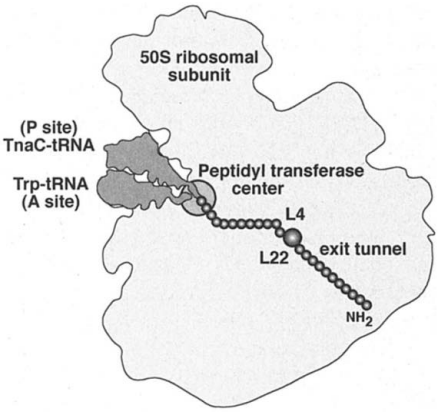

Fig. 3. Schematic representation of the 50S ribosomal subunit with TnaC-peptidyl-tRNA^{Pro} in the P site and a decoding Trp-tRNA^{Trp} in the A site. A segment of the peptidyl portion of TnaC-peptidyl-tRNA^{Pro} and the tryptophanyl moiety of Trp-tRNA^{Trp} are placed in the peptidyltransferase center. We assume the narrowest part of the exit tunnel formed with ribosomal proteins L4 and L22 (*1*, *28*, *30*) responds to the segment of TnaC containing the crucial residue W12 (enlarged circle) by altering features of the peptidyltransferase center, creating a tryptophan induction site.

gation factor Tu, guanosine triphosphate, and/or the associated tRNA^{Trp} contribute to Trp-tRNA^{Trp} binding in the ribosomal A site; this would account for the effectiveness of Trp-tRNA^{Trp} at lower concentrations than free tryptophan. The induction site could be a newly created site that specifically recognizes tryptophan. Alternatively, the peptidyltransferase center could be displaced or altered during synthesis of TnaC-peptidyl-tRNA^{Pro}; thus, when tryptophan enters the center, peptidyltransferase activity is inhibited. It is unlikely that the induction site is formed in the ribosomal D site, a newly identified site proposed to participate in the initial step in decoding (*23*). Our findings strongly suggest that information inherent in the sequence of the nascent TnaC peptide chain is communicated to the translating ribosome and that this information is used to mediate a response to tryptophan binding at the ribosomal A site. Peptide-ribosome interactions of this type could regulate the rate of peptide chain elongation, facilitate cotranslational protein folding (*24*), or, as in the *tna* operon, allow tryptophan to compete with a release factor and force ribosome stalling at a transcript site required for Rho factor binding.

Our findings and those of other investigators therefore attribute to the translating ribosome the ability to sense features of a nascent peptide and of responding by altering one or more events in ribosome action (Fig. 3). Inhibition of peptidyl-tRNA transfer or cleavage may also occur during translation of the uORF (upstream open reading frame) preceding an *arg* gene of fungi (*25*), the uORF2 preceding a gene of the human cytomegalovirus (*26*), and the uORF preceding the coding region for mammalian *S*-adenosylmethionine decarboxylase (*27*). Nascent peptides have also been described that inhibit translation elongation (*6*, *28*, *29*). These examples illustrate ribosomal versatility in mediating regulatory decisions.

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18. S-30 reaction conditions have been described (*15*). A circularized DNA fragment bearing the intact *tna* promoter and the *tnaC* coding region, followed by the *rpoC* terminator sequence, was the template (*15*). Mutations in the *tnaC* coding region were introduced by PCR.
19. This radiolabeled peptidyl-tRNA is ~25 kD judged by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). Its position is distinct from that of the free peptide; it migrated comparably to TnaC-peptidyl-tRNA^{Pro} previously identified by ribonuclease treatment and RT-PCR (*15*). Labeled TnaC-peptidyl-tRNA^{Pro} is shown in Fig. 1B.
20. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Potential Regulatory Function of Human Dendritic Cells Expressing Indoleamine 2,3-Dioxygenase

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Antigen-presenting cells (APCs) can induce tolerance or immunity. We describe a subset of human APCs that express indoleamine 2,3-dioxygenase (IDO) and inhibit T cell proliferation in vitro. IDO-positive APCs constituted a discrete subset identified by coexpression of the cell-surface markers CD123 and CCR6. In the dendritic cell (DC) lineage, IDO-mediated suppressor activity was present in fully mature as well as immature CD123^{+} DCs. IDO^{+} DCs could also be readily detected in vivo, which suggests that these cells may represent a regulatory subset of APCs in humans.

Professional APCs, in particular DCs, are key regulators of the choice between tolerance and immunity (*1*). It has been proposed that immature or resting (steady state) DCs may present antigen in a tolerogenic fashion, whereas mature (activated) DCs drive T cell immunity. Immature DCs may promote tolerance in part by presenting antigens without the costimulatory signals required for full T cell activation (*2*). Alternatively, it has been hypothesized that a specialized subset of mature DCs might actively divert T cell responses toward tolerance (*3*). However, in humans no such subset has been defined, and the molecular mechanisms by which such cells might function remain unclear. We and others have recently shown that

cells expressing the tryptophan-catabolizing enzyme IDO are capable of inhibiting T cell proliferation in vitro (*4*, *5*) and reducing T cell immune responses in vivo (*6*–*10*). In the current study, we explored whether expression of IDO might define a particular subset of regulatory human APCs.

Using an IDO-specific antibody [fig. S1 and supporting online text (*11*)], we found by flow cytometry that fresh human monocytes expressed low to undetectable levels of the protein (Fig. 1A). Monocyte-derived macrophages (Mϕs) (*4*) up-regulated IDO upon activation with interferon-γ (IFN-γ). Expression of IDO in these cells was confined to a particular subset of cells coexpressing CD123 [the interleukin-3 (IL-3) receptor α chain] and the chemokine receptor CCR6 (Fig. 1A). Similarly, monocyte-derived DCs (*12*) expressed IDO, which was also confined to a $CD123^+$, $CCR6^+$ subset.

Because serum factors are known to influence DC maturation (*13*), we derived DCs in both bovine calf serum (BCS)–based medium and serum-free medium (SFM). Both systems yielded IDO^+ DCs with the same phenotype, but >90% of the IDO^{NEG} cells in SFM were tightly adherent. This allowed facile enrichment of the nonadherent IDO^+ population to >90% purity (*14*). The IDO^+ cells expressed cell-surface markers ($CD14^{NEG}$, $CD83^+$, $CD80^+$, $CD86^{HI}$, $HLA\text{-}DR^{HI}$) (Fig. 1B) and morphology consistent with mature DCs (Fig. 1C). Adherent cells lacked CD83 and displayed residual levels of CD14, consistent with an immature or transitional phenotype. Although IDO^+ cells in DC cultures expressed DC-specific lineage markers, and the IDO^+ Mϕs expressed Mϕ-lineage markers (Fig. 1D), in both types of cells the IDO^+ subset could be specifically identified by expression of CD123 and CCR6.

As shown previously by our group (*4*), resting macrophage colony-stimulating factor (MCSF)–derived Mϕs did not express high concentrations of IDO until they received a triggering signal such as IFN-γ (Fig. 2A). In contrast, IDO could be detected constitutively in $CD123^+$ DCs (Fig. 2B). However, activation with IFN-γ was still required for expression of functional enzymatic activity (Fig. 2E), which suggests that the IDO protein could exist in both enzymatically active and inactive states (*15*). Because the maturational status of DCs may affect a number of functional attributes of these cells, we asked whether maturation affected IDO expression by $CD123^+$ DCs. Although maturation itself had no effect on the constitutive (basal) expression of IDO protein, subsequent activation of mature DCs with IFN-γ resulted in complete down-regulation of IDO. This was a consistent observation in 16 experiments with 10 different donors and was confirmed by flow cytometry (Fig. 2C), enzymatic activity (Fig. 2E), and mRNA (*16*).

Interleukin 10 (IL-10) is a regulatory cytokine that has been associated with the development of tolerogenic DCs (*17*). The presence of IL-10 during maturation prevented IFN-γ–induced down-regulation of IDO, resulting in sustained expression of functional IDO even in mature, IFN-γ–activated DCs

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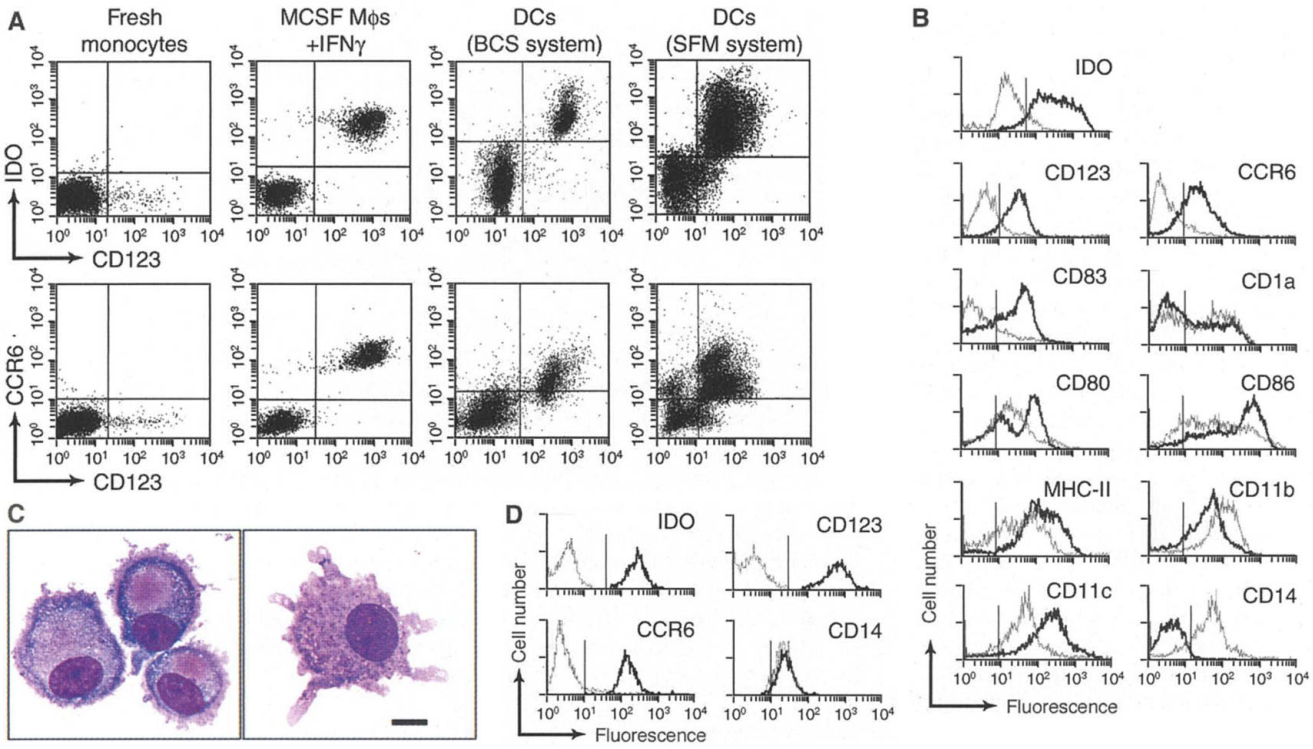

Fig. 1. Expression of IDO by APCs. (**A**) Human monocytes were analyzed without culture (fresh, $n = 12$); cultured (*14*) for 7 days in MCSF with IFN-γ added for the final 18 hours (Mϕs + IFNγ, $n = 8$); or cultured in granulocyte-macrophage CSF + IL-4 (DCs) in BCS medium ($n = 34$) or SFM ($n = 24$). (Upper) IDO versus CD123; (lower) CCR6 versus CD123 on the same triple-stained cells. Negative control for IDO staining was the primary antibody preadsorbed with the immunizing peptide. (**B**) Immunophenotype of nonadherent (IDO^+) DCs (dark lines) versus adherent cells (light lines) from SFM cultures [matured with tumor necrosis factor–α/IL-1β/IL-6/prostaglandin E_2 (*14*)]. (**C**) Morphology of adherent (left) and nonadherent (right) cells (cytocentrifuge preparations, Wright's stain; scale bar, 10 μm). (**D**) Immunophenotype of MCSF-derived Mϕs, gated separately on the $CD123^+$ (dark lines) and $CD123^{NEG}$ (light lines) populations.

(Fig. 2, D and E). [We observed similar results when transforming growth factor–β was present during maturation (*16*).] Taken together, these data raised the possibility that expression of IDO by mature DCs might be determined by the prevailing regulatory influences during maturation.

We next tested IDO$^+$ DCs for their ability to stimulate T cells in allogeneic mixed-leukocyte reactions (MLRs). In Fig. 2, B to E, recombinant IFN-γ was added to simulate signals from activating T cells (*4*), but for MLRs the DCs received no exogenous IFN-γ (*11*). Immature DCs, selected and enriched to >90% purity for IDO expression (Fig. 1B), stimulated very little T cell proliferation (Fig. 2F). However, in most donors proliferation could be significantly enhanced by addition of 1-methyl-tryptophan (1MT), a competitive inhibitor of IDO (donor-to-donor variability and the use of 1MT are discussed in (*11*). After maturation, enriched IDO$^+$ DCs displayed one of two patterns: in 4 of 45 experiments the mature DCs lost their IDO-mediated inhibitory activity (Fig. 2F), whereas in 41 of 45 experiments they maintained potent inhibitory activity despite maturation, which was reversed by the addition of 1MT (Fig. 2G). Continued expression of IDO by mature DCs in the latter experiments was confirmed by flow cytometry on MLR cultures and by measurement of tryptophan and kynurenine in supernatants (*16*). The two different patterns observed in MLR were suggestive of the different patterns observed when mature DCs were tested in isolation (Fig. 2, C and D), as discussed in (*11*).

We performed the experiments in Fig. 2G with highly enriched IDO$^+$ DCs from SFM cultures. In contrast, BCS medium yielded a mixture of nonadherent IDO$^+$ and IDO^{NEG} cells, with IDO^{NEG} cells typically in the majority. Under these conditions, T cell activation predominated, and 1MT had little detectable effect (Fig. 2H). However, when the IDO$^+$ DCs were enriched from such mixtures by sorting for CD123 expression, they displayed inhibitory activity comparable to the IDO$^+$ DCs from SFM (Fig. 2H).

To verify the specificity of 1MT as an inhibitor of IDO, we added 1MT to MLRs containing APCs that did not express inhibitory amounts of IDO (adherent cells from SFM cultures, <10% IDO$^+$). Under these conditions, T cell proliferation was not inhibited, and 1MT had no effect on T cell proliferation (Fig. 2I).

In vivo, we detected few IDO$^+$ cells in normal lymphoid tissue (fig. S3). However, human tonsils displaying features of chronic inflammation often possessed intense focal infiltrates of IDO$^+$ cells (Fig. 3A), which were morphologically distinct from Ham56$^+$ macrophages (*18*) or S100$^+$ interdigitating DCs (*19*). Some IDO$^+$ cells coexpressed CD83, a marker of mature DCs, and some expressed CD123 and CCR6 (Fig. 3B). However, not all CD83$^+$ (or

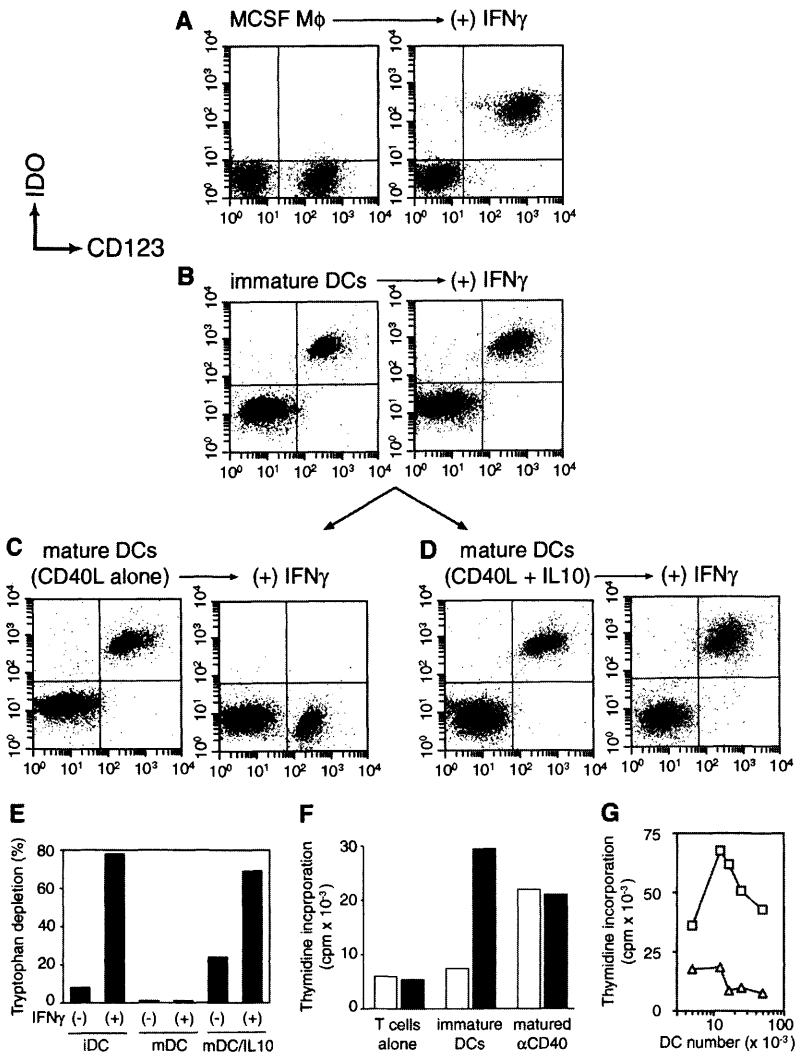

Fig. 2. Effect of DC maturation on IDO expression. (**A**) Monocyte-derived Mϕs (additional groups from Fig. 1A) were analyzed with (left) or without (right) IFN-γ added for the final 18 hours. (**B**) DCs (BCS system), with or without IFN-γ for 18 hours. (**C**) DCs matured with antibody to CD40 on days 5 to 7 (*14*), with or without IFN-γ for 18 hours. (**D**) As in (C), but with IL-10 added during the maturation step (*14*). (**E**) Functional enzymatic activity. Depletion of tryptophan from the culture medium (*4*) (expressed as a percentage of the starting tryptophan concentration in fresh medium, 25 μM) by DCs with or without IFN-γ for 18 hours. Immature DCs (iDC), CD40-matured DCs (mDC), and CD40-matured DCs in the presence of IL-10 (mDC/IL10) were generated, with or without IFN-γ activation, as in (B) to (D). (**F**) Allogeneic MLRs using enriched IDO$^+$ DCs (nonadherent cells, SFM system, without added IFN-γ). DCs were either immature or matured with antibody to CD40. APC/T cell ratio = 1: 20. White bars, without 1MT; black bars, with 1MT. (**G**) MLR with a titration of enriched IDO$^+$ DCs (nonadherent cells, SFM system, no IFN-γ) matured with cytokine-containing supernatant from activated monocytes (*14*). Similar results were observed when DCs were matured with the cytokine regimen used in Fig. 1B (*16*). Responder T cell number, 5×10^5 (highest APC/T cell ratio = 1:10) without 1MT (triangles),and with 1MT (squares). (**H**) Immunomagnetic sorting (*14*) of CD123$^+$ DCs from a mixed DC preparation [BCS system, tumor necrosis factor–α (TNF-α) matured, no IFN-γ]. Unfractionated (pre-sort), sorted CD123$^+$ cells (>80% purity), and CD123-depleted cells. APC/T cell ratio = 1: 10. White bars, with 1MT (DL-racemic mixture); black bars, without 1MT. (**I**) Adherent cells (<10% IDO$^+$) from SFM cultures, matured with TNFα/IL1β/IL6/prostaglandin E_2, used as stimulators in allogeneic MLRs. (White squares) without 1MT, (black squares) with 1MT. For comparison, nonadherent (IDO$^+$) cells from the same culture are shown without 1MT (triangles). Representative of six experiments.

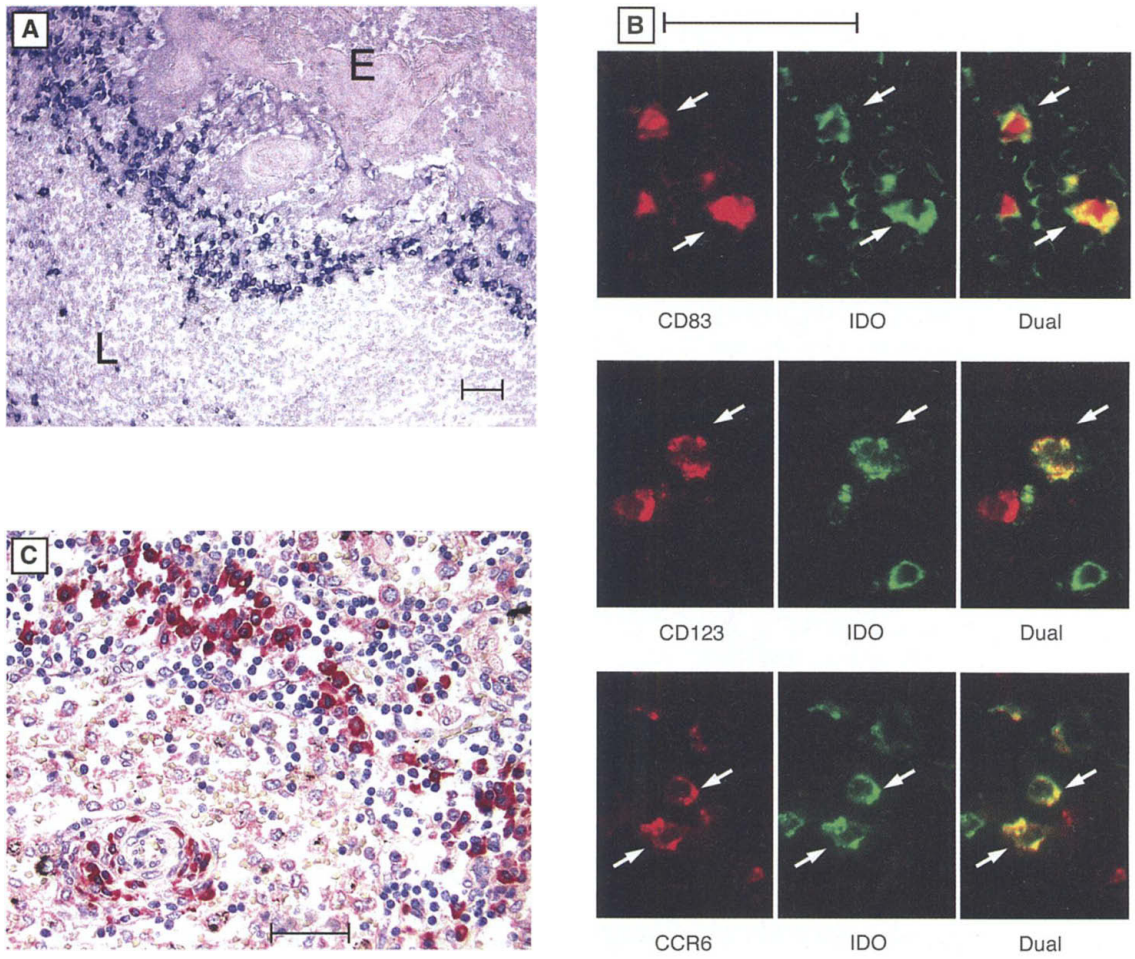

Fig. 3. IDO-expressing human cells in vivo (*14*). (**A**) Chronically inflamed tonsil (actinomycosis) showing accumulation of IDO$^+$ cells (blue) in lymphoid tissue (L) underlying the mucosal epithelium (E). (**B**) Expression of IDO by cells in tonsil coexpressing CD83, CD123, and CCR6 (dual exposure, merged image at right; arrows mark coexpressing cells). (**C**) Lymph node from a representative patient with malignant melanoma, showing accumulation of IDO-expressing cells (red). Scale bars, 50 μm.

CD123$^+$ or CCR6$^+$) cells expressed IDO, and no single marker identified all IDO$^+$ cells, which suggests that IDO may be expressed by more than one population in vivo (*11*). Analyses of regional and sentinel (first draining) lymph nodes (*11*) taken from patients with malignant melanoma revealed that 25 of 66 patients had one or more nodes with abnormal accumulation of IDO$^+$ cells (Fig. 3C). In many of these patients, accumulation of IDO$^+$ cells in the sentinel node occurred before overt metastasis (*11*). Similar accumulation of IDO$^+$ cells was found in nodes from patients with breast, colon, lung, and pancreatic cancers (*16*). The association of IDO$^+$ cells with draining lymph nodes of tumors is being further investigated by our laboratory.

In this study, we describe a subset of human monocyte-derived DCs that use IDO to inhibit T cell proliferation in vitro. In both DC and Mϕ lineages, IDO$^+$ cells could be characterized by coexpression of CD123 and CCR6 (despite the expression of otherwise distinct lineage-specific markers), which suggests that the IDO$^+$ population may represent a discrete subset of professional APCs. IDO$^+$ DCs expressed major histocompatibility complex class II and costimulatory molecules and were effective stimulators of T cell proliferation when IDO was blocked by 1MT, which suggests that these cells could act as competent APCs. We hypothesize that this may reflect a regulatory subset of APCs specialized to cause antigen-specific depletion (*20*) or otherwise negatively regulate the responding population of T cells. In light of the finding that large numbers of such cells are present in a proportion of tumor-draining lymph nodes, we speculate that IDO$^+$ APCs may participate in the state of apparent immunologic unresponsiveness displayed by many cancer patients toward tumor-associated antigens. However, the extent to which IDO-expressing APCs might influence immunologic unresponsiveness in vivo remains to be determined.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5588/1867/DC1
Materials and Methods
SOM Text
Figs. S1 to S3

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