The Retinoid Receptor (RXR) Selective Agonist IRX4204 Promotes Differentiation of Human aTreg Cells and Inhibits Differentiation of Human Th17 Cells

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Introduction

Retinoid receptors have been shown to be involved in several important immune pathways, in particular in the generation of anti-inflammatory adaptive T regulatory cells (aTreg). Retinoid X receptor is a nuclear receptor that is activated by 9-cis retinoic acid and dimerizes with other nuclear receptors. IRX4204 is an orally available second generation highly selective RXR agonist. It has shown activity against a variety of human cancers in vitro and in animal models, including prostate, breast, pancreas, and lung. Preclinical studies have also demonstrated potential for the drug to be active in Alzheimer's disease, Parkinson's disease, and multiple sclerosis.

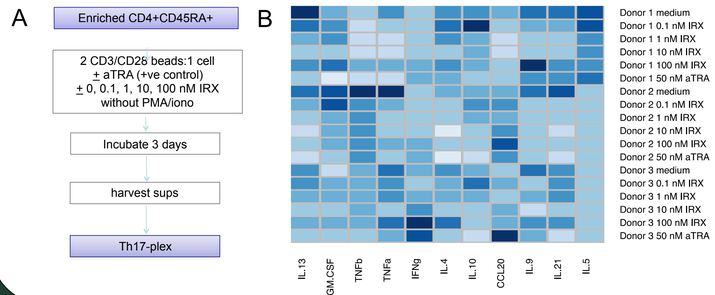
Furthermore, IRX4204 has been demonstrated in preclinical mouse studies to have a potent effect on the *in vitro* differentiation of T cells and *in vitro* cytokine production by both T cells and monocytes. To gain a further understanding of mechanism of action of IRX4204, we examined the effect of the agonist in several *in vitro* analyses with a cohort of healthy control subjects. IRX4204 reduced IL-17A/F secretion from T cells incubated under Th17 skewing conditions. The presence of IRX4204 reduced the level of several inflammatory cytokines from naive T cells stimulated with anti-CD3/CD28, a polyclonal activator. When naive CD4+ T cells were cultured under aTreg skewing conditions, addition of IRX4204 gave rise to increased numbers of aTreg. Taken together, these results indicate that IRX4204 may be a promising anti-inflammatory therapeutic.

Methods

Blood was drawn from 3 healthy individuals. PBMCs were isolated and naïve CD4+ T cells were isolated using magnetic beads. Naïve CD4+ T cells were cultured in the following conditions:

Naïve CD4+ activation: anti-CD3/anti-CD28 beads (2 cells/bead), IL-2 (50U/ml)
Th17 Skewing: AIM V without serum, IL-2/IL-1beta/IL-6/IL-23, anti-IFNg/anti-IL-4, anti-CD3/anti-CD28 beads (4 cells/bead)
Adaptive T_{reg} skewing: TGFbeta (0, 0.1, 1, 2 ng/ml), IL-2 (50U/ml), anti-CD3/anti-CD28 beads (4 cells/bead), 2.5% human serum
All cultures were treated with a range of IRX4204, from 0-100nM. Supernatants were analyzed by ELISA or a Th17 multiplex kit, and cells were phenotyped by flow cytometry.

Results



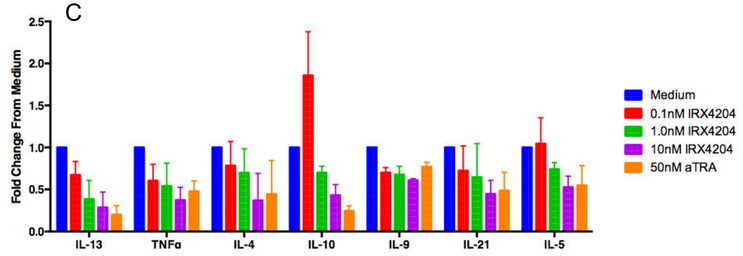
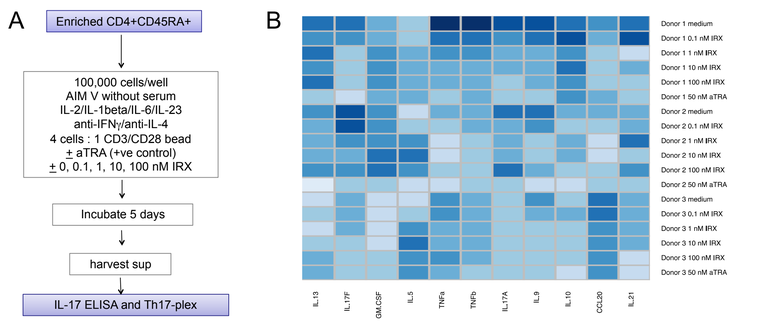


Figure 1. IRX4204 alters cytokine secretion by naïve CD4+ T cells cultured in activating conditions. (A, at left) Experimental design. PBMCs were isolated from whole blood, then enriched using magnetic beads to a >95% pure CD4+CD45RA+ naïve T cell fraction. Cells were stimulated as indicated and supernatants collected following 3 days in culture. (B, at left) Heat map showing results from a multiplex analysis of cellular supernatants. Darker blue indicates greater cytokine levels. (C) Fold change of cytokine expression normalized to medium. The mean and SD of three individuals is shown.



No effect seen on GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL13, IL-15, IL-25, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33

Figure 2. IRX4204 reduces Th17-associated cytokine secretion by cells cultured in Th17 skewing conditions. (A) Experimental design. PBMCs were isolated from whole blood, then magnetically separated to enrich a ~95% pure CD4+CD45RA+ naïve T cell fraction. Cells were stimulated as indicated and supernatants collected following 5 days in culture.
(B) Heatmap of multiplex analysis of day 5 supernatants. Darker blue indicates greater cytokine levels. (C) Fold change of cytokine expression normalized to medium. The mean and SD of three individuals is shown. Notable trends with IRX4204 in the culture. A decrease in IL-17A/F, TNFalpha, TNFbeta, IL-9 and CCL20 was seen.

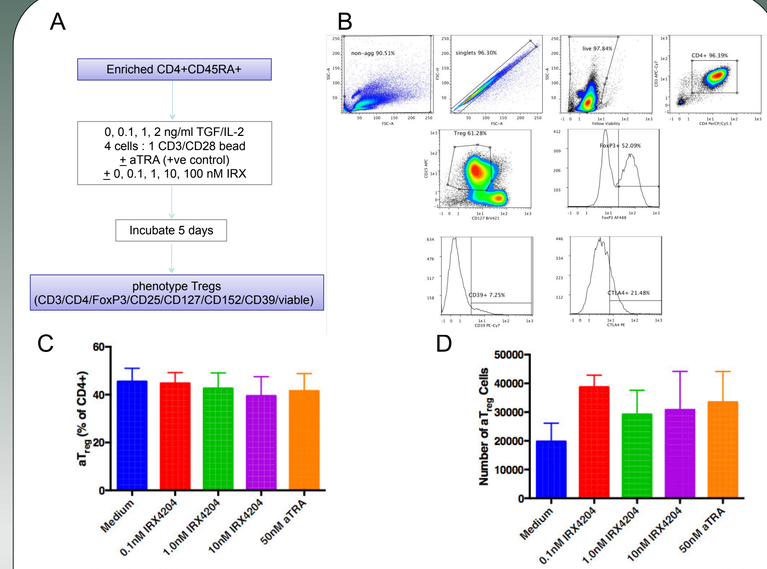


Figure 3. IRX4204 increases aTreg conversion and proliferation of naïve CD4+ T cells in aTreg skewing conditions. (A) Experimental design. PBMCs were isolated from whole blood, then magnetically separated to enrich a ~95% pure CD4+CD45RA+ naïve T cell fraction. Cells were stimulated as indicated and analyzed via flow cytometry after 5 days in culture. (B) Flow cytometry gating strategy. Non-aggregate, then live, then singlet cells were gated. The CD3+CD4+ cells were gated and analyzed for the frequency of CD127loCD25+ cells. These cells were then analyzed for FoxP3, CD39, and CD152. (C) Frequency of CD4+FoxP3+CD25+ cells. (D) Absolute number of aTreg cells from cells cultured in skewing conditions with 1ng/ml TGFβ. 0.1nM of IRX4204 greatly increased the number of aTreg cells. The mean and SD of three individuals is shown.

Conclusions

Our results show:

- IRX4204 inhibits *ex vivo inflammatory* cytokine production by activated human naïve CD4+ T cells
- IRX4204 induces a strong, dose-dependent decrease in cytokines produced by *in vitro* skewed human Th17 cells
- IRX4204 shows a strong effect on human aT_{reg} proliferation

These results show that IRX4204 can alters the responses of naïve human CD4+ T cells. IRX4204 may prove to be a potent therapeutic in inflammatory or autoimmune diseases.

Acknowledgements

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