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Data Article

Anti-inflammatory cytokine stimulation of HMC3 cells: Proteome dataset

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ABSTRACT

The immunoprotective functions of microglia in the brain are mediated by the inflammatory M1 phenotype. This phenotype is challenged by anti-inflammatory cytokines which polarize the microglia cells to an immunosuppressive M2 phenotype, a trait that is often exploited by cancer cells to evade immune recognition and promote tumor growth. Investigating the molecular determinants of this behavior is crucial for advancing the understanding of the mechanisms that cancer cells use to escape immune attack. In this article, we describe liquid chromatography (LC)-mass spectrometry (MS)/proteomic data acquired with an EASY-nanoLC 1200-Q Exactive™ Orbitrap™ mass spectrometer that reflect the response of human microglia cells (HMC3) to stimulation with potential cancer-released anti-inflammatory cytokines known to be key players in promoting tumorigenesis in the brain (IL-4, IL-13, IL-10, TGFB and MCP-1). The MS files were processed with the Proteome Discoverer v.2.4 software package. The cell culture conditions, the sample preparation protocols, the MS acquisition parameters, and the data processing approach are described in detail. The RAW and processed MS files associated with this work were deposited in the PRIDE partner repository of the ProteomeXchange Consortium with the dataset identifiers PXD023163 and PXD023166, and the analyzed data in the Mendeley Data cloud-based repository

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with DOI 10.17632/fvhw2zwt5d.1. The biological interpretation of the data can be accessed in the research article "Systems-Level Proteomics Evaluation of Microglia Response to Tumor-Supportive Anti-inflammatory Cytokines" (Shreya Ahuja and Iulia M. Lazar, *Frontiers in Immunology* 2021 [1]). The proteome data described in this article will benefit researchers who are either interested in re-processing the data with alternative search engines and filtering criteria, and/or exploring the data in more depth to advance the understanding of cancer progression and the discovery of novel biomarkers or drug targets.

Specifications Table

Subject	Cell biology, proteomics.
Specific subject area	Cell based model system, immune cells, anti-inflammatory cell treatments, proteomic analysis approaches.
Type of data	Tables, charts, figures, MS/RAW and processed files.
How the data were acquired	The data were acquired with a nano-LC (EASY-nLC 1200) system interfaced via electrospray ionization (ESI) to a hybrid quadrupole-Orbitrap™ mass spectrometer (Q Exactive™) from ThermoFisher Scientific, by using the XCalibur data acquisition software (v4.2 SP1). The MS instrument was operated in positive (+) ion mode, and tandem MS data were collected by utilizing a data dependent analysis (DDA) approach on the top 20 most intense ions. The RAW data were processed with the Proteome Discoverer software package (v.2.4) and the Sequest HT algorithm by setting the target false discovery rate (FDR) thresholds to 0.01/0.03 (medium/high).
Data format	RAW, processed, analyzed.
Description of data collection	Serum-depleted (48 h) and non-depleted HMC3 cells were treated with anti-inflammatory cytokines (IL-4, IL-13, IL-10, TGFB) and MCP-1 for 24 h. Three biological replicates of treated and non-treated cells were lysed, and the nuclear and cytoplasmic cell fractions were enzymatically digested with trypsin and analyzed by LC-ESI-MS/MS. The MS/RAW data were processed and changes in abundance were assessed based on normalized peptide spectrum matches (PSM) by using a t-test.
Data source location	Virginia Tech Blacksburg, VA 24061 USA
Data accessibility	Repository name: PRIDE/ProteomeXchange Data identification number: PXD023163, PXD023166. Direct URL to data: https://www.ebi.ac.uk/pride/archive/projects/PXD023163 [2] https://www.ebi.ac.uk/pride/archive/projects/PXD023166 [3] Repository name: Mendeley Data Data identification number: 10.17632/fvhw2zwt5d.1 Direct URL to data: https://data.mendeley.com/datasets/fvhw2zwt5d [4]
Related research article	S. Ahuja, I.M. Lazar, Systems-Level Proteomics Evaluation of Microglia Response to Tumor-Supportive Anti-inflammatory Cytokines, *Front. Immunol. 12* (2021), Article 646043. https://www.frontiersin.org/articles/10.3389/fimmu.2021.646043/full [1]

Value of the Data

- The data described in this manuscript comprise eight proteome datasets of human fetal microglia cells (HMC3) grown in the presence and absence of anti-inflammatory cytokines, under serum-deprived and serum-rich culture conditions, enriched in nuclear and cytoplasmic cell fractions. The datasets provide a systems-level landscape of a microglia phenotype that can be used to gain insights into the immunosuppressive activities mounted by microglia in the presence of brain cancer.
- The HMC3 anti-inflammatory proteome profiles, the cell-membrane proteins that trigger signaling pathways in cells, and the biological processes that are associated with these proteins can be utilized in comparative studies that aim at characterizing microglia responses to different experimental conditions.
- Unlike earlier literature reports that present data describing the behavior of various types of microglia in a range of animal and macrophage research models, the present datasets describe a much less studied model system, i.e., the human fetal microglia.
- The HMC3 proteome profiles will benefit researchers who are interested in studying the behavior of immune cells, the molecular mechanisms that drive cancer development in the brain, and the cell-membrane protein networks that can facilitate the discovery of novel therapeutic targets.
- The MS/RAW files can be re-processed with other search engines that use different algorithms for peptide/protein identifications, or by using other human databases that contain protein isoforms or mutated sequences, to produce complementary results and provide additional insights into the behavior of HMC3 cells.
- The tandem MS data of previously un-identified peptides can be used in the generation of reference spectral libraries that have value in a variety of mass spectrometry applications (e.g., for targeted peptide identification and quantitation, data-independent analysis, etc.).

1. Objective

Mass spectrometry technologies were used to generate comprehensive proteome profiles of HMC3 cells reflective of how microglia are activated in response to anti-inflammatory cytokines released from cancer cells. The biological interpretation of results is described in a related research manuscript published in *Frontiers in Immunology* [1]. The data presented in this article include additional qualitative and quantitative details that will enable researchers to broaden the premise for the interpretation of results.

2. Data Description

The RAW, processed and analyzed proteomic datasets that are described in this manuscript comprise:

(a) RAW and msf mass spectrometry files of HMC3 cells, nuclear and cytoplasmic fractions, generated from cells cultured in the presence and absence of fetal bovine serum (FBS), with or without treatment with anti-inflammatory cytokines (IL-4, IL-13, IL-10, TGFB) and chemokine MCP-1 (files shared in the PRIDE/ProteomeXchange repository).

(b) Processed RAW files with Proteome Discoverer v.2.4/Sequest HT that include the UniProt protein IDs, Protein Description, Protein Sequence, FASTA title line, Protein Coverage (%), # Peptides (distinct peptide sequence matches to the protein group), # PSMs per protein, # Protein Unique Peptides (peptide sequences unique to the protein group‘), # AAs (amino acids) per protein, MW, Calculated pI, and the following parameters that characterize each protein in each dataset and biological replicate: Protein FDR Confidence, Experimental q-value, Sequest HT score, Coverage (%), PSMs, and Peptides (Supplemental file 1).

(c) Processed RAW files with Proteome Discoverer v.2.4/Sequest HT that include the information from (b), for proteins that contained at least two peptides and that past the t-test for 2-fold change (FC) in PSM counts upon treatment with anti-inflammatory cytokines (increased or decreased) for serum-free and serum-treated cells (Supplemental files 2-5), and that align the proteins with increased/decreased spectral counts with controlled vocabulary terms (Supplemental file 6).

(d) Lists of enriched (FDR≤5 %) up- and down-regulated GO biological processes and pathways (KEGG, Reactome, Wiki) that were represented by the combined lists of proteins from (c) with increased (1296 proteins) and decreased (775 proteins) PSMs (Supplemental files 7 and 8).

(e) Figures that visualize the experimental setup, the microglia functions in the brain, the qualitative results and reproducibility of proteomic data, and, for supporting the interpretation of the data, a summary of the main biological processes that were affected by the proteins that changed expression level or function in response to the treatment with cytokines: Fig. 1 provides a schematic of the experimental cell culture conditions; Fig. 2 highlights the main physiological roles of microglia in the brain, and therefore, the premise of the study; Fig. 3 provides (A) the identified protein counts per cell state and per biological replicate, and (B) the number of cell membrane and non-cell membrane proteins involved in the main up/down regulated biological processes; Fig. 4 highlights the important immunological processes that were upregulated in the cytokine treated HMC3 cells, as represented by the list of 1296 protein with increased PSMs.

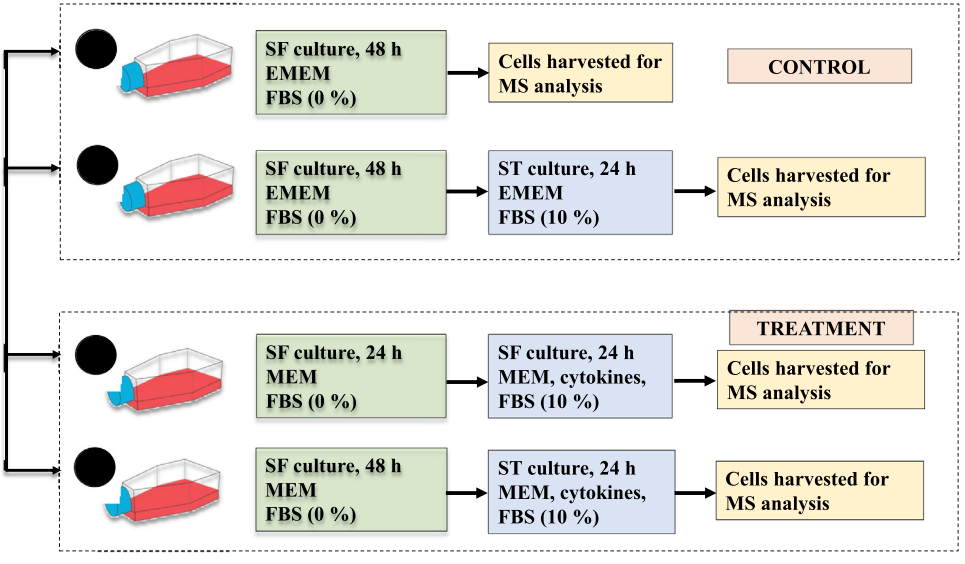

Fig. 1. Schematic diagram of the HMC3 cell culture protocol.

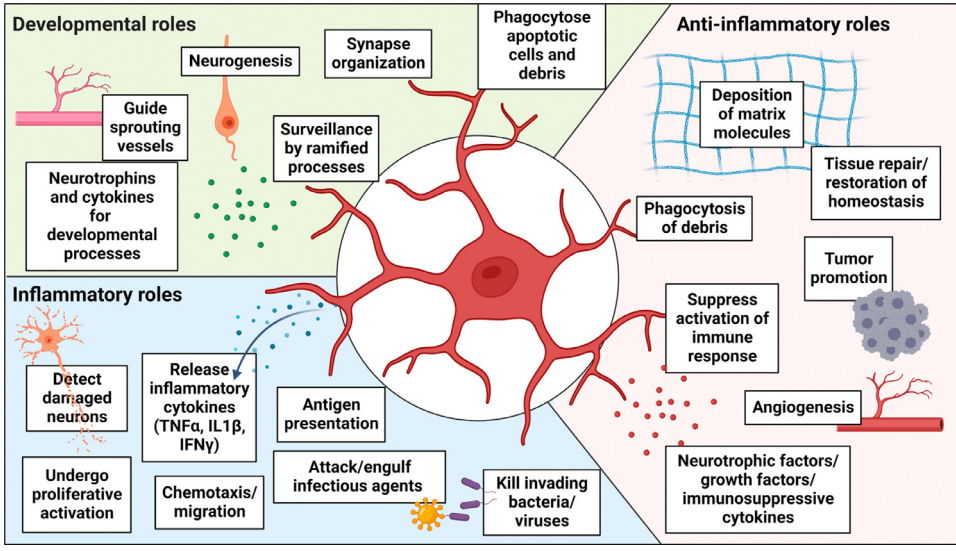

Fig. 2. Main physiological roles of microglia in the brain.

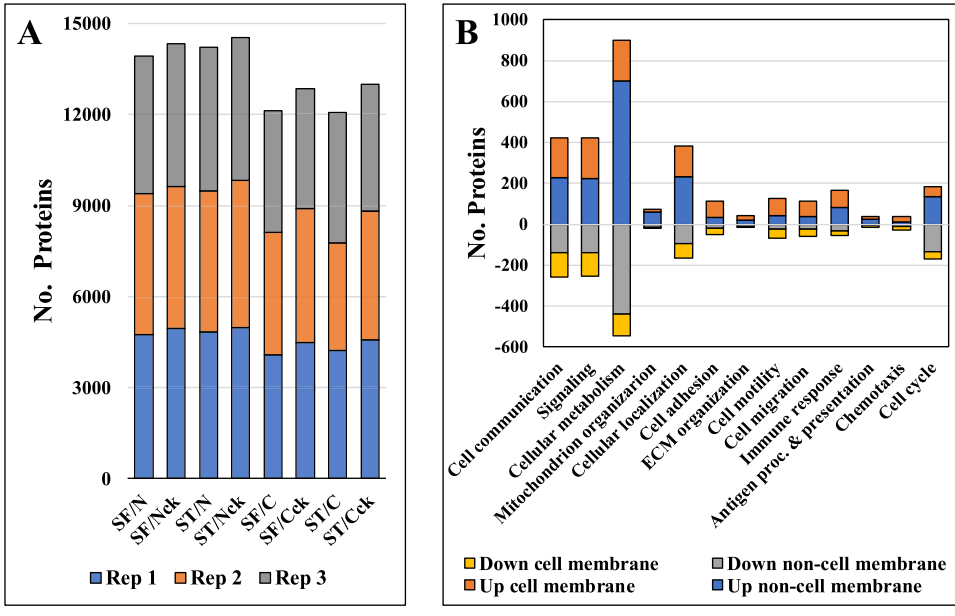

Fig. 3. Stacked bar charts representing protein identifications and biological processes affected by cytokine stimulation. (A) Number of detected proteins in HMC3 cytokine treated (ck) and control cells, nuclear (N) and cytoplasmic (C) fractions, in the presence (ST) and absence (SF) of serum. (B) Main biological processes affected by the cytokine stimulation of HMC3 cells as represented by non-membrane and cell-membrane proteins that initiate these responses (all proteins matched by two distinct peptide sequences, 2-FC in spectral counts, $p<0.05$).

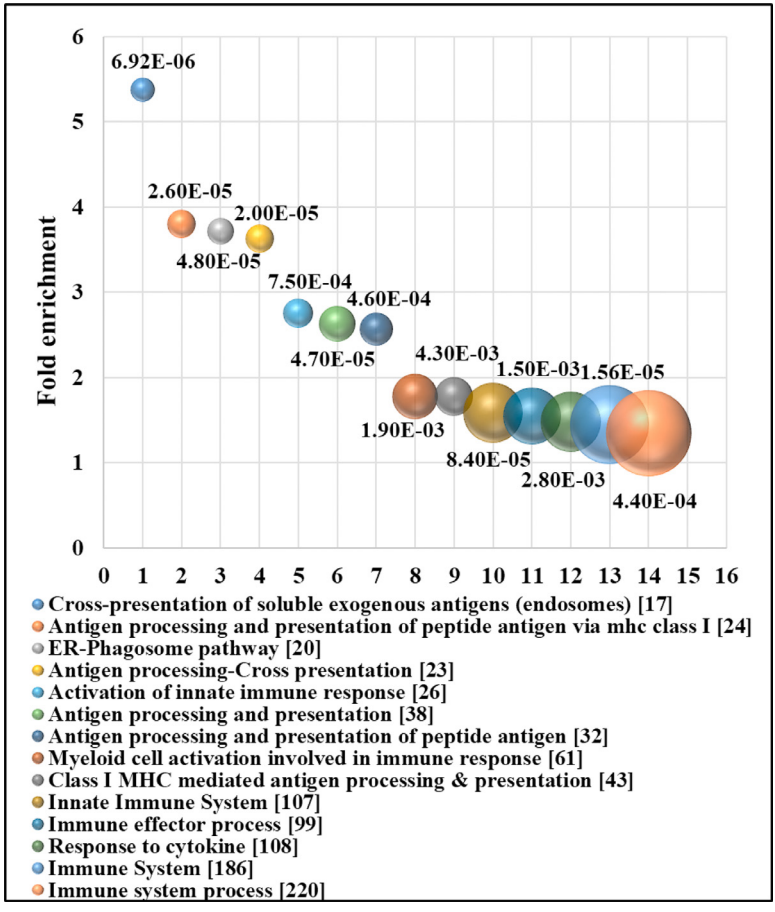

Fig. 4. Bubble chart representing up-regulated immune response processes in HMC3 cells treated with anti-inflammatory cytokines.

3. Experimental Design, Materials and Methods

3.1. Reagents and Materials

Cells (HMC3), EMEM, and PenStrep were purchased from ATCC (Manassas, VA). Other media and reagents necessary for cell culture such as phenol red/glutamine-free MEM, L-glutamine, DPBS, and trypsin-EDTA were procured from Gibco (Gaithersburg, MD), and FBS from Gemini Bio Products (West Sacramento, CA). The recombinant human cytokines (IL-4, IL-10, IL-13, CCL2, TGFβ1/TGFβ2-both HEK293 derived) were purchased from Peprotech (Rocky Hill, NJ). Propidium iodide for performing FACS analysis was bought Invitrogen (Carlsbad, CA). Reagents for sample preparation such as CH_3COOH, CF_3COOH, NH_4HCO_3, urea, DTT, phosphatase inhibitors (Na_3VO_4, NaF), protease inhibitor cocktail (P8340), RNase, Triton X-100, and the nuclear and cytoplasmic cell extraction kit (Cell Lytic^{TM} NuCLEAR^{TM}) were acquired from Sigma Aldrich (St. Louis, MO). Sequencing grade trypsin was from Promega (Madison, WI). BSA standards and the Bradford reagent were secured from Biorad (Hercules, CA). Pipette tips for sample cleanup (SPEC-PTC18 and SPEC-PTSCX) were purchased from Agilent technologies (Santa Clara, CA). HPLC-grade solvents for sample preparation (CH_3OH, CH_3CN) were supplied by Fischer Scientific (Fair Lawn, NJ)

and ethanol by Decon Laboratories (King of Prussia, PA). Water, high-purity, for sample solution preparations and HPLC analysis, was prepared by distillation from de-ionized water, in-house.

3.2. HMC3 Culture

HMC3 cells were retrieved from liquid nitrogen, thawed, and cultured in EMEM with FBS (10 %) in an incubator with 5 % CO_2 atmosphere that was maintained at 37 °C. An outline of the cell culture conditions is provided in Fig. 1 [1]. Cell cultures that were used as control were either (a) starved of FBS for 48 h, or (b) starved for 48 h and then released with FBS (10 %) for 24 h. Stimulation with anti-inflammatory cytokines was performed for 24 h by using the following conditions: (a) HMC3 cells were first starved for 24 h in MEM (phenol red-free) supplemented with glutamine (2 mM), and then starved for another 24 h but with the cytokine cocktail added to the culture medium; and (b) HMC3 cells were starved for 48 h, then stimulated with cytokines in the presence of FBS (10 %) for 24 h. The concentration of cytokines used for stimulation was chosen based on literature reports [5–7] and was: IL-4 (40 ng/mL), IL-10 (20 ng/mL), IL-13 (20 ng/mL), TGFβ1 (20 ng/mL), TGFβ2 (20 ng/mL), and CCL2, i.e., MCP-1 (40 ng/mL). All cell culture media contained PenStrep (0.5 %) to prevent bacterial contamination. Cell harvesting was performed by trypsinization, and the cells were flash frozen at -80 °C until further processing.

Data for serum starved cells (stimulated or non-stimulated with cytokines) were collected to enable an assessment of the HMC3 response to the cytokine treatment without interference from the FBS proteins. Three batches of cells, retrieved from liquid nitrogen and processed independently, were used as biological replicates for the control and the stimulated cells.

3.3. FACS

FACS analysis was performed with the FACSCalibur system (BD Biosciences, San Jose, CA) for assessing the cell cycle stage of the serum-starved and serum-treated cells. In preparation for analysis, the fresh cell cultures were fixed in 70 % EtOH, stained with propidium iodide (0.02 mg/mL) in a PBS solution containing RNase (0.2 mg/mL) and Triton X-100 (0.1 %), and then incubated at room temperature for 30 min.

3.4. Cell Extract Preparation and Processing

All cell states and treatments were fractionated into nuclear (N) and cytoplasmic (C) cellular subfractions. The manufacturer-recommended protocol was followed for cell lysis and processing. Hypotonic lysis buffer (10X) and Igepal from the Cell Lytic™ NuCLEAR™ extraction kit were used for lysing the cells, and the Extraction buffer for performing the extraction of nuclear proteins [8–10]. The cell and nuclear lysis reagents were supplemented with phosphatase (Na_3VO_4, NaF) and protease inhibitor cocktails. The concentration of protein extracts was determined with the Bradford assay. For MS analysis, 500 µg of protein extracts were first denatured and reduced at 57°C, for 1 h, in the presence of urea (8 M) and DTT (5 mM). After 10-fold dilution with NH_4HCO_3 (50 mM), the samples were subjected to overnight enzymatic digestion with trypsin (50:1 protein/enzyme ratio) at 37 °C. The enzymatic reaction was quenched with glacial acetic acid (10 µL/mL proteolytic digest). Buffers and cell lysis components from the proteolytic digests were removed with the SPEC-PTC18/SCX sample clean-up cartridges, and the resulting peptides were re-suspended in a concentration of 2 µg/µL in a solution of H_2O:CH_3CN:CF_3COOH (98:2:0.01). The samples were frozen at -80°C until further analysis.

3.5. LC-MS Analysis

Nano-liquid chromatography mass spectrometry was performed with an Easy-nLC 1200 ultra-high pressure LC system and a Q Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometer

(ThermoFisher Scientific, USA) interfaced via an EASY-Spray™ ion source [1]. The separations were performed on ES802A columns (250 mm long x 75 µm i.d.) packed with C18/silica particles (2 µm), with an eluent gradient of ~2 h. The eluent flow was 250 nL/min, with solvent A being prepared from H_2O:CH_3CN:TFA (96:4:0.01, v/v), and solvent B from H_2O:CH_3CN:TFA (10:90:0.01, v/v). The composition of the eluent gradient was as follows: 7 % B (0-2 min), 7-30 % B (2-107 min), 30-45 % B (107-109 min), 45-60 % B (109-110 min), 60-90 % B (110-111 min), 90 % B (111-121 min), 90-7 % B (121-122 min), and 7 % B (122-127 min). The separation column was heated at 45 °C, ESI was established at 2 kV, and the MS ion inlet capillary was heated at 250 °C. The data were acquired by using a DDA approach, with MS acquisition occurring over a range of m/z=400-1600. The MS acquisition parameters were pre-set to 70,000 resolution, AGC target 3E6, and IT maximum of 100 ms. The isolation window for the quadrupole filter was set to m/z=2.4. Tandem MS was performed by using higher energy collision dissociation (HCD) with a normalized collision energy of 30 %, and MS2 spectra were acquired on the top 20 ions with a resolution of 17,500, AGC target 1E5, and IT maximum of 50 ms. Charge exclusion was enabled for +1 ions and ions with undetermined charge states, selecting ions with peptide-like isotope distribution, isotope exclusion on, apex trigger 1-2 s, minimum AGC trigger 2E3, and dynamic exclusion of 10 s.

3.6. MS/RAW Data Processing

The RAW mass spectrometry files were interpreted with the Proteome Discoverer (v.2.4) software package (ThermoFisher Scientific) by using the Sequest HT search engine and a UniProt *Homo sapiens* database [11] of 20,404 reviewed and non-redundant protein entries (2019). A target-decoy processing workflow was used for matching the experimental tandem mass spectra to the theoretical peptides generated from the UniProt database. The following parameter settings were used in the search: fully tryptic peptides with precursor masses of 400-5,000 Da and a minimum of 6 amino acids, maximum 2 tryptic missed cleavage sites, tolerances of 15 ppm for precursor ions and 0.02 Da for fragment ions, all b/y/a ions considered in the search, and dynamic modifications allowed on the N-terminus (42.0106 Da/acetyl) and methionine (15.9949 Da/oxidation). For PSM validation, a forward/reverse concatenated database was used (maximum Delta Cn 0.05, maximum rank 1). Only rank 1 peptides were counted, and only for top scoring proteins, with the strict parsimony principle being enabled for the protein grouping node. The probability threshold for the peptide modifications was set to 75, and all peptide/protein level FDRs were set at confidence thresholds of 0.03 (relaxed)/0.01 (stringent).

3.7. Bioinformatics MS Data Analysis

A total of eight datasets were created, i.e., two cell states of serum-free and serum treated cells (SF/ST), nuclear and cytoplasmic fraction (N/C), with cytokine (ck) stimulation or without stimulation (control). For each of the eight cell conditions, three LC-MS/MS technical replicates were generated with results combined in one multiconsensus Proteome Discoverer v.2.4 report. Three biological replicates were created for all of the above to enable statistical evaluations of changes in protein expression (Fig. 3A). For a complete proteome profile, a combined multiconsensus report was generated from all 72 experimental RAW files (8 datasets x 3 technical replicates x 3 biological replicates) (Supplemental file 1). List of proteins that changed PSMs, reflective of differential protein expression between cytokine-treated *vs.* non-treated cells, were generated by processing and filtering the qualitative protein lists according to the following criteria: (a) The PSM count data for each protein were normalized based on the average of total spectral counts for each of the three biological replicates of cytokine-treated and non-treated (control) cell states that were compared; (b) One spectral count was added to each protein in each data set to account for missing values; (c) Only proteins that were identified by two distinct

peptide sequences were accepted for quantitative comparisons; (d) A two-tailed t-test was performed for each protein to assess the statistical significance of the change in PSMs; (e) Proteins that displayed a 2-FC in spectral counts, i.e., with Log2(Cytokine-treated cells/Non-treated cells) $\geq$0.9 or $\leq$(-0.9), and that passed the significance threshold set by p-value$<$0.05, were included in the final lists (Supplemental files 2-5). Controlled vocabulary terms from UniProt were used to extract *Homo sapiens* proteins with functionally related roles (Fig. 3B and Supplemental file 6). Enriched up-/down-regulated biological processes and pathways (FDR$<$0.05) represented by the above lists of proteins with change in spectral counts (1296 proteins with increased PSMs, 775 proteins with decrease PSMs) were inferred based on GO [12], KEGG [13], REACTOME [14] and WIKI [15] bioinformatics tools enabled via the STRING [16] website (Supplemental files 7-8). The representation of the biological roles of microglia was created with tools provided by BioRender.com (Fig. 2). Bar- and bubble charts were generated with Microsoft Excel (Figs. 3 and 4).

Ethics Statement

Not applicable.

Data Availability

Proteomic analysis of human microglia cells (HMC3) stimulated with anti-inflammatory cytokines using serum-deprived culture conditions (Original data) (PRIDE)

Proteomic analysis of human microglia cells (HMC3) stimulated with anti-inflammatory cytokines using serum-enriched culture conditions (Original data) (PRIDE)

Anti-Inflammatory Cytokine Stimulation of HMC3 Cells: Proteome Dataset (Original data) (Mendeley Data)

CRediT Author Statement

Shreya Ahuja: Data curation, Formal analysis, Investigation, Writing – review & editing; **Iulia M. Lazar:** Data curation, Formal analysis, Funding acquisition, Investigation, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2023.109433.

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