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Kynurenine Pathway Enzymes in Dendritic Cells Initiate Tolerogenesis in the Absence of Functional IDO

Maria L. Belladonna,* Ursula Grohmann,* Paolo Guidetti,† Claudia Volpi,* Roberta Bianchi,* Maria C. Fioretti,* Robert Schwarcz,† Francesca Fallarino,* and Paolo Puccetti2*

Dendritic cell (DC) tryptophan catabolism has emerged in recent years as a major mechanism of peripheral tolerance. However, there are features of this mechanism, initiated by IDO, that are still unclear, including the role of enzymes that are downstream of IDO in the kynurenine pathway and the role of the associated production of kynurenines. In this study, we provide evidence that 1) murine DCs express all enzymes necessary for synthesis of the downstream product of tryptophan breakdown, quinolinolate; 2) IFN-γ enhances transcriptional expression of all of these enzymes, although posttranslational inactivation of IDO may prevent metabolic steps that are subsequent and consequent to IDO; 3) overcoming the IDO-dependent blockade by provision of a downstream quinolinate precursor activates the pathway and leads to the onset of suppressive properties; and 4) tolerogenic DCs can confer suppressive ability on otherwise immunogenic DCs across a Transwell in an IDO-dependent fashion. Altogether, these data indicate that kynurenine pathway enzymes downstream of IDO can initiate tolerogenesis by DCs independently of tryptophan deprivation. The paracrine production of kynurenines might be one mechanism used by IDO-competent cells to convert DCs lacking functional IDO to a tolerogenic phenotype within an IFN-γ-rich environment.

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CD8+ DCs had effects comparable to pharmacologic inhibition of the enzyme. Perhaps of greater interest, initiating the kynurenine pathway in CD8+ DCs downstream of IDO led to the production of QUIN and imparted tolerogenic properties to those cells in the absence of tryptophan consumption. Thus, similar to CD8+ DCs, their CD8+ counterparts are competent for the portion of the pathway that is subsequent to IDO, and can further metabolize the initial kynurenine when provided externally. This suggests that the paracrine production of kynurenines could be one mechanism whereby IDO-competent cells affect the activity of DCs in which functional IDO is blocked postranslationally.

Materials and Methods

Mice and reagents

Female DBA/2J (H-2d) mice were obtained from Charles River Breeding Laboratories. Synthetic kynurenines (used in vitro at 10 or 100 μM according to the experimental design) and racemic 1-MT (27) were synthesized and purified as described previously (22, 25). The CD8+ (27) fraction was −45% CD4+ and typically contained <0.5% contaminating CD8+ cells. Less than 1% CD8+ and <5% CD8+ DCs expressed the B220 marker, respectively (26). DCs were exposed to 100 ng/ml IL-12 (CD8+ fraction was >99%) or 200 μM IFN-γ (CD8+ or CD8− DCs) for 24 h at 37°C in the presence or absence of 4 μM 1-MT (27). For immunization, cells were washed between and after incubations during the last 18 h of incubation. Cells were then re-covered, washed, and immediately used for in vitro or in vivo experiments. Control treatment consisted of Negative Control siRNA (Ambion).

DC preparation, treatments, and immunization

Splenic DCs were purified by magnetic-activated sorting using CD11c MicroBeads and MidiMacs (Miltenyi Biotec) (22, 25). The CD8+ (27) fraction was −45% CD4+ and typically contained <0.5% contaminating CD8+ cells. Less than 1% CD8+ and <5% CD8+ DCs expressed the B220 marker, respectively (26). DCs were exposed to 100 ng/ml IL-12 (CD8+ DCs) or 200 μM IFN-γ (CD8+ or CD8− DCs) for 24 h at 37°C in the presence or absence of 4 μM 1-MT (27). For immunization, cells were washed between and after incubations before peptide loading (5 μM, 2 h at 37°C), irradiation, and i.v. injection into recipient hosts. A total of 3 × 105 IL-12-treated CD8− DCs was injected in combination with 5% IFN-γ-treated CD8+ or CD8− DCs.

Small interfering RNA (siRNA) synthesis and transfection

The siRNA sequences specific for murine Indo (sense, 5′-GGAGGUCUCUCUCUCGUCUUC-3′; antisense, 5′-AGAGAGAGAAGAAGAGCCT-3′) were selected, synthesized, and annealed by the manufacturer (Ambion). For transfection, siRNAs (6.7 μg) in 30 μl of transfection buffer (20 mM HEPEs, pH 7.4) were pipetted into a sterile Eppendorf tube. In a separate polystyrene tube, 6.7 μg of 1,2-dioleoyl-3-trimethyl-ammonium-propane were mixed with 30 μl of transfection buffer, and then both solutions were mixed gently by pipetting several times. After incubation at room temperature for 20 min, the mixture was added to 1 ml of complete medium containing 105 DCs and incubated for 4 h at 37°C, with or without Indo-γ during the last 18 h of incubation. Cells were then recovered, washed, and immediately used for in vitro or in vivo experiments.

PCR analysis

RT-PCR analysis was performed (30 cycles, annealing temperature of 60°C) using sense and antisense primers, as follows: Indo sense, 5′-GAAGGATCTTGAGAGACAC-3′; Indo antisense, 5′-GAAGGTCGGGATTTCCACA-3′; Sod2 sense, 5′-AGAGCTGCGTATCCGACTATG-3′; Sod2 antisense, 5′-AGGGAAATTAGAGCTGTACG-3′. Real-time PCR were run on a Chromo4 Four-Color Real-Time Detector (Bio-Rad) using specific primers as follows: Indo sense, 5′-GAAGGATCTTGAGAGACAC-3′; Indo antisense, 5′-GAAGGTCGGGATTTCCACA-3′; Kynu sense, 5′-AAATTCTCCTGGGCTCTAAAC-3′; Kynu antisense, 5′-GCGGTGTTGTACATCAGTG-3′; Kynu sense, 5′-GAAGGATCTTGAGAGACAC-3′; Kynu antisense, 5′-GAAGGTCGGGATTTCCACA-3′; Kynu sense, 5′-GCGGTGTTGTACATCAGTG-3′; Kynu antisense, 5′-GAAGGATCTTGAGAGACAC-3′; Kynu sense, 5′-GAAGGATCTTGAGAGACAC-3′; Kynu antisense, 5′-GAAGGATCTTGAGAGACAC-3′. PCR products were normalized to murine Gapdh using specific primers (sense, 5′-GCCTTGCCGTGTTTCCACCC-3′; antisense, 5′-CAGTGGGGCCCTCAGATGTC-3′).

Kynurenine and quinolinic acid measurements

These procedures, using gas chromatography with electron capture negative ionization mass spectrometry (GC/MS), have previously been described in detail (28, 29). Briefly, 10 μl of culture supernatant was diluted (1/5, v/v) in water. Fifty microliters of internal standard (100 nM 3,5-dihydroxybenzonic acid and 200 nM homophenylalanine) were added, and proteins were precipitated with HCl (25 μl, 5 N). Fatty compounds were removed by extraction with chloroform (100 μl). After centrifugation (10 min, 10,000 × g), 100 μl of the acidic supernatant were added to a glass tube containing 50 μl of 62.5 mM tetrabutylammonium hydrogen sulfate. The mixture was lyophilized and resuspended in 25 μl of acetone containing 7.5% of disopropylthylamine and 3% pentafluorobenzyl bromide. The samples were incubated in scintillation vials at 37°C for 15 min at 60–65°C. Fifty microliters of decane and 750 μl of water were then added, the samples were thoroughly mixed, and the decane phase was removed. One microliter of the decane phase was injected into a GC/MS system consisting of a Trace GC coupled with a Trace MS quadrupole mass spectrometer (ThermoFinnigan). Chromatographic separation was achieved using a 30-m Rtx-5MS capillary column (0.25-mm internal diameter, 0.25-μm film thickness; Restek) with helium as a carrier gas. A split/splitless injection port (1-μl injection volume) was used. The temperature program was as follows: 155°C for 1.25 min, 40°C/min to 275°C, 10°C/min to 320°C, 1 min at 320°C, injection port 228°C. The GC/MS uses electron capture negative ion chemical ionization mass spectrometry with methane as the reagent gas. The ion source temperature was 220°C. Selected ion monitoring was performed by recording signals of characteristic (M−PFBB−) ions.

Skin test assay

A skin test assay was used for measuring class I-restricted delayed-type hypersensitivity responses to synthetic peptides as described previously (22, 30). Results were expressed as the increase in footpad weight of peptide-injected footpads over that of vehicle-injected counterparts. Values (mean ± SD) are compiled from independent experiments with at least six mice per group per experiment. The statistical analysis was performed using Student’s paired t test by comparing the mean weight of experimental footpads with that of control counterparts.

Induction of apoptosis by IDO+ DCs

Conditions for inducing and assaying IDO-dependent apoptosis have been described previously (32). Briefly, CD8+ or CD8− DCs (2 × 105), treated overnight with IFN-γ in the presence or absence of 10 μM Indo-γ-kynurenine (KYN), were washed and cultured for 4 h with 4 × 105 F76 cells, a Th1-type P815AB-specific CD4+ T cell clone, in the presence of 5 μM P815AB peptide. At the end of the coculture, the CD11c+ cells were removed by the use of CD11c MicroBeads (Miltenyi Biotec), and the remainder of the population was surface stained with anti-CD4-PE and FITC-labeled annexin V and propidium iodide (BD Pharmingen). For measurement of apoptosis, a gate was set on CD4+ T cells, and the percentage of cells in the very early stages of apoptosis was determined by annexin V staining excluding PI+ cells.

Transwell DC conditioning

DC conditioning by IDO-competent cells was performed using a 6.5-mm Transwell system (Corning) with a 0.4-μm pore polycarbonate membrane insert. CD8− DCs (105) were added to the lower chamber, while CD8+ DCs (105) were added to the upper chamber. Cultures were set up in the presence of 200 μM IFN-γ, and, after 18 h, CD8+ DCs were recovered, extensively washed, and used for in vivo sensitization.

Results

Indo silencing and 1-MT have comparable effects on functional IDO in vitro and in vivo

IDO catalyzes the initial and rate-limiting step in tryptophan degradation to QUIN through the sequential formation of KYN, 3-hydroxykynurenine (3-HK) or alternatively antrahnic acid (AA), and 3-HAA (Fig. 1). Thus, pharmacologic blockade of IDO by the use of 1-MT results in impaired formation of KYN and the downstream metabolites including QUIN. siRNA technology is a means of posttranscriptional gene silencing that can be used to modulate gene expression in murine DCs (25, 27). To comparatively examine the effects of 1-MT and posttranscriptional Indo silencing, splenic DCs were fractionated and the CD8+ fraction, treated or not with IFN-γ, was transfected with Indo-specific or control
siRNA, resulting in efficient and selective inhibition of *Indo* transcript expression at 48 h of transfection (Fig. 2A). This was accompanied by inhibition of *IDO* function as measured by the production of KYN by IFN-γ-treated CD8⁺ DCs (Fig. 2B) as well as by their ability to induce apoptosis of a Th1 clone, as documented previously (32, 33) (Fig. 2C). All of these functional effects in vitro were mimicked by the use of 1-MT in place of *Indo* siRNA.

FIGURE 1. Kynurenine pathway of tryptophan catabolism in mammalian cells, in which IDO catalyzes the initial and rate-limiting step under transcriptional regulation by IFN-γ. Indicated in the figure are the enzyme activities, and the corresponding genes, leading to the production of immunologically relevant metabolites.

DC populations in the spleens of DBA/2 mice consist of CD8⁻ (~90%) and CD8⁺ (~10%) fractions that mediate the respective immunogenic and tolerogenic presentation of the synthetic tumor/self nonapeptide P815AB. Upon transfer into recipient hosts, peptide-loaded CD8⁻ DCs initiate immunity, and CD8⁺ DCs initiate anergy, when Ag-specific skin test reactivity is measured at 2 wk after cell transfer (30, 31). On cotransferring cytokine-conditioned DCs of the two subsets, the IDO-inducing tolerogenic potential of IFN-γ acting on CD8⁺ DCs enables these cells to prevail over the adjuvant properties of IL-12 acting on CD8⁻ DCs, resulting in lack of skin test reactivity (22, 34–36). P815AB-pulsed, IL-12-treated CD8⁻ DCs were injected in combination with 5% CD8⁺ DCs, either untreated or treated with IFN-γ, with or without concomitant 1-MT or *Indo* siRNA treatment (Fig. 2D). The results showed that IFN-γ was an absolute requirement for the occurrence of suppressive effects by CD8⁺ DCs cotransferred with IL-12-primed CD8⁻ DCs. However, the effect was equally ablated by treatment of the tolerogenic fraction with 1-MT or *Indo* siRNA before DC cotransfer. This confirms that IFN-γ is a major pharmacologic target of racemic 1-MT in our experimental setting of CD8⁺ DC conditioning in vitro with IFN-γ.

Detection of transcriptional but not functional expression of kynurenine pathway enzymes in CD8⁻ DCs treated with IFN-γ

Using RT-PCR, we previously showed that IFN-γ activates *Indo* transcription in both CD8⁻ and CD8⁺ DCs, resulting in comparable expressions of IDO protein in the two subsets (21, 23). In this

FIGURE 2. *Indo* silencing has effects comparable to 1-MT on functions mediated by IDO in vitro and in vivo. A, Kinetic RT-PCR analysis of *Indo* expression in CD8⁻ DCs treated with *Indo*-specific siRNA in the presence or absence of IFN-γ. Control cells were treated with negative control (nc) siRNA. Expression of the IFN-γ-inducible *Sod2* gene was also assayed as a specificity control. B, Effect of IFN-γ on tryptophan (100 μM) conversion to KYN in CD8⁻ DCs previously subjected to 1-MT treatment or *Indo* silencing by siRNA for 48 h. KYN levels in 5-h supernatants were measured by GC/MS, and the results are mean ± SD of three different experiments, each performed in triplicate. *p < 0.005 (IFN-γ vs medium treatment). C, Effect of IFN-γ silencing on IDO-dependent apoptosis mediated by IFN-γ-treated CD8⁻ DCs. Apoptosis was measured in Ag-specific CD4⁺ T cells cultured with CD8⁻ DCs pre-exposed to IFN-γ with or without *Indo* siRNA or 1-MT. Representative dot plots are shown of annexin V staining of gated CD4⁺ PI⁻ cells. Numbers within boxes indicate percentages of CD4⁺ apoptotic cells in one experiment representative of three. The fold increase values in apoptosis rate induced by IFN-γ in the remaining experiments were 2.9 and 2.5, respectively, and these values decreased to <1.1 upon *Indo* silencing. D, *Indo* silencing ablates the tolerogenic potential of CD8⁻ DCs. Combinations of IL-12-treated CD8⁻ and CD8⁺ DCs subjected to various treatments (indicated) were loaded with P815AB and injected into recipient mice to be assayed at 2 wk for skin test reactivity to the eliciting peptide. *, p < 0.0001 (experimental vs control footpads). Compiled data from three independent experiments.
study, we used real-time PCR for quantitative and comparative assessment of Indo expression in CD8⁻ and CD8⁺ DCs and extended the examination to a series of genes coding for enzymes downstream of IDO, namely Kno (coding for kynurenine 3-monooxygenase), Kynu (kynureninase), and Haao (3-hydroxyanthranilate 3,4-dioxygenase) (Fig. 3A). The results confirmed enhanced transcriptional activity of Indo by IFN-γ in CD8⁻ DCs, to an extent comparable to CD8⁺ counterparts. Similar to Indo albeit to a lesser extent, the expressions of Kmo, Kynu, and Haao were all enhanced by IFN-γ in both DC subsets. Despite the increase in gene transcription, IFN-γ induced little or no production of KYN and QUIN in CD8⁻ DCs (Fig. 3B), possibly accounting for the failure of the recombinant cytokine to promote tolerogenic properties in this subset (21, 23, 35). In contrast, in CD8⁺ DCs, IFN-γ resulted in early (5 h) production of KYN, followed by later (18 h) detection of high levels of QUIN. Collectively, these data indicated that DCs are endowed with the whole enzymatic machinery necessary for the production of one of the end products of the kynurenine pathway, QUIN. However, these data do not clarify whether the inability of IFN-γ to activate the pathway in the CD8⁻ subset of DCs is due solely to an inefficient rate-limiting step (i.e., the one mediated by IDO) or to a combination of the latter with posttranscriptional regulation of downstream enzymes.

**KYN conversion into QUIN occurs in CD8⁺ DCs, resulting in tolerogenic properties**

We investigated whether the enzyme response induced by IFN-γ downstream of IDO in CD8⁻ DCs is associated with the production of QUIN when the precursor KYN is added to the culture medium. We first validated the model by adding KYN to a culture of Indo siRNA-treated CD8⁺ DCs and found that the addition of the external precursor was indeed associated with the production of QUIN when the cells had been exposed to IFN-γ (Fig. 4A). Similar to the latter cells, CD8⁻ DCs produced high levels of QUIN if cotreated with IFN-γ and KYN. The ability of CD8⁺ DCs to release QUIN in response to IFN-γ and the externally added precursor prompted us to investigate whether synthesis of QUIN was associated with the acquisition of properties typical of the tolerogenic CD8⁺ DC subset. In the experimental setting illustrated above, we measured apoptosis of CD4⁺ cells cultured with CD8⁻ DCs either untreated or treated with IFN-γ and KYN, singly or in combination (Fig. 4B). The results revealed an ability of the CD8⁻ DCs to induce apoptosis in ~25% of Th1 target cells upon coculture with IFN-γ and KYN. We also asked whether the CD8⁺ DCs conditioned by KYN and IFN-γ treatment would be capable of suppressing properties of IFN-γ in vivo when cotransferred with otherwise immunogenic DCs. In an experimental model system similar to that of Fig. 2D, P815AB-pulsed and IL-12-primed CD8⁻ DCs were injected in combination with 5% CD8⁺ DCs cotreated with a mixture of IFN-γ and KYN, either alone or together with PNU 156561, a selective inhibitor of kynurenine 3-monooxygenase (Fig. 5A). The results showed that the combined effects of IFN-γ and KYN turned CD8⁻ DCs from immunogenic to suppressive, thus mimicking the behavior of the CD8⁺ DC subset. However, inhibiting the conversion of KYN into 3-HK, and thus preventing synthesis of QUIN, abolished the onset of suppressive properties in the CD8⁻ DC fraction. Of interest, the mice sensitized with the DC population containing 5% IFN-γ/KYN-treated CD8⁻ DCs were not amenable to subsequent immunogenic priming to the peptide when performed 1 mo after the first exposure (Fig. 5B). Again, this effect is similar to that observed with IFN-γ-treated CD8⁺ DCs (35) and indicates the onset of an Ag-specific tolerant state. Taken together, these data suggest that even in the absence of functional IDO, kynurenine pathways enzymes, if activated by IFN-γ in the presence of an appropriate precursor, catalyze the synthesis of QUIN, and the effect is associated with DC acquisition of tolerogenic properties.

**IDO-competent DCs transfer tolerogenic potential across a cell-impermeable membrane**

The finding that kynurenine pathway enzymes downstream of IDO can initiate tolerogenesis in DCs lacking functional IDO prompted us to investigate whether IDO-competent cells might turn otherwise immunogenic DCs into tolerogenic cells via soluble molecules. CD8⁻ DCs were exposed overnight to IFN-γ, and CD8⁻ DCs were added to the lower chamber of a Transwell system. The CD8⁻ cells had been treated with either negative control or Indo-specific siRNAs. The CD8⁻ DCs were then recovered and assayed in vivo for their ability to suppress induction of skin test reactivity.

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**FIGURE 3.** CD8⁻ DCs express kynurenine pathway enzymes but produce little or no KYN and QUIN. A. Induction of Indo, Kmo, Kynu, and Haao transcripts in CD8⁻ and CD8⁺ DCs by IFN-γ. Sorted CD8⁻ and CD8⁺ DCs were exposed to IFN-γ for 4 h. At the end of the culture, mRNA levels of genes related to the kynurenine pathway were quantified by real-time PCR using Gapdh normalization. Data (means ± SD of three experiments using triplicate samples) are presented as normalized transcript expression in the samples relative to normalized expression in the respective control cultures, i.e., cells unexposed to IFN-γ but maintained in medium alone (fold change = 1; dotted line). B. Conversion of tryptophan (100 μM) to KYN and QUIN by CD8⁻ vs CD8⁺ DCs in response to exposure to IFN-γ for 5 or 18 h. Values are means ± SD of three independent experiments performed in triplicate. * Undetectable levels.
Discussion

The kynurenine pathway is a major route of l-tryptophan catabolism, resulting in the production of nicotinamide adenine dinucleotide and other neuroactive (37, 38) and immunomodulatory (10) intermediates. Although all kynurenines are found in high concentration in urine, none of them has so far been assigned an important physiological function in peripheral organs. In the brain, the kynurenine pathway yields several intermediates, including the free radical generator 3-HK, the excitotoxic N-methyl-D-aspartate receptor agonist QUIN as well as its antagonist, kynurenic acid. Astrocytes, neurons, and microglia all express IDO, but only microglial cells are capable of producing substantial quantities of QUIN (39). Although KYN can be produced in the brain to a

FIGURE 5. The combined effects of IFN-γ and KYN confer tolerogenic potential in vivo to CD8^+ DCs. Combinations of IL-12-treated CD8^+ DCs and a minority fraction (5%) of the same cells not treated with IL-12 but exposed to 200 U/ml IFN-γ and 10 μM KYN were loaded with P815AB. A group of IFN-γ/KYN-treated CD8^+ DCs was also exposed to 20 μM PNU 156561, a selective inhibitor of kynurenine 3-monoxygenase. The cells were then transferred into recipient hosts. Mice were assayed for skin test reactivity on day 15 after cell transfer (A) or, alternatively, were treated on day 30 with an otherwise effective priming consisting of P815AB-pulsed CD8^+ DCs, to be assayed for skin test reactivity on day 45 (B). Compiled data from three experiments. *, p < 0.0001 (experimental vs control footpads).

FIGURE 6. IDO-competent DCs added across a Transwell to a culture of nontolerogenic cells induce suppressive properties in the latter via IDO-dependent mechanisms. CD8^+ DCs (10^6) were added to the upper chamber of a Transwell system, the bottom chambers of which contained an equal number of CD8^- DCs. rIFN-γ was present in the cultures at 200 U/ml, and the CD8^- DCs had been transfected with Indo siRNA or negative control (nc) siRNA. After 18 h, the recovered CD8^- DCs were pulsed with P815AB and added to a majority fraction (95%) of peptide-pulsed, IL-12-treated DCs. The cells were then transferred into recipient mice that were assayed at 2 wk for P815AB-specific skin test reactivity. The contents of the lower and upper chambers of the Transwell are indicated in the figure. Compiled data from three experiments. *, p < 0.0001 (experimental vs control footpads).
The pathway regulatory enzyme IDO in CD8
by DCs. The kynurenine 3-monooxygenase-dependent conversion
glia (37), our current data demonstrate that KYN is taken up
addition, similar to what was observed with astrocytes and micro-
tolerogenic phenotype with the function of a coordinately regu-
first to demonstrate the presence of a functional kynurenine path-
tolerogenic properties in vivo. Thus, the current study may be the
Activation of the pathway was accompanied by the appearance of
presence of KYN, a downstream precursor of the end product.
expression of kynurenine path-
tryptophan to QUIN (46, 47); yet, no information was previously
we have provided evidence that 1) IDO is the major relevant target
IDO inhibition consequent to IDO function; and 4) bypassing the IDO-
mediators of immunoregulation (18, 33, 42–44). In this study,
addition to IDO function, and the remainder pathway enzymes to immunosuppression (41),
several mechanistic features of the pathway that await further clar-
Among these are the relative contributions of IDO and the remainder pathway enzymes to immunosuppression (41),
IDO mechanism is quite general in nature, in that, for example, not only DCs but also CD4+ T cells (40) and neutrophils
IDO-competent DCs (IDO−) driven by IFN-γ-rich environment, either
by T cells (2, 22, 26, 27). Unlike soluble or cell-bound CTLA-4, IFN-γ is unable per se to induce functional IDO expression in CD8− DCs (21). Our current data demonstrate that, in the presence of IFN-γ, CD8− DCs can be rendered tolerogenic by the nearby production of kynurenines that are taken up by the cells and then metabolized to downstream products of the pathway. This mech-
mechanism is qualitatively different from that of CTLA-4, which acts on CD8− DCs to activate IDO, making it functionally responsive to IFN-γ up-regulation (21). On the one hand, the bulk of these data demonstrates that tryptophan consumption may not always be an absolute requirement for the DCs to exert suppressive properties.
On the other hand, it is possible that, to meet the needs of flexi-
ability and redundancy, the paracrine production of kynurenines is exploited by IFN-γ-competent DCs to convert DCs that are not com-
petent to a tolerogenic phenotype.
A model of cross-talk of DCs and T cells in tryptophan catab-
within an IFN-γ-rich environment is illustrated in Fig. 7. The model, far from detracting from the role of tryptophan star-
vation in mediating specific effects of the IDO mechanism (16), reinforces the concept that tryptophan deprivation and tryptophan

human macrophages are endowed with the ability to convert tryptophan to QUIN (46, 47); yet, no information was previously available regarding the functional expression of kynurenine pathway enzymes other than IDO in human or murine DCs. We found that the splenic CD8+ subset of murine DCs could be induced by IFN-γ to release high levels of QUIN. Despite functional lack of the pathway regulatory enzyme IDO in CD8− DCs, these cells could nevertheless be induced by IFN-γ to produce QUIN in the presence of KYN, a downstream precursor of the end product.
Activation of the pathway was accompanied by the appearance of tolerogenic properties in vivo. Thus, the current study may be the first to demonstrate the presence of a functional kynurenine pathway in murine DCs and, interestingly, to specifically associate the tolerogenic phenotype with the function of a coordinately regulated set of enzymes, as observed upon treatment with IFN-γ. In addition, similar to what was observed with astrocytes and microglial cells (37), our current data demonstrate that KYN is taken up by DCs. The kynurenine 3-monooxygenase-dependent conversion of KYN into QUIN by DCs further demonstrates that specific intracellular metabolism of kynurenines occurs in those cells. Of interest in this regard, we have also found that similar to KYN,
synthetic 3-HAA is also taken up by DCs, resulting in the production of QUIN (data not shown).

DCs have several modes at their disposal to mediate tolerogenic functions, and metabolic pathways of immune regulation are but one possibility (48). A distinctive feature of tryptophan catabolism as an effector mechanism of suppression is that its function is compatible with a directive role of Tregs in orchestrating peripheral tolerance (30). We have previously shown that the default immunogenic or tolerogenic program of Ag presentation by DC subsets is highly flexible, and that otherwise immunogenic DCs can be rendered tolerogenic by the actions of inhibitory ligands expressed by T cells (2, 22, 26, 27). Unlike soluble or cell-bound CTLA-4, IFN-γ is unable per se to induce functional IDO expression in CD8− DCs (21). Our current data demonstrate that, in the presence of IFN-γ, CD8− DCs can be rendered tolerogenic by the nearby production of kynurenines that are taken up by the cells and then metabolized to downstream products of the pathway. This mechanism is qualitatively different from that of CTLA-4, which acts on CD8− DCs to activate IDO, making it functionally responsive to IFN-γ up-regulation (21). On the one hand, the bulk of these data demonstrates that tryptophan consumption may not always be an absolute requirement for the DCs to exert suppressive properties. On the other hand, it is possible that, to meet the needs of flexibility and redundancy, the paracrine production of kynurenines is exploited by IFN-γ-competent DCs to convert DCs that are not competent to a tolerogenic phenotype.

A model of cross-talk of DCs and T cells in tryptophan catabolism within an IFN-γ-rich environment is illustrated in Fig. 7. The model, far from detracting from the role of tryptophan starvation in mediating specific effects of the IDO mechanism (16), reinforces the concept that tryptophan deprivation and tryptophan

![FIGURE 7. A model of cross-talk of DCs and T cells in tryptophan catabolism. In local tissue microenvironments, the activity of IDO competent DCs (IDO+) driven by IFN-γ will result in the sustained production of kynurenines (Kyn). These, in turn, could recruit other cell types to the regulatory response, including DCs in which the function of IDO, but not other kynurenine pathway enzymes, is inhibited posttranslationally (IDO−). The combined effects of tryptophan (Trp) starvation (16), caused by IDO− DCs, and the high kynurenine production, resulting from the actions of IDO+ and IDO− DCs, is expected to induce a great variety of effects on target T cells (18, 33, 44) and other cell types (42, 43). Tregs would have a crucial role in establishing an IFN-γ-rich environment, either via CTLA-4/B7-dependent interaction with DCs (30) or by direct production of the cytokine (49). Because the combined effects of tryptophan starvation and tryptophan catabolites include the induction of a regulatory phenotype in naive CD4+ T cells (51), an IDO-based positive feedback loop might be locally operative, resulting in Treg generation by Tregs (dotted line).](http://www.jimmunol.org/)

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catabolites may act singly or in combination to establish a regulatory environment most suitable to the control of local inflammatory or systemic T cell-mediated responses. The model also emphasizes the likely dual role of tryptophan catabolism both as an effector system (30, 49) and an inducer of Treg activity (7, 44, 50, 51). Because I-MT has been shown to act synergistically with cytoreductive chemotherapy in an experimental tumor setting (9), the identification of proper enzyme targets for pharmacologic inhibition of the kynurenine pathway may pave the way to improved strategies of antitumor chemotherapy (13). More generally, understanding the molecular mechanisms that preside over control of IDO expression by DCs may provide new opportunities for designing ways to induce or abrogate tolerance in physiopathologic conditions (21, 25, 52).

Disclosures

The authors have no financial conflict of interest.

References


