Microbiome-microglial crosstalk in graft-versus-host disease of the central nervous system

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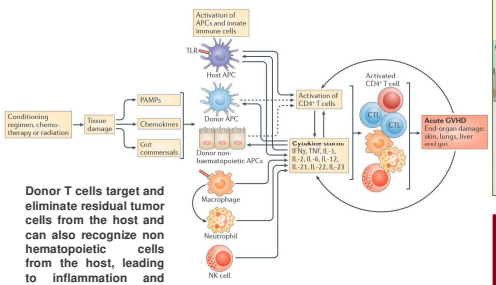
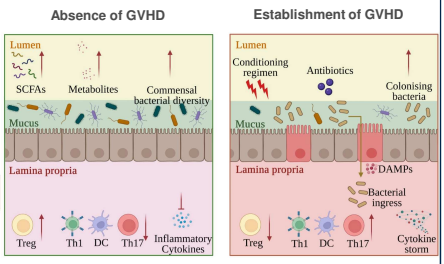
Introduction

Allogenic hematopoetic cell transplant (Allo-HCT) is a treatment modality for various hematological disorders. In patients, Graft-versus-host disease (GVHD) is the major clinical complication arising after Allo-HCT.



Donor T cells target and eliminate residual tumor cells from the host and can also recognize non hematopoietic cells from the host, leading to inflammation and tissue damages or GVHD.

Adapted from Blazar BR *et al*, *Nature Reviews Immunology*, 2012



Does gut-microbial manipulation in murine GVHD have an effect on microglial activation in the central nervous system?

Methods

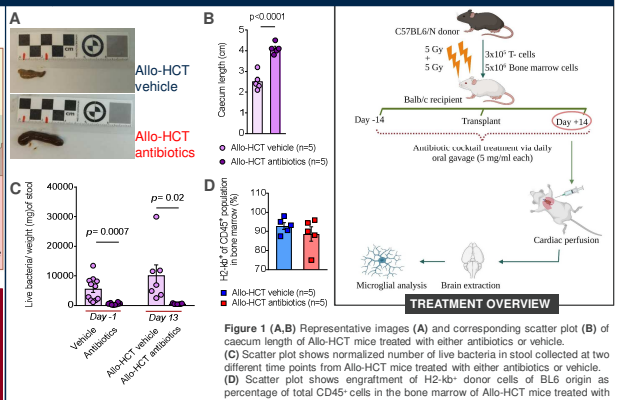


Figure 1 (A,B) Representative images **(A)** and corresponding scatter plot **(B)** of caecum length of Allo-HCT mice treated with either antibiotics or vehicle. **(C)** Scatter plot shows normalized number of live bacteria in stool collected at two different time points from Allo-HCT mice treated with either antibiotics or vehicle. **(D)** Scatter plot shows engraftment of H2-kb+ donor cells of BL6 origin as percentage of total CD45+ cells in the bone marrow of Allo-HCT mice treated with either antibiotics or vehicle.

Microbiome depletion drives T-cell towards activation and pro-inflammatory phenotype

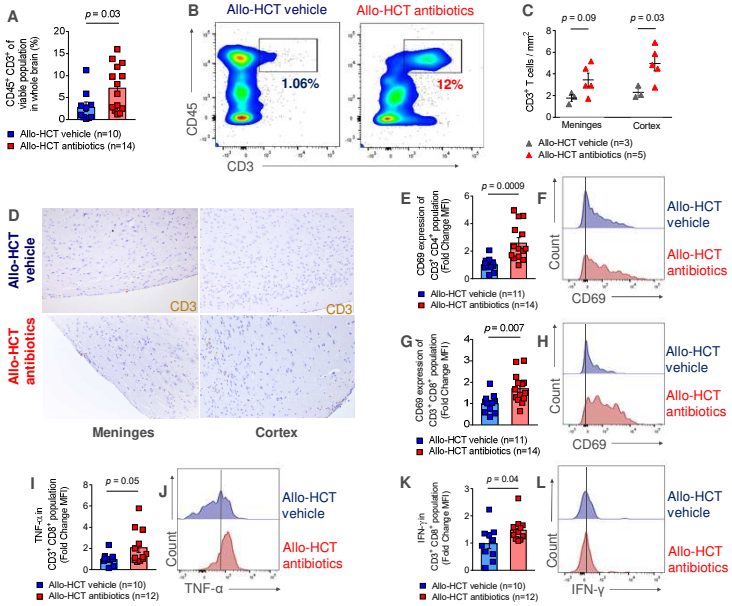


Figure 2 (A,B) : Scatter plot **(A)** shows quantification (percentage of total viable population) of T cells ($CD45^+$ $CD3^+$) in the CNS of Allo-HCT mice treated with either antibiotics or vehicle. **(B)** Representative population plot visualizes T cells in the CNS by plotting CD3 (x-axis, log2) versus CD45 (y-axis, log2).
(C,D) Histology of brain samples immunostained for $CD3^+$ T cells (brown) from Allo-HCT mice treated with either antibiotics or vehicle as indicated. **(D)** A representative image from each group is shown. Scale bars: 50 µm. The scatter plot **(C)** shows the number of $CD3^+$ T cells (per mm2) in cerebral meninges and cortex.
(E-F) Scatter plot **(E)** and representative population plot **(F)** shows quantification of CD69 expression on $CD3^+$ $CD4^+$ from the CNS of Allo-HCT mice treated with either antibiotics or vehicle.
(G-H) Scatter plot **(G)** and representative population plot **(H)** shows quantification of CD69 expression on $CD3^+$ $CD8^+$ from the CNS of Allo-HCT mice treated with either antibiotics or vehicle.
(I-L) Scatter plot and representative flow cytometry plot shows expression of TNF-α **(I,J)** and IFN-γ **(K,L)** expression $CD3^-$ $CD8^+$ from the CNS of Allo-HCT mice treated with either antibiotics or vehicle.

Microbiome depletion upregulates p38 MAPK and NF-κb pathways in microglia

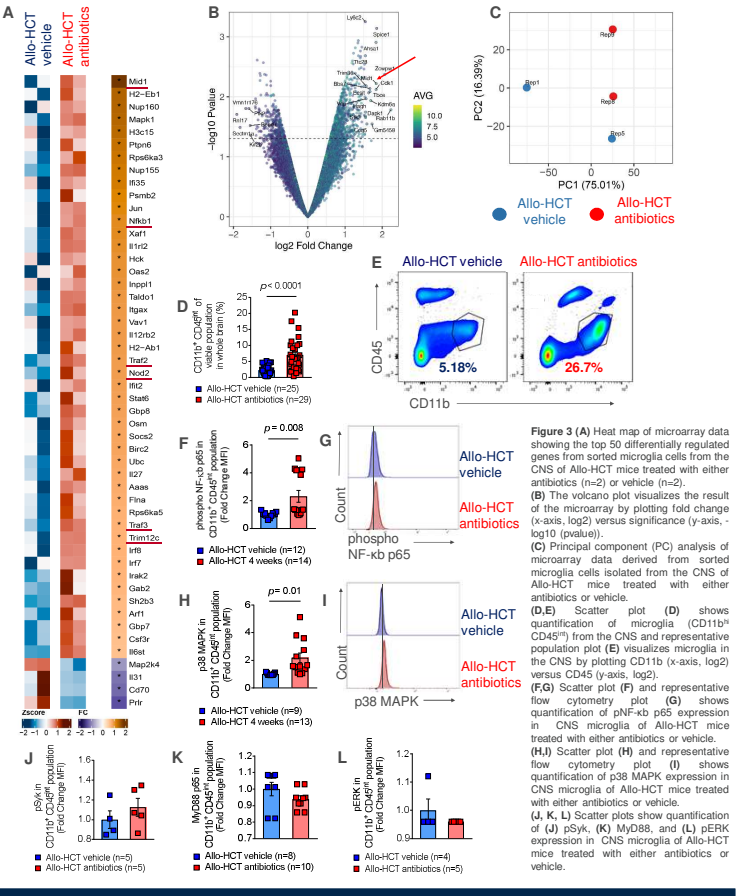


Figure 3 (A) Heat map of microarray data showing the top 50 differentially regulated genes from sorted microglia cells from the CNS of Allo-HCT mice treated with either antibiotics (n=2) or vehicle (n=2).
(B) The volcano plot visualizes the result of the microarray by plotting fold change (x-axis, log2) versus significance (y-axis, -log10 (pvalue)).
(C) Principal component (PC) analysis of microarray data derived from sorted microglia cells isolated from the CNS of Allo-HCT mice treated with either antibiotics or vehicle.
(D,E) Scatter plot **(D)** shows quantification of microglia ($CD11b^{hi}$ $CD45^{int}$) from the CNS and representative population plot **(E)** visualizes microglia in the CNS by plotting CD11b (x-axis, log2) versus CD45 (y-axis, log2).
(F,G) Scatter plot **(F)** and representative flow cytometry plot **(G)** shows quantification of pNF-kb p65 expression in CNS microglia of Allo-HCT mice treated with either antibiotics or vehicle.
(H,I) Scatter plot **(H)** and representative flow cytometry plot **(I)** shows quantification of p38 MAPK expression in CNS microglia of Allo-HCT mice treated with either antibiotics or vehicle.
(J, K, L) Scatter plots show quantification of **(J)** pSyk, **(K)** MyD88, and **(L)** pERK expression in CNS microglia of Allo-HCT mice treated with either antibiotics or vehicle.

Microbiome depletion upregulates activation markers on microglia

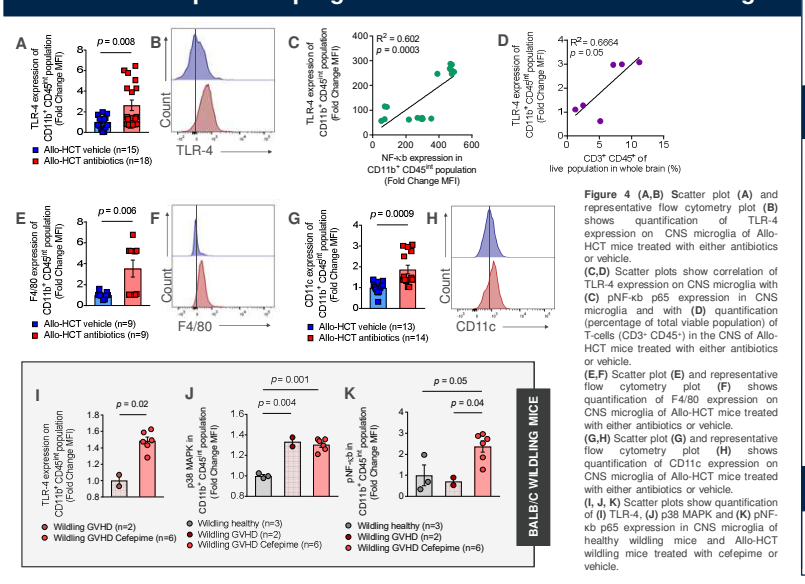


Figure 4 (A,B) Scatter plot **(A)** and representative flow cytometry plot **(B)** shows quantification of TLR-4 expression on CNS microglia of Allo-HCT mice treated with either antibiotics or vehicle.
(C,D) Scatter plots show correlation of TLR-4 expression on CNS microglia with **(C)** pNF-κb p65 expression in CNS microglia and with **(D)** quantification (percentage of total viable population) of T-cells ($CD3^+$ $CD45^-$) in the CNS of Allo-HCT mice treated with either antibiotics or vehicle.
(E,F) Scatter plot **(E)** and representative flow cytometry plot **(F)** shows quantification of F4/80 expression on CNS microglia of Allo-HCT mice treated with either antibiotics or vehicle.
(G,H) Scatter plot **(G)** and representative flow cytometry plot **(H)** shows quantification of CD11c expression on CNS microglia of Allo-HCT mice treated with either antibiotics or vehicle.
(I, J, K) Scatter plots show quantification of **(I)** TLR-4, **(J)** p38 MAPK and **(K)** pNF-kb p65 expression in CNS microglia of healthy wildling mice and Allo-HCT wildling mice treated with cefepime or vehicle.

Microbiome depletion worsens GVHD severity

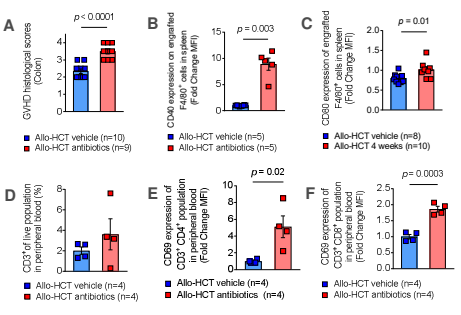


Figure 5 (A) Colon from Allo-HCT mice treated with either antibiotics or vehicle was cut into 3 µm sections and stained with hematoxylin and eosin. GVHD scoring was performed by unbiased pathologists in a blinded fashion. Healthy tissue was considered as 0, whereas mild and moderate tissue damage were graded with 1–2. Severe tissue damage was considered as 3.
(B, C) Scatter plots show quantification of **(B)** CD40 and **(C)** CD80 expression on engrafted ($H2\text{-}kb^+$) $F4/80^+$ cells in the spleen of Allo-HCT mice treated with either antibiotics or vehicle.
(D) Scatter plot shows quantification (percentage of total viable population) of $CD3^+$ cells in peripheral blood collected from Allo-HCT mice treated with either antibiotics or vehicle.
(E, F) Scatter plots show quantification of CD69 expression on **(E)** $CD3^+$ $CD8^+$ and on **(F)** $CD3^+$ $CD4^+$ in peripheral blood collected from Allo-HCT mice treated with either antibiotics or vehicle.

References

1. Mathew NR, Vinnakota JM *et al, Journal of Clinical Investigation*, 2019
2. Erny D *et al, Nature Neuroscience*, 2015
3. Zeiser R and Blazar BR, *New England Journal of Medicine*, 2017

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