El feto expresa durante el embarazo antígenos procedentes del padre que no provocan el rechazo como cualquier otro injerto semi-allogénico. Entre los sistemas implicados en la inducción de la tolerancia materno-fetal, se encuentra la enzima indolamina 2,3-dioxygenasa (IDO) que inicia la degradación del triptófano. Al consumir el triptófano provoca una inhibición de la respuesta de las células T que no pueden superar un punto de control de la fase G1 del ciclo celular. Numerosos tumores pueden expresar la enzima IDO de forma ectópica con lo que evitan el ataque inmune. Dendritic cells can also express IDO and thus, induce a tolerogenic response. The knowledge of tolerogenic capacity of tumours helps not only in the understanding of cancer growth but also in developing new therapeutic approaches.

PALABRAS CLAVE: Tolerancia/ Indolamina/ Inmunosupresión/ Triptófano/ 1-metil-triptófano.

ABSTRACT
During pregnancy, the foetus expresses paternal antigens that do not provoke rejection by the mother, as would any other semi-allogeneic graft. In this tolerance, several systems are implicated, including the enzyme indolamine 2,3-dioxygenase (IDO), which catalyses the first step in the tryptophan degradation pathway. IDO-mediated tryptophan depletion provokes an inhibition of T-cell responses, which cannot get through a checkpoint in the G1 phase of cell cycle. Several tumours can express IDO ectopically, enabling them to evade the immune attack. The knowledge of tolerogenic capacity of tumours helps not only in the understanding of cancer growth but also in developing new therapeutic approaches.

KEY WORDS: Tolerance/ Indolamine/ Immunosuppressive/ Tryptophan/ 1-methyl-tryptophan.
INTRODUCTION

Tumour cells should evade the selective immune pressure that limits their growth. Multiple alterations in oncogenes and tumour suppressor genes and the lack of sensitivity to signals of growth-inhibition and apoptosis allow these cells to proliferate. Tumour cells also release factors to the medium that promote the growth of blood vessels that provides enough nutrients and oxygen for the expanding malignant cell population. In addition, these malignant cells should evade the immune system, which can potentially kill them.(1)

The idea of tumour evasion of the immune system implies that the tumour can be immunogenic and that it can be destroyed by an immune response. Most of the patients suffering from cancer are fully immunocompetent at the moment of presentation. However, tumour cells transplanted to express alloantigens can avoid the T cell attack that is produced by normal tissues expressing the same antigen. Similarly, cross-presentation of tumour antigens by antigen presenting cells induces tolerance(2). This situation resembles the tolerogenic situation during pregnancy where the foetal genes express antigens foreign to the mother, which are tolerated. In fact, mammals owe their existence to a tolerogenic situation that prevents foetal rejection by the mother. Munn et al. have recently shown the important role of indoleamine 2,3-dioxygenase (IDO) in regulating maternal T-cell immunity during pregnancy. Application of the IDO inhibitor 1-methyl-tryptophan provoked high rates of abortions in a mouse model(3). Furthermore, Uyttenhove et al.(4) have shown that IDO is implicated in the tolerogenic mechanism in cancer. In this review we study the immunosuppressive action of IDO and its implications in cancer biology.

TRYPTOPHAN METABOLISM

Tryptophan is an essential amino acid, and it is the rarest of the 20 amino acids found in proteins. Tryptophan can be hydroxylated to be converted into the neurotransmitter serotonin or catabolized through the kynurenine pathway to produce adenosine nucleotides (Fig. 1)(5). Two different enzymes catalyse the oxidative cleavage of the indole ring of tryptophan, one is tryptophan 2,3-dioxygenase (TDO, EC 1.13.11.11) and the other is indoleamine 2,3-dioxygenase (IDO, EC 1.13.11.17)(5,6). TDO is a constitutive hepatic enzyme.
of 320 kDa with a low affinity for tryptophan, and thus, hepatic metabolism of this amino acid is more active when its levels are high, such as after a meal. IDO is a 45 kDa enzyme with a high affinity for tryptophan, so once expressed, it eagerly catabolizes this amino acid. While TDO is not induced or regulated by immunological signals, IDO expression is inducible in antigen presenting cells and its expression is tightly regulated(17).

The IDO gene, which is a very ancient gene that has been conserved up to 600 million years, codifies a heme-containing protein and is evolutionarily adapted to various applications, acting as a tryptophan degradation enzyme or as an oxygen-transporter protein(8). IDO has ligand binding properties similar to myoglobin and its activity can be inhibited by heme ligands, such as nitric oxide(9).

**CELLULAR INDUCTION OF IDO**

IDO was initially found in the rabbit intestine(10), and in humans it is constitutively expressed in some tissues such as placenta, intestine, spleen thymus and lymph nodes(11). Most researches have focused on the induction of IDO in antigen presenting cells, particularly in macrophages and dendritic cells, where IFN-γ is a potent inducer(12). The inflammatory cytokines IL-1 and TNF-α as well as LPS act synergistically with IFN-γ to induce IDO(13). It has been shown that LPS can also induce IDO by a TNF-α-dependent mechanism(14). Other cytokines also modulate IDO expression and, for example, TGF-β inhibits IFN-γ-induced IDO expression in fibroblasts(15). This indicates that IDO expression is tightly regulated in different cell types.

There are two interferon stimulated response elements (ISRE), an interferon-γ activating site, and a MCH-II X,Y box-like motif in the promoter of the *Indo* gene that confers responsiveness to interferon via the JAK-STAT signalling pathway(16). Both type I IFN and IFN-γ induce IDO in monocytes, but the IFN-γ is the most potent because the ISRE region is less stimulated by type I IFN(17). However, this response can be different in certain cells and, for example, a subset of dendritic cells characterized by expressing CD19 possess IDO activity upon stimulation with type I IFN, but not with type II IFN(18).

Professional antigen presenting cells (monocytes, macrophages and dendritic cells) express IDO after exposure to IFN-γ(12, 18, 20), and maturation of monocytes to macrophages increases the capacity to induce IDO upon stimulation with IFN-γ. Dendritic cells comprise a heterogeneous population, where the culture conditions to differentiate and mature are very important in obtaining IDO mediated suppression, and not all the types of dendritic cells have IDO activity(20, 21). Even though IDO is one of the genes highly induced during monocyte-derived dendritic cell maturation, full IDO activation needs further stimulation (unpublished results, A.G.)21,22. Grohman et al. showed that even though mouse dendritic cells express IDO(20, 26), after IFN-γ treatment only the CD8+ subset has IDO activity with the capacity to suppress T cells(23). This implies a post-translational regulation, in which nitric oxide might be implicated, among other substances(27). Similarly, in humans there is a non-adherent subset of plasmacytoid dendritic cells expressing CD123 and CCR6 that possess IDO activity and can suppress T cell proliferation. However, other authors have not found in these non-adherent CD123+ cells the capacity to suppress mixed lymphocyte reactions(22, 27). There is controversy on this issue since the existence of a dendritic cell expressing IDO in basal conditions would give rise to immune tolerance. More so if we take into account that, apparently, such putative IDO+ DC population tends to be differentiated from monocytes in serum free medium. However, in our experience at generating clinical grade DC we cannot detect basal IDO expression, suggesting that, at least in our hands, the therapeutic DC are not expected to be tolerogenic on the basis of IDO activity.

Grohman et al. reported that the tolerogenic property of cytolytic T lymphocyte associated antigen 4 (CTLA4) is due to IDO activation in dendritic cells(28). Binding of CTLA4-immunoglobulin fusion to the B7-1 or B7-2 receptor in dendritic cells provokes the autocrine production of IFN-γ and, as a result, IDO induction. This in turn causes tryptophan depletion and immune suppression. The IDO inhibitor 1-methyl-tryptophan can abrogate this effect. More recently, these authors reported that CD200R ligation by soluble CD200-Ig fusion protein induces IDO in plasmacytoid dendritic cells through the type I IFN signalling pathway(29). We have shown that 1-methyl-tryptophan increases the expression of the non classical MHC class I antigen HLA-G in antigen presenting cells(30), causing decreased NK cytotoxicity and T cell responses(31).

Nitric oxide can inactive the activator of transcription 1 (STAT1), which interferes with the induction of IDO by IFN-γ and can impede the tolerance to self-antigens(32). The pathological implications were demonstrated in predisposed non-obese diabetic (NOD) mice, where the high expression of peroxynitrites in dendritic cells impairs the IFN-γ-STAT1 pathway-mediated induction of IDO(32).

**IDO AS AN IMMUNOSUPPRESSIVE ENZYME**

IDO expression by macrophages and dendritic cells inhibits T-cell proliferation in mixed lymphocyte reactions(33).
The suppression of allogeneic responses by IDO is not related to other tolerogenic molecules such as HLA-G(34). Even though some metabolites of the kynurenine pathway are biologically active causing apoptosis in lymphocytes(35), the effect of IDO in suppression is mainly due to tryptophan depletion, and not to those metabolites. However, cellular anergy caused by IDO can not be restored by repleting again with tryptophan(36), but only if a second round of T cell receptor signalling is provided in the presence of tryptophan. T cells are unable to proliferate in the absence of tryptophan due to a tryptophan-sensitive check-point in the middle of the G1 phase of the cell cycle(36). Recently, the GCN2 kinase pathway has been implicated as the mediator of IDO suppression in T cells(37). GCN2 is a stress-response kinase that is activated by elevations in uncharged transfer RNA (tRNA) because GCN2 contains a regulatory domain that binds to the uncharged form of tRNA. Amino acid deficiency causes a rise in uncharged tRNA, which activates the GCN2 kinase and initiates a downstream signalling pathway(38), that triggers cell-cycle arrest or apoptosis(39).

**IDO EXPRESSION BY TUMOUR CELLS**

It is known since the middle of last century that in malignancy there is an increase of tryptophan metabolism(40). An initial hypothesis considered that the capacity of IDO to deplete tryptophan in the medium could be an IFN-induced mechanism that inhibits tumour proliferation(41). However, more recently Munn et al.(42) have suggested that this inhibitory effect can be compensated by tumour cells suppressing the immune response in a similar way as the mechanism that avoids foetal rejection. In support of this theory, it was reported that 1-methyl-tryptophan, administered *in vivo*, caused a growth delay of the Lewis lung carcinoma tumour in syngeneic mice(43). Obviously, to get this advantage, tumour cells should be less sensitive
to tryptophan deprivation than T lymphocytes. As IDO is an intracellular enzyme, an efficient transport is essential to allow tryptophan to enter into the cell\(^{44, 45}\). This has been demonstrated in some tumours, such as the MDA-MB-231 human breast cancer cell line\(^{46}\). Alterations or blockade of tryptophan transporter affects its intracellular degradation through IDO activity, even without a direct effect in enzymatic function. Interestingly, 1-methyl-tryptophan not only inhibited IDO activity, but also stimulated tryptophan efflux from these cells\(^{44, 45}\).

The expression of IDO has been shown both in human tumour cell lines\(^{44, 46}\) and in primary tumours\(^{45}\) even though the percentage of IDO-positive specimens and IDO-positive tumour cells varied greatly among the different types of tumours. Uyttenhove demonstrated that IDO expression was nearly 100% positive in prostate, colorectal, pancreatic and cervical primary human tumours, but almost absent in choriocarcinomas and testicular seminomas\(^{46}\). The reason for the upregulation of IDO in tumours seems to emerge from the study of the suppression of the Bin1-Amphiphysin2 gene in tumour cells\(^{47}\). This is a cancer suppressor gene that is frequently lost in advanced melanoma, breast, prostate, and colon cancer. Muller et al. observed that when \textit{in vitro} transformed Bin1-null and Bin1-expressing primary mouse embryo keratinocytes were grafted into syngeneic animals, the Bin1-null cells formed large tumours, whereas the Bin1-expressing cells formed only indolent nodules. However, Bin1-expressing cells produced rapidly growing tumours when introduced into either athymic nude mice or syngeneic mice depleted of CD4\(^+\)/CD8\(^+\) T cells. For this reason, the difference in the immune behaviour provoked by Bin1-null or Bin1-expressing tumours is the result of different IDO expression. Bin-adapter proteins can affect the traffic of signalling proteins such as STAT or NF-\(\kappa\)B directed to the nucleus\(^{47, 48}\). Deletion of Bin1 results in overexpression of IDO (Fig. 1), and addition of the IDO inhibitor 1-methyl-tryptophan suppresses the outgrowth of Bin1-null cells in syngeneic mice\(^{47}\). Transfection of the \textit{Indo} gene into tumour cell lines provides them the capacity to inhibit T cell responses\(^{49}\). Uyttenhove et al.\(^{46}\) also demonstrated that transfecting the immunogenic mouse tumour cell line P815 with IDO made it resistant to rejection even in pre-immunized mice. This effect was accompanied by a lack of accumulation of specific T cells at the tumour site, and such a state can be partially reverted by systemic treatment of mice with the inhibitor of IDO 1-methyl-tryptophan, which delays tumour outgrowth. Interestingly, the effect that is observed with this IDO inhibitor was dependent on the presence of functional T cells.

**IDO EXPRESSION BY ANTIGEN PRESENTING CELLS IN TUMOURS**

Apart from the expression of IDO by tumour cells themselves, the recruitment of APC expressing IDO can be another mechanism by which tumours evade the immune response (Fig. 2). This confers another advantage to the tumour in order to escape the immune response. This is because although IDO expression by tumour cells could suppress local activation of T cells, there is still capacity to develop an immune response outside the tumour itself\(^{50}\). Up to now there is no evidence that IDO expression by tumour cells can induce systemic tolerance, although it has been suggested that IDO expressing tumour cells could induce regulatory T cells\(^{51}\). Antigen presentation by dendritic cells seems to be a crucial event for both CD8\(^+\) and CD4\(^+\) cell responses\(^{52}\). Therefore, the expression of IDO by these dendritic cells could be a really efficient mechanism to overcome the adaptive immunity and enhance tumour survival\(^{53}\). This could be the case of human melanoma, where the presence of APC expressing IDO in tumour draining lymph nodes has been shown\(^{54}\), even in the absence of tumour cells. In tumour-draining lymph nodes, there is a subpopulation of B220\(^+\) plasmacytoid dendritic cells that can be defined by the B-cell marker CD19, which is responsible for the IDO-mediated suppression\(^{53}\). The presence of IDO positive infiltrating cells has been demonstrated immunohistochemically in some tumours such as hepatocarcinoma\(^{54}\), or breast, colon\(^{30}\) and small cell lung carcinomas\(^{55}\). In the latter type of tumour the IDO\(^+\) cells were identified as eosinophil granulocytes. In a study with 40 melanoma patients without detectable lymph node metastases at the time of diagnosis, Munn et al.\(^{56}\) showed that the presence of plasmacytoid dendritic IDO\(^+\) cells in the sentinel lymph node at the time of initial diagnosis correlated with a significantly worse long-term outcome. The accumulation of IDO-expressing cells might thus constitute an early and adverse prognostic factor in addition to the presence of tumour cells in the sentinel node.

Measurement of the relation of kynurenine to tryptophan in serum can be a method to identify the activation of IDO-dependent pathway in cancer patients\(^{56}\). A study with patients suffering from gynecological cancer showed that there was a decrease of tryptophan but not kynurenine, compared to healthy controls\(^{57}\). However, there are not clear results in this sense, as IDO expression is not a generalized event and tryptophan depletion takes place in a very local milieu. It should be noted that there are other sources of kynurenine outside the tumour itself (unpublished results, A.G.).
A survey of the literature evidences an apparent discrepancy, since different studies demonstrate that IDO favours tumour expansion\(^{4}\), while other show that IDO causes tumour inhibition\(^{32}\). However, one should consider that there is a complex interaction between the darwinian-selected tumour and the immune system, in a process called cancer immunoediting. According to this concept, selective pressure causes an initial cancer cell elimination, which induces new tumour cell phenotypes that can evade tumour surveillance\(^{58}\). IFN-\(\gamma\) has been implicated in immunoediting with solid genetic evidence\(^{41}\). It is thus conceivable that IDO can participate in these processes even if initially noxious to tumour cells, because of becoming useful to avoid the immune attack at later stages\(^{59}\).

**EFFECT OF IMMUNOTHERAPY ON IDO EXPRESSION**

As indicated above, both tumours and APC can express IDO, either naturally or after cytokine stimulation, which can drive to immune suppression. For this reason, therapeutic approaches to treat cancer patients should overcome the tolerizing tools employed by tumour cells. Interestingly, a clear increase of IDO in monocytes, accompanied with approaches to treat cancer patients should overcome the tolerizing tools employed by tumour cells. This situation can be common to different cancer cells types independently of the mutations acquired to have a tumour behaviour\(^{60}\). The knowledge of how tumour cells actively and passively evade the immune system will provide in the next future a new, and probably safer, therapeutic approach to treat cancer patients\(^{47}\).

**REFERENCES**


