Appendix

IL-1 signaling is critical for expansion but not generation of autoreactive GM-CSF^{+} Th17 cells

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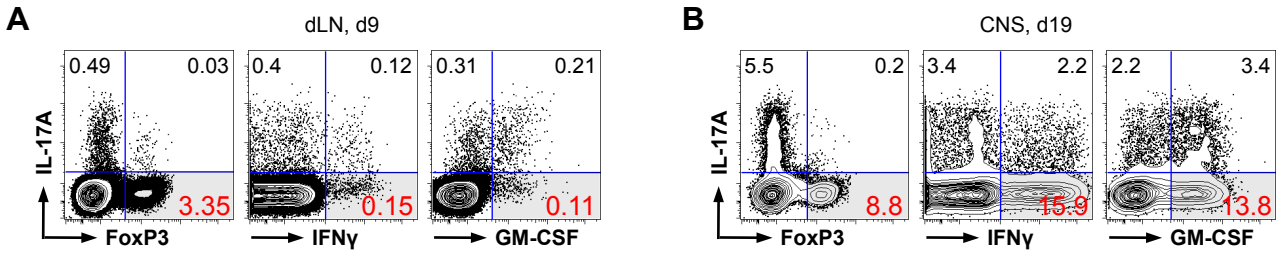
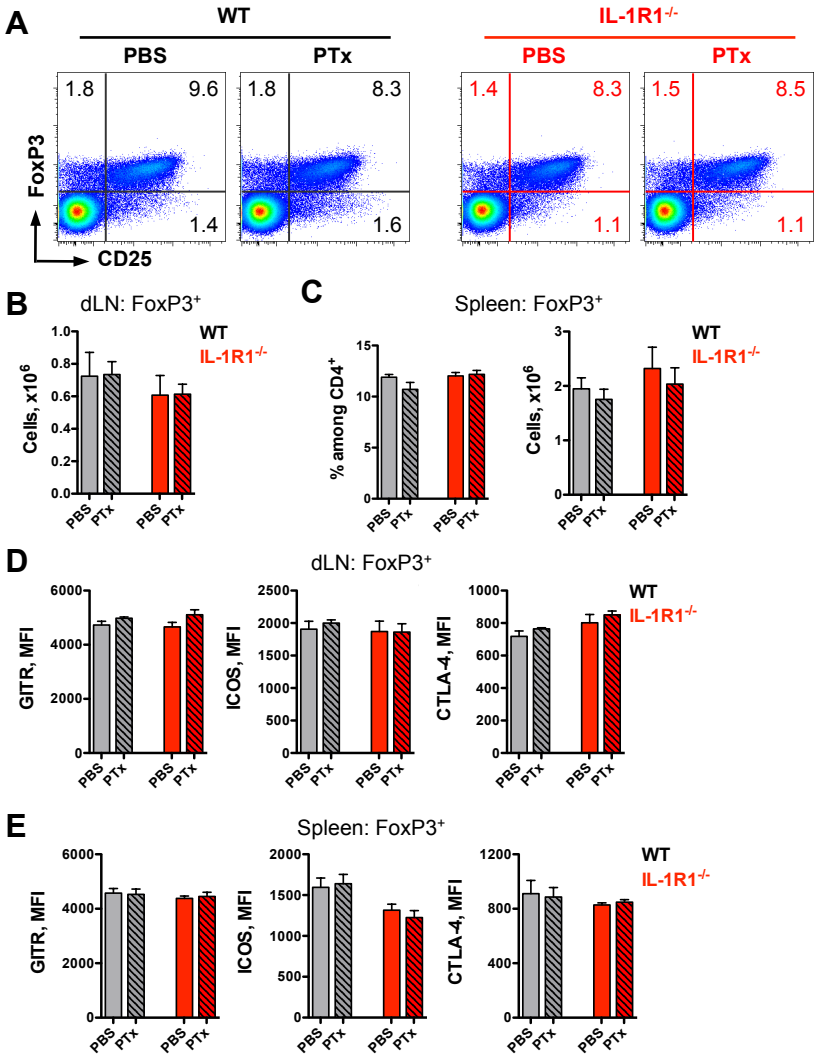


Appendix Fig S1. Analysis of CD4 T cell development after EAE induction.

A-B – Analysis of cytokine and FoxP3 expression by CD4 T cells isolated from (A) dLN and (B) CNS at the peak of disease. Filled subpopulations were further analyzed for IL-1R1 expression depicted in Fig 2A and C.

Cells were isolated (A) at day 9 after immunization and (B) at the peak of EAE and restimulated in the presence of MOG for 6 h. Data are representative FACS plots, gated on $VD^{-}TCR\beta^{+}CD4^{+}$ cells with mean frequencies per group and consist of n=4 wild type mice immunized with MOG/CFA/PTx. Experiments were performed at least twice with similar results.



Appendix Fig S2. Treg cell development is not dependent on IL-1 signaling and PTx administration.

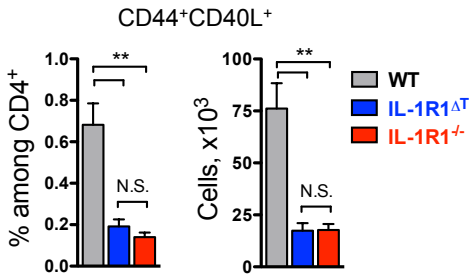
A - Analysis of Treg cells isolated from dLN. Data are representative FACS plots, gated on VD^{-}TCRβ^{+}CD4^{+} cells with mean frequencies per group.

B - Total numbers (mean +SEM) of Treg cells isolated from dLN shown in (A).

C - Frequencies and total numbers (mean +SEM) of Treg cells isolated from the spleen.

D-E - Mean fluorescent intensity (mean +SEM) of GITR, ICOS and CTLA-4 staining by Treg cells isolated from the dLN (D) and spleen (E).

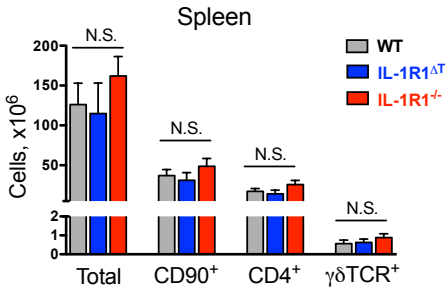
Cells (A-E) were isolated at day 9 after immunization. Data consist of n=5 WT PBS, n=4 WT PTx, n=4 IL-1R1$^{-/-}$ PBS, n=4 IL-1R1$^{-/-}$ PTx, MOG/CFA immunized mice. Experiments were performed twice with similar results.



Appendix Fig S3. T cell specific deletion of IL-1R1 impairs MOG-specific CD4 T cell expansion.

Frequencies and total numbers (mean +SEM) of $CD44^+CD40L^+$ CD4 T cells isolated from the spleen (depicted in Fig 4B).

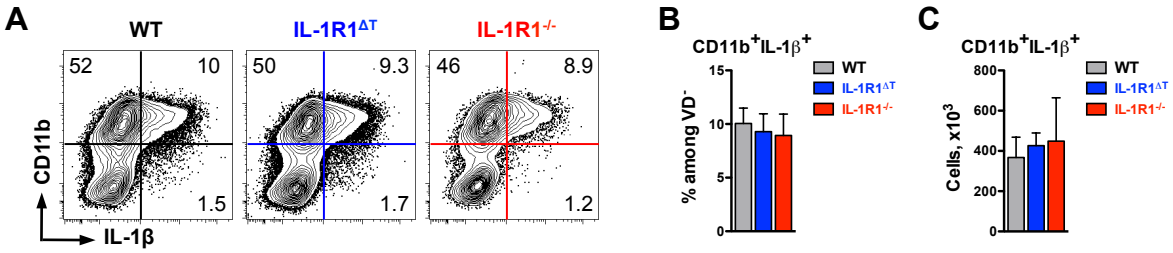
Cells were isolated at day 9 after MOG/CFA/PTx immunization and restimulated in the presence of MOG for 6 h. Data consist of n=5 mice of each genotype. $^{**}p < 0.01$, $^{***}p < 0.001$, N.S. – not significant; two-tailed unpaired t-test. Experiments were performed twice with similar results.



Appendix Fig S4. IL-1R1 deficient mice display non-impaired total cellularity in the spleen after MOG/CFA/PTx immunization.

Total numbers (mean +SEM) of different cell types isolated form the spleen of mice after EAE induction (depicted in Fig 5D).

Data consist of n=4 WT, n=3 IL-1R1$^{\Delta T}$, n=3 IL-1R1$^{-/-}$ mice. N.S. – not significant; two-tailed unpaired t-test. Experiments were performed at least twice with similar results.



Appendix Fig S5. Analysis of IL-1β expression within the inflamed CNS during adoptive transfer EAE.

A - Analysis of IL-1β expression by cells isolated from the CNS of Rag1$^{-/-}$ EAE diseased mice which received IL-23 polarized cells of the indicated genotypes (depicted in Fig 6F). Data are representative FACS plots with mean frequencies per group, gated on VD^{-} cells.

B – Frequencies (mean +SEM) of CD11b^{+}IL-1β^{+} cells isolated from the CNS shown in (A).

C - Total numbers (mean +SEM) of CD11b^{+}IL-1β^{+} cells isolated from the CNS shown in (A).

Data (A-C) consist of Rag1$^{-/-}$ mice which received WT (n=6), IL-1R1$^{\Delta T}$ (n=3) and IL-1R1$^{-/-}$ (n=4) cells polarized in the presence of IL-23 prior to transfer. Experiments were performed twice with similar results.

Appendix Table S1. Anti-mouse FACS antibodies used for surface staining.

Ag	Supplier
CD11c	eBioscience
CD11c	BD Biosciences
CD11b	eBioscience
Ly6G	Biolegend
Ly6C	BD Biosciences
TCR-β	BD Biosciences
TCR-β	Biolegend
CD44	Biolegend
CD44	BD Biosciences
CD4	Biolegend
CD4	BD Biosciences
CD25	BD Biosciences
ICOS	eBioscience
GITR	Biolegend
CD45.2	eBioscience
CD90.2	eBioscience
CD90.2	Biolegend
B220	Biolegend
TCR-γδ	eBioscience
CD19	Biolegend
CD3	BD Biosciences

Appendix Table S2. Anti-mouse FACS antibodies used for intracellular staining.

Ag	**Supplier**
IL-17A	eBioscience
CD40L	Biolegend
IFNγ	BD Biosciences
IFNγ	eBioscience
GM-CSF	eBioscience
pro-IL-1β	eBioscience
IL-1α	Biolegend
IL-1R1	Biolegend
FoxP3	eBioscience
CTLA4	BD Biosciences
CCR6	Biolegend
Isotype:	
Armenian Hamster IgG	Biolegend