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Different Partners, Opposite Outcomes: A New Perspective of the Immunobiology of Indoleamine 2,3-Dioxygenase

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Indoleamine 2,3-dioxygenase (IDO), a metabolic enzyme that catalyzes tryptophan conversion into kynurenines, is a crucial regulator of immunity. Altered IDO activity is often associated with pathology, including neoplasia and autoimmunity. IDO is highly expressed in dendritic cells (DCs) that exploit the enzyme's activity and the production of tryptophan catabolites to regulate immune responses by acting on several cell types, including T lymphocytes, of which they promote a regulatory phenotype. IDO also contains immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that, once bound by distinct molecular partners, will either promote degradation or initiate signaling activity and self-maintenance of the enzyme. We here discuss how ITIM-dependent molecular events can affect the functional plasticity of IDO by modifying the protein half-life and its enzymic and nonenzymic functions.

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INTRODUCTION

The enzyme indoleamine 2,3-dioxygenase (IDO) is recognized as one of the prominent mediators of immune regulation by metabolic pathways. IDO catabolizes the essential amino acid tryptophan, an effect originally traced to the need for inhibiting replication of pathogens in local tissue microenvironments dominated by the cytokine interferon-γ (IFN-γ) (1). The original view of IDO as a mere tryptophan-depleting enzyme has now been revisited in several immunological contexts (2). The enzyme activity of IDO is indeed accompanied by the production of a series of immunoregulatory metabolites, collectively known as "kynurenines," which can exert direct effects on immune cells, but also propagate messages of immune tolerance from one cell type to another. As a result, IDO controls and fine-tunes both innate and adaptive immune responses (3) under a variety of conditions, ranging from pregnancy (4) and transplantation (5,6) to infection (7), chronic inflammation (8), autoimmunity (9) and neoplasia (10).

Among immune cells, dendritic cells (DCs) are known to express the highest level of IDO activity in response to IFN-γ (11). DCs are considered the most potent antigen-presenting cells (APCs), capable of orchestrating antigen-specific immune responses in an either immunogenic or tolerogenic manner. Expression of the gene coding for IDO (*IDO1* in humans; *Ido1* in mice) confers a potent immunoregulatory phenotype on DCs, operating in both innate and adaptive immunity via multiple mechanisms, including the regulatory T (Treg) responses (12). Thus, the IFN-γ/IDO axis in DCs will arrest the growth of microorganisms but also avoid potentially harmful inflammatory responses in the host. However, whereas the transient, though intense, expression of the enzyme induced by IFN-γ can explain the immunoregulatory effects of IDO in acute inflammation, this mechanism does not explain the long-term effects of IDO, known to be at work in the maintenance of immune homeostasis, as required by pregnancy (4) and tolerance to self (13).

Transforming growth factor-β (TGF-β) is a cytokine with the prominent function of inducing and maintaining T-cell tolerance to self via direct effects on the differentiation and homeostasis of both effector and Treg cells (14). In DCs, autocrine or paracrine TGF-β was found to induce long-term IDO-dependent effects that imply a functional conversion of DCs to a stably regulatory phenotype as well as the spreading of regulatory functions to cells other than DCs, including T lymphocytes, a phenomenon known as "infectious tolerance" (15). We recently demonstrated that TGF-β provides IDO with a novel, nonenzymic function that relies on signaling events required for sustaining a stable, regulatory phenotype in DCs (16).

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We also found that the proinflammatory cytokine interleukin (IL)-6 and suppressor of cytokine signaling 3 (SOCS3) can promote IDO proteasomal degradation and interrupt its functioning (17,18). Indeed, the wide range of the suppressive effects of IDO does require a counterregulatory mechanism capable of opposing IDO activity, via proteasomal degradation, as dictated by changes in environmental conditions.

In this review, we discuss the molecular mechanisms underlying either TGF-β–induced IDO signaling pathways or, in alternative, IL-6–driven IDO proteasomal degradation, and how they influence each other through their opposing roles under different physiologic and pathologic conditions.

IMMUNORECEPTOR TYROSINE-BASED INHIBITORY MOTIFS AND IMMUNE REGULATION

Immunomodulatory mechanisms widely use negative regulators in the form of signaling proteins bearing one or more immunoreceptor tyrosine-based inhibitory motifs (ITIMs). A prototypic ITIM is a highly conserved consensus amino acid sequence (I/V/L/SxYxxL/V/F) where x denotes any amino acid (19). ITIMs were first identified in the cytoplasmic tail of FcγRIIb receptors expressed by B lymphocytes, where they participate in the negative signaling leading to inhibition of B-cell activation (20). Most ITIM-containing proteins are represented by transmembrane receptors that usually act in balance with immunoreceptor tyrosine-based activatory motif–containing counterparts (21). A common feature of ITIMs is that, upon tyrosine phosphorylation operated by the Src family protein tyrosine kinases, they can act as docking sites for phosphotyrosine phosphatases, such as Src homology 2 (SH2) domain–containing phosphatase (SHP-1), SHP-2 and inositol-phosphatase SHIP. Phosphotyrosine phosphatases directly remove phosphate groups from various proteins. These include a series of proteins involved in activating cellular pathways, such as protein tyrosine kinases, adaptor molecules and effector enzymes, in which switching off leads to cell inhibition (22). In addition to the classic signaling role, tyrosine-phosphorylated ITIMs can bind the SH2 domain of SOCS proteins, which will target the inhibitory receptor for proteasome degradation (23,24).

Because inhibitory receptors appear to be involved in a considerable number of physiopathological processes, the molecular events involved downstream of ITIM tyrosine phosphorylation may represent new potential pharmacologic targets.

IDOS, ITIMs AND EVOLUTION

Although the link between immune regulation and tryptophan catabolism has been crucially linked to IDO, other enzymes can catalyze the same reaction along the kynurenine pathway (3). Those enzymes include the ancestral enzyme tryptophan 2,3-dioxygenase (TDO) (also present in bacteria) and a paralogue of IDO, IDO2 (25). Originally thought as a constitutive enzyme mainly confined to liver and brain, TDO (encoded by *TDO2* in humans and *Tdo2* in mice) has recently been found to be expressed in several human tumors (26,27). In addition, in preclinical models, TDO expressed by tumors prevents their rejection by immunized mice. Remarkably, administration of a specific inhibitor of TDO catalytic activity induces tumor rejection by tumor-bearing mice (27).

IDO (inducible under inflammatory conditions) has been found in several organs and tissues, including neoplastic cells and brain but also lung, gut, placenta, pancreatic islets and lymphoid organs. In contrast, the expression and function of IDO2, particularly in immune cells, has not yet been clearly defined. To date, *IDO1* has only been found in mammals and yeasts, with the latter having possibly coevolved with the former organisms to guarantee a more proficient commensalism (3,28). In contrast, *IDO2* is also present in lower vertebrates, such as chicken, fish and frog. Thus, IDO may have arisen from IDO2 (29), by gene duplication occurring before the divergence of mammals.

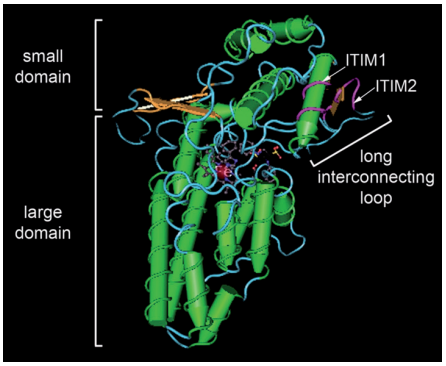

Figure 1. Structure view of human IDO. Putative ITIM1 and ITIM2 in human IDO are localized close to each other outside the catalytic domain. Human IDO folds into a catalytic large C-terminal domain, a noncatalytic small N-terminal domain and a long loop connecting the two domains. Green cylinders represent α-helices and orange arrows indicate β-sheets. ITIM1 and ITIM2, highlighted in pink, are located within the α-D helix of the small domain and the β-sheet of the interconnecting loop, respectively. Image (PDB code 2D0T_A) was generated using a Cn3D structure viewer.

The definition of the crystal structure of human IDO (30) has revealed a folding into a catalytic large C-terminal domain, a noncatalytic small N-terminal domain and a long connecting loop (Figure 1). Apart from covering the top of the heme-binding site, the role of the noncatalytic small domain is unclear. Interestingly, TDO has a homotetrameric structure, in which each monomer, when aligned to IDO, appears to contain the large catalytic but not the small domain (31). Although not crystallized yet, the protein sequence of IDO2 suggests that it also possesses the small domain (29).

We recently demonstrated that IDO contains two functional ITIMs (16,17), which, once tyrosine phosphorylated, can bind either SOCS3 or SHP-1/2 tyrosine phosphatase, depending on environmental stimuli. By mapping the human IDO structure, IDO ITIMs, although spanning quite distant portions of the primary sequence (Figure 2), are found to be positioned close to each other in an

exposed surface region of the protein (see Figure 1). In particular, ITIM1 and ITIM2 are located in the small domain and in the interconnecting loop of the two domains, respectively. Alignment of human, dog, rat and mouse proteins indicate that ITIM1 and ITIM2 IDO sequences are well conserved in mammals (see Figure 2). In contrast, in mammalian IDO2 as well as its chicken and fish counterparts, only a putative ITIM2 is present, owing to a tyrosine substitution with phenylalanine in ITIM1. In addition, in the deposited mouse IDO2 sequence, the +3 position of ITIM2 is occupied by a nonhydrophobic alanine, thus suggesting that ITIM2 may also be lacking. However, the current scenario of immune regulation on the basis of tryptophan catabolism clearly involves the ancestral enzyme TDO, particularly in tumor settings (26,27), TDO-mediated immunosuppression appears to be entirely on the basis of its catalytic activity, that is, by the production of L-kynurenine, which activates the aryl-hydrocarbon receptor (26).

Thus, the appearance of an IDO isoform with an ITIM-mediated signaling ability in placental organisms may represent the result of novel evolutionary constraints necessary to allow the coexistence with a foreign individual during pregnancy.

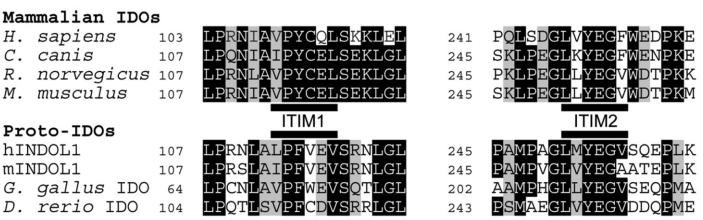

Figure 2. Multiple alignments of the amino acid sequences of IDOs. Mammalian IDOs (*H. sapiens*, accession number P14902; *M. musculus*, P28776; *R. norvegicus*, Q9ERD9; *C. canis*, XM_532793) and proto-IDOs (hIDO2, human IDO2, NP_919270; mIDO2, mouse IDO2, NP_666061; *G. gallus*, XM_424397; *D. rerio*, BAF45469). The alignments focus on the stretch of amino acids containing the putative ITIM1 and ITIM2 motifs. Conserved residues (black boxes) and conservative replacements (gray boxes) are indicated.

IL-6 AND ITIM-MEDIATED REGULATORY PROTEOLYSIS OF IDO

The identification of two ITIMs in the amino acid sequence of IDO was a turning point in our understanding of IDO-mediated immune regulation. Like most ITIM sequences characterizing the cytoplasmic domain of several immunoreceptors, IDO ITIMs can, once phosphorylated, act as docking sites for different protein partners. Among IDO-interacting proteins, SOCS3 represents the first ligand identified so far capable of binding the ITIM-docking sites of the enzyme (17).

SOCS3 was initially described as a feedback inhibitor of IL-6 proinflammatory effects. In fact, upregulated by the cytokine itself, SOCS3 is capable of switching off the signaling pathway of IL-6 by inhibiting the activity of signal transducers and activators of transcription, via still unclear mechanisms (32). In the regulation of immunity and tolerance driven by DCs, IL-6–induced SOCS3 is responsible for inhibiting the transcriptional expression of *Ido1* induced by IFN-γ (33). Intriguingly, an inverse correlation between SOCS3 and IDO expression was observed, such that, in DCs lacking SOCS3, the immunoadiuvant effect provided by otherwise immunostimulant CD28-Ig treatment evokes the same IDO-mediated effect as that induced by cytotoxic T lymphocyte–associated antigen 4–immunoglobulin (CTLA-4-Ig), an immunosuppressive drug also known as "abatacept" (34). The biunivocal association between immunogenicity and SOCS3 function in DCs was further confirmed after demonstration of proteasomal degradation of the IDO protein induced by direct binding of SOCS3 (17). In fact, SOCS proteins possess a Src homology 2 (SH2) domain, which binds phosphotyrosine-containing sequences, and a SOCS box, which recruits the enzymatic complex E3 ubiquitin ligase, which can transfer molecules of ubiquitin on bound proteins. In particular, the SH2 domain of SOCS3, by anchoring the phosphorylated ITIMs of IDO, brings the enzyme close to the E3 ubiquitin complex that ubiquinates and targets IDO for proteasomal degradation. In the ubiquitin/proteasome-mediated degradation of IDO initiated by SOCS3, the crucial role of the ITIM sequences of IDO is supported by the observation that mutations of the ITIM phosphorylable tyrosines abolish IDO association with SOCS3 and prevent IDO ubiquitination (17).

Ubiquitin-mediated proteasomal degradation controls several important biological processes, including cell cycle progression, apoptosis, DNA repair and immune cell signaling. In 2003, the U.S. Food and Drug Administration approved bortezomib as a drug to treat multiple myeloma (35), and several other promising proteasome inhibitors are currently in clinical trials for treating cancer. In DCs, the proteasome activity has a well-recognized role in antigen processing. However, a novel function in the turning on/off of transcription factors, such as nuclear factor (NF)-κB and IFN regulatory factors, has recently been described (36). Ubiquitin-driven, proteasomal degradation is a hallmark of activation of transcription factors belonging in the NF-κB family, which can be activated via a canonical (normally proinflammatory) or noncanonical (antiinflammatory) pathway (37). Interestingly, IDO expression in DCs requires activation of the noncanonical NF-κB pathway, which involves the upstream intervention of the IκB kinase α (IKKα) and the downstream nuclear translocation of p52-reticuloendotheliosis viral (v-rel) oncogene (p52-RelB) dimers (38). Thus, the inhibition of the proteasome in DCs could prevent degradation of IDO on the one hand, but, on the other, could also affect activation of the

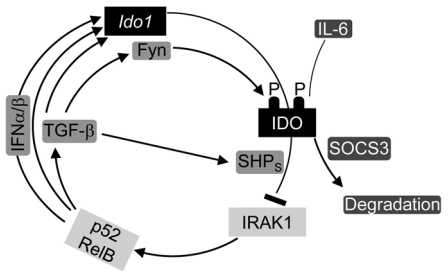

Figure 3. Schematic representation of ITIM-mediated signaling function of IDO. Tyrosine-phosphorylated IDO ITIMs (P) act as docking sites for tyrosine phosphatases (SHPs) and SOCS3. Association of IL-6–induced SOCS3 promotes IDO proteasomal degradation. TGF-β, by inducing SHPs and activating the tyrosine kinase Fyn that phosphorylates IDO ITIM domains, promotes the association of IDO with SHPs. IDO/SHPs complexes, via inhibition of IRAK1, activate the noncanonical NF-κB pathway (p52/RelB), which in turn induces the production of type I IFNs (IFNα/β) and TGF-β, which synergize with noncanonical NF-κB in upregulating *Ido1* expression.

signaling pathways, leading to expression of IDO. Nevertheless, proteasome inhibition in DCs may still represent an effective tool in the prevention or therapy of immune disorders characterized by defective IDO expression (8,13), since the expression level of SOCS3, the main mediator of IDO proteolysis, can critically switch the DC capacity to promote either immunity or tolerance (17,34).

TGF-β AND ITIM-MEDIATED SIGNALING FUNCTION OF IDO

In the cell, enzymes are the best-informed molecules of environmental changes. Thus, it is not surprising that several ancestral metabolic enzymes have learned a "moonlighting" (that is, second) function, to meet the newly presenting survival needs imposed by phylogenesis (39). IDO catalytic activity is pivotal in innate/inflammatory as well as adaptive responses. In inflammation, IFN-γ is the primary IDO inducer to prevent hyperinflammatory responses, yet the enzyme is also responsible for longer-term self-tolerance effects. Treatment of mouse DCs, either conventional (cDCs) (40) or plasmacytoid (pDCs) (16), with TGF-β, but not with IFN-γ, will switch on a regulatory program capable of inducing long-term tolerance *in vivo* (40). Furthermore, TGF-β confers regulatory effects on IDO that are mechanistically separable from its enzymic activity (16,41). In fact, IDO-dependent tolerogenic effects induced by TGF-β in pDCs are abrogated by *Ido1* gene silencing, but not by the use of 1-methyltryptophan, the gold standard inhibitor of IDO enzyme activity.

Recently, molecular dissection of the TGF-β/IDO pathway in pDCs led to the discovery of a series of events, as depicted in Figure 3. Briefly, via small mother against decapentaplegic (Smad)-independent but phosphatidylinositol 3-kinase–dependent signaling, TGF-β activates Fyn (a tyrosine kinase belonging in the Src family) that phosphorylates the ITIM domains in IDO. Once phosphorylated, ITIMs of IDO act as docking sites for the tyrosine phosphatases SHP-1 and SHP-2, for which expression—but not that of SOCS3 (ML Belladonna and MT Pallotta, unpublished data)—is greatly upregulated by TGF-β in pDCs. The TGF-β/IDO/SHP axis activates the noncanonical NF-κB pathway by inhibiting the IL-1 receptor–associated kinase 1 (IRAK1), known to be involved in the activation of the canonical NF-κB pathway and in the upregulation of inflammatory cytokines such as IL-6 (42). In turn, the noncanonical NF-κB pathway induces the production of type I IFNs and TGF-β, which synergize with noncanonical NF-κB in upregulating *Ido1* expression (16,38). Thus, in a microenvironment dominated by TGF-β, IDO performs a moonlighting, signaling task that implies a positive feedback loop capable of perpetuating long-term IDO expression and activity in pDCs.

Thus, IDO is not only pivotal in limiting potentially exaggerated inflammatory reactions in response to danger signals (8) and in assisting Treg effector function (38), but also an important component of a regulatory system that presides over long-term control of immune homeostasis, by stably switching DCs to a tolerogenic phenotype, as may be required by pregnancy (4) and tolerance to self (13). Central to the maintenance of homeostasis in placental mammals (3), the ancestral metabolic enzyme IDO may have eventually acquired a moonlighting function (that is, a signaling activity capable of perpetuating a long-term IDO expression and activity) that may be much more efficient in controlling adaptive immune responses.

ITIM-REGULATED EXPRESSION OF IDO AND FUNCTIONAL PLASTICITY OF DCs: A DUET BY TGF-β AND IL-6

The presence of two ITIMs in the small domain of IDO leads to two opposite outcomes with respect to the half-life of IDO. On the one hand, tyrosine phosphorylated ITIMs provide IDO with a role as a signal transducer that translates into an upregulation and maintenance of the enzyme expression. On the other hand, the same motifs drive proteasomal degradation of IDO, accelerating the turnover of the enzyme. Interestingly, the presence of two or more ITIMs activates more efficiently the phosphatase activity of SHPs by simultaneous engagement of both SH2 domains present in SHP-1 and SHP-2 (22), whereas only one ITIM is required for SOCS3 binding (43,44). Thus, since the more ancient IDO2 sequence contains only one putative ITIM (see Figure 2), the presence of two ITIMs and consequent IDO signaling activity via SHPs may represent an evolutionary response to new constraints associated with placental mammals physiology (that is, the needs imposed by long-term maternal immune tolerance). As a matter of fact, IDO2 does not bind or activate SHPs (16).

In the dichotomic fate of IDO driven by ITIMs, the cytokines IL-6 and TGF-β play opposite but fundamental roles. IL-6 upregulates SOCS3 (but not SHPs; M T Pallotta, unpublished data) and promotes SOCS3 binding to ITIMs of IDO (phosphorylated under these conditions by still nonclarified mechanisms), which leads to

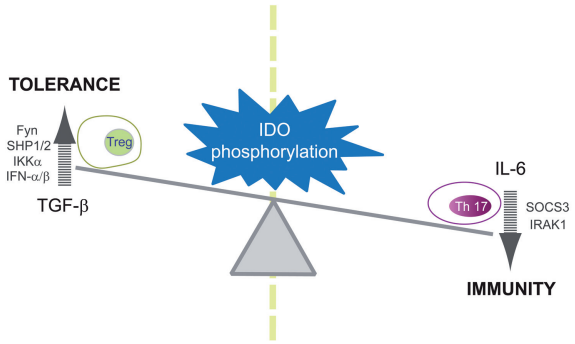

Figure 4. Schematic model of IDO ITIM-mediated regulation of the balance of Treg/Th17 cells. In a TGF-β–driven microenvironment, phosphorylation of IDO ITIMs initiates a positive regulatory loop that involves a specific set of molecules (Fyn, SHP-1/2, IKKα, IFN-α/β) and results in an amplified differentiation of Treg cells, which in turn promotes immune tolerance (TOLERANCE). This molecular loop is opposed by IL-6, which induces/activates a different set of molecules (SOCS3, IRAK1) and, via the same phosphorylated IDO ITIMs, favors the differentiation of Th17 cells that contribute to immune activation (IMMUNITY).

a contraction of the half-life of IDO (17). Because IL-6 is a typical proinflammatory cytokine, an early inflammatory context would indeed require that IDO be quickly degraded in DCs. In contrast, later inflammatory or noninflammatory conditions would require efficient and durable immune regulatory mechanisms, such as those provided by IDO and SHPs, which initiate a tolerogenic program in DCs culminating in the continuous *de novo* synthesis of IDO itself (16). However, ITIM-mediated mechanisms (that is, either SOCS3- or SHP-mediated) may not be mutually exclusive over time, but rather finely integrating, to provide IDO with different degrees of expression capable of quickly accomplishing distinct immunological needs. Thus, the choice in associating with SOCS3 or SHP proteins may depend on a cross-talk between the proinflammatory IL-6 and the "Jack of all trades" TGF-β (Figure 4). In fact, TGF-β is required for the development of Treg cells, but, in the presence of IL-6, can favor the differentiation of the inflammatory T helper 17 (Th17) subset (45). In addition, Treg and Th17 cells are highly plastic and can interconvert, depending on the environmental signals (46), including kynurenines, that can tip the balance in favor of Treg cells (47–49). In a TGF-β–dominated environment, IDO$^+$ pDCs participate in the spread of the immune tolerance by maintaining sustained IDO expression, via ITIM/SHP signaling, and by producing TGF-β, which can act in a positive feedback loop on pDCs themselves, but also by inducing antigen-specific, long-acting CD4$^+$Foxp3$^+$ Treg cells *in vivo* (16). As a consequence, the activation of the TGF-β/IDO/SHPs axis will block the production of proinflammatory cytokines such as IL-6, via IRAK1 inhibition, limiting SOCS3-mediated degradation of IDO. The molecular mechanisms described above may also provide a key to explaining the constitutively high number of Treg cells observed in *Irak1*$^{-/-}$ mice (50). In inflammatory conditions, high levels of IL-6 can break down IDO, a critical molecule in DC tolerogenic signaling, by rapidly inducing the alternative IDO partner SOCS3, which may act as a competitor with SHPs for ITIM binding. Thus, IL-6–induced SOCS3 in tolerogenic DCs may interrupt the regulatory feedback loop between Treg cells and DCs, by both competing with SHPs for ITIM-anchoring and directly disrupting IDO-ITIM anchors. IDO/SOCS3 association in DCs represents a molecular mechanism whereby IDO$^+$ DCs, expressing a tolerogenic phenotype, can revert to immunostimulatory APCs (17). In this perspective, IL-6–conditioned DCs could contribute to the differentiation of Th17 cells, by turning IDO$^+$ pDCs from a tolerogenic to an immunogenic phenotype, via SOCS3-mediated proteolysis of IDO. Interestingly, *in vitro* pretreatment of murine pDCs with IL-6 induces a Th17 phenotype in naive CD4$^+$ T cells (F Fallarino, unpublished data). On the other hand, silencing SOCS3 expression in DCs can turn the immunoadjuvant properties of IL-6–treated DCs into regulatory and IDO dependent (34). Moreover, SOCS3 deficiency in DCs is known to result in an enhanced induction of Foxp3$^+$ Treg cells, mostly dependent on a higher production of TGF-β by *Socs3*$^{-/-}$ DCs (51). Therefore, the opposite signals activated by ITIMs of IDO in DCs, exposed either to TGF-β alone or in the presence of IL-6, allow for the role of DCs as chief regulators of the balance between tolerance and immunity (Figure 5). According to the variety of pathophysiologic contexts that DCs must face, the same ITIM domains in IDO, recruiting either SOCS3 or SHPs, may therefore switch toward an immunogenic or tolerogenic pathway, providing DCs with the adequate flexibility to guarantee homeostasis in distinct immunologic conditions.

PHYSIOPATHOLOGICAL SETTINGS READ OVER THE ITIM-RELATED FUNCTIONS OF IDO

Most of the data indicate that IDO is one of the main causes of immune unresponsiveness in neoplasia and viral infection (7,52–56). In contrast, IDO deficiency has been revealed in autoimmune (13,57) and chronic inflammatory diseases (8). Maneuvers aimed at modulating IDO induction and/or activity (either in a negative or positive fashion) may therefore represent an important therapeutic option, although pharmacologic blockade of IDO will induce fulminant pancreatitis with severe lymphocyte infiltration and hyperglycemic coma in rhesus macaques infected with the simian immunodeficiency virus (58). The identification of new molecular keys associated with IDO/ITIM-mediated pathways may allow for revisiting the pathogenesis of several diseases possibly

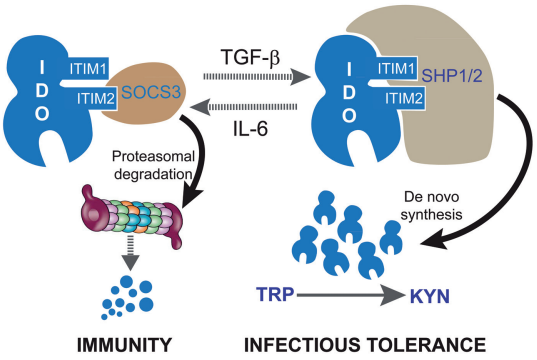

Figure 5. ITIM-regulated expression of IDO. TGF-β and IL-6 regulate IDO expression in a positive and negative fashion, respectively. TGF-β promotes anchoring of SHP-1/2 to phosphorylated IDO ITIMs, initiating a molecular pathway that culminates in *de novo* synthesis of IDO and amplified tryptophan (TRP) conversion into kynurenine (KYN). This TGF-β–induced mechanism contributes to the maintenance and spreading of immune tolerance (INFECTIOUS TOLERANCE). IL-6 induces SOCS3 and favors its association with IDO, targeting the enzyme for proteasomal degradation. This mechanism, by affecting IDO lifespan, subverts the tolerogenic program of the DC, promoting immune activation (IMMUNITY).

linked to IDO and thus open new and safer therapeutic perspectives aimed at regulating IDO function in a more physiologic fashion, as detailed below.

Fyn, A Tyrosine Kinase Phosphorylating IDO

Fyn-deficient mice exhibit a number of immunological abnormalities (59) and develop autoimmune uveoretinitis (60). In addition, Fyn genetic ablation exacerbates pulmonary allergic inflammation in an experimental mouse model of asthma (61). Fyn regulates autoimmune disease and massive lymphadenopathy in MLR/lpr mice (62). Fyn is upregulated in prostate cancer and cancer neurodegenerative diseases (63) and is thought to be involved in both progression and metastasis (64). Fyn and SHP-1/2 mediate the signaling triggered by human immunodeficiency virus (HIV) upon binding of the ITIM-containing DC immunoreceptor (DCIR), which behaves as an HIV-1 attachment factor (65).

SHP-1 and SHP-2, Two Tyrosine Phosphatases Binding IDO

The biological importance of SHP-1 is underscored by the motheaten mutant strain, characterized by immunological disorders involving multiple organs and by the close association of aberrant SHP-1 expression in several human diseases (66). SHP-1 expression controls the development of allergic airway inflammation (67), but is strongly upregulated in prostate, ovarian and breast cancer (68). Mutations in SHP-2 have been identified in the Noonan syndrome, a human developmental disorder that is sometimes associated with juvenile myelomonocytic leukemia (69). SHP-1 and SHP-2 expressions positively correlate with the progression of Condyloma acuminatum and cervical cancer after infection by human papilloma virus (70).

IRAK1, a Proinflammatory Kinase Inhibited by IDO Signaling

Irak1$^{-/-}$ mice are protected from developing experimental autoimmune encephalomyelitis (EAE) (50,71), and an IRAK1 haplotype containing a functional 196F variant was found to significantly associate with systemic sclerosis susceptibility (72). IRAK1 is also closely associated with the pathogenesis of diverse inflammatory diseases, including atherosclerosis (73). The *IRAK1* gene is a bona fide target of miR-146a, an important negative regulator of inflammation, myeloid cell proliferation and cancer (74). A new oncogenic pathway in lymphomagenesis was discovered that involves phosphorylated IRAK1 (75).

IKKα and p52/RelB, Inducers of IDO Expression

In the noncanonical pathway (37), activation of IKKα results in the processing of p100 to p52 and consequent formation of p52-RelB dimers, which translocate into the nucleus and activate an antiinflammatory gene program (76,77). IKKα expression increases in early pregnancy, whereas IKKβ is downregulated, thus suggesting that immunosuppressive mechanisms may prevail at this time (78). In contrast, lack of IKKα activation inhibits prostate cancer metastasis (79) and retards tumor development in response to carcinogens (80). Downstream RelB/p52 complexes also promote carcinogenesis (81).

SOCS3, an Inducer of IDO Proteasomal Degradation

Although strong evidence exists to support SOCS3 as a crucial regulator of many disease processes, further studies are needed to elucidate its overall function within each disease state (82). In fact, conflicting functional effects have been reported. On one hand, overexpression of SOCS3 in DCs increases the proliferation of autoreactive Th cells in an EAE model (83), and transgenic mice overexpressing *Socs3* show multiple features of asthma (84). In addition, preclinical observations unveil a relationship between SOCS3 overexpression in tumor cells and *in vivo* tumor growth inhibition (85,86). On the other hand, injection of autoantigen-pulsed *Socs3*-transduced DCs suppresses the development of EAE (87), and splenic APCs overexpressing *Socs3* prevent the development of collagen-induced arthritis (88). Furthermore, monocytes from patients with relapsing-remitting multiple sclerosis express less SOCS3 during relapse than remission (89).

As a whole, the bulk of literature data suggests that the expression and activity of critical molecules involved in ITIM-

mediated function of IDO correlate very well in the same (Fyn, SHP-1/2, IKKα-p52/RelB) or inverse (IRAK1) direction with abnormal expression of IDO in several disease conditions and thus could be used as alternative pharmacologic targets to normalize IDO expression. In contrast, the pathogenic role of SOCS3 appears to be more variable in terms of possible correlations with IDO expression. Although still speculative, this incongruence might be because SOCS3 is endowed with more than one function (that is, inhibition of IL-6–driven signaling and inflammatory pathways as well as proteasomal degradation of target proteins such as IDO) that could have a different hierarchy of importance in distinct physiopathologic conditions.

CONCLUSIONS

Analogous to many novel pharmacologic targets, knowledge of IDO biology is rapidly evolving. Recently discovered ITIM-related functions of IDO open a wide panorama of non-IDO molecular targets for modulating indirectly IDO functional expression and activity. Because of the relevant physiopathological role of IDO in distinct conditions, including pregnancy, therapeutics acting on ITIM-anchored partners of IDO and "beyond" may thus represent a less direct but more physiologic modality for manipulating the enzyme, with a possibly better assurance of mammalian homeostasis. In fact, the dissection of molecular events downstream of IDO ITIM phosphorylation does suggest direct partners of IDO, SOCS3 and SHP-1/2 as potential molecular targets but also other molecules, some of which are already identified (Fyn, SHP-1/2, IKKα-p52/RelB and IRAK1), and others possibly to be involved (arylhydrocarbon receptor [26,90,91]). Biological systems (that is, the ubiquitin-proteasome system) should be considered when developing therapeutic interventions on the basis of IDO modulation.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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