Toll-Like Receptor 7/8 Activation of Immune and Non-Immune Cells in Muscle by RNA-Containing Immune Complexes can Contribute to Inflammation and the Pathogenesis of Myositis

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CONCLUSIONS

- **TLR7/8 is activated by RNA in immune complexes from patients with IIM, particularly those with Jo-1-positive DM**
- **TLR7/8 activation can lead to inflammation in muscles with deleterious effects**

The efficacy and safety of enpatoran in IIM (DM/PM) are currently being evaluated in the Phase II NEPTUNIA trial (NCT05650567); the results presented here provide important mechanistic insights supporting this approach

INTRODUCTION

- **IIM** is a heterogeneous group of connective tissue disorders, primarily characterized by **muscle weakness**, which is classified into several subtypes including **DM, PM and IBM**[1]
- **Tissue inflammation is a major disease driver** in IIM, but the **upstream pathways** causing inflammation are **poorly characterized**[1]
- Activation of endosomal TLRs is one possible driver of inflammation in IIM; **hallmarks of TLR activation** are observed in some patients, including high Type I IFN and the presence of RNA-containing immune complexes[2]

OBJECTIVE

To evaluate whether TLR7/8 activation contributes to inflammation in IIM

METHODS

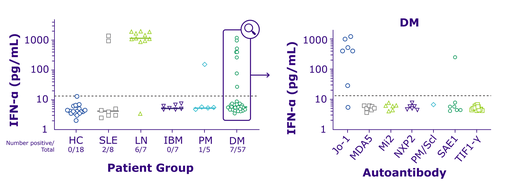
IgG isolated from patients with IIM or lupus and healthy controls was combined with necrotic cell lysate to form immune complexes that were added to healthy donor PBMCs at 37°C for 24 hours

- Cytokine production was measured by AlphaLISA
- NanoString gene expression analysis was performed
- IgG was profiled for autoreactivity using an array with 1581 human antigens
- RNA molecules were custom synthesized, incubated with LyoVec, added to PBMCs at a final RNA concentration of 1 µg/mL, and cytokine production was measured
- Human myoblasts and satellite cells were treated with supernatants from TLR7/8-activated PBMCs and gene expression was evaluated by NanoString

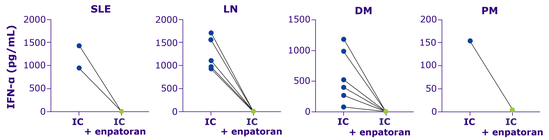
RESULTS

Immune complexes derived from patients with PM and DM activate TLR7/8

- **IFN-α production** was stimulated by immune complexes generated using IgG from patients with **DM and PM**, but not IBM
- **DM samples** were grouped based on the presence of myositis-specific autoantibodies; the majority were **positive for Jo-1** autoantibodies, which are associated with anti-synthetase syndrome



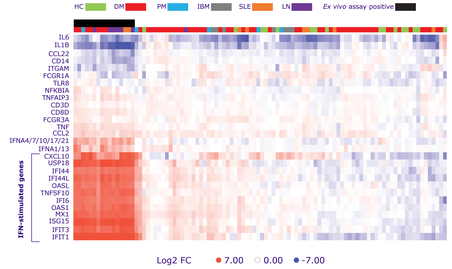
- Pre-treatment with the TLR7/8 inhibitor **enpatoran** (1 µM, 30 minutes) completely **inhibited the production of IFN-α**, suggesting IFN-α production was mediated by TLR7/8



RESULTS

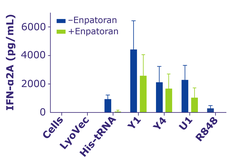
TLR7/8 activation induced by immune complexes had a greater effect on the IRF pathway than the NF-κB pathway

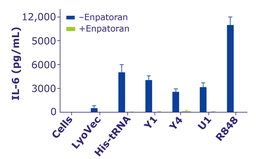
- Gene expression analysis on the PBMC lysates showed that the samples that stimulated IFN-α protein production (shown by black shading) also induced the expression of **IFN-stimulated genes**
- A comparable induction of **NF-κB cytokines**, including *IL6, IL1B* and *TNF*, or the **NF-κB activation markers** *TNFAIP3* and *NFKBIA*, was not observed



RNAs associated with immune complexes stimulate cytokine production via TLR7/8

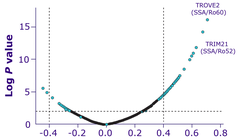
- Several autoantibodies that were associated with TLR7/8 activation are known to react with RNA-binding proteins and have associated RNA; anti-Jo-1 likely contains His-tRNA, anti-Ro60 contains Y1 and Y4 RNA, and anti-Smith contains U1 RNA
- These **RNA ligands** were found to **induce the production of IFN-α2A and IL-6** in PBMCs
- **IFN-α2A** production induced by His-tRNA was **effectively blocked** by enpatoran, but this was less effective for Y4, U1 and Y1
- Enpatoran **effectively reduced** the production of **IL-6** induced by all of the RNAs



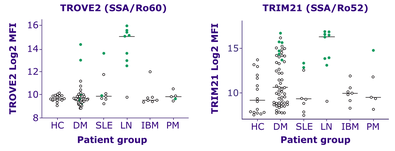


Several autoreactivities may activate TLR7/8

- To identify autoreactivities that may be associated with TLR7/8 stimulation, IgG samples were profiled for autoreactivity, and correlation analysis was performed to identify the reactivities that correlated with positivity in the *ex vivo* assay
- **Several auto-reactivities** were identified that **strongly correlated with TLR7/8 activation**

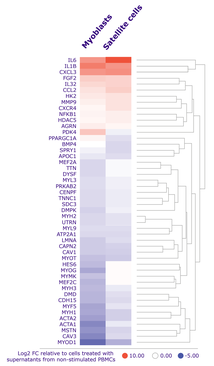


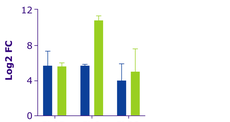
- **TROVE2 (SSA/Ro60)** was mainly elevated in **DM and LN** samples and the majority of samples that induced IFN-α in the *ex vivo* assay (shown in green) had high TROVE2
- **TRIM21 (SSA/Ro52)** was elevated in **several of the patient groups** and all of the samples that were positive in the *ex vivo* assay (shown in green) had high TRIM21

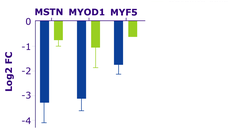


Supernatants from TLR7/8-activated PBMCs impact human myoblasts and satellite cells

- To determine if myoblasts and satellite cells are affected by immune cells activated by TLR7/8 stimulation, gene expression was evaluated following treatment with supernatants from PBMCs that had been stimulated with the TLR7/8 agonist R848 (3 µM) for 24 hours
- Increased expression of **inflammatory genes** and decreased expression of **muscle cell markers** (which was more dramatic in myoblasts than satellite cells) was observed







Abbreviations: DM, dermatomyositis; **FC**, fold change; **HC**, healthy control; **His-tRNA**, histidyl-transfer RNA; **IBM**, inclusion body myositis; **IC**, immune complex; **IFN**, interferon; **IFN-α**, interferon-alpha; **IFN-α2A**, interferon-alpha 2A; **IgG**, immunoglobulin G; **IIM**, idiopathic inflammatory myopathies; **IL-6**, interleukin-6; **IRF**, interferon regulatory factor; **LN**, lupus nephritis; **MDA5**, melanoma differentiation-associated protein 5; **MFI**, mean fluorescence intensity; **NF-κB**, nuclear factor-kappa B; **NXP2**, nuclear matrix protein 2; **PBMCs**, peripheral blood mononuclear cells; **PM**, polymyositis; **RNA**, ribonucleic acid; **SAE1**, small ubiquitin-like modifier activating enzyme 1; **SLE**, systemic lupus erythematosus; **TIF1-γ**, transcription intermediary factor 1-gamma; **TLR**, toll-like receptor; **TRIM21**, tri-partite motif-containing-21; **TROVE2**, TROVE domain family member 2; **SSA**, Sjögren's-syndrome-related antigen A

References: 1. Lunderberg IE, et al. *Nat Rev Dis Primers.* 2021;7(1):86. 2. Ekholm L, et al. *Scand J Immunol.* 2016;84(2):100–9.

YW, **AD**, **NG and CCS** are employees of EMD Serono. **VS, PV and ATB** were employees of EMD Serono when the study was conducted. **DF** has received consultancy fees from Acelyrin, Amgen, Argenyx, Biogen, Bristol Myers Squibb, Janssen Gilead, Pfizer, UCB, and the healthcare business of Merck KGaA, Darmstadt, Germany, as well as funding for contracted research from Argenyx, EMD Serono and Pfizer. **KYS** has received consultancy fees from Aeovian, Dermbiont and Phoenicis, and advisor fees from Almirall and NFlection Therapeutic.

This study was sponsored by the healthcare business of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945), who funded medical writing support by Bioscript Group Ltd, Macclesfield, UK. The antigen array was performed by Oncimmune, Nottingham, UK.

Presented at the ACR Convergence | 10 – 15 November, 2023 | San Diego, California

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