

## Theme Issue Article

# Antimicrobial and immunoregulatory effector mechanisms in human endothelial cells

## Indoleamine 2,3-dioxygenase versus inducible nitric oxide synthase

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### Summary

In infectious diseases, interferon-gamma (IFN- $\gamma$ ) is generally accepted as one of the most important inducers of antimicrobial and immunoregulatory effects, and both seemingly contradictory effects, can be mediated by the same effector molecules. In detail, several IFN- $\gamma$  induced enzymes such as the inducible nitric oxide synthase (iNOS) as well as the indoleamine 2,3-dioxyge-

nase (IDO) also exert this double function. In this review we focus on antimicrobial and immunoregulatory properties of both enzymes expressed by human endothelial cells, which are prominent players in infectious diseases, tumour immunology and transplant medicine.

### Keywords

Endothelial cells, inducible nitric oxide synthase, indoleamine 2,3-dioxygenase, interferon-gamma, effector mechanisms

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## Introduction

Endothelial cells play a major role in a number of physiological and pathological situations. Here we focus on the role of endothelial cells in infections and on the immunoregulatory effects mediated by these cells during solid organ transplantation and tumour progression (1).

Endothelial cells can function as primary host cells for several pathogens (for example *Bartonella spec.* [2] and *Rickettsia spec.* [3]). On the other hand endothelial cells can phagocytose extracellular bacteria, which may then proliferate within the endothelial cells (for example *Staphylococcus spec.* [4] or *Pseudomonas spec.* [5]). Moreover endothelial cells present a barrier to tissue invasion for several pathogens (such as *Plasmodium spec.* [6] and *Candida albicans* [7]).

Endothelial cells can respond to pathogens in several aspects. Pathogens, or their products (8), can modify the activity of several genes in endothelial cells. For example, it has been shown by array analysis (9) that infection of human brain microvascular endothelial cells (HBMEC) with *Cryptococcus neoformans* results in a change in the mRNA concentrations derived from ap-

proximately 50% of the 12000 genes analysed. Furthermore endothelial cells can respond to cytokines produced by T cells, macrophages or granulocytes. For example, interferon-gamma (IFN- $\gamma$ ) and tumour necrosis factor-alpha (TNF- $\alpha$ ) produced by T cells that have been stimulated with Cytomegalovirus (CMV) proteins induce a profound fractalkine production (as a marker for the initiation of an inflammatory response) by co-cultured human umbilical vein endothelial cells (HUVEC) (10).

In terms of their antimicrobial effector capacity, endothelial cells restrict the growth of parasites (e.g. *Toxoplasma gondii* [11]), bacteria (e.g. *Staphylococcus aureus* [12], *Pseudomonas aeruginosa* [5], *Rickettsia rickettsii* [13]) and viruses (e.g. Herpes simplex virus [14]). A number of different antimicrobial effector mechanisms have been shown to be inducible in endothelial cells. In this review, we will mainly focus on antimicrobial and immunoregulatory effects, mediated by endothelial cells expressing indoleamine 2,3-dioxygenase (IDO), an enzyme that converts the essential amino acid tryptophan to kynurenine. In addition the role of nitric oxide synthesis will be discussed. Since there is ample evidence that antimicrobial effector mechanisms active in murine and human cells differ strongly

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from each other (15), we will focus on mainly human endothelial cells. But concomitant with the restriction to the human system we have to note that most data were obtained *in vitro* only. Furthermore, the majority of the experiments were performed with immortalised endothelial cells or with HUVEC, making it difficult to estimate how representative the data are. For example, the discrimination between micro- and macrovascular endothelial cells could be critical as microvascular endothelial cells have special properties (16). They are directly associated with inflamed tissue, regulate diapedesis, and influence the blood supply of tumours as well as acting as major target cells for the cellular immune response during transplantation.

## Nitric oxide-dependent antimicrobial effects mediated by endothelial cells

Nitric oxide (NO) mediates three major actions in the endothelial cell: 1. vasodilatation, 2. apoptosis and 3. antimicrobial effects. Currently three isoforms of nitric oxide synthases (NOS) are known and named according to the cell type from which they were first described or by their kind of regulation. The neuronal NOS (nNOS or NOS-1) and the endothelial NOS (eNOS or NOS-3) are both constitutively expressed (cNOS) while iNOS (inducible NOS or NOS-2) is an inducible isoform, mainly expressed in murine macrophages. A lot of data have been published concerning the role of iNOS in the antimicrobial defence in model systems using rodents or rodent cells (17). The functional role of iNOS in the human system is more controversial. There are only few publications describing iNOS activity and iNOS mediated effects in human endothelial cells in infections.

For example it was shown that vascular endothelial growth factor-A (VEGF-A), which usually is involved in proliferation and migration of endothelial cells and plays an important role in angiogenesis, is capable of inducing iNOS and eNOS protein in HUVEC as well as in porcine endothelial cells. In this publication the VEGF-receptor 2 appeared to be the main receptor involved in the induction of both iNOS and eNOS in porcine endothelial cells, whereas VEGF-A induced iNOS expression in HUVEC was weak (18). However, the effect of pro-inflammatory cytokines, which are important inducers of NO production, was not analysed in parallel (18). Therefore a final interpretation of these data with respect to inflamed tissue is not possible. In a more recent work, iNOS induction in HUVEC was demonstrated after infection of cell cultures with *Pseudomonas aeruginosa* strains transfected with genes coding for a type three secretion system (19). The effect of NO produced by HUVEC in this setting was not considered to be a protective one, since NO is involved in the induction of apoptosis in HUVEC as shown by the use of the iNOS inhibitor L-arginine-methyl-ester (L-NAME). A comparable deleterious iNOS up-regulation and induction of apoptosis was found in human lung endothelial cells after contact with red blood cells infected with *Plasmodium falciparum* (20).

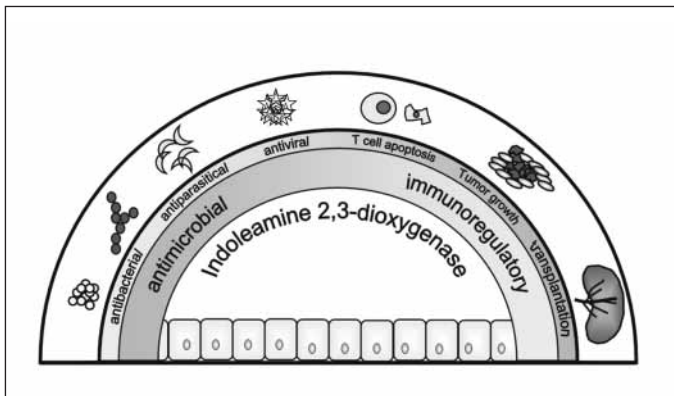
Using an infection model with Junin virus (a member of the *Arena virus* family and the causative agent of Argentine haemorrhagic fever) a striking production of NO by HUVEC was shown (21). This NO production was due to the up-regulation of

eNOS and was seemingly relevant to the *in vivo* situation, since nitrite concentration in serum samples from patients with a Junin virus infection was found to be four- to five-fold higher than in samples from healthy controls. However, Gomez et al. (21) were unable to detect iNOS in HUVEC even after co-stimulation with a cocktail of pro-inflammatory cytokines. The authors suggest that the increased production of NO might contribute to the haemorrhagic diathesis characteristic of Argentine haemorrhagic fever. In this respect the involvement of iNOS in coagulation homeostasis must also be considered (22). In addition, an inducibility of NO production in HUVEC by a simultaneous stimulation with IFN- $\gamma$ , TNF- $\alpha$  and interleukin-beta (IL-1 $\beta$ ) has been demonstrated (23). However this NOS activity was found to be calcium-dependent and was membrane-associated. The authors conclude that eNOS was responsible for the NO production detected. Therefore, it is evident that the inducible NOS is not always responsible for NO production after stimulation with pro-inflammatory cytokines (23).

The discrepancies in the inducibility of NO production in HUVEC cells might at least be explained by individual factors: Feng et al. (24) described that only one in five HUVEC preparations produce large amounts of NO after stimulation with pro-inflammatory cytokines. Furthermore, data published by Orpana et al. (25) indicate that the quantity of iNOS mRNA and of NO produced by HUVEC after stimulation with pro-inflammatory cytokines, largely depends on the culture conditions used. In their cultures HUVEC express a high iNOS activity, even when they are plated at low cell density, on a reconstituted extracellular matrix (Matrigel) while the same amount of HUVEC cultured with gelatine substrate produced considerably less NO.

All manuscripts cited in the previous sections discuss the induction of iNOS as analysed by the determination of NO in culture supernatants or the detection of the iNOS mRNA or protein in endothelial cells. However, there are only limited data available on the antimicrobial effect of NO in human endothelial cell cultures. Recently published data by Woods and Olano (13) show a surprisingly high production of NO by human endothelial cells and, using the NOS inhibitor L-NAME, it was demonstrated that this NO-production is responsible for an antibacterial effect directed against *Rickettsia rickettsii*, an obligate intracellular bacterium, infection with which leads to Rocky Mountain Spotted Fever. In this paper (13) immortalised human cerebral endothelial cells were used instead of HUVEC and these cells were stimulated with a cytokine cocktail containing IFN- $\gamma$ , TNF- $\alpha$  and IL-1 $\beta$ . However, no data were shown concerning the type of the NOS responsible for the observed NO production. Furthermore, the authors mentioned that their endothelial cell medium contained 100  $\mu\text{g/ml}$  of heparin. Heparin is often present in endothelial cell cultures since it is described that heparin enhances the activity of the endothelial cell growth factor, however heparin antagonises or blocks completely the actions of IFN- $\gamma$  (26, 27). Therefore, we suggest that the role of IFN- $\gamma$  in the induction of NO production should be re-observed using preferentially microvascular endothelial cells and heparin-free conditions. In summary, the role of iNOS in the antimicrobial defence mediated by human endothelial cells is not well understood and future experiments are needed to clarify this point.





**Figure 1: A general overview of immunologic IDO properties: One enzyme mediates antimicrobial and immunoregulatory functions.**

### IDO-dependent antimicrobial and immunoregulatory effects mediated by endothelial cells

Indoleamine 2,3-dioxygenase (IDO) is an IFN- $\gamma$  inducible enzyme initiating the kynurenine pathway by the oxidative cleavage of the indole ring of tryptophan. This enzyme mediates antiparasitic effects originally described by Pfefferkorn et al. in 1984 (28). In addition, IDO has immunoregulatory properties as firstly shown by Munn et al. in 1998 (29). In general, IDO activity is currently accepted as an antimicrobial and an immunoregulatory effector mechanism. In endothelial cells tryptophan derived metabolites, accumulating after IDO activation, might have a range of additional functions. For example, it has been proposed that kynurenine, the breakdown product of tryptophan, might be a novel endothelium derived vascular relaxing factor (30). Furthermore, tryptophan and the tryptophan degradation products kynurenine and 3-hydroxy-kynurenine are of importance as UV-filters in the eye lens (31, 32). In widening the analysis of the role of IDO in UV-protection, it has been shown that murine corneal endothelial cells, after UV-irradiation, display an increased IDO expression (33). As a consequence of IDO-activation in corneal endothelial cells a reduced apoptosis rate after UV-exposition was observed. However, in the following chapters we will focus on the 'classical' role of IDO in antimicrobial defence and in the regulation of the immune response as shown in Figure 1.

### Antimicrobial effects mediated by IDO-positive endothelial cells

Various human cell types expressing IDO are able of restricting the growth of several bacteria such as *Staphylococcus aureus* (12), *Streptococcus suis* (34), group B Streptococci (35), Enterococci (36), Chlamydia (37) and Rickettsia (13). Human endothelial cells from different origins are able to express IDO mRNA, IDO protein and/or show IDO activity after stimulation with IFN- $\gamma$  in the absence or presence of TNF- $\alpha$  and/or IL-1 $\beta$ . For example, Maghzal et al. (30) detected IDO activity in human aortic endothelial cells and Beutelspacher et al. (38) found IDO ac-

tivity in HUVEC whereas human endothelial cells derived from the saphenous vein (HSVEC), the radial artery or from the internal mammary artery are not able to initiate the kynurenine pathway after stimulation with IFN- $\gamma$ . It was suggested that the lack of IDO-induction in HSVEC was due to an upregulation of SOCS-3 (suppressor of cytokine synthesis-3), which was not observed in HUVEC (38). We observed that IFN- $\gamma$  activated HBMEC exhibit IDO activity. We also found that HBMEC are capable of restricting the growth of *Staphylococcus aureus* by an IDO-dependent mechanism, since the supplementation of IFN- $\gamma$  activated HBMEC cultures with excess amounts of tryptophan abolishes the observed anti-staphylococcal effect (12). However endothelial cells seem to mediate several effects in the control of staphylococcal growth. For example an antimicrobial effect independent of tryptophan or iron depletion, but dependent on phagocytosis of Staphylococci was discovered (4). The ability of endothelial cells to restrict the growth of Staphylococci is possibly of patho-physiological relevance since endothelial cells have been shown to phagocytose these usually extracellular bacteria. These internalised Staphylococci are not completely killed and remaining ones can persist and even replicate within endothelial cells. It was assumed that these intracellular bacteria might contribute to the persistent and recurrent nature of staphylococcal infections and might facilitate the development of systemic staphylococcal diseases (4). Recent data show that endothelial cells are antimicrobial effector cells mediating IDO dependent (12) and independent (4) effects. Furthermore endothelial cell-mediated processes which have an indirect antibacterial effect, such as enhanced expression of adhesion molecules or of cytokines have been described.

Therefore, the reduction of the local tryptophan concentration is an antibacterial effector mechanism but the final outcome of IDO-induction *in vivo* is difficult to understand, since IDO acts also as an immunosuppressive effector-mechanism resulting in a damping of T-cell activation and IFN- $\gamma$  production as discussed later on. However, recently we found that the tryptophan requirement of bacteria is higher than the tryptophan requirement of T cells. *In vivo* this allows IDO to act as an antibacterial effector mechanism prior to the mediation of contra-regulatory immunosuppressive effects (39).

Impressive data concerning IDO expression *in vivo* were achieved in an experimental system using CBA/T6 and C57Bl/6 mice infected with *Plasmodium berghei*. Hunt et al. (40) demonstrated a surprisingly intense IDO-immunoreactivity in endothelial cells from Plasmodium-infected mice. The role of IDO in the defence against this particular pathogen is still unclear, but future analysis with IDO-deficient mice infected with Plasmodia might answer this question definitively. Despite the unclear role of IDO in the defence against Plasmodia, *in vitro* data clearly show the effectiveness of IDO in the defence against the obligate intracellular parasite *Toxoplasma gondii*. While Woodman et al. (41) detected an IFN- $\gamma$ -mediated, seemingly IDO-independent, antiparasitic effector mechanism in HUVEC, we found an IDO-dependent inhibition of toxoplasma growth by HBMEC (11). One of the major clinical problems after *Toxoplasma gondii* infection is the development of encephalitis, especially in immunocompromised patients. In the setting of toxoplasmic encephalitis HBMEC are relevant and we suggest a double role of IDO in

the defence against *Toxoplasma*. Firstly endothelial cells, via tryptophan degradation, are capable of restricting the growth of this particular parasite. Secondly, IDO-expressing endothelial cells, which comprise the blood-brain barrier, reduce the influx of tryptophan into the brain parenchyma and may act in concert with IDO-positive cells in the brain e.g. astrocytes to restrict toxoplasma growth by limiting the local availability of tryptophan (14).

The same double function might be ascribed to endothelial cells in viral infections, since IDO mediates antiviral effects against Vaccinia virus (42), Measles virus (43) and several Herpes viruses (44, 45). We found that HBMEC mediate IDO-dependent antiviral effects against Herpes simplex virus (46). Herpes simplex virus is the most frequent cause of viral encephalitis in humans resulting in hospital treatment. In immunosuppressed patients it is imaginable that *Toxoplasma gondii* might cause encephalitis or *Staphylococcus aureus* might cause brain abscesses at the same time. It is well described that viruses have developed escape mechanisms to block IFN- $\gamma$ -induced antiviral effects. One of these mechanisms, used by Herpes viruses, is the blockade of IFN- $\gamma$  signalling (47). This would result in the inhibition of all IFN- $\gamma$ -induced effects, including IDO-activation. And indeed, in preliminary experiments, we have found that an infection with Herpes simplex virus, prior to the stimulation with IFN- $\gamma$ , results in a profound inhibition of IDO activity and completely blocks the capacity of these cells to restrict the growth of Staphylococci (own unpublished results).

In summary, a lot of *in vitro* and *in vivo* data argue for an active role of IDO-expressing endothelial cells in the defence against several microorganisms. The efficiency of these IDO-mediated effects depends on the amount of IFN- $\gamma$ , co-stimulatory effects mediated by other pro-inflammatory cytokines and on a possible interaction of different pathogens *in vivo*.

## IDO-dependent immunoregulatory effects mediated by endothelial cells

Since the observation that the application of 1-methyl-DL-tryptophan, a competitive inhibitor of IDO, was fatal for pregnancy in mice carrying allogeneic foetuses, but not for syngeneic concepti, IDO has been recognised as having an immunoregulatory function (29). Due to the role of IDO during pregnancy, IDO expression and activation were analysed in the inner organs of the female reproductive tract and especially in the placenta (48, 49). It has been shown by different groups that IDO is expressed by endothelial cells in human and murine placenta. However, to date no conclusive data concerning the functional role of IDO in endothelial cells during human pregnancy are available. However, a substantial expression of IDO in vascular endothelial cells was recently found in the endometrium and the decidua of non-human primates (50). The functional role of IDO in an *in vivo* experimental animal system, which is closer to the human situation than the rodent model, may provide essential missing data.

In addition to the role of IDO during pregnancy the immunoregulatory role of IDO in autoimmune diseases like multiple sclerosis (51, 52) or diabetes (53), in transplant medicine and in tumour immunology has gained much attention. The following

chapters will focus on IDO expression in endothelial cells in tumour tissue as well as on the role of endothelial IDO in transplant medicine.

## Endothelial IDO expression in tumour immunology

An IFN- $\gamma$ -inducible IDO activity in tumour cell lines was described by Ozaki et al. in 1988, and the authors suggested that the antiproliferative action of tryptophan degradation might be an effector mechanism against tumour cells (54). The recognition of IDO as an immunosuppressive effector mechanism was central to this point of view and it was suggested that the IDO activity of dendritic cells or tumour cells might result in an inhibition of the anti-tumour response and might therefore be a tumour-protective effect (55). Indeed, it has been demonstrated that tumour cells can induce tolerance towards their own antigens, and host antigen presenting cells expressing IDO activity might contribute to an immunosuppressive milieu in tumour draining lymph nodes. Therefore, IDO expression in tumour tissue (56) might locally inhibit effector T cell function, while IDO-expressing professional antigen-presenting cells like dendritic cells, presenting tumour antigens, might induce a peripheral tolerance against tumour antigens (57). In both models of IDO function in tumour immunology, the immunosuppressive effect of IDO seems to mediate the observed tumour protective effect.

An additional point of view is derived from recently published data from urogenital tumours: A profound IDO-immunoreactivity was detected in tumour tissue from patients with prostatic hyperplasia and in patients with prostate cancer. Immunohistological studies indicate that IDO in these patients is expressed mainly by endothelial cells in the tumour tissue. This is a surprising finding since initially it was suspected that IDO would be expressed in the tumour cells or dendritic cells only. On the other hand, IDO protein was found only in a small number of tumour cells (about 5%) in some patients (58). Interestingly, a high IDO expression in the tumour tissue is correlated with a change in the tryptophan/kynurenine ratio in the serum, indicating that this local IDO activity is capable of influencing the systemic tryptophan homeostasis (58). In addition to these data from patients with prostatic disease, a strong IDO expression in microvascular endothelial cells within tumour tissue was recognised in patients with renal carcinoma (59). In addition to the localisation of IDO in endothelial cells, the finding that a strong IDO-immunoreactivity in endothelial cells correlates with a higher survival rate of patients was most important. This finding is surprising and is contrary to observations made by several other groups mentioned above. However, additional immunohistological studies indicated that tumour cells adjacent to IDO-positive endothelial cells express lower amounts of tumour proliferation marker Ki67. Therefore, the authors suggest that IDO-expressing endothelial cells cause a reduced influx of tryptophan into tumour tissue, which results in an inhibition of tumour growth.

In conclusion, IDO has both tumour-protective and tumour-destructive properties and the outcome of IDO activation might depend on the tumour-type, tryptophan requirement of tumour cells and the strength of the anti-tumour immune response.

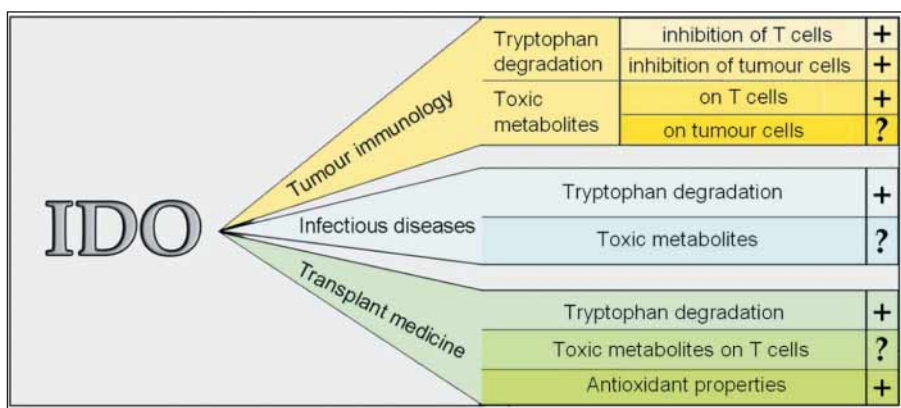
## Endothelial IDO expression in transplant medicine

In the context of allogeneic solid organ transplantation, endothelial cells are the first foreign cells which come into contact with alloreactive T cells from the recipient. Damage to these endothelial cells, either due to T cell alloresponse or due to an overwhelming pro-inflammatory reaction, is fatal for the function of the transplant. Therefore, it is of clinical interest to analyse the capacity of endothelial cells to interact with T cells and to regulate the T cell response. The ability of endothelial cells to activate naïve and memory T cells was studied by different groups. For example, Ma and Probey (60) showed that HUVEC stimulated cytokine secretion by naïve as well as by memory T cells after lectin-driven polyclonal T-cell activation. McDouall et al. (61) found that human heart microvascular endothelial cells, as well as endothelial cells derived from the aorta or the pulmonary artery, can induce alloproliferation of purified CD4+ T cells.

Furthermore IFN- $\gamma$ -stimulated endothelial cells can negatively influence the T cell response. In this respect we discovered that HBMEC are able to block T cell responses induced by lectin, as well as by superantigen, in an IDO-dependent manner (14). The involvement of IDO in this inhibition of T cell responses was shown by the antagonistic effect of supplemental L-tryptophan on the IFN- $\gamma$ -induced inhibition of T cell responses. Subsequently, it was found by others that IFN- $\gamma$ -activated HUVEC are also capable of expressing IDO activity in amounts sufficient to block an alloantigen-driven T cell response, which could be restored by the inhibition of IDO activity using 1-methyl-tryptophan (38). This T cell regulatory effect was confirmed using IDO-transfected endothelial cells from the umbilical cord, as well as from the saphenous vein (38). Additional detailed analysis of the mechanism of the inhibition of T cell responses by IDO-positive endothelial cells indicate that the effect is mediated by the induction of T cell anergy, T cell apoptosis and by the activation of antigen-specific regulatory T cells. These *in vitro* data have been confirmed by several *in vivo* analyses. For example, Beutelspacher et al. (62) found that, after allogeneic cornea transplantation in mice, a significant up-regulation of IDO in the cornea occurs. However, the magnitude of this pathophysiologic induction of IDO activity was not sufficient to block the alloresponse *in vivo*. Furthermore, cytokine activated murine cornea endothelial cells express only low amounts of IDO *in*

*vitro* and only modestly influence allogeneic T-cell activation. In contrast, murine endothelial cells which were transfected with murine IDO strongly inhibit allogeneic T cell responses *in vitro*. Additionally, the same group found that IDO-transfected endothelial cells present in the cornea transplant mediate a significant prolongation of the allograft survival (62). These data indicate that IDO expression in endothelial cells has an immunoregulatory function *in vivo*. Comparable data were also obtained in other animal models for organ transplantation: Allogeneic lung transplants expressing recombinant IDO (63), especially in epithelial and endothelial cells, induce a dramatically reduced acute cellular rejection. At the same time the functional parameters of the allogeneic lung transplants were significantly better than those from IDO-negative lung transplants. Furthermore, support for the causative role of IDO activity in this experimental setting was provided by treatment of rats with 1-methyl-tryptophan which was shown to block this transplant-preserving effect of IDO. The authors suggest that, in this transplant model, IDO-mediated immunoregulatory effects are at least in part responsible for the observed tolerance against the allogeneic tissue, but, in addition, they propose that the antioxidant function of IDO might co-determine the clinical outcome. This additional cytoprotective effect of IDO expression in endothelial and epithelial cells was analysed in a further experimental system. Using syngeneic lung transplants, Liu and Visner (64) showed in a subsequent paper that syngeneic lung transplants carrying IDO-positive endothelial cells display a reduced rate of endothelial apoptosis, reduced vascular permeability and leukocyte extravasation and an overall improved graft function. In these experiments a non-viral gene transfer approach was used to enhance IDO activity in lung cells. They suggest that in this syngeneic transplant situation a direct cytoprotective effect of IDO in combination with its antioxidant action might be responsible for the better clinical outcome.

In summary, IDO expression in endothelial cells can mediate several functions as listed in Figure 2. The effect of IDO in the different clinical settings is always mediated by its enzymatic function but the result of tryptophan degradation or of the tryptophan metabolites depends on the target cells. For example, in tumour immunology a tryptophan starvation results in an inhibition of tumour cell growth while at the same time tryptophan starvation prevents a correct T cell activation directed against tumour antigens, thus favouring the outgrowth of tumour cells.



**Figure 2: A detailed analysis of described (+) consequences of tryptophan degradation by IDO in tumour immunology, infectious diseases and transplant medicine.**

Similar contradictory properties of IDO activity were also observed in transplant medicine. In this clinical setting tryptophan metabolites might be toxic for the foreign tissue while some of these metabolites counteract the pro-inflammatory response observed during reperfusion. *In vivo*, all IDO-mediated effects are dependent on the magnitude of tryptophan depletion or on concentration of tryptophan metabolites in the specific micro-environment. The cellular composition, and therefore the capacity to express IDO activity, is different in various tissues and determines the clinical outcome.

### Abbreviations

CMV, cytomegalovirus; cNOS, constitutive nitric oxide synthase; eNOS, endothelial nitric oxide synthase; HBMEC, human brain microvascular endothelial cells; HUVEC, human umbilical vein endothelial cells; HSVEC, human saphenous vein endothelial cells; IDO, indoleamine 2,3-dioxygenase; IFN $\gamma$ , interferon gamma; IL-1 $\beta$ , interleukin 1 beta; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; TNF- $\alpha$ , tumour necrosis factor-alpha.

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