Tumoral Immune Resistance Mediated by Enzymes That Degrade Tryptophan

Nicolas van Baren1,3 and Benoît J. Van den Eynde1,2,3

Abstract

Cancer patients mount T-lymphocyte responses against antigens expressed selectively by their malignancy, but these responses often fail to control their disease, because tumors select mechanisms that allow them to resist immune destruction. Among the numerous resistance mechanisms that have been proposed, metabolic inhibition of T cells by tryptophan catabolism deserves particular attention, because of the frequent expression of tryptophan-degrading enzymes in human tumors, and because in vitro and in vivo studies have shown that their enzymatic activity can be readily blocked by pharmacologic inhibitors, thereby restoring T-cell–mediated tumor cell killing and paving the way to targeted therapeutic intervention. In view of recent observations, and taking into account the differences between human and mouse data that differ in several aspects, in this Cancer Immunology at the Crossroads article, we discuss the role of the three enzymes that have been proposed to control tryptophan catabolism in tumoral immune resistance: indoleamine 2,3-dioxygenase 1 (IDO1), tryptophan 2,3-dioxygenase (TDO), and indoleamine 2,3-dioxygenase 2 (IDO2). Cancer Immunol Res; 3(9): 978–85. ©2015 AACR.

Introduction

Infectious agents and tumors, in order to survive and proliferate in their host, must interfere with the host immune reaction that they trigger. This resistance to immune destruction can be either intrinsic or adaptive. Intrinsic resistance is actively caused by the pathogen or the tumor and includes mechanisms such as loss or downregulation of antigens and production of immunosuppressive molecules. Adaptive resistance is a consequence of the immune reaction, in which tumor cells or pathogens take advantage of the negative feedback of the immune response, a physiologic process aimed at preventing potentially harmful inflammatory and autoimmune processes resulting from excessive immune reaction. The molecular mechanisms of adaptive resistance are multiple and only partially elucidated. The best characterized is the PD-1/PD-L1 immune checkpoint pathway, which can be reverted in cancer patients by therapy with blocking antibodies, leading to reactivation of T cells and subsequent tumor rejection (1). This clinical success highlights the importance of adaptive resistance against antitumor immunity.

Another important mechanism of immune resistance in tumors involves tryptophan catabolism controlled by dedicated enzymes. As discussed hereafter, it can be either intrinsic or adaptive. Local degradation of tryptophan results in T-cell inhibition through two different, possibly complementary mechanisms. First, the depletion of tryptophan in the extracellular environment is sensed in nearby T lymphocytes by kinase Gcn2, which detects uncharged tRNA molecules and thus amino acid shortage, and subsequently induces a stress response that includes cell-cycle arrest (2, 3). Parallel inactivation of the mTOR pathway may also contribute to proliferation arrest (4). Second, apoptosis of effector T lymphocytes, or their differentiation into regulatory T cells (Treg), can be triggered by molecules such as kynurenine and its derivatives, which are catabolites of enzymatic L-tryptophan degradation (5–7). These molecules are thought to act by binding to and activating the aryl hydrocarbon receptor (AhR; ref. 8).

Tryptophan degradation via the kynurenine pathway is enzymatically controlled at its first step, the oxidative breakdown of the indole group of L-tryptophan. Two enzymes, indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase (TDO), have this property. Both IDO1 and TDO are expressed in human tumors and are thought to be involved in immune resistance. Furthermore, both IDO1 and TDO can be enzymatically inhibited by synthetic molecules, which are now in preclinical and clinical development. The ability of some of these pharmacologic inhibitors to restore antitumor immunity against IDO1- and TDO-expressing tumors has been demonstrated in murine models.

Recently, indoleamine 2,3-dioxygenase 2 (IDO2), a protein with high homology to IDO1, was identified and proposed as a third enzyme controlling tryptophan catabolism, with possible implications in tumoral immune resistance. IDO1, TDO, and IDO2 each have distinct characteristics regarding their structure, their enzymatic properties, and their pattern and regulation of expression in normal and cancerous tissues. In this Cancer Immunology at the Crossroads perspective, we discuss these characteristics in view of their presumed role in tumoral resistance against T-cell–mediated attack, and recent experimental observations.

Indoleamine 2,3-dioxygenase 1

IDO1 was identified many years ago as a tryptophan-degrading enzyme. It was thought that the function of IDO1 was primarily to...
Table 1. Main features of human IDO1, TDO, and IDO2

<table>
<thead>
<tr>
<th>Gene and transcript</th>
<th>IDO1</th>
<th>TDO2</th>
<th>IDO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symbol</td>
<td>IDO1</td>
<td>TDO2</td>
<td>IDO2</td>
</tr>
<tr>
<td>Chromosomal location</td>
<td>8p11.21</td>
<td>4q32.1</td>
<td>8p11.21</td>
</tr>
<tr>
<td>Transcription</td>
<td>Single transcript, 10 exons</td>
<td>Single transcript, 12 exons</td>
<td>1 transcript, 11 exons, encoding the full length protein, at least 4 alternative transcripts encoding shorter isoforms (35)</td>
</tr>
<tr>
<td>Regulation of gene expression</td>
<td>By IFNγ, other proinflammatory cytokines and DC maturation signals (e.g., LPS, PGE2; ref. 39)</td>
<td>Constitutive expression in liver cells</td>
<td>By IFNγ (controversial; ref. 40)</td>
</tr>
<tr>
<td>Relevant functional polymorphisms</td>
<td>None</td>
<td>None</td>
<td>R248W (reduced enzymatic activity) and Y359X (truncated, enzymatically inactive; ref. 35)</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein size</td>
<td>403 amino acids, 45 kDa</td>
<td>406 amino acids, 167 kDa (as tetramer)</td>
<td>420 amino acids, 47 kDa</td>
</tr>
<tr>
<td>Homology at the amino acid level</td>
<td>Very little homology with IDO1 and IDO2</td>
<td>Very little homology with IDO1 and IDO2</td>
<td>43% with IDO1</td>
</tr>
<tr>
<td>Conformation</td>
<td>Monomer</td>
<td>Homotetramer</td>
<td>Monomer</td>
</tr>
<tr>
<td>Heme-containing</td>
<td>Yes, active ferrous form (Fe2⁺), inactive ferric form (Fe3⁺)</td>
<td>Yes, 2 hemes per tetramer, active ferrous form (Fe2⁺), inactive ferric form (Fe3⁺)</td>
<td>Yes</td>
</tr>
<tr>
<td>Substrate specificity</td>
<td>L-tryptophan, D-tryptophan, 5-hydroxytryptophan, tryptamine, serotonin (41)</td>
<td>L-tryptophan</td>
<td>Unknown</td>
</tr>
<tr>
<td>L-tryptophan catalytic activity (Kcat)</td>
<td>Yes (20 μmol/L; ref. 42)</td>
<td>Yes (190 μmol/L; ref. 43)</td>
<td>Very weak or absent (6,800 μmol/L; ref. 42)</td>
</tr>
<tr>
<td>Enzyme inhibitors</td>
<td>1-methyl-L-tryptophan (39), INCB024360 (44), NLG919 (45), MMG358 (46)</td>
<td>N.B. 1-methyl-L-tryptophan (indoximod), which is currently being tested in clinical trials as an &quot;IDO-pathway inhibitor&quot; does not inhibit the enzymatic activity of IDO1 (37)</td>
<td>Not relevant</td>
</tr>
<tr>
<td>Expression in normal tissues</td>
<td>Epithelial cells in female genital mucosa, myeloid DCs in lymphoid organs and tissues, endothelial cells in placenta and lung (14)</td>
<td>Liver (very strongly; ref. 15)</td>
<td>Liver, tests, and thyroid (weakly; ref. 15)</td>
</tr>
<tr>
<td>Expression in tumors</td>
<td>At various levels and frequencies in most tumor types (14, 15)</td>
<td>Hepatocarcinoma (very strongly), many other tumor types (weakly; ref. 15)</td>
<td>Almost none (15)</td>
</tr>
<tr>
<td>Cell types expressing the protein</td>
<td>Tumor cells, myeloid DCs in lymphoid areas, endothelial cells (14)</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

IDO1, an IFNγ-inducible enzyme, is mainly expressed in inflammatory and in lymphoid tissues. IDO1 was also found to be expressed constitutively in the placenta, and its function during pregnancy was investigated. A landmark article demonstrated that IDO1 played a major role in protecting murine fetuses against rejection by the maternal immune system (9). The same team later showed that IDO1 was expressed in some dendritic cells (DC), and that this expression rendered the DCs tolerogenic and incapable of activating T lymphocytes (10, 11). Our group showed that IDO1 was expressed constitutively in human tumors and tumor cell lines, and that its forced expression in an immunogenic tumor cell line rendered tumors derived from this cell line resistant to immune rejection in a murine tumor model. Importantly, when the transplanted mice received 1-methyl-L-tryptophan, a tryptophan analogue that binds IDO1 and blocks its enzymatic activity, the IDO1-positive tumors grew less rapidly, thereby providing the proof of principle that the tumor-protective effect of IDO1 can be reversed by pharmacologic inhibition (12). Numerous subsequent studies have extended this observation to different mouse tumor models and to other inhibitory drugs and have confirmed the expression of IDO1 in various human tumor types, showing that IDO1 expression is often negatively correlated with disease outcome (13). As a gene induced by IFNγ, IDO1 is strongly expressed during the late phase of inflammatory reactions, in which its immunosuppressive role contributes to the physiologic retrocontrol of the immune response. Tumors often hijack this negative feedback mechanism for their own benefit.

The main known features of IDO1 are summarized in Table 1 (human) and Table 2 (mouse). Herein we briefly review those that are relevant to antitumor immunity, specifically pertaining to cellular and tissue expression of IDO1 in cancerous and non-cancerous tissues. We have recently profiled IDO1 expression in human tissues by immunohistochemistry using a highly specific murine monoclonal antibody that we have developed and validated (14). The observed expression pattern sheds new light and raises questions about prior knowledge of IDO1 expression. This updated expression profile will help in selecting patients who will...
van Baren and Van den Eynde

Table 2. Distinct features of mouse IDO1, TDO and IDO2

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Idol</th>
<th>Tdo2</th>
<th>Idol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal location</td>
<td>8A2</td>
<td>3E3</td>
<td>8A2</td>
</tr>
<tr>
<td>Transcription</td>
<td>1 transcript, 11 exons, encodes the full protein, another transcript with alternative first exon encodes a shorter isoform</td>
<td>Single transcript, 12 exons</td>
<td>Single transcript, 11 exons</td>
</tr>
<tr>
<td>Regulation of gene expression</td>
<td>Constitutive expression in liver cells; upregulation by L-tryptophan and glucocorticoids (in rats; ref. 27)</td>
<td>By IFNγ (49)</td>
<td>Normal embryologic development, no major phenotypic abnormalities, increased sensitivity to the induction of inflammatory and autoimmune reactions (29)</td>
</tr>
<tr>
<td>Gene knockout mice</td>
<td>Normal embryologic development, no major embryologic abnormalities</td>
<td>Increased L-tryptophan plasma levels, increased neurogenesis, reduced anxiety-related behavior, increased sensitivity to LPS-induced endotoxaemia (29, 28)</td>
<td>Normal embryologic development, no major phenotypic abnormalities, defects in IDO-mediated T-cell regulation and inflammatory responses (50, 56)</td>
</tr>
<tr>
<td>Protein</td>
<td>407 amino acids, 45 kDa</td>
<td>406 amino acids, 167 kDa (as tetramer)</td>
<td>405 amino acids, 45 kDa</td>
</tr>
<tr>
<td>l-tryptophan catalytic activity (Km)</td>
<td>Yes (28 μmol/L; ref. 51)</td>
<td>Yes (210 μmol/L; ref. 52)</td>
<td>Very weak or absent (12,000 μmol/L; ref. 51)</td>
</tr>
<tr>
<td>Protects tumors against immune rejection in vivo</td>
<td>Yes (12)</td>
<td>Yes (32)</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Tumor protection relieved by pharmacologic inhibition</td>
<td>Yes</td>
<td>Yes</td>
<td>Not investigated</td>
</tr>
<tr>
<td>Expression in normal tissues</td>
<td>Placenta, colon, heart, liver, epididymis, prostate, endometrium, kidney, lymph nodes, spleen, thymus, gut, pancreas, lung, muscle (18, 26)</td>
<td>Liver</td>
<td>Liver, kidney, epididymis, testis, brain, placenta (34, 53)</td>
</tr>
<tr>
<td>Cell types expressing the protein</td>
<td>Hepatocytes</td>
<td>Hepatocytes, bile ducts, kidney tubules, spermatozoa, neurons, DCs (53)</td>
<td>Interstitial antigen-presenting cells, plasmacytoid DCs, epithelial cells, smooth muscle cells, vascular cells, trophoblast cells</td>
</tr>
</tbody>
</table>

benefit from inhibitory therapy, and better anticipating possible side effects of this therapeutic approach.

IDO1 mRNA and protein are not expressed or are very weakly expressed in most healthy human tissues with the exception of the placenta, the mucosa of the female genital tract, the lungs, and the lymphoid organs (14, 15). In the placenta, IDO1 is found in endothelial cells of blood vessels present both at the fetal and the maternal interface (14, 16). This contrast with mouse Ido1, which appears to be mainly expressed in trophoblast cells (17, 18). The functional consequence of this differential cellular expression, particularly in terms of fetal resistance against maternal immune rejection, is not known. Placental IDO1 is enzymatically active, as indicated by the increased kynurenine-to-tryptophan gradient in umbilical cord blood (16). How fetal development copes with reduced tryptophan is another mystery. The other organ in which endothelial cells express IDO1 is the lung (14). Here, the function of IDO1 also is unclear. By reducing tryptophan concentration in the alveolar compartment, it could help protect against inhaled pathogens. It could also be involved in the development of lung vasculature, as Ido1 knockout mice have a reduced blood vessel density in the lungs (19). In the female genital tract, IDO1 is restricted to mucosal cells in the cervix, endometrium, and fallopian tube (14, 20). It has been proposed that IDO1 helps prevent genital infections. In lymphoid organs, IDO1 is detected in lymph nodes, tonsils, Peyers’s patches, the spleen, the lamina propria in the gut, and the medulla of the thymus (14). Its expression is restricted to scattered interstitial cells, often associated with lymphocyte clusters, consistent with DCs. Double-staining experiments confirmed that these IDO1+ cells express mature myeloid DC markers DC-LAMP and CD83, whereas markers of other DCs (including plasmacytoid DCs) or myeloid subtypes were negative (14). In vitro, IDO1 expression is induced in monocyte-derived DCs following stimulation with bacterial endotoxin or a cocktail of cytokines, but only at the late stages of DC maturation. In human lymph nodes, only about half of the mature DCs were found to be IDO1 positive. Together, these observations support the view that Ido1 acts as a late, negative feedback of T-cell activation in lymphoid organs, occurring after the initial immunostimulatory effect of newly activated DCs. In the mouse, the tolerogenic function of Ido1-expressing DCs has been studied in detail (21, 22). Interestingly, in plasmacytoid DCs, part of this Ido1-dependent immunosuppressive effect occurred independently of tryptophan-degrading catalytic activity, through a signaling mechanism triggered by TGFβ (22). It is unclear whether this finding applies to human plasmacytoid DCs, which do not express IDO1 (14).

IDO1 is expressed in several types of human malignancies. In most reports that made a link with disease outcome, IDO1 expression was associated with a worse prognosis (13). In a large series of human tumors, we recently confirmed IDO1 expression in more than half of the samples, and at much higher levels than in most noncancerous tissues. Importantly for data validation, the expression profile of the IDO1 protein matched remarkably with that of the IDO1 transcript in The Cancer Genome Atlas (TCGA) database, in terms of hierarchical proportion of positive samples per tumor type (14, 15). The level of expression, the proportion of positive samples, and the cellular types that expressed IDO1 were...
not equally spread among tumors of different histologic types. Some tumors, such as endometrial and cervical carcinomas, were very frequently positive, and therefore appear to be preferred targets for IDO1 blockade therapy, whereas others, such as glioblastomas, were most often negative. IDO1 protein was detected in tumor cells, in stromal interstitial cells associated with lymphocytes, or in endothelial cells. The contribution of this cellular specificity to immune resistance is not known.

In tumor cells, two patterns of expression were noticed. First, in some tumors, particularly in cervical carcinomas, the IDO1+ tumor cells were often concentrated in marginal areas in contact with a lymphocyte-rich stroma. This pattern is compatible with adaptive resistance, whereby activated T lymphocytes contacting the tumor would produce IFNγ, which in turn would induce IDO1 expression in neighboring tumor cells (Fig. 1). IDO1 would regulate in a feedback manner and block further T-cell activity. In other tumors, such as many endometrial carcinomas, the IDO1+ tumor cells were more diffusely distributed, without systematic stromal contacts and T-cell infiltrates, suggesting that IDO1 is constitutively expressed in these tumors (Fig. 1). This pattern, indicative of intrinsic resistance, is in line with the observation that some human tumor cell lines spontaneously express IDO1. Whether targeted IDO1 inhibition would have a different outcome between these two tumor expression patterns is not known.

The IDO1+ interstitial DC-like cells often present in the tumor stroma look very similar to the IDO1+ mature DCs that had been observed in lymphoid tissues and are assumed to be late activated DCs that have become tolerogenic. Besides IFNγ-induced IDO1 expression in tumor cells, this pattern is likely to represent another mechanism of adaptive immunity in tumors. It has been observed in most types of IDO1+ tumors, with particular abundance in colorectal carcinomas.

Finally, the last cellular pattern of expression of IDO1 in tumors is the most intriguing. IDO1+ endothelial cells in small venules and capillaries can be observed in many different types of tumors, but they are particularly abundant in renal cell carcinoma, in which they are often present in the absence of any stromal or tumoral expression of IDO1, suggesting a distinct mechanism of induction. As in placenta and lung, the functional consequence of this endothelial IDO1 expression in tumors is unknown. In mice, Ido1-expressing endothelial cells were shown to cause vasodilation and decreased blood pressure in inflammatory conditions (23). Of note, in one published series of kidney tumors, vascular IDO1 expression was correlated with a relatively better prognosis (24), as opposed to what has been reported in other IDO1+ malignancies.

Tumor-draining lymph nodes (TDLN) have been the subject of particular attention by tumor immunologists, because it is thought that they play an important role in the induction of adaptive antitumor immune responses. It has been hypothesized that, given its mode of action, IDO1 may be involved in immunosuppressive activities in TDLNs. Several reports in mouse models and in human TDLN series have shown an increased proportion of IDO1+ cells in TDLNs as compared with that in normal lymph nodes (11, 25). However, our immunohistochemical analysis of 30 TDLNs from melanoma and breast cancer patients with our validated anti-IDO1 antibody failed to confirm this difference (14). We therefore believe that, at least in humans, the main immunosuppressive activity mediated by IDO1 is located in the tumor microenvironment.

There are marked differences between the pattern of expression of human IDO1 and its mouse homolog (see Table 1 and Table 2). Murine Ido1 appears to be more widely expressed in normal tissues, as it was observed in the colon, heart, liver, prostate, endometrium, kidney, lymph nodes, spleen, thymus, gut, pancreas, lung, and muscle, and is particularly abundant in the epididymis, which in humans does not express IDO1 (14, 18, 26). In addition, endothelial IDO1 expression in human placenta and lung has no murine counterpart. Finally, no murine tumor cell line has been reported to express IDO1 constitutively. As a consequence, caution should be taken when extrapolating on-target toxicity as well as efficacy profiles of IDO1 inhibitors from mice to humans.

**Tryptophan 2,3-dioxygenase**

The main characteristics of human and mouse TDO are shown in Table 1 (human) and Table 2 (mouse). TDO is a liver enzyme that is responsible for maintaining homeostatic tryptophan levels in the blood. In the rat liver, TDO is expressed in hepatocytes, and its enzymatic activity is enhanced by increased tryptophan concentration, consistent with its physiologic function, as well as by glucocorticoids (27). In the mouse, deletion of the Tdo2 gene results in much higher concentrations of blood t-tryptophan than in wild-type mice. This is accompanied by neurologic and behavioral perturbations, in line with the fact that tryptophan is a precursor of the neurotransmitter serotonin, whose concentration is also increased in the blood and brain of Tdo2−/− mice (28). Apart from these abnormalities, Tdo2−/− mice do not display major developmental or phenotypic alterations. Interestingly, these mice, as well as wild-type mice treated with the Tdo2 inhibitor 680C91, show increased sensitivity to endotoxin-induced shock, indicating that the tryptophan-degrading activity of Tdo is involved in dampening inflammatory reactions (29).

It is tempting to make a causal link between this anti-inflammatory effect of TDO and the fact that the liver is often regarded as an immunoprivileged organ. Indeed, donor-recipient HLA matching is less important in liver transplantation, which also requires less immunosuppressive drugs to prevent allogeneic rejection as compared with other allografted organs (30).

As compared with IDO1, TDO has a lower affinity for t-tryptophan. Its Km of 190 μmol/L is consistent with its homeostatic function, which is to maintain the physiologic t-tryptophan concentration to around 80 μmol/L. It is insufficient to deplete this amino acid to levels around or below 1 μmol/L, which causes T-cell inhibition in vitro (2). Thus, it is likely that the anti-inflammatory and tumor-protective effects of TDO are mediated by the production of tryptophan catabolites such as kynurenine, rather than by tryptophan depletion.

Apart from the liver, TDO is not expressed or is very weakly expressed in normal tissues, with the possible exception of the placenta (31). In human tumors, the TDO2 gene is highly expressed in hepatocarcinomas, consistent with its liver-specific expression (15). In many other malignancies, it is more weakly expressed, albeit at higher levels than in the corresponding normal tissues (15, 32). The nature of TDO-expressing cells in tumors is not known. We and others have detected TDO expression in some human tumor cell lines, suggesting that tumor cells may constitutively express this enzyme (32, 33). Identification of the cellular source of TDO will be important to understanding whether and how TDO affects tumor development.
Figure 1.
A scenario for the role of IDO1 in tumoral immune resistance. In the top portion of the figure, IDO1 is not expressed in the tumor microenvironment. Appropriate T-cell stimulation by mature DCs (mDC) results in cytolytic T cells (CTL) acquiring the full range of effector mechanisms, including local proliferation, migration into the tumor, cytokine secretion, and lysis of tumor cells. In the middle portion, the same scenario initially occurs, but, in a second step, the IFNγ produced by activated T cells induces IDO1 expression in nearby cells, including tumor cells. IDO1 is also acquired by DCs upon further maturation. By depleting tryptophan (Trp) and producing kynurenine (Kyn), IDO1 represses further T-cell activation, either directly or through the induction of Treg. In the bottom portion, IDO1 is constitutively expressed by tumor cells, with similar T-cell suppression as shown above. IDO1 can also be expressed by endothelial cells, with unknown consequences on antitumor immune responses. iDC, immature dendritic cells.
The proof of principle that TDO can protect tumors against immune rejection has been established in two experimental models. In an in vitro human tumor model, cultured glioblastoma cells were found to express TDO, which produced kynurenine upon tryptophan degradation. This kynurenine activated the AhR pathway in an autocrine and paracrine fashion, resulting in enhanced tumor cell survival and motility, as well as a reduced capacity to be recognized and killed by immune cells (33). In a second model, a first group of mice immunized against cancer–germline antigen P1A and then challenged with the P1A-expressing P815 mastocytoma cell line rejected the transplanted tumor, whereas a second group of mice challenged similarly with a TDO-expressing P815 cell line gave rise to growing tumors. Administration of the TDO inhibitor LM10 to a third group of mice restored their capacity to reject the TDO-expressing tumor, indicating that the tumor-protective effect of TDO can be counteracted pharmacologically (32).

**Indoleamine 2,3-dioxygenase 2**

Much less is known about IDO2 than about IDO1 and TDO (Tables 1 and 2). IDO2 was identified a few years ago simultaneously by several groups as an IDO1 homolog, based on similarities in nucleic acid sequence between the corresponding genes (34, 35). The human IDO1 and IDO2 genes are located next to each other on chromosome 8p11, suggesting that they resulted from a gene duplication event during evolution.

The function of the IDO2 protein is not known. Because of its structural analogy with IDO1, it has been hypothesized that IDO2 could have similar functions, including tryptophan catabolism, with impaired T-cell responses and tumoral immune resistance as possible consequences. However, convincing experimental data that support this view are missing. Ido2 knockout mice are viable and devoid of severe phenotypic deviations. Subtle functional assays are required to observe differences in terms of inflammatory or immune responses, as compared with wild-type mice. Ido2−/− mice show less severe inflammation of joints in a model of rheumatoid arthritis (36). These mice also have less intense hypersensitivity reactions and a reduced capacity to produce inflammatory cytokines and Tregs. Except for the latter, these observations point toward a proinflammatory rather than an immunosuppressive role for Ido2. In addition, arguments in favor of an L-tryptophan-degrading capacity of IDO2 are scarce. Even though IDO2 was shown to degrade L-tryptophan in vitro, it is very unlikely that this reaction takes place in vivo, as the corresponding Ki (> 4,000 μmol/L) is much higher than that of IDO1 and TDO (20 and 190 μmol/L, respectively) and is far above the physiologic L-tryptophan concentrations (around 80 μmol/L in the serum).

The pattern of expression of the human IDO2 mRNA and protein is still a matter of debate. In normal tissues, IDO2 gene expression has been reported in the liver, small intestine, spleen, placenta, thymus, lung, brain, kidney, and colon (35); in tumor samples, IDO2 gene expression was found in gastric, colon, and renal carcinoma, as well as in several tumor cell lines, after they were incubated with IFNγ (37, 38). However, the RT-PCR conditions chosen to assess this expression did not always take into consideration that the IDO2 gene encodes several alternative transcripts, not all of which give a full, presumably functional protein. In addition, two frequent SNPs in the IDO2 gene result in a truncated or altered protein. Ido2 protein expression was detected in a small series of pancreatic tumors, by immunohistochemistry with an unvalidated polyclonal antibody (38). From the publicly available GTex and TCGA RNA-Seq databases, we observed that the IDO2 transcripts were absent or very weakly expressed in normal human tissues, except in the liver, thyroid and testis, and in the vast majority of more than 8,000 human tumor samples, with less than 1% showing relevant IDO2 gene expression (15).

Taken together, these observations support an argument against the hypothesis that IDO2 is a tryptophan-degrading enzyme that contributes to tumoral resistance against immune rejection.

**Conclusions**

Recent experimental observations help to better understand the respective role of IDO1, TDO, and IDO2 in tumor resistance to immune-mediated rejection. IDO1 is frequently expressed in many, but not all, human cancers. It contributes to tumoral resistance by at least two mechanisms, tryptophan depletion and production of tryptophan catabolites in the tumor environment, both of which interfere with T-cell effector activities. The proof of principle for the antitumor effect of its pharmacologic inhibition has been demonstrated in mouse models. The predicted risk of toxicity associated with this approach should take into account the expression profile and function of IDO1 in normal tissues, particularly in the lung vasculature. TDO is highly expressed in the liver and hepatocarcinomas, weakly in most other tumors, and not at all in other normal tissues. It may contribute to tumoral immune resistance by producing tryptophan catabolites locally, although its precise cellular expression in tumors and interaction with immune cells requires further investigation. Like IDO1, TDO can be blocked by inhibitory drugs. IDO2 is unlikely to play a significant role in tumoral resistance, because it has no relevant enzymatic activity and is not expressed in human tumors.

**Disclosure of Potential Conflicts of Interest**

B.J. Van Den Eynde serves as chairman of the scientific committee, has ownership interest (including patents), and is a consultant/advisory board member for iTeos Therapeutics. No potential conflicts of interest were disclosed by the other author.

**Authors’ Contributions**

Conception and design: N. van Baren, B. J. Van den Eynde

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. van Baren, B. J. Van den Eynde

Writing, review, and/or revision of the manuscript: N. van Baren, B. J. Van den Eynde

Study supervision: B. J. Van den Eynde

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