eMethods

Sampling and flow cytometric analysis of PBMC

Freshly thawed or stimulated PBMC were centrifuged at 300×*g* for 5 min. Then, PBMC were resuspended in phosphate buffered saline (PBS, Sigma-Aldrich) supplemented with 2% heat-inactivated fetal bovine serum (FBS, GE Healthcare) and 2 mM ethylenediaminetetraacetic acid (EDTA, Sigma-Aldrich) and incubated with fluorochrome-conjugated antibodies at 4°C for 30 min. Chemokine receptor staining was performed at 37°C for 30 min. When indicated, PBMC were washed and additionally stained with the Zombie NIR or Aqua Fixable Viability Kit (Biolegend) according to the manufacturer's manual to distinguish between living and dead cells. For staining of intracellular proteins, components from the BD Cytofix/Cytoperm Kit (BD Biosciences) were used according to the manufacturer's manual. PBMC were washed and resuspended in PBS/FBS/EDTA and analyzed by flow cytometry using a CytoFlex Flow Cytometer (Beckman Coulter). The antibodies used for comprehensive immune cell phenotyping are summarized in eTable 5.

Sampling and flow cytometric analysis of CSF cells

CSF samples were collected by lumbar puncture and were processed within 60 min as previously described.[e1] In our study, we did not use blood-tinged CSF. Afterwards, CSF cells were obtained by centrifugation (15 min, 290×*g*, 4°C). Subsequently, the cells were incubated with VersaLyse buffer for 10 min. After washing, CSF cells were stained using the α-human antibodies depicted in eTable 6 for 30 min and analyzed using a Navios flow cytometer (Beckman Coulter).

Cell culture

For analysis of intracellular cytokine production, freshly thawed PBMC were centrifuged at 300×*g* for 5 min and were cultured in X-Vivo 15 medium (Lonza) over night. On the next day, PBMC were restimulated with the Leukocyte Activation Cocktail (LAC; phorbol 12-myristate 13-acetate, ionomycin, and Brefeldin A; BD Biosciences) at a concentration of $5x10^6$ PBMC/ml and incubated at 37°C and 5% CO_2 for 4 h to assess cytokine production of T cells. For analysis of monocyte-specific cytokine production, PBMC were stimulated with 100 ng/ml lipopolysaccharide (LPS, Sigma-Aldrich) at a concentration of $5x10^6$ PBMC/ml and incubated at 37°C and 5% CO_2 for 2 h. Afterwards, PBMC were washed and stained with respective antibodies for flow cytometry.

Degranulation assay

To assess the release of cytotoxic granules of CD8 T cells in response to polyclonal stimulation by α-CD3-loaded on P815 cells.[e2] PBMC were mixed in a 1:1 ratio with P815 target cells and incubated in the presence of 10 µl/ml Golgi plug (BD Biosciences), 0.0625 µg/ml α-CD3 (OKT3, Biolegend), and 0.25 µg/ml CD107a-FITC antibody

(H4A3, BD Biosciences) for 3 h at 37 °C/5% CO_2. Following incubation, cells were centrifuged at 300×*g* for 10 min at room temperature and stained for lineage-defining surface markers for 30 min at 4 °C. Following washing, samples were acquired by flow cytometry. Degranulation of CD8 T cells was assessed as the percentage of CD107a-expressing CD8 T cells.

Gating strategy

Immune cell subsets in the PB of study subjects (cohort A) were identified using the following markers:

Lymphocytes: FSC vs. SSC

B cells: $CD19^+$ $CD3^-$ Lymphocytes

T cells: $CD3^+$ $CD56^-$ Lymphocytes

CD4: $CD4^+$ $CD8^-$ T cells

CD8: $CD8^+$ $CD4^-$ T cells

CD4/CD8 naive: $CD45RO^-$ $CD27^-$

CD4/CD8 memory: $CD45RO^+$

CD4/CD8 central memory: $CD45RO^+$ $CD27^+$

CD4/CD8 effector memory: $CD45RO^+$ $CD27^-$

TEMRA: $CD45RO^-$ $CD27^-$ $CD4^+$ or $CD8^+$ T cells

Monocytes: $CD14^+$ HLA-DR^+ $CD3^-$ $CD56^-$ $CD19^-$ $CD66b^-$

Classical monocytes: $CD14^{high}$ $CD16^-$ Monocytes

Intermediate monocytes: $CD14^{high}$ $CD16^+$ Monocytes

Non-classical monocytes: $CD14^+$ $CD16^{high}$ Monocytes

For analysis of the CSF of study subjects (cohort B) the following gating strategy was obtained as described previously[e3]:

Lymphocytes: FSC vs. SSC of $CD45^+$ cells

Monocytes: FSC vs. SSC of $CD45^+$ cells

T cells: $CD3^+$ $CD56^-$ Lymphocytes

CD4: $CD4^+$ $CD8^-$ T cells

CD8: $CD8^+$ $CD4^-$ T cells

CD4/CD8 HLA-DR: HLA-DR^{+} CD4 or CD8 T cells

NK cells: CD56^{+} CD3^{-} Lymphocytes

CD56^{dim}: CD56^{dim} CD16^{+} NK cells

CD56^{bright}: CD56^{bright} CD16^{-} NK cells

B cells: CD19^{+} Lymphocytes

Plasma cells: CD138^{+} Lymphocytes

Serum neurofilament light chain (sNfL) measurements

Our sNfL measurement protocol was previously described in detail.[e4,5] Briefly, sNfL levels were determined in duplicates by single molecule array with a SiMoA HD-1 (Quanterix, USA) using the Nf-Light Advantage Kits (Quanterix) according to manufacturer's instructions. Resorufin-β-D-galactopyranoside (RGP) was incubated at 33°C for 60 min prior to running the assay. All coefficients of variation (CV) of the two replicates were below 20%, resulting in a mean intra-assay CV of 6.6%. Low and high controls, consisting of recombinant human NfL antigen, were included in each sample run to monitor plate-to-plate variation (low: mean 3.7 pg/ml, inter-assay CV 6.7%; high: mean 195.1 pg/ml, inter-assay CV 8.1%). sNfL measurements were performed in a blinded fashion without information about clinical data.