

Review

Interaction of indoleamine-2,3-dioxygenase and CD4⁺CD25⁺ regulatory T cells in tumor immune escape

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There are numerous factors involved in the tumor evasion from immune surveillance. Recently, considerable attention has been given to indoleamine 2,3-dioxygenase (IDO) and CD4⁺CD25⁺ regulatory T cells (Tregs). The interaction between the two may play a crucial role in tumor immune escape. This article reviews the correlation between IDO and Tregs in immune escape of tumors.

Immune escape is a key mechanism in formation of malignant tumors, which allows the tumor to escape from immune surveillance. Moreover, immune escape determines the course of tumor invasion and metastasis, as well as affects the efficacy of clinical treatment. Indoleamine-2,3-dioxygenase (IDO) is an important negative regulator on immune system which attracts much attention in recent years. IDO was first identified in rabbit intestines in 1963, which is the only rate-limiting enzyme outside liver to catalyze oxidative cracking of the indole ring of tryptophan along the kynurenine pathway. IDO can degrade tryptophan into L-kynurenine, picolinic acid, quinolinic acid and many other metabolites,¹ a process critically related to tumor escape. Further study reveals that IDO induces the production of a new type of regulatory T cells, CD4⁺CD25⁺ regulatory T cells (Tregs), through which it mediates immune tolerance. The correlation between IDO and Tregs has become a new hot spot for exploring the mechanism of immune tolerance, which may provide novel insights for the mechanism of tumor immune escape.

Role of IDO in Immune Escape of Tumors

Initial studies on the role of IDO in tumors focused on the inhibitory effects of IDO on the local immune function of

tumors. Uyttenhove et al.² first reported that IDO is continuously expressed in the majority of tumor cell lines, which was confirmed by immunohistochemical examine. Thereafter, studies have found a close correlation of IDO overexpression to poor prognosis of non-small cell lung cancer, ovarian cancer, cervical cancer, colon and liver cancer,²⁻⁷ implying active participation of IDO in the malignant progress of tumors. T cells in the mid-G1 phase are very sensitive to tryptophan deficiency. Overexpression of IDO in tumor cells inevitably results in deficiency of tryptophan. As a result, T cells can neither effectively proliferate nor amplify via clonal expansion. Moreover, T cell proliferation is difficult to be re-activated once it is inhibited.⁸ On the other hand, toxic tryptophan metabolites can directly inhibit the function of T cells, even induce apoptosis of T cells. This toxic effect differs from that of tryptophan deficiency in that it is selective and is only effective on activated T cells, but has no significant impact on resting cells.⁹ Munn et al.¹⁰ identify a special subgroup of dendritic cells (DC) in tumor-draining lymph nodes (TDLNs) that stably express IDO and have the ability to inhibit T cells. These DC suppress T cells to produce specific immune response against tumor antigen, thus to play important roles in inducing tumor immune tolerance.

Production of IDO, either by tumor cells or by DC from TDLNs, would lead to tryptophan depletion and dysfunction of T lymphocytes, which may account for IDO-mediated local immune tolerance. More importantly, tumors usually cause systemic immune tolerance. The function of IDO in inducing systemic immune tolerance remains unclear. Recent study has shown that, IDO could induce the production of new Tregs, which may be one of the important mechanisms for IDO-mediated systemic immune tolerance in tumors.¹¹

Role of Tregs in Immune Escape of Tumors

Tregs are a subgroup of T cells identified recently, which are mainly generated by thymus. They are important in maintaining immune homeostasis and modulating immune responses. They have two major functional features, immune incompetence and immunosuppression, which make Tregs an important component in maintaining self-tolerance in the body. Besides inhibition on immune response, Tregs are actively involved in immune pathology, graft immune tolerance, prevention of autoimmune reaction and maintenance of immune balance of the whole body.

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Tregs can secrete transforming growth factor (TGF)- β , Interleukin (IL)-10 and other inhibitory cytokines through autocrine, to inhibit the cellular function of antigen-specific CD4⁺CD25⁺ T cells, cytotoxic CD8⁺ T cells and natural killer T (NKT) cells. The cell surface molecule CTLA-4 (CD152) of Tregs participates in negative regulation of immune responses as a co-stimulatory signal molecule. A high level of CTLA-4 is expressed on the cell surface of Tregs shortly after the activation of T-cell receptor (TCR). After binding to CD80/CD86 on the activated T cells, CTLA-4 inhibits the secretion of IL-2 induced by T cells, thereby inhibits proliferation of T cells.¹² Mouse CTLA-4 can also stimulate CD4⁺ T cells to secrete TGF- β , which further inhibits the immune response ability of effector cells. CTLA-4 on the surface of Tregs is found to bind to CD80/CD86 (B7-1/B7-2) on the DC surface, resulting in inhibition of maturation and differentiation of DC, as well as reduction of MHC II expression. It is believed that the indirect inhibitory effect of Tregs on effector T cells is mediated through inhibiting the synthesis of antigen presenting cells (APC).¹³

Additionally, binding of Tregs to the B7 molecule on the surface of DC could also increase IDO expression, and thereby inhibit the function of effector T cells.¹⁴ This finding provides a new point of view to study the immune escape of tumors.

Relationship Between IDO and Tregs

The key functions of IDO and Tregs in immune escape of tumors have been studied by many researchers. Mutual regulation and stimulation between IDO and Tregs have been confirmed. During the mediation of tumor immune tolerance, while Tregs can induce the expression of IDO, IDO can induce the production of more Tregs.

Tregs-induced expression of IDO. Many studies show that Tregs could stimulate the expression of IDO on DC, mainly through expression of CTLA-4 on the surface of Tregs and secretion of cytokines, such as IFN- γ and so on.

Grohmann et al.¹⁵ reveal that binding of CTLA-4 to B7-1/B7-2 could stimulate the expression of a high level of IDO in mouse DC in vitro, and stimulate catabolism of tryptophan, thus inhibit the clonal expansion of T cells. When an IDO inhibitor, 1-methyl-tryptophan (1MT), was used to inhibit the enzyme activity of IDO, the immune response of T cells is restored. When autoantigens are presented to Tregs by DC, signals are received through the surface molecule CTLA-4, which can bind to B7-1/B7-2 on DC and activate the signaling transduction pathway to upregulate the protein content and enzyme activity of IDO, leading to overexpression of IDO. This signaling pathway involves p38 mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF κ B), two key pathways to produce interferon (IFN)- γ and activate IDO. However, this process is highly selective, since CTLA-4-Ig cannot induce high expression of IDO in all types of DCs in vivo. Grohmann et al.¹⁵ also believe that the major role of CTLA-4 is to induce the expression of IDO in CD8 α ⁺ DC. Meanwhile, Mellor et al.¹³ have discovered that, CTLA-4-Ig and CTLA-4-positive Tregs upregulate the expression of IDO in B220⁺ DC. Although Tregs cannot induce IDO expression in all

subgroups of DC, increased expression of IDO in CD8 α ⁺ DC or B220⁺ DC is sufficient to inhibit the immune response of effector T cells, and therefore execute the immunosuppressive function of Tregs. An increased amount of Tregs is found in tumor cells and one group of TDLNs.¹⁶ In addition, one type of regulatory DC (regsDC) is upregulated in TDLNs. These regsDC can induce anergy of T cells and immune deviation, as well as promote apoptosis of effector T cells to mediate immune tolerance. Recently, a study on monkeys infected with SIV supports the theory that Tregs induces the expression of IDO.¹⁷ It is noticed that CTLA-4⁺ Tregs in these monkeys could inhibit the anti-SIV immune response. In the meantime, a high level of IDO with high activity was identified in multiple organs of these monkeys. When these monkeys were treated by an antibody specifically blocking CTLA-4, a reduced expression of IDO mRNA was detected in tissues. Although this result may be explained otherwise, it is consistent with the hypothesis that upregulation of IDO in APC cells is achieved through CTLA-4⁺ Tregs. In order to confirm this, further genetic studies using knockout or transgenic mice are necessary.

Tregs can also increase IDO expression by stimulating the production of IFN- γ , thus inducing apoptosis of effector T cells to maintain the state of immune tolerance. Wood and Sawitzki¹⁸ believe that Tregs stimulated by CD3 monoclonal antibody and Lipopolysaccharide (LPS) could promote the secretion of IFN- γ , which would greatly increase the expression of IDO when Tregs are co-cultured with DC.

In contrast, Thuere et al.¹⁹ raised a different opinion based on a study on pregnant mice. They propose that Tregs could not stimulate the expression of IDO, because they only appear on maternal decidua, while IDO is expressed in placenta. They find that Tregs appear during early pregnancy, but high expression of IDO starts from day eight of pregnancy. Therefore, Tregs could not induce high expression of IDO. Although their study does not cover all aspects, it raises new challenges for studying the relationship between Tregs and IDO, as well as provides new directions for future studies on these two molecules.

IDO-Induced Activation of Tregs

The impact of Tregs on IDO was firstly noticed. Later on, it was found that IDO also has an effect on Tregs.

As discussed earlier, an increase in IDO expression leads to tryptophan degradation in micro-environment, which results in tryptophan deficiency and an increase of kynurenine. This micro-environment can induce production of Tregs. Fallarino et al.²⁰ reveal that, in medium with a low tryptophan and a high kynurenine (LT-K) concentration, or during co-culture with IDO⁺DC, CD4⁺CD25⁺Foxp3⁻ cells could be transformed to CD4⁺CD25⁺Foxp3⁺ cells, accompanied by an increase of surface markers of Tregs, such as CD69⁺, CD45RB^{low}, CD62L⁺, CTLA-4⁺, BTLA^{low} and GITR⁺. Hill et al.²¹ exhibit that LPS could stimulate mature DC to express IDO at a high level, which subsequently increases the number of Tregs. Curti et al.²² find that human acute myeloid leukemia (AML) cells express IDO and have the ability to induce CD4⁺CD25⁺ Tregs in vitro. Co-culture of CD4⁺CD25⁺ T cells with IDO⁺ AML could transform CD4⁺CD25⁺ T cells to

CD4⁺CD25⁺ T cells. However, such an effect is abrogated after the treatment with 1MT. Moreover, intradermal injection of AML cells in mice increased the number of Tregs. Yu et al.²³ used adenovirus to transfect IDO into bone marrow DC (BMDC) to treat mice. IDO⁺ DC not only caused expansion of native Tregs, but also induced the transformation of CD4⁺CD25⁻ T cells to Tregs. Tryptophan metabolites catalyzed by IDO enzyme, including 3-hydroxykynurenine (3OHK), hydroxyanthranilic acid (3-HAA), as well as the downstream product quinolinic acid (QUIN), can all stimulate the activity of Tregs, all of which confirm that IDO could induce the production of Tregs.

Sharma et al.¹¹ investigated the mechanism of IDO-induced activation of Tregs. They believe that activation of Tregs by IDO⁺pDC is mediated by activation of GCN2 signaling pathway of Tregs cells and degradation of tryptophan. In normal cells, activation of GCN2 pathway leads to cell cycle arrest and cell anergy. Activated GCN2 pathway results in eIF2α phosphorylation, and phosphorylated eIF2 can inhibit biosynthesis of the majority of proteins. Meanwhile, activation of GCN2 also changes the translation initiation site of the ribosome, promotes ATF4 translation and CHOP transcription, thus to cause functional changes of downstream genes, subsequently leading to inhibition of cell growth. When increased IDO activity causes a decrease in tryptophan, the GCN2 pathway of Tregs cells would be activated. This could not only activate activity of Tregs, but also induce the transformation of CD4⁺CD25⁻ T cells to CD4⁺CD25⁺ T cells. On the other hand, IDO could activate GCN2-eIF2α pathway through altering expression of some key cytokines, such as IL-10 or TGF-β, to stimulate Treg function. Although IDO could increase the expression of Foxp3 on immature CD4⁺ T cells, upregulation of Foxp3 is not essential for the generation of Tregs, because not all Foxp3⁺ cells produced by CD4⁺ T cells are transformed into Tregs after IDO induction. However, the key point is that IDO can promptly stimulate mature Tregs to become active.

At present, all the above conclusions are deduced based on in vitro observations. If similar finding are confirmed in vivo, then IDO and Tregs would be revealed as a closely coupled positive-feedback system, with Tregs inducing IDO and IDO promoting production of Tregs in the process of immune escape of tumors (Fig. 1).

Application of IDO and Tregs in tumor immunotherapy. Wobser et al.²⁴ claim that melanoma patients transfused with autologous DC vaccine show positive Foxp3 expression at the vaccine inoculation site, implying the generation of Tregs. In addition, the 11 patients underwent immunotherapy deteriorated and had shortened survival time, suggesting the negative regulation of immune function by Tregs mediated through IDO. This shows the limitation of DC vaccine in tumor treatment, and diverts the attention of researchers to IDO and Tregs for immunotherapy.

Since IDO plays such an important role in immune tolerance of tumors, it is adopted as a new target in anti-tumor treatment, among which a IDO inhibitor, 1MT, entered the clinical trials in 2007.²⁵

Changing the number and function of Tregs are expected to serve as the target for tumor immune therapy. Some researchers used anti-IFN-γ, anti-CD25, CTLA-4 antibodies, IL-2 toxin

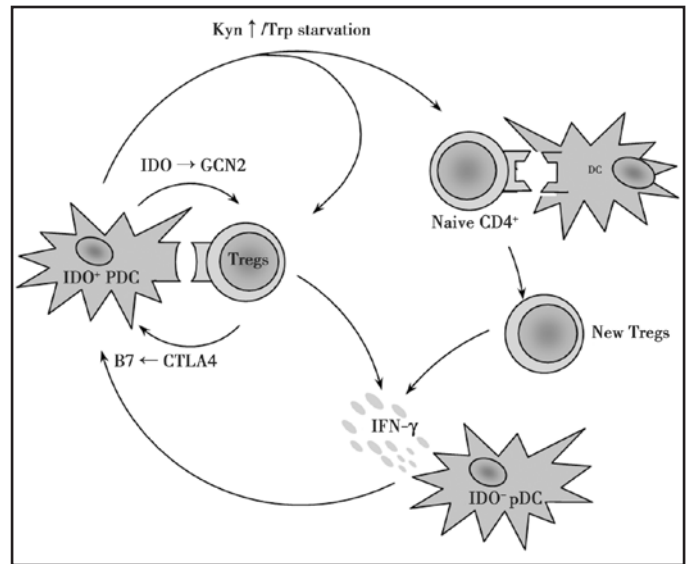


Figure 1. Model of the interaction between indoleamine 2,3-dioxygenase (IDO) and CD4⁺CD25⁺ regulatory T cells (Tregs).

chimeric protein or glucocorticoid-induced tumor necrosis factor receptor (GITR) as anticancer drugs due to their inhibition of Treg function.²⁶⁻²⁹ Although enhanced anti-tumor immunity is observed, these effects are still limited to in vitro studies or in the mouse model.

With better understanding of the essential roles and mechanisms of IDO and Tregs in immune tolerance of tumors, as well as the interaction between the two, application of them in anti-tumor immunotherapy is promising. For example, altering the interaction between Tregs and DC by inhibiting IDO activity, or reducing the number of Tregs in DC-based immunotherapy may enhance the efficacy of anti-tumor therapy. An increasing amount of studies on tumor immunotherapy are focusing on altering IDO or Tregs to eliminate their negative regulation on immune function during tumor treatment.

Future Prospects

Breaking immune tolerance of tumors has become an urgent issue in tumor immunotherapy. Currently, mechanisms of the involvement of IDO and Tregs in immune escape of tumors are still controversial. One crucial problem is to control the interaction between IDO and Tregs, reverse the immune tolerance mediated by them and, therefore, inhibit tumor growth. The answer to this question may provide a new treatment strategy for the prevention and treatment of tumors.

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