



# Indoleamine 2,3-dioxygenase and tumor-induced tolerance

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**Tumors arise from normal cells of the body through genetic mutation. Although such genetic mutation often leads to the expression of abnormal antigens, the immune system fails to respond effectively to these antigens; that is, it is tolerant of these antigens. This acquired state of tolerance must be overcome for cancer immunotherapy to succeed. Indoleamine 2,3-dioxygenase (IDO) is one molecular mechanism that contributes to tumor-induced tolerance. IDO helps create a tolerogenic milieu in the tumor and the tumor-draining lymph nodes, both by direct suppression of T cells and enhancement of local Treg-mediated immunosuppression. It can also function as an antagonist to other activators of antitumor immunity. Therefore, strategies to block IDO might enhance the effectiveness of tumor immunotherapy.**

## Introduction

It is now clear that tumors express many antigens to which the immune system can respond (1–3). Tumor-bearing hosts also possess high-avidity T cells that are specific to these antigens (4). Despite this, however, the host fails to reject the tumor. This occurs because each tumor evolves ways to escape immune surveillance (1). The net result of these escape mechanisms is that the host immune system fails to respond to tumor-associated antigens in a way that causes rejection. In immunologic terms, the host is tolerant of the tumor.

Immunologic tolerance to tumors is not simply a passive process. Although in some cases the immune system seems unaware of the presence of tumor antigens (5, 6), in many cases the immune system is aware of these antigens but is somehow actively rendered tolerant of them. This has been most clearly shown in experiments in which tumors were engineered to express a strongly immunogenic foreign antigen; typically, in such cases, the tumors are not rejected but simply create tolerance of the new antigen (7, 8). Perhaps even more daunting, once this acquired tolerance has been established, immunization with tumor-associated antigens can merely serve to intensify antigen-specific immunosuppression (9, 10). Therefore, tumor-induced tolerance is an acquired state, reflecting active mechanisms of tolerance induction, and it can represent an enormous impediment to the effectiveness of immunotherapeutic approaches to treating cancer.

The molecular mechanisms underlying tumor-induced tolerance are currently the subject of active research, and a number of contributing mechanisms have been identified (11). In this Review, we consider the role of the enzyme indoleamine 2,3-dioxygenase (IDO) as an immunosuppressive and tolerogenic mechanism in cancer. IDO catalyzes the rate-limiting step of tryptophan degradation along the kynurenine pathway (12), and both the reduction in local tryptophan concentration and the production of immunomodulatory tryptophan metabolites contribute to the immunosuppressive

effects of IDO (Figure 1). Studies of serum biomarkers (tryptophan and kynurenine concentration) indicate that IDO is chronically activated in many patients with cancer (13) and that IDO activation correlates with more extensive disease (14). However, the mechanistic role of IDO in tumor immunology and its links with other tolerogenic mechanisms have only recently begun to be elucidated.

## IDO as a mechanism of acquired tolerance

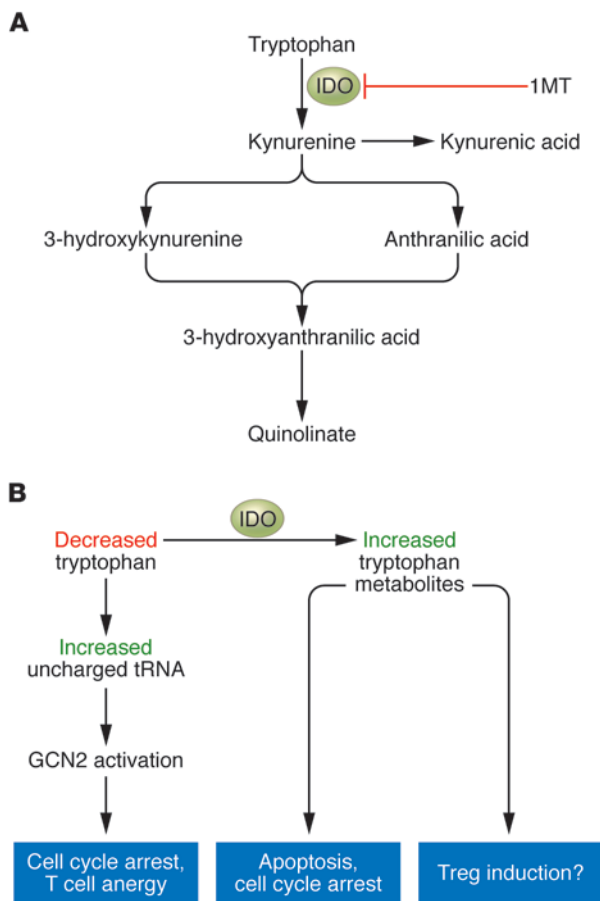
Acquired tolerance of tumors is undesirable and harmful to the host, but acquired tolerance of other classes of antigens (such as fetal antigens and harmless foreign antigens at mucosal surfaces) is beneficial and necessary (15). IDO has been implicated as a normal, endogenous mechanism of peripheral tolerance and immunosuppression in a number of settings. It was originally described as contributing to maternal tolerance toward the fetus, as shown by the fact that mice treated early in pregnancy with 1-methyl-tryptophan (1MT), which is an inhibitor of IDO, underwent immune-mediated rejection of allogeneic concepti (16–18). The fetus represents an example of a set of foreign antigens to which the immune system is forced to remain tolerant and therefore is conceptually analogous to tumors in this regard.

More generally, it has now been observed that mice treated with IDO inhibitors become refractory to acquired tolerance induction in a number of settings. For example, blocking IDO with 1MT prevents the induction of tolerance to islet cell allografts by the fusion protein cytotoxic T lymphocyte-associated antigen 4-Ig (CTLA4-Ig) (19, 20), and 1MT blocks the tolerance that normally occurs when foreign antigens are introduced into the anterior chamber of the eye (an immunologically privileged site) (21). In settings where self tolerance has already been disrupted (for example, autoimmune disorders), pharmacologic inhibition of IDO causes marked exacerbation of inflammation and worsened symptoms of disease, as shown in models as diverse as inflammatory bowel disease (22), EAE (23), and experimental allergic asthma (24). Conversely, ectopic overexpression of IDO by gene transfer results in suppression of immune responses. For example, MHC-mismatched lung allografts transfected with IDO are protected from rejection without further immunosuppression (25, 26), and similar results have been reported in corneal transplants (27). Therefore, in vivo, IDO functions as a molecular mechanism contributing to acquired peripheral tolerance.

**Nonstandard abbreviations used:** CpG ODN, oligodeoxynucleotide(s) containing one or more unmethylated CpG dinucleotides; CTLA4, cytotoxic T lymphocyte-associated antigen 4; GCN2, general control nonderepressible 2; IDO, indoleamine 2,3-dioxygenase; 1MT, 1-methyl-tryptophan.

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**Figure 1**

Molecular mechanisms of IDO-induced immunosuppression. **(A)** IDO catalyzes the initial and rate-limiting step in the degradation of tryptophan along the kynurenine pathway. Tryptophan metabolites have been shown to have immunomodulatory activity, alone or in combination with the GCN2 signaling pathway. **(B)** IDO enzymatic activity results in the local depletion of tryptophan and a local increase in the concentration of downstream metabolites. The decrease in tryptophan can cause a rise in the level of uncharged transfer RNA (tRNA) in neighboring T cells, resulting in activation of the amino acid–sensitive GCN2 stress-kinase pathway. In turn, GCN2 signaling can cause cell cycle arrest and energy induction in responding T cells. The local increase in tryptophan metabolites can cause cell cycle arrest, apoptosis, and (in conjunction with GCN2 signaling) differentiation of new Tregs from uncommitted CD4<sup>+</sup> T cells.

functional IDO enzymatic activity is tightly regulated by specific maturation and activation signals (31–36).

Conceptually, this ability to upregulate or downregulate IDO in response to external stimuli seems logical, given the need for APCs to sometimes present antigens in an activating fashion and sometimes in a tolerizing fashion, depending on the context. However, this plasticity somewhat complicates the study of IDO-competent APCs in tumor-bearing hosts since both the relevant APC subsets and the specific signals that turn IDO expression on or off in these cells must be identified. It is even less well understood how IDO expression might be regulated in the tumor cells themselves (for example, in response to local inflammatory mediators such as IFN- $\gamma$ ; ref. 37) and whether IDO expressed by tumor cells has the same properties as that expressed by DCs and macrophages.

**Molecular mechanisms of IDO-mediated immune suppression**

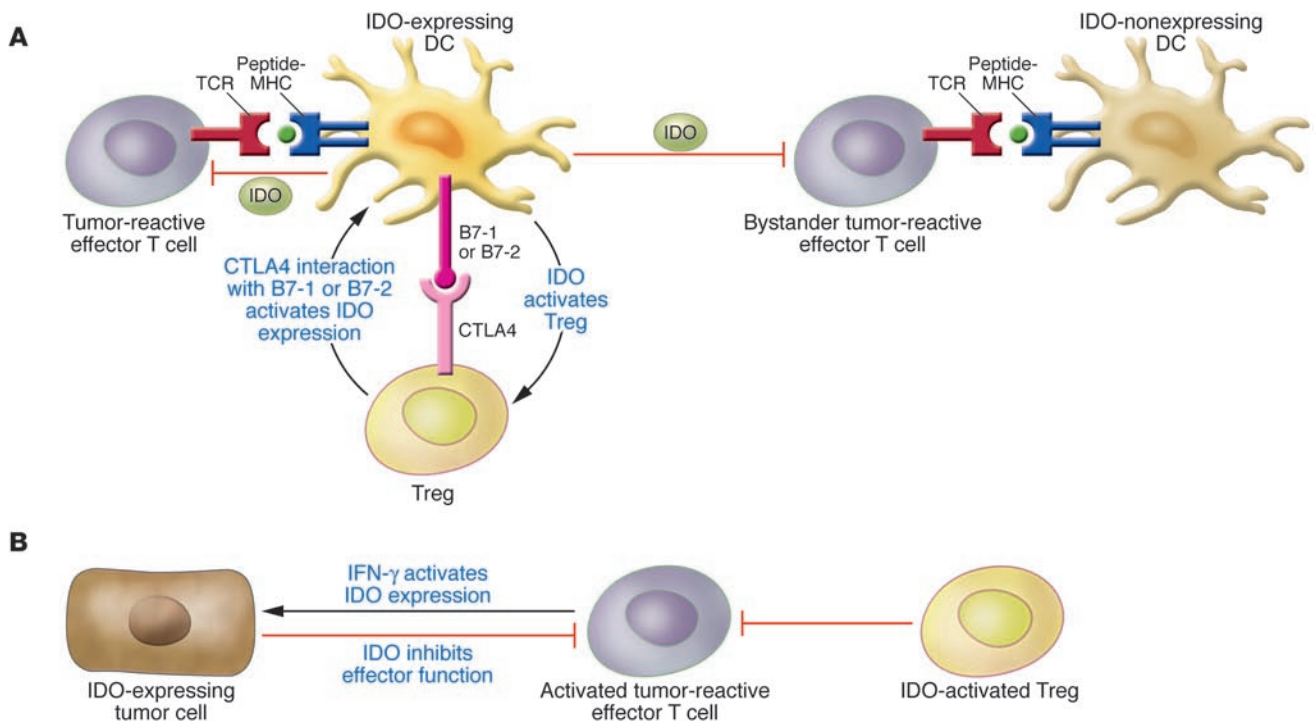
IDO initiates the degradation of tryptophan along the kynurenine pathway (12). IDO and the downstream enzymes in this pathway produce a series of immunosuppressive tryptophan metabolites (Figure 1A). Some of these metabolites suppress T cell proliferation in vitro or cause T cell apoptosis (38–41), and some can affect NK cell function (42). Even if IDO itself is not present, enzymes downstream of IDO in the kynurenine tryptophan degradation pathway can create immunosuppressive metabolites if supplied with kynurenine (43). In addition, in vivo, rats treated with a mixture of tryptophan metabolites showed prolonged graft survival (44); and the drug *N*-[3',4'-dimethoxycinnamoyl] anthranilic acid (Tranilast), a synthetic derivative of the tryptophan metabolite anthranilic acid, has been shown to reduce inflammation and reverse paralysis in mice with EAE (45). Therefore, it seems that a number of tryptophan metabolites are immunosuppressive. The molecular mechanism by which these compounds exert their immunologic effects is not known, but at least one recent report has described a receptor able to bind a specific metabolite of tryptophan (kynurenic acid) (46). The biologic function of this orphan G protein-coupled receptor, GPR35, is unknown, but its expression was highest in cells of the immune system and the gut, sites where IDO is known to be expressed (46). Whether there are other such receptors for other tryptophan metabolites and what the biologic effects of such receptors might be in vivo are important questions that deserve further investigation.

In addition to the immunosuppressive effects of tryptophan metabolites, the cellular stress imposed by local depletion of tryptophan also seems to mediate some of the immunosuppressive effects of IDO (Figure 1B). This was first suggested by the

However, IDO does not seem to be required for the constitutive maintenance of tolerance to self. This is shown by the fact that mice genetically modified to lack IDO (*Ido*<sup>-/-</sup> mice) do not develop lethal autoimmune or lymphoproliferative disorders (20) and mice treated systemically for up to 28 days with pharmacologic IDO inhibitors have not been observed to develop spontaneous autoimmunity (22, 28). Therefore, in certain settings, IDO can be very important for tolerance, but the effects of IDO are selective and are narrowly focused on specific forms of acquired peripheral tolerance. This specificity is potentially an advantage when contemplating the clinical use of pharmacologic IDO inhibitors since these would not be predicted to have severe spontaneous autoimmunity as a limiting side effect.

**Regulation of IDO expression**

IDO can be expressed by multiple cell types in response to inflammation. The regulation of IDO expression (gene, protein, and functional activity) is complex and remains a subject of active investigation. However, two important points have emerged from the literature. First, within the immune system, certain types or subsets of APCs seem to be preferentially disposed to express functional IDO when challenged with proinflammatory stimuli or exposed to signals from activated T cells. In mice, these “IDO-competent” APCs include a subset of plasmacytoid DCs (29), CD8 $\alpha$ <sup>+</sup> splenic DCs (or a subset thereof) (30, 31), and doubtless other subsets of DCs and macrophages as well. Second, even in APCs that are IDO competent, the actual presence or absence of

**Figure 2**

Model of the effects of IDO in tumors and tumor-draining lymph nodes. **(A)** In tumor-draining lymph nodes, IDO-expressing DCs directly suppress and anergize tumor-reactive effector T cells responding to antigens presented by IDO<sup>+</sup> DCs. Also, through bystander suppression, IDO can inhibit T cell responses to antigens presented by neighboring APCs. One mechanism that can induce IDO expression in DCs is reverse signaling mediated by B7-1 or B7-2 molecules expressed on DCs binding to CTLA4 expressed on Tregs. Reciprocally, IDO expression by DCs can also activate Tregs and drive the differentiation of new Tregs from uncommitted CD4<sup>+</sup> T cells. **(B)** In the tumor microenvironment, tumor cells can either express IDO constitutively or upregulate IDO in response to inflammatory signals generated by activated effector T cells. IDO expression by tumor cells inhibits effector T cells and also activates Tregs to further contribute to the suppressive microenvironment within the tumor.

observation that some effects of IDO on T cells are reversed by the addition of excess tryptophan *in vitro* (25, 29, 33, 47). Recently, the stress-responsive kinase general control nonderepressible 2 (GCN2) has been identified as a signaling molecule that enables T cells to sense and respond to stress conditions created by IDO (48, 49). The kinase activity of GCN2 is triggered by a rise in the amount of uncharged transfer RNA (tRNA) in the cell (50); therefore, insufficiency of any amino acid (such as occurs when IDO depletes tryptophan) will activate GCN2 kinase activity and initiate a downstream signaling pathway (51). This results in repression of most protein translation but causes selective upregulation of a small subset of genes that are responsive to signaling by GCN2 (52). These GCN2-responsive genes are different for each cell type, and exactly how this signal transduction pathway modulates immune responses is still under investigation; however, T cells from mice lacking GCN2 are resistant to IDO-induced inhibition of proliferation, and they do not acquire the state of antigen-specific unresponsiveness (anergy) normally induced by exposure to IDO (48). Furthermore, CD4<sup>+</sup> T cells from GCN2-deficient mice fail to undergo IDO-induced differentiation into Tregs (48, 49).

Local tryptophan depletion would obviously be a short-range immunosuppressive phenomenon since the total body pool of tryptophan could not be depleted. However, since IDO-expressing APCs and responding T cells are obliged to be in close physical contact, it might be that tryptophan concentrations can be lowered sufficiently in T cells interacting with the IDO-express-

ing APCs to activate the GCN2 signaling pathway. However, as with the effects of tryptophan metabolites, much of this model of the mechanisms by which local tryptophan depletion elicits immunosuppression remains speculative. It seems probable that both the GCN2 pathway and the metabolite pathways function synergistically to create the full biologic effects of IDO, as has been recently suggested (49).

### Potential sites of action for IDO in mediating tolerance to tumors

Multiple mechanisms contribute to tumor-induced tolerance. These can operate in the tumor itself or in key sites where the immune system encounters tumor antigens, such as the tumor-draining lymph nodes. In combination, these tolerogenic mechanisms act to block the initial response to tumor antigens (53, 54), inhibit the ability of activated T cells to kill tumor cells (55), and enhance the suppressive activity of Tregs (56). Emerging evidence suggests that IDO has the potential to contribute to each of these processes, both in the tumor and in the tumor-draining lymph nodes (Figure 2).

### IDO expression in tumor-draining lymph nodes

Most studies suggest that naive, resting T cells become aware of tumor antigens primarily through presentation of tumor-derived peptides by host APCs rather than by direct presentation of the peptides by the tumor cells themselves (57–61). Classically, anti-



gen presentation to resting T cells occurs in draining lymph nodes (62). Usually, this is thought to lead to T cell activation, but studies now show that this can also lead to potent, antigen-specific tolerance induction, particularly when antigens are presented in lymph nodes draining normal uninflamed tissues or mucosal surfaces (63–65). These studies emphasize that it is often the nature of the lymph node where the antigen is initially presented rather than the nature of the antigen itself, that dictates the choice between tolerance and immunity (66). Since most tumor antigens are first presented in the tumor-draining lymph nodes, these nodes are pivotally positioned to influence the immune response to tumor antigens (67, 68).

IDO is expressed in tumor-draining lymph nodes of humans (32, 36, 69, 70) and mice (29). In humans, the specific cell type expressing IDO has not yet been definitively characterized, but it is clear that these are frequently host cells (not metastatic tumor cells) and that they often display a “plasmacytoid” morphology (69). In mice, the immunosuppressive IDO-expressing cells in tumor-draining lymph nodes are phenotypically similar to mouse plasmacytoid DCs (expressing the cell surface markers CD11c, B220, and Ly6C) but with additional coexpression of the B cell-lineage marker CD19 (29). Similar CD19<sup>+</sup> plasmacytoid DCs that express IDO have also been observed in nontumor models of tolerance induction (71, 72), so the CD19<sup>+</sup> phenotype seems to represent an IDO-competent subset of DCs. IDO<sup>+</sup> DCs from tumor-draining lymph nodes have been shown to suppress T cell responses *in vitro* and induce antigen-specific T cell anergy when adoptively transferred into new hosts (29, 48). This ability to induce anergy might be conceptually similar to the ability of mouse splenic IDO-expressing CD8 $\alpha$ <sup>+</sup> DCs to create systemic unresponsiveness to antigens *in vivo* in other experimental models of tolerance induction (30).

Taken together, these studies suggest that IDO-expressing host DCs found in tumor-draining lymph nodes might help suppress the initiation of immune responses to tumor-derived antigens and perhaps help create systemic tolerance to these antigens. Further work is needed to determine whether tumor-derived antigens are indeed presented by these DCs (or possibly by other DCs within an immunosuppressive milieu created by these IDO-expressing DCs) and whether this leads to tolerance induction.

*Prognostic significance of IDO expression in sentinel lymph nodes.* In humans, IDO-expressing cells have been observed by immunohistochemistry in lymph nodes from patients with melanoma and breast, lung, and colon cancer as well as other tumors (ref. 29 and our unpublished observations). To date, however, the only studies of IDO expression in radiographically mapped sentinel lymph nodes (where it is known that the lymph node in question actually drains the tumor) have been performed in patients with malignant melanoma (29, 70). In two studies, IDO expression was associated with substantially worse clinical prognosis (29, 70). However, it will require larger studies to determine whether IDO expression in tumor-draining lymph nodes constitutes an independent prognostic factor in individuals with melanoma and other tumors. For the purposes of therapy, however, it would not be necessary that IDO be statistically independent of other known risk factors: the relevant point is that IDO represents a functional molecular target for which inhibitors exist. Therefore, the key question is whether blocking IDO will improve the immunologic response to tumors, not whether IDO is independent of other risk factors.

### **Suppression in the tumor microenvironment**

A second potential site of IDO activity is the tumor microenvironment itself. Uyttenhove and colleagues have shown by immunohistochemistry that various human tumors express IDO (28). IDO expression by tumor cells has been shown to correlate with a poor clinical prognosis in ovarian carcinoma (73), endometrial carcinoma (74), and colon carcinoma (75). It is not yet known whether this worsened prognosis compared with that of individuals whose tumor cells lack IDO reflects the immunosuppressive effects of IDO or a more general alteration in the biology of the tumor that is associated with IDO expression. However, on the basis of mouse models, it is reasonable to speculate that IDO expression by tumor cells could create an immunosuppressive microenvironment in the tumor. Consistent with this hypothesis, Uyttenhove et al. showed that mouse tumor cells transfected with IDO became resistant to immunologic rejection, even in mice that had been preimmunized against the tumor and that would have fully rejected the same tumor cells not expressing IDO (28). Conceptually, this is analogous to the lung transplant studies cited above, in which ectopic expression of IDO protected antigenically foreign tissues from rejection by the host immune system (25, 26). IDO might be expressed constitutively by tumor cells as part of the genetic changes involved in malignant transformation (18). Alternatively, IDO is known to be inducible in many tumor cell lines by IFN- $\gamma$  and other inflammatory mediators (37); therefore, IDO might be secondarily induced in tumor cells or in host stromal cells in response to inflammatory cytokines from the initial host response against the tumor (76).

### **IDO, Tregs, and CTLA4**

Tregs are emerging as a key component of acquired tolerance to tumors. Increased Treg activity facilitates tumor growth (77) whereas depletion of Tregs allows effective antitumor immune responses that would otherwise be undetectable or ineffectual to occur (4, 8, 78, 79). IDO has been implicated as a possible immunosuppressive effector mechanism of Tregs. Grohmann and colleagues showed that Tregs can trigger high levels of functional IDO expression in mouse DCs *in vitro* (80). This occurred through binding of CTLA4 on Tregs to B7-1 and B7-2 on DCs, which transduced a signal in the DCs that upregulated IDO protein expression and functional enzymatic activity (19, 80). A similar ability of CTLA4–B7-1 and/or B7-2 interactions to induce IDO has been shown in human monocyte-derived DCs (31, 33). Therefore, IDO might function as one downstream mechanism by which CTLA4<sup>+</sup> Tregs mediate immunosuppression (81).

Some support for this hypothesis comes from recent studies of primates infected with SIV. Infected monkeys have CTLA4<sup>+</sup> Tregs that inhibit anti-SIV immune responses, and these same animals also show high levels of systemic IDO expression and functional activity (82). When SIV-infected animals were treated with a blocking antibody specific for CTLA4, they showed a substantial reduction in IDO mRNA in tissues and decreased amounts of IDO metabolites in plasma (82). These results would be consistent with the hypothesis that IDO expression by APCs can be upregulated by CTLA4 expressed on Tregs (although other interpretations are also possible). Further studies in genetically defined knockout and transgenic mice are needed to definitively address this question. However, since tumor-draining lymph nodes can contain many activated Tregs (83–85), Treg-mediated induction of IDO through CTLA4 offers one potential explanation for the increased level of IDO expression in tumor-draining lymph nodes.



Recently, *in vitro* studies have indicated that IDO expression by DCs can also promote the differentiation of new Tregs from naive CD4<sup>+</sup> T cells (49, 86). At present, however, this remains an *in vitro* observation, and additional studies are needed to demonstrate whether this pathway has an *in vivo* role in tumor immunology. If this is shown to occur *in vivo*, however, then IDO and Tregs would be revealed as a closely coupled positive-feedback system, with Tregs inducing IDO and IDO driving differentiation of new Tregs.

### IDO inhibitors and chemotherapy

Cytotoxic chemotherapy is now known to place a substantial stress on the state of established tolerance created by the tumor. This is, in part, because chemotherapy causes dying tumor cells to release a wave of tumor-associated antigens, which can then enter the antigen-presentation pathway (87, 88). In addition, many chemotherapy regimens induce a period of transient lymphopenia and homeostatic recovery, during which T cells seem to be more receptive to breaking tolerance (89–92). Finally, certain chemotherapy regimens can at least partially deplete or transiently inactivate tumor-protective Tregs (4, 93–96). Chemotherapeutics have been shown to enhance the effectiveness of immunotherapy in a number of different mouse models of cancer (4, 88, 97–100), and chemotherapy has begun to be incorporated as an element of immunotherapy regimens in some recent clinical trials (101, 102).

Despite these potential benefits, however, most chemotherapeutic agents do not seem to trigger a detectable protective immune response against established human tumors. One possible reason for this failure could be that the tumor is able to rapidly reestablish tolerance to itself following each cycle of chemotherapy. Tumor antigens released by chemotherapy are presented in tumor-draining lymph nodes, and mouse studies show that IDO expression in draining lymph nodes can convert them into an immunosuppressive and tolerance-inducing milieu (29). Therefore, inhibiting IDO in the post-chemotherapy period could potentially delay or disrupt the reacquisition of tolerance to tumor antigens.

Preclinical studies in mouse tumor models demonstrate that 1MT has substantial synergy when combined with a number of chemotherapy drugs (18, 103). This was first demonstrated by Muller and colleagues using a stringent model of autochthonous breast tumors arising in mice engineered to overexpress the oncogenic protein HER2/neu in breast tissue (18). An important feature of this model is that, as in humans, each tumor is genetically different, arising through its own unique series of genetic mutations (104), and therefore must develop its own strategy to escape immune surveillance. Despite this genetic diversity, tumors consistently regressed in size when treated with 1MT in combination with any one of several chemotherapeutics (18). Effective chemotherapeutics in this model included cyclophosphamide, doxorubicin, paclitaxel, and cisplatin. These findings have recently been extended in a second report (103). The synergy between 1MT and chemotherapy was immune mediated, as shown by the fact that the effect of 1MT was lost when tumors were grown in immunodeficient RAG1-deficient hosts (103). Therefore, by a functional definition of tolerance, the combination of 1MT and chemotherapy was able to break tolerance to established tumors, allowing therapeutic antitumor immune responses that formerly would not have been possible to occur. The molecular mechanism of the synergy between IDO inhibitors and chemotherapeutics is still a subject of active investi-

gation. However, from a clinical standpoint, the ability of IDO inhibitors to function in combination with chemotherapy offers a substantial practical advantage because it means that immunotherapy regimens can be tested in clinical trials without denying patients the benefits of standard chemotherapy.

### Choosing among available IDO inhibitors

In preclinical studies, the most widely used IDO inhibitor has been 1MT, which exists as both D and L isomers. Most studies to date have employed the racemic mixture (that is, a mixture of the D and L isomers of 1MT), thereby leaving open the question of which isomer would be better suited as an immune-modulating agent in combination with chemotherapy. The L isomer is the more potent inhibitor of IDO activity when tested in assays using the purified IDO enzyme (105) or IDO expressed in cell lines (103). However, using *in vitro* assays based on T cell responses to IDO-expressing APCs, we and others have found that the D isomer of 1MT is at least equally efficacious (32, 33, 86, 106, 107), a finding that would not have been predicted based solely on assays using the purified enzyme. Direct comparison of the D and L isomers *in vitro*, and also *in vivo* in mouse models of cancer, suggested that the D isomer is more effective at reversing the suppression of T cells created by IDO-expressing DCs in the models tested (103). The racemic mixture of 1MT at high concentrations has been reported to display off-target effects (108), and the advantage of the D isomer is perhaps due to reduction in the off-target inhibition of T cell responses that was seen with the L isomer and racemic mixture (103). Furthermore, *in vivo* mouse tumor studies suggested that the target of the D isomer of 1MT is authentic IDO since the antitumor efficacy of D-1MT was lost when tumors were grown in IDO-deficient (*Ido*<sup>-/-</sup>) mice (103).

### IDO as a counterregulatory mechanism in cancer immunotherapy

One of the biologic functions of IDO seems to be as a counterregulatory mechanism to suppress excessive immune activation. Such counterregulatory pathways are important in the immune system because uncontrolled immune responses can cause unacceptable damage to the host. At sites of inflammation, IDO expression is not limited to DCs and macrophages but can be found in epithelial cells (27, 109, 110), eosinophils (111), endothelial cells (112), and possibly other cell types as well. Blocking this inflammation-induced IDO can substantially worsen local tissue damage, sometimes to the point of lethality (22). Therefore, IDO is an important endogenous counterregulatory mechanism that helps protect the host. However, virtually all tumor-immunotherapy strategies aim to induce inflammation and immune activation. If endogenous IDO is induced by this therapeutic immune activation (as it is during the physiologic response to pathogens and inflammation), then IDO could potentially antagonize the desired effects of immunotherapy. Emerging evidence suggests that this might be more than a theoretical concern.

### Induction of IDO by CpG oligonucleotides

One well-known inducer of IDO is bacterial LPS, a TLR4 ligand (113). Recently, it has been shown that IDO is also induced by ligands for TLR9 (oligodeoxynucleotide containing one or more unmethylated CpG dinucleotides [CpG ODN]) (72, 114). CpG ODN are potent immunologic activators (115) and are currently in phase III clinical trials as an immunostimulatory agent in patients



with lung cancer (116, 117). Recently, however, it has been shown that in mice, high-dose intravenous administration of CpG ODN can cause upregulation of IDO expression in the spleen with potent IDO-induced immunosuppression (72, 114). This effect seemed to be mediated by autocrine and/or paracrine production of IFN- $\alpha$  since mice lacking the receptor for IFN- $\alpha$  did not induce IDO in response to CpG ODN (72). Therefore, although CpG ODN are potent immunostimulatory agents, under certain circumstances they can also induce immunosuppressive IDO expression. Blocking this unwanted IDO activity (for example, by coadministration of an IDO inhibitor) might enhance the efficacy of CpG ODN as an immunostimulatory agent in cancer.

**Induction of IDO by IFNs and vaccine adjuvants**

In addition to TLR ligands, IDO is known to be inducible by IFN- $\gamma$  and IFN- $\alpha$ , one or both of which are frequently found at sites of inflammation (118). Vaccine adjuvants, which are critically important for enhancing responses to tumor and other vaccine antigens, typically function by inducing inflammation, and adjuvants often include TLR ligands (119). It has not yet been reported whether clinically relevant vaccine adjuvants might induce IDO. However, it has been shown that bacteria such as *Mycobacterium bovis* bacillus Calmette-Guérin and *Listeria monocytogenes*, both of which have been used as vaccine adjuvants, are also potent inducers of IDO in vivo (120, 121). Therefore, like CpG ODN, other vaccine adjuvants have the potential to induce counterregulatory IDO and might potentially show enhanced efficacy if IDO were blocked.

**Induction of IDO by 4-1BB**

The costimulatory molecule 4-1BB (also known as CD137) is a member of the TNF receptor superfamily with potent T cell-activating effects in certain mouse models of cancer (122). Agonistic monoclonal antibodies specific for 4-1BB have recently entered

phase I clinical trials in solid tumors. Somewhat unexpectedly, however, systemic ligation of 4-1BB has been reported to induce IDO in vivo (123, 124). Treatment of mice with an agonistic 4-1BB-specific antibody was shown to suppress disease severity in autoimmune arthritis (124) and also in a model of uveitis (123), in both cases related to the collateral induction of IDO (mediated through elevated levels of IFN- $\gamma$ ). Therefore, it is possible that the antitumor efficacy of agents targeting 4-1BB might also be limited by counterregulatory induction of IDO.

**Conclusion**

Successful immunotherapy of cancer will require a combination of multiple immunomodulatory agents and strategies (125). Such combinations will need to be rationally designed, based on a mechanistic understanding of the goals to be accomplished and barriers to be overcome. Key steps include breaking preexisting tolerance to the tumor (especially removing the suppression mediated by Tregs); providing a suitable set of antigens (whether from the tumor itself or a vaccine); ensuring that these antigens are presented in an activating milieu (rather than the tolerizing milieu that seems to be the case in most tumor-draining lymph nodes); and providing sufficient adjuvant and immunostimulatory signals to drive a potent, curative antitumor immune response. As discussed in this Review, IDO might antagonize these desirable goals at a number of points. Therefore, inhibition of the IDO pathway might enhance the efficacy of various immunotherapeutic and chemotherapeutic strategies for the treatment of individuals with cancer.

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