Immunomodulation through indoleamine 2,3-dioxygenase

M.H.S. Kema (s1661035)
B.Sc. Life Science & Technology
Faculty of Mathematics and Natural Sciences
University of Groningen

Prof. dr. A.J.M. van Oosterhout
Department of Pathology and Medical Biology
University Medical Center Groningen

April 23, 2009

BACHELOR THESIS

Summary

Indoleamine 2,3-dioxygenase (IDO) is an enzyme involved in tryptophan (Trp) catabolism and is principally induced by interferon-gamma. Trp, one of the essential amino acids, is required for adequate T-cells functioning. By depleting local Trp resources IDO facilitates in, among others, induction of T-cell proliferation arrest. Furthermore, IDO is involved in tolerization through antigen-presenting cells and induction of T-cell anergy. Such interdependence allows modulation of the immune response within a specific microenvironment through IDO. This strategy is useful in multiple scenarios: it prevents an adaptive immune reaction from escalating, but also averts rejection of developing fetuses by pregnant women due to foreign MHC and antigens. Conversely, cancer utilizes IDO to permit malignant growth and avoid the host's immune system. Insights into mechanisms -such as the IDO pathway- allows better understanding of pathophysiology of tumors, which might then provide either new or additional starting points for treatment.

Contents

1. Introduction

The theory stating that the immune system discriminates between self ('which is safe') and nonself ('which should be destroyed') provides a decent explanation for many of the body's defense responses. However, it fails to explain why pregnant women are able to give birth in stead of rejecting the developing fetus or how tumors are able to escape the host's immune response. Additional concepts, like immunoediting and tolerance, are required to account for such events.[1-3] Immunomodulation (or immunoediting) refers to changes in the immune system that can either stimulate or suppress its function.[3] The term tolerance is used to describe the absence of an immune response to an antigen.[4]

Over the past decade researchers have taken a key interest in the intracellular enzyme indoleamine 2,3-dioxygenase (IDO). IDO is involved in tryptophan (Trp) catabolism, which in turn is linked to maintaining the adaptive immune system.[2] This thesis' main focus is on the role of immunomodulation via IDO in tumoral immune escape, and its potential for future clinical application.

1.1 Tryptophan and indoleamine 2,3-dioxygenase

L-Trp is one of the eight essential amino acids, and serves as a precursor in the biosynthesis of serotonin (5-HT), melatonin, and niacin. In humans merely 1% of the available L-Trp is used for formation of serotonin, however, the majority (>95%) of L-Trp is metabolized along the kynurenine (Kyn) pathway.[5,6] A recent study indicated that blood of healthy adults should show a 19.0-49.8 Trp-to-Kyn ratio. Samples should be obtained prior to breakfast due to variation in Trp throughout the day.[7]

During the first (and also rate-determining) step in the Kyn pathway L-Trp is converted into *N*-Formylkynurenine. This reaction is catalyzed by either IDO or tryptophan 2,3-dioxygenase (TDO), *see Fig. 1*.[5,6] Despite showing functional and structural similarities -IDO and TDO both catalyze the same catabolic reaction and both contain a heme group- there are several important differences between these two enzymes. TDO is expressed primarily in the liver, and is induced by among others L-Trp, tyrosine, and histidine. In contrast, IDO is regulated by several immunological signals such as interferon-gamma (IFN- γ), and is expressed ubiquitously.[8]

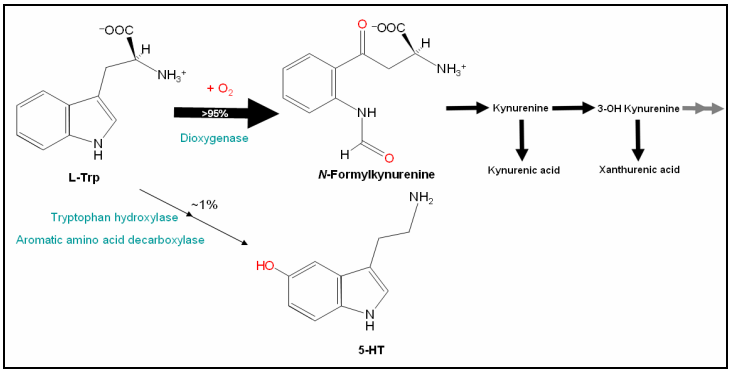

Figure 1: Overview of L-Trp linked metabolic conversions (*modified from: Berg et al, 2006 and Takikawa, 2005*). *The figure depicts the first step in the Kyn pathway and the synthesis of 5-HT, both resulting in a reduced L-Trp level. Several other catabolites along the Kyn pathway are also shown. The combination of O_2 and an oxygenase enzyme, such as IDO or TDO, is required to break an aromatic ring in the L-Trp molecule en route to formation of N-Formylkynurenine. 5-HT biosynthesis occurs via tryptophan hydroxylase and aromatic amino acid decarboxylase*.[6,9]

IFN-γ is one of the principal inducers of IDO, yet there are several more factors involved in IDO regulation. Transcription of *INDO* (the IDO gene) is controlled by Janus kinase-1 (Jak1) and signal transducer and activator of transcript 1 (STAT1). STAT1 binds the *INDO* promoter region at GAS sites, but also activates IFN-regulatory factor 1 (IRF-1), which subsequently binds to two IFN-stimulated response element sites within the *INDO* promoter region.[2,10] Nuclear factor-κB (NF-κB) is also implicated to be connected to this pathway. It was proposed that non-canonical NF-κB activation serves to maintain functioning of regulatory T cells.[11] Furthermore, cyclooxygenase 2 (COX-2) was found to contribute to induction of IDO (in a/o macrophages) via production of proinflammatory molecule prostaglandin E2 (PGE2). Interestingly it was observed that *in vitro* blockage of COX-2 also leads to reduced IDO activity. In addition, soluble CTLA4-immunoglobulin fusion protein (CTLA4-Ig) was reported to be another inducer of IDO in dendritic cells (*see Fig. 4a*). Conversely, NO decreases IDO activity. NO binds directly to the heme group, causing inactivation and eventual degradation of the enzyme. In fibroblasts it was observed that transforming growth factor-β (TGF-β) antagonizes IDO activation via IFN-γ.[2,10] Yet, Belladonna and colleagues reported that TGF-β promotes IDO in T cells.[12]

1.2 T-cell proliferation

Nutrient depletion is a strategy, employed by both simple and complex organisms, to control proliferation. Based on observations *in vitro*, it was thought for many years that IDO solely served as a cytostatic against Trp-dependent microbes, by reducing the Trp concentration in the local microenvironment. However, about ten years ago it was reported that IDO-expressing macrophages are able to suppress T-cell proliferation using the same mechanism.[13] This takes place when monocytes differentiate into macrophages while in the presence of macrophage colony-stimulating factor (MCSF). It appears that the Trp shortage subsequently occurring results in proliferation arrest in T cells.[13]

T cells are classified as part of the adaptive cell-mediated immunity. The adaptive immune response has several distinct features contributing to the host's defense against nonself. With regard to immunomodulation the goal is to keep everything as it is, apart from some specific (local) changes. For naïve T cells the following coherence is assumed: specificity and diversity allow recognition of millions of different antigens, when a foreign antigen is recognized clonal expansion (proliferation) occurs, followed by among others specialization and development of memory.[4]

If a subsided T-cell response to a foreign antigen is required then, in the ideal scenario, the complementary receptor would be absent. As a direct consequence, this would involve altering the maturation process. Immunoediting (in e.g. cancer or pregnancy) is done through a different strategy. After a naïve T-cell is activated, proliferation is necessary en route to an adequate immune response. However, there are some conditions to be fulfilled before cell division can take place, like sufficient availability of nutrients.[4] This is where IDO intervenes, by causing depletion of the essential amino acid Trp. Proliferation arrest occurs because of a Trp sensitive checkpoint in the G1 phase. Once Trp shortage is detected, a Gcn2-dependant stress signaling pathway is activated. Increased Gcn2 leads to phosphorylation of eIF2α. Subsequently, phosphorylated eIF2α largely restrains the initiation of translation, causing decreased expression of most genes (*see Fig. 2*). After disruption, proliferation can only resume through a second round of T-cell receptor signaling, in the presence of Trp. The latter is evidently hindered due to high IDO activity in the local microenvironment. The

IDO pathway is thus able to interfere at the very onset of the adaptive immune response. As a result neither an adequate response, nor further specialization and formation of memory can take place. In addition, IDO is involved in both tolerization of T cells and induction of T-cell anergy, causing further increase of immunosuppression (*see 2.3*).[3,14]

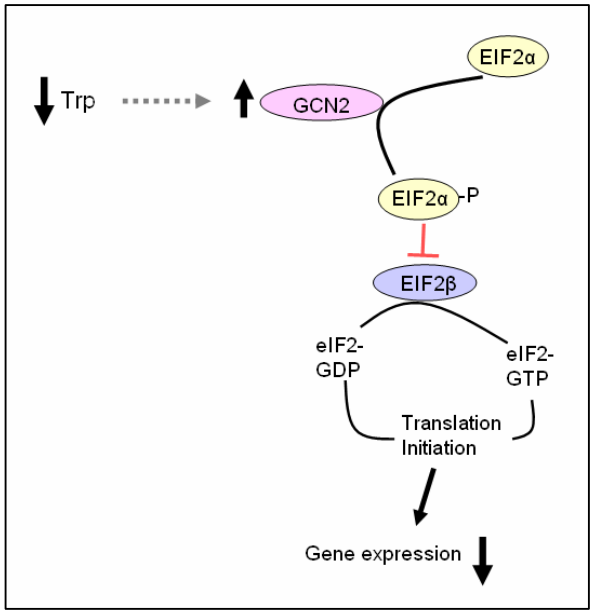

Figure 2: Schematic overview of Gcn2-dependend stress signaling (*modified from: Katz et al, 2008*).
Trp reduction induces an increase in Gcn2 activity, causing phosphorilation of eIF2α. Subsequently, eIF2α blocks eIF2β, which is connected to initiation of translation. Through this route IDO is thus able to reduce gene expression, ultimately leading to proliferation arrest.[3]

2. Cancer

Cancer annually kills over 7.6 million people worldwide, and is predicted to become the most common cause of death by 2010.[15] The disease is characterized by uncontrolled and anomalous cellular growth, usually initiated by an accumulation of genetic mutations in somatic cells. The malignant cells damage adjacent tissue by disturbing the surrounding environment, but can also metastasize throughout the body. Cancer genes are generally divided into two groups: proto-oncogenes and tumor suppressor genes, most of which are involved in regulation of cell division or DNA repair. Possible treatment for cancer involves among others: radiation, chemotherapy, immunotherapy, surgery, or a combination.[16]

2.1 Immunomodulation in cancer evolution

Genetic mutations are mainly responsible for the onset of cancer, and remain of great importance throughout the early stages. However, during the process of oncogenic evolution the tumor microenvironment becomes of the utmost importance in development of the disease. This particularly applies to interventions by the host's immune cells. Mutated cells containing multiple epigenetic alterations, resulting in genomic instability, show deviant protein expression on their cell surface, herewith drawing attention from the immune system. This results in short term gain since many anomalies will be wiped out. However, this competition will eventually become the driving force behind oncogenic evolution, pushing the remaining tumor cells to develop methods for immune evasion. This process is labeled immunoediting, which can ultimately lead to a progressive disease as the tumor escapes the immune system. The latter is considered a fundamental trait of cancer. In between short term gain by immune surveillance and long term pain when the tumor escapes, a state of immune equilibrium exists. At equilibrium ('tumor dormancy') the tumor can fend off, but not overcome the immune system, and vice versa (*see Fig. 3*). It has been proposed that further disruption of the processes aimed at restoring immunoresponsiveness is crucial at equilibrium, in order to realize successful immune escape.[2,3]

Tumor cells execute several different direct and indirect strategies during immunomodulation, like the IDO pathway which will be discussed later (*see 2.2*). Other approaches involve regulatory dendritic cells (DCs), mast cells, and regulatory T cells (Tregs). It was reported that mast cells are able to influence formation of Tregs, which subsequently contribute to epithelial tumorgenesis. Furthermore, regulatory DCs present an antigen attached to MHC Class II proteins and B7 (a costimulatory molecule) to CD4+ T cells. B7 connects to a CD4+ T cell via either the CD28 ligand, providing either stimulatory signals ('causing activation'), or CTLA4 providing inhibitory signals ('which tolerize'). In the tumor microenvironment, regulatory DCs often instruct CD4+ cells through inhibitory signals, making them insensitive to the presented antigen (*see Fig. 4a*). The precise manner in which tumors manage to reconfigure both regulatory DCs and Tregs, and the role of IDO in this process, is still to be unraveled. Nevertheless, there is mounting evidence to suggest B7 signaling pathways play an important role in immune escape.[2,3,11]

Insights in the mechanisms underlying immune escape and the progression of oncogenic evolution are key to understanding cancer pathophysiology. Additional research on this subject will potentially provide starting points for additional treatment.

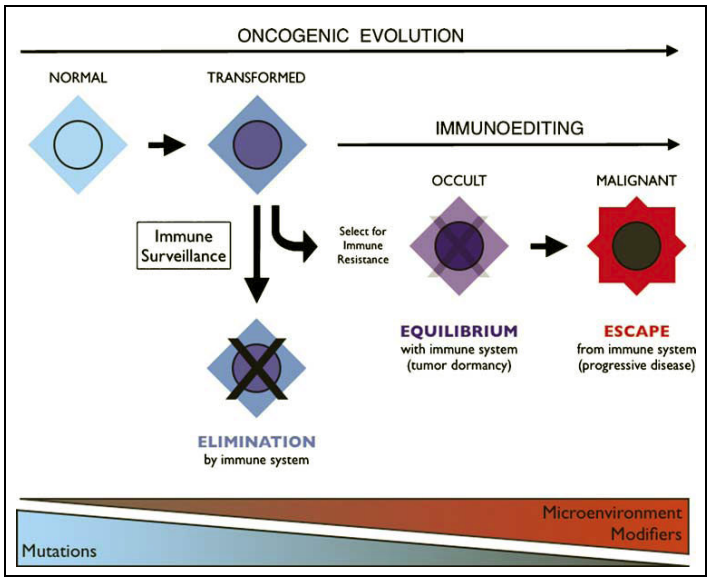

Figure 3: Overview of the process of oncogenic evolution.[3]
The figure depicts different phases in the development of cancer. At the outset, deviant cells (normal cells transformed via e.g. mutations) are partially cleared by the immune system. By eliminating many tumor cells, the immune system eventually drives oncogenic evolution until equilibrium is reached. At this point, neither the tumor cells, nor the immune system is able to gain ascendancy. Successful immunoeduting by cancer would ultimately allow tumor escape, allowing the disease to enter a progressive stage.[3]

2.2 IDO and tumoral immune escape

Several surveys among cancer patients provided insight into the impact of IDO activity on survival rates. Overexpression of IDO of cells in the tumor microenvironment correlates inversely with patient survival. In addition, there is positive correlation between frequency of liver metastases and IDO activity.[2]

IDO is principally linked to immunomodulation by its role in Trp catabolism, which in turn is connected to maintaining the adaptive immune system.[2] However, studies by Uyttenhove et al. confirmed expression and activity of IDO in various types of tumors. Expression levels were most prominent in prostatic, pancreatic and colorectal carcinomas. They also showed IDO activity in these tumor cell lines was equal or higher to placental IDO activity, suggesting sufficient availability for *in vivo* immunoediting. Experiments showed an increased speed of tumor growth in mice with high IDO expression in tumor cells, compared to mice with lower IDO expression. Additional tests also showed effects of preimmunization (e.g. rejection) in mice were undone when tumor cells expressed IDO.[17]

T cells show the most severe response to IDO activation. Proliferation arrest occurs when local Trp supplies are marginalized, impairing further T-cell activation (*see 1.2*). Furthermore, both T cells and natural killer cells are affected by Kyn and other products originating from Trp metabolism (*see Fig. 1 and Fig. 4b*).[17,18] Findings regarding the alleged connection between T-cell apoptosis and catabolites along the IDO pathway are contradicting.[17] However, the combination of those catabolites and Trp deficiency is said to play a part in formation of Tregs and immune suppression.[17] Moreover, research in IDO knockout mice indicated tumor immune escape requires IDO activation in either tumor cells or nodal regulatory DCs. With regard to the latter, it was proposed that IDO facilitates in improving the tumor microenvironment, by promoting formation of either Tregs via regulatory DCs or vice versa (*see Fig. 4a*). Interestingly, such actions by IDO don't result in autoimmunity, which suggests the

enzyme is involved only in tolerance to nonself antigens. This characteristic is important to cancer, because it allows modification of the host's immune system without any direct adverse effects.[3] Furthermore, the immune response itself can also trigger IDO expression, since part of its activation occurs via IFN-γ. This implies IDO-negative tumor cells might also profit from the immunomodulation through IDO (*see Fig. 4b*).[17]

The increased odds for survival are, however, partly compensated by scarcity of the essential amino acid Trp. But even though proliferation of T cells is obstructed in absence of Trp, tumor cells only show reduced growth rates *in vitro*. The explanation for this possibly lays in enzyme kinetics, since the K_m of tryptophanyl-tRNA synthetase for Trp is lower than that of IDO, indicating superior binding capacities. In addition, tumor cells don't appear to have a checkpoint for Trp in the G1 phase of the cell-cycle either.[2,17]

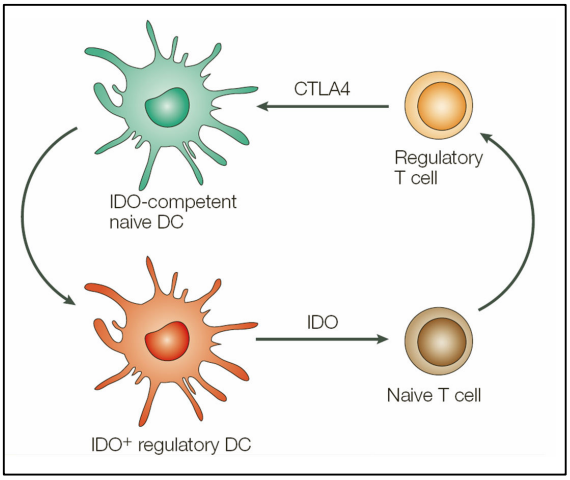

Figure 4a: Reciprocity between T cells and DCs via IDO (simplified).[10]

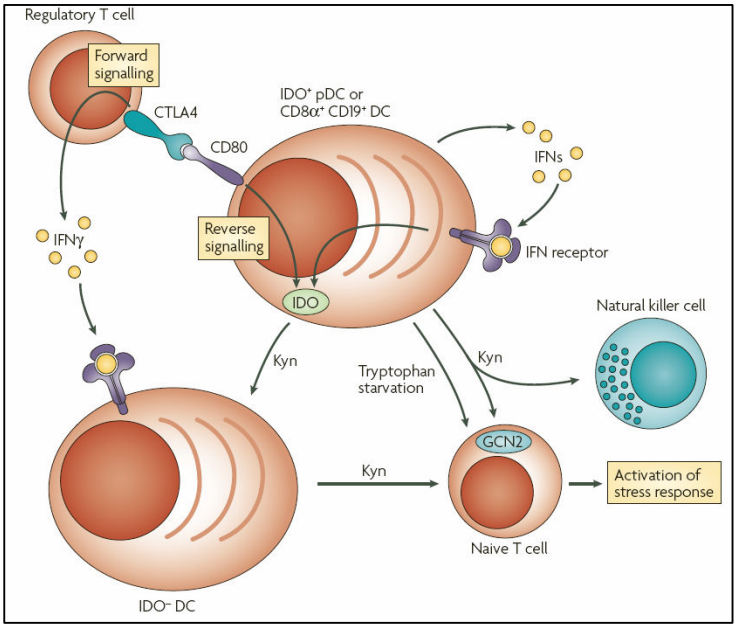

Figure 4b: Proposed interactions between T cells, DCs, and metabolites originating from Trp catabolism.[11]
The figure depicts production of IDO induced via both autocrine and paracrine signaling. Subsequently, the increase in IDO causes depletion of Trp and elevated levels of Kyn. These, in turn, affect cellular immunity.[11]

2.3 Clinical application of IDO and IDO2

The previous emphasizes the crucial contribution of immunomodulation in development of cancer. Because of its divergent character, the IDO pathway is of great importance to this process. There is an increasing support for the idea that for immunotherapeutic strategies to be successful several immunosuppression mechanisms (e.g. tolerization) should be counteracted. Recent findings indicate small-molecule inhibitors of IDO can boost efficacy of standard treatment, such as chemotherapy. Standalone treatment of cancer with an IDO inhibitor showed poor results in several preclinical tests. However, regression was achieved when administering both an IDO inhibitor and a chemotherapeutic drug. This improvement could not be fully explained by a synergism, because no comparable outcomes were observed in absence of CD4+ T cells. Whatever the underlying mechanism, reports regarding the cooperation noted in these experiments labeled IDO as a target in drug development.[19]

The IDO molecule has multiple traits making it an appropriate target for drug development. There is sufficient knowledge of the enzyme's biochemical properties; and development of molecular inhibitors is relatively well doable compared to other targets in cancer. Secondly, TDO is likely to (partially) compensate for possible accumulation of Trp. In addition, preclinical validation tools exist and pharmacodynamical analysis of the inhibitory molecules is feasible.[19]

The small-molecule compound 1-methyl-tryptophan (1MT) is reported to be a competitive IDO inhibitor. Initial studies used a racemic mixture of 1MT. Although L-1MT is structurally similar to Trp, the D isomer is more active in some biological systems. Despite L-1MT proving a more suitable inhibitor against IDO, D-1MT functions better as an *in vivo* anti-tumor compound.[2,3,20]

Through extensive genomic studies indoleamine 2,3-dioxygenase 2 (IDO2) was identified, downstream of the IDO gene *INDO*. The two proteins, IDO and IDO2, show a relatively low degree of homology (43% identity), yet crystallographic and mutagenesis studies confirmed structural conservation in the part critical to catalytic activity. Furthermore, expression of IDO2 is limited when compared to the ubiquitous distribution of IDO. Experiments by Metz et al. showed some inhibition of IDO via L-1MT and none by D-1MT. Conversely, IDO2 was solely inhibited by the D isomer. Difficulties regarding the interpretations of such results will remain as mice and humans show different results to both 1MT variants. However, small-molecule IDO inhibitors have entered phase I clinical trials at the end of 2007.[2,3,20]

Because of its central role in immunomodulation there are more reasons why IDO blockage (via e.g. 1MT) can contribute to greater therapeutic results. Many of the current strategies make use of the host's immune response to treat a specific condition. However, signaling involved in regulation of an immune response can induce IDO expression, leading to a subsided reaction. Another problem arises when considering cancer vaccination is related to the adjuvant. The adjuvant is required to boost immune response against specific antigen(s). However, it was shown that several bacteria, such as *Mycobacterium bovis* and *Listeria monocytogenes*, cause *in vivo* IDO activation when used as an adjuvant. It is unclear whether this applies to many clinically relevant adjuvants. This possibility should be taking into consideration when developing vaccines, because such counterregulatory side effects would severely hinder the goal of the procedure itself.[21] Furthermore, several studies suggest a coherence between psychopathologies and distorted Trp levels (Trp is a precursor of the neurotransmitter 5-HT, *see 1.1*). Again a direct relation between the previous and IDO inhi-

bition is uncertain, however the potential consequences of further altering Trp metabolism should be prepared for.[22-24]

3. **P**regnancy

Chapter two strongly emphasizes the IDO pathway and the way it is utilized by cancer, having harmful consequences to the host. However, there are also circumstances under which immunomodulation through IDO positively influences the host. Altering the immune response can be particularly helpful during pregnancy. The concept of local immunosuppresion elucidates the paradox why pregnant women's immune systems don't attack a developing fetus ('foreign body').[1]

Munn and colleagues reported that cells of fetal origin express IDO in human placenta. They first hypothesized MCSF is involved in induction of IDO in macrophages, leading to proliferation arrest in T cells.[25] It was shown that proliferation arrest occurs due to IDO activity leading to Trp shortage (*see 1.2*).[14] Their findings also suggested a connection between Trp catabolism by IDO+ cells and maternal tolerance.[25] This well-cited publication provided the basic insights into fetal immune evasion through IDO.

The proposed contributions of IDO to successful pregnancy were supported further by reports that fetal survival was reduced severely in absence of activated IDO. Furthermore, studies have shown that activation of maternal T cells is required to provoke complement activation and subsequent fetal rejection. It was shown that the stand-alone inhibitory factor Crry (complement receptor-related protein y) can not prevent complement deposition, because of high local availability of complement components. This implies that T-cell suppression by the IDO barrier also hinders complement activation.[26]

Studies in mice during normal pregnancy have shown an increase in Tregs in multiple organs as of day 2, compared to abortion-prone mice. IDO expression initiated on day 8 and was limited to the placenta. The increase in Tregs occurs before IDO is expressed, implying induction of Tregs in mice doesn't occur through the IDO pathway. The latter is in contrast to the mechanism as proposed in humans; such interspecies differences should be taken into consideration when extrapolating from animal studies.[27]

Furthermore, it was concluded that IDO is involved in the induction of maternal tolerance to extravillous fetal trophoblast (EVT) invasion. Trophoblasts form a layer of cells that enclose the blastocyst. There is a mechanism in place to ensure both limited invasion of EVT, as well as maternal response to 'foreign' EVT. First, apoptosis is induced in EVTs (restraining invasion). Subsequently the apoptotic bodies are processed by antigen-presenting cells, followed by tolerization of T cells via the IDO pathway. Tolerization is crucial because fetal cells express both foreign MHC and antigens.[28]

In addition, mesenchymal stem cells (MSC), extracted from human placental tissue, were shown to have T-cell suppressing capabilities. It was also observed that this is another process mediated by IDO.[29]

The preceding illustrates how several mechanisms connected to IDO contribute to local alteration of the immune system. These strategies are crucial in order to avoid a rejection of the fetus, while maintaining an adequate response to other pathogens.

4. Conclusion and future research

The previous chapters illustrate how the Trp converting enzyme IDO is profoundly involved in immunomodulation. Through depletion of the essential amino acid Trp in the microenvironment it locally subsides the immune response. In the first place this mechanism serves to maintain homeostasis, by preventing an escalated reaction of the adaptive immune system. However, the IDO pathway plays a key role in several other scenarios.[2,3,13] During pregnancy cells from the developing fetus express MHC as well as a variety of other proteins, which are foreign to the maternal immune system. In order to prevent rejection, the maternal immune system should be altered, but without compromising its ability to fight off other pathogens. IDO facilitates this process, allowing fetal maturation without immunological interference.[25,26] Conversely, cancer employs the IDO pathway to execute an equivalent strategy, yet to permit malignant growth. Research has provided many insights into the consequences of IDO activation. T-cell proliferation arrest occurs due to Trp shortage. Furthermore, the enzyme is involved in induction of T-cell anergy, as well as Tregs. Catabolites resulting from the Kyn pathway were also reported to affect cellular immunity. In addition, IDO influences complement deposition.[2,3,17,26] It is important to further understand the pathways underlying immunomodulation, because this knowledge can lead to new or alternative treatments for diseases -such as cancer- that disrupt the regular immune response. Moreover, findings resulting from research on pregnancy regarding IDO can contribute to a better understanding of cancer, and vice versa, because both employ comparable strategies. Extrapolation of results is not limited to pregnancy and cancer, but might also make contributions to the field of transplantation immunology, allergies et cetera.

With regard to cancer treatment, it was suggested that several immunosuppression mechanisms (e.g. tolerization) should be counteracted. Furthermore, it was shown that IDO can be blocked via the competitive inhibitor 1MT.[19,20] Deducing from chapter two, one of the immune system's main issues in responding to cancer, is that suitable lymphocytes (i.e. complimentary to cancer antigens) are impeded in activation. Yet *in vivo* modifications through drugs include high risk of causing further damage to an already weakened patient. Therefore, it might be worthwhile to perform such actions *in vitro*, once a tumor sample is obtained by either surgery or a biopsy. Excess 1MT should be added to the sample to ensure inhibition of all available IDO. Subsequently, apoptosis should be induced via a mild dose of chemo or another suitable alternative. Hereupon, an extract containing a sample of the patient's antigen-presenting cells and lymphocytes should be added. If it is possible to harvest -either activated or tolerized- T cells afterwards, this procedure might be eligible for testing in appropriate models.

5. **R**eferences

1. Bonney EA, Matzinger P. Much IDO about pregnancy. Nature Medicine. 1998; 4; 10: 1128-1129.
2. Katz JB, Muller AJ, Prendergast GC. Indoleamine 2,3-dioxygenase in T-cell tolerance and tumoral escape. Immunological Reviews. 2008; 222: 206-221.
3. Prendergast GC. Immune escape as a fundamental trait of cancer: focus on IDO. Oncogene (Nature Publishing Group). 2008: 1-12.
4. Abbas AK, Lichtman AH. Basic Immunology: Functions and Disorders of the Immune System, 3rd edition. Saunders Elsevier. 2008. 3-20, 42, 103-111, 173-178.
5. Berg JM, Tumoczko JL, Styer L. Biochemistry, 6th edition. W.H. Freeman & Company. 2006. 671-672.
6. Takikawa O. Biochemical and medical aspects of the indoleamine 2,3-dioxygenase-initiated L-tryptophan metabolism. Biochemical and Biophysical Research Communications. 2005; 338: 12-19.
7. De Jong WHA, Smit R, Bakker SJL, De Vries EGE, Kema IP. Plasma tryptophan, kynerunine and 3-hydroxykkynurenine measurement using automated online solid-phase extraction HPLC-tandem mass spectrometry. Journal of Chromotography B. 2009; 877: 603-609.
8. Macchiarulo A, Camaioni E, Nuti R, Pellicciari R. Highlights at the gate of tryptophan catabolism: a review on the mechanisms of activation and regulation of indoleamine 2,3-dioxygenase (IDO), a novel target in cancer disease. Amino Acids (Springer). 2008.
9. Fernstrom JD. Role of Precursor Availability in Control of Monoamine Biosynthesis in Brain. Physiological Reviews. 1983; 63; 2: 484-546.
10. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nature Reviews | Immunology. 2004; 4: 762-774.
11. Puccetti P, Grohmann U. IDO and regulatory T cells: a role for reverse signaling and non-canonical NF-κB activation. Nature Reviews | Immunology. 2007; advance online publication.
12. Belladonna ML, Orabona C, Grohmann U, Puccetti P. TGF-β and kynurenines as the key to infectious tolerance. Trends in Molecular Medicine. 2009; 15; 2; 41-49.
13. Mellor AL, Munn DH. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation. Immunology Today.1999; 20; 10: 469-473.
14. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T Cell proliferation by Macrophage Tryptophan Catabolism. J. Exp. Med. 1999; 189; 9: 1363-1372.
15. American Cancer Society, reviewed online at http://www.cancer.org/ (April 4, 2009)
16. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell, 4th edition. Garland Science. 2002. 1313-1362.
17. Uyttenhove C, Pilotte L, Théate I, Stroobant V, Colau D, Parmentier N, Boon T, Van den Eynde BJ. Nature Medicine. 2003; 9; 10: 1269-1274.
18. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrare GB. Tryptophan-drived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,,3-dioxygenase. Journal of Experimental Medicine. 2002; 196; 4: 459-468.

19. Muller AJ, Prendergast GC. Marrying Immunotherapy with Chemotherapy: Why Say IDO. Cancer Research. 2005; 65; 18: 8065-8068.
20. Metz R, DuHadaway JB, Kamasani U, Laury-Kleintop L, Muller AJ, Prendergast GC. Novel Tryptophan Catabolic Enzyme IDO2 Is the Preferred Biochemical Target of the Antitumor Indoleamine 2,3-Dioxygenase Inhibitory Compound D-1-Methyl-Tryptophan. Cancer Research. 2007; 67; 15: 7082-7087.
21. Munn DH, Mellor AL. Indoleamine 2,3-dioxygenase and tumor-induced tolerance. The Journal of Clinical Investigation. 2007; 117; 5: 1147-1154.
22. Russo S, Boon JC, Kema IP, Willemse PHB, Den Boer JA, Korf J, De Vries EGE. Patients With Carcinoid Syndrome Exhibit Symptoms of Aggressive Impulse Dysregulation. Psychosomatic Medicine. 2004; 66: 422-425.
23. Russo S, Kema IP, Fokkema R, Boon JC, Willemse PHB, De Vries EGE, Den Boer JA, Korf J. Tryptophan as a Link between Psychopathology and Somatic States. Psychosomatic Medicine. 2003; 65: 665-671.
24. Dantzer R, O'Connor JC, Freund GC, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nature Reviews | Neuroscience. 2008; 9: 46-57.
25. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL. Prevention of Allogeneic Fetal Rejection by Tryptophan Catabloism. Science Magazine. 1998; 281: 1191-1193.
26. Mellor AL, Sivakumar J, Chandler P, Smith K, Molina H, Mao D, Munn DH. Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism. Nature Immunology. 2001; 2; 1: 64-68.
27. Thuere C, Zenclussen ML, Schumacher A, Langwisch S, Schulte-Wrede U, Teles A, Paeschke S, Volk HD, Zenclussen AC. Kinetics of Regulatory T Cells During Murine Pregnancy. American Journal of Reproductive Immunology. 2007; 58: 514-523.
28. Von Rango U. Fetal tolerance in human pregnancy – A crucial balance between acceptance and limitation of trophoblast invasion. Immunology Letters. 2008; 115: 21-32.
29. Jones BJ, Brooke G, Atkinson K, McTaggart SJ. Immunosuppression by Placental Indoleamine 2,3-dioxygenase: A Role for Mesenchymal Stem Cells. Placenta. 2007; 28: 1174-1181.