The Journal of Immunology

RESEARCH ARTICLE | JUNE 15 2003

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J Immunol (2003) 170 (12): 5809–5813.
https://doi.org/10.4049/jimmunol.170.12.5809

THE JOURNAL OF IMMUNOLOGY

BRIEF REVIEWS

Tryptophan Catabolism and Regulation of Adaptive Immunity

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The immune system discriminates among multiple stimuli allowing some to provoke immune responses, leading to immunity, and preventing others from doing so, leading to tolerance. Immunological discrimination must emerge from processes that integrate contextual information from local tissue microenvironments to elicit appropriate responses to particular stimuli. Nevertheless, this network of integrated control mechanisms is not foolproof. Chronic infectious diseases, autoimmune disorders, and the pathologic state of tolerance displayed toward tumors all suggest a breakdown in these fundamental immunoregulatory processes. The potential to deliver improved clinical care to patients suffering from chronic inflammatory diseases and cancer provides considerable impetus to elucidate fundamental mechanisms underlying immune discrimination and regulation.

Immunoregulatory paradoxes—the keys to immunotherapy

Progress in elucidating cellular, molecular, and biochemical processes that regulate immune responses provides increasingly plausible explanations for the normal status of tolerance to self Ags that guards most humans from Ehrlich's imagined horror autotoxicus (1). Emerging data on regulatory T cells (Tregs)[2] and regulatory APC (APCregs) provide fertile ground for resolving some perplexing immunological paradoxes. (2–4). Thus, most people harbor potentially autoreactive T and B cells without suffering autoimmune diseases. This situation is reminiscent of the paradox of pathogen and tumor persistence in immunocompetent hosts. Unresponsiveness in these situations is puzzling, raising hopes that immunotherapeutic interventions might reverse tolerance. Tregs and APCregs may be the culprits in these cases, perhaps exploited by mechanisms evolved by pathogens, or acquired by tumors, to protect them from host immunity. Tumor survival and pathogen persistence in immunocompetent hosts has a counterpart in mammalian reproduction. Fetal tissues that express paternally inherited genes (foreign to the mother) are tolerated by the maternal immune system despite intimate association between maternal and fetal tissues during gestation. Recent reviews on Tregs and APCregs (cited above) provide excellent summaries of progress to elucidate their roles in immunoregulatory processes. However, molecular mechanisms underlying immunoregulatory phenomena remain elusive.

Clearly, no single mechanism can completely explain immune discrimination and regulation under a variety of circumstances. Nevertheless, in this review we focus exclusively on one specific mechanism that appears to play a key role in regulating T cell responses. This mechanism has, as its centerpiece, catabolism of the essential amino acid, tryptophan, a simple biochemical process that does not have immediately obvious mechanistic links to immunoregulatory processes. Tryptophan catabolism does not involve interactions between ligands and cell surface receptors and, at first sight, it might seem unlikely that tryptophan catabolism could contribute to discriminatory processes driving appropriate immune responses to specific stimuli. However, a growing body of experimental evidence points to the relevance of this biochemical process to immunoregulation. Moreover, these studies provide strong hints that exploiting this mechanism may permit novel immunotherapeutic interventions to alleviate chronic infectious diseases, cancer, autoimmune diseases, as well as therapies to suppress tissue allograft rejection. We have structured this review to summarize experimental evidence supporting the notion that tryptophan catabolism is mechanistically linked to immunoregulatory processes. This is followed by descriptions of working models to explain these links and we end by providing speculative views of the potential biological significance of tryptophan catabolism for understanding the mechanistic basis of some immunoregulatory processes.

Why is tryptophan catabolism relevant to regulation of adaptive immune responses?

We first posed this question while attempting to make sense of data from studies on human (macrophage colony stimulating factor-derived) macrophages that prevented T cell proliferation (5). An unusual feature of this system was that no tryptophan remained after macrophage-T cell coculture. Tryptophan is a rare but essential amino acid with features consistent with a role in control processes. Mammals possess two intracellular heme-containing enzymes, indoleamine 2,3-dioxygenase (IDO, EC 1.13.11.42) and tryptophan dioxygenase (TDO, EC 1.13.11.11) that catalyze oxidative catabolism of tryptophan

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Received for publication March 6, 2003. Accepted for publication April 9, 2003.

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[2] Abbreviations used in this paper: Treg, regulatory T cell; APCreg, regulatory APC; IDO, indoleamine 2,3-dioxygenase; TDO, tryptophan dioxygenase; DC, dendritic cell.

0022-1767/03/$02.00

(6). IDO is evolutionarily related to the myoglobin family of enzymes and oxygen-transporter proteins. Genes encoding TDO and IDO have distinct patterns of expression. IDO is expressed at basal levels in epididymis, thymus, gut, lung, and the maternal-fetal interface during gestation and is up-regulated in response to infection and tissue inflammation. TDO is expressed in hepatic cells and regulates homeostatic serum tryptophan concentrations, although TDO transcripts are present in the uterus during gestation (7). Recent data shows that IDO is expressed prominently in some subsets of myeloid cells including $CD11c^+CD8\alpha^+$ DCs in mice and $CD123^+$ DCs in humans (8, 9). Literature on IDO published before 1997 suggested that the biological role of IDO was to suppress microbial infections, by reducing tryptophan availability in infected tissues (6).

The tryptophan depletion hypothesis—immunoregulation by starvation?

Applying information about IDO to our macrophage-T cell system, we developed a model called the tryptophan depletion hypothesis to explain T cell unresponsiveness (5, 10). Basic features of this working model are that IDO activity in mature APCs does not affect T cell entry into cell cycle but reduced access to free tryptophan blocks cell cycle progression. Cell cycle arrest will prevent clonal expansion and may promote T cell death by apoptosis, induce T cell ignorance, anergy, and deviation, or generate Tregs. These notions lead to three key predictions: 1) APC subsets express IDO, 2) IDO^+ APCs block T cell cycle progression but not cell cycle entry, and 3) reduced access to tryptophan impedes T cell cycle progression. These predictions are amenable to experimental verification and evidence supporting the tryptophan depletion hypothesis follows.

Prediction 1: APC subsets express IDO. Several laboratories have documented IDO expression in cells from humans (9, 11–13) or mice (8, 14–17). IDO expression in cultured human dendritic cells (DCs) is restricted to nonadherent DCs that coexpress CD123 (IL-3R α-chain) and CCR6 (9). Unlike human macrophage colony stimulating factor-macrophages, $CD123^+$ DCs constitutively express IDO. Under standard laboratory culture conditions (GM-CSF, IL-4) a significant proportion of human DCs express IDO (9). In mice, IDO expression is associated with $CD11c^+CD8\alpha^+$ splenocytes, a DC subset that mediates immunoregulatory phenomena (15, 18). IDO protein expression in DCs does not always mean that IDO is enzymatically active (8, 9). Two-dimensional gel analyses of cell lysates from human IDO^+CD123^+ DCs revealed several isoforms of IDO protein suggesting that posttranslational modifications may influence IDO enzyme activity (9). Biochemical factors, such as nitric oxide, heme biosynthesis, and redox potential also affect IDO activity in cells (19, 20). These or other factors may explain why IDO enzyme activity was detected in murine $CD11c^+CD8\alpha^+$ but not $CD11c^+CD8\alpha^-$ DCs, even though similar amounts of IDO protein were detected in both subsets (8).

Recent reports reveal that IDO expression is up-regulated by ligands or Abs that bind and cross-link surface molecules expressed by APCs, including CD80/86, CD200R, and FcϵRI (13, 17, 21). Seminal discoveries that the immunomodulatory reagent CTLA4-Ig induces IDO expression in murine DCs via B7 ligation and that 1-methyl-tryptophan abrogates CTLA4-Ig-mediated suppression of islet allograft rejection (17) suggest that costimulatory blockade is not the sole mechanism by which CTLA4-Ig can mediate immunosuppression. Moreover, this discovery may provide a key clue to the biological significance of the IDO mechanism if cells expressing CTLA4, such as Tregs, induce IDO in APCs, effectively converting them into APCregs (22). Indeed, this mechanism could explain how Tregs suppress responses by other (naive) T cells indirectly without direct Treg-T cell interactions.

Prediction 2: IDO+ APCs block T cell clonal expansion. IDO expression in APCs correlates with weak T cell proliferation, enhanced apoptosis, and weak responses in vivo (5, 9, 11, 23, 24). In studies with human IDO^+ DCs and macrophages addition of the IDO inhibitor 1-methyl-tryptophan or excess tryptophan restored T cell proliferation (9, 11) 1-methyl-tryptophan enhanced T cell responses during murine gestation or following adoptive transfer of alloreactive T cells and abrogated regulatory processes that suppressed T cell responses to tumor-associated Ags, autoantigens in EAE, and liver allografts (15, 25–28). Genetic approaches complement studies using 1-methyl-tryptophan. Hence, cells transfected or transduced to over-express IDO acquire the ability to inhibit T cell proliferation in vitro and to suppress T cell responses to cell and tissue allografts in vivo (29–31). Preliminary data show that IDO up-regulation in transgenic mice protects male skin grafts from rejection by female recipients and that grafted females become tolerant to male-specific alloantigens (our unpublished data). Collectively, these observations support the notion that IDO activity promotes tolerance to tissue allo- and autoantigens in these systems. If, as has been suggested, IDO is active in cancer and HIV infection, inhibition of IDO activity should promote responses to tumor-specific and pathogen-encoded Ags (10).

As proposed, a key feature of the tryptophan depletion hypothesis is that IDO activity in mature APCs does not prevent T cell entry into cell cycle but prevents entry into S-phase by inducing cell cycle arrest. Experimental evidence supporting this notion comes from studies on T cells cultured with IDO^+ APCs or activated in tryptophan-free medium (5, 9, 16, 29). In these circumstances, T cells expressed early (CD69, CD25) but not late (CD71, cyclin D3, cdk4) markers of T cell activation. In addition, maturation of human $CD123^+$ DCs using a mixture of cytokines and other factors (TNF-α, IL-1β, IL-6, PGE_2) increased their underlying T cell stimulatory capability, but significant T cell proliferation was observed only if 1-methyl-tryptophan was present (9). Treating human $CD123^+$ DCs with anti-CD40 Abs down-regulated IDO and restored T cell proliferation in a minority of human PBLs but CD40 ligation alone was insufficient in most cases to overcome the inhibitory effects of IDO. Thus, maturation of $CD123^+$ DCs increased their underlying T cell stimulatory properties but did not render them less inhibitory, unless IDO activity was also down-regulated (9). This conclusion is not consistent with the simple paradigm that immature DCs always promote tolerance while mature DCs always promote immunity. Rather, IDO activity in some mature DCs appears to over-ride innate T cell stimulatory properties.

Prediction 3: tryptophan deficiency blocks T cell activation. Naive (primary) human and murine T cells activated in chemically defined tryptophan-free media entered the cell cycle but cell cycle progression arrested at the approximate mid-point of G_0-S phase transition (5, 16). In contrast, T cells entered S-phase before succumbing to the effects of amino acid deprivation when activated in media containing no leucine or isoleucine, even though the frequency of these two amino acids is

~10% while tryptophan accounts for only ~1% of amino acids. These outcomes suggest that T cells may be specifically sensitive to low levels of tryptophan during G_0-S phase transition, rather than generally susceptible to amino acid deprivation.

The mechanisms by which T cells sense and respond to low levels of tryptophan are not known. Originally, we proposed that IDO$^+$ APCs created a tryptophan-free zone in their microenvironment that would affect all bystander T cells, even those not in contact with APCs (10). However, it is difficult to envisage how Ag-specific effects would emerge from this model. We refined the model by proposing a requirement for intimate association between T cells and IDO$^+$ APCs, perhaps via the immunologic synapse. This refinement provides a potential explanation for directed, Ag-specific effects of IDO$^+$ APCs if tryptophan transport occurs through the synaptic region from T cells to APCs where it is catabolized by IDO. We have shown that IDO-transfected tumor cell lines inhibited T cell proliferation without completely depleting tryptophan from medium (29) suggesting that IDO-mediated suppression can occur in microenvironments where T cells have access to tryptophan. Further studies to address the subcellular location of cytoplasmic IDO and tryptophan transporters in APC-T cell conjugates are needed to shed light on these issues.

Human and murine T cells activated in tryptophan-free media display unique gene expression patterns relative to T cells activated in tryptophan-sufficient media when comprehensive gene-expression profiling is performed using DNA microarrays (our unpublished data). One potential mechanism is that T cells exploit the mammalian homologue of the yeast GCN2 pathway (32). GCN2 senses the ratio of amino acid charged to uncharged tRNA. Uncharged tRNA activates the kinase domain of GCN2, which phosphorylates the ribosomal initiation factor eIF2α (33), which in turn initiates a downstream stress-response pathway termed the Integrated Stress Response (34). Thus, the response to amino acid withdrawal is not simply metabolic shutdown, but an active, regulated and specific signaling pathway.

Alternative models—substrate deprivation or metabolite generation

The tryptophan depletion hypothesis is one of several models that could explain links between IDO activity and inhibition of T cell proliferation (35). IDO inhibits replication of intracellular pathogens and tumor cells by depleting tryptophan (19, 36–38). However, IDO also produces downstream metabolites (6) and high concentrations (250–1000 μM) of some tryptophan metabolites are toxic to T cells in vitro (12, 23, 39). However, these concentrations are considerably higher than actual metabolite concentrations produced by IDO$^+$ APCs, and higher than total precursor (tryptophan) concentration available in vitro or in vivo. In contrast, supplementing cultures containing IDO$^+$ APCs with excess (10× normal) tryptophan abrogates IDO-mediated inhibition of T cell proliferation (5, 9, 13, 19, 27). These outcomes appear to rule out significant contributions from toxic metabolites since more tryptophan yields more metabolites, yet it restores T cell proliferation. However, downstream metabolites might be selectively toxic for T cells following cell cycle arrest, or altered redox potentials due to consumption of reactive oxygen species (ROS) by IDO$^+$ APCs might influence T cell responses (20). Further research is needed to address these possibilities.

The biological significance of IDO expression—a way to reprogram immune responses?

Intriguingly, IDO is a common feature in immunological paradoxes mentioned previously when tolerance to fetal, tumor, and pathogen Ags occurs for obscure reasons. IDO expression is associated with chronic inflammation; persistent parasitic, viral, and bacterial infections; tumor growth; and pregnancy (6, 10). However, IDO is clearly not the only mechanism capable of regulating T cell responsiveness in these diverse situations. Rather, we propose that the IDO mechanism is one of several mutually reinforcing ways in which T cell responses are regulated according to the context in which T cells encounter Ags on APCs. However, tolerogenic properties of CTLA4-Ig may be IDO-dependent (17) and skin allografts were protected by upregulated IDO expression in CTLA4-Ig treated and IDO-transgenic mice (our unpublished data). These findings support the notion that IDO activity in APCs induced in response to tissue inflammation can have potent immunoregulatory effects that promote tolerance. Hence, the notion that IDO induction is a natural immunomodulatory process now rests on a firm foundation of evidence from several sources.

In this final section, we attempt to synthesize experimental evidence linking IDO activity and T cell suppression with current hypotheses to explain immunoregulatory phenomena. Of necessity, this exercise is speculative and relies on interpreting controversial issues in particular ways. Consequently, our aim is to provide one perspective on key and unresolved issues to stimulate further research into this immunoregulatory mechanism. To this end, we consider four hypothetical scenarios in which APCs acquire Ags and present them to naive T cells (Fig. 1).

In scenario A, APCregs present tissue Ags under homeostatic conditions, producing tolerant outcomes. Mechanistically, T cells may fail to divide, becoming anergic or ignorant to Ag encounter, undergoing apoptosis (via AICD), or differentiating into Th2 T cells or Tregs. Until recently, the dominant paradigm to explain tolerogenic outcomes under homeostatic conditions has been that immature APCs present Ags to naive T cells (1). However, recent reports suggest that some mature DC subsets may promote tolerogenic outcomes under homeostatic conditions, though the mechanisms used to promote tolerance rather than immunity are not yet known (4). Hence, IDO and

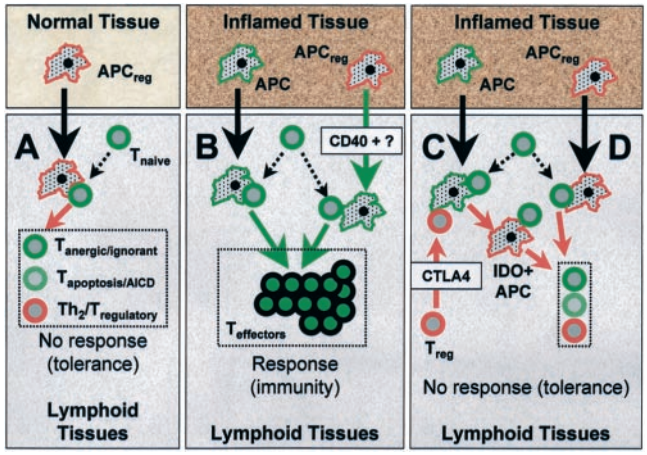

FIGURE 1. Immune outcomes following APC migration from normal (*A*) or inflamed (*B—D*) tissues to lymphoid tissues where they encounter naive T cells. Cells (symbols) and processes (arrows) are colored red or green, contingent upon whether they promote tolerance or immunity, respectively.

factors influencing DC maturation may promote tolerant outcomes during homeostasis but more research is needed to address this issue.

In scenario B, mature activated (stimulatory) APCs migrating from inflamed tissues promote naive T cells to proliferate and differentiate into effector T cells. In this case, IDO expression by APCs would not be desirable as this could compromise rapid elicitation of efferent responses needed to combat infections. Hence, IDO-dependent regulatory functions of APCregs may be abrogated by factors promoting APC maturation and IDO down-regulation, such as CD40 ligation and signaling through Toll-like receptors in combination with other factors that have yet to be defined.

In scenario C, we envisage that pre-existing Tregs expressing high levels of CTLA4 may interact with APCs generated during tissue inflammation and induce IDO expression in APCs via B7 ligation. In effect, this reprograms APCs to become APCregs. In turn, APCregs may promote naive T cells encountering Ags displayed by APCregs to become tolerized via mechanisms analogous to those proposed for tolerance maintenance during tissue homeostasis. A similar scenario was proposed by Finger and Bluestone (22) when considering the biological implications of CTLA4-Ig-mediated IDO up-regulation via B7 ligation on murine APCs. Verification of these notions will require experimental demonstration that $CTLA4^+$ T cells alter the functional status of APCs in the same way that CTLA4-Ig reprograms APC functions by up-regulating IDO (17).

Finally, in scenario D, we envisage that committed APCregs might act as a counterregulatory pathway to suppress T cell responses, even during episodes of tissue inflammation. In principle, this notion might explain why IDO expression is induced by inflammatory stimuli, such as IFNs (10). Even under inflammatory conditions, a population of IDO^+ APCs might help to reduce the risk of excessive responses to autoantigens. However, this beneficial immunosuppressive property of IDO^+ APCs could provide potential opportunities for exploitation by pathogens and tumors. These might evolve or acquire the ability to promote IDO expression in APCs or to selectively recruit them to sites of infection or tumor growth. For example, HIV infection induces IDO expression in macrophages (40), and both human and murine tumors appear to recruit IDO^+ cells into tumor-draining lymph nodes (9, 28). Selective recruitment of IDO^+ APCs may be beneficial to prevent autoimmunity but it could be the Achilles' heel that explains tumor and pathogen persistence in immunocompetent hosts.

In summary, analysis of these scenarios provides speculative explanations for the biological significance of IDO-dependent suppression of T cell responses, particularly under inflammatory conditions when thresholds for response are lowered and the need for discrimination between foreign and autoAgs is crucial. We surmise that the IDO mechanism may provide ways to help discriminate between innocuous and dangerous foreign Ags encountered under inflammatory conditions. IDO may also offer a molecular mechanism by which $CTLA4^+$ Tregs inhibit other T cells by reprogramming APC functions to favor tolerant outcomes. The danger in this system is that tumors and pathogens may have evolved ways to exploit this immunoregulatory mechanism for their own ends, to the detriment of patients.

Acknowledgments

We thank Pandelakis Koni for critical comments on this manuscript.

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