Immunomodulatory effects of Atractylenolide II from Yu-Ping-Feng Formula

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INTRODUCTION

Yu-Ping-Feng (YPF) formula, a classical prescription in traditional Chinese medicine (TCM), is renowned for its immunomodulatory properties. Comprising Astragali Radix (Huang-Qi), Atractylodis Macrocephalae Rhizoma (Bai-Zhu), and Saposhnikoviae Radix (Fang-Feng), YPF has been used for centuries to enhance immunity and treat respiratory and immune-related disorders, including asthma, chronic obstructive pulmonary disease, and cancers. Recent studies have highlighted its ability to modulate immune responses by regulating cytokine production, immune cell proliferation, and the cellular microenvironment in primary Sjögren syndrome (pSS) [1]. Among bioactive components in YPF, atractylenolide II (Atr II), a sesquiterpene lactone derived from Atractylodis Macrocephalae Rhizoma (AMR), has emerged as a potential contributor to immunomodulatory effects of YPF formula[1]. Despite its significance, the specific mechanisms by which Atr II influences immune responses remain underexplored. This study aims to investigate the immunomodulatory effects of Atr II using flow cytometry and enzyme-linked immunosorbent assay (ELISA) to assess its impact on immune cell subsets and cytokine profiles. By elucidating role of Atr II, this research seeks to provide a scientific basis for its therapeutic application in immune-related disorders.

METHODOLOGY

Database mining and molecular docking were utilized to ensure genetic and molecular linkage between Atr II and pSS, using the online program Venny 2.1.0 (from Juan Carlos Oliveros, BioinfoGP, CNB-CSIC). Relevant immune cells were extracted from lymph tissues of C57BL/6 mice and were cultured in R10 solution for at least 72 hours. Cells were treated with Atr II solution at concentrations of 0, 6.25, 12.5, 25 umol/L for 72 hours. Flow cytometry was used for quantification of cell presented. Cell culture supernatants was collected to measure cytokines (IL-6, and IL-21) using ELISA kits. Absorbance of the samples was measured at 450 nm using spectrometer.

RESULTS & FIGURES

Cell culture and Flow Cytometry

Naïve $CD4^+$ T cells were extracted from C57BL/6 mice and purified. Then, the cells are subjected to TFH polarization in the Atractylenolides II addition and control. The quadruplicate cell cultures yield the positive results. With Atr II presented, smaller cell counts was obtained. Moreover, cytometric analysis also distinguished the effect of different concentration of Atr II on $CD4^+$ cells.

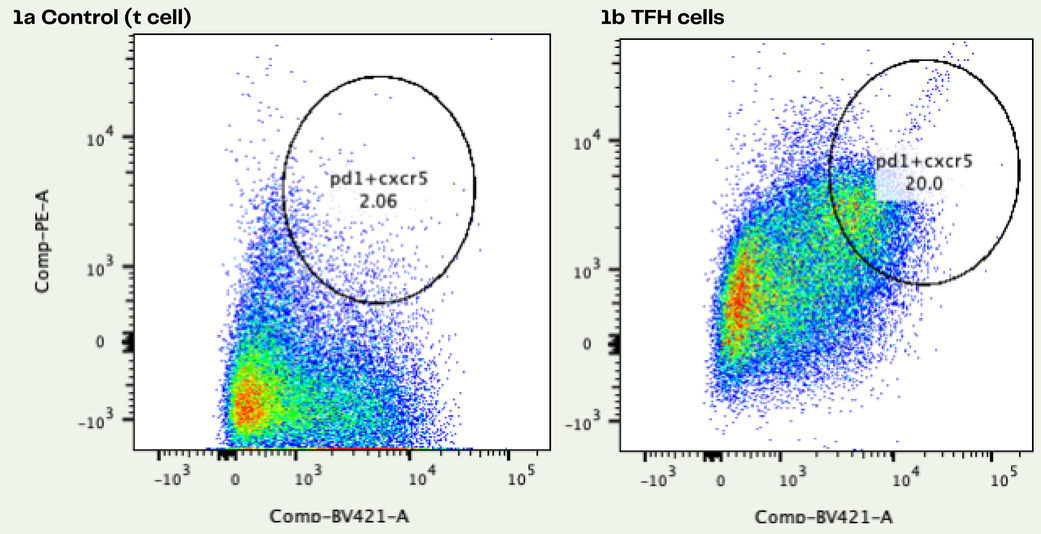

Figure 1a and 1b showed the flow cytometry graph of the control, $CD4^+$ T cells with no differentiation, and $CXCR5^+$ $PD1^+$ TFH subset of $CD4^+$ T cells. The of amount of TFH cells in original source without any Atr II addition is 38519.
Figure 2a, 2b, 2c and 2d showed the flow cytometry graph of $CXCR5^+$ $PD1^+$ TFH subset of $CD4^+$ T cells at different concentration of Atr II culture.

Figure 1a &1b. Flow cytometry graph of $CD4^+$ T and $CXCR5^+$ PD^+ TFH cells without Atr II addition

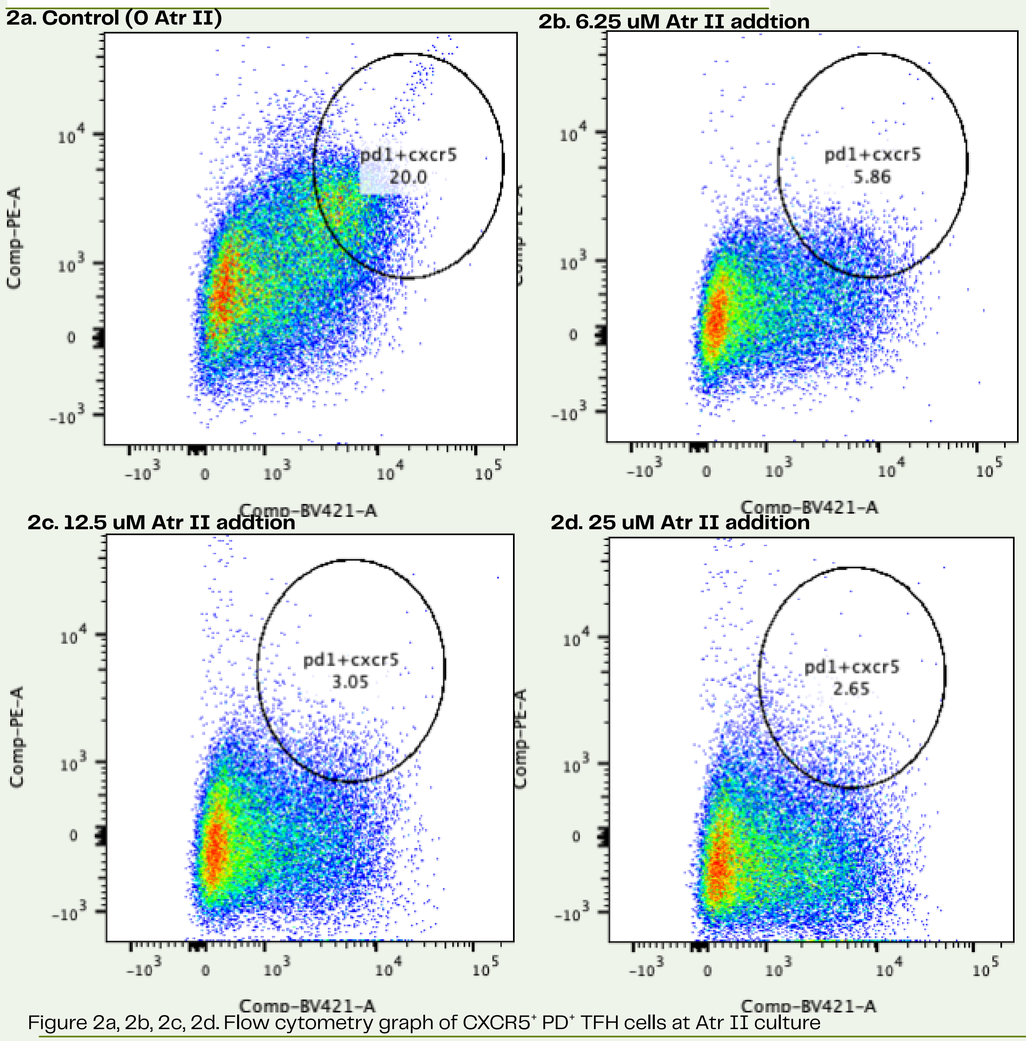

Figure 2a, 2b, 2c, 2d. Flow cytometry graph of $CXCR5^+$ PD^+ TFH cells at Atr II culture

The quantity of Atr II-treated cells range from 833 to 1454, thus, Atr II induce a percentage decrease in TFH cells by over 95%. This illustrate the inhibitory effect of Atr II.

3 different concentrations of Atr II, 6.25, 12.5, 25 uM were considered and utilized. Although all concentrations of could yield a decrease in TFH cell counts, 25 uM of Atr II encountered the greatest decrease in TFH cells number, illustrating in the Figure 3. Therefore, concentration of 25 umol/L would be the most optimal concentration to generate the inhibitory effect to TFH cells.

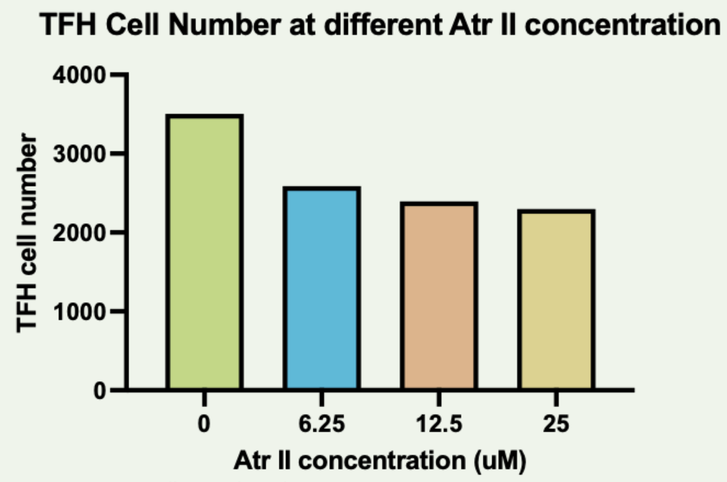

Figure 3. TFH cells number decrease at increasing Atr II concentrations

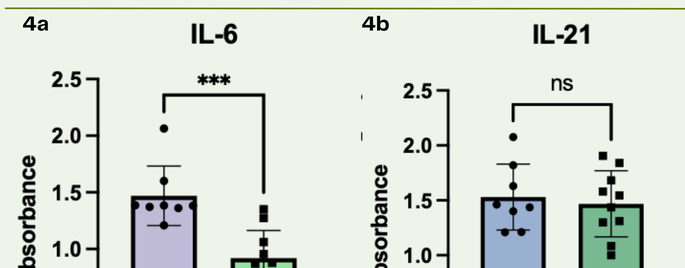

Figure 4a & 4b. ELISA results of IL-6 and IL-21 content in cell culture

ELISA

The content of cytokines IL-6 and IL-21 was measured using ELISA kits and expressed as absorbance difference. Atr II can inhibit the expression of both IL-6 and IL-21, with much greater effect on IL-6 expression, coupled with significant unpaired t-test result and p=0.0003. Nonobvious reduction is found in IL-21 amount, with insignificant result (p=0.6712). ELISA results further confirm anti-inflammatory properties of Atr II, as evidenced by decreased levels of pro-inflammatory cytokines IL-6 and IL21.

Network pharmacology analysis of Atractylenolides II

To illustrate the potential mechanisms behind the immunomodulatory effects of the chemicals in AMR, a network pharmacology approach was employed to identify overlaps between the bioactive components of the AMR and the target proteins involved in the pathogenesis of pSS. A total of 1203 genes related to SS were gathered from the GeneCards human gene database and the Online Mendelian Inheritance in Man (OMIM) database, and twelve drug target proteins associated with the Atr II extracted from Traditional Chinese Medicine Systems Pharmacology Database and the Encyclopedia of Traditional Chinese Medicine. After eliminating duplicates using a Venn diagram tool, four shared targets between the Atr II and pSS were identified, including XBP1, CA2, CA1, and PRKCA.

Among these 4 common targets, XBP1 is significant for the IL-6, IL-21, and IL-17 signal pathway[2][3]. The activation of XBP1 allows its binding to the IL-6 promoter[2]. This enhances IL-6 transcription and hence trigger immune response, B-cell differentiation and any acute- phase reactions[2], then T follicular helper cells and other immune cells that produce IL-21. Besides, XBP1 is also substantial in the activation of T Helper 17 (Th17) cell differentiation and IL-17 production[3] and amplifies inflammatory response. Specifically, XBP1 can upregulate genes involved in Th17 cell function, including those promote IL-17 expression such as RORγt, a key transcription factor for Th17 differentiation[3]. Effect of Atr II on IL-17 has been determined in Yu et al. [1].

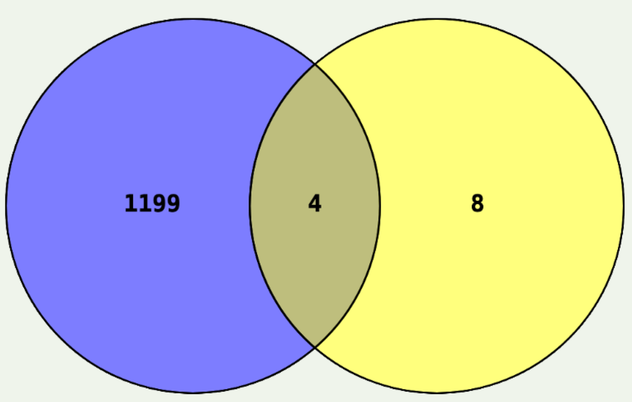

Figure 5. Venn diagram for interactions of component targets

Gene (Full Name)	Function
XBP1 (X-Box Binding Protein 1)	Transcription factor involved in the unfolded protein response (UPR) during endoplasmic reticulum stress
PRKCA (Protein Kinase C Alpha)	Encodes a serine/threonine kinase involved in signal transduction pathways
CA1 (Carbonic Anhydrase 1)	Encodes an enzyme similar to CA2, primarily expressed in red blood cells, aiding CO_2 transport
CA2 (Carbonic Anhydrase 2)	Encodes an enzyme that catalyzes the reversible hydration of carbon dioxide to bicarbonate and protons

Figure 6. Table of infomation of the four common targets

CONCLUSION AND FUTURE PLANS

This study has effectively explained how Atr II in AMR modulate the immune system by the network pharmacology and the biological makeup of pSS to find commonalities with target proteins. By combining a vast dataset of 1203 genes from databases along with drug target proteins from Atr II, four common targets were found, which are also key in the immune pathways. Importantly, XBP1 activation was seen to be crucial for the signalling pathways of IL-6, IL-21, and IL-17. This not only enlarge immune responses and B-cell differentiation but also affects follicular helper T cells and other immune cells that produce IL-21. These results hint that targeting XBP1 and its related pathways, might open up new ways to treat pSS by tweaking key inflammatory and immune responses. Future research should focus on confirming the role of the four relevant gene in immuno-signaling pathway, to create more targeted treatments for autoimmune diseases in clinical field.

Reference

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