Identification of a T Cell Immunomodulatory Domain in Histidyl-tRNA Synthetase

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Abstract

Histidyl-tRNA synthetase (HARS) is the autoantigen target of Jo-1 antibodies, which occur in the major form of anti-synthetase syndrome. These patients are characterized by an autoimmune myositis and interstitial lung disease. Circulating extracellular HARS is detected in healthy individuals, but is reduced or undetectable in Jo-1-positive individuals. Administration of ATYR1940, a recombinant form of HARS, ameliorates lung fibrosis and reduces T cell cytokine production in the bleomycin-induced lung injury model. Similar effects were observed with the N-terminal domain of HARS (the iMod domain) conjugated to IgG Fc, suggesting that this domain confers the immunomodulatory activity of HARS.

To confirm primary immune effects of ATYR1940 and ATYR1923 (iMod.Fc), human T cells were isolated from PBMC from healthy individuals and stimulated with anti-CD3/anti-CD28. Proteins containing the HARS iMod domain reduced *in vitro* activation of human CD4$^+$ and CD8$^+$ T cells, as evidenced by reduced secretion of IL-2, IFNγ, TNFα, IL-17, IL-13, and granzyme B, as well as decreased upregulation of activation markers such as CD69 and CD40L. ATYR1940 and ATYR1923 also inhibited cytokine release after *ex vivo* stimulation of human memory T cells in a NSG mouse xenogeneic GVHD model. T cell inhibition by ATYR1940 was dependent on its iMod domain, as demonstrated using an iMod-specific blocking monoclonal antibody. The ATYR1940-induced T cell gene signature reflected a general inhibitory effect on activation as well as on cell cycle protein expression. These results suggest that circulating levels of HARS may act to control the threshold stimulatory signal required to activate T cells. We propose circulating HARS as a soluble immune set-point modulator.

Introduction

- A number of non-canonical functions of proteins generated from tRNA synthetase genes have been reported, demonstrating diverse roles for these proteins outside of protein synthesis (Wakasugi & Schimmel, 1999; Park et al., 2008; Arif et al., 2017).
- Proteins derived from the histidyl-tRNA synthetase (HARS) gene are found extracellularly and are detected in the serum of all healthy donors.
- Patients with anti-synthetase syndrome that are positive for anti-HARS (Jo-1) antibodies are often characterized by inflammatory infiltrates in skeletal muscle and lung.
- In these individuals, circulating HARS is reduced or undetectable (unpublished results).

Hypothesis: Extracellular HARS may exert immunomodulatory functions

Material and Methods

Cell Culture:

- Peripheral blood mononuclear cells (PBMC) were isolated from the blood of healthy donors and T cells, CD4$^+$ T cells and CD8$^+$ T cells were purified by negative selection using magnetic beads.
- T cells were incubated in medium alone (unstimulated) or were stimulated with plate-bound anti-CD3 antibodies at 1.25 – 5 μg/mL and with soluble anti-CD28 antibodies at 1 μg/mL in the presence of ATYR1940, iMod-Fc, iMod or vehicle.
- After 24 hours of stimulation, cytokine and granzyme B release was measured in the supernatant by ELISA, Luminex Milliplex and/or MSD immunoassays and cells were analyzed for expression of surface activation markers by flow cytometry.

Graft-versus-Host Disease (GvHD) model:

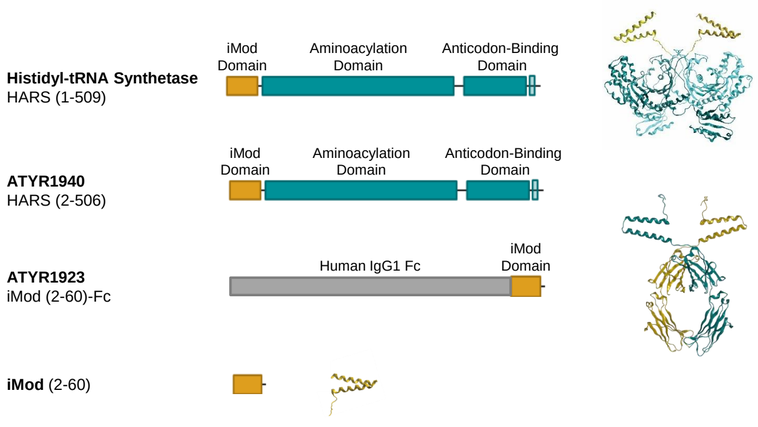
- Human PBMC were injected into NSG (NOD *scid* gamma) mice and spleens collected 11 days later.
- Splenocytes were analyzed by flow cytometry to confirm effector/memory phenotype, and cultured with anti-human CD3 antibodies at 2.5 μg/mL and anti-human CD28 antibodies at 1 μg/mL in the presence of vehicle or ATYR1940.
- Cytokine release was measured using Luminex Milliplex immunoassays.

Gene profiling:

- Gene profiling was performed on unstimulated T cells and on T cells stimulated with 2.5 μg/mL of anti-CD3 antibodies and 1 μg/mL of anti-CD28 antibodies in presence of ATYR1940, ATYR1923 or vehicle for 24 hours. RNA sequencing was done by GENEWIZ. Gene expression was also measured using QuantiGene Plex assays (Thermo Fisher Scientific).

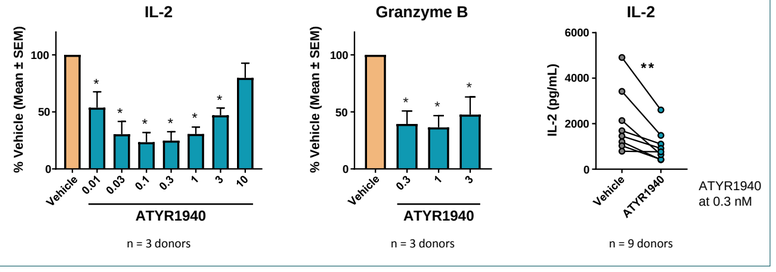
Statistics: One-way ANOVA (Dunnett's post-hoc test) was used to compare each condition to the stimulated vehicle control. ****p < 0.0001; ***p <0.001; **p < 0.01; *p <0.05.

Figure 1. Generation of HARS-Derived Proteins



The N-terminal domain of HARS, the iMod domain, consisting of the first 59 amino acids, is sometimes referred to as the WHEP domain, a specialized version of the helix-turn-helix motif, that is responsible for forming complexes with other proteins (Rho et al., 1999).

Figure 2. ATYR1940 Inhibits IL-2 and Granzyme B Release



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- Wakasugi K & Schimmel P (1999) Two distinct cytokines released from a human aminoacyl-tRNA synthetase. *Science* 284, 147-151.

Figure 3. ATYR1940 Inhibits Upregulation of T Cell Activation Markers

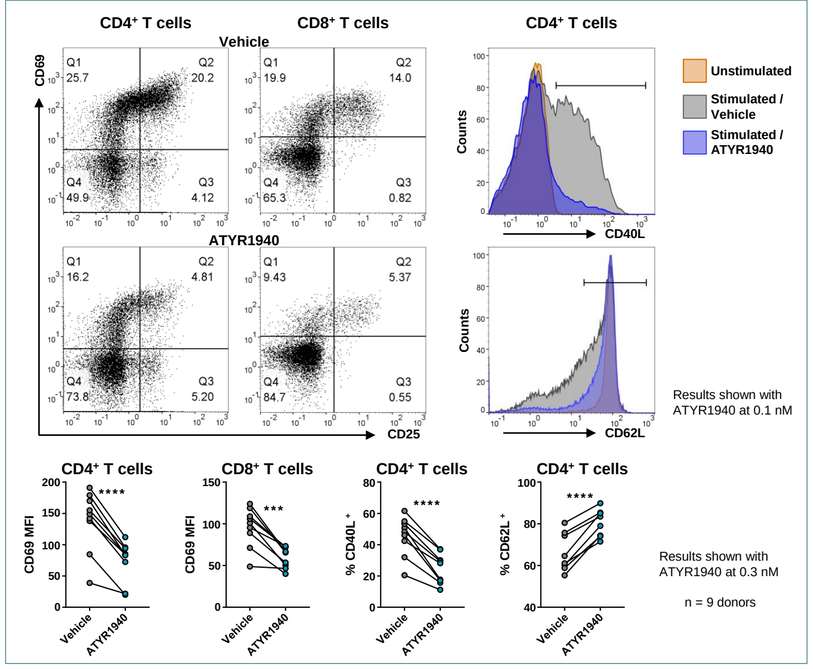


Figure 4. ATYR1940 Decreases Cytokine Release from Stimulated T Cells

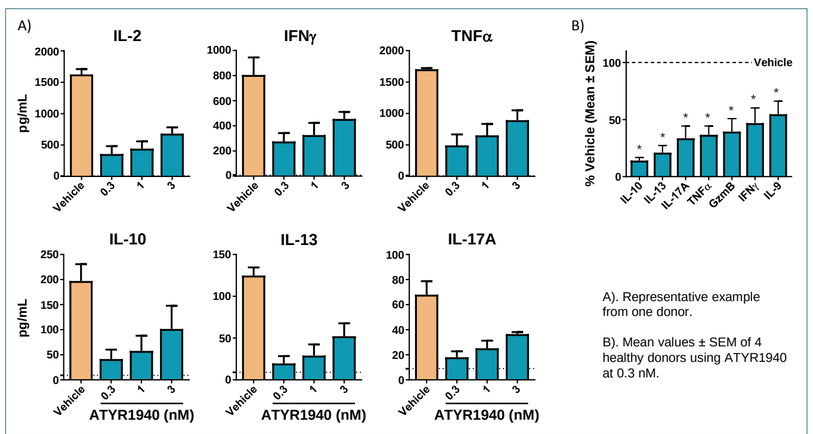


Figure 5. ATYR1940 Regulates Activation of CD4$^+$ and CD8$^+$ T Cells

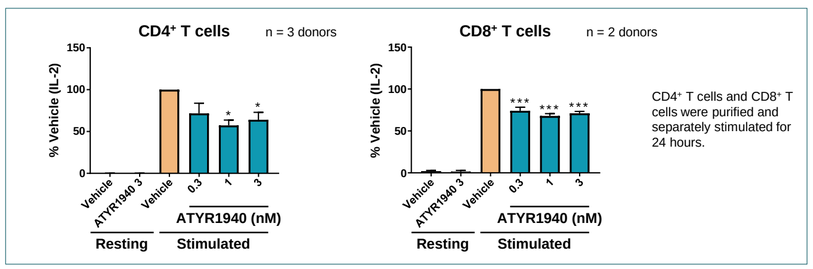


Figure 6. Memory T cells Respond to ATYR1940 Treatment

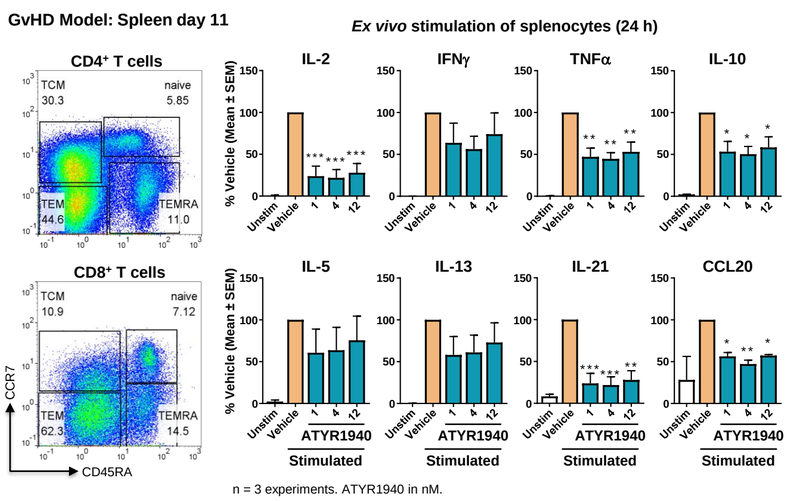


Figure 7. RNA Signature Confirms ATYR1940's Activity in T Cells

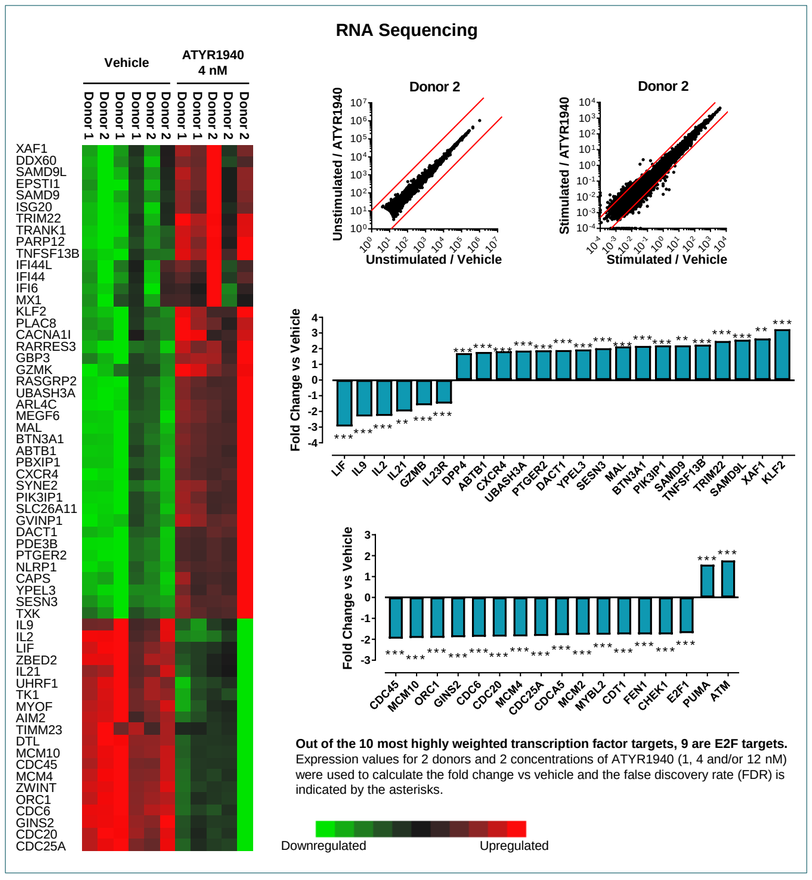
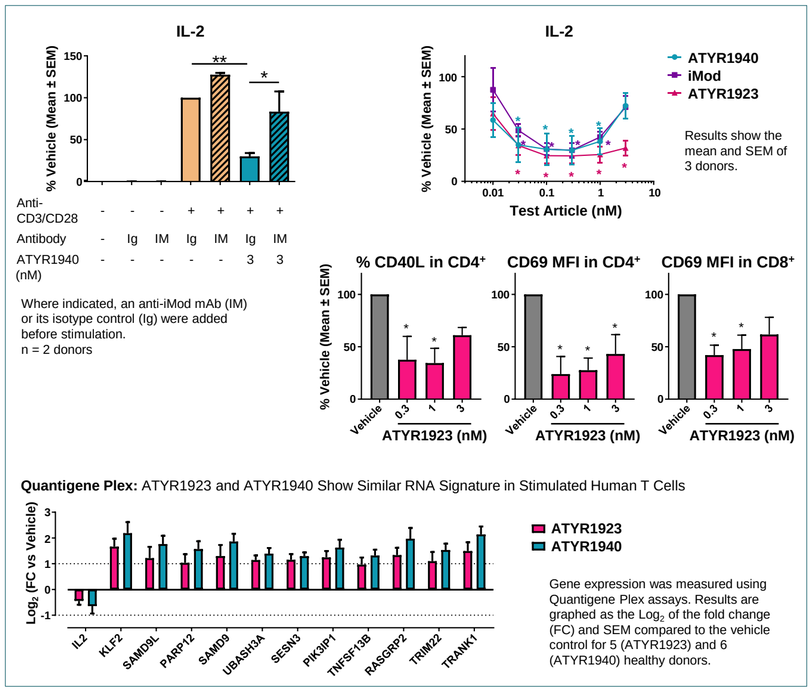


Figure 8. The iMod Domain Has Immunomodulatory Functions



Conclusions

- A non-canonical function of HARS was discovered based on studies with ATYR1940, a recombinant form of the protein.
- ATYR1940 reduced human T cell activation as indicated by:
  - Lower surface expression of activation markers
  - Decreased release of Th1, Th2, and Th17 cytokines.
- Effects by ATYR1940 were observed on naïve and effector/memory T cells as well as on CD4$^+$ and CD8$^+$ subsets.
- Gene profiling studies confirmed that ATYR1940 reduced T cell activation and sustained expression of genes that maintain T cells in a resting state.
- The iMod domain of ATYR1940 was responsible for mediating the immunomodulatory function of ATYR1940:
  - A blocking antibody abrogated the activity of ATYR1940.
  - iMod and ATYR1923 also reduced T cell activation and cytokine release from stimulated T cells.
  - ATYR1940 and ATYR1923 induced a similar RNA signature in stimulated T cells.

These results suggest that HARS may function as a circulating immune set-point modulator through action by its iMod domain.