Mouse strain-specific differences in cytokine, chemokine, and growth factor expression revealed by multiplex immunoassay

Rick Wiese, Harold Steiner III, Sasha Williams, Brooke Gilliam, Munmun Banerjee, and Anthony Saporita

Introduction

The use of different mouse strains in laboratory research enhances the ability to model human diseases, understand genetic influences, and explore biological processes. Cytokines, chemokines, and growth factors are critical mediators of immune system function in laboratory mice, making their study critical in many research applications. The ability to simultaneously measure these proteins in biological samples by multiplexed immunoassay provides an ideal tool to evaluate protein profiles across different mouse strains. Here we simultaneously measured the expression of 68 cytokines, chemokines, and growth factors in serum and plasma samples from four strains (CD1, Swiss-Webster, C57BL/6, and Balb/c) and identified proteins with baseline differences in apparently healthy mice. Further, we evaluated immune response in CD1 mice challenged with LPS over an eight-hour time course to characterize the induction of these 68 immune factors. Several analytes, including IL-6 and MCP-1, exhibited 100-fold average increases in concentration in response to *in vivo* LPS challenge. Induction patterns differed amongst analytes, with proteins such as IL-10 and GM-CSF peaking at the two-hour LPS-stimulation time point, while others reached maximal induction at later points. In summary, this mouse cytokine, chemokine, and growth factor multiplex kit identified differences in protein expression amongst four healthy mouse strains and characterized the immune response to LPS stimulation.

Methods

Mouse Strains: Serum and plasma was collected from Swiss-Webster, BALB/C, C57BL/6, and CD-1 mice by a third-party biorepository. To characterize the response to immunostimulatory challenge, CD-1 mice were injected with lipopolysaccharide and sample was collected at 2, 4, 6, and 8 hours post-administration.

Immunoassays and Data Analysis: The MILLIPLEX® Mouse Cytokine Expanded Panel 1 68-Plex (Cat. No. MCYT1-190K) was used to evaluate all samples according to the kit protocol on the xMAP® INTELLIFLEX® instrument. Data was acquired via xPONENT® v. 4.3 software and analysis was performed for all immunoassays using the Belysa® Immunoassay Curve Fitting Software (Cat. No. 40-122). Figures were prepared in GraphPad Prism and Microsoft Excel.

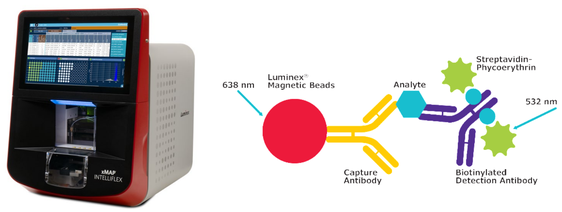

Figure 1. MILLIPLEX® immunoassay format. MILLIPLEX® assays use magnetic microspheres (beads) conjugated to capture antibodies. Each set of beads is distinguished by different ratios of two internal dyes yielding a unique fluorescent signature to each bead set, allowing researchers to simultaneously measure the analytes targeted by the capture antibodies. Native protein is analyzed by means of a "sandwich" immunoassay, pairing the capture beads with a biotinylated detection antibody.

Results

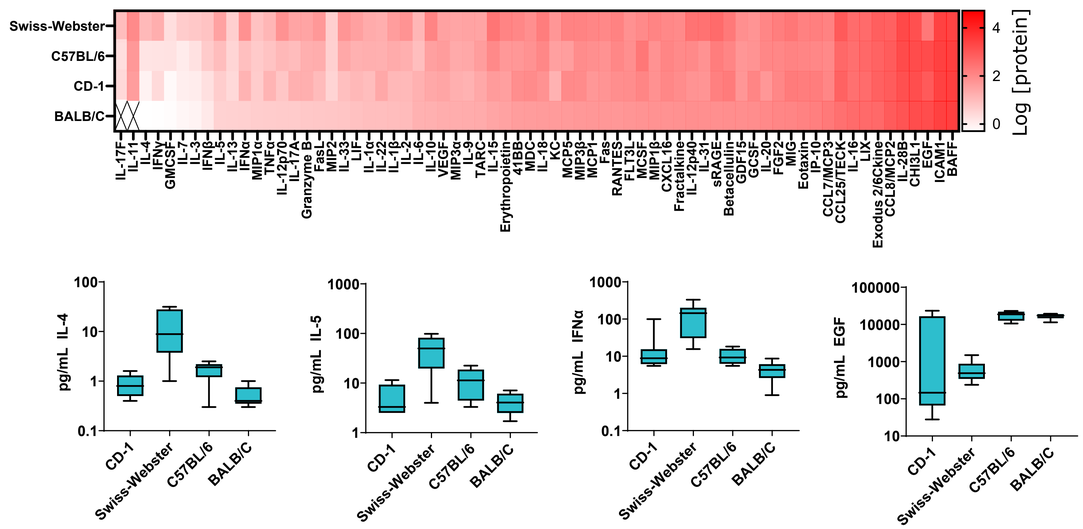

Figure 2. Measurement of 68 mouse proteins in serum and plasma samples from four strains. Four serum and four plasma samples from healthy mice were tested with the MILLIPLEX® Mouse Cytokine Expanded Panel 1 for each of the following mouse strains: CD-1, Swiss-Webster, C57BL/6, and BALB/C. The log values of average concentration (pg/mL) for each analyte are displayed in the heat map above. Several analytes including IL-4, IL-5, and IFNα showed significant elevation in Swiss-Webster samples compared to the other 3 strains. In contrast, EGF was significantly elevated in C57BL/6 and BALB/C mice relative to Swiss-Webster.

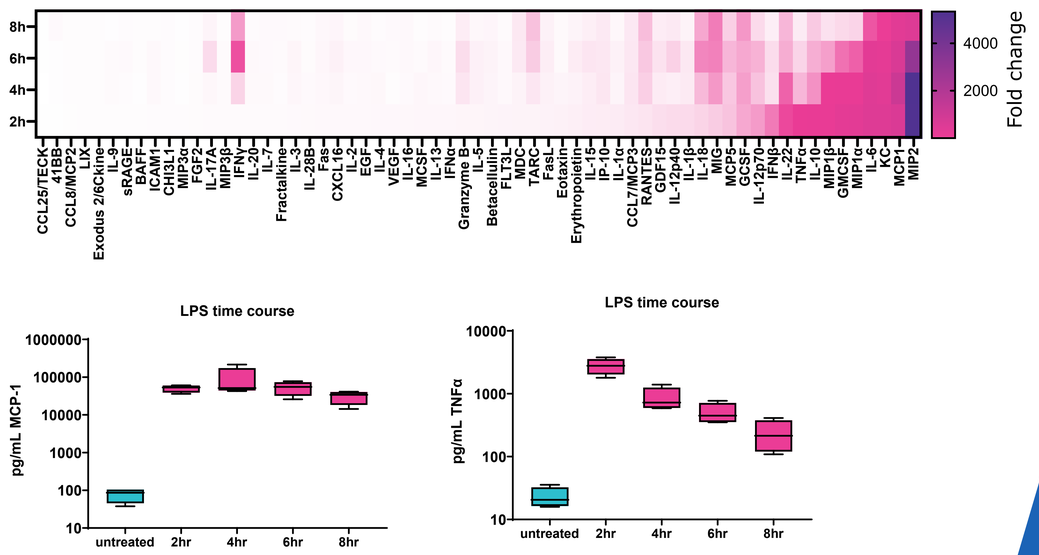

Figure 3. *In vivo* LPS challenge stimulates cytokine expression in CD-1 mice. CD-1 mice were subjected to inoculation with lipopolysaccharide (LPS) and serum was collected every 2 hours following injection (n=4 at each time point). Stimulated CD-1 mouse serum was then compared to unstimulated controls using the MILLIPLEX® Mouse Cytokine Expanded Panel 1. A heat map of the average fold change in protein concentration during the LPS time course is displayed above. The profiles of two strongly induced analytes, MCP-1 and TNFα, are shown above.

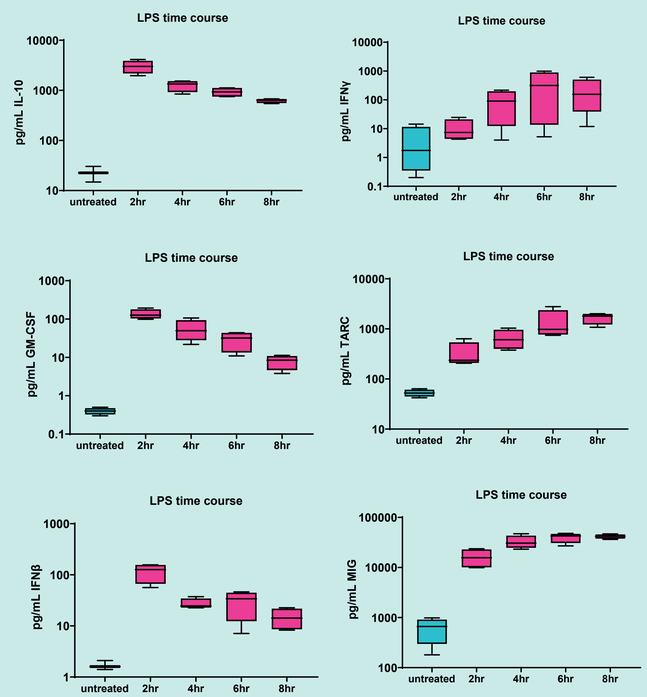

Figure 4. *In vivo* LPS induction patterns in CD-1 mice. Following LPS challenge, some proteins such as IL-10, GM-CSF, and IFNβ peaked early, whereas others such as IFNγ, TARC, and MIG reached peak elevation at later time points.

Summary

The MILLIPLEX® Mouse Cytokine/Chemokine/Growth Factor Expanded Panel 1 enables the simultaneous measurement of 68 immune-related proteins in just 25 µL of 1:2-diluted murine serum and plasma samples. Here we used this multiplex kit to interrogate strain-specific differences in cytokines, chemokines, and growth factors in healthy CD-1, Swiss-Webster, C57BL/6, and BALB/C mice. Several proteins with differential expression between strains were identified, including IL-4, IL-5, IFNα, and EGF. Additionally, we characterized the response to LPS challenge in CD-1 mice, enabling the identification of analytes which displayed peak elevation at the earliest time point (2 hours) versus those that continued to increase throughout the time course. In conclusion, these results highlight the utility of the MILLIPLEX® Mouse Cytokine/Chemokine/Growth Factor Expanded Panel 1 for profiling 68 key regulators of immune function in mice.

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