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The immunoregulatory role of IDO-producing human dendritic cells revisited

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Following the finding that indoleamine 2,3-dioxygenase (IDO), an enzyme expressed in the placenta, prevents rejection of allogeneic fetuses in mice, many studies have focused on the role of IDO in the regulation of the immune response. Most arguments for an immunoregulatory role of IDO *in vivo* are based on observations in mice. Here, we critically examine the arguments for and against a function of IDO-expressing human dendritic cells (DCs) and conclude that proof for an immunoregulatory role *in vivo* is still lacking.

Introduction

Elucidation of the various mechanisms by which the immune system regulates its reactions not only helps us to understand better the etiopathogenesis of certain diseases, but also leads to the development of new therapeutic strategies. In the past few years, an immunoregulatory mechanism mediated by an enzyme that induces the catabolism of tryptophan has attracted the attention of the scientific community.

Indoleamine 2,3-dioxygenase and tryptophan metabolism: the beginnings

Two rate-limiting enzymes, tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), are known to initiate the catabolism of tryptophan, leading to a common downstream metabolic pathway (Figure 1) [1]. Whereas TDO is mainly located in the liver and has high specificity for tryptophan, IDO can be synthesized by many cell types, has less substrate specificity and can be induced during the immune response [2]. Although a causal link between inflammation and upregulation of IDO was postulated early on [2], the role of this enzyme in the immune defense mechanism remained elusive. In 1984, Pfefferkorn [3] observed that interferon-γ (IFN-γ) – a cytokine that induces IDO – blocked the growth of *Toxoplasma gondii* in human fibroblasts in an IDO-dependent way. The author proposed two possible mechanisms for this effect: the parasites are either destroyed by toxic tryptophan metabolites or starved of the essential amino acid tryptophan. Based on his experimental findings, Pfefferkorn favored the second hypothesis. A subsequent series of *in vitro* studies confirmed the anti-infectious effect of IDO [4–7].

IDO prevents rejection of the fetus in pregnant mice: an old mechanism in a new biological context

In 1998, a publication appeared that revolutionized the view of the role of IDO in living beings. Munn *et al.* [8] showed that IDO can prevent rejection of the fetus during pregnancy in mice. Extending Pfefferkorn's previous hypothesis [3], the authors [8] proposed that IDO inhibits the maternal T-cell attack by destroying tryptophan. This was a turning point in the 'history' of IDO, one that led to an avalanche of experiments trying to elucidate the role of the enzyme in regulation of the immune response.

Grohmann *et al.* [9] showed that cytotoxic T-lymphocyte antigen-4 immunoglobulin (CTLA-4Ig) upregulates IDO in murine dendritic cells (DCs) by ligation to B7 antigen. Interestingly, long-term survival of pancreatic islet allografts induced by CTLA-4Ig can be reversed by treatment with the inhibitor of IDO 1-methyl tryptophan (1-MT) – a finding that supported an immunoregulatory role for IDO *in vivo* [9]. The same group observed that regulatory $CD4^+CD25^+$T cells induce active IDO in murine DCs through a CTLA-4-dependent mechanism [10]. If, by contrast, CD28 interacted with B7, the DCs expressed interleukin (IL)-6 and IDO production induced by IFN-γ was abrogated – a mechanism that also appeared to work *in vivo* [11]. Apparently, binding of B7 molecules on murine DCs by CD28 resulted in stimulatory DCs, whereas binding of the same molecules by CTLA-4 resulted in tolerogenic DCs. But the story did not end there. If expression of suppressor of cytokine signaling 3 (SOCS3) in DCs was silenced, CD28 induced IDO activity and thus turned from a stimulatory into a suppressive molecule [12].

Another interesting study pointed to a role for IDO in the promotion of tumor growth [13]. Expression of IDO by immunogenic tumor cells in mice prevented rejection in preimmunized recipients, an effect partly reversed by systemic treatment with the IDO inhibitor 1-MT. IDO also inhibited graft rejection in certain transplant models [14,15]. Taken together, these observations, relying on experiments in mice, support the hypothesis that IDO has an immunoregulatory role.

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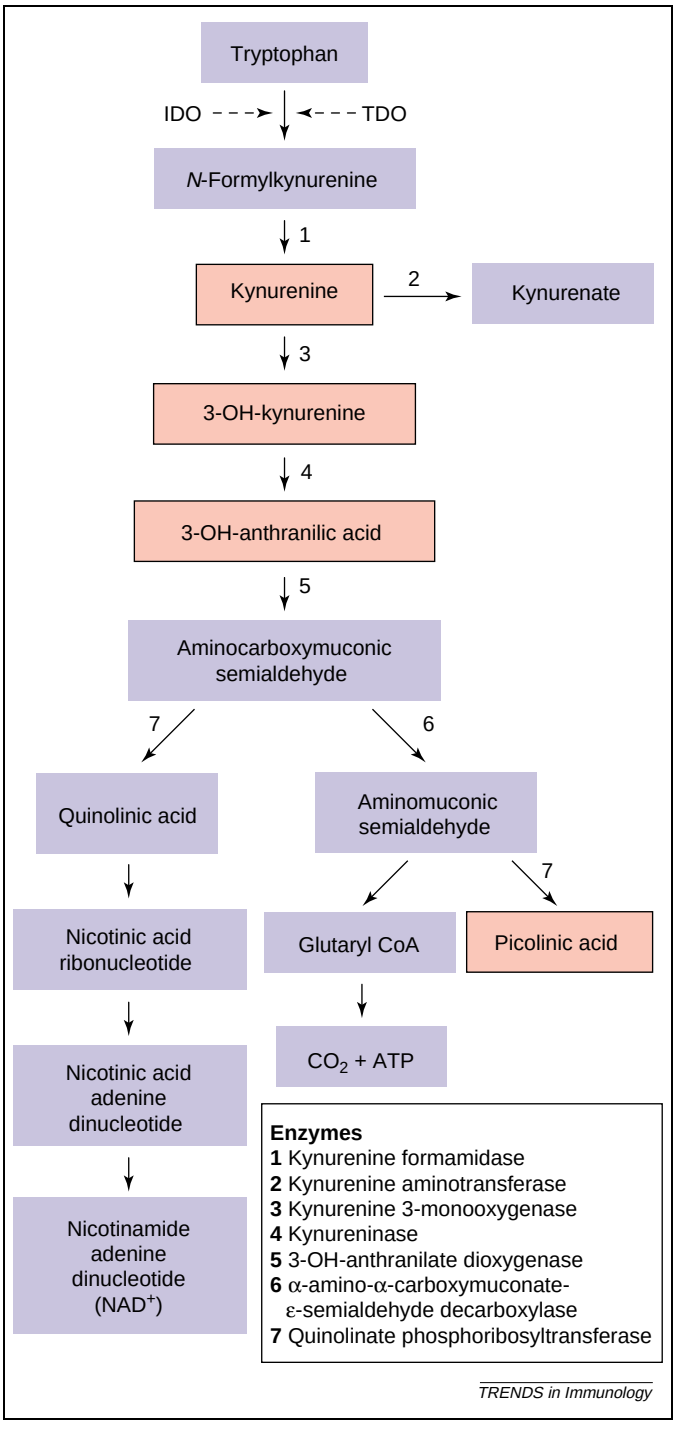

Figure 1. Pathway of IDO-induced tryptophan catabolism. Immunosuppressive catabolites are in orange. Numbers indicate enzymes, which are listed in the box.

The immunoregulatory role of human IDO-producing DCs: speculation, facts and artifacts

Expression of constitutive and inducible IDO in human DCs

The findings presented above demonstrate that murine DCs can express IDO, but further studies showed that human DCs can also produce this enzyme. Similar to many other cell types, DCs express IDO on exposure to IFN-γ [16,17], an effect enhanced by natural ligand of CD40 (CD40L) and lipopolysaccharide (LPS) [16]. It was reported that ligation of the co-stimulatory proteins CD80 and CD86 by CTLA-4 and CD28 expressed on $CD4^+$ T cells triggers IDO activity in human DCs [18], suggesting that, similar to the findings in mice, CTLA-4 is involved in the induction of IDO activity in humans (Figure 2a, yellow). This has recently been confirmed by experiments showing that soluble CTLA-4 induces functionally active IDO in LPS-matured monocyte-derived human DCs [19].

When trying to identify tolerogenic DCs, the question arose as to whether there is a subpopulation of human DCs that constitutively express IDO. One study reported a subset of human DCs that do constitutively express IDO and suppress the T-cell response [20]. These cells were generated from blood monocytes and defined as a nonadherent subpopulation expressing IL-3 receptor α-chain (CD123) and chemokine CC-motif receptor 6 (CCR6), in addition to the classic DC phenotype. If the DCs were matured in serum-free medium, >90% of the nonadherent fraction was IDO-positive [20]. In an attempt to repeat this study, it was concluded that, although a DC subpopulation expressing these surface markers exists, it does not express IDO or suppress the T-cell response (Figure 2a, green) [17]. A factor that might have been responsible for generating misleading results in the original work on the suppressive IDO-positive DC subpopulation [20] was the use of a highly polyreactive rabbit antihuman IDO antibody for identifying expression of IDO in DCs using fluorescence-activated cell-sorting (FACS) analysis. This antibody was also used in other studies for detection of DCs that constitutively express IDO [18,21,22].

The finding that nonadherent $CD123^+CCR6^+$ DCs do not express IDO is not proof that DCs constitutively expressing IDO do not exist in humans; it indicates only that these surface markers are not a 'signature' for such a subpopulation of cells.

Can IDO-expressing human DCs suppress the T-cell response?

Although the existence of DCs that constitutively express IDO could not be confirmed [20], the question as to whether IFN-γ-induced IDO-producing DCs can inhibit the T-cell response was addressed. Human DCs were treated with IFN-γ [17] and, as expected, strong IDO activity was obtained. Surprisingly, however, the IDO-producing DCs did not suppress, or only marginally suppressed, the T-cell response (Figure 2a, green). Vacca *et al.* [19] recently showed that human DCs matured with CD40L are refractory to induction of IDO by CTLA-4Ig and do not inhibit the T-cell response. This observation cannot explain our findings, because the generated DCs expressed IDO after exposure to IFN-γ [17].

While analyzing the properties of IDO-producing DCs, some technical aspects were noted that might have influenced the previous conclusions. In some experiments, the inhibitor of IDO 1-MT was used as a 'gold standard' for demonstrating the suppressive function of IDO-positive DCs [18,20,23]. When T-cell proliferation increased on addition of 1-MT, it was concluded that this happened because the suppressive action of IDO was blocked. 1-MT clearly abrogates IDO activity. However, 1-MT also inhibits the tryptophan transporter [24] and, thus, has