthesized, the IDO holoenzyme catalyzes the oxidative cleavage of the pyrrole ring of L-tryptophan to generate *N*-formyl-kynurenine which is metabolized to formic acid and the stable-end product, kynurenine. IDO has high affinity for L-tryptophan (Km~0.02 mM) and therefore can rapidly catabolize it to create a local tissue microenvironment devoid of this essential amino acid. From a biochemical standpoint, the structural analysis of IDO has addressed a gap in understanding of heme chemistry as it pertains to dioxygenase catalytic mechanisms, possibly contributing to the design of potent and specific small molecule inhibitors.[1]

IDO Expression Pattern in the Body

IDO is expressed intracellularly in a constitutive or inducible manner in different cells and tissues. Other than in the male epididymis (the significance of which is unknown), IDO is constitutively expressed only in the lower gastrointestinal tract.[4] Interferon-gamma (IFN-γ) is a potent inducer of IDO expression in placenta,[5] macrophages,[6] dendritic cells,[7] cultured fibroblasts,[8] and many cancer cell lines.[9] IDO has been also detected in other cells which may be important to allergic inflammation including eosinophils[10], endothelial cells [11, 12], and lung epithelial cells.[13]

Regulation of IDO Expression

IDO in mice and humans is encoded by a single gene, termed *Indo*, with 10 exons spread over ~1.5 kbp of DNA located on the short arm of chromosome 8 (8p12–8p11)[14] and, as shown in murine and human dendritic cells (DCs), it was found to be co-regulated by a limited number of genes [15]. Gene transcription, in general, occurs in response to inflammatory mediators, most prominently IFN-γ, or toll-like receptor (TLR) ligation (e.g. through lipopolysaccharide).[16] In fact IDO gene is regulated by a promoter that contains a single IFN-γ-activated site specific for IFN- γ, as well as two nonspecific IFN-stimulated response elements, which can respond to IFN-α and IFN-β as well as IFN-γ. Depending on the cell type being cultured, IFN-γ has been described as being up to 100 times more potent at inducing IDO expression than IFN-α or IFN-β 8. In contrast, T helper 2 (Th2) cytokines such as IL-4 and IL-13 inhibit the expression of IDO.[17,18]

Intracellular signaling following ligation of IDO inducers occurs along the JAK-STAT pathway and nuclear factor κB (NFκB)[16,19] to finally result in expression of the monomeric, cytosolic, 45 kDa IDO glycoprotein [20]. Furthermore, some of the IDO-dependent effects are initiated by CpG-rich oligodeoxynucleotides and rely on cell signaling through TLR9, [21,22] whose activation typically results in type I IFN release by plasmacytoid DCs.[23]

In the immune system, certain types or subsets of APCs seem to be preferentially disposed to express functional IDO when challenged with proinflammatory stimuli or exposed to signals from activated T cells. In mice, these "IDO-competent" APCs include a subset of plasmacytoid DCs,[24] $CD8\alpha^+$ splenic DCs (or a subset thereof),[15,25] and doubtless other subsets of DCs and macrophages as well.[26] On the other hand, even in

APCs that are IDO competent, the actual presence or absence of functional IDO enzymatic activity is tightly regulated by specific maturation and activation signals.[27-29] Conceptually, this ability to upregulate or downregulate IDO in response to external stimuli seems logical, given the need for APCs to sometimes present antigens in an activating fashion and sometimes in a tolerating fashion, depending on the context.[30]

THE IMMUNOMODULATORY FUNCTION OF IDO

IDO has recently emerged as an important immunomodulator of T cell function and inducer of tolerance. Expression of IDO plays 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.[16] The induced expression of IDO by dendritic cells may suppress T cell responses and promote tolerance either through direct effects on T cells (mediated by tryptophan depletion or tryptophan metabolites) or through effects of IDO on the dendritic cell. In addition to the potential role of IDO in promoting tolerance in pregnancy, transplantation, and autoimmunity, its role in modulating allergic responses has more recently been investigated, raising the possibility that IDO and its metabolites may be novel targets for immunomodulation in allergy and asthma.[31]

IDO has been implicated as a possible immunosuppressive effector mechanism of Regulatory T cells (Tregs). Grohmann et al.[32] showed that Tregs could trigger high levels of functional IDO expression in mouse DCs in vitro. This occurred through binding of CTLA4 on Tregs to B7-1 and B7-2 on DCs, which transduced a signal in the DCs that upregulated IDO protein expression and functional enzymatic activity.[32,33] A similar ability of CTLA4-B7-1 and/or B7-2 interactions to induce IDO has been shown in human monocyte-derived DCs [15, 34]. Therefore, IDO might function as a downstream mechanism through which $CTLA4^+$ Tregs mediate immunosuppression.[35]

One of the biologic functions of IDO seems to be a counter regulatory mechanism to suppress excessive immune activation. Such counter regulatory pathways are important in the immune system because uncontrolled immune responses can cause unacceptable damage to the host. At sites of inflammation, IDO expression is not limited to DCs and macrophages but can be found in epithelial cells,[36,37] eosinophils,[10] endothelial cells,[12] and possibly other cell types as well. Therefore, IDO is an important endogenous counter-regulatory mechanism that helps protect the host.

IDO and Pregnancy

According to immunological concepts, a fetus, due to parentally acquired genes encoding antigens foreign to mother's immune system, should not survive gestation. This fascinating paradox was first raised by the Nobel Laureate Sir Peter Medawar in 1953. Endogenous IDO has been implicated as one mechanism that helps maintain maternal tolerance towards the fetus as shown by the fact that mice treated early in pregnancy with 1-methyl-tryptophan (1MT), which is an inhibitor of IDO, underwent immune-mediated rejection of allogeneic concepti.[38-40] These findings suggest that IDO expressing trophoblastic cells provide an immunosuppressive barrier protecting semi-allogeneic fetus tissue from maternal T-cell immunity.

Molecular Mechanisms of IDO-Mediated Immune Suppression

The molecular mechanism(s) by which IDO suppresses T cells are still being elucidated. It appears that some of the biological effects of IDO can be mediated via local depletion of tryptophan, whereas others are mediated via immunomodulatory tryptophan metabolites. As mentioned earlier, IDO initiates the degradation of tryptophan along the kynurenine pathway. IDO and the downstream enzymes in this pathway produce a series of immunosuppressive tryptophan metabolites (Figure 1). Some of these metabolites suppress T cell proliferation in vitro or cause T cell apoptosis, and some can affect NK cell function.[41-44] The molecular mechanism by which tryptophan metabolites exert their immunologic effects is not known, but at least one recent report has described a receptor able to bind a specific metabolite of tryptophan (kynurenic acid).[45] The biologic function of this orphan G protein–coupled receptor, GPR35, is unknown, but its expression was highest in cells of the immune system and the gut, the sites where IDO is known to be expressed.[45] Whether there are other such receptors for other tryptophan metabolites and what the biologic effects of such receptors might be *in vivo* are important questions that deserve further investigation.

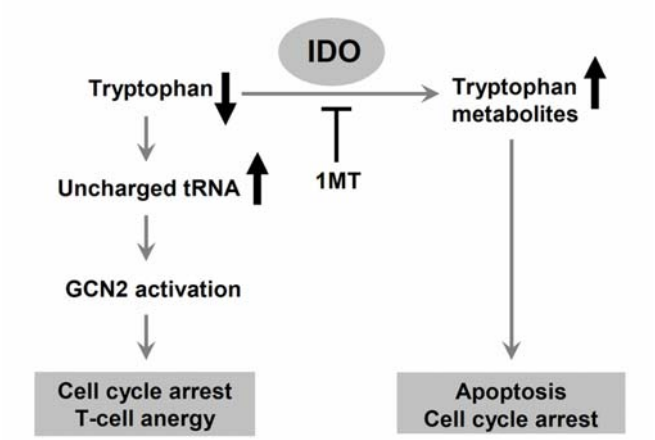

Figure 1. Molecular mechanisms of IDO- induced immunosuppression. IDO catalyzes the initial and rate-limiting step in the degradation of tryptophan along the kynurenine pathway. 1-methyl-tryptophan (1MT) can function as a specific inhibitor of IDO activity. Tryptophan metabolites have been shown to have immunomodulatory activity, alone or in combination with the GCN2 signaling pathway. IDO enzymatic activity results in the local depletion of tryptophan and a local increase in the concentration of downstream metabolites. The decrease in tryptophan can cause a rise in the level of uncharged transfer RNA (tRNA) in neighboring T cells, resulting in activation of the amino acid-sensitive GCN2 stress-kinase pathway. In turn, GCN2 signaling can cause cell cycle arrest and anergy induction in responding T cells. The local increase in tryptophan metabolites can cause cell cycle arrest and apoptosis.

In addition to the immunosuppressive effects of tryptophan metabolites, the cellular stress imposed by local depletion of tryptophan also mediate some of the immunosuppressive effects of IDO (Figure 1).

This was first suggested by an observation that some effects of IDO on T cells are reversed by the addition of excess tryptophan *in vitro*.[24, 46, 47] Recently, the stress-responsive kinase general control non-derepressible 2 (GCN2) has been identified as a signaling molecule that enables T cells to sense and respond to stress conditions created by IDO[48,49] which will be discussed in more detail in the following section.

GCN2 Pathway as a Potential Downstream Mechanism for IDO Function

As mentioned earlier, one possible mechanism for immunoregulatory effect of IDO is through depleting the essential amino acid tryptophan in the microenvironment to the level that T cells are unable to proliferate[16] so IDO might affect pathways known to respond to amino acid metabolism. There are two known amino-acid-responsive signal-transduction pathways through which T cells might sense decreased levels of free tryptophan. One of these pathways is the essential amino acid deficiency antagonizing signaling through the mammalian target of rapamycin mTOR kinase pathway.[16,50,51] mTOR signalling is required for normal initiation of ribosomal translation. This pathway is important for growth-factor signaling, and T cells are particularly sensitive to inhibitors of mTOR, such as the immunosuppressant drug rapamycin.[52,53] However, it has been found that inhibitors of mTOR such as rapamycin did not recapitulate the profound proliferative arrest seen with IDO-mediated suppression.[54] In yeast, there exists a second amino acid-sensitive pathway that is mediated by the kinase GCN2.[55] GCN2 contains a regulatory domain that binds the uncharged form of transfer RNA (tRNA). Amino acid insufficiency causes a rise in uncharged tRNA, which activates the GCN2 kinase domain and initiates downstream signaling.[56] Recently, the mammalian homolog of GCN2 has been identified and shown to have similar signaling properties.[57,58] GCN2 is one of a family of four related kinases (GCN2, PERK, HRI, and PKR), which share as their only known substrate the alpha subunit of translation eukaryotic Initiation Factor 2 (eIF2α). Indeed, GCN2 was originally identified as a regulator of translation control in response to starvation for one of many different amino acids.[59] Uncharged tRNA that accumulates during amino-acid depletion binds to a GCN2 regulatory domain homologous to histidyl–tRNA synthetase enzyme, triggering eIF2α kinase activity.[58] The activation of eIF2α kinase can provide a signal transduction pathway linking eukaryotic cellular stress in response to alterations in the control of gene expression at the translational level.[60,61] Therefore, GCN2 participates in nutritional stress management that guides food selection for survival. Animals faced with a diet lacking in essential amino acids quickly reject the imbalanced food and forage for complete or complementary sources of protein.[62,63] Activation of GCN2 kinase pathway, which has been termed the integrated stress response (ISR), can trigger cell-cycle arrest, differentiation, compensatory adaptation, or apoptosis, depending on the cell type and the initiating stress.[64-67] It has been found that expression of IDO by APCs activates the GCN2 kinase pathway in responding T cells, generating an intracellular signal

that mediates key biologic effects of IDO. Specifically, it has been shown GCN2 is required for $CD8^+$ T cells to sense and respond to conditions created by IDO. T cells lacking GCN2 proliferated normally in the presence of IDO^+ APCs both *in vitro* and *in vivo* and were not susceptible to IDO-induced anergy. [48]

Exactly how IDO creates the stress that activates GCN2 in T cells is not yet known. In the published literature, the only well-characterized and proven stimulus for GCN2 activation is a rise in uncharged tRNA, as would occur if IDO depleted the T cells of tryptophan. The fact that IDO-mediated suppression was reversed by addition of 10 times tryptophan might also be consistent with a mechanism of tryptophan depletion. However, we cannot exclude the alternative possibility that a downstream metabolite produced by IDO might interfere with the acylation reaction by which tryptophan is ligated to its tRNA. Although speculative, such an inhibitory metabolite would also cause a rise in uncharged $tRNA^{TRP}$ (by inhibiting the charging reaction), and its effect might be overcome by high tryptophan concentration. Finally, our focus on the role of GCN2 in T cells does not mean that T cells are the only possible sites of action for GCN2. GCN2 is widely expressed, and other cell types (including the APC itself) might respond to IDO via their own endogenous GCN2 pathway. It has been shown that IDO can mediate potent cell-autonomous effects (i.e., effects that modify the biology of the IDO-expressing cell).[68-70] Thus, the IDO/GCN2 pathway might influence the biology of the APC itself, and perhaps even other bystander cells as well.

In brief, studies thus identify GCN2 as a downstream mediator for several key effects of IDO. This constitutes the first elucidation of a specific molecular target for the immunoregulatory action of IDO in T cells.

IDO, Self Tolerance and Autoimmune Conditions

IDO also regulates the severity of a variety of experimental autoimmune disorders.[71-74] In autoimmunity, apoptosis of potentially autoreactive lymphocytes by IDO-expressing DCs activated by IFN-γ could represent a crucial means of maintaining peripheral tolerance.[2] According to the model suggested by Grohmann *et al*,[72] IFN-γ acts on tolerogenic DCs to activate the expression of IDO which leads to the induction of immunosuppressive tryptophan catabolism and to the onset of specific self tolerance.

Furthermore, Gurtner *et al.*[71] showed that IDO plays an important role in the down-regulation of Th1 responses within the gastrointestinal tract and may play a similar role in human inflammatory bowel disease. Consistent with a role for IDO in immune regulation in gut and lung, in settings where self tolerance has already been disrupted (for example, autoimmune disorders), pharmacologic inhibition of IDO causes marked exacerbation of inflammation and worsened symptoms of disease, as shown in models as diverse as inflammatory bowel disease,[71] experimental allergic asthma[13] and experimental autoimmune encephalomyelitis (EAE).[74] Thus, IDO might play a physiological role in regulating immune responses against self antigens.

The Role of IDO in Host Defence against Infection

IDO forms part of the innate host defense against certain infections. Although most microorganisms can synthesize their own tryptophan, some depend on exogenous tryptophan (auxotrophs). Such organisms are sensitive to the tryptophan-depleting activity of IDO. Examples include *Chlamydia pneumoniae, Toxoplasma gondii* and certain bacteria, such as group B streptococci and mycobacteria.[6,75,76] These are pathogens that are either intracellular or live in intimate association with a host cell, and induction of IDO expression by the host cell inhibits pathogen replication *in vitro*. Similarly, during viral infection, IDO inhibits the replication of cytomegalovirus and herpes simplex virus *in vitro*.[77] In all of these examples, the effect of IDO was found to be specifically due to its ability to deplete tryptophan, because adding supra-physiological levels of exogenous tryptophan restored pathogen or viral replication.

Although IDO has an effect on pathogen replication *in vitro*, however, the biological relevance of IDO in controlling infections *in vivo* remains unclear. IFN-γ-deficient mice, which fail to induce IDO during infection, are less able to control *C. pneumoniae* and *T. gondii* infection *in vivo;*[78] however, it is unknown whether this is due specifically to the lack of IDO expression or to one of the many other effects of IFN-γ. Further studies are therefore required to address the specific contribution of IDO to host defense against infection. This question is particularly relevant, given that one (highly undesirable) consequence of inducing IDO expression by the innate

system might be the suppression of T cell responses by the adaptive system.[79]

One may speculate that slowing pathogen replication by IDO *in vitro* does not necessarily mean that it would be a useful host defense *in vivo* (particularly in the case of chronic, slow-growing infections such as those described). So, like the T helper 2 -cell bias in chronic leishmaniasis,[80] it is possible that IDO could benefit the pathogen more than the host. However, in general the current body of knowledge surrounding the relation between IDO and infectious diseases is in the favor of the fact that IDO induction should be regarded as an important mechanism of antimicrobial resistance to intracellular organisms, such as parasites and bacteria.

IDO AND ITS POTENTIAL ROLE IN TRANSPLANTATION IMMUNOLOGY AND TOLERANCE

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, 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 [81]. In fact it has been recently suggested that cells expressing IDO might contribute to the underlying mechanism of donor-specific tolerance without the use of immunosuppressive drugs. Several experiments, mostly carried out *in vitro*, corroborated the evidence that IDO activity possesses the potential to down-regulate allo-responses.[7,82] In an *in vivo* study, recipient mice exposed to 1-methyl-tryptophan (1MT), a specific inhibitor of IDO activity, rejected the murine liver allografts at the time of engraftment.[83] Further evidence on immunoprotective role of IDO in cell and tissue allografts comes from a study using adenoviral transfection to increase IDO expression in donor islet cells. The study reported prolonged survival of transplanted allogeneic pancreatic islet cells.[84]

Our recently published data provide compelling evidence in supporting our working hypothesis that the expression of IDO in bystander fibroblasts through either IDO genetic modification or IFN-γ treatment suppresses immune cell proliferation.[61,85-88] In our most recent study, we have been able to show that bystander IDO-expressing syngeneic fibroblasts have the ability to suppress the allogeneic lymphocytes proliferation which normally occurs in response to allogeneic pancreatic islets.[85]

This hypothesis has been substantiated by the fact that co-culturing IDO genetically modified fibroblasts with different types of immune cells, significantly increased the number of damaged bystander human peripheral blood mononuclear cells (PBMC), $CD4^+$ T cells, Jurkat cells, THP-1 monocytes, as well as $CD8^+$ and B cell-enriched lymphocytes, relative to those of controls (Figure 2).

This bystander effect proved to be due to IDO induction of a tryptophan deficient cell culture environment. In addition, the finding of this study further demonstrated that, by an unknown mechanism, only immune, but not primary skin cells are sensitive to IDO induced low tryptophan environments.[88] This selective effect of IDO may be due to different tryptophan metabolism pathways in immune versus non immune cells.

Incorporation of tryptophan into protein is initiated by tryptophanyl-transfer RNA synthetase (WRS).WRS is the only aminoacyl synthetase that responds to inflammatory mediators, such as IFN-γ,[89] and overexpression of WRS has been postulated to help cells that express IDO to compensate for the reduction in intracellular tryptophan.

It might therefore be important that T cell lines do not show induction of WRS expression in response to IFN-γ.[90] In another study,[68] we have demonstrated a significant down-regulation of cell membrane associated MHC class I antigen in IDO genetically modified keratinocytes relative to that of either non-transfected or empty vector transfected cells. Further experiments showed that an addition of tryptophan or IDO inhibitor markedly restored the expression of MHC class I on IDO transfected keratinocytes (Figure 3). Thus, the findings of this study suggest for the first time that down-regulation of MHC class I expression by IDO might be one of the mechanisms through which IDO mediates local immunosuppression.

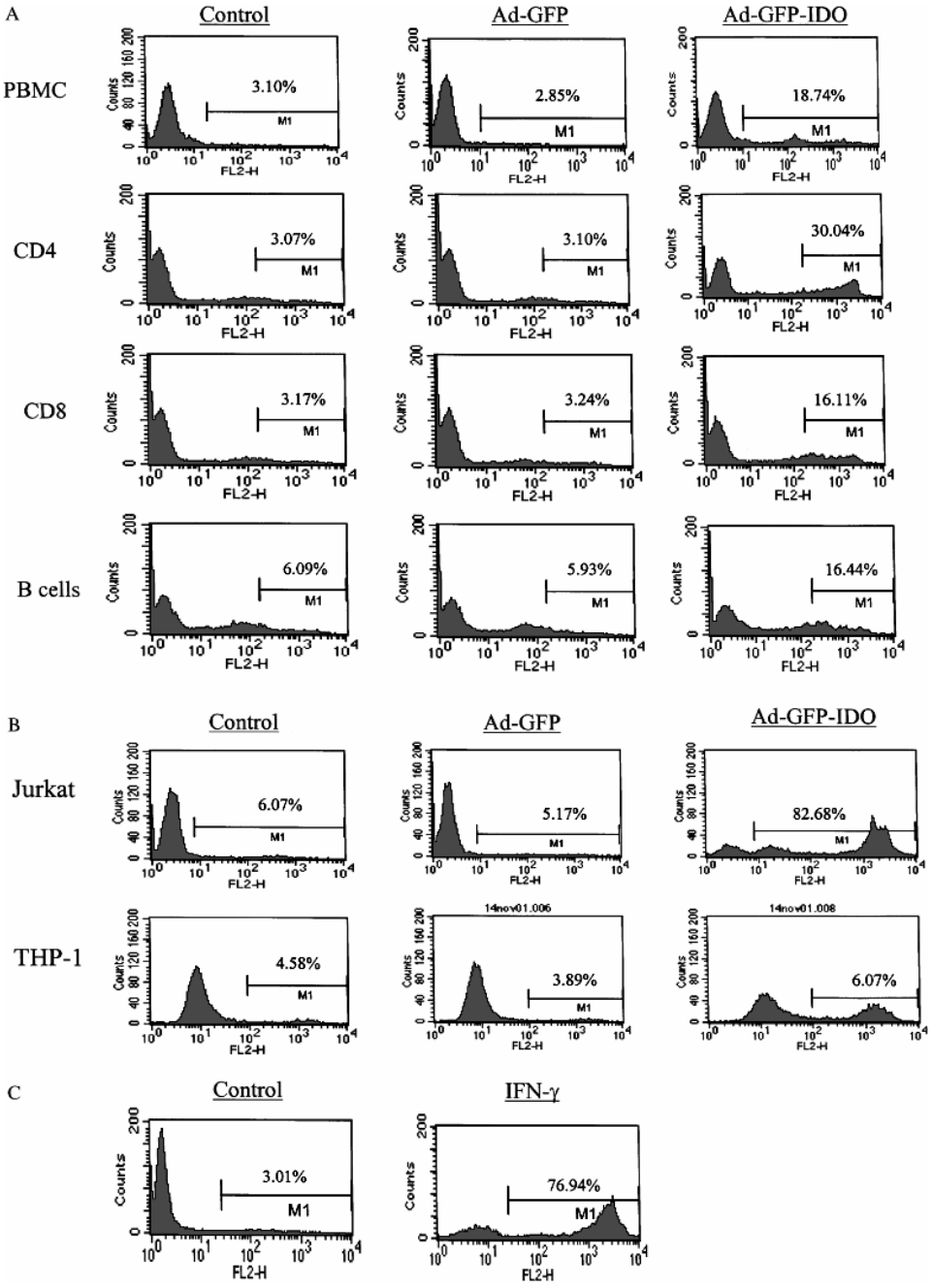

Figure 2. FACS analysis of Propidium Iodine positive bystander immune cells. (*A*) Non-viral-infected (control) and pre-infected fibroblasts with either an empty vector (Ad-GFP) or IDO adenoviral vector (Ad-GFP-IDO) were co-cultured with either human PBMC, CD4+-, CD8+-, or B cell-enriched immune cells for 5 days, respectively. The immune cells were then harvested and stained with 10 µg per mL of PI for 10 min and analyzed by FACS. (*B*) Non-viral-infected (control) and pre-infected fibroblasts with either an empty vector (Ad-GFP) or IDO adenoviral vector (Ad-GFP-IDO) were co-cultured with either CD4+ Jurkat cells or THP-1 monocytes for 3 days, respectively. The bystander immune cells were then harvested and stained with 10 µg per mL of PI for 10 min and analyzed by FACS. (*C*) CD4+ Jurkat cells were co-cultured with either non-treated fibroblasts or IFN-γ pre-treated fibroblasts for 4 days. Jurkat cells were then harvested and stained with 10 µg per mL of PI for 10 min and analyzed by FACS. FACS, fluorescence-activated cell sorting; PI, propidium iodide; GFP, green fluorescent protein; IDO, indoleamine 2,3-dioxygenase; PBMC, peripheral blood mononuclear cells; IFN, interferon. [Figure obtained from J Invest Dermatol 2004 Apr;122(4):953-64.]

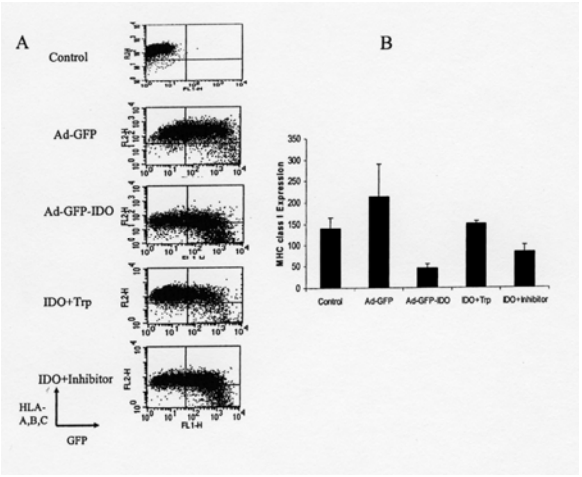

Figure 3. IDO down regulates class I MHC proteins in keratinocytes and addition of tryptophan and IDO inhibitor partially restored this downregulation. Keratinocytes were transfected with either Ad-GFP or Ad-GFP-IDO for 30 hours. Free viral particles were removed and the fresh medium with either 250 µM of tryptophan or 800 µM of 1-methyl-DL-tryptophan (IDO inhibitor) was added. Cells received vehicle were also included as negative control. Cells were harvested and stained with PE-conjugated anti-HLA-A, -B, -C at day 5 post transfection. MHC class I protein expression was determined by FACS. Panel A illustrates the results from two-color (PE and GFP) channel analysis; whereas panel B depicts the quantitative analysis of HLA expressing levels determined from the PE fluorescence intensity. Data presented here are one representative of triplicate experiments (panel A). Data of panel B are means ± standard deviation, from three separate experiments. Abbreviations: Ad-GFP = adenoviral vector carry GFP; Ad-GFP-IDO = adenoviral vector carrying GFP and IDO; GFP = green fluorescent protein; HLA= human leukocyte antigen; IDO=indoleamine 2,3-dioxygenase; MHC=major histocompatibility complex [Figure obtained from Hum Immunol 2004 Feb;65(2):114-23.]

In another series of studies by Sarkhosh et al,. [86,87,91] we provided compelling evidence that IFN-γ induced IDO expression suppresses the proliferation of immune cells co-cultured with IDO-expressing fibroblasts (Figure 4).

This finding was supported by the fact that addition of an IDO-inhibitor (1MT) reversed the suppressive effects of IDO on PBMC proliferation in a dose-dependent fashion. As an alternative method to genetic modification, we have used a temperature-sensitive polymer, conjugated IFN-γ as a slow release system in a skin substitute to further prolong the effect of IFN-γ on IDO expression in skin cells. We, therefore, concluded that IDO-expressing allogeneic fibroblasts embedded within a collagen gel suppress the proliferation of allogeneic immune cells, while they still remain viable in this IDO-induced tryptophan-deficient environment.

In human immunology a potential relevance for IDO induction by reverse signaling from T cells to DCs was provided by Munn *et al.* [28] 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 a mixed lymphocyte reaction. These DCs were found to be able to suppress the proliferation of allogeneic T cells and suppression was reversed by addition of 1MT.

Overall, these data suggest a potentially dominant role of IDO governing allo-reactivity and 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. 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 does not necessarily mean more immunoregulatory activity in the direction of tolerance.

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.

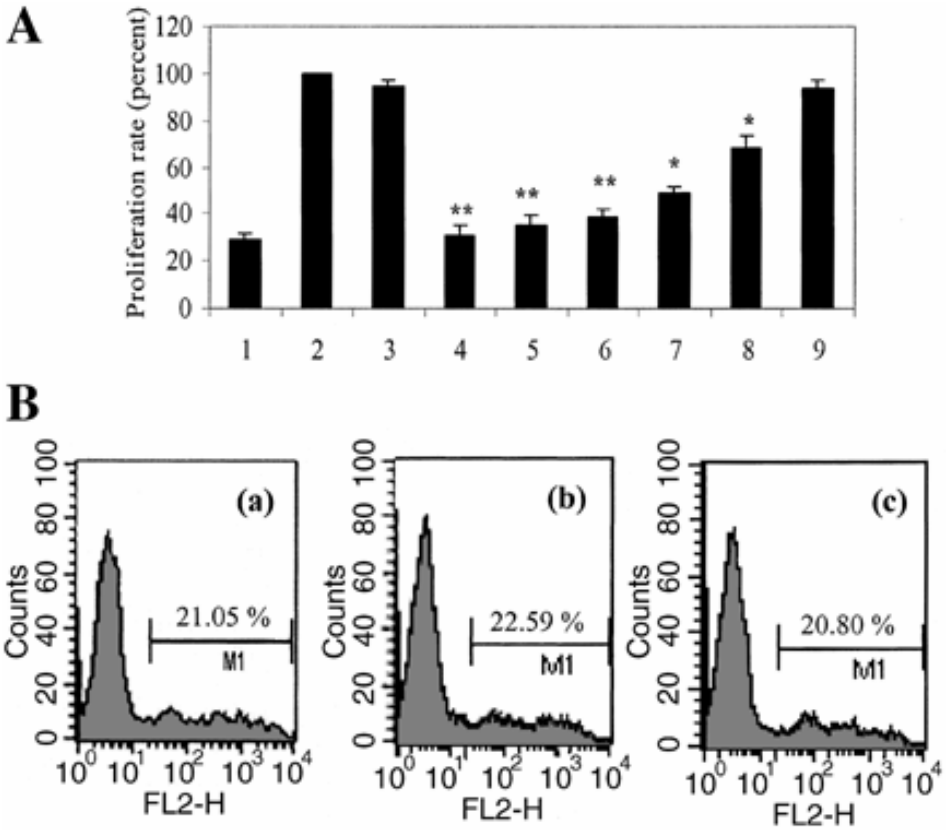

Figure 4. The proliferation of PBMC cocultured with IDO-expressing dermal fibroblasts was suppressed. Fibroblasts were treated with 2,000 U of IFN-γ for 48 h. To eliminate the effects of IFN-γ, after this period, conditioned medium was replaced with fresh medium with no IFN-γ. IFN-γ treated or untreated fibroblasts were then cocultured with isolated human PBMC for a period of 5 days. ^{3}H-thymidime was then added to each coculture sample at a final concentration of 2 µCi/mL and 16 h later, the floating PBMC were harvested, washed twice with PBS, and their radioactive count was measured. In panel A, PBMC were cultured either alone (lane 1), with non-IDO-expressing fibroblasts (lane 2), with non-IDO-expressing cells plus 200 µM of IDO inhibitor (1-methyl-d-tryptophan) (lane 3), or with IDO-expressing fibroblasts in the absence of IDO inhibitor (lane 4), or in presence of various concentrations of IDO inhibitor at final concentrations of 50, 100, 200, 400, or 800 µM (lanes 5-9). Data represents the mean ± SD for three separate experiments. The asterisks (*, **P < 0.05 and <0.001, respectively) denote a significant difference in proliferation of PMBC in control samples (cocultured with non-IDO-expressing fibroblasts) and those either cocultured with IDO-expressing fibroblasts (lane 2 vs. 4) or IDO-expressing cells in the presence of various concentration of IDO inhibitor (lane 2 vs. 5, 6, 7, and 8). PBMC proliferation was restored at 800 µM concentration of 1-methyl tryptophan (lane 9), where no significant difference was observed in rate of cocultured PBMC proliferation between control and IDO producing fibroblasts (lane 2 vs. 9). Panel B: To evaluate the viability of PBMC, cells were stained with PI and the number of positive PBMC was measured in the samples cultured either alone (a), cocultured with no IDO-expressing fibroblasts (b), or cocultured with IDO-expressing fibroblasts (c) for 5 days. [Figure obtained from J Cell Biochem. 2003 Sep 1;90(1):206-17]

CONCLUSION

There is convincing evidence that tryptophan metabolism through IDO dependent pathway plays an important role in immunomodulation in physiologic, paraphysiologic, and pathologic states in mammals. There are several reports that cells expressing IDO can suppress T cell responses and promote tolerance. Furthermore, differential sensitivity observed between immune and primary cells to an IDO-generated tryptophan-deficient environment can be exploited in development of allogeneic grafts such as a non-rejectable allogeneic skin substitute or wound coverage. The local immunosuppressive properties of IDO can have significant role in the development of a local immunosuppressive barrier for grafted cells and tissues without any severe effect on host's immune system.

REFERENCES

1. Muller AJ, Prendergast GC. Indoleamine 2,3-dioxygenase in immune suppression and cancer. Curr Cancer Drug Targets 2007; 7(1):31-40.
2. Grohmann U, Fallarino F, Puccetti P. Tolerance, DCs and tryptophan: much ado about IDO. Trends Immunol 2003; 24(5):242-8.
3. Sugimoto H, Oda Si, Otsuki T, Hino T, Yoshida T, Shiro Y. Crystal structure of human indoleamine 2,3-dioxygenase: Catalytic mechanism of O2 incorporation by a heme-containing dioxygenase. PNAS 2006; 103(8):2611-6.
4. Yoshida R, Urade Y, Nakata K, Watanabe Y, Hayaishi O. Specific induction of indoleamine 2,3-dioxygenase by bacterial lipopolysaccharide in the mouse lung. Arch Biochem Biophys 1981; 212(2):629-37.
5. von Rango U, Krusche CA, Beier HM, Classen-Linke I. Indoleamine-dioxygenase is expressed in human decidua at the time maternal tolerance is established. J Reprod Immunol 2007; 74(1-2):34-45.
6. Carlin JM, Borden EC, Sondel PM, Byrne GI. Interferon-induced indoleamine 2,3-dioxygenase activity in human mononuclear phagocytes. J Leukoc Biol 1989; 45(1):29-34.
7. Hwu P, Du MX, Lapointe R, Do M, Taylor MW, Young HA. Indoleamine 2,3-dioxygenase production by human dendritic cells results in the inhibition of T cell proliferation. J Immunol 2000; 164(7):3596-9.
8. Taylor MW, Feng GS. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J 1991; 5(11):2516-22.
9. Yoshida R, Oku T, Imanishi J, Kishida T, Hayaishi O. Interferon: a mediator of indoleamine 2,3-dioxygenase induction by lipopolysaccharide, poly(I) X poly(C), and pokeweed mitogen in mouse lung. Arch Biochem Biophys 1986; 249(2):596-604.
10. Odemuyiwa SO, Ghahary A, Li Y, Puttagunta L, Lee JE, Musat-Marcu S, et al. Cutting Edge: Human Eosinophils Regulate T Cell Subset Selection through Indoleamine 2,3-Dioxygenase. J Immunol 2004; 173(10):5909-13.
11. Varga J, Yufit T, Hitraya E, Brown RR. Control of extracellular matrix degradation by interferon-gamma. The tryptophan connection. Adv Exp Med Biol 1996; 398:143-8.
12. Beutelspacher SC, Tan PH, McClure MO, Larkin DF, Lechler RI, George AJ. Expression of indoleamine 2,3-dioxygenase (IDO) by endothelial cells: implications for the control of alloresponses. Am J Transplant 2006; 6(6):1320-30.
13. Hayashi T, Beck L, Rossetto C, Gong X, Takikawa O, Takabayashi K, et al. Inhibition of experimental asthma by indoleamine 2,3-dioxygenase. J Clin Invest 2004; 114(2):270-9.
14. Burkin DJ, Kimbro KS, Barr BL, Jones C, Taylor MW, Gupta SL. Localization of the human indoleamine 2,3-dioxygenase (IDO) gene to the pericentromeric region of human chromosome 8. Genomics 1993; 17(1):262-3.
15. Orabona C, Puccetti P, Vacca C, Bicciato S, Luchini A, Fallarino F, et al. Toward the identification of a tolerogenic signature in IDO-competent dendritic cells. Blood 2006; 107(7):2846-54.
16. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol 2004; 4(10):762-74.
17. Chaves AC, Ceravolo IP, Gomes JA, Zani CL, Romanha AJ, Gazzinelli RT. IL-4 and IL-13 regulate the induction of indoleamine 2,3-dioxygenase activity and the control of Toxoplasma gondii replication in human fibroblasts activated with IFN-gamma. Eur J Immunol 2001; 31(2):333-44.
18. Musso T, Gusella GL, Brooks A, Longo DL, Varesio L. Interleukin-4 inhibits indoleamine 2,3-dioxygenase expression in human monocytes. Blood 1994; 83(5):1408-11.
19. Thomas SR, Stocker R. Redox reactions related to indoleamine 2,3-dioxygenase and tryptophan metabolism along the kynurenine pathway. Redox Rep 1999; 4(5):199-220.
20. Tone S, Takikawa O, Habara-Ohkubo A, Kadoya A, Yoshida R, Kido R. Primary structure of human indoleamine 2,3-dioxygenase deduced from the nucleotide sequence of its cDNA. Nucleic Acids Res 1990; 18(2):367.
21. Mellor AL, Baban B, Chandler PR, Manlapat A, Kahler DJ, Munn DH. Cutting edge: CpG oligonucleotides induce splenic CD19+ dendritic cells to acquire potent indoleamine 2,3-dioxygenase-dependent T cell regulatory functions via IFN Type 1 signaling. J Immunol 2005; 175(9):5601-5.
22. Wingender G, Garbi N, Schumak B, Jungerkes F, Endl E, von Bubnoff D, et al. Systemic application of CpG-rich DNA suppresses adaptive T cell immunity via induction of IDO. Eur J Immunol 2006; 36(1):12-20.
23. Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. Nat Immunol 2004; 5(12):1219-26.
24. Munn DH, Sharma MD, Hou D, Baban B, Lee JR, Antonia SJ, et al. Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. J Clin Invest 2004; 114(2):280-90.
25. Grohmann U, Fallarino F, Bianchi R, Belladonna ML, Vacca C, Orabona C, et al. IL-6 inhibits the tolerogenic function of CD8 alpha+ dendritic cells expressing indoleamine 2,3-dioxygenase. J Immunol 2001; 167(2):708-14.

26. Braun D, Longman RS, Albert ML. A two-step induction of indoleamine 2,3 dioxygenase (IDO) activity during dendritic-cell maturation. Blood 2005; 106(7):2375-81.
27. Grohmann U, Bianchi R, Orabona C, Fallarino F, Vacca C, Micheletti A, et al. Functional plasticity of dendritic cell subsets as mediated by CD40 versus B7 activation. J Immunol 2003; 171(5):2581-7.
28. Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, et al. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science 2002; 297(5588):1867-70.
29. von Bergwelt-Baildon MS, Popov A, Saric T, Chemnitz J, Classen S, Stoffel MS, et al. CD25 and indoleamine 2,3-dioxygenase are up-regulated by prostaglandin E2 and expressed by tumor-associated dendritic cells in vivo: additional mechanisms of T-cell inhibition. Blood 2006; 108(1):228-37.
30. Munn DH, Mellor AL. Indoleamine 2,3-dioxygenase and tumor-induced tolerance.J Clin Invest 2007; 117(5):1147-54.
31. Le AV, Cho JY, Miller M, McElwain S, Golgotiu K, Broide DH. Inhibition of allergen-induced airway remodeling in Smad 3-deficient mice. J Immunol 2007; 178(11):7310-6.
32. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, et al. Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol 2003; 4(12):1206-12.
33. Grohmann U, Orabona C, Fallarino F, Vacca C, Calcinaro F, Falorni A, et al. CTLA-4-Ig regulates tryptophan catabolism in vivo. Nat Immunol 2002; 3(11):1097-101.
34. Munn DH, Sharma MD, Mellor AL. Ligation of B7-1/B7-2 by human CD4+ T cells triggers indoleamine 2,3-dioxygenase activity in dendritic cells. J Immunol. 2004; 172(7):4100-10.
35. Finger EB, Bluestone JA. When ligand becomes receptor--tolerance via B7 signaling on DCs. Nat Immunol 2002; 3(11):1056-7.
36. Beutelspacher SC, Pillai R, Watson MP, Tan PH, Tsang J, McClure MO, et al. Function of indoleamine 2,3-dioxygenase in corneal allograft rejection and prolongation of allograft survival by over-expression. Eur J Immunol 2006; 36(3):690-700.
37. Barceló-Batllori S, André M, Servis C, Lévy N, Takikawa O, Michetti P, et al. Proteomic analysis of cytokine induced proteins in human intestinal epithelial cells: implications for inflammatory bowel diseases.Proteomics 2002; 2(5):551-60.
38. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, et al. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 1998; 281(5380):1191-3.
39. Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E, Prendergast GC. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. Nat Med 2005; 11(3):312-9.
40. Mellor AL, Sivakumar J, Chandler P, Smith K, Molina H, Mao D, et al. Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism during pregnancy. Nat Immunol 2001; 2(1):64-8.
41. Terness P, Bauer TM, Röse L, Dufter C, Watzlik A, Simon H, et al. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. J Exp Med 2002; 196(4):447-57.
42. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J Exp Med 2002; 196(4):459-68.
43. Fallarino F, Grohmann U, Vacca C, Bianchi R, Orabona C, Spreca A, et al. T cell apoptosis by tryptophan catabolism. Cell Death & Differ 2002; 9(10):1069-77.
44. Della Chiesa M, Carlomagno S, Frumento G, Balsamo M, Cantoni C, Conte R, et al. The tryptophan catabolite L-kynurenine inhibits the surface expression of NKp46- and NKG2D-activating receptors and regulates NK-cell function. Blood 2006; 108(13):4118-25.
45. Wang J, Simonavicius N, Wu X, Swaminath G, Reagan J, Tian H, et al. Kynurenic Acid as a Ligand for Orphan G Protein-coupled Receptor GPR35. J Biol Chem 2006; 281(31):22021-8.
46. Swanson KA, Zheng Y, Heidler KM, Mizobuchi T, Wilkes DS. CDllc+ Cells Modulate Pulmonary Immune Responses by Production of Indoleamine 2,3-Dioxygenase. Am J Respir Cell Mol Biol 2004; 30(3):311-8.
47. Beatty WL, Belanger TA, Desai AA, Morrison RP, Byrne GI. Tryptophan depletion as a mechanism of gamma interferon-mediated chlamydial persistence. Infect Immun 1994; 62(9):3705-11.
48. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 Kinase in T Cells Mediates Proliferative Arrest and Anergy Induction in Response to Indoleamine 2,3-Dioxygenase. Immunity 2005; 22(5):633-42.
49. Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, et al. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. J Immunol 2006; 176(11):6752-61.
50. Gao X, Zhang Y, Arrazola P, Hino O, Kobayashi T, Yeung RS, et al. Tsc tumour suppressor proteins antagonize amino-acid-TOR signalling. Nat Cell Biol 2002; 4(9):699-704.

51. Rohde J, Heitman J, Cardenas ME. The TOR kinases link nutrient sensing to Cell growth. J Biol Chem 2001; 276(13):9583-6.
52. Fingar DC, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. Oncogene 2004; 23(18):3151-71.
53. Grolleau A, Bowman J, Pradet-Balade B, Puravs E, Hanash S, Garcia-Sanz JA, et al. Global and specific translational control by rapamycin in T cells uncovered by microarrays and proteomics. J Biol Chem 2002; 277(25):22175-84.
54. Fox CJ, Hammerman PS, Thompson CB. The Pim kinases control rapamycin-resistant T cell survival and activation. J Exp Med 2005; 201(2):259-66.
55. Hinnebusch AG. The eIF-2 alpha kinases: regulators of protein synthesis in starvation and stress. Semin Cell Biol 1994; 5(6):417-26.
56. Drevet JR. The antioxidant glutathione peroxidase family and spermatozoa: a complex story. Mol Cell Endocrinol 2006; 250(1-2):70-9.
57. Harding HP, Novoa I, Zhang Y, Zeng H, Wek R, Schapira M, et al. Regulated translation initiation controls stress-induced gene expression in mammalian cells. Mol Cell 2000; 6(5):1099-108.
58. Sood R, Porter AC, Olsen D, Cavener DR, Wek RC. A Mammalian Homologue of GCN2 Protein Kinase Important for Translational Control by Phosphorylation of Eukaryotic Initiation Factor-2alpha. Genetics 2000; 154(2):787-801.
59. Wek RC, Jackson BM, Hinnebusch AG. Juxtaposition of domains homologous to protein kinases and histidyl-tRNA synthetases in GCN2 protein suggests a mechanism for coupling GCN4 expression to amino acid availability. Proc Natl Acad Sci U S A 1989; 86(12):4579-83.
60. Clemens MJ. Initiation factor eIF2 alpha phosphorylation in stress responses and apoptosis. Prog Mol Subcell Biol 2001; 27:57-89.
61. Li Y, Tredget EE, Ghaffari A, Lin X, Kilani RT, Ghahary A. Local expression of indoleamine 2,3-dioxygenase protects engraftment of xenogeneic skin substitute. J Invest Dermatol 2006; 126(1):128-36.
62. Gietzen DW, Magrum LJ. Molecular mechanisms in the brain involved in the anorexia of branched-chain amino acid deficiency.J Nutr 2001; 131(3):851S-855S.
63. Wek RC, Jiang HY, Anthony TG. Coping with stress: eIF2 kinases and translational control. Biochem Soc Trans 2006; 34(Pt 1):7-11.
64. Anthony TG, McDaniel BJ, Byerley RL, McGrath BC, Cavener DR, McNurlan MA, et al. Preservation of liver protein synthesis during dietary leucine deprivation occurs at the expense of skeletal muscle mass in mice deleted for eIF2 kinase GCN2. J Biol Chem 2004; 279(35):36553-61.
65. Crosby JS, Chefalo PJ, Yeh I, Ying S, London IM, Leboulch P, et al. Regulation of hemoglobin synthesis and proliferation of differentiating erythroid cells by heme-regulated eIF-2alpha kinase. Blood 2000; 96(9):3241-8.
66. Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D. Perk is essential for translational regulation and cell survival during the unfolded protein response. Mol Cell 2000; 5(5):897-904.
67. Niwa M, Walter P. Pausing to decide. Proc Natl Acad Sci U S A 2000; 97(23):12396-7.
68. Li Y, Tredget EE, Ghahary A. Cell surface expression of MHC class I antigen is suppressed in indoleamine 2,3-dioxygenase genetically modified keratinocytes: implications in allogeneic skin substitute engraftment. Hum Immunol 2004; 65(2):114-23.
69. Marshall B, Keskin D, Mellor A. Regulation of prostaglandin synthesis and cell adhesion by a tryptophan catabolizing enzyme. BMC Biochem 2001; 2:5.
70. van Wissen M, Snoek M, Smids B, Jansen HM, Lutter R. IFN-gamma amplifies IL-6 and IL-8 responses by airway epithelial-like cells via indoleamine 2,3-dioxygenase. J Immunol 2002; 169(12):7039-44.
71. Gurtner GJ, Newberry RD, Schloemann SR, McDonald KG, Stenson WF. Inhibition of indoleamine 2,3-dioxygenase augments trinitrobenzene sulfonic acid colitis in mice. Gastroenterology 2003; 125(6):1762-73.
72. Grohmann U, Fallarino F, Bianchi R, Orabona C, Vacca C, Fioretti MC, et al. A defect in tryptophan catabolism impairs tolerance in nonobese diabetic mice. J Exp Med 2003; 198(1):153-60.
73. Bertazzo A, Punzi L, Bertazzolo N, Pianon M, Pozzuoli A, Costa CV, et al. Tryptophan catabolism in synovial fluid of various arthropathies and its relationship with inflammatory cytokines. Adv Exp Med Biol 1999; 467:565-70.
74. Kwidzinski E, Bunse J, Aktas O, Richter D, Mutlu L, Zipp F, et al. Indolamine 2,3-dioxygenase is expressed in the CNS and down-regulates autoimmune inflammation. FASEB J 2005; 19(10): 1347-9.
75. Gupta SL, Carlin JM, Pyati P, Dai W, Pfefferkorn ER, Murphy MJ, Jr. Antiparasitic and antiproliferative effects of indoleamine 2,3-dioxygenase enzyme expression in human fibroblasts. Infect Immun 1994; 62(6):2277-84.
76. Pfefferkorn ER. Interferon gamma blocks the growth of Toxoplasma gondii in human fibroblasts by inducing the host cells to degrade tryptophan. Proc Natl Acad Sci U S A 1984; 81(3):908-12.
77. Bodaghi B, Goureau O, Zipeto D, Laurent L, Virelizier JL, Michelson S. Role of IFN-gamma-induced indoleamine 2,3

dioxygenase and inducible nitric oxide synthase in the replication of human cytomegalovirus in retinal pigment epithelial cells. J Immunol 1999; 162(2):957-64.

78. Fujigaki S, Saito K, Takemura M, Maekawa N, Yamada Y, Wada H, et al. L-tryptophan-L-kynurenine pathway metabolism accelerated by Toxoplasma gondii infection is abolished in gamma interferon-gene-deficient mice: cross-regulation between inducible nitric oxide synthase and indoleamine-2,3-dioxygenase. Infect Immun 2002; 70(2):779-86.
79. Widner B, Weiss G, Fuchs D. Tryptophan degradation to control T-cell responsiveness. Immunol Today 2000; 21(5):250.
80. Rogers KA, DeKrey GK, Mbow ML, Gillespie RD, Brodskyn CI, Titus RG. Type 1 and type 2 responses to Leishmania major. FEMS Microbiology Letters 2002; 209(1):1-7.
81. Hainz U, Jurgens B, Heitger A. The role of indoleamine 2,3-dioxygenase in transplantation. Transpl Int 2007; 20(2):118-27.
82. Bertera S, Alexander AM, Crawford ML, Papworth G, Watkins SC, Robbins PD, et al. Gene combination transfer to block autoimmune damage in transplanted islets of Langerhans. Exp Diabesity Res 2004; 5(3):201-10.
83. Miki T, Sun H, Lee Y, Tandin A, Kovscek AM, Subbotin V, et al. Blockade of tryptophan catabolism prevents spontaneous tolerogenicity of liver allografts. Transplant Proc 2001; 33(1-2):129-30.
84. Alexander AM, Crawford M, Bertera S, Rudert WA, Takikawa O, Robbins PD, et al. Indoleamine 2,3-dioxygenase expression in transplanted NOD Islets prolongs graft survival after adoptive transfer of diabetogenic splenocytes. Diabetes 2002; 51(2):356-65.
85. Jalili RB, Rayat GR, Rajotte RV, Ghahary A. Suppression of islet allogeneic immune response by indoleamine 2,3 dioxygenase-expressing fibroblasts. J Cell Physiol 2007; 213(1):137-43.
86. Sarkhosh K, Tredget EE, Karami A, Uludag H, Iwashina T, Kilani RT, et al. Immune cell proliferation is suppressed by the interferon-gamma-induced indoleamine 2,3-dioxygenase expression of fibroblasts populated in collagen gel (FPCG). J Cell Biochem 2003; 90(1):206-17.
87. Sarkhosh K, Tredget EE, Li Y, Kilani RT, Uludag H, Ghahary A. Proliferation of peripheral blood mononuclear cells is suppressed by the indoleamine 2,3-dioxygenase expression of interferon-gamma-treated skin cells in a co-culture system.Wound Repair Regen 2003; 11(5):337-45.
88. Ghahary A, Li Y, Tredget EE, Kilani RT, Iwashina T, Karami A, et al. Expression of indoleamine 2,3-dioxygenase in dermal fibroblasts functions as a local immunosuppressive factor. J Invest Dermatol 2004; 122(4):953-64.
89. Frolova LY, Grigorieva AY, Sudomoina MA, Kisselev LL. The human gene encoding tryptophanyl-tRNA synthetase: interferon-response elements and exon-intron organization. Gene 1993; 128(2):237-45.
90. Fleckner J, Martensen PM, Tolstrup AB, Kjeldgaard NO, Justesen J. Differential regulation of the human, interferon inducible tryptophanyl-tRNA synthetase by various cytokines in cell lines. Cytokine 1995; 7(1):70-7.
91. Sarkhosh K, Tredget EE, Uludag H, Kilani RT, Karami A, Li Y, et al. Temperature-sensitive polymer-conjugated IFN-gamma induces the expression of IDO mRNA and activity by fibroblasts populated in collagen gel (FPCG).. J Cell Physiol 2004; 201(1):146-54.