Current Trends in Immunology
Vol. 11, 2010

Review

Immunomodulatory role of IDO in physiological and pathological conditions

Azadeh Hosseini-Tabatabaei, Malihe-Sadat Poormasjedi-Meibod, Reza Baradar Jalili, and Aziz Ghahary*

Department of Surgery, University of British Columbia, Vancouver, BC, Canada

ABSTRACT

Indoleamine 2,3-dioxygenase (IDO) is a cytosolic hemoprotein enzyme responsible for the rate-limiting step in catabolism of tryptophan to N-formylkynurenine. The importance of this immunomodulatory protein in mammalian pregnancy was first described in 1998 by Munn *et al.* They showed that in contrary to transplantation rules, the fetus that carries both maternal and paternal genes is protected in mother's immune system during gestation due to high levels of IDO expression in placenta. Since then, IDO has also been shown to have important roles in immunoregulation of cancer, inflammation and allergy, autoimmune diseases, and allotransplantation. Two main mechanisms have been suggested to be responsible for IDO immunosuppressive effects: either local tryptophan starvation, formation of toxic catabolites or both resulted from IDO activity, which both affect T cell-mediated immune response. In this article, we will discuss the biological characteristics of IDO along with its immunomodulatory functions in physiological and pathological conditions.

*Corresponding author and reprint requests to:
Dr. Aziz Ghahary,
Director, Burn and Wound Healing Research Lab,
Rm 351, Jack Bell Research Centre,
2660 Oak Street, Vancouver, BC,
V6H 3Z6, Canada.
aghahary@interchange.ubc.ca

KEYWORDS: indoleamine 2,3-dioxygenase, tryptophan catabolism, kynurenine, immuno-regulation, immunosuppression

ABBREVIATIONS

IDO	Indoleamine 2,3-dioxygenase
NAD+	Nicotinamide adenine dinucleotide
TDO	Tryptophan 2,3-dioxynegase
LPS	Lipopolysaccharides
IFNs	Interferons
DCs	Dendritic cells
INDOL1	Indoleamine 2,3-dioxygenase-like-protein 1
JAK/STAT1	Janus kinase/signal transducer and activator of transcription 1
IFNγR1	IFNγ receptor subunit 1
ISREs	Interferon stimulatory response elements
GAS	γ-activating sequences
TGF-β	Transforming growth factor-β
PI3K	Phosphatidylinositol 3-kinase
NF-κB	Nuclear factor- κB
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
JNK	C-Jun-N-terminal kinase
TLR	Toll-like receptor
TCR	T cell receptor
GCN2	General control nonderepressible 2
T regs	Regulatory T cells
Th	T helper

NKT cells	Natural killer T cells
APCs	Antigen presenting cells
HLA-G	Human leukocyte antigen-G
1-MT	1-methyl tryptophan
NK	Natural killer
NKG2D	NK cell activation receptor
tRNA	Transfer RNA
PBMC	Peripheral blood mononuclear cell
T1D	Type 1 diabetes
IDDM	Insulin-dependent diabetes mellitus
NOD	Nonobese diabetic
EAE	Experimental autoimmune encephalomyelitis

INTRODUCTION

The complex role of immune system in regulating the balance between inflammation and tolerance has been the subject of great interest for many years. Many studies have recently focused on the importance of the immunregulatory pathway of tryptophan metabolism and its role in modulation of peripheral tolerance. For almost a decade, the key enzyme of this pathway, indoleamine 2,3-dioxygenase (IDO), and its far-reaching immunomodulatory roles in different traits including neoplasia, pregnancy, autoimmune diseases, transplantation and infectious diseases, have been investigated. Many studies implicate IDO for induction of immune tolerance or as a host innate immune defense against pathogens [1]. The enormous increase in the number of literatures about IDO and its activities, confirms the importance of this enzyme in different aspects of immune response. This article explains the biological and molecular characteristics of IDO. Moreover, we have summarized the current data on the immunoregulatory roles of IDO and tryptophan metabolism in modulation of immune response. Because of space restrictions, we will only focus on effects of IDO on immune cells and its role in physiological and pathological conditions including pregnancy, cancer, autoimmune diseases and allotransplantation.

Tryptophan breakdown and the role of IDO

L-tryptophan is the least abundant of all essential amino acids in human body. This amino acid not only serves as a building block in protein biosynthesis, but also is a precursor for synthesis of serotonin and nicotinamide adenine dinucleotide (NAD+) [2]. While tryptophan hydroxylase initiates degradation of tryptophan in the serotonin pathway, indoleamine-2,3 dioxygenase 1 (IDO1), or tryptophan 2,3-dioxynegase (TDO) are the rate-limiting enzymes that initiate catabolism of tryptophan through kynurenine pathway [2, 3]. IDO1 catalyses tryptophan to N-formylkynurenine which then rapidly is degraded to form a series of metabolites named kynurenines and finally NAD+ [2, 4]. Serotonin can also further be converted to melatonin via activities of N-acetyltransferase and 5-hydroxyindole-O-methyltransferase [5].

The expression of TDO is mainly restricted to the liver, however, this enzyme has also been found in other sites, like brain, mucous membranes and epididymis [3]. On the other hand, IDO1 is widely expressed intracellulary in different cells and tissues specially the immune privileged sites. Examples of above include epididymis [6], placenta [7, 8], anterior chamber of the eye, mucosa of the gut (distal ileum and colon) [6], lung [9], primary and secondary lymphoid organs [2, 4]. In addition to constitutive expression of IDO1 in the gut, lipopolysaccharides (LPS), type I interferons (IFNs) (IFN-α and IFN-β) and more potently, type II IFNs (IFN-γ) can induce IDO1 expression in variety of cells [10]. For instance, IDO1 is strongly induced by IFN-γ in monocytes and macrophages, dendritic cells (DCs), trophoblasts, cultured fibroblasts [4] and pancreatic β-cells [3]. Interestingly, type I and II IFNs are reported to have equal potency in IDO induction in case of plasmacytoid DCs [11].

IDO2 is another enzyme with IDO1-like activity, (also known as indoleamine 2,3-dioxygenase-like-protein 1 (INDOL1)) [12], which has not only been identified in mammals but also in lower invertebrates [3]. The enzymatic activity of IDO1 and IDO2 are quite similar, however, their expression patterns in tissues are different [12].

IDO expression

IDO1 is coded by a gene located on the chromosome 8p 12 in humans, named *INDO* or *IDO1* gene [13]. The promoter of this gene

contains a specific site for IFNγ-, and two non-specific sites for IFNα and IFNβ-reponsive elements [11]. IFNγ mainly regulates *INDO* transcription through Janus kinase/signal transducer and activator of transcription 1 (JAK/STAT1) pathway [13]. Upon binding to the receptor, IFNγ stimulates the oligomerization of the receptor and activates JAK proteins. Activated JAKs phosphorylate tyrosine 440 of the IFNγ receptor subunit 1(IFNγR1), providing a docking site for STAT1. Upon getting phosphorylated on tyrosine 701 and serine 727, STAT1 is diamerized and translocates to the nucleus [14]. Then it binds to interferon stimulatory response elements (ISREs) and γ-activating sequences (GAS) in the *IDO* promoter and regulates expression of *IDO* gene [3].

Recently, it was reported that transforming growth factor- β (TGF-β) is also able to activate *INDO* transcription through phosphatidylinositol 3-kinase (PI3K)/Akt and non-canonical nuclear factor- κB (NF-κB). This activity, leads to transformation of immunogenic $CD8^-$ DCs to tolerogenic ones [15]. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and LPS are also reported to induce IDO activity through NF-κB activity [16]. Moreover, a recent study showed that LPS induces IDO expression in primary murine macroglia, independent of IFNγ-mediated pathway and through c-Jun-N-terminal kinase (JNK) signaling pathway [17]. CpG-rich oligodeoxynucleotides is also another inducer of IDO1 expression whose engagement with Toll-like receptor (TLR) 9 results in the secretion of type I IFNs by plasmocytiods DCs [18]. Other inducers of *INDO* transcription include poly (I:C) (TLR3 ligand) [19], endogenous thymosin α1 [20] and hormones like chorionic gonadotropin and estrogens [21, 22].

IDO structure, biochemical characteristics and regulation of expression

IDO1 is an intracellular monomeric oxydo-reductase containing a heme prosthetic group. This enzyme consists of 407 amino acids and the mature IDO1 has a molecular weight of 42-45 kDa [13]. Like IDO1, the IDO2 enzyme is a hemoprotein and its crystal structure has been identified to be 43% similar to IDO1 [3]. Using X-ray crystallography, it is revealed that IDO protein is folded into a large alpha-helical catalytic domain located at the C-terminal of the protein, and a small alpha-helical non-catalytic domain in N-terminal [3, 4, 23], and a long loop connecting these two domains. The heme prosthetic group is positioned between the two domains [23].

The existence of inactive enzyme shows that IDO1 might go under post-translational modifications, which have not been defined yet. However, incorporation of the heme prosthetic group, oxidation and reduction status of the cell, and nitric oxide production induced by proinflammatory cytokines are factors known to control IDO1 activity post-translation [16].

IDO and modulation of immune response

The mechanisms by which IDO modulate the immune response are still being elucidated. However, the immunoregulatory effects of IDO are in part due to broad suppression of cell proliferation caused by tryptophan starvation. Additionally, other studies suggest that downstream tryptophan metabolites could be directly responsible for some of the observed effects.

IDO and T cells

Local tryptophan deprivation has been shown to provoke G1 phase-cell cycle arrest in effector T cells [13]. Interestingly, recapturing tryptophan does not guarantee the completion of activation process and therefore, a second signal from T cell receptor (TCR) is also required [24]. It is elucidated that activation of a stress-responsive kinase named as general control nonderepressible 2 (GCN2) in T cells, not only is involved in their proliferative arrest but also induces anergy in these cells [4, 25, 26], which will be further discussed in detail. Other consequences of tryptophan starvation include inhibition of T cell proliferation, induction of FoxP3 and generation of regulatory T cells (T regs), down regulation of TCR-ζ chain $CD8^+$, defective formation of memory T cells (Tan and Bharath 2009), and suppression of alloreactive T cells [1]. Interestingly, it is revealed that suppressive effects of IDO on $CD8^+$ T cells are more than $CD4^+$

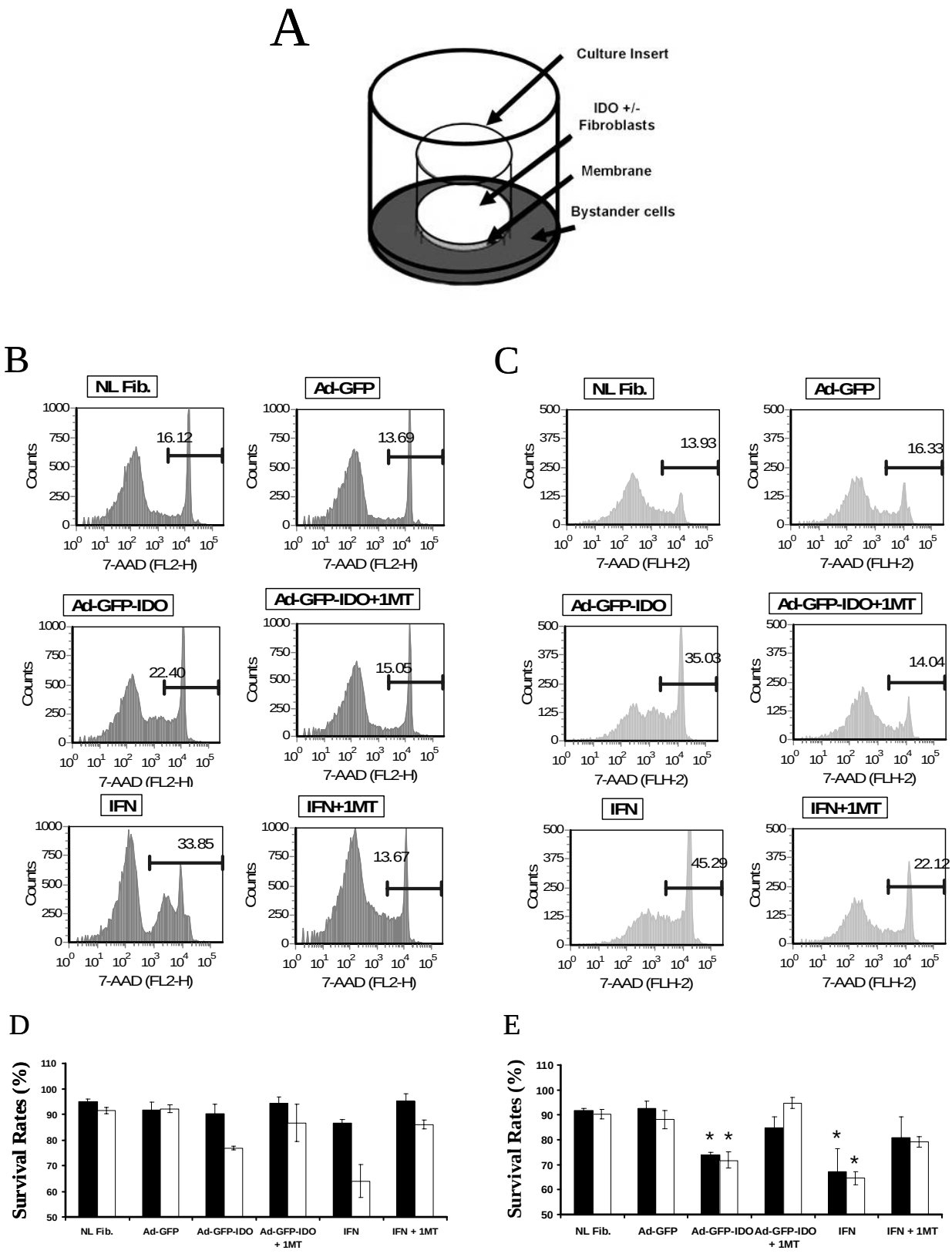
A
Culture Insert
IDO +/-
Fibroblasts
Membrane
Bystander cells
B
NL Fib.
16.12
Ad-GFP
13.69
Ad-GFP-IDO
22.40
Ad-GFP-IDO+1MT
15.05
IFN
33.85
IFN+1MT
13.67
Counts
7-AAD (FL2-H)
C
NL Fib.
13.93
Ad-GFP
16.33
Ad-GFP-IDO
35.03
Ad-GFP-IDO+1MT
14.04
IFN
45.29
IFN+1MT
22.12
7-AAD (FLH-2)
D
Survival Rates (%)
NL Fib.
Ad-GFP
Ad-GFP-IDO
Ad-GFP-IDO + 1MT
IFN
IFN + 1MT
E
Survival Rates (%)
NL Fib.
Ad-GFP
Ad-GFP-IDO
Ad-GFP-IDO + 1MT
IFN
IFN + 1MT

Figure 1

T cells in terms of proliferation (Figure 1) [26]. In fact, the antiproliferative property of IDO resulted from catabolism of tryptophan, can be an explanation to its anti-tumor characteristics and direct antimicrobial effects on tryptophan-dependent intracellular pathogens and tumor cells during the course of diseases.

The second proposed model for IDO-induced immunomodulation is based on accumulation of kynurenines, the downstream metabolites of tryptophan catabolism pathway. It is believed that these metabolites can remarkably induce apoptosis in thymocytes and T helper (Th)1 lymphocytes. In addition to their direct toxicity to Th1 cells, tryptophan catabolites inhibit and induce tolerance in Th2 cells. Thus, it seems that IDO activity leads helper-T cells towards Th2 profile [13, 27]. Furthermore, a recent study showed that 3-hydroxykynurenine suppressed $CD4^+$ T cell proliferation, while induced development of T regs leading to better corneal allograft survival [28]. Another study showed that IDO activity and the presence of tryptophan metabolites resulted in a shift in cytokine responses of invariant natural killer T cells (NKT cells), an immunoregulatory subclass of T cells, towards Th2 profile [29].

IDO and antigen presenting cells (APCs)

Tolerogenic DCs are known as the main source of IDO in the body. IDO is strongly induced by IFNα and IFNγ in plasmacytoid DCs. A combination of these two cytokines in suboptimal concentrations also induces IDO1 expression in these cells, due to observed synergic or additive effects of these cytokines [11]. While expressed by tolerogenic DCs in response to type I/II IFNs, IDO regulates expression of type I IFNs (mainly IFNα) from $CD19^+$ DCs as a positive feedback. Such effect leads to the stimulation of IDO expression from DCs [11].

In addition to these effects, there is evidence that 3-hydroxyanthranilic acid, a downstream metabolite of tryptophan catabolism, induces cell surface expression and secretion of human leukocyte antigen-G (HLA-G) in DCs. This compound has also been shown to increase HLA-G surface expression in macrophages, but not in monocytes [30]. Therefore, one can suggest that IDO and HLA-G are two molecules involved in immunosuppressive properties of tolerogenic DCs [30, 31]. Furthermore, a separate study by Sekkai *et al.* [32] revealed that 3-hydroxyanthralinic acid not only inhibits the induction of nitric oxide synthesis in macrophages, but also decreases the activity of this enzyme in those cells. Since nitric oxide has been identified as an inhibitor of IDO activity, it seems that the downstream metabolites of kynurenine pathway reinforce IDO function by decreasing the generation of nitric oxide.

It has previously been shown that IFNγ is able to induce *INDO* transcription both in immunogenic $CD8^-$ and tolerogenic $CD8^+$ DCs. However, posttranslational inactivation of IDO in $CD8^-$ DCs interferes with the action of enzymes consequent to IDO, which results in nontolerogenic properties of these DCs. It has been reported that addition of quinolinic acid, a downstream metabolite of tryptophan catabolism, reactivates the pathway and converts nontolerogenic $CD8^-$ DCs to suppressive $CD8^-$ DCs following treatment with IFN-γ [33].

Legend to Figure 1. Differential immunosuppressive effect of IDO on primary human $CD4^+$ and $CD8^+$ T cells. IDO expression was induced in dermal fibroblasts, by IFNγ (1000U/ml) or gene transfection using adenoviral vector (Ad-GFP-IDO). Cells were then rinsed and co-cultured with purified primary human $CD4^+$ and $CD8^+$ T cells (either resting (solid bars) or stimulated (open bars) by 1 μg/ml of anti-CD3) in two chamber co-culture system for 4 days (**A**), in the absence or presence of 1-MT (800 μM), an inhibitor of IDO activity. The viability of $CD4^+$ (**B** and **D**) and $CD8^+$ (**C** and **E**) T cells was determined by FACS analysis using 7-AAD staining. * denotes significant difference with a P-value <0.001 (n=3). The data shows that in contrary to resting $CD4^+$ T cells, stimulated $CD4^+$ T cells were sensitive to IDO-induced low tryptophan environment (**Fig 2B** and **2D**). However, the survival of both resting and stimulated $CD8^+$ T cells decreased significantly in the IDO mediated microenvironment (**Fig 2C** and 2E). Moreover, treatment of the cells with 1-MT, markedly reversed the effect of IDO expression induced by both Ad-GFP-IDO and IFNγ on $CD4^+$ and $CD8^+$ T cells (**Fig 2D** and **2E**).

IDO and T regulatory cells

Mature plasmacytoid DCs are able to induce IL-10-producing T regs, who can subsequently modulate immune responses. Therefore, it seems that IDO contributes to peripheral expansion of T regs population [34]. Moreover, it is well documented that T regs expressing CTLA-4 can provoke IFNγ release and subsequently high levels of IDO expression and activity by tolerogenic B7-expressing DCs [16]. This could be one of the mechanisms by which CTLA-4^+ T regs modulate immune response.

IDO and B cells

Although many studies have confirmed the suppressive effects of IDO1 mainly on T cells survival and proliferation, some researchers have suggested a more complex role for IDO1. A research study by Scott *et al.* 2009 showed that blockage of IDO1 activity using 1-methyl tryptophan (1-MT), ameliorates B cell-mediated autoimmune responses in rheumatoid arthritis [35]. In contrast, using an experimental model of autoimmune myasthenia gravis it was shown that IFNγ-treated DCs, not only decreased the number of plasma cells, but also suppressed the function of B cells [36]. Moreover, it is shown that treatment with tryptophan metabolies like kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, induces B cells death [37].

IDO and NK cells

Research into the effect of IDO on natural killer (NK) cells has demonstrated that tryptophan deprivation and its metabolites impair the lysing activity of NK cells [37, 38]. In addition, the downstream products of tryptophan metabolism, induce death of NK cells [37]. Interestingly, it has been revealed that IDO expression results in downregulation of NK cell activation receptor (NKG2D) via JNK pathway. This has been suggested as a mechanism underlying Epstein-Barr virus escape from NKG2D-mediated immune attack, since this virus is able to induce IDO expression in B cells [39].

Molecular mechanism underlying immunomodulatory effects of IDO

As described before, IDO plays a critical role in modulation of T cell-mediated immune response. However the molecular mechanism underlying its immunoregulatory effects is not well understood. Since IDO catabolizes tryptophan, there is a possibility that it affects the pathways responding amino acid metabolism. Several studies have shown the profound role of GCN2 kinase pathway, an amino acid sensitive pathway in IDO-mediated responses. Tryptophan deficiency, resulted from IDO activity, causes an increase in uncharged form of transfer RNA (tRNA). The uncharged tRNA binds to the regulatory domain of GCN2 kinase leading to activation of this enzyme and triggers the downstream signaling pathway. Known as an integrated stress-response, GCN2 kinase activation can initiate cell-cycle arrest, apoptosis, differentiation and compensatory adaptation. In fact these effects vary depending on the cell type and the nature of the triggering stress [25]. Anergy is an important GCN2 kinase-mediated response that extends the immunomodulatory effects of IDO. Studies have shown that the IDO-mediated anergy is antigen-specific and IDO/GCN2 pathway only affects T cells that are concomitantly activated with their specific antigen [25].

The enzymatic reactions through kynurenine pathway start with conversion of tryptophan to N-formylkynurenine by TDO or IDO that is subsequently degraded to kynurenine. In addition to kynurenine there are two other metabolic intermediates within this pathway that are 3-hydroxyanthranilate and quinolinate. It has been shown that the level of the major metabolites of kynurenine pathway increases in the blood and body fluids following immune stimulation. Although tryptophan breakdown to kynurenine is almost a common response to IFNγ treatment in various cell types, it seems that the conversion of 3-hydroxyanthranilate to quinolinate is only seen in hepatocytes and some immune cells. Finding highly specific localization of quinolinate in immune cells, it was suggested that this metabolite plays an immunomodulatory role [2]. As described before, kynurenine, 3-hydroxykynurenine and 3-hydroxyanthranilate were shown to have additive inhibitory effects on T cell proliferation by inducing apoptotic cell death [37]. Furthermore, quinolinate has been also found to induce apoptosis in HL-60, a human leukemia cell line, through a caspase-mediated mechanism [40]. Morita *et al.* [41] have reported

that 3-hydroxyanthranilate induces apoptotic cell death in monocytes via overproduction of hydrogen peroxide. Considering the data from these studies, induction of apoptosis is apparently the main mechanism underlying the inhibitory effects of kynurenine pathway metabolites on immune cells.

Role of IDO in pregnancy, cancer, transplantation and autoimmunity

During the last decade numerous studies were conducted to elucidate immunomodulatory roles of IDO. As described before, the initial function of this enzyme was described as tryptophan depleting ability which explains its important role in innate immunity against certain infections such as HIV [42], *Chlamydia pneumonia* [43], *Toxoplasma gondii* [44] and certain bacteria. In 1998, Munn *et al.* discovered the immunosuppressive effect of IDO in protecting allogeneic fetus in mammalian pregnancy [45], which inspired sequential studies in this area. This enzyme is also involved in pathological conditions where normal adaptive immune responses are disrupted such as autoimmune diseases, chronic infections [46], and neoplasia [47-49], which will be discussed in subsequent sections.

IDO and pregnancy

In 1953, Sir Peter Medawar [50] considered three different mechanisms that might explain the immunological paradox of fetal survival, including: 1) mother-fetus anatomic separation, 2) fetus antigenic immaturity, and 3) mother's immunologic tolerance. Considering the fact that systemic tryptophan concentration during pregnancy is reduced [51] due to IDO expression by syncytiotrophoblast cells in human placenta [52], Munn and Mellor investigated the function of IDO in preventing T cell-mediated allogeneic fetus rejection in pregnant mice [45]. Treatment of pregnant mice with 1-MT drastically increased abortion rate in allogeneic but not syngeneic mating combinations suggesting the immuno-suppressive role of IDO in inducing materno-fetal tolerance [45]. Subsequent studies by Mellor *et al.* clarified the mechanisms of IDO immuno-suppression by locally depleting tryptophan, producing toxic tryptophan catabolites and suppressing T-cell dependent, antibody independent induction of C3 deposition at feto-maternal interface [53, 54]. Later studies by Baban *et al.* in 2004, using IDO-deficient mice, demonstrated that although IDO activity is a key mechanism for protecting allogeneic fetus against maternal T cells in normal mice, it is not the sole mechanism, since other redundant processes can compensate the loss of IDO activity during pregnancy [55].

IDO and cancer

Despite the systemic presence of high avidity T cells responsive to tumour-associated antigens, each tumour develops ways to evade the local immune destruction. Multiple mechanisms are suggested to be responsible for tumour induced immunological tolerance. These mechanisms may include IDO expression by tumour cells, tumour associated DCs and macrophages. Clinical investigations have determined that IDO is chronically activated in tumour bearing hosts [56] and that IDO activation serves as a marker of poor clinical prognosis in ovarian carcinoma and colon carcinoma [47, 49]. IDO contributes to tolerance against tumours by blocking the initial response to tumour associated antigens, inhibiting the action of immune effector cells in tumour microenvironment, inducing tumour-specific T cell anergy [1, 48, 57] and promoting the differentiation and suppressive activity of T regs [58-61]. In this regard, recent studies showed the constitutive expression of IDO in a population of host APCs in tumour draining lymph nodes of humans [62] and mice [63]. Presentation of tumour derived peptides to naive, resting T cells by these IDO-expressing APCs might result in a state of local immunosuppression in tumour draining lymph nodes besides inducing systemic tolerance to tumour antigens. In addition, IDO expression by various human tumours provides a microenvironment that protects these cells against tumour infiltrated activated effector T cells. This assumption is supported by the data from Uyttenhove and colleagues [64] showing that the T cells are more sensitive to the antiproliferative effects of IDO than the tumour cells.

Besides suppressing the activity of effector T cells, IDO can stimulate naive $CD4^+$ T cells to differentiate into $Foxp3^+$ regulatory T cells [59, 65]. These cells are physiologically engaged in inducing immunological self-tolerance and suppressing antitumor immune responses. Different groups reported the presence of a large number of $Foxp3^+$ CD 25^+ $CD4^+$ T regs in tumours and draining lymph nodes in tumour bearing animals and patients with lung, liver, gastrointestinal tract, head and neck, breast, ovary or prostate cancer. These cells may be recruited to the area by recognizing inflammatory signals in tumours or differentiate from non-T regs because of high levels of IDO and TGF-β secreted by tumour cells and/or host DCs. T regs can induce high levels of IDO expression in DCs through binding of CTLA-4 on T regs to B7-1 and B7-2 on DCs [66] which may act as a positive feedback in maintaining the T reg population. Preclinical studies in mouse tumour models demonstrated that combination of chemotherapy medications and 1-MT results in the development of more effective chemotherapeutic regiments, which break tolerance towards established tumours by inhibiting IDO activity in both tumour cells and host APCs [63, 64, 67, 68].

IDO and transplantation

The role of IDO in inducing tolerogenic mechanisms after transplantation is raised from a study by Miki *et al.* in 2001, in which orthotopic murine liver allografts, normally tolerated without using immunosuppressive drugs, were rejected after recipient mice received 1-MT treatment [69]. Moreover, overexpression of IDO by genetic modifications, using different vectors, resulted in prolonged survival of cardiac [70], lung [71], corneal [72] and islet allografts [73].

Our published data provides substantial evidence supporting the immunosuppressive roles of IDO expressed by bystander fibroblasts following either gene transfection or IFN-γ treatment [74-76]. Using co-culture systems we showed that IDO expression by genetically-modified fibroblasts selectively suppressed the activity of the bystander human peripheral blood mononuclear cell (PBMC), $CD4^+$ T cells, Jurkat cells, TPH-1 monocytes. In addition we have shown that only immune but not primary skin cells or pancreatic islets are sensitive to the IDO induced low tryptophan environments [74, 76-80]. As mentioned before, the activation of GCN2 pathway, has been identified as a potential mechanism responsible for the IDO-induced suppressive effect on T cells. In our study we showed that GCN2 signalling pathway is not activated in mouse islets in response to IDO exposure while it is intensively activated in mouse lymphocytes (Fig. 2) [65, 80], which may result in the selective responsiveness of mouse lymphocytes to IDO exposure.

In our recent publication [81] we used a novel approach for studying the immunosuppressive effect of IDO in islet transplantation. Bystander syngeneic fibroblasts were used as the gene transfer target instead of islets, to reduce the risk of cytotoxicity and loss of islet function [82]. Islets were embedded within a collagen matrix which improves islet viability and function [83]. The findings of this study showed that local IDO expression prevents cellular and humoral alloimmune responses against islet and significantly prolongs islet allograft survival without administration of systemic immunosuppressive agents. Local IDO expression suppressed T cells at the graft site, induced a Th2 immune response shift, generated an anti-inflammatory cytokine profile, delayed alloantibody production, and increased number of regulatory T cells in draining lymph nodes, which resulted in antigen-specific impairment of T cell priming [81].

IDO and autoimmunity

Loss of peripheral or central tolerance against self-antigens leads to an uncontrolled immune system activation, and destruction of cells and tissues, causing chronic and debilitating diseases. The activation of $CD4^+$ T cells by autoantigens is a common process seen in autoimmune disorders. Regarding the immunomodulatory roles of IDO, this enzyme may be involved in maintaining immunological self-tolerance. Type 1 diabetes (T1D), previously called insulin-dependent diabetes mellitus (IDDM), is resulted from the destruction of insulin producing beta cells in pancreatic islets of Langerhans by autoreactive

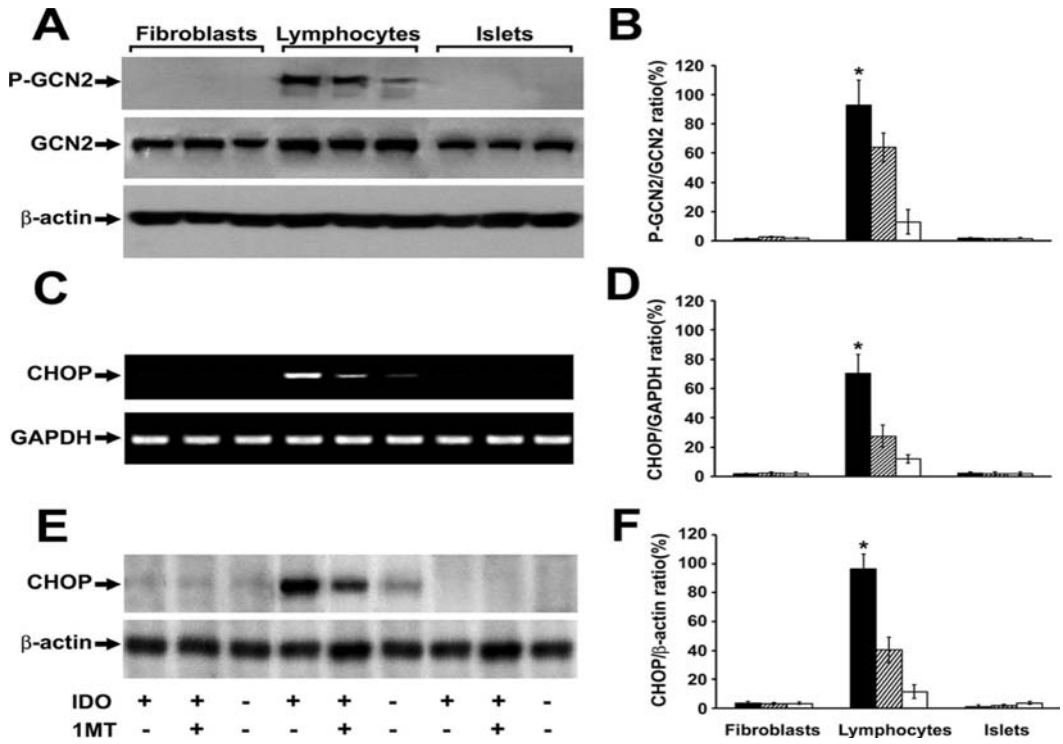

Figure 2. Selective activation of GCN2 and CHOP expression in immune cells in comparison to islets and fibroblasts. GCN2 phosphorylation and CHOP induction was measured after co-culturing the stimulated mouse lymphocytes, fibroblasts, or islets with IDO-expressing or control fibroblasts for 48 hours. IDO activity was inhibited by 1-MT in one set of IDO-expressing co-cultures. **A**: phosphorylated-GCN2 (upper row) and total GCN2 (middle row) Western blot. **C**: CHOP RT-PCR result. **E**: result of Western blot analysis for CHOP. Upper arrow shows a 29 kDa band corresponding to CHOP. **B**, **D** and **F**: the mean ratio of densities of phospho-GCN2, CHOP message, and protein bands to those of total GCN2, glyceraldehyde-3-phosphate dehydrogenase-1, and β-actin bands, respectively, in cells co-cultured with either IDO expressing (solid bars), IDO expressing plus 1-MT (hatched bars), or control (open bars) fibroblasts. * denotes significant difference in phospho- GCN2 and CHOP level between the IDO exposed and the control lymphocytes (n= 3, $P < 0.001$). P-GCN2: phosphorylated GCN2. This data shows that an IDO-mediated tryptophan deficient microenvironment selectively induces activation of GCN2 kinase pathway activation and subsequent CHOP expression in mouse lymphocytes, but not in pancreatic islets and dermal fibroblasts.

T cells. Shinomia *et al.* Study in 1999 provided evidence for IDO role in inducing self-tolerance. They showed that IFN- γ stimulated DCs transferred into nonobese diabetic (NOD) mice can induce a long lasting insulin independence in the recipients [84]. This might be resulted from the immunoregulatory effects of IDO expressed in response to IFN- γ stimulation in DCs. This idea is further supported by the finding that DCs from NOD mice which are prone to developing autoimmune diabetes have defect in IDO transcription which make them nonresponsive to the tolorgenic effects of IFN- γ [85]. In another study it has been shown that IDO inhibition with 1-MT accelerates the progression of T1D [86]. The role of IDO in controlling autoimmunity was further studied in other autoimmune diseases. Data obtained from murine model of experimental autoimmune encephalomyelitis (EAE), confirmed the immunosuppressive effect of IDO in different of stages of this disease as well. In this study, Platten *et al.* showed that inhibiting IDO activity by 1-MT aggravates the tissue damage, inflammation and accelerates the progression of the disease [87].

SUMMARY AND FUTURE DIRECTIONS

This review summarizes current data on IDO activity and its interrelation with immunomodulation and tolerance. Two mechanisms have been suggested to explain how IDO and tryprophan catabolism modulate immune response and advantage tolerance. Either tryptophan starvation inducing cell cycle arrest in T cells, or accumulation of downstream metabolites of tryptophan catabolism that provoking apoptosis in certain immune cells. IDO not only has been recognized as a part of the innate immune defense against certain pathogens, but also has been shown to regulate adaptive T cell immunity [1]. The recognition of IDO as a local immunoregulatory factor in mammalian pregnancy was a critical step in further defining the importance of IDO in modulation of immune response. Since then, enormous number of literatures has investigated the role of IDO in immunology aspect of physiologic, paraphysiologic and pathologic conditions like pregnancy, cancer, infections, autoimmune diseases and transplantation. Growing evidence indicates that augmentation of IDO activity has been implicated to prevent allograft rejection and induce allo-tolerance [4, 75, 88], and also treatment of autoimmune diseases [88] and allergic disorders [9, 88]. On the contrary, inhibiting IDO activity has been suggested as a strategy for management of neoplasia [13, 88] and chronic infections [88].

There is no doubt that the exact role of IDO and tryptophan catabolism in immune response is not fully discovered. Moreover, it should be taken to account that there are species differences in IDO expression and tryptophan catabolism during the immune response [2]. Demonstrating promising results in initial animal studies, it seems that IDO could be a novel therapeutic strategy for treatment or management of many diseases and conditions. However, due to the widespread roles of IDO in regulation of the immune response, this possibility should be further examined.

REFERENCES

1. Mellor, A. L. and Munn, D. H. 2004, Nat. Rev. Immunol., 4, 762-74.
2. Moffett, J. R. and Namboodiri, M. A. 2003, Immunol. Cell Biol., 81, 247-65.
3. Grohmann, U. and Bronte, V. 2010, Immunol. Rev., 236, 243-64.
4. Jalili, R. B., Forouzandeh, F., Bahar, M. A., and Ghahary, A. 2007, Iran J. Allergy Asthma Immunol., 6, 167-79.
5. Klein, D. C. 1999, Adv. Exp. Med. Biol., 460, 5-16.
6. Yoshida, R., Urade, Y., Nakata, K., Watanabe, Y., and Hayaishi, O. 1981, Arch. Biochem. Biophys., 212, 629-37.
7. Honig, A., Rieger, L., Kapp, M., Sutterlin, M., Dietl, J., and Kammerer, U. 2004, J. Reprod. Immunol., 61, 79-86.
8. von Rango, U., Krusche, C. A., Beier, H. M., and Classen-Linke, I. 2007, J. Reprod. Immunol., 74, 34-45.
9. Hayashi, T., Beck, L., Rossetto, C., Gong, X., Takikawa, O., Takabayashi, K., Broide, D. H., Carson, D. A., and Raz, E. 2004, J. Clin. Invest., 114, 270-9.
10. Taylor, M. W. and Feng, G. S. 1991, FASEB J., 5, 2516-22.
11. Puccetti, P. 2007, Eur. J. Immunol., 37, 876-9.
12. Maiwald, S., Wehner, R., Schmitz, M., Bornhauser, M., Loeb, S., and Wassmuth, R. 2010, Tissue Antigens, 77, 136-42.
13. Soliman, H., Mediavilla-Varela, M., and Antonia, S. 2010, Cancer J., 16, 354-9.
14. Gough, D. J., Levy, D. E., Johnstone, R. W., and Clarke, C. J. 2008, Cytokine Growth Factor Rev., 19, 383-94.
15. Belladonna, M. L., Volpi, C., Bianchi, R., Vacca, C., Orabona, C., Pallotta, M. T., Boon, L., Gizzi, S., Fioretti, M. C., Grohmann, U., and Puccetti, P. 2008, J. Immunol., 181, 5194-8.
16. Thomas, S. R., Terentis, A. C., Cai, H., Takikawa, O., Levina, A., Lay, P. A., Freewan, M., and Stocker, R. 2007, J. Biol. Chem., 282, 23778-87.
17. Wang, Y., Lawson, M. A., Dantzer, R., and Kelley, K. W. 2010, Brain Behav. Immun., 24, 201-9.
18. Colonna, M., Trinchieri, G., and Liu, Y. J. 2004, Nat. Immunol., 5, 1219-26.
19. Suh, H. S., Zhao, M. L., Rivieccio, M., Choi, S., Connolly, E., Zhao, Y., Takikawa, O., Brosnan, C. F., and Lee, S. C. 2007, J. Virol., 81, 9838-50.

20. Fallarino, F., Volpi, C., Zelante, T., Vacca, C., Calvitti, M., Fioretti, M. C., Puccetti, P., Romani, L., and Grohmann, U. 2009, J. Immunol., 183, 6303-12.
21. Zhu, W. H., Lu, C. Z., Huang, Y. M., Link, H., and Xiao, B. G. 2007, Mult. Scler., 13, 33-40.
22. Ueno, A., Cho, S., Cheng, L., Wang, J., Hou, S., Nakano, H., Santamaria, P., and Yang, Y. 2007, Diabetes, 56, 1686-93.
23. Sugimoto, H., Oda, S., Otsuki, T., Hino, T., Yoshida, T., and Shiro, Y. 2006, Proc. Natl. Acad. Sci. USA, 103, 2611-6.
24. Munn, D. H., Shafizadeh, E., Attwood, J. T., Bondarev, I., Pashine, A., and Mellor, A. L. 1999, J. Exp. Med., 189, 1363-72.
25. Munn, D. H., Sharma, M. D., Baban, B., Harding, H. P., Zhang, Y., Ron, D., and Mellor, A. L. 2005, Immunity, 22, 633-42.
26. Forouzandeh, F., Jalili, R. B., Germain, M., Duronio, V., and Ghahary, A. 2008, Wound Repair Regen., 16, 379-87.
27. Fallarino, F., Grohmann, U., Vacca, C., Bianchi, R., Fioretti, M. C., and Puccetti, P. 2002, J. Immunol., 169, 1182-8.
28. Zaher, S. S., Germain, C., Fu, H., Larkin, D. F., and George, A. J. 2011, Invest. Ophthalmol. Vis. Sci., 52, 2640-8.
29. Molano, A., Illarionov, P. A., Besra, G. S., Putterman, C., and Porcelli, S. A. 2008, Immunol. Lett., 117, 81-90.
30. Lopez, A. S., Alegre, E., Diaz-Lagares, A., Garcia-Giron, C., Coma, M. J., and Gonzalez, A. 2008, Immunol. Lett., 117, 91-5.
31. Lopez, A. S., Alegre, E., LeMaoult, J., Carosella, E., and Gonzalez, A. 2006, Mol. Immunol., 43, 2151-60.
32. Sekkai, D., Guittet, O., Lemaire, G., Tenu, J. P., and Lepoivre, M. 1997, Arch. Biochem. Biophys., 340, 117-23.
33. Belladonna, M. L., Grohmann, U., Guidetti, P., Volpi, C., Bianchi, R., Fioretti, M. C., Schwarcz, R., Fallarino, F., and Puccetti, P. 2006, J. Immunol., 177, 130-7.
34. Ito, T., Yang, M., Wang, Y. H., Lande, R., Gregorio, J., Perng, O. A., Qin, X. F., Liu, Y. J., and Gilliet, M. 2007, J. Exp. Med., 204, 105-15.
35. Scott, G. N., DuHadaway, J., Pigott, E., Ridge, N., Prendergast, G. C., Muller, A. J., and Mandik-Nayak, L. 2009, J. Immunol., 182, 7509-17.
36. Adikari, S. B., Lian, H., Link, H., Huang, Y. M., and Xiao, B. G. 2004, Clin. Exp. Immunol., 138, 230-6.
37. Terness, P., Bauer, T. M., Rose, L., Dufter, C., Watzlik, A., Simon, H., and Opelz, G. 2002, J. Exp. Med., 196, 447-57.
38. Frumento, G., Rotondo, R., Tonetti, M., Damonte, G., Benatti, U., and Ferrara, G. B. 2002, J. Exp. Med., 196, 459-68.
39. Song, H., Park, H., Kim, J., Park, G., Kim, Y. S., Kim, S. M., Kim, D., Seo, S. K., Lee, H. K., Cho, D., and Hur, D. 2011, Immunol. Lett., 136, 187-93.
40. Ogata, S., Takeuchi, M., Fujita, H., Shibata, K., Okumura, K., and Taguchi, H. 2000, Biosci. Biotechnol. Biochem., 64, 327-32.
41. Morita, T., Saito, K., Takemura, M., Maekawa, N., Fujigaki, S., Fujii, H., Wada, H., Takeuchi, S., Noma, A., and Seishima, M. 2001, Ann. Clin. Biochem., 38, 242-51.
42. Grant, R. S., Naif, H., Thuruthyil, S. J., Nasr, N., Littlejohn, T., Takikawa, O., and Kapoor, V. 2000, Redox. Rep., 5, 105-7.
43. Rottenberg, M. E., Gigliotti Rothfuchs, A., Gigliotti, D., Ceausu, M., Une, C., Levitsky, V., and Wigzell, H. 2000, J. Immunol., 164, 4812-8.
44. Dai, W., Pan, H., Kwok, O., and Dubey, J. P. 1994, J. Interferon. Res., 14, 313-7.
45. Munn, D. H., Zhou, M., Attwood, J. T., Bondarev, I., Conway, S. J., Marshall, B., Brown, C., and Mellor, A. L. 1998, Science, 281, 1191-3.
46. Makala, L. H., Baban, B., Lemos, H., El-Awady, A. R., Chandler, P. R., Hou, D. Y., Munn, D. H., and Mellor, A. L. 2011, J. Infect. Dis., 203, 715-25.
47. Okamoto, A., Nikaido, T., Ochiai, K., Takakura, S., Saito, M., Aoki, Y., Ishii, N., Yanaihara, N., Yamada, K., Takikawa, O., Kawaguchi, R., Isonishi, S., Tanaka, T., and Urashima, M. 2005, Clin. Cancer Res., 11, 6030-9.
48. Gajewski, T. F., Meng, Y., Blank, C., Brown, I., Kacha, A., Kline, J., and Harlin, H. 2006, Immunol. Rev., 213, 131-45.
49. Huang, A., Fuchs, D., Widner, B., Glover, C., Henderson, D. C., and Allen-Mersh, T. G. 2002, Br. J. Cancer, 86, 1691-6.

50. Medawar, P. 1953, Evolution, 7, 320.
51. Schrocksnadel, H., Baier-Bitterlich, G., Dapunt, O., Wachter, H., and Fuchs, D. 1996, Obstet. Gynecol., 88, 47-50.
52. Kamimura, S., Eguchi, K., Yonezawa, M., and Sekiba, K. 1991, Acta. Med. Okayama., 45, 135-9.
53. Mellor, A. L. and Munn, D. H. 1999, Immunol. Today, 20, 469-73.
54. Mellor, A. L. and Munn, D. H. 2001, J. Reprod. Immunol., 52, 5-13.
55. Baban, B., Chandler, P., McCool, D., Marshall, B., Munn, D. H., and Mellor, A. L. 2004, J. Reprod. Immunol., 61, 67-77.
56. Schrocksnadel, K., Wirleitner, B., Winkler, C., and Fuchs, D. 2006, Clin. Chim. Acta., 364, 82-90.
57. Fallarino, F., Grohmann, U., Vacca, C., Bianchi, R., Orabona, C., Spreca, A., Fioretti, M. C., and Puccetti, P. 2002, Cell Death Differ., 9, 1069-77.
58. Sharma, M. D., Hou, D. Y., Baban, B., Koni, P. A., He, Y., Chandler, P. R., Blazar, B. R., Mellor, A. L., and Munn, D. H. 2010, Immunity, 33, 942-54.
59. Sharma, M. D., Baban, B., Chandler, P., Hou, D. Y., Singh, N., Yagita, H., Azuma, M., Blazar, B. R., Mellor, A. L., and Munn, D. H. 2007, J. Clin. Invest., 117, 2570-82.
60. Chen, W., Liang, X., Peterson, A. J., Munn, D. H., and Blazar, B. R. 2008, J. Immunol., 181, 5396-404.
61. Puccetti, P. and Fallarino, F. 2008, Blood Cells Mol. Dis., 40, 101-5.
62. Lee, J. H., Torisu-Itakara, H., Cochran, A. J., Kadison, A., Huynh, Y., Morton, D. L., and Essner, R. 2005, Clin. Cancer Res., 11, 107-12.
63. Munn, D. H., Sharma, M. D., Hou, D., Baban, B., Lee, J. R., Antonia, S. J., Messina, J. L., Chandler, P., Koni, P. A., and Mellor, A. L. 2004, J. Clin. Invest., 114, 280-90.
64. Uyttenhove, C., Pilotte, L., Theate, I., Stroobant, V., Colau, D., Parmentier, N., Boon, T., and Van den Eynde, B. J. 2003, Nat. Med., 9, 1269-74.
65. Fallarino, F., Grohmann, U., You, S., McGrath, B. C., Cavener, D. R., Vacca, C., Orabona, C., Bianchi, R., Belladonna, M. L., Volpi, C., Santamaria, P., Fioretti, M. C., and Puccetti, P. 2006, J. Immunol., 176, 6752-61.
66. Grohmann, U., Orabona, C., Fallarino, F., Vacca, C., Calcinaro, F., Falorni, A., Candeloro, P., Belladonna, M. L., Bianchi, R., Fioretti, M. C., and Puccetti, P. 2002, Nat. Immunol., 3, 1097-101.
67. Hou, D. Y., Muller, A. J., Sharma, M. D., DuHadaway, J., Banerjee, T., Johnson, M., Mellor, A. L., Prendergast, G. C., and Munn, D. H. 2007, Cancer Res., 67, 792-801.
68. Muller, A. J., DuHadaway, J. B., Donover, P. S., Sutanto-Ward, E., and Prendergast, G. C. 2005, Nat. Med., 11, 312-9.
69. Miki, T., Sun, H., Lee, Y., Tandin, A., Kovscek, A. M., Subbotin, V., Fung, J .J., and Valdivia, L. A. 2001, Transplant Proc., 33, 129-30.
70. Li, J., Meinhardt, A., Roehrich, M. E., Golshayan, D., Dudler, J., Pagnotta, M., Trucco, M., and Vassalli, G. 2007, Am. J. Physiol. Heart Circ. Physiol., 293, H3415-23.
71. Liu, H., Liu, L., Fletcher, B. S., and Visner, G. A. 2006, FASEB J., 20, 2384-6.
72. Beutelspacher, S. C., Pillai, R., Watson, M. P., Tan, P. H., Tsang, J., McClure, M. O., George, A. J., and Larkin, D. F. 2006, Eur. J. Immunol., 36, 690-700.
73. Alexander, A. M., Crawford, M., Bertera, S., Rudert, W. A., Takikawa, O., Robbins, P. D., and Trucco, M. 2002, Diabetes, 51, 356-65.
74. Li, Y., Tredget, E. E., Ghaffari, A., Lin, X., Kilani, R. T., and Ghahary, A. 2006, J. Invest. Dermatol., 126, 128-36.
75. Jalili, R. B., Rayat, G. R., Rajotte, R. V., and Ghahary, A. 2007, J. Cell Physiol., 213, 137-43.
76. Ghahary, A., Li, Y., Tredget, E. E., Kilani, R. T., Iwashina, T., Karami, A., and Lin, X. 2004, J. Invest. Dermatol., 122, 953-64.
77. Sarkhosh, K., Tredget, E. E., Uludag, H., Kilani, R. T., Karami, A., Li, Y., Iwashina, T., and Ghahary, A. 2004, J. Cell Physiol., 201, 146-54.
78. Sarkhosh, K., Tredget, E. E., Karami, A., Uludag, H., Iwashina, T., Kilani, R. T., and Ghahary, A. 2003, J. Cell Biochem., 90, 206-17.
79. Sarkhosh, K., Tredget, E. E., Li, Y., Kilani, R. T., Uludag, H., and Ghahary, A. 2003, Wound Repair Regen., 11, 337-45.

80. Jalili, R. B., Forouzandeh, F., Moeenrezakhanlou, A., Rayat, G. R., Rajotte, R. V., Uludag, H., and Ghahary, A. 2009, Am. J. Pathol., 174, 196-205.
81. Jalili, R. B., Forouzandeh, F., Rezakhanlou, A. M., Hartwell, R., Medina, A., Warnock, G. L., Larijani, B., and Ghahary, A. 2010, Diabetes, 59, 2219-27.
82. Barbu, A. R., Akusjarvi, G., and Welsh, N. 2002, Mol. Med., 8, 733-41.
83. Wang, R. N. and Rosenberg, L. 1999, J. Endocrinol., 163, 181-90.
84. Shinomiya, M., Fazle Akbar, S. M., Shinomiya, H., and Onji, M. 1999, Clin. Exp. Immunol., 117, 38-43.
85. Grohmann, U., Fallarino, F., Bianchi, R., Orabona, C., Vacca, C., Fioretti, M. C., and Puccetti, P. 2003, J. Exp. Med., 198, 153-60.
86. Saxena, V., Ondr, J. K., Magnusen, A. F., Munn, D. H., and Katz, J. D. 2007, J. Immunol., 179, 5041-53.
87. Platten, M., Ho, P. P., Youssef, S., Fontoura, P., Garren, H., Hur, E. M., Gupta, R., Lee, L. Y., Kidd, B. A., Robinson, W. H., Sobel, R. A., Selley, M. L., and Steinman, L. 2005, Science, 310, 850-5.
88. Tan, P. H. and Bharath, A. K. 2009, Expert Opin. Ther. Targets, 13, 987-1012.