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Dendritic cells, indoleamine 2,3 dioxygenase and acquired immune privilege

Lei Huang¹, Babak Baban², Burles A Johnson III, and Andrew L. Mellor^{3,*}

¹Immunotherapy Center and Departments of Radiology, Medical College of Georgia, Augusta GA 30912. USA

²Pathology, Medical College of Georgia, Augusta GA 30912. USA

³Medicine, Medical College of Georgia, Augusta GA 30912. USA

Abstract

Dendritic cells (DCs) are specialized to stimulate T cell immunity. Paradoxically some DCs suppress T cell responses, and activate regulatory T cells. In this review we focus on a potent counter-regulatory pathway mediated by plasmacytoid DCs (pDCs) expressing the immunosuppressive enzyme indoleamine 2,3 dioxygenase (IDO). IDO-expressing pDCs inhibit effector T cell responses, activate regulatory T cells, and attenuate pro-inflammatory responses in settings of chronic inflammation that manifest in clinical syndromes such as infectious, allergic and autoimmune diseases, cancer, and transplantation. Thus IDO-expressing pDCs create immune privilege, and provide novel opportunities to improve immunotherapy in multiple disease syndromes.

1. Introduction

1.1 Immune stimulatory inflammation

Dendritic cells (DCs) are uniquely specialized to acquire, process, and present antigens to naïve and resting memory T cells leading to T cell activation, clonal expansion and differentiation of effector T cells. However some DCs suppress T cell responses after antigen presentation and T cell activation by promoting T cell apoptosis, and enhancing the development of T cells with regulatory functions, including CD4⁺ Foxp3-lineage T cells (Tregs). During homeostasis *immature* tissue DCs continuously acquire antigens, and migrate to local draining lymph nodes (dLNs) where they induce tolerance by stimulating abortive T cell responses, and activating Tregs (Fig. 1A). The default tolerogenic pathway is interrupted by local tissue inflammation caused by wounding or infection (Fig. 1B). In such settings DCs respond to inflammatory signals such as pro-inflammatory cytokines, and ‘danger’ signals that ligate Toll-Like Receptors (TLRs) causing DCs to mature and acquire potent T cell stimulatory functions that promote effective and lasting T cell immunity (1). However, it is unclear how self/non-self discrimination is maintained since mature DCs present self (tissue), as well as non-self (e.g. pathogen derived) antigens under T cell stimulatory conditions. Thresholds for immune responses to non-self antigens may be lower than for self antigens due to the absence of pre-existing tolerance. Counter-regulatory processes also predominate in late phases of inflammatory lesions, and tolerance to self antigens may be re-imposed at this stage, in parallel with generation of long term memory to non-self antigens.

*Corresponding Author: Andrew L. Mellor Ph.D. Director, Immunotherapy Center, Medical College of Georgia, Augusta GA 30912.

1.2 Counter-regulatory inflammation

Paradoxically, inflammation in some physiologic settings creates local suppression and counter-regulation that promotes tolerance, and attenuates pro-inflammatory processes to block natural (and vaccine-induced) immunity to all antigens presented in these settings. Even during homeostasis tissues with large mucosal surface areas such as the gut, lungs and uterus during pregnancy, – and associated lymphoid tissues – exhibit multiple features of inflamed tissues, including DC maturation, lymphocyte activation and proliferation, and cytokine production above baseline levels. However, antigens introduced into such constitutively inflamed tissues often elicit weak or attenuated immune responses. Attenuated immunity in such settings makes sense because mucosal surfaces are exposed continuously to innocuous substances such as allergens, harmless commensal microbes in the gut, and allogeneic fetal tissues in the uterus of pregnant females. Thus counter-regulation prevents excessive T cell immunity to innocuous substances encountered constitutively at mucosal surfaces during normal tissue functions.

Unfortunately counter-regulation induced in other settings of chronic inflammation promotes disease. Chronic inflammation associated with developing tumors and induced by some persistent pathogen infections is notoriously resistant to natural, as well as vaccine-induced immunity. Resistance to T cell immunity is a significant barrier to successful immunotherapy to treat cancer, and chronic infections such as HIV1, tuberculosis, and malaria. Moreover most therapeutic vaccines (administered to patients with pre-existing conditions) have little effect on slowing disease progression, let alone stimulating pathogen clearance, or tumor regression. Thus, local counter-regulatory processes that protect pre-malignant lesions and sites of infection are hurdles to effective immunotherapy. The well-established paradigm that mature DCs generated at sites of inflammation are T cell stimulatory is not consistent with these observations. A key question is whether mature DCs present antigens in a T cell stimulatory, or T cell regulatory fashion in inflammatory settings where counter-regulation is predominant.

1.3 Acquired immune privilege

Early research on transplantation hinted that natural T cell regulation was active constitutively in some tissues because the potencies of elicited host immune responses to allografts varied according to the local sites where allografts were placed (2). The term ‘immune privilege’ was coined to describe this phenomenon. We, and others expanded the original notion of immune privilege to encompass all inflammatory settings where active local suppression predominates over pro-inflammatory processes that would otherwise stimulate robust effector T cell responses (3, 4). For the purpose of this review we hypothesize that acquired immune privilege is mediated, in part, by DCs expressing the enzyme indoleamine 2,3 dioxygenase (IDO), since IDO-expressing DCs possess potent T cell regulatory functions (Fig. 1C). IDO-expressing DCs are not the only mechanism that can create immune privilege. However IDO-expressing DCs are present in many settings of chronic inflammation associated with clinical disease, and IDO inhibitors exacerbate destructive T cell mediated immunity in mouse models of autoimmune and allergic diseases, tumor bearing mice and fetal development. Genetically enhanced IDO activity also reduced T cell mediated pathology in models of allo-transplantation. DCs expressing IDO may regulate T cell responses to all antigens encountered in such settings. In the rest of this review we discuss how IDO-expressing DCs may induce and maintain local immune privilege (Section 3), and how this mechanism suggests novel approaches to improve immunotherapy for chronic inflammatory disease syndromes (Section 4). Other cell types (non-DCs) expressing IDO may also help mediate T cell regulation in some syndromes, but this issue is beyond the scope of this review. In the next section we provide a brief overview

of IDO, and evidence supporting the hypothesis that cells expressing IDO regulate T cell immunity in some settings of inflammation.

2. IDO and regulation of T cell immunity

2.1 A brief summary of IDO molecular genetics and biology

Mammalian IDO genes are highly conserved, and encode cytosolic heme-containing enzymes that catalyze oxidative catabolism of compounds containing an indole ring, such as the essential amino acid tryptophan and the neurotransmitter serotonin (5, 6). Heme-IDO complexes chelate superoxide anion radicals, a reactive oxygen species (ROS) that breaks indole rings in substrate molecules. A recent study suggested that cytochrome b5 may be the major IDO reductant in human cells (7). X-ray structural analyses of IDO complexed with substrates (or inhibitors) have yielded critical insights into IDO enzymology (6). Mice and humans possess two closely related genes (IDO1, IDO2) tightly linked in a syntenic region of chromosome 8 in each species (8, 9). IDO1 and IDO2 may respond to different signals in distinct cell types as their patterns of gene expression are not identical. Intact IDO1 genes encode functional IDO in murine pDCs sufficient for certain induced T cell regulatory functions because they were abrogated in IDO1-deficient (IDO1-KO) mice possessing intact IDO2 genes (10-13). For this reason we will not discuss IDO2 further, though IDO2 may encode functional IDO proteins in other cell types, and in DCs of other mammals.

IDO gene expression occurs in a range of tissues, especially those with large mucosal surfaces such as the gastro-intestinal (GI) tract and lungs. However such broad patterns of expression are misleading because cells expressing functional IDO are rare under normal physiologic conditions. Moreover, IDO gene expression may not correlate with IDO enzyme activity due to post-translational requirements for enzyme co-factors such as hemin, appropriate redox potentials, and modifications to IDO proteins necessary for enzyme function. IDO enzyme activity is also attenuated by natural immunomodulators such as nitric oxide (NO), which binds to the heme tetrapyrrole group. IDO activity in mice with normal gut flora is elevated in the GI-tract (relative to gnotobiotic mice), probably due to increased 'homeostatic inflammation' at mucosal surfaces harboring commensal microorganisms.

Response elements for interferon type I (IFN α , IFN β) and type II (IFN γ) signaling (ISRE and GAS elements, respectively) are present in promoter regions of mammalian IDO genes, providing a rationale for the strong correlation between inflammation and IDO expression. The distinct patterns of IFN type I and type II production – largely produced by activated cells of the innate immune system and T cells, respectively – during innate and adaptive immune responses means that cells competent to express IDO will respond in different ways, unless IDO genes respond to signals from both IFN classes. Also a number of cell types are competent to express IDO, including stromal (endothelial, epithelial), hematopoietic (monocytic, eosinophils) cells and tumor cells, but few reports describe lymphoid cells that express IDO (14-19). IFN γ is widely considered to be the major inducer of IDO in most cells, but some IDO-competent cells respond exclusively to signals from type I IFNs. For example, IFN α – not IFN γ – was the obligate, upstream IDO inducer for murine IDO-competent splenic CD19⁺ pDCs when TLR9 and B7 (CD80/86) ligands were used to induce DCs to express IDO (11, 20). IDO expression and enzyme activity is also enhanced or attenuated by other transcriptional control mechanisms (e.g. SOCS3, DAP12, IRF8, NF κ B), other cytokines (e.g. IL-10, TGF β , IL-6), and other surface ligand/receptors (e.g. CD200, GITR, 4-1BB) and the eicosanoid prostaglandin E2 (PGE2), though these factors may influence IDO expression indirectly by modulating IFN production, as for TLR and B7 ligands (4, 21-26).

Several artificial immunomodulatory reagents induce IDO-competent DCs in mice and/or humans to express IDO including TLR ligands (LPS, TLR4; CpGs, TLR9; Resiquimod, TLR7/8), soluble CTLA4 and CD40 (CTLA4-Ig, CD40-Ig), dexamethasone (via GPCR ligand), and histone deacetylase (HDAC) inhibitors (11, 20, 27-32). The immunomodulatory attributes of some forms of soluble CTLA4 may depend, at least in part, on their ability to induce IDO in DCs, as well as mediating T cell co-stimulatory blockade. It remains to be seen if other immunomodulatory reagents also induce DCs to express IDO, and whether the ability of such reagents to modulate immune outcomes depends, at least in part, on reagent-induced changes in IDO activity in DCs.

2.2 IDO-mediated suppression of T cell immunity

Potent T cell Regulatory effects of IDO have been described in a range of chronic inflammatory disease syndromes of clinical relevance. The most frequent approach used was to treat rodents predisposed to spontaneous or induced disease with the IDO-specific inhibitor 1-methyl-tryptophan (1MT), and monitor disease progression. This approach is analogous to studies that identified IDO as a protective mechanism for fetal allografts during murine pregnancy, when 1MT treatment caused fetal allograft rejection by maternal T cells (33, 34). Subsequently, 1MT was shown to enhance T cell immunity, and exacerbate the severity of autoimmune, allergic and infectious diseases in mouse models (4, 25). Recent reports provide additional support for the hypothesis that IDO activity is a key factor in autoimmune and allergic disease progression, though it is not always clear if IDO activity slows disease progression, or is an irrelevant consequence of chronic inflammation in such syndromes (35, 36); indeed, IDO activity exacerbated, rather than attenuated, B cell mediated immunity in a murine model of rheumatoid arthritis (37).

Though abundant evidence supports the notion that IDO regulates T cell mediated immunity in clinically-relevant models of disease, mice developmentally deficient for IDO1 did not develop spontaneous (autoimmune) disease, and pregnant IDO1-KO mice carried allogeneic fetuses to term (10), and unpublished data). However abortifacient effects of 1MT did not manifest in pregnancies involving IDO1-KO parents, confirming that IDO enzyme encoded by the IDO1 gene was the relevant pharmacologic target of IDO inhibitor, and suggesting that redundant regulatory mechanisms compensated for developmental IDO1 ablation. Contradictory roles for IDO were also reported in murine models of allergy since pharmacologic IDO inhibition exacerbated allergy in one report, and genetic IDO ablation alleviated allergic inflammation in another study (18, 38). IDO2 genes may compensate for IDO1 ablation during pregnancy, but this premise has not been tested (IDO2-deficient mice are under development). IDO-independent regulatory mechanisms may also compensate for loss of IDO in other syndromes.

IDO1-KO mice exhibited a robust tumor resistant phenotype in a standard model of inflammation-driven carcinogenesis, suggesting that IDO1 protects pre-malignant lesions from immune surveillance in this model (12). Moreover T cell mediated graft-versus-host disease (GvHD) and pathology were exacerbated in IDO1-KO recipient mice, and counter-regulatory effects of histone-deacetylase (HDAC) inhibitors in GvHD models were lost in IDO1-KO mice (31, 39). Thus, intact IDO1 genes are essential for immune regulatory functions in a number of clinically relevant syndromes, implying that IDO activity may be manipulated to improve immunotherapy in chronic inflammatory diseases (Section 4).

3. IDO-competent dendritic cells and immune privilege

3.1 IDO-competent dendritic cells

Though the phenomenon of IDO-dependent T cell suppression has been described in a range of chronic inflammatory diseases, the contributions of particular IDO-expressing cell types

to pathologic syndromes is poorly understood. DCs occupy a unique and critical niche connecting afferent and efferent arms of the immune system. Thus DCs may have pivotal roles in eliciting T cell responses by collating local information - in addition to presenting antigens - and adopting functional states appropriate to prevailing circumstances. Where inflammation mediates T cell regulatory outcomes we hypothesize that DCs with regulatory phenotypes block or attenuate T cell clonal expansion and effector T cell differentiation, and that IDO is a regulatory mechanism exploited by DCs in such settings (Fig 1C).

DCs competent to express IDO in response to specific signals have been described in humans and mice (24, 26). A key finding in both species is that competency to express IDO is a feature of only some tissue DCs. Flow cytometry (FACS) was used to sort purified populations of phenotypically defined DC subsets before assessing their T cell stimulatory properties in the presence or absence of IDO inhibitor *ex vivo*. In mice competency to express IDO following treatment with TLR9 and B7 ligands was restricted to a rare subset of splenic plasmacytoid DCs (pDCs) expressing the lymphoid DC marker CD8 α , the pDC marker B220, the B cell marker CD19, and relatively high levels of the DC marker CD11c (reviewed in (26). Murine CD19⁺ pDCs did not express the pDC marker mPDCA1, further distinguishing them from conventional (CD19^{neg}) pDCs (11), and unpublished data). Analogous studies to identify IDO-expressing DCs in inflamed draining lymph tumor (dLNs) associated with melanoma growth were consistent with findings from spleen as constitutive IDO expression in tumor dLNs was restricted to CD19⁺ pDCs, which exhibited potent T cell suppressor activity dependent on IDO (40). IDO-expressing pDCs with potent T cell suppressor activity were also present in inflamed skin dLNs of mice exposed to a chemical (phorbol ester, PMA) that stimulates intense local inflammation, and promotes tumor progression after carcinogen exposure (12). These findings from three different physiologic settings support the hypothesis that CD19⁺ pDCs are a distinct subset of murine pDCs that suppress (or stimulate) T cell responses contingent on whether CD19⁺ pDCs express IDO (or not) in settings of inflammation.

The unusual phenotypic profile of murine IDO-competent pDCs calls into question their developmental origins. Splenic CD19⁺ pDCs co-express markers shared with other DC subsets (CD11c), T cells (CD8 α) and B cells (B220, CD19). CD19⁺ pDCs in tumor dLNs expressed the B cell lineage commitment factor Pax5, and non-productive (D-J) DNA recombination events were detected in immunoglobulin genes in CD19⁺ pDCs, suggesting that CD19⁺ pDCs may develop from B cell progenitors, not myeloid progenitors. Splenic CD19⁺ pDCs from untreated mice expressed high levels of surface MHC (I/II) and B7 co-stimulatory molecules (CD80/86), and were potent T cell stimulators, revealing that CD19⁺ pDCs exhibit features of mature DCs before treatment with IDO inducers or reagents that stimulate DC maturation (20, 41). CD19⁺ pDCs also produced 5-10 times more IFN α per cell than conventional pDCs when treated with TLR9 ligands *ex vivo*, indicating that CD19⁺ pDCs have low thresholds to activation signals via TLR9 (42). Further studies are needed to elucidate the developmental origins of IDO-competent CD19⁺ pDCs, and molecular mechanisms that induce them to acquire T cell regulatory phenotypes in inflamed tissues.

Similar findings were reported for splenic DC populations enriched by antibody-based cell separation techniques (MACS) followed by DC adoptive transfer to evaluate T cell regulatory functions in a classical DTH model, though CD19 was not evaluated as a marker of IDO-competent pDCs, and IDO-competent pDCs expressed mPDCA1 (24). Differences in the methods used to purify DCs, and assess their T cell stimulatory properties are potential reasons why such discrepancies emerged from these two approaches. Lack of competency to express IDO in the majority of splenic DCs is caused by negative transcriptional regulation of cytokine signaling (SOCS3, DAP12) since interventions to block SOCS3 and DAP12 expression resulted in many more DCs expressing functional IDO

in response to IDO inducers (43, 44). Moreover manipulating upstream transcriptional control elements in this way converted soluble CD28 (CD28-Ig) from a T cell co-stimulatory ligand into a T cell regulatory ligand due to reduced thresholds to induce functional IDO expression in DCs (45, 46). Under homeostatic conditions the regulatory cytokine TGF β helps maintain DCs in a default T cell regulatory phenotype dependent on IDO (47). Collectively, studies on murine IDO-competent pDCs support the hypothesis that IDO-competent pDCs regulate T cell responses to antigens encountered in normal and inflamed tissues.

Studies on IDO expression in human DCs focus on DCs cultured from blood monocyte precursors as direct access to human tissue DCs is not usually feasible (32, 48-54). As in mice, human pDCs mediated IDO dependent differentiation of T cells with regulatory phenotypes in most studies. IDO expression has also been evaluated in patients with cancer, infectious, autoimmune and allergic diseases (55-60). IDO stained cells with plasmacytoid morphologies, and co-stained with human DC markers were described in some studies, and accumulation of expanded cohorts of IDO-expressing cells correlated with worse prognosis for cancer patients in some cases. However IDO-mediated regulatory effects on human T cells in physiologic settings are difficult to evaluate as no phenotypic markers specifically associated with IDO-mediated regulatory processes have been reported. Hence, the hypothesis that human DCs expressing IDO contribute to normal or pathologic disease is largely based on extrapolations from animal models. It remains to be seen if IDO inhibitors administered to cancer patients in ongoing Phase I experimental trials will be effective in enhancing anti-tumor immunity. Based on outcomes in preclinical studies (52, 61), evidence of clinical efficacy may not emerge until future Phase II trials involving use of IDO inhibitor in combination with other cancer treatments are concluded (Section 4).

3.2 IDO-dependent T cell suppression

IDO activity in DCs has profound effects on the ability of T cells to respond to antigenic stimulation. IDO may attenuate the ability of DCs to stimulate effective T cell responses in a number of ways. T cells activated by DCs expressing IDO recognized antigen and entered the cell cycle, but IDO activity blocked subsequent cell cycle progression, and enhanced T cell apoptosis (14, 62, 63). IDO did not induce DCs to lose APC (T cell stimulatory) functions; rather, activated T cells were susceptible to metabolic changes caused by IDO activity in DCs. IDO enzyme activity has three biochemical effects; tryptophan withdrawal, production of tryptophan metabolites (known collectively as 'kynurenines'), and altered redox potentials due to consumption of superoxide radicals. T cells with defective GCN2-dependent amino acid stress response pathways were resistant to the regulatory effects of IDO activity (64, 65). Certain kynurenines produced naturally by IDO-expressing cells also possess T cell regulatory properties; for example, 3-hydroxyanthranilic acid blocked T cell responses, and promoted T cell apoptosis by inhibiting PDK1, which is an essential mediator of CD28-induced NF κ B activation (66). T cells may also be sensitive to changes in redox potential mediated by IDO in DCs, but there are no reports of such effects. Reactive oxygen species (ROS) are produced in inflamed tissues and have major effects on cell metabolism and biological functions. Indeed mice with a genetic defect that blocks NADPH oxidase dependent ROS production exhibited defective IDO activity that contributed to T cell hyperactivity in a model of pulmonary aspergillosis (67), analogous to patients susceptible to chronic granulomatous disease (CGD).

It is not clear if the T cell regulatory effects of IDO in physiologic settings are limited to (a) DCs that present antigens directly to T cells, or (b) whether local IDO activity in uninvolved (bystander) DCs also affects T cells activated by DCs not expressing IDO; bystander suppression via IDO-activated Tregs may be important (see Section 3.3). The key point is that T cell regulatory effects would be confined to antigens presented by IDO-expressing

DCs in scenario (a), while IDO would mediate T cell suppression to all antigens in a local microenvironment in scenario (b). Another key issue is that IDO activity may modify APC and non-APC functions of DCs in ways that influence pro-inflammatory and immune responses indirectly. Thus human DCs matured in the presence of IDO promoted preferential Treg differentiation (68), and IDO activity was essential for mouse splenic DCs to produce IFN α after treatment with CTLA4-Ig to ligate B7 molecules (42).

3.3 IDO-activated regulatory T cells (Tregs)

IDO-competent CD19⁺ pDCs are a small fraction of murine splenocytes (0.1-0.5%), comprising about 10% of total splenic DCs. Yet when induced to express IDO, CD19⁺ pDCs mediate potent T cell suppression that predominates over the T cell stimulatory properties of other DCs (11, 12, 20, 40). Dominant suppression is unlikely to result from direct interactions between IDO-expressing DCs and T cells because T cell stimulatory DCs greatly outnumber CD19⁺ pDCs. Though CD19⁺ pDCs may accumulate (or differentiate *in situ*) in inflamed LNs over time, considerable evidence supports the hypothesis that IDO-expressing DCs enhance suppression mediated by Foxp3-lineage Tregs in certain settings of chronic inflammation. Tregs from normal (non-inflamed) tissues such as spleen must be activated *ex vivo* – usually with a T cell mitogen such as anti-CD3 mAb – to stimulate bystander suppression dependent on IL-10 and TGF β (69). In contrast, Tregs prepared from inflamed tumor dLNs possessed constitutive T cell suppressor activity that was abrogated in tumor bearing mice exposed to IDO inhibitor (70). IDO-activated Tregs from tumor dLNs were significantly more potent suppressors than mitogen-activated Tregs, and suppression was not dependent on IL-10 and TGF β , but was dependent on interactions between the negative co-stimulatory molecule Programmed Death-1 (PD-1), and its ligands PD-L1 and PD-L2. IDO-activated Tregs were also detected in inflamed LNs draining skin exposed to the pro-inflammatory chemical phorbol ester (12), and unpublished data). Similarly, splenic Tregs from mice treated for 24 hours with high dose TLR9 ligands (to induce CD19⁺ pDCs to express IDO) mediated potent PD-1-dependent suppression *ex vivo* (13). However, splenic Tregs did not mediate suppression if IDO was ablated genetically or pharmacologically before TLR9 ligation. Collectively, these reports reveal that IDO-expressing pDCs activate Tregs in some settings of inflammation. Thus cooperative interactions between IDO-expressing pDCs and Tregs provides a potential explanation for dominant T cell suppression in such settings. The mechanisms of IDO-mediated Treg activation and PD-1/L-dependent suppression require more study. However, experiments performed *in vitro* using FACS-sorted Tregs, pDCs and other cell components suggest that IDO $\rightarrow$ GCN2, MHCII $\rightarrow$ TCR signals from IDO-expressing pDCs to Tregs, and CTLA4 $\rightarrow$ B7 signaling from Tregs to pDCs are essential for Treg activation via IDO (70), and experiments with genetically-deficient mice suggest that similar mechanisms were required when Tregs were activated via IDO *in vivo* following high-dose TLR9 ligand treatment (13). IDO-expressing DCs may also promote local suppression by stimulating naïve T cells to differentiate into T cells with regulatory phenotypes – including Foxp3⁺ Tregs – at sites in inflammation. Support for this hypothesis has emerged from studies on T cells cultured with IDO-expressing DCs from mice and humans (32, 54, 71-74). These studies provide additional support for the hypothesis that IDO-expressing DCs create local conditions that favor T cell regulation.

Studies on IDO-activated Tregs yielded other key insights into the role of IDO-expressing DCs as mediators of counter-regulation. Under conditions of IDO ablation resting Tregs converted uniformly into T_H17-like effector T cells expressing the pro-inflammatory cytokine IL-17 in tumor dLNs, and in spleen following high dose TLR9 ligand treatment (13, 75). Splenic Treg conversion into T_H17-like T cells occurred rapidly (less than 9 hours after TLR9 ligation), affected pre-formed Foxp3-lineage Tregs only, and was IL-6 and

TGF β dependent (13), and unpublished data), analogous to the requirements for generation of T_H17 T cells from studies in other model systems (76). Induced IL-6 expression in spleen was blocked completely when IDO was co-induced using TLR9 ligands. Thus IDO-expressing CD19⁺ pDCs block pro-inflammatory cytokine production, as well as activating Tregs. Moreover, IDO ablation in tumor-bearing mice treated with anti-tumor vaccines led to significant increases in the proportions of T_H17-like T cells in tumor dLNs, and in tumor lesions. Hence improved tumor clearance in mice treated with IDO inhibitor correlated with enhanced IL-6 expression, consequent Treg conversion into T_H17-like effector T cells in addition to blockade of constitutive Treg suppression at local sites of tumor growth (75).

3.4 IDO and acquired immune privilege

Creating and maintaining immune privilege may be essential to prevent autoimmune T cell mediated pathology during homeostasis in some tissues, especially those with large areas of mucosal surface. DCs in lymphoid tissues associated with mucosal and systemic tissues exhibit distinct functional properties correlating with differential tendencies to regulate or stimulate T cells, respectively (77). DCs in gut-associated lymphoid tissues (GALT) tend to suppress effector T cells and promote regulatory T cells, including Tregs, and IDO-expressing DCs in GALT may be important in preventing autoimmune syndromes in the GI-tract (78-80). Interestingly, IDO-competent pDCs in mouse spleen express high levels of the integrin CD103 (unpublished data), and DCs expressing CD103 from the GI-tract of mice and humans display conserved T cell regulatory functions that may prevent inflammatory bowel disease (79, 81). Type I autoimmune diabetes (T1D) progression in NOD female mice was exacerbated by IDO inhibitors and pDC depletion (82). Moreover, defective IDO expression in pre-diabetic NOD female mice may pre-dispose mice to T1D (83). IDO also attenuated experimental autoimmune encephalitis (EAE), a murine model of multiple sclerosis, though it is not clear if CNS myeloid cell types mediated T cell regulation in this model (84, 85). Interestingly, stem cells expressing IDO also attenuated EAE (86). However IDO activity in CNS tissues may have detrimental effects on neuronal tissues since some kynurenines are potent neurotoxins (87, 88). IDO activity induced by chronic infection and LPS treatment also caused symptoms of neurologic depression, though it is not known if DCs expressing IDO mediated such neuro-immunologic effects (89).

4. IDO and Immunotherapy

Immune privilege establishes local conditions that protect tissues from undesirable pathology caused by autoimmune and immune responses to innocuous agents. Moreover interventions to induce mechanisms that create and maintain immune privilege are likely to enhance tissue allograft survival. On the other hand immune privilege benefits developing tumors and established infections by protecting these lesions from natural and vaccine induced immunity. The knowledge that DCs expressing IDO promote T cell regulation that help establish and maintain immune privilege provides novel opportunities to improve immunotherapy to treat these syndromes.

4.1 Use of IDO inhibitors to attenuate immune privilege

Phase I experimental clinical trials are currently underway at two US medical centers to test if the IDO-specific inhibitor (1-methyl-[D]-tryptophan, D-1MT) is a cancer vaccine adjuvant. D-1MT - rather than the L stereoisomer L-1MT - was selected for clinical trials based on extensive pre-clinical analyses of the ability of each stereoisomer to rescue T cell responses that were subject to IDO-dependent suppression mediated by DCs (90). For example, D-1MT is a more effective inhibitor of IDO expressed by physiologic DCs from tumor dLNs in mice, and in slowing tumor progression in transplantable melanoma and autochthonous breast carcinoma models in mice (52). D-1MT was also an effective inhibitor

of IDO activity in human DCs cultured from monocyte precursors (32, 50-54, 91). Moreover, abnormally large cohorts of IDO-expressing DCs in tumor dLNs from melanoma patients correlated positively with poor prognosis (92). Numerous epidemiological studies suggest that similar correlations between poor prognosis for patients and elevated IDO activity also apply for a range of different cancers (93-97); however, these descriptive studies do not identify the cell types expressing IDO – DCs or other cell types, including tumor cells themselves – responsible for poor prognosis in cancer patients.

The choice of D-1MT (over L-1MT) by the NCI and corporate sponsors promoting the clinical application of IDO inhibitors as cancer vaccine adjuvants has generated some controversy (98), in large part because D-1MT barely inhibits IDO activity in cell lines – including tumor cells, while L-1MT inhibits IDO activity in such cells (9, 99). More research will be necessary to discover the biochemical reasons for this discrepancy. Outcomes from clinical trials using D-1MT alone (ongoing Phase I), and D-1MT in combination with other standard and novel cancer therapies (planned future Phase II trials, contingent on no evidence of toxicity in Phase I trials) will provide critical information about the efficacy of D-1MT as an IDO inhibitor in humans, and the role of DCs expressing IDO in tumor progression and response to therapy. Not surprisingly, much effort and resources are being devoted to the search for alternative IDO inhibitors (100). Other potential applications of IDO inhibitors are to enhance natural and vaccine induced T cell immunity to treat patients with established chronic infectious diseases, and to enhance protective immunity to pathogens induced by prophylactic vaccines.

4.2 Use of IDO inducers to create and sustain immune privilege

Chronic inflammation in autoimmune and allergic diseases, and at sites of allo-transplantation is often associated with enhanced levels of IDO activity. Indeed, monitoring changes in IDO activity may provide early warnings of episodes of increased disease activity or transplant relapse in patients with autoimmune and allergic diseases or transplants (101). Such observations – though at first sight inconsistent with the notion that IDO activity creates immune privilege – are consistent with the more nuanced paradigm that IDO activity slows inflammatory disease progression, even if IDO does not prevent disease progression. An appropriate analogy might be that fires always attract firefighters, irrespective of whether firefighters ultimately succeed in preventing extensive damage caused by fires. If correct, therapies that induce and stabilize IDO activity will be beneficial in regulating immune-mediated pathology in autoimmune and allergic diseases, and will help promote tissue allograft survival.

As mentioned above some artificial immunomodulatory reagents are IDO inducers, and some reagents – such as soluble CTLA4 (CTLA4-Ig) and TLR ligands – induce DCs to express IDO by stimulating IFN production in lymphoid tissues of mice. It is unclear if equivalent reagents (e.g. soluble CTLA4 marketed as Orencia™) approved for clinical use also induce IDO and, whether induced IDO explains the observed immunomodulatory effects of reagents in clinical settings. More research is needed to evaluate optimal methods to stimulate IDO in clinical settings. Importantly, CTLA4-Ig mediated IDO induction in murine splenic DCs were dependent on the combination of mouse strains and CTLA4-Ig isotypes used (20, 41), and unpublished data). Moreover, as humanized CTLA4-Ig isotypes used in many mouse pre-clinical studies to evaluate co-stimulatory blockade did not induce IDO in mice (unpublished data), it is possible that the immunomodulatory effects of these reagents in clinical settings may be mediated, at least in part, by IDO.

Cellular therapies with human DCs induced to express IDO *ex vivo* offer a potential alternative approach that has the potential advantage of allowing defined antigens to be introduced into patients with heightened T cell responsiveness that drives autoimmune and

allergic disease progression, and allograft rejection. However this approach is likely to prove difficult to apply in practice due to technical difficulties in preparing clinical grade 'tolerogenic' DCs uniformly expressing IDO, and the potential for DCs to exacerbate disease progression unless IDO predominates over the innate T cell stimulatory properties of DCs.

Therapies with immunomodulatory reagents that induce IDO, and with DCs expressing IDO have the inherent disadvantage of stimulating transient bursts of IDO activity; this is likely to be a problem with DCs, which are few in number and are short lived. Hence, transient bursts of IDO activity in DCs are unlikely to have major effects on reducing the severity, or slowing the progression of established T cell mediated hyper-immune syndromes, even if reagents were administered at the time of initial clinical presentation. Regular dosing with IDO-inducing reagents may be feasible to address this problem, as long as no toxic side effects manifest as a consequence of repeated therapy with IDO inducers.

An alternative approach that may circumvent the problem of transient IDO elevation is to develop genetic methods to express IDO ectopically in long-lived stromal (non-DC) cell types, or by introducing vectors into undifferentiated progenitor cells that continuously replenish DC populations, or other cell types that can suppress T cell mediated immunity when induced to express IDO. Some promising results have already emerged from this type of approach. Adenovirus and transposon based vectors engineered to express IDO ectopically protected donor lung allografts from T cell mediated rejection in rodent models, even in the complete absence of global immunosuppression (102, 103). In a recent development, Liu and colleagues introduced naked DNA encoding human IDO into rats prior to transferring their lungs into recipient mice, and showed that this genetic manipulation increased donor lung allograft survival significantly due to enhanced IDO activity in donor lungs (104). Moreover, IDO activity reduced - but did not completely prevent - the generation of allo-specific effector T cells that homed to donor lungs, and also impaired the cytotoxic functions of T cells in lung tissues. These outcomes suggest that IDO acts at two distinct stages in elaborating host T cell responses to lung allografts by (a) reducing T cell clonal expansion and effector T cell differentiation in lung-associated lymphoid tissues and, (b) impairing the ability of effector T cells to cause pathology in target tissues. DCs expressing IDO are likely to regulate T cell clonal expansion in lung LNs, while stromal cell types are more likely to regulate the cytotoxic functions of effector T cells in lung tissues.

4.3 A general role for IDO during vaccination?

A major theme in this review has been the role of IDO-expressing DCs in creating immune privilege in chronically inflamed tissues. A recent study by Guilloneau and colleagues provides a novel perspective on IDO as a suppressor of primary T cell responses following prophylactic vaccination (105). The iNKT cell activator, α -galactosylceramide (α -galcer) was used as an adjuvant in mice vaccinated with inactivated Influenza A virus (IAV). However, α -galcer induced IDO in a CD1d-dependent manner - suggesting that iNKT cells mediate IDO induction, perhaps by releasing IFN γ . Induced IDO attenuated primary effector T cell responses to IAV, as IDO inhibitor (1MT) enhanced vaccine-induced responses. Nevertheless, adjuvant-induced IDO did not affect the generation of CTL memory responses, implying that independent mechanisms regulated primary and memory CTL responses, and that IDO may inhibit clonal expansion of effector cells without impacting the creation of a new memory T cell pool. These findings expand the potential significance of IDO-mediated T cell regulation to prophylaxis in individuals with no pre-existing inflammatory conditions. Hence, IDO may have an under-appreciated role in attenuating primary T cell responses, that may interfere with the generation of memory T cell repertoires that provide long term protective immunity. Speculatively, IDO-mediated

subversion of T cell responses to vaccines may help explain why effective T cell vaccines to influenza have not materialized, despite significant efforts in this direction in recent years. Findings that TLR ligands induce IDO, as well as pro-inflammatory responses that stimulate T cell immunity, may also be related to this novel paradigm. If so, it may be necessary to re-evaluate the role of IDO-mediated T cell regulatory responses to vaccines and vaccine adjuvants, since combination therapies with IDO inhibitors has the potential to enhance beneficial immunity required for clinical efficacy for vaccines and adjuvants that failed to meet these criteria when used alone.

5. Summary and Future directions

IDO is active in a range of animal models of human disease, and manipulating IDO for clinical benefit is a promising new strategy to improve immunotherapeutic interventions. These facts are driving intense interest in elucidating the role of IDO in DCs and other cell types, and the mechanisms that explain the phenomenon of IDO-mediated T cell regulation that contributes to the creation and maintenance of immune privilege in tissues subject to chronic inflammation. In the near future, we can expect better insights into the underlying biochemical mechanisms that stimulate and maintain IDO enzyme activity in specific cell types, and improved methods to control IDO activity for therapeutic applications.

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Figure 1. Dendritic cells (DCs) acquire antigens and collate inflammatory signals from local tissue microenvironments to elicit distinctive T cell responses

A. During tissue homeostasis immature DCs present antigens in a T cell tolerogenic fashion, and regulatory cytokines such as TGFβ and IL-10 help maintain default counter-regulation in such settings, **B.** T cell stimulatory inflammation in which DCs mature, and acquire potent T cell stimulatory properties that overcome default T cell regulation and tolerance. Cytokines such as IL-6 promote pro-inflammatory outcomes, including conversion of resting regulatory T cells (Tregs) into helper/effector TH17-like T cells expressing IL-17. **C.** T cell regulatory inflammation creates immune privilege in which mature DCs stimulate abortive T cell responses, activate Treg suppressor functions, and block pro-inflammatory cytokine production, in part by promoting the IDO mechanism in DCs.