Poster #: PB065

Interferon induced transcriptomic and proteomic changes in CD14 monocytes

Erik Layton[1], Paige Bouvatte[1, 2], Susan Ludmann[1], Catalina Sakai[1], Ariela Mitchell[1], Neel Kaul[1], Nicholas Moss[1], Troy R. Torgerson[1], Emma L. Kuan[1],
[1]Allen Institute for Immunology, Seattle, USA. [2]Allen Institute Postbac Program, Seattle, USA.

ABSTRACT:

Interferons (IFNs) are paramount for normal and pathogenic immune responses. Despite the importance of the IFN response, we do not fully understand how different human immune cell subsets respond to the three major types of IFN. To allow us to disentangle the various IFN-induced transcriptional signatures, we generated a new single-cell RNA sequencing dataset that profiles responses of human peripheral blood mononuclear cell (PBMC) subsets to IFN-α2A, IFN-β, IFN-γ, and IFN-λ1. Using this data, we were able to compare responsiveness of major immune cell subsets to the various IFNs and subsequently derive cell-type specific, IFN-driven transcriptional signatures for each. One cell-type specific gene, transcriptionally induced in CD14 monocytes by IFN-α stimulation, is cathepsin L (CTSL), a lysosomal protease. Despite transcriptional induction of the CTSL gene, we did not see a significant change in cathepsin L protein expression in activated CD14 monocytes by flow cytometry. In contrast, super-resolution confocal microscopy showed IFN-α inducible condensation and colocalization of CTSL protein with the lysosome, suggesting that it is either being actively degraded or secreted upon IFN activation. Since CTSL is one of many IFN-stimulated genes (ISGs) that encode secreted proteins, we are analyzing collected supernatants from IFN-treated monocytes. While the role of CTSL in immune responses from a systems standpoint is unclear, we aim to evaluate the IFN-α induced secretome of CD14 monocytes and the functional effects through co-culture assays utilizing multiple myeloma cell lines.

Figure 3.

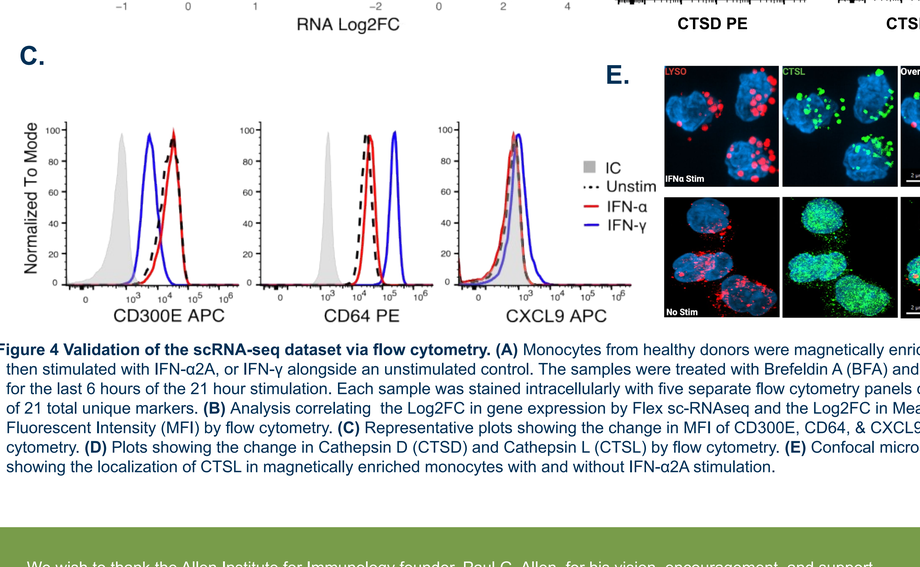

Figure 3 IFN stimulated signature seen in cohort of newly diagnosed multiple myeloma patients. (A) PBMCs and serum from a cohort of Newly Diagnosed Multiple Myeloma (NDMM) and healthy controls were analyzed using sc-RNAseq and Meso Scale Discovery (MSD) assays. **(B)** MSD results comparing the plasma concentration of IFN-α2A, IFN-β, & IFN-γ of NDMM patients and healthy controls.**(C)** sc-RNAseq analysis comparing the Log2FC of the NDMM cohort relative to the healthy control (Y axis) and the Log2FC of PBMCs stimulated with IFN-α2A relative to unstimulated PBMCs (X axis).
More information about this cohort can be found on poster # PB055

Figure 4.

Figure 4 Validation of the scRNA-seq dataset via flow cytometry. (A) Monocytes from healthy donors were magnetically enriched and then stimulated with IFN-α2A, or IFN-γ alongside an unstimulated control. The samples were treated with Brefeldin A (BFA) and monensin for the last 6 hours of the 21 hour stimulation. Each sample was stained intracellularly with five separate flow cytometry panels consisting of 21 total unique markers. **(B)** Analysis correlating the Log2FC in gene expression by Flex sc-RNAseq and the Log2FC in Mean Fluorescent Intensity (MFI) by flow cytometry. **(C)** Representative plots showing the change in MFI of CD300E, CD64, & CXCL9 by flow cytometry. **(D)** Plots showing the change in Cathepsin D (CTSD) and Cathepsin L (CTSL) by flow cytometry. **(E)** Confocal microscopy showing the localization of CTSL in magnetically enriched monocytes with and without IFN-α2A stimulation.

RESULTS

Figure 1.

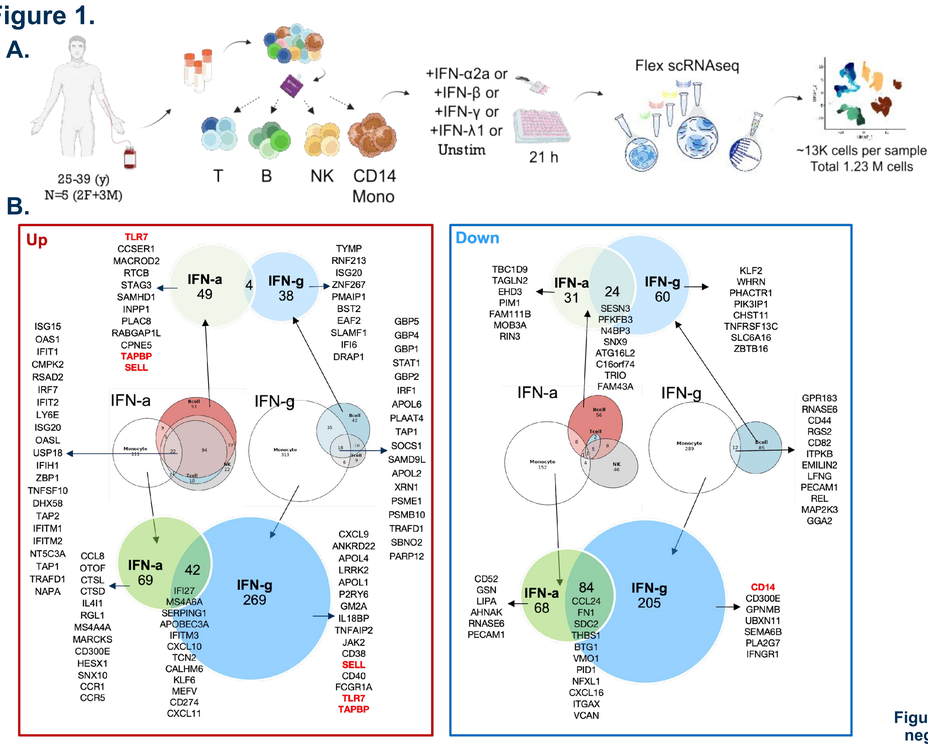

Figure 1. Transcriptomic response of different immune cell subsets to IFN stimulation. (A) PBMCs were isolated from healthy controls and magnetic enrichment was used to isolate T cells, B cells, NK cells, and CD14 monocytes. The cells were then stimulated IFN-α2A, IFN-β, IFN-γ, or IFN-λ1 and analyzed using Flex sc-RNAseq with unstimulated cells serving as a control. **(B)** Comparison of the differentially expressed genes (DEGs) after IFN-α2A, or IFN-γ stimulation by cell type.

Figure 2.

A.

Procedure	Purity	Yield
Standard	<80%	25%
+TCRab	97-98%	25-30%
+TCRab +Wash	91-98%	~45-70%

B.

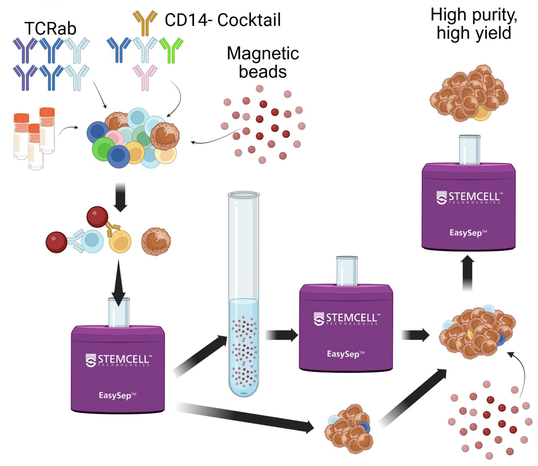

Figure 2. Optimized procedure for the magnetic enrichment of CD14 monocytes using negative selection. (A) Purity and yield using the standard monocyte enrichment procedure, when adding the TCRab positive selection cocktail, and by adding an extra wash step. **(B)** Both the CD14- selection and the TCRab+ selection cocktails are added to PBMCs, and the monocytes are isolated as normal. After pouring off the first monocyte fraction the beads are washed with fresh buffer and the magnet is applied, and a second fraction of monocytes is isolated. The first and second fraction are combined and more magnetic beads are added before a final magnetic incubation to yield high purity monocytes with significantly higher yield.

Figure 5.

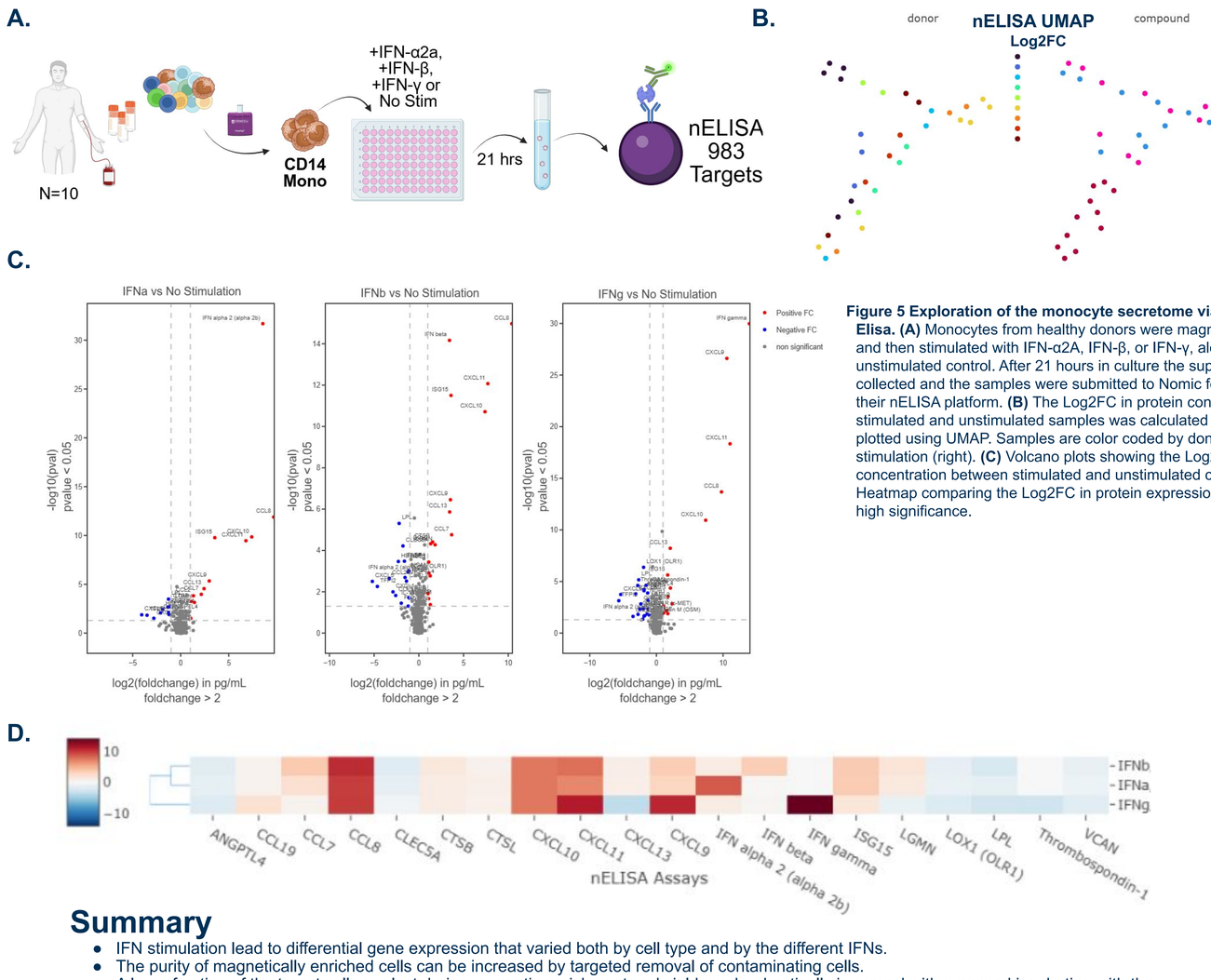

Figure 5 Exploration of the monocyte secretome via bead based Elisa. (A) Monocytes from healthy donors were magnetically enriched and then stimulated with IFN-α2A, IFN-β, or IFN-γ, alongside an unstimulated control. After 21 hours in culture the supernatant was collected and the samples were submitted to Nomic for analysis through their nELISA platform. **(B)** The Log2FC in protein concentration between stimulated and unstimulated samples was calculated for each donor and plotted using UMAP. Samples are color coded by donor (left) and by stimulation (right). **(C)** Volcano plots showing the Log2FC in protein concentration between stimulated and unstimulated conditions. **(D)** Heatmap comparing the Log2FC in protein expression in assays with high significance.

Summary

- IFN stimulation lead to differential gene expression that varied both by cell type and by the different IFNs.
- The purity of magnetically enriched cells can be increased by targeted removal of contaminating cells.
- A large fraction of the target cells are lost during magnetic enrichment and yield can be drastically improved with a second incubation with the magnet.
- PBMCs from NDMM patients have an expression profile similar to that of IFN stimulated cells.
- The gene expression of monocytes largely correlates with protein expression analyzed via flow cytometry, with several notable differences.
- Secretome analysis shows UMAP clustering that appears to be driven largely by stimulation and by donor.
- Changes in protein concentration is similar between each stimulation condition especially between the Type 1 (IFN-α2A & IFN-β) stimulations.

- On average there is a similar trend seen across each of these assays, but significant differences are seen in individual markers.
  - There is a large increase in CXCL9 RNA expression, while flow cytometry shows only a slight increase, and a huge increase in the secreted protein.
  - CTSL gene expression and supernatant concentrations increase with IFN stimulation, while flow cytometry sees no change at all, and microscopy shows a dramatic shift in protein localization.

We wish to thank the Allen Institute for Immunology founder, Paul G. Allen, for his vision, encouragement, and support.