Influence of Itraconazole on T-cell Immune Deviation

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ABSTRACT

**Objective:** The objective of this study was to determine if the presence of itraconazole can alter the activation and differentiation pattern of T-cells. Proliferation assays utilizing immunofluorescence and flow cytometry were used to determine the presence, number, and type of T-cell subpopulations within each experimental group. Also, we sought to verify that the presence of itraconazole can alter the production of Th2 mediated cytokines. RT-PCR was employed to measure relative numbers of gene transcripts for various characteristic cytokines associated with T-cell subpopulations.

**Design:** Prospective, controlled experiment.

**Subjects:** Samples of whole blood were taken from healthy volunteers. Samples of whole blood were taken from healthy volunteers.

**Intervention(s):** Following isolation of naive T cells, cells were divided into 4 groups and immune depleted in the following fashion. Controls cell lines were activated with anti-CD3 and CD28 and additional cytokines and antibodies were added to deviate towards Th1 and Th2 conditions. Experimental groups consisted of similar conditions with the addition of itraconazole.

**Results:** Successful immune deviation of controls was performed. With the addition of itraconazole to Th2 deviating conditions, IL-5, IL-13, and IFN gamma were all noted to decrease (both by PCR and supernatant assay).

**Conclusions:** Itraconazole inhibits the deviation of naive T cells towards the Th2 mediated pathway.

**METHODS AND MATERIALS**

**CD45RA + Naive T Cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
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<tbody>
<tr>
<td>No-Additive</td>
<td>CD45RA+ Naive T Cells</td>
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<tr>
<td>Anti-IgE</td>
<td>Anti-IgE and Anti-IL-4</td>
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<tr>
<td>Anti-IL-4</td>
<td>Anti-IgE and Anti-IL-4</td>
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Cells were incubated in respective solutions for 96 hours. Following incubation, T cells were then harvested into 2 groups. Group 1 cells were stained and fixed in preparation for flow cytometry to determine the number of each T-cell population (Th1 vs Th2). Real time PCR was then performed on the group 2 cells, to determine the relative number of mRNA transcripts for IFN-γ, IL-4, IL-5, and IL-13 as compared to a reference protein.

**Aim 1:** Successfully deviate naïve T cells into the Th1 and Th2 conditions.

**Result:** Protein assays (Bioplex) show production of related cytokines, including IL-4, IL-5, IL-13, and IFN gamma.

**RESULTS**

**HYPOTHESIS**

Itraconazole will inhibit the differentiation of Th cells into Th2 populations. This inhibition will be demonstrated by a skewing of the resultant population into Th1 activation, noted on flow cytometry analysis. Further, the cytokine profile of the resultant populations should also show a marked reduction in the production of IL-4 and IL-5.

**INTRODUCTION**

Chronic rhinosinusitis (CRS) is a chronic inflammatory condition affecting the paranasal sinuses and nasal cavities which has a significant adverse impact on the quality of life and daily functioning of its sufferers.1,2 No one specific etiology has been defined and it is more likely that this term represents either multiple different diseases with or without various stages of severity along their spectra.

Certain subsets of CRS have been strongly associated with a Th2 mediated inflammatory process and, in turn, it has been theorized that at least a portion of these subsets are associated with an abnormal reaction to the presence of fungi in the nasal cavity and paranasal sinuses.3,4 Previous work has demonstrated the ability of itraconazole to mitigate the production of Th2 associated cytokines when non-specific T-cell cultures are grown in its presence. Additionally, there has been some data regarding the clinical efficacy of treating CRS patients with itraconazole.5,6 However, no work has been done evaluating the potential role that it may have in affecting the initial differentiation process of the Th0 cell upon activation.

Several studies have related anti-myocotics to the suppression of T cell (IL-2) and Th2-mediated cytokines (IL-4 and IL-5). Bruseder demonstrated that itraconazole suppressed the cytokine-driven proliferation of human AML cell lines.7 Kanda et al showed results that anti-myocotics reduced the anti-CD3/CD28-induced mRNA expression and promoter activities for interleukin-4 and interleukin-5 in atopic and normal volunteer human T-cells.8,9

**DISCUSSION**

Successful immune deviation of naïve CD 45 RA + T cells into Th1 and Th2 conditions was demonstrated by production of respective Th1/Th2 cytokines under directional stimulation. This proved the technique for immune deviation to be valid prior to the addition of itraconazole to the experimental design.

The addition of itraconazole to cells stimulated in both the TH1 and TH2 pathways showed pan-inhibition with cytokines, showing that the percentage of IL-5, IL-13, and IFN-γ producing cells were reduced. This information may point to the direct inhibition by itraconazole of the initial T-cell differentiation pathway.

Perhaps as an inhibitor of IL-4 and IFN-γ message generation, itraconazole is indirectly reducing the number of viable cells by blocking pro-differentiation/anti-apoptotic cytokines. Further study is warranted with longer incubation periods to determine if a more pronounced effect can be seen as has been previously demonstrated on a mature T-cell population.

**CONCLUSION**

Itraconazole inhibited the differentiation of naive T cells into Th2 pathway. This was demonstrated by the skewing of the naïve T-cell population with the addition of itraconazole to the stimulate naive T cell. Further, the cytokine profile of the resultant populations should also demonstrated reduction in the production of Th2 stimulatory cytokines, IL-4 and IL-5.

**REFERENCES**


**ADDITIONAL INFORMATION**

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