Proteomic Analysis of Microglia During LPS-Induced Acute Neuroinflammation

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Introduction

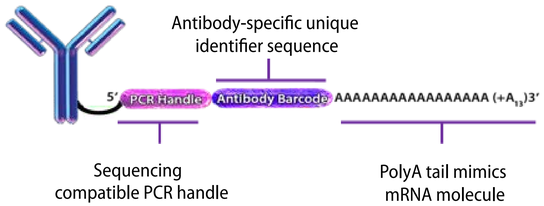
Microglia are the major tissue resident macrophages in the brain and function as sentinels in maintaining CNS homeostasis. Dysregulation of microglial function has been associated with neuroinflammation and neurodegenerative disorders. To gain better understanding of microglial function, we assessed their proteomic profile during systemic inflammation, using BioLegend's proprietary technology, TotalSeq™, which allows simultaneous analysis of both the transcriptome and proteome at the single-cell level. We utilized a panel of 200 TotalSeq™ antibody-oligonucleotide conjugates, including well-know microglial markers, to analyze differentially expressed proteins under normal and LPS-induced inflammation in mice.

Materials and Methods

Systemic inflammation induction: Adult C57BL/6 mice (7-8 weeks) were injected intraperitoneally with either PBS, low (25 µg) or high (250 µg) dosages of LPS daily for 4 days. Animals were sacrificed and single-cell suspensions of microglia were prepared using trypsin digestion followed by a 70/37/30% percoll gradient to remove myelin. Microglial marker expression was analyzed by flow cytometry (FC) using antibodies against CD11b, CD45, CX3CR1, and P2RY12.

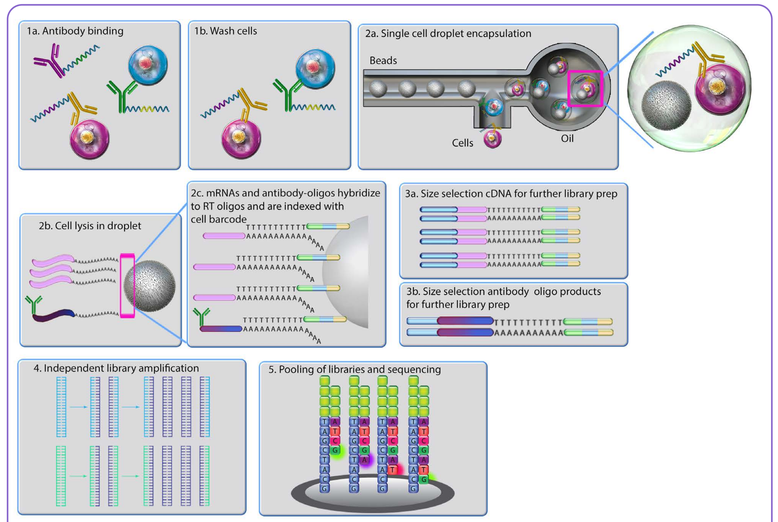
TotalSeq™ Oligo-conjugated antibody preparation and staining: Each highly specific, FC-tested monoclonal antibody (mAb) was directly conjugated to an oligonucleotide containing a 15-bp barcode. The 5' and 3' ends of the barcode are flanked by universal sequences used for library amplification. Single-cell suspensions of microglia were incubated with antibody-oligo conjugates, washed, and subsequently partitioned into droplets using the 10x Genomics Chromium Controller. Sequencing libraries were then generated (Chromium Single-Cell 3' v3.1 Kit) and sequenced on an Illumina sequencer.

1. Diagram of TotalSeq™ antibody conjugates



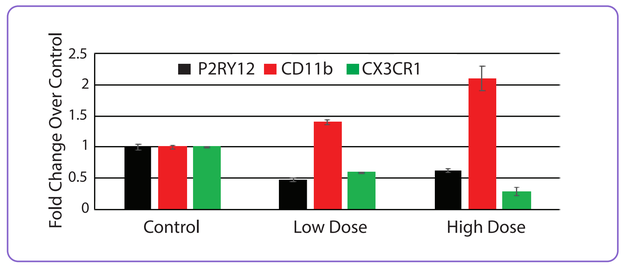
Each TotalSeq™ oligo-antibody conjugate contains a barcode, a PCR handle and a poly-A tail. The barcode is a unique antibody identifier consisting of a 15-bp sequence. The PCR handle is used for library amplification. The poly-A tail mimics an mRNA molecule and is a capture sequence for any poly (dT) capture system. The poly-A tail confers compatibility with 3' single-cell sequencing protocols.

2. General TotalSeq™ workflow



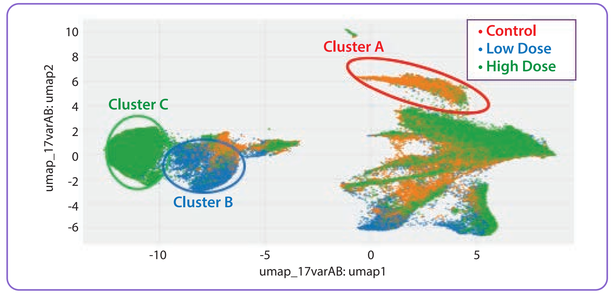
Cells are stained with TotalSeq™ oligo-antibody conjugates and partitioned into individual droplets. Capture beads will hybridize with TotalSeq™ antibodies as well as cellular mRNA. Two libraries are prepared, one containing the protein barcodes, and another containing the mRNA identifiers. The two libraries are separated and sequenced.

3. LPS-induced alterations in microglial markers assessed by FC



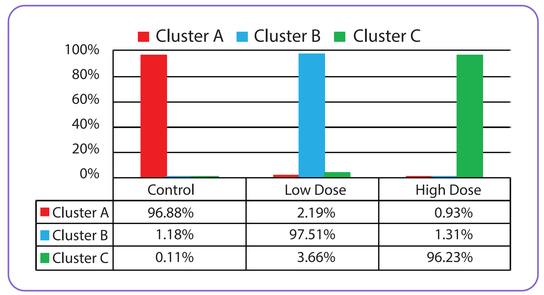
Flow cytometric quantification of microglia markers gated on CD45 showed a significant increase in CD11b$^+$ cells in both low and high dose LPS-treated mice compared to the control untreated (PBS) group. Conversely, low and high dose LPS led to a significant reduction in P2RY12 and CX3CR1 expression in these cells. Fluorescence intensity is expressed as a fold change over control for each marker.

4. Microglial protein expression profile using TotalSeq™



UMAP of microglial cell populations clustered based on the top 18 differentially expressed proteins from a TotalSeq™ antibody panel consisting 200 antibodies, including P2RY12, CD11b, and CX3CR1. Three distinct cell populations, clusters A-C, corresponding to control or LPS treatment groups were identified.

5. Composition of cell populations in clusters A-C



	Control	Low Dose	High Dose
Cluster A	96.88%	2.19%	0.93%
Cluster B	1.18%	97.51%	1.31%
Cluster C	0.11%	3.66%	96.23%

Analysis of cell population compositions demonstrates that the majority of cells in cluster A, B, and C belong to the untreated, low or high LPS treatment groups, respectively.

6. Microglial marker expression intensity in clusters A-C

Intensity of microglial marker expression in clusters A, B, and C correlates well with their expression levels measured by FC under control or LPS treatment conditions. P2RY12 and CX3CR1 expression levels are higher in cluster A, while CD11b expression is higher in clusters B and C.

Conclusion

Our study demonstrates that TotalSeq™ reagents provide a great solution for comprehensive and reliable analysis of a wide range of markers including those that are cell-type specific.

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