Elucidating the Role of 2-(1' H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) on Immune Cell Differentiation

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Abstract

The association of aryl hydrocarbon receptor (AhR) activation has consistently been associated with immunological changes, specifically in T cells. The prototypic ligand, (TCDD), has been shown to induce the anti-inflammatory subset of Foxp3+ T cells known as T-regulatory cells. However, this ligand has been shown to be toxic at higher doses. In this study, the nontoxic tryptophan derivative and endogenous AhR ligand, 2-(1' H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) was used to explore its effects on T-regulatory cell induction and cytokine secretion. Splenocytes were isolated and plated for 24 hours in the absence or presence of Concanavalin A, a polyclonal T cell mitogen. ITE was also added to cell cultures at a dose of 10 μM or 100 μM. Our results showed that ITE increases secretion of IL-10, the signature cytokine for T-regulatory cells, and IL-6 at the lower dose, but not at the higher 100 μM dose. Further, there was a trend of upregulation of Foxp3 expression upon ITE exposure at this 10 μM concentration. However, this was not observed in cells activated with ConA. Taken together, these results support ITE's ability to skew T cell differentiation towards an immunosuppressive T-regulatory phenotype at low doses. Future studies should explore the effects associated with high dose exposure to ITE.

Introduction

- ITE is a nontoxic, tryptophan-derived, endogenous AhR ligand that has recently been found to be efficient in containing inflammatory diseases
- It has demonstrated an increase in the production of T-regulatory cells
- Concanavalin A has proven to mimic a strong immune reaction in mice by activating T cells polyclonally.

Methodology

Female C57BL/6 mice aged 10 weeks were humanely euthanized and splenocytes were isolated. Cells were cultured in a 24-well plate in complete RPMI with or without Concanavalin A at a dose of 5 μg/mL and with or without ITE at a dose of 10 μM or 100 μM. After 24 hours, cells were collected and stored in Qiazol until RNA isolation. Supernatants were also collected and used to conduct ELISAs.

Results

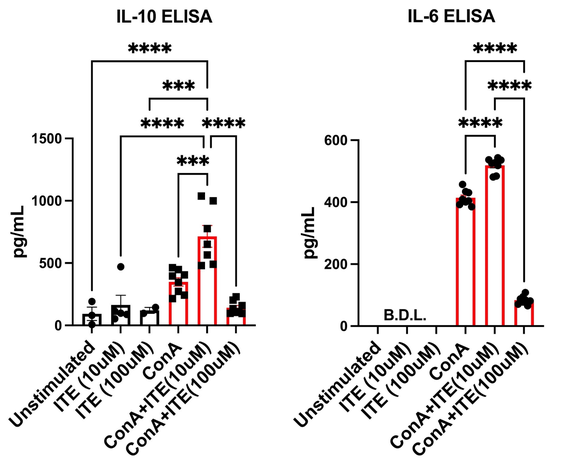

Figure 1: 10 μM ITE increases IL-10 and IL-6 production

ELISAs show that IL-10 production is increased upon treatment with 10 μM ITE but decreased upon treatment with 100 μM (A). IL-6 production is only detected upon ConA activation and is again increased upon treatment with 10 μM ITE (B).

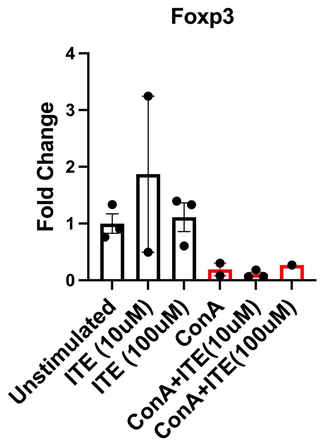

Figure 2: ITE increases Foxp3 expression

PCR shows trends of ITE increasing Foxp3 expression even in the absence of ConA stimulation.

Conclusions

- ITE is capable of increasing production of both IL-10 and IL-6 when used at a concentration of 10 μM
- At a dose of 100 μM, these effects of ITE are diminished
- PCR results show a trend of upregulating Foxp3 expression when exposed to ITE
- Taken together, these results support ITE's ability to skew T cell differentiation towards an immunosuppressive T-regulatory phenotype at a dose of 10 μM, but not at the higher dose of 100 μM

Acknowledgements

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