Indoleamine 2,3-Dioxygenase and Immunological Tolerance during Pregnancy

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ABSTRACT

Indoleamine 2,3-dioxygenase (IDO), an enzyme involved in the catabolism of tryptophan, is expressed by a variety of cells and tissues such as macrophages, dendritic cells, cells of the endocrine system and by the placenta. IFN-γ is the main inducer of this enzyme. IDO acts as an important defense mechanism of innate immunity against pathogens. It also has tumor suppressive activity and prolongs the survival of allograft. One of the interesting functions of IDO is prevention of the allogenic fetus rejection during pregnancy by inhibiting alloreactive T cells. It was shown that inhibition of IDO activity by IDO inhibitor, 1-methyl tryptophan, during mouse pregnancy causes fetal rejection. The main mechanism by which IDO protects fetus is through reducing the tryptophan level and suppressing the T cell activity in the feto-maternal interface. In this review the biological functions of IDO with emphasis on its role in allogeneic fetus protection have been discussed.

Key words: Immunology, Indoleamine 2,3-Dioxygenase, Pregnancy, Tolerance

ABBREVIATIONS

IDO: Indoleamine 2,3 Dioxygenase,TDO: Tryptophan 2,3 Dioxygenase, IFN: Interferon, NAD: Nicotineamide dinucleotide, LPS: Lipopolysaccharide, TNF: Tumor necrosis factor, IL: Interleukin, HIV: Human immunodeficiency virus, HTLV: Human T lymphotropic virus, CMV: Cytomegalovirus, MHC: Major Histocompatibility Complex, APC: Antigen Presenting Cell, M-CSF: Monocyte Colony Stimulating Factor, AICD: Activation induced cell death, MLR: Mixed lymphocyte reaction, PHA: Phytohemagglutinin, RAG: Recombination activating gene, PC: Post Coitus, TCR: T cell receptor, KO: Knockout, uNK: Uterine Natural Killer (cell), TGF: Transforming growth factor

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INTRODUCTION

Inadequate intake of tryptophan, one of the essential amino acids, may cause negative nitrogen balance and loss of muscle mass leading to weight loss (1). Tryptophan is mainly catabolized through two distinct pathways:
1) Tryptophan 5-hydroxylase mediates the conversion of tryptophan to 5-hydorxy tryptophan which then transforms into serotonin (5-hydroxy tryptamine).
2) Tryptophan can also be converted to N-formylkynurenine and then to kynurenine by the action of either Tryptophan 2,3 Dioxygenase (TDO or Tryptophan Pyrrolase) or Indoleamine 2,3 Dioxygenase (IDO)(2).
TDO is predominantly synthesized by liver cells and its function is regulated by glucocorticoids. Administration of glucocorticoids induces TDO activity and thereby decreases free tryptophan levels in circulation (3,4). IDO, on the other hand, is expressed by a variety of cells such as macrophages and cells of the endocrine and central nervous system (5,6). In contrast to TDO whose only natural substrate is L-tryptophan (7), IDO catabolizes a broad range of substrates including L-tryptophan, 5- hydroxy tryptophan and serotonin (2,8). The other distinctive feature of IDO is its inducibility by Interferons, especially Interferon γ (IFN-γ). Indeed, the mediator of IFN-γ action in many cells has been recognized to be IDO (9). IDO has much wider range of expression than TDO; it has been so far reported to be expressed in lung (9), small intestine and placenta of mammals including rabbits (10), rats (11), mice (12) and humans (13).
Kynurenine, the catabolite of tryptophan by the action of either IDO or TDO, is further metabolized to a series of compounds, some of which with potential neurotoxic or neuroprotective properties (14). For example, quinolinic acid causes hyperexcitation and convulsion (15). This compound is, in turn, metabolized in liver to nicotinic acid which enters the nicotineamide dinucleotide (NAD) metabolism pathway and finally provides energy (16,17). Kynurenine production is constitutively active in the liver and therefore, there exists a basal serum level of this compound (18), which should be kept in mind for designing studies aimed at assessment of IDO or TDO activity by the measurement of their end product in the serum (19).
Interferons are potent inducers of IDO (2). Other stimuli such as LPS, TNF-α and IL-2 exert their action on increasing IDO level probably through increased expression of IFN-γ (20). In general, IFN-γ is the most potent stimulus, known so far, for IDO induction (21).

IDO AND HOST DEFENSE AGAINST PATHOGENS

IDO operates as an important host defense mechanism of innate immunity against a number of viral pathogens such as HIV, HTLV and CMV (22-26). Numerous studies have shown that intracellular multiplication of *Toxoplasma gondii* (27,28), *Chlamydia psittaci* (29), *Chlamydia pneumoniae* (30,31) and some other parasites living preferably inside cells (32) is inhibited by IFN-γ and in most cases it is restored by addition of tryptophan to culture media. There are also reports of increased levels of IDO in central nervous system during chronic infection by HIV

(26) and HTLV (33).
The main mechanism by which IDO controls infections is tentatively through tryptophan catabolism; by decreasing the available levels of this essential amino acid, IDO suppresses protein synthesis and inhibits the proliferation of surrounding cells. Suppression of protein synthesis in the host cell disrupts replication cycle of viruses and intracellular parasites (34).
Interferon-γ is among the cytokines that are released in most cell-mediated immune responses. It has been observed to mediate macrophage activation and enhancement of MHC expression of these cells during most inflammatory responses (35).
Paradoxically, IFN-γ has been shown to have immunosuppressive properties particularly in the course of chronic activation of immune system that usually accompany infections such as HIV (36). In the course of chronic HIV infection, increased levels of IFN-γ is reported to be associated with lack of proper T cell response (37,38).
Since IFN-γ is a potent stimulus for IDO induction, it seems quite reasonable to conclude that IDO mediates, at least some of the antiproliferative effects of IFN-γ (39). Many investigations have supported this tenet, as tryptophan catabolism occurs at the site of tissue inflammation. IDO expression in these sites significantly suppressed the inflammation and reduced subsequent tissue injury (40-42). One such mechanism is mediated by inhibition of tissue expression of metalloproteinases which are among inflammatory mediators (39,43). Therefore, it seems that not only does IDO prevent the proliferation of microorganisms and infected cells but also confines the potentially hazardous uncontrolled immune responses. In this way IDO both attacks the invaders and guards the normal tissue against damage. IDO may also have a role in inflammation control by acting as a scavenger of free oxygen radicals as it needs superoxide for tryptophan oxidation (44,45).

IDO AND TUMOR IMMUNITY

IDO possesses tumor suppressive function. A few studies have shown that transplantation of tumor tissue to allogenic animal induces IDO expression (46,47) and it seems that antiproliferative effects of IFN-γ on tumor cells is mediated through IDO expression (2). IDO deprives tumor cells of tryptphan and in this way, it causes tumor regression. However, this mechanism may act as a double-edge sword, in that tumor cells may evade immune system by attraction of IDO-expressing cells to the tumor site and result in suppression of immune cell proliferation (42). This theory may provide an explanation for resistance of some tumors to the host immunity (48,49).
Antigen presenting cells (APCs) such as machrophages (50) and dendritic cells (51) that express IDO have been shown to suppress anti-tumor responses in-vivo. Therefore, it seems plausible to enhance anti-tumor immune responses by blocking IDO action (52-54). In this approach, it is predicted that specific T-cell responses to tumor antigens would be strengthened and amplified following IDO inhibition.

IDO AND ALLOGRAFT TRANSPLANTATION

Liver transplantation is a suitable candidate for investigating the role of IDO in allograft transplantation. As a whole, allogenic liver transplants are well tolerated in most strains of mouse whereas heart and other solid organ transplants are definitely rejected (55). Interestingly, IDO is not constitutively expressed by liver or syngenic liver transplants, while allogenic liver transplants express IDO with peak of expression in the seventh day of transplantation when the most severe rejection reactions are expected to occur and its expression continues till the day 30 (56,57). It has also been demonstrated that treatment of the liver recipient with IDO inhibitor, 1-methy1 tryptophan results in transplant rejection (56,58). These findings clearly indicate the importance of IDO in preventing rejection of liver transplant, because 1-methy1 tryptophan acts as a specific IDO inhibitor with no effect on TDO, the hepatic enzyme with similar function (59). Another recent study provided support for this notion, in which prolonged survival of transplanted allogenic pancreatic islet cells was observed following IDO gene transfer in the recipient by adenoviral vector (60). Accordingly, it could be suggested that IDO expression can be utilized for induction of tolerance to allogenic transplants regarding its role in suppression of proliferative responses of T-cells and in establishment of anergic state in these cells which conduct the central part in transplantation rejection.

IDO AND SUPPRESSION OF T CELL RESPONSES

There is increasing evidence that APCs are able to suppress T cell proliferation by IDO production. Monocytes transformed to macrophges under the influence of M-CSF acquire the ability to inhibit T cell proliferation by selective catabolism of tryptophan through the action of IDO. Induction of IDO can be achieved by treating macrophges with IFN-γ and CD40 ligation, both of which are T-cell derived stimuli. Inhibiting IDO with 1-methyl tryptophan prevents the suppressive effect on T cell proliferation (50). It is reported that deprived of tryptophan, activated T cells express IL-2 receptor and synthesize IL-2, nevertheless, the cell cycle progression halted at mid G1 and was not restored even by tryptophan addition to the culture media (50).

Other reports indicated that transgenic dendritic cells expressing IDO inhibited in-vitro proliferation of allogenic T cells (61,51). Furthermore, some but not all of tryptophan metabolites including kynurenine, 3-hydroxy kynurenine and 3-hydroxy anthranilic acid, have the ability to suppress proliferative T cell responses (34). These T cells, whose cell cycles had been interrupted, lost the ability to resume normal cycle. In addition, tryptophan metabolites had toxic effects on CD3+ T cells and this effect was more pronounced on activated T cells (61). The anti-proliferative effects of tryptophan deprivation were specific to tryptophan and not due to inhibition of protein synthesis. This implies that T cells may possess a tryptophan-sensitive checkpoint in G1 phase that determines whether T cells should finally proliferate or not.

CD11c+ cells of murine spleen are among cells that express IDO and it is interesting

that most of these IDO+ cells are CD8+. Exposure of these cells to IDO inhibitors abolishes their ability to suppress DTH and anti-tumor responses (52-54). Although both CD8+ and CD8- dendritic cells have been shown to express IDO protein, metabolism of tryptophan by IDO is only functional in the CD8+ population, suggesting that post-translational regulations take place in these cells (62).
Upon encountering the antigens, resting T cells become activated and transform into effector cells. Effector T cells then undergo Activation Induced Cell Death (AICD) after a couple of division. Murine T cells that become activated in the absence of tryptophan, show extreme sensitivity to Fas-mediated AICD and undergo apoptosis before they enter the first cycle of cell division. Similarly, CD8+ T cells co-cultured with IDO+ cells occasionally undergo division (34). All these observations indicate that activated T cells need an ample source of tryptophan for successful completion of first G1 phase. IDO has also been reported to inhibit T cell proliferation in response to PHA or even in allogenic MLR (63). A practical implication of these findings would be in control of those autoimmune conditions in which T cells play the central role (e.g. Myasthenia Gravis or Rheumatoid Arthritis) by limiting tryptophan concentration in local tissue microenvironment.
Most studies revealed that IDO was mainly localized to a subset of cells of immune system morphologically resembling macrophages or dendritic cells. In other words, the major source of IDO was found to be antigen presenting cells (64-66). These IDO expressing cells were detected in many immunoprivileged sites such as thymus, intestinal epithelium, epidydimis, placenta and anterior chamber of eye (13,64,67,68). It, therefore, seems that the presence of such cells is associated with induction of immunological tolerance. The reason for why APCs should express IDO simultaneously to antigen presentation lies in the fact that activated T cells are more sensitive to free tryptophan available in their microenvironment than resting T cells (50). Antigen presenting cells sensitize T cells to diminished tryptophan concentration by activating them through their T cell receptors and at the same time, at least a subset of them, regulate their responses by IDO expression.

IDO AND PREGNANCY

In 1953, Medawar proposed three mechanisms for survival of semi-allograft transplant of fetus (69):
1. Trophoblast acts as an anatomic barrier, separating fetus and mother physically.
2. Fetus is anitgenically inert or is unable to elicit an immune response.
3. Mother becomes immunologically tolerant to fetus.
According to the evidence that fetal cells are traced many years after delivery in mother's blood (70) and that maternal immune system recognizes fetal MHC alloantigens (71,73) the first two hypotheses are not acceptable any more. Therefore, explanation of mechanisms underlying the third one, i.e. the immunological tolerance, has become the focus of attention.
Regarding the role of IDO in suppression of proliferative T cell responses and the expression of IDO by cells of syncytiotrophoblasts (59,74-77), it is conceivable that IDO expression at the maternal-fetal interface contributes to the suppression of

maternal immune responses and hence to the survival of semi-allograft transplant of fetus.

Decline in systemic concentration of tryptophan during pregnancy provided some further clues to the issue (78-80). This hypothesis was first formulated by David Munn and Andrew Mellor and was tested in an elegantly designed study (74). They studied IDO expression and response to IDO inhibitor (1-methyl tryptophan) in syngenic (CBA x CBA) and allogenic (CBA x C57BL/6) mouse pregnancies. They showed that IDO mRNA appeared in all concepti from day 7.5 to day 9.5 post coitus (PC), but IDO expression remained restricted to the placenta from day 10.5-13.5 PC with detectable IDO mRNA in neither the deciduas nor the embryo. The pregnant mice were then treated with 1-methyl tryptophan, from day 4.5 PC corresponding to the implantation time. No allogenic concepti remained after day 9.5 PC and histological studies showed inflammation and hemorrhage surrounding these concepti. In contrast, syngenic concepti remained intact after treating with IDO inhibitor. When RAG deficient (RAG-/-) female mice (with CBA background) were mated with male mice and then treated with 1-methyl tryptophan, they had totally normal litters. Since RAG -/- mice are defective in the development of lymphocytes and lack functional lymphocytes, it was concluded that maternal lymphocytes contributed to the rejection of allogenic concepti after inhibition of IDO. The reverse was true in case of syngenic pregnancy, in which the concepti was not detected as foreign tissue by the maternal immune system and hence, IDO inhibition did not induce rejection.

In their next step to characterize the nature of the cellular interactions and the effector mechanisms mounting in fetal rejection, they studied two groups of MHC-matched and mHC-mismatched matings. It revealed that classic MHC class I and some minor histocompatibility antigens were responsible for immune responses resulting in fetal rejection when IDO activity is blocked during allogenic pregnancies (81). Since these antigens are mainly recognized by maternal T cells, the critical event for saving allogenic fetus after blastocyst implantation would be T-cell inhibition by IDO which is expressed mainly on APCs located either at the maternal-fetal interface, or in lymphoid tissues draining the uterus. Analysis of IDO mRNA by in situ hybridization method showed that a cohort of maternal cells expressing IDO migrated from metrical gland toward the developing fetus and their number increased until they surrounded the entire conceptus 6 days after implantation in mouse (82).

As for the immediate mechanism causing fetal rejection in allogenic concepti, Munn and Mellor showed in their study that administration of 1-methyl tryptophan in mice carrying allogenic concepti led to the extensive C3 precipitation on tissues surrounding embryo and on trophoblasts and perivascular areas of deciduas (81). Normally, complement deposition on allogenic cocepti is prevented by action of an inhibitory factor (crry) in mice. This finding raised the possibility that IDO inhibition, allowing the elaboration of unfavorable T cell responses, activated the complement system so extensively that it overcame the natural inhibition.

To further elucidate the underlying mechanism for complement deposition in mice susceptible to IDO inhibitor, they mated female mice of CBA-strain genetic background with H-2K^b transgenic males (81). Female mice had a monoclonal H-2K-spcific CD8+ T cells with no B cell or CD4+ T cells as a result of transgenic TCR

backcrossing onto RAG1-/- background. Inhibition of IDO with use of 1-methyl tryptophan mounted an extensive inflammation and complement deposition into the maternal-fetal interface. This latter investigation clearly showed that lethal activation of complement at the maternal-fetal interface depended upon specific T cell response to paternal antigens and it occurred through the alternative pathway, as no antibody was present in this model. Activation of C3 in the alternative pathway takes place mainly by the action of properdin on alternative C3 convertase (factor Bb/C3b complex). Interestingly, properdin was shown to be secreted by myeloid cells as well as T lymphocytes under certain circumstances. It is, thus, conceivable that T cells recognizing paternal antigens at the maternal-fetal interface activate alternative pathway of complement by in situ release of properdin. There is, however, another possibility that activated T cells may trigger properdin release by myeloid cells at the site and hence, indirectly trigger complement activation (42). There is another issue that which one of effector T cell function or complement deposition causes fetal rejection. Some recent evidence showed that the immediate underlying mechanism is complement activation, as mice deficient in murine complement inhibitory factor (crry) rejected all their fetuses (82). Therefore, complement deposition without any enforcement by effector T cells is a potent and efficient mechanism capable of causing fetal rejection. But complement is tightly regulated at the maternal-fetal interface and once T cells are activated, they have the necessary force to drive complement cascade regardless of regulatory mechanisms.

Many attempts have been made to characterize the nature of IDO+ cells at the maternal-fetal interface. These studies showed that IDO+ cells lack mouse macrophage marker of F4/80 and they exist in NK KO mice (34). In human, it was demonstrated that IDO expression in syncytiotrophoblasts is mainly cytoplasmic and does not occur at their maternal facing border membrane (75). IDO expression has not been detected in the first trimester human placenta, its expression, however, starts at around 14th week of gestation and continues through term pregnancy. In term placenta, IDO was irregulary localized to the mesenchymal core and isolated areas of syncytiotrophoblasts (77). Kudo et al. showed that in-vitro stimulation of chorionic villous explants of both early and term placenta enhance the tryptophan degradation, an indirect indicator of IDO expression (76). Although, most studies focused on IDO expression in pregnancy, some investigators found IDO transcripts in endometrial glandular and epithelial cells and demonstrated that IDO expression increased during menstrual cycle. IDO was also reported to be expressed in epithelium of cervical glands and of fallopian tubes (77). Recently, another enzyme involved in tryptophan catabolism, Tryptophan 2,3-Dioxygenase (TDO), has been shown to be expressed at the maternal-fetal interface (59,83). This enzyme acts similar to IDO and produces the same metabolites as kynurenin. TDO mRNA has been found in deciduas of pregnant mice at implantation time (around 4.5 days PC). Also, TDO expression was induced in mouse uterine by deciduoma formation or embryo transfer but not by ovarian steroid hormones (83). In another study, Suzuki and coworkers demonstrated that tryptophan catabolism that occurred in early pregnancy before IDO being expressed, could be attributed to the TDO activity. They also found that TDO mRNA was detectable in early concepti in days 5.5-10.5 of mouse pregnancy (59). Interestingly, TDO activity is not inhibited by IDO

inhibitor i.e. 1-methyltryptophan. Therefore, it seems reasonable to conclude that both TDO and IDO activities contribute to the strict regulation of tryptophan level at the site of placenta development. In other words, the fact that TDO expression takes place mainly in early and IDO expression in midterm pregnancy indicates that tryptophan level in maternal-fetal interface is tightly brought under control by these two enzymes with different time spans.

The question that arises here is that what function IDO may perform in pregnant mice carrying syngenic fetuses. Munn and Mellor showed that IDO expression suppress T cell response to paternal allogenic antigens and hence prevent fetal rejection, whereas in syngenic pregnancies, there was basically no need to such suppression because no foreign antigen exists at maternal-fetal interface (84). In analogy, syngenic liver transplants were found not to induce IDO expression in contrast to what is usually observed in allogenic transplants (56,57). All these data suggest that IDO at maternal-fetal interface may have other physiological functions than solely suppression of the mothers' immune responses.

Increased levels of tryptophan have inhibitory effects on in-vitro development of 1-cell embryo to blastocysts (85). TDO and IDO expression, besides their roles in control of harmful maternal immune responses, prevent this toxic effect of high levels of tryptophan on the newly implanted blastocyst.

IDO needs superoxide anion for catabolism of tryptophan, in this way it acts as a scavenger to protect the fetus against deleterious effects of free radicals (84). Moreover, IDO may decrease vasoconstriction by metabolism of serotonin which is a potent vasoactive substance (84). Another potential role proposed for IDO is regulation of uterine NK cell (uNK) activity. It was shown that in early pregnancy the number of uNK cells increases significantly in deciduas (86). These cells contribute importantly to achievement of successful pregnancy. uNK cells mainly act in maternal-fetal interface to finely regulate the growth of placenta by operating two seemingly opposite functions which are restriction of unregulated growth of invasive trophoblasts as well as, secretion of immunotrophic cytokines for optimum growth and development of placenta. Increased number and uncontrolled activity of these cells have been implicated in the etiology of fetal resorption in abortion-prone mating of CBA/j x DBA/2mice (87). It has been shown that metabolites of tryptophan have toxic effects on NK and B cells, as well as CD3+ cells (61). Therefore, IDO expression in maternal-fetal interface, along with HLA-G expression on syncytiotrophoblasts and extravillous trophoblasts, may have a role in regulation of uNK activity.

Little is known about the regulating mechanisms of IDO expression. So far, IFN-γ is known as the major provoking factor for IDO expression (2). IFN-γ is the hallmark of Th1 cytokine profile. However, Th2 profile, including cytokines such as IL-10 and IL-4, predominates in the maternal-fetal interface in most of pregnancy duration. The paradox exists as IFN-γ that induces IDO expression to prevent fetal rejection, may itself cause abortion at high levels (88-90). On the other hand, IL-4, IL-10 and TGF-β which favor successful pregnancy, prevent IDO expression (23,90-92). It seems plausible that effect of such cytokines as IL-4 on IDO expression may be prevention of the overexpression of IDO with drastic consequences.

CONCLUSION

Evidence exists that maternal T cells recognize paternal foreign antigens during an allogenic pregnancy, but due to a number of mechanisms including IDO activity and tryptophan catabolism, they remain in an anergic state. This means that in contrast to what was thought before, the fetus is not anitgenically inert because maternal lymphocytes specific for fetal antigens have been detected whose effector function leads to fetal rejection following inhibition of such anergy-inducing mechanisms as IDO. Finally, it seems that allogenic fetus is basically no different from other allogenic transplants; what especially distinguishes fetal transplantation is the ability of fetus-originated tissues, most significantly tissues, most significantly trophoblasts and syncytiotrophoblasts, to actively suppress the maternal T cell responses through multiple mechanisms including IDO expression and therefore, to ensure its survival in the potentially hostile environment of mothers uterus.

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