**LGI1-/CASPR2 autoimmune encephalitis is associated with loss of regulatory MAIT cells**

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**Introduction**

Anti-Leucine-rich glioma inactivated-1 (LGI1) and anti-contactin-associated-protein-2 (CASPR2) associated autoimmune encephalitis (AIE) variants are characterised by directly pathogenic autoantibodies present in serum and CSF. The dynamics and drivers of intrathecal and systemic autoantibody production are incompletely understood. We aimed to elucidate the immunologic basis of the LGI1-/CASPR2-associated AIE variants by performing a transcriptomic profiling of CSF/blood in untreated patients. We validated findings by flow cytometry in independent cohorts and confirmed functionality using rodent immunization.

**Experimental set up**

**Results**

1. **Characterising LG11-/CASPR2 AIE transcriptionally**

   - UMAP clustering across (A) 75,496 CSF and 86,864 blood cells of 8 LG11-AIE and 5 CASPR2 AIE patients. The subclustered (B) all T cells spanned a transcriptional gradient.

2. **LG11-/CASPR2 AIE show a loss of MAIT and gdT cells in the CSF compared to healthy controls**

   - Comparing the proportions of T cell subclusters, we surprisingly identified a reduction in the proportion of cells annotated as MAIT (e.g. CXCR6, KLRB1/CDD161, ZBTB16) and γδ T cells (cluster named gdT; e.g. TRD3) in CSF, which reached the significance threshold in (A) LG11-AIE, but not in (B) CASPR2-AIE compared to controls, likely due to differences in statistical power (pH vs. H5). Volcano plots depicting differential abundance in the CSF of T cell sub-clusters. The log2 fold change of the differential abundance was plotted against the negative log10 of the adjusted p values.

3. **Cross compartment loss of MAIT cells in LG11-/CASPR2 AIE**

   - Cryopreserved PBMCs analyzed by flow cytometry of an independent additional cohort of LG1-AIE (n = 14), CASPR2-AIE (n = 11) and matched controls (n = 14) showing a loss of MAIT cells but not γδ T cells in the blood of CASPR2/LGI1 AIE patients. The proportion of (A) all MAIT cells and (B) CD8+ MAIT cells among all T cells in patients with AIE is visualized in box plots and categorized by disease group. The boxes represent the lower quartile, median and upper quartile. Whiskers include 1.5 times the interquartile range.

4. **MAIT deficient mice show higher rates of LG11-/CASPR2 Antibodies after active immunisation**

   - To functionally confirm that MAIT cells repress systemic humoral anti-LGI1-/CASPR2 autoimmunity we actively immunized mice with the recombinant extracellular portion of LG11 and CASPR2 proteins. After 15 days, 25% (1 of 4) of CASPR2+ mice showed detectable LG1 / CASPR2 autoantibodies in serum compared to 83.3% (5 of 6) of immunized immunomes genetically deficient in the invariant MR1 antigen presenting molecule that lack MAIT cells (left). In addition to antibody prevalence, antibody titers were also higher in MR1-deficient mice than in MR1-competent controls (middle) as measured by indirect immunoassay (right, red shift on LG1 and CASPR2 transfected cell lines). Statistical significance was determined by Fisher’s exact test.

**Conclusion**

Cross compartment loss of regulatory mucosa-associated invariant T (MAIT) cells and gamma delta T cells in the CSF are shared features of LG1-/CASPR2-AIE. We validated the functional role of these invariant T cells using a novel murine active immunization paradigm including both autoantigens: MAIT cells suppressed systemic formation of LG1 and CASPR2-specific anti-neuronal antibodies. We propose that MAIT cells could lose their autoantibody-suppressive capacity in LG1-/CASPR2-AIE in direct or indirect contact with danger signals in barrier-tissue. A loss of systemic and intrathecal regulatory mechanisms mediated by innate-like T cells could thus promote the known plasma cell expansion and autoantibody production as a shared mechanism in AIE.

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