REVIEW

Contribution of IDO to human respiratory syncytial virus infection

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Abstract
IDO is an enzyme that participates in the degradation of tryptophan (Trp), which is an essential amino acid necessary for vital cellular processes. The degradation of Trp and the metabolites generated by the enzymatic activity of IDO can have immunomodulating effects, notably over T cells, which are particularly sensitive to the absence of Trp and leads to the inhibition of T cell activation, cell death, and the suppression of T cell effector functions. Noteworthy, T cells participate in the cellular immune response against the human respiratory syncytial virus (hRSV) and are essential for viral clearance, as well as the total recovery of the host. Furthermore, inadequate or non-optimal polarization of T cells is often seen during the acute phase of the disease caused by this pathogen. Here, we discuss the capacity of hRSV to exploit the immunosuppressive features of IDO to reduce T cell function, thus acquiring relevant aspects during the biology of the virus. Additionally, we review studies on the influence of IDO over T cell activation and its relationship with hRSV infection.

KEYWORDS
indoleamine-2,3-dioxygenase, tryptophan (Trp), hRSV, T cells

1 | INTRODUCTION

IDO is an enzyme that is encoded by the gene IDO1 (INDO) in mammals.1 In humans, IDO1 is located in the short arm of chromosome 8 and encodes a ~45 kD polypeptide that is composed of 403 amino acids.2,3 The primary function of IDO is to catalyze the degradation of tryptophan (Trp), which decreases the availability of this amino acid in the cell and promotes the production of kynurenine (Kyn) and other metabolites (e.g., kynurenic acid, tryptamine) associated to this amino acid in IDO-expressing cells.4,5 It is known that most human cells have little or no constitutive IDO expression, however, the expression of this enzyme can be induced by IFN-γ-induced signaling pathways in several cell types,4,5 such as dendritic cells (DCs) and macrophages (Mφs).6–8 Other cell types, such as astrocytes, neurons, microglia, and respiratory tract epithelial cells can also express IDO after stimulation by IFN-γ or TNF-α.9,10

Trp is necessary within cells for vital cellular functions, including protein synthesis, and the absence of this amino acid can suppress the proliferation of lymphocytes by an arrest of the cell cycle at a mid-G1,11 which promotes cell apoptosis.12 Hence, IDO activity is thought to modulate immune response by controlling the proliferation of lymphocytes.13,14 Another main effect associated with IDO activity is that this enzyme promotes immunological tolerance by inhibiting T cell responses and promoting the proliferation of regulatory T cells (Tregs).15

The availability of Trp is also fundamental for the replication of intracellular pathogens such as viruses, as they are obligated parasites and need the host cellular machinery and cellular components to sustain their replication cycle.16–20 During infections produced by viruses, bacteria, parasites, and fungi, IDO can be activated on antigen-presenting cells (APCs) residing at the infected tissues to reduce Trp availability and deprive pathogens of this amino acid, as an ancient innate host defense mechanism aimed at controlling pathogen proliferation and dissemination (Fig. 1).21 However, it has been suggested that
IDO1 is an IFN-stimulated gene (ISG), and IFN-γ has been recognized as the most important inducer of IDO in several cell types, as mentioned above. According to in vitro experiments with epithelial A549 cells, IFN-γ is a more potent inducer of IDO than type I IFN. Hundreds of ISGs have been described, but only a handful of them have been shown to display an antiviral activity in vivo. Besides IFN-γ, other cytokines can also stimulate IDO expression, such as IL-6 and TNF-α. On the other hand, IL-4 and IL-13, two cytokines characteristic of Th2 response, are recognized as inhibitors of IDO.

IFN-γ induces IDO activity particularly in DCs and Mφs. This interferon has been reported to induce the activation of IDO1 through the JAK/STAT pathway. Binding of IFN-γ to its receptor results in the phosphorylation and activation of the JAK1 and JAK2, which in turn leads to the phosphorylation and subsequent activation of STAT1. The phosphorylated form of this transcription factor (STAT1) then translocates from the cytosol to the nucleus and binds to IFN-γ activation sites (GAS) promoting the initiation of the transcription of different ISGs, including IDO1. Additionally, it was reported that mature DCs might constitutively express IDO, but its enzymatic activity would be productive only after IFN-γ stimulation, which suggests two different enzymatic states for this protein: one active and another inactive.

Gene expression of IDO can also be induced through a mechanism that is independent of IFN-γ, which depends on the activity of the transcription factors NF-κB and the p38 MAPK. Interestingly, Fujigaki et al. showed that inhibitors of p38 MAPK and NF-κB could inhibit the activity of IDO. Both, p38 MAPK and NF-κB are also necessary for the production of IFN-γ and the subsequent transcriptional regulation of IDO1. However, the precise mechanism mediating this regulation is not fully understood yet. As IDO activity is up-regulated in APCs in response to IFN-γ secreted by activated T cells (Fig. 1), it has been suggested that a negative feedback loop may exist in which this enzyme might be involved in regulating T cell activation.

3 | IMMUNOMODULATORY FEATURES OF IDO ACTIVITY

As mentioned above, IDO activity impacts the immune system in a “double-branch” manner: first, by decreasing the amount of Trp available to the cell and second by increasing the quantity of Kyn affecting both, IDO-expressing APCs and T cells interacting with them. This immunoregulation mechanism leads to the suppression of T cell function, generating an immunosuppressive microenvironment around the cells that display IDO activity (Fig. 2). The lack of Trp caused by IDO activity allows an accumulation of uncharged Trp-tRNAs in the cell, which in turn activates GCN2 (eukaryotic translation initiation factor 2 alpha kinase 4) and inhibits the mammalian target of rapamycin (mTOR). GCN2 is one of the 4 stress-sensitive kinases that phosphorylate serine 51 in the α subunit of mTOR.
subunit of the translation eukaryotic initiation factor 2 (eIF2α), and this route can prevent the cell activation. After eIF2α is phosphorylated (and thus inhibited) by GCN2, cell growth and global translation are blocked. This kind of inhibition is thought to protect the cell from a lack of nutrients. It has been reported that the depletion of Trp mediated by IDO in T cells inhibits the G1 to S phase transition. Studies made using in vivo models have shown that T cells with altered GCN2 are not susceptible to this effect.

On the other hand, mTOR is an evolutionarily conserved kinase that can sense the nutritional status of the cell by integrating environmental signals to control cell proliferation, cell homeostasis, and many other cellular functions. Also, it is known that mTOR is necessary for an adequate activation and proliferation of effector T cells, restraining the development of Tregs and other immune functions. Thus, mTOR is an essential regulator of the immune response.

Another immune-modulating effect of IDO activity is mediated through Tregs. IDO-expressing plasmacytoid DCs have been shown to activate resting Tregs and thus initiating a potent suppressor activity. In vitro conditions, naïve CD4+ T cells can differentiate into Foxp3+ Tregs promoted by IDO activity, with such T cells promoting the expression of programmed cell death 1 ligand 1 (PD-L1) and PD-L2 in DCs. Additionally, IDO has been proposed to be a critical controller, keeping an equilibrium between suppressor and effector functions of T cells. Due to the activity of IDO, suppressor Tregs acquire a stable suppressor phenotype that blocks their eventual reprogramming into Th17-like effector T cells.

Different products from the Trp degradation generated by IDO activity such as Kyn, kynurenic acid, and tryptamine participate in other mechanisms of immunomodulation. These metabolites can bind to the aryl hydrocarbon receptor (AhR) and act as ligands for this molecule. AhR works as a transcription factor that can modulate the differentiation of immune cells after detecting endogenous ligands. Once activated, AhR migrates to the nucleus to regulate the expression of genes, such as CYP1A1, which is a member of the cytochrome P450 superfamily of enzymes. CYP1A1 has an essential role in the metabolism of drugs and chemicals, and polymorphisms in this gene have been associated with different tumors.

On the other hand, binding of Kyn to AhR leads to the activation of an AhR-dependent differentiation pathway for T cells, triggering the modulation and proliferation of Foxp3+ Tregs, which can influence the Th1-Th2- or Th17 subsets. Moreover, the Kyn-AhR interaction induced the expression of IDO and IL-10 by DCs, as well as the inhibition of T cell activation (Fig. 2). Also, AhR was reported to induce the transcription of IL-22 and mediate the differentiation of Tregs, thus showing antimicrobial and anti-inflammatory roles, respectively, and contributing to immune homeostasis. IL-10 has a crucial role in the maintenance of persistent viral infections (Fig. 2). IL-10 suppresses Ag presentation to CD4+ T cells by DCs and Mφs, which could increase the overall immunosuppressive effect of IDO activity.

T cells are sensitive to a decrease of Trp. This sensibility can also be considered a condition that promotes viral persistence and disease severity. However, T cells may be protected from Trp absence and the subsequent stop at the mid-G1 phase through the expression of the IFN-γ-induced enzyme tryptophanyl-tRNA-synthetase (WARS), which produces a reserve of Trp in the form of a complex Trp-tRNA, which IDO cannot catabolize. It has been suggested that CD8+ T cells are less sensitive than CD4+ T cells to the lack of Trp, likely...
because when stimulated with recombinant CTLA-4 under in vitro conditions, CD4+ T cells express IDO and WARS, but CD8+ T cells only express WARS.76

It has been proposed that under physiological conditions, IDO plays different roles in the regulation of Th1 and Th2 responses. In DCs, IDO activity has a suppressive effect over Th1 responses, likely to avoid over-reaction of cellular responses that could damage the host.79 In vitro and in vivo models have shown that the activity of the enzyme is associated with the apoptosis of proliferating T cells, but this effect occurs preferably in Th1 cells. This phenomenon generates an imbalance in the proportion of Th1/Th2 cells, favoring a Th2, over a Th1 profile of cytokines.80,81 An example of this cellular imbalance comes from a recent study that has shown that enhanced activity of IDO in human gastric mucosa infected with Helicobacter pylori down-regulates Th1 and Th17 but up-regulates a Th2 response.82 The reason for this selectivity that causes a Th1/Th2 imbalance is still unknown.

Additionally, the proliferation of memory T cells is subject to regulations by the catabolism of Trp mediated by IDO activity. By evaluating allograft survival in mice, along with inhibition and induction of IDO, it has been established that Trp catabolism mediated by IDO controls the generation of memory CD8+ T cells. When IDO activity is up-regulated, central and effector memory CD8+ T cells are diminished, but these cells proliferate when IDO is suppressed.83

4 | INHIBITION OF IDO ACTIVITY DURING VIRAL INFECTIONS

Besides hRSV, different viruses such as the human metapneumovirus, adenoviruses, bocaviruses, coronavirus, and enteroviruses can also cause acute respiratory infections,84 but to date, there are no studies on the relationship between infection by such pathogens and IDO activity by the host. However, such a relationship has been explored with other viruses that do not cause respiratory disease. For example, IFN-γ and TNF-α induce IDO activity that inhibits HSV-2 and HSV-1 replication, but an increase in Trp abrogates this antiviral effect.17,19 The same antiviral effect due to IFN-γ and IDO activity was seen against measles virus18 and vaccinia virus.16 It has been reported that during HIV infection, IDO activity is also increased due to viral infection and that this alteration may be contributing to the persistence of the virus.85

It has been suggested that the inhibition of IDO activity may improve the immune response against some viral pathogens.86 Inactivation of IDO can reverse the immunosuppressive effects induced by its activity. 1-Methyl-tryptophan (1MT) is a drug that can inhibit IDO activity and has been mainly tested in the treatment of different tumors. However, 1MT has also been reported to be able to restore the host immune response after infections with pathogens. In an in vivo model of influenza virus, the infection was accompanied with an increase in both, a Th1 and a Th17 immune response along with a robust response of specific T lymphocytes against the virus when IDO activity was inhibited.87

Similarly, it has been shown that by decreasing the activity of IDO with 1MT during the primary response to influenza virus infection, the response of memory T cells is improved by increasing the production of IFN-γ.88 After mice were primed with influenza A strain X31 (H3N2), treated with 1MT and challenged with PR8 (H1N1) 28 days p.i., Sage and colleagues evaluated the impact of IDO inhibition on the memory T cell response to influenza virus. The number of memory Th1 (IFN-γ+) cells and lung virus-specific CD8+ IFN-γ+ T cells showed to be higher in the bronchoalveolar lavage (BAL) from the group of mice treated with 1MT compared to the control group (without 1MT treatment). Interestingly, all these cellular changes were accompanied by an accelerated repair of the lung tissue. At day 7 post-challenge the authors described less necrosis, alveolar exudate, and neutrophil recruitment in the lungs of mice treated with 1MT compared to control mice. Although the number of Tregs (CD4+ Foxp3+) responding to the virus challenge was not altered by 1MT treatment, the inhibition of IDO resulted in fewer Tregs expressing CTLA-4 on the cell surface according to the median fluorescence intensity (MFI).88 These data suggest an essential role for IDO during the initial response of the host against the pathogen. This finding could be exploited for the development of vaccines or act as a stimulus for vaccines with weak immune activation to improve T memory responses and reduce disease-associated morbidity.88,89

5 | hRSV-INFECTION AND IDO ACTIVITY

hRSV is the most important etiological agent of acute lower respiratory tract infections in infants and young children worldwide, as well as an important pathogen for the elderly.90 It has been estimated that hRSV infects 50% of children during their first years of life, and 100% of children under 3 years old.91,92 Usually, 30–75% of infants infected before 12 months of age will be re-infected before the age of 2.93 This effect is due to an incomplete protective immune response elicited by the host against hRSV because the virus can interfere with the development of both, effector and memory CD8+ T cells in the lungs.94–96 Furthermore, re-infection can occur throughout life; with the elderly and immunosuppressed patients being a high-risk group.97–99 Probably, the susceptibility of the host to hRSV-infections in the elderly is due to a low number of specific memory T cells found in this population100 and could be explained by the fact that hRSV induces IDO expression, which in turn controls the generation of memory CD8+ T cells as discussed above.

During the acute phase of the disease caused by hRSV, systemic CD4+ T cells and CD8+ T cells counts are low while viral loads, neutrophils counts, and disease symptoms reach their peak.101 Also, there is suppression of memory CD8+ T cell differentiation mediated by mTOR, apoptosis of T cells, and an up-regulation of PD-1 (T cell inhibitory molecule). As neutrophils begin to decrease, pulmonary CD8+ T cells begin to increase along with viral clearance and symptoms decrease.101

CD8+ T cells play an essential role in the immune response against viruses and frequently help the host reach complete viral clearance.102 Consistently, hRSV titers in the lungs of infected mice are reduced...
after the adoptive transfer of CD8+ T cells. It has been shown that CD8+ T cells induce apoptosis of virus-infected cells by cell-cell contact through the interaction of surface molecules, such as Fas/FasL and TRAIL/DR4,5 or because of the secretion of perforin and granzymes. Also, CD8+ T cells can secrete IFN-γ and TNF-α, which have proinflammatory effects. An essential role for CD8+ T cells is also evidenced by the fact that the absence of CD8+ T cells generates a persistent infection with hRSV. On the other hand, the central antiviral role of CD4+ T cells seems to be supporting the activation of B cells that produce antiviral antibodies as plasma cells.

IFN-γ has significant antiviral activity and is related to the modulation of Th1- or Th2-like immune responses, as IFN-γ affects the differentiation of naïve T cells into either Th1 or Th2 cells. Importantly, it is known that a Th1 response is characterized by the activation of CD8+ T cells.

A Th1 or a balanced Th1/Th2 immune response is necessary for an adequate response by cytotoxic T cells (CTL) and IFN-γ secreting CD4+ T cells, which are demonstrated to contribute to hRSV clearance. However, during the acute phase of hRSV infection, the immune response of the host is biased towards a Th2 type, which is not able to eliminate the infection caused by hRSV, on the contrary, it can be harmful to the host. This bias would be contributing to the disease pathogenesis.

The data discussed above indicate that the degradation of Trp, has also been observed for several other viruses, including vaccinia virus, HSV-2, and measles virus. Regarding the immunosuppressive effects of IDO, it has been shown that epithelial cells can suppress antigen-specific and antigen-non-specific T cell activation (i.e., T cell proliferation and IFN-γ production) through IDO activity. The immunomodulatory activity of IDO has been shown to affect immune responses associated with hRSV, and the inhibition of specific inflammatory cytokines and chemokines (IL-6, IL-8, CCL4, and CXCL10) that were induced by IFN-γ. The study also reported that IDO was able to decrease CXCL10 levels induced by infection and was not associated with IFN-γ.

In a recent study oriented to counteract the lack of Trp and the subsequent antiviral effects of IDO, Rabbani et al. showed that it is possible to restore viral growth in cells infected with hRSV, as well as parainfluenza virus type 3, through the restoration of Trp levels. The results indicate that the cause of the inhibitory effect over viral growth would be due to a lack of Trp, rather than the products of catabolism of this amino acid. Also, several hRSV proteins, such as N, P, M, and M2-1, were affected by IDO, and the restitution of Trp restored such viral protein levels. The inhibition of viral replication due to IDO up-regulation, as well as the suppression of this effect by the addition of Trp, has also been observed for several other viruses, including vaccinia virus, HSV-2, and measles virus.

Regarding the immunosuppressive effects of IDO, it has been shown that epithelial cells can suppress antigen-specific and antigen-non-specific T cell activation (i.e., T cell proliferation and IFN-γ production) through IDO activity. In 2015, it was reported for the first time that hRSV induces the expression of IDO1 in human monocyte-derived DCs, which depended on viral replication but was independent of IFN-γ. Rabbani et al. showed in both, human lung cells and BALB/c mice that IDO contributed to the inhibition of viral replication associated with IFN-γ, and the inhibition of specific inflammatory cytokines and chemokines (IL-6, IL-8, CCL4, and CXCL10) that were induced by IFN-γ. The study also reported that IDO was able to decrease CXCL10 levels induced by infection and was not associated with IFN-γ.

As mentioned at the beginning of this review, IDO1 is an ISG, and some of these genes display antiviral activity. Rajan and colleagues showed in both, human lung cells and BALB/c mice that IDO expression is induced by hRSV infection; the results suggest that IDO and IFN-γ would affect viral replication and host cellular responses upon infection. The authors indicated that IDO contributed to the inhibition of viral replication associated with IFN-γ, and the inhibition of specific inflammatory cytokines and chemokines (IL-6, IL-8, CCL4, and CXCL10) that were induced by IFN-γ. The study also reported that IDO activity contributes to a direct relationship between the replication of the virus and the expression of IDO. Likely, hRSV induces IDO activity through the

**FIGURE 3** hRSV-induced IDO activity contributes to Th1/Th2 imbalance. During the acute phase of hRSV-infection, the activity of IDO increases independent of the presence of IFN-γ, but in a hRSV-replication-dependent manner (probably through RIG-I and NF-κB signaling). The activity of IDO induces the death of T cells, contributing to an imbalance in the proportion of Th1 and Th2 cells. IL-4 and IL-13 cytokines are characteristic from a Th2 response and inhibit the expression of IDO, which would probably be part of a regulatory mechanism of IDO.
RIG-I pathway via NF-κB and p38 MAPK (Fig. 3). Further, the authors showed that the induction of IDO activity was dependent on virus infection and replication. Interestingly, T cell activation was partially recovered when TLR signaling was activated in epithelial cells after the addition of LPS and poly I:C, mimicking bacterial and viral infections, respectively. To date, the immunosuppressive effect of IDO induced by hRSV has only been demonstrated in vitro. Thus, it would be necessary to corroborate this effect in an in vivo model of infection in order to propose this enzyme as a target for therapy.

The increase in the expression of IDO can be up to 70-fold in mesenchymal stem cells at 72 h after infection with hRSV, as compared to uninfected cells and the use of anti-IFN-β reduced the expression of IDO. Importantly, the overexpression of IDO in hRSV-infected mesenchymal stem cells negatively affects the proliferation of lymphocytes, which could be related to the lack of immune protection against subsequent hRSV infections.

Because IDO up-regulation occurs immediately after infection (24 h p.i.), as determined in an in vivo model of infection with influenza virus, it is likely that this enzyme is similarly induced shortly after hRSV infection and that it may be able to promptly shift the immune response toward a Th2 response (IL-4, IL-13), altogether down-regulating IDO expression (Fig. 3).

It is known that hRSV can promote IDO expression during viral infections and that this virus promotes Th2 polarization that causes significant secretion of IL-4, IL-5, and IL-13 that contributes to airway inflammation. Additionally, type 2 Innate Lymphoid Cells (ILC-2), have been associated with triggering an exacerbated inflammatory response in the airways contributing to pathology after the hRSV infection. This effect promotes a higher cytokine amount and eosinophils recruitment in the lungs associated with respiratory diseases such as asthma. In a study with patients with asthma, it was found that eosinophils from these individuals displayed high levels of IDO expression, which sustained the polarization of a Th2 response. Also, this Th2 polarization could be modulated by DCs. In this context, it was possible to suggest that hRSV infection through IDO expression could contribute to ILC-2 activation and promote allergy-like pulmonary responses and diseases that are typically associated with hRSV.

Regarding B cells, it was recently reported that hRSV could infect human neonatal regulatory B cells (nBreg). In this study, it was found that the F-protein hRSV can bind to the B cell receptor and that G-protein hRSV interacts with the chemokine receptor CX3CR1, which allows viral entry. Importantly, nBreg cell infection promoted IL-10 secretion that inhibits Th1 polarization of T cells, thus increasing the viral loads in the infected tissue and disease severity. Although no evidence suggests a role for IDO in the regulation of B cells infected with hRSV, it has been shown that IDO can regulate humoral immunity in response to T cell-independent Ags. According to this study, intrinsic ido1 in B cells controls the hypersensitivity of these cells to self T cell-independent Ags and thereby, prevents the development of autoimmune responses. Thus, considering these findings, we can speculate that it is possible that hRSV-infected B cells increase the secretion of IL-10, favoring the Th1/Th2 unbalanced response commonly observed during hRSV pathology. However, further research is required to demonstrate that these associations take place.

6 | CONCLUDING REMARKS

IDO is an enzyme that participates in the catabolism of Trp, which is an essential amino acid for vital cellular functions. The enzyme is activated during infections to deprive pathogens of Trp. Nonetheless, the catabolism of Trp and the metabolites it generates can also induce an immunosuppressive environment. It has been suggested that the dominant nature of the role of IDO during infections is pathogen-specific, i.e., antimicrobial or immunoregulatory. Some reports show both, antiviral and immunosuppressive effects by the activity of IDO during hRSV infection. However, currently, there is not enough information in the literature about the relationship between IDO expression and hRSV infection. Indeed, the immunoregulatory effects of IDO need to be demonstrated in vivo to generate more robust conclusions.

Two relevant characteristics of hRSV infection are a Th1/Th2 imbalance and both, effector and memory CD8+ T cells impairment in the lungs. hRSV-induced IDO activity contributes to this cell proportion imbalance in favor of the survival of Th2 over Th1 cells, yet this selectivity remains unexplained. From this point of view, hRSV-induced IDO activity could be participating as a mechanism to avoid Th1 immune responses. On the other hand, the up-regulation of IDO results in a decrease in both, central and effector memory CD8+ T cells, which could explain hRSV-reinfections or infections later on in life, as older adults show a lower number of hRSV-specific memory T cells. Increased activity of IDO induced by hRSV seems to be an early event (acute phase) during infection. Likely, the virus takes advantage of the immunosuppressive effects of IDO activity in order to avoid the immune response mediated by T cells, suggesting that, after a Th2 immune response is set against the virus, both IL-4 and IL-13 regulate IDO activity, and this change could contribute to clear the virus in a later phase of infection. However, this idea needs further empirical confirmation. New therapeutic strategies, as the use of complementary anti-IDO treatment, could improve both, the immunological memory and the cytokine profile of the host during viral infection. Thus, it would be interesting to assess if 1MT can work as an "adjuvant push" for emerging vaccines, such as vaccines against hRSV and hMPV, which are currently under development. An anti-IDO treatment or adjuvant therapy for hRSV infection would require corroborating the up-regulation of IDO and the subsequent inhibition of T cell proliferation and activation in an in vivo model as a first step. Different viruses cause acute respiratory infections; however, there is little information on the relationship between IDO activity and infection by different pathogens within that category. To date, most studies assess hRSV and influenza virus. However, the development of new prophylactic and therapeutic approaches against viral diseases requires understanding the pathogenesis of the disease to improve vaccine responses and the design of treatments to prevent and
potentially cure chronic viral infections. It may be important to focus on studying the role of IDO during respiratory infections caused by other pathogens.

AUTHORSHIP

All authors have made substantial, direct, and intellectual contribution to the work and approved it for publication. F.B. was associated with conceptualization, writing original draft, review, editing, and revision of the final version. J.S., M.P.O., K.B., P.G., and S.B. edited and revised the final version. A.K. was associated with conceptualization, revision of original draft, editing, and revision of final version.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Ball HJ, Sanchez-Perez A, Weiser S, et al. Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice. Gene. 2007;396:203-213.
2. Tone S, Takikawa O, Habara-Ohkubo A, Kadoya A, Yoshida R, Kido R. Primary structure of human indoleamine 2,3-dioxygenase deduced from the nucleotide sequence of its cDNA. Nucleic Acids Res. 1990;18:367.
3. Dal W, Gupta SL. Molecular cloning, sequencing and expression of human interferon-γ-inducible indoleamine 2,3-dioxygenase cDNA. Biochem Biophys Res Commun. 1990;168:1-8.
4. Ozaki Y, Edelstein MP, Duch DS. Induction of indoleamine 2,3-dioxygenase: a mechanism of the antitumor activity of interferon gamma. Proc Natl Acad Sci USA. 1988;85:1242-1246.
5. Takikawa O, Kuroiwa T, Yamazaki F, Kido R. Mechanism of interferon-gamma action. Characterization of indoleamine 2,3-dioxygenase in cultured human cells induced by interferon-gamma and evaluation of the enzyme-mediated tryptophan degradation in its anticytotoxic activity. J Biol Chem. 1988;263:2041-2048.
6. Popov A, Schultz JL. IDO-expressing regulatory dendritic cells in cancer and chronic infection. J Mol Med. 2008;86:145-150.
7. Schulz S, Landi A, Garg R, Wilson JA, van Drunen Littel-van den Hurk S. Indolamine 2,3-dioxygenase expression by monocytes and dendritic cell populations in hepatitis C patients. Clin Exp Immunol. 2015;180:484-498.
8. Carlin JM, Borden EC, Sondel PM, Byrne GI. Interferon-Induced indoleamine 2,3-dioxygenase activity in human mononuclear phagocytes. J Leukoc Biol. 1989;45:29-34.
9. van Wissen M, Snoek M, Smids B, Jansen HM, Lutter R. IFN-γ amplifies IL-6 and IL-8 responses by airway epithelial-like cells via indoleamine 2,3-dioxygenase. J Immunol. 2002;169:7039-7044.
10. Guillemín Gilles J, Smythe G, Takikawa O, Brew Bruce J. Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes, and neurons. Glia. 2004;49:15-23.
11. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mel- lor AL. Inhibition of T Cell Proliferation by Macrophage Tryptophan Catabolism. J Exp Med. 1999;189:1363-1372.
12. Lee GK, Park HJ, Macleod M, Chandler P, Munn DH, Mellor AL. Tryptophan deprivation sensitizes activated T cells to apoptosis prior to cell division. Immunology. 2002;107:452-460.
13. Munn DH, Mellor AL. Indoleamine 2,3-dioxygenase and metabolic control of immune responses. Trends Immunol. 2013;34:137-143.
14. Kim SB, Choi JY, Uyangaa E, et al. Blockage of indoleamine 2,3-dioxygenase regulates Japanese encephalitis via enhancement of type I/II IFN innate and adaptive T-cell responses. J Neuroinflamm. 2016;13:79.
15. Mehrav V, Routy J-P. Tryptophan catabolism in chronic viral infections: handling uninvited guests. Int J Tryptophan Res. 2015;8:41-48.
16. Terajima M, Leporati AM. Role of indoleamine 2,3-dioxygenase in antiviral activity of interferon-γ against vaccinia virus. Viral Immunol. 2005;18:722-729.
17. Adams O, Besken K, Oberdörfer C, MacKenzie CR, Rüßing D, Däubener W. Inhibition of human herpes simplex virus type 2 by interferon-γ and tumor necrosis factor α is mediated by indoleamine 2,3-dioxygenase. Microbes Infect. 2004a;6:806-812.
18. Obojes K, Andres O, Kim KS, Däubener W, Schneider-Schaulies J. Indoleamine 2,3-dioxygenase mediates cell type-specific anti-measles virus activity of gamma interferon. J Virol. 2005;79:7768-7776.
19. Adams O, Besken K, Oberdörfer C, MacKenzie CR, Takikawa O, Däubener W. Role of indoleamine-2,3-dioxygenase in alpha/beta and gamma interferon-mediated antiviral effects against herpes simplex virus infections. J Virol. 2004;78:2632-2636.
20. Lopez S, Arias C. How viruses hijack endocytic machinery. Nat Educ. 2010;3:16.
21. Barth H, Raghuraman S. Persistent infectious diseases say – IDO. Role of indoleamine-2,3-dioxygenase in disease pathogenesis and implications for therapy. Crit Rev Microbiol. 2014;40:360-368.
22. Schmidt SV, Schultzle JL. New insights into IDO biology in bacterial and viral infections. Front Immunol. 2014;5:384.
23. Loughman JA, Hunstad DA. Induction of indoleamine 2,3-dioxygenase by uropathogenic bacteria attenuates innate responses to epithelial infection. J Infect Dis. 2012;205:1830-1839.
24. Zelante T, Fallarino F, Bistoni F, Puccetti P, Romani L. Indoleamine 2,3-dioxygenase in infection: the paradox of an evasive strategy that benefits the host. Microbes Infect. 2009;11:133-141.
25. Divanovic S, Sawtell NM, Trompette A, et al. Opposing biological functions of tryptophan catabolizing enzymes during intracellular infection. J Infect Dis. 2012;205:152-161.
26. Ajamian F, Wu Y, Ebeling C, et al. Respiratory syncytial virus induces indoleamine 2,3-dioxygenase activity: a potential novel role in the development of allergic disease. Clin Exp Allergy. 2015;45:644-659.
27. Chon SY, Hassanain HH, Gupta SL. Cooperative role of interferon regulatory factor 1 and p91 (STAT1) response elements in interferon-γ-inducible expression of human indoleamine 2,3-dioxygenase gene. J Biol Chem. 1996;271:17247-17252.
28. Rabbani MAG, Ribaudo M, Guo JT, Barik S. Identification of interferon-stimulated gene proteins that inhibit human parainfluenza virus type 3. J Virol. 2016;90:11145-11156.
29. Xue Y, Xiao H, Guo S, et al. Indoleamine 2,3-dioxygenase expression regulates the survival and proliferation of Fusobacterium nucleatum in THP-1-derived macrophages. Cell Death Dis. 2018;9:355.
30. Mackenzie C, Hadding U, Däubener W. Interferon-gamma-induced activation of indoleamine 2,3-dioxygenase in cord blood monocyte-derived macrophages inhibits the growth of group B streptococci. J Infect Dis. 1998;178:875-878.

31. Mackenzie CR, González RG, Kniep E, Roch S, Däubener W. Cytokine-mediated regulation of interferon-gamma-induced IDO activation. Adv Exp Med Biol. 1999;467:533-539.

32. Chaves ACL, Cerávolo IP, Gomes JAS, Zani CL, Romanha AJ, Gazzinelli RT. IL-4 and IL-13 regulate the induction of indoleamine 2,3-dioxygenase activity and the control of Toxoplasma gondii replication in human fibroblasts activated with IFN-γ. Eur J Immunol. 2001;31:333-344.

33. Takikawa O, Tagawa Y, Iwakura Y, Yoshida R, Truscott RJ. Interferon-γ-dependent/independent expression of indoleamine 2,3-dioxygenase. Studies with interferon-gamma-knockout mice. Adv Exp Med Biol. 1999;467.

34. Hwu P, Du MX, Lapointe R, Do M, Taylor MW, Young HA. Indoleamine 2,3-dioxygenase production by human dendritic cells results in the inhibition of T cell proliferation. J Immunol. 2000;164:3596.

35. Horvath CM. The Jak-STAT pathway stimulated by interferon γ. Clin Exp Immunol. 2004:164:3596.

36. Chon SY, Hassanain HH, Pine R, Gupta SL. Involvement of two regulatory elements in interferon-γ-regulated expression of human indoleamine 2,3-dioxygenase gene. J Interferon Cytokine Res. 1995;15:517-526.

37. Konan KV, Taylor MW. Importance of the two interferon-stimulated response element (ISRE) sequences in the regulation of the human indoleamine 2,3-dioxygenase gene. J Biol Chem. 1996;271:19140-19145.

38. Platianis LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol. 2005;5:375.

39. Schroder K, Hertzog Paul J, Ravasi T, Hume David A. Interferon-γ: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004;75:163-189.

40. Darnell J, Kerr I, Stark G. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science. 1994;264:1415-1421.

41. Decker T, Kovarik P, Meinke A. GAS elements: a few nucleotides not essential for the induction of indoleamine 2,3-dioxygenase by human dendritic cells expressing indoleamine 2,3-dioxygenase. Cytokine Res. 2002;297:1867.

42. Fujigaki H, Ishiguro H, Sudo K, et al. The signal transducer and activator of transcription 1α and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-κB pathways, and synergistic effect of several proinflammatory cytokines. J Biol Chem. 2006;139:655-662.

43. Munn DH, Sharma MD, Lee JR, et al. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science. 2002:297:1867.

44. Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E, Prendergast GC. Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. Nat Med. 2005;11:312.

45. Muller AJ, DuHadaway JB, Jaller D, Curtis P, Metz R, Prendergast GC. Immunotherapeutic suppression of IDO and tumor growth with ethyl pyruvate. Cancer Res. 2010;70:1845-1853.

46. Muller AJ, Smith C, Metz R, Prendergast GC. IDO in immune escape: regulation and therapeutic inhibition. Cancer Immunotherapy. 2nd ed. San Diego, CA: Academic Press; 2013:565-581.

47. Muller AJ, DuHadaway JB, Jaller D, Curtis P, Metz R, Prendergast GC. Immunotherapeutic suppression of IDO and tumor growth with ethyl pyruvate. Cancer Res. 2010;70:1845-1853.

48. Muller AJ, Smith C, Metz R, Prendergast GC. IDO in immune escape: regulation and therapeutic inhibition. Cancer Immunotherapy. 2nd ed. San Diego, CA: Academic Press; 2013:565-581.

49. Berthon C, Fontenay M, Corm S, et al. Metabolites of tryptophan catabolism are elevated in sera of patients with myelodysplastic syndromes and inhibit hematopoietic progenitor amplification. Leuk Res. 2013;37:573-579.

50. Mellor AL, Munn DH. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation. Immunol Today. 1999;20:469-473.

51. Metz R, Rust S, DuHadaway JB, et al. IDO inhibits a tryptophan sufficiency signal that stimulates mTOR: a novel IDO effector pathway targeted by D-1-methyl-tryptophan. Oncoimmunology. 2012;1:1460-1468.

52. Lageix S, Rothenburg S, Dever TE, Hinnebusch AG. Enhanced interaction between pseudokinase and kinase domains in Gen2 stimulates eLF2α phosphorylation in starved cells. PLoS Genet. 2014;10:e1004326.

53. Donnelly N, Gorman AM, Gupta S, Samali A. The eLF2α kinases: their structures and functions. Cell Mol Life Sci. 2013;70:3493-3511.

54. Munn DH, Sharma MD, Baban B, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. Immunity. 2005;22:633-642.

55. Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. Annu Rev Immunol. 2012;30:39-68.

56. Weichhart T, Hengstschläger M, Linke M. Regulation of innate immune cell function by mTOR. Nat Rev Immunol. 2015;15:599-614.

57. Weichhart T, Säemann MD. The multiple facets of mTOR in immunity. Trends Immunol. 2009;30:218-226.

58. Fallarino F, Grohmann U, You S, et al. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor ζ-chain and induce a regulatory phenotype in naïve T cells. J Immunol. 2006;176:6752.

59. Sharma MD, Baban B, Chandler P, et al. Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs via indoleamine 2,3-dioxygenase. J Clin Invest. 2007;117:2570-2582.

60. Baban B, Chandler PR, Sharma MD, et al. IDO activates regulatory T cells and blocks their conversion into T(H)17-like T cells. J Immunol. 2009;183:2475-2483.

61. DiNatale BC, Murray IA, Schroeder JC, et al. Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. Toxicol Sci. 2010;115:89-97.

62. Opitz CA, Litzenberger UM, Sahn F, et al. An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. Nature. 2011;478:197.

63. Tsujii N, Fukuda K, Nagata Y, et al. The activation mechanism of the aryl hydrocarbon receptor (AhR) by molecular chaperone HSP90. FEBS Open Bio. 2014;4:796-803.

64. Mimura J, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. The activation mechanism of the aryl hydrocarbon receptor (AhR) by molecular chaperone HSP90. FEBS Open Bio. 2014;4:796-803.

65. Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. A novel mechanism of regulation of Ah (dioxin) receptor function. Genes Dev. 1999;13:20-25.

66. Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. The aryl hydrocarbon receptor regulates Stat1 activation and partic-ipation in the development of Th17 cells. Proc Natl Acad Sci USA. 2008;105:9721-9726.

67. Quintana FJ, Basso AS, Iglesias AH, et al. Control of Treg and TH17 cell differentiation by the aryl hydrocarbon receptor. Nature. 2008;453:65.

68. Klifii R, Messaoud O, Rebai A, Hamza-Chaffai A. Polymor-phisms in the human cytochrome P450 and arylamine N-acetyltransferase: susceptibility to head and neck cancers. BioMed Res Int. 2013;2013:582768-582768.

69. Meng F-d, Ma P, Sui C-g, Tian X, Jiang Y-h. Association between cytochrome P450 1A1 (CYP1A1) gene polymorphisms and the risk of renal cell carcinoma: a meta-analysis. Sci Rep. 2015;5:8108.

70. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlington WJ, Bradfield CA. An interaction between kynurenine and the aryl...
hydrocarbon receptor can generate regulatory T cells. J Immuno l. 2010;185:3190-3198.

70. Stevens EA, Mezrich JD, Bradfield CA. The aryl hydrocarbon receptor: a perspective on potential roles in the immune system. Immunology. 2009;127:299-311.

71. Nguyen NT, Kimura A, Nakahama T, et al. Aryl hydrocarbon receptor negatively regulates dendritic cell immunogenicity via a kynurenine-dependent mechanism. Proc Natl Acad Sci USA. 2010;107:19961-19966.

72. Vogel CFA, Goth SR, Dong B, Pessay IN, Matsumura F. Aryl hydrocarbon receptor signaling mediates expression of indoleamine 2,3-dioxygenase. Biochem Biophys Res Commun. 2008;375:331-335.

73. Zelante T, Iannitti RG, Fallarino F, et al. Tryptophan feeding of the IDO1-AhR axis in host–microbial symbiosis. Front Immunol. 2014;5:640.

74. Jones S. Viral persistence: IL-10 is the key. Nat Rev Immunol. 2006;6:878.

75. Mittal SK, Roche PA. Suppression of antigen presentation by IL-10. Curr Opin Immunol. 2015;34:22-27.

76. Favre D, Mold J, Hunt PW, et al. Tryptophan catabolism by indoleamine 2,3-dioxygenase 1 alters the balance of Th17 to regulatory T cells in HIV disease. Sci Transl Med. 2010;2:32ra36.

77. Terness P, Bauer TM, Röse L, et al. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase—expressing dendritic cells: mediation of suppression by tryptophan metabolites. J Exp Med. 2002;196:447-457.

78. Boasso A, Herbeuval J-P, Hardy AW, Winkler C, Shearer GM. Regulation of indoleamine 2,3-dioxygenase and tryptophanyl-tRNA-synthetase by CTLA-4-Fc in human CD4+ T cells. Blood. 2005;105:1574-1581.

79. Xu H, Zhang G-X, Ciric B, Rostami A. IDO: a double-edged sword for immune regulation. Curr Drug Metab. 2003;5:527.

80. Larussa T, Leone I, Suraci E, et al. Enhanced Expression of indoleamine 2,3-dioxygenase in Helicobacter pylori-infected human gastric mucosa modulates Th1/Th2 pathway and interleukin 17 production. Helicobacter. 2014;20:41-48.

81. Liu Z, Dai H, Wan N, et al. Suppression of memory CD8 T cell generation and function by tryptophan catabolism. Cell Deathiffer. 2002;9:1069.

82. Boasso A, Shearer GM. How does indoleamine 2,3-dioxygenase contribute to HIV-mediated immune dysregulation. Curr Drug Metab. 2007;8:217-223.

83. Cheung MB, Sampayo-Escobar V, Green R, Moore ML, Mohapatra SS. Respiratory syncytial virus-infected mesenchymal cells regulate immunity via interferon beta and indoleamine-2,3-dioxygenase. PLoS One. 2016;11:e0163709.

84. Fox JM, Sage LK, Huang L, et al. Inhibition of indoleamine 2,3-dioxygenase enhances the T-cell response to influenza virus infection. J Gen Virol. 2013;94:1451-1461.

85. Fox JM, Sage LK, Poore S, Johnson S, Tompkins SM, Tripp RA. Drug analog inhibition of indoleamine 2,3-dioxygenase (IDO) activity modifies the memory T cell response to influenza challenge. Viral Immunol. 2014;27:112-123.

86. Fox JM, Sage LK, Poore S, Johnson S, Tompkins SM, Tripp RA. Drug analog inhibition of indoleamine 2,3-dioxygenase (IDO) activity modifies pattern recognition receptor expression and proinflammatory cytokine responses early during influenza virus infection. J Leukoc Biol. 2014;96:447-452.
112. Schoggins JW, Rice CM. Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol*. 2011;1:519-525.

113. Rajan D, Chinnadurai R, Keefe EO, et al. Protective role of indoleamine 2,3 dioxygenase in respiratory syncytial virus associated immune response in airway epithelial cells. *Virology*. 2017;512:144-150.

114. Aldajani WA, Salazar F, Sewell HF, Knox A, Ghaemmaghami AM. Expression and regulation of immune-modulatory enzyme indoleamine 2,3-dioxygenase (IDO) by human airway epithelial cells and its effect on T cell activation. *Oncotarget*. 2016;7:57606-57617.

115. Schauer U, Hoffjan S, Rothoeft T, et al. Severe respiratory syncytial virus infections and reduced interferon-gamma generation in vitro. *Clin Exp Immunol*. 2004;138:102-109.

116. Stier MT, Bloodworth MH, Toki S, et al. Respiratory syncytial virus infection activates IL-13-producing group 2 innate lymphoid cells through thymic stromal lymphopoietin. *J Allergy Clin Immunol*. 2016;138:814-824.e11.

117. Klein Wolterink RGJ, Hendriks RW. Type 2 innate lymphocytes in allergic airway inflammation. *Curr Allergy Asthma Rep*. 2013;13:271-280.

118. Scanlon ST, McKenzie ANJ. Type 2 innate lymphoid cells: new players in asthma and allergy. *Curr Opin Immunol*. 2012;24:707-712.

119. Drake LY, Iijima K, Kita H. Group 2 innate lymphoid cells and CD4+ T cells cooperate to mediate type 2 immune response in mice. *Allergy*. 2014;69:1300-1307.

120. Wen T, Rothenberg ME. The regulatory function of eosinophils. *Microbiol Spectr*. 2016;4. https://doi.org/10.1128/microbiolspec.MCHD-0020-2015.

121. Jacobsen EA, Zeilner KR, Colbert D, Lee NA, Lee JJ. Eosinophils regulate dendritic cells and Th2 pulmonary immune responses following allergen provocation. *J Immunol*. 2011;187:6059-6068.

122. Zhivaki D, Lemoine S, Lim A, et al. Respiratory syncytial virus infects regulatory B cells in human neonates via chemokine receptor CX3CR1 and promotes lung disease severity. *Immunity*. 2017;46:301-314.

123. Shinde R, Shimoda M, Chaudhary K, et al. B cell-intrinsic IDO1 regulates humoral immunity to T cell-independent antigens. *J Immunol*. 2015;195:2374-2382.

124. Palavecino CE, Céspedes PF, Gómez RS, Kalergis AM, Bueno SM. Immunization with a recombinant bacillus Calmette–Guérin strain confers protective Th1 immunity against the human metapneumovirus. *J Immunol*. 2014;192:214.

125. Bueno SM, González PA, Cautivo KM, et al. Protective T cell immunity against respiratory syncytial virus is efficiently induced by recombinant BCG. *Proc Natl Acad Sci USA*. 2008;105:20822-20827.

126. Cautivo KM, Bueno SM, Cortes CM, Wozniak A, Riedel CA, Kalergis AM. Efficient lung recruitment of respiratory syncytial virus-specific Th1 cells induced by recombinant bacillus Calmette-Guérin promotes virus clearance and protects from infection. *J Immunol*. 2010;185:7633.

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