Peer Review File

Article Information: http://dx.doi.org/10.21037/atm-20-3594

Reviewer A:

Comment 1:

The abbreviation should not be used in headings.

Reply 1:

We have modified the abbreviation in the headings.

Changes in the text:

See page 6, line 131; page 7, line 133; page 9, line 179; page 9, line 187; page 10, line 204; page 11, line 224; page 12, line 261.

Comment 2:

There are few grammatical mistakes.

Reply 2:

We have modified the grammatical mistakes throughout the manuscript.

Reviewer B:

Comment 1:

There are several grammatical issues required for English proofreading.

Reply 1:

We have modified the grammatical mistakes throughout the manuscript.

Comment 2:

The authors did not clearly describe the roles of IDO/TDO in liver diseases (Table 1).

Reply 2:

We described the exact role of IDO/TDO in the table 1.

Changes in the text:

See page 25, table 1 “Role of IDO/TDO in liver diseases”.

Reviewer C:

Comment 1:

The authors should discuss about the mechanisms explaining how IDO1 which is mainly expressed in extrahepatic tissues could play a role in liver diseases.

Reply 1:

We discussed the mechanisms as follows. “The capacity of TDO in the liver to catalyze Trp is much stronger than that of IDO under basal conditions. However, IDO expressed in the extrahepatic tissues is markedly induced by the abovementioned cytokines under some pathophysiological circumstances of immune activation including bacterial and viral infection in the liver, as well as nonpathogenic inflammation including autoimmune liver diseases, liver tumors and liver transplantation, which means IDO has a wider range of effects. That’s the reason why IDO could play a role in the liver diseases.”

Changes in the text:

See page 4-5, line 86-93.

Comment 2:

IDO, there are two isoforms, the authors should precise which isoform of IDO by using IDO1 or IDO 2.

Reply 2:

We explained the obscure term as follows. “Two forms of IDO, IDO1 and IDO2 are constitutively and locally expressed, respectively(1).The capacity of IDO2 to metabolize Trp is much smaller than that of IDO1, so we mainly focus on IDO1, and use IDO instead (IDO in the following article all refers to IDO1)”.

Changes in the text:

See page 4, line 78-81.

Comment 3:

line 57, the capacity of TDO to catalyze Trp is more important than that of IDO. This is true under homeostatic conditions but not under inflammatory conditions. The authors should discuss this point

Reply 3:

We discussed the point as follows. “The capacity of TDO in the liver to catalyze Trp is much stronger than that of IDO under basal conditions. However, IDO expressed in the extrahepatic tissues is markedly induced by the abovementioned cytokines under some pathophysiological circumstances of immune activation including bacterial and viral infection in the liver, as well as nonpathogenic inflammation including autoimmune liver diseases, liver tumors and liver transplantation, which means IDO has a wider range of effects. That’s the reason why IDO could play a role in the liver diseases.”

Changes in the text:

See page 4-5, line 86-93.

Comment 4:

line73 “IDO may has opposite antimicrobial effect when excessive Trp is available”. They should also discuss works on the role of IDO in metabolic diseases through shaping gut microbiota (i.e. DOI: 10.1038/s41591-018-0060-4)

Reply 4:

We discussed the role of IDO in the metabolic diseases as follows. “Moreover, genetic deficiency of IDO could promote the metabolic health through shaping the intestinal microbiota. Depletion of microbiota in the high fat diet(HFD)-fed wildtype(WT) and IDO-knockout(KO) mice

discarded the body weight difference between these two groups of mice in which IDO-KO mice were protected from metabolic complications. Co-housing these two groups of mice made the phenotype of WT mice similar to IDO-KO mice in the separated cages. What's more, feeding the WT mice with the feces from L-1methyl tryptophan (1MT)-treated ob/ob mice made the WT mice gain less body weight. The bacterial components of feces indicated that the decrease of Clostridiales Lachnospiraceae may be beneficial to control inflammation in the HFD-fed IDO-KO and 1MT-treated ob/ob mice. All these phenomena demonstrated that the intestinal microbiota of IDO-KO mice indeed protected the mice against obesity(20)."

Changes in the text:

See page 6, line 115-126.

Comment 5:

the role of IDO in viral infections described page 6 is unclear.

Reply 5:

We modified the role of IDO in other viral infection as follows. "When it comes to other hepatitis virus infections, blockage of TDO and IDO lead to different outcomes. In a study of mouse hepatitis virus (MHV-A59) model, blocking TDO with LM10, a derivative of 3-[2-(pyridyl) ethenyl] indole, decreased the level of anti-MHV antibody and inhibited autoimmune responses, indicating that TDO may be a new perspective and target to treat certain kind of viral infection(27). Nevertheless, Vega et al(28) expressed the opposite opinion, indicating that application of L-MT, which blocked IDO without affecting TDO, did not protect the mice from MHV infection but instead augmented detrimental effects of the virus action, including hypergammaglobulinemia, anti-MHV Ab and uric acid release, as well as liver fibrosis."

Changes in the text:

See page 8-9, line 169-178.

Comment 6:

IDO in NAFLD diseases. In the paper mentioned, the authors used high fat diet to induce liver steatosis. This part is uncomplete and needs discussion. Indeed, the authors conclude that "IDO plays a protective role in NAFLD" but another paper (DOI: 10.1038/s41591-018-0060-4) showed opposite result and this was not discussed in this review.

Reply 6:

We modified this part as follows. "Non-alcoholic fatty liver disease (NAFLD) is one of the metabolic diseases with high prevalence among contemporary humans. It was reported that IDO-KO mice were more prone to liver inflammation and fibrosis induced by a high fat diet compared to IDO-wildtype(WT), suggesting that IDO may be beneficial in NAFLD(30). Moreover, a study conducted among obese women showed that the increased activity of IDO in the liver and adipose tissue was inversely correlated with balance of Th17 relative to regulatory T cells(Treg) in subcutaneous fat(31). However, Laurans et al(32) demonstrated that upregulated activity of IDO was correlated with obesity. They found that lack of IDO ameliorated the state of insulin resistance, improved the intestinal permeability, and protected mice from liver steatosis. In addition, another study demonstrated that kynurenine, the metabolite of Trp by IDO, was a known aryl hydrocarbon receptor (AhR) agonist and resulted in obesity. Blocking IDO or AhR prevented longer-term, diet-induced liver steatosis(33). Moreover, a high fat diet which facilitated proinflammatory environment suppressed the activity of TDO in the liver, but enhanced the

extrahepatic metabolism of Trp by IDO(34). Therefore, IDO plays the complex roles in the NAFLD which needs further research."

Changes in the text:

See page 9-10, line 188-203.

Comment 7:

the authors sometimes over interpreted the results. For example, in the paper cited as ref 32, the authors showed that IDO activity increases in obese women but this does not mean that this increase "compensate for obesity-associated inflammation". In that paper, the authors described an inverse correlation between IDO activity and Th17/Treg balance.

Reply 7:

We modified the explanation of this reference as follows. "Moreover, a study conducted among obese women showed that the increased activity of IDO in the liver and adipose tissue was inversely correlated with balance of Th17 relative to regulatory T cells(Treg) in subcutaneous fat(32)."

Changes in the text:

See page 9, line 191-194.

Comment 8:

line 137, what does it mean serum IDO? IDO is expressed by immune cells and epithelial cells and what we can measure in serum is Trp metabolites not IDO itself?

Reply 8:

In the reference" Indoleamine 2,3-dioxygenase 1 defciency attenuates CCl4-induced fbrosis through Th17 cells down-regulation and tryptophan 2,3-dioxygenase compensation (DOI:10.18632/oncotarget.17119)", authors used IDO1(Cusabio, China) ELISA KIT to detect serum samples from human participants and mice. Although in many other literature, authors used the ratio of kynurenine/trptophan(KTR) to measure the activity of IDO.

Reviewer D:

Comment 1:

Please include the name tryptophan pyrrolase as well for tryptophan 2.3 dioxygenase since that was the name used in the earlier literature.

Reply 1:

We added the name tryptophan pyrrolase for tryptophan 2.3 dioxygenase as follwows. "TDO，also known as tryptophan pyrrolase".

Changes in the text:

See page 3, line 64-65.

Comment 2:

Line 83, "IDO expression on hepatocytes was elevated due to activation of HBV…" Please

specify the cell types IDO is present in the liver. Is it expressed in the hepatocytes or is it expressed only in Kuffer cells? If present in hepatocytes, how is regulated? Is it regulated by steroids or is it regulated by interferon γ?

Reply 2:

In the reference "Upregulation of indoleamine 2,3-dioxygenase in hepatocyte during acute hepatitis caused by hepatitis B virus-speci¢c cytotoxic T lymphocytes in vivo(DOI:10.1111/j.1478-3231.2008.01748.x)", the authors injected HBV-specific CTLs into HBV Tg mice, making the direct contact between HBsAg-positive hepatocytes and HBV-specific CTLs. Then HBV-specific CTLs produced a large amount of IFN-γ which directly acted on hepatocytes to induce the expression of IDO in this acute hepatitis model. Therefore, we modified the sentence as follows. "Activated HBV and HCV-specific cytotoxic T lymphocytes produced IFN-γ which acted on hepatocytes, making the hepatocytes express IDO in patients with the corresponding type of viral hepatitis(21, 22)"

Changes in the text:

See page 7, line 135-137.

Comment 3:

Line 148: "Taken together, IDO is correlated with liver disease". Positively or negatively? Please be more specific.

Reply 3:

We modified the sentence as follows. "Taken together, the opposite effects of IDO on liver fibrosis are associated with different circumstances. It exacerbated liver fibrosis and cirrhosis in some cases, and in other cases, it may ameliorate the lesions."

Changes in the text:

See page 11, line 221-223.

Comment 4:

Line 188: "IDO expressing DC group showed milder symptoms…….". Are there dendritic cells (DC) in the liver? Or are the DCs mentioned here are in immune organs like spleen?

Reply 4:

In the reference" IDO-Competent-DCs Induced by IFN-γ Attenuate Acute Rejection in rat Liver Transplantation (DOI:10.1007/s1087-012-9681-4)", the authors used recombinant rat IFN-γ to treat the cultured DCs which were separated from the spleen. The IFN-γ-treated DCs were able to produce IDO. In the IFN-γ-DC treatment group, the DCs were re-infused into the recipient animals via the portal vein. Therefore, we modified the sentence as "Moreover, Sun et al(56) demonstrated that IFN-γ-treated IDO-expressing DCs re-infused rat group showed milder symptoms of acute rejection compared to the control group".

Changes in the text:

See page 13, line 267-269.

Comment 5:

Is the depletion of tryptophan the primary mechanism, or the kynurenine and the metabolites play the major role, or both mechanisms play major roles in the immune regulation? Please give a brief overview of the state of the art on this issue.

Reply 5:

We gave a brief overview about this issue as follows. “Indeed, it is both the depletion of Trp and the production of kynurenine and other metabolites that play the roles in immune regulation. Firstly, catabolizing Trp itself in the local microenvironment triggers amino acid-sensing signal transduction pathways. Secondly, the downstream metabolites of Trp reduce activities of innate cells like natural killer (NK) cells, DCs, macrophages; inhibit Th1 cells proliferation; while promote Th2-phenotype polarization; induce transforming growth factor-β(TGF-β) production and subsequent regulatory T cells (Tregs) differentiation. Tregs activate myeloid-derived suppressor cells (MDSCs), leading to T cells inhibition. The metabolites also act to block differentiation of type 17 T helper (Th17) cells(5, 12-19).”

Changes in the text:

See page 5, line 100-110.