Supplemental information

Cold exposure protects from neuroinflammation through immunologic reprogramming

Martina Spiljar, Karin Steinbach, Dorothée Rigo, Nicolas Suárez-Zamorano, Ingrid Wagner, Noushin Hadadi, Ilena Vincenti, Nicolas Page, Bogna Klimek, Mary-Aude Rochat, Mario Kreutzfeldt, Claire Chevalier, Ozren Stojanović, Olivia Bejuy, Didier Colin, Matthias Mack, Dilay Cansever, Melanie Greter, Doron Merkler, and Mirko Trajkovski
Figure S1

A

B

C

D

E

F

BM-myeloid

BM-progenitors
Figure S1. Cold housing temperature causes immunological alterations, Related to Figure 1

(A) Multidimensional scaling and principal component analysis of the bone marrow from mice after 2 weeks of cold exposure at 10°C compared to room temperature mice.

(B) 10 most enriched Metacore Metabolic Networks of mice as in (A).

(C) Metacore Pathway Maps analysis identifying the 10 most differentially regulated pathways of mice as in (A).

(D) 1 of 4 heatmaps that were consistently changed (> 80% of genes in the same direction) from the top 10 deregulated Metacore Pathway Maps of mice as in (A).

(E) Absolute cell numbers within the bone marrow of mice as in Figure 1F as determined by flow cytometry. Shown is one experiment or for total bone marrow cells a pool of 5 experiments, mean ±SD and significance was calculated using student’s t test, * p<0.05, ** p<0.01.

(F) Bone marrow monocytes were gated as live, single, CD45+, Ly6G-, CD11b+, CD115+, Ly6C+ cells and MHCII+ population determined. Bone marrow progenitors were all gated as single, live, CD45+, Lin- (NK1.1, CD3, CD19, Ter119, Ly6G) and CD11b-, Sca1+, CD117hi, CD115+, Ly6Ch (cMoP), CD11b-, Sca1+, CD117hi, CD115+, Ly6C- (MDP), CD11b-, Sca1+, CD117-, CD115+, CD135+ (MDP), CD117+, CD127+, Sca-1+ (CLP), Sca-1+, CD117+ (LSK).

(C-D) The cutoff on differentially regulated genes considered for the pathway analysis is p<0.05 and pathways are considered deregulated with p<0.05. Shown are –log(p-value).
**Figure S2. Cold modulates blood monocytes, Related to Figure 2**

(A) Gating strategy for the FACS (CD115-PE+) sort after MACS (anti-PE) sort before RNA sequencing from blood of 2 weeks cold exposed (10°C) compared to room temperature mice. Sorted were DAPI-, single, CD115+ cells.

(B) Multidimensional scaling (left) and principal component analysis (right) after RNA sequencing of monocytes as in (A).

(C-E) 10 most differentially regulated Metacore Pathway Maps without p-value cutoff on considered genes (C), Metacore Metabolic Networks (D) and Gene Ontology Cellular Components (E). Genes were considered as differentially regulated genes with p<0.05 and pathways are considered deregulated or enriched with p<0.05. Shown are –log(p-value).

(F) For flow cytometry analysis, blood monocytes were gated as Ly6G−, CD11b+, CD115+, Ly6Chigh/int/low and MHCII+ positive population was gated.

(G) Absolute cell numbers within the blood of mice as in Figure 2D as determined by flow cytometry. Shown is a pool of three experiments and mean ±SD.
Figure S3. Cold reduces monocyte MHCII, Related to Figure 3

(A) Peritoneal fluid cells were analyzed by flow cytometry 24h after i.p. injection with thioglycollate into two weeks cold exposed (10°C) or room temperature mice. Shown is mean ±SD.

(B) Flow cytometry analysis of lymph node draining cells 12 and 18 hours after FITC skin painting on one flank of 2 weeks cold exposed (10°C) or room temperature mice. Percentage of panDCs (upper) and Ly6C<sup>hi</sup> monocytes (bottom), their FITC uptake and MHCII expression. Shown is mean ±SD, two-way ANOVA. Every dot represents one animal and the experiment is representative of two.
Figure S4

A. EAE score over time for RT and CE groups.

B. Mac3+ / mm² and CD3 / mm² cell counts over time for RT and CE groups.

C. APP per WM mm² over time for RT and CE groups.

D. EAE score (peak) over time for RT and CE groups.

E. Mac3+ / mm² over time for RT and CE groups.

F. CD3 / mm² over time for RT and CE groups.

G. Pathway Maps

H. Table showing n, cumulate score mean, and p-values for different groups.

I. Leading logFC dim1 over Leading logFC dim2 for RT and CE groups.

J. List of pathways and their significance levels.

K. EAE score over time for RT and WE groups.

L. Body weight over time for RT and WE groups.

M. Food intake (g) for 2 mice.

N. Body weight (g) over time for d6-WE.

O. % Survival over time for RT and WE groups.

P. Onset day over time for RT and WE groups.
Figure S4. Cold alleviates, while warm worsens neuroinflammation, Related to Figure 4

(A) Individual EAE curve (as in Figure 4A) of each room temperature and cold-exposed mouse.

(B-G) Histology on sagittal spinal cord sections of cold-exposed and room temperature peak EAE mice. Number of lesions that were found in both LFB-PAS and APP staining were quantified in APP staining (B). Immunohistochemistry using anti-mac3 (C) and anti-CD3 antibody (D). APP staining was quantified within areas identified as demyelinated in LFB-PAS staining and presented as percentage per white matter area (E). EAE disease score at sacrifice of mice in which histology was performed (F). LFB-PAS stain shows demyelinated areas within the white matter in purple (first panel), anti-mac3 stains mac3+ macrophages (second panel), anti-CD3 stains T cells in brown (third panel) and amyloid precursor protein (APP) accumulates at axons with impaired axonal transport, a surrogate for acute axonal damage (fourth panel). Arrows identify punctuated APP-positive axonal spheroids. (G). Anti-mac3, anti-CD3 and anti-APP stainings were developed with DAB and nuclei counterstained with hemalum.

(H) Table of cumulative EAE scores ±SD and number of mice of each individual experiment.

(I, J) Multidimensional scaling analysis of spinal cord from mice as in Figure 4J (I). Top 35 Metacore Metabolic Network pathways of mice with EAE, genes with p<0.05 were included for analysis (J).

(K-P) EAE disease curve of warm exposed (34°C, started one week before immunization) or room temperature mice. Arrows indicate individual mice that died or had to be sacrificed, which received a score of five on the day of death (K). Body weight during the course of EAE (L). Food intake of two mice per cage within 24 h on
day 6 of warm exposure (M) and body weight on day six of warm exposure (N), before immunization. Survival of mice during EAE (O) and day of EAE onset (P). (B-F, K-P) Shown is mean ±SEM, two-way ANOVA (K, L); Mantel-Cox (O), student’s t test (B-F, L-M, P) with mean ±SD, *p < 0.05, **p < 0.01, ***p < 0.001.
Figure S5

A

B

C

D

E

F

G

Days after EAE induction

Days after EAE induction

EAE Score

Body weight (g)
Figure S5. Metabolic profiling of cold-exposed mice after immunisation, Related to Figure 5

(A) Glycemia on day 8 of EAE after 3 hours of fasting. Mice were cold exposed for 2 weeks before immunisation and during EAE or steady state. Control mice were at room temperature for the same duration.

(B-D) Adipose tissue weights of inguinal subcutaneous adipose tissue (B), perigonadal visceral adipose tissue (C) o, or interscapular brown (D) of mice as in (A).

(E-F) mRNA expression of browning markers relative to B2m and 36b4 in interscapular brown (E) and inguinal subcutaneous adipose tissue (F) of mice as in (A) as determined by qPCR.

(G) Symptoms of EAE (left) and body weight (right) in Ucp1-knockout (KO) and their respective wildtype (WT) littermate controls housed at room temperature or cold for 1 week before and during EAE. Shown is 1 representative (n=10-15 per group) of 2 experiments.

(A-G, I) Multiple t-test with Holm-Sidak correction (A,E,F), students t-test (B-D), two-way ANOVA (G). (A-F) show mean ±SD; (G) shows mean ±SEM. *p < 0.05, **p < 0.01, ***p < 0.001.
Figure S6. Cold reduces monocyte and T cell pathogenicity during neuroinflammation, Related to Figure 6

(A) Gating strategy for the FACS sort after MACS sorting before RNA sequencing (as in Figure 6A). Sorted were DAPI-, CD115+, single cells.

(B) Multidimensional scaling and principal component analysis of FACS sorted blood monocytes at EAE onset as in Figure 6A.

(C) Gating strategy for MHCII expression on Ly6C^{hi} blood monocytes of mice as in Figure 6A. Cells were gated as single, Ly6G-, CD115^{+}CD11b^{+}, Ly6C^{hi}, MHCII^{+}.

(D) Absolute cell numbers within the blood of mice as in Figure 5D as determined by flow cytometry. Shown is a pool of three experiments and mean ±SD. Pool of 3 experiments. (E-F) Blood (E) and lymph node (F) immune cells of mice as in Figure 6A on different days after immunization were visualized using UMAP and clustered using the FlowSOM algorithm in R. Heatmap shows median relative expression of all panel markers.

(G) Gating strategy for MHCII expression on microglia of mice as in Figure 6A. Cells were gated as single, live, Ly6G-, CD45^{int}, MHCII^{+}.

(H) Flow cytometry analysis of mesenteric LN (upper panel) or spleen cells (lower panel) of mice as in Figure 6A at EAE onset. Percentage of cytokine expression in CD4^{+} T cells. Shown is mean ±SD, significance was calculated using student’s t test, * p<0.05.

(I) Flow cytometry analysis of draining LN of mice as in Figure 6A at EAE onset. Percentage of Foxp3+ Tregs of total CD4^{+} T cells. Shown is mean ±SD.
Figure S7

(A) Schematic diagram showing the experimental setup with RT, CERT, and CE groups.

(B) Graph showing the EAE score over days after EAE induction.

(C) Graph showing the percentage of MHCI of Ly6Chi M cells over d0, d3, and d7 of EAE.

Legend: ns, *p=0.069.
Figure S7. Transferring cold-exposed mice to room temperature prior to T cell priming limits the beneficial cold-induced effects, Related to Figure 7

(A-B) Scheme showing experimental setup for cold exposure and EAE (A). Cold exposure either started two weeks before and continued during EAE (blue, CE) or switched to room temperature from the day of immunization (violet, CE-RT) and compared to room temperature mice (red, B). EAE disease curve shown as mean ±SEM, two-way ANOVA, *p < 0.05.

(C) Percentage of MHCII expression of Ly6C\textsuperscript{hi} blood monocytes via flow cytometry on day zero, day three and day seven after EAE induction. Multiple t-test with Holm-Sidak correction with mean ±SD, *p < 0.05, **p < 0.01, ***p < 0.001.