ATYR1923 Reduces Neutrophil Infiltration in an Acute Lipopolysaccharide (LPS) Lung Injury Model

Suzanne Paz, Clara Polizzi, Dalena Chu, Lauren Guy, Christoph Burkat, Ryan Adams, David King, Kathleen Ogilvie, Sanna Rosengren
A Tyr Pharma, San Diego, CA, USA

Abstract

A number of aminoacyl tRNA synthetases have evolved non-canonical functions including the tRNA synthetase for histidine, HARS. HARS downregulates immune responses via its N-terminal domain, which we have termed the Imod (immunomodulatory) domain. The Imod domain was fused to human IgG Fc to generate ATYR1923, which is currently in clinical evaluation for pulmonary sarcoidosis. ATYR1923 binds to neuropilin-2 (NRP-2), a pleiotropic co-receptor participating in several pathways including class III lymphopines/plexins and VEGF-C/VEGFR3. To date, little is known about the role of NRP-2 in immune regulation, although growing evidence indicates that NRP-2 influences myeloid cell biology such as activation and recruitment to inflammatory sites. For instance, NRP-2 expression on alveolar macrophages regulates airway inflammatory responses to inhaled LPS (Immormino et al. 2016). To determine whether ATYR1923 was able to influence myeloid cell trafficking, ATYR1923 or a pentameric Imod construct, Imod-COMP, were administered intravenously to C57BL/6 mice 24h prior to LPS challenge, either by intraperitoneal (IP) injection to induce a systemic inflammatory response or by airway administration to generate acute lung inflammation. Multi-color flow cytometry was used for immunophenotyping analysis and detection of NRP-2 levels on surfaces of various immune cell populations. In vitro, mouse bone-marrow derived macrophages (BMDM), human THP-1 monocytic cells, and primary human dendritic cells (DCs) were used to confirm NRP-2 cell surface expression. Results indicated that LPS stimulation in vitro or in vivo upregulated NRP-2 on a variety of myeloid cells including macrophages (splenic & alveolar), DCs and neutrophils. Notably, prophylactic administration of ATYR1923 or Imod-COMP led to a significant and dose-dependent reduction in LPS-induced neutrophil infiltration in the mouse lung. This finding appeared to be specific to neutrophil recruitment, since the number of monocytes, alveolar macrophages, or other myeloid cells was not altered. Altogether, these results suggest that the activity of ATYR1923 includes inhibition of neutrophil migration to inhibit lung inflammation.

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, Arti et al., 2017). Proteins derived from the histidyl-tRNA synthetase (HARS) gene are found extracellularly and are thought to play a role in regulating key cells in the immune system to ensure appropriate control of immune responses. ATYR1923 is a clinical stage immunomodulatory protein. ATYR1923 comprises the Imod domain of HARS fused to human IgG Fc (Fig. 1.) to extend plasma half-life. NRP-2 was identified to be a binding partner of ATYR1923. ATYR1923 exerts some of its immunomodulatory functions by affecting T cell activation & cytokine release (data presented at ASH 2018 by E. Mertsching). NRP-2 was shown to play a role in airway inflammatory responses to inhaled LPS (Immormino et al. 2018).

In vivo Experimental Procedure

Table 1. In vivo study design

<table>
<thead>
<tr>
<th>Group</th>
<th>Test Article (TA)</th>
<th>Dose (mg/kg)</th>
<th>TA Route</th>
<th>LPS Treatment (µL)</th>
<th>Time Point (hrs)</th>
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<tbody>
<tr>
<td>1</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>0/IV</td>
<td></td>
<td></td>
<td>24</td>
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<tr>
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<td>0/IV</td>
<td></td>
<td></td>
<td>10/24</td>
</tr>
<tr>
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<td>ATYR1923</td>
<td>1/IV</td>
<td></td>
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<td>10/24</td>
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<tr>
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<td>3/IV</td>
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<td>10/IV</td>
<td></td>
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<td>10/24</td>
</tr>
<tr>
<td>7</td>
<td>Imod-comp</td>
<td>3/IV</td>
<td></td>
<td></td>
<td>10/24</td>
</tr>
</tbody>
</table>

Day 1: Body weights (BW) for TA administration were recorded & animals were dosed with TA at 5mg/kg according to the table above. Day 0: Mice in groups 3-7 were anaesthetised at 2.4% isoflurane (3L/min) & injected with 10 µg LPS in 50 µL PBS. Grp 2 received 50 µL PBS only. Grp 1 mice were naive to induction & treatment.

Day 1: BWs were recorded and mice were euthanized with lethal ketamine/xylazine cocktail (~300/30 mg/kg) at 24 hours post LPS induction. Blood was collected from the abdominal veins, processed for serum for pathway analysis (cytokine, ATYR1923 & NRP-2 levels). The lung and trachea were exposed & perfused for BALF (bronchoalveolar lavage) collection using 0.6 ml PBS through cannulated trachea. Collected BALF was placed on ice and volume recorded. BALF cells were collected by centrifugation and supernatant was retained for potential measurement of biochemical inflammatory markers (HARS pathway proteins, cytokines and/or ATYR1923 etc.). RBC lysis was applied to BALF cells & stained for flow cytometry analysis. Results from flow analysis were analyzed using FlowJo & statistical analysis performed using Prism.

In vitro Experimental Procedure

Fig. 3. Generation of mouse BMDM

Fig. 4. Generation of Human Macrophages from THP1 Cell Line

Fig. 5. Generation of Human Primary Macrophages

Fig. 6. Generation of Human Primary Dendritic Cells

Fig. 7. ATYR1923 binds Human and Mouse NRP-2

Fig. 8. In Vitro NRP-2 Expression

Fig. 9. ATYR1923 Reduces Neutrophil Infiltration in BALF

Results

Conclusions

- ATYR1923 binds to both human and mouse NRP-2.
- NRP-2 is detected on the cell surface of myeloid cells both in vitro and in vivo.
- NRP-2 was induced following activation of TLR found on the cell surface (mainly TLR1, 2, 4, 5 & 6), but not endosomal TLR ligands (TLR3, 7, 8, & 9).
- In an acute LPS lung injury model, a significant increase in immune cell infiltration and NRP-2 expression was observed in positive control group.
- ATYR1923 significantly decreased the CD11b+ population following LPS installation in the lung, which was ascribed to an inhibitory effect on neutrophil infiltration.
- These findings highlight the potential of ATYR1923 to regulate myeloid cell biology during lung inflammation.