Blood-CSF-Barrier Mediated Immune Cell Entry into the CNS

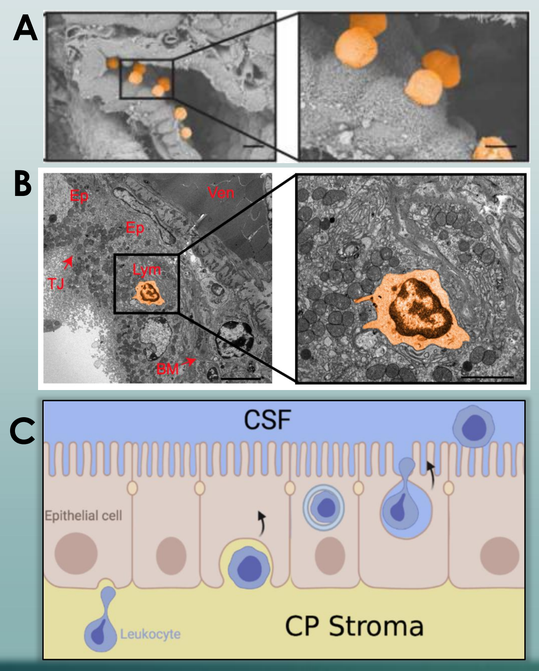
Ron Refaeli, Sivan Gelb, Ayal Ben Zvi

Department of Developmental Biology and Cancer Research, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem 91120, Israel

ABSTRACT

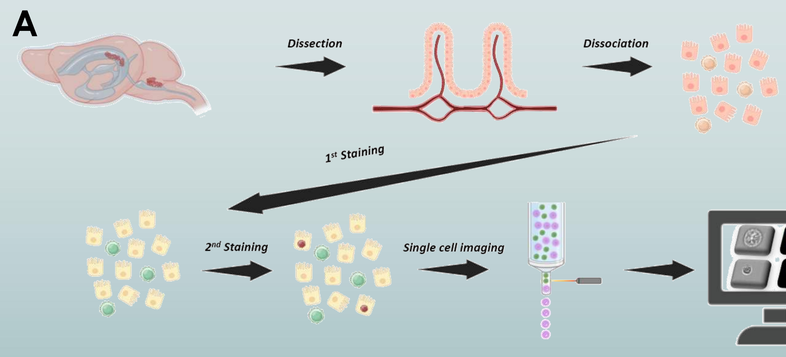
Neuropsychiatric lupus (NPSLE) involves neuropathic antibodies in the brain, once attributed to blood–brain barrier (BBB) disruption. Mouse models instead implicate dysfunction of the blood–cerebrospinal fluid barrier (BCSFB) at the choroid plexus (CP), which actively mediates immune cell migration into cerebrospinal fluid via regulated trans-epithelial processes. The CP shows marked inflammation and tertiary lymphoid structure formation. Using imaging-flow cytometry and single-cell RNA sequencing, we identify epithelial cell types and immune subsets—predominantly specific T cells—crossing the CP epithelium, likely driven by altered epithelial or immune cell properties. These findings may guide strategies to block inappropriate immune entry in CNS autoimmunity and reduce cognitive decline from CNS autoreactivity.

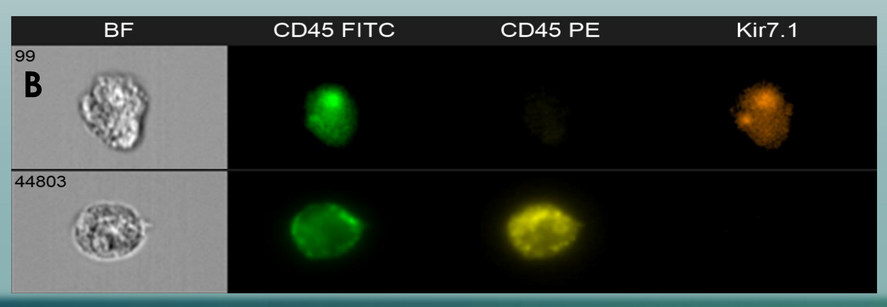
Abnormal BCSFB Immune Cell Crossing – Dominant Transepithelial



Previous work from our lab demonstrated; **A.** Immune cells abnormally located on the brain side (CSF) of the BCSFB in the lupus mouse model (SEM image, Scale bar 100µm, 10µm inset)[1] **B.** Immune cells shuttle through the epithelial layer of the ChP (TEM image, Scale bar 5µm)[1]. **C.** Schematic representation of transepithelial migration.

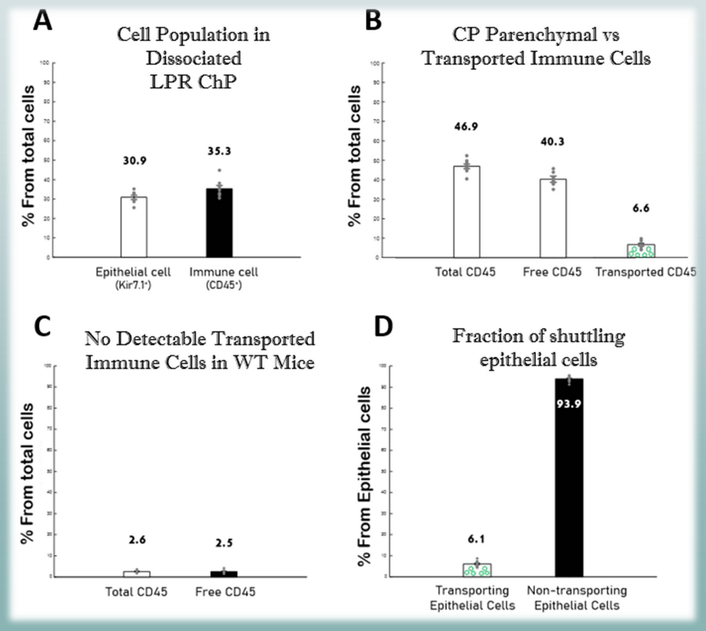
High Throughput Approach for Analyzing BCSFB Crossing Events





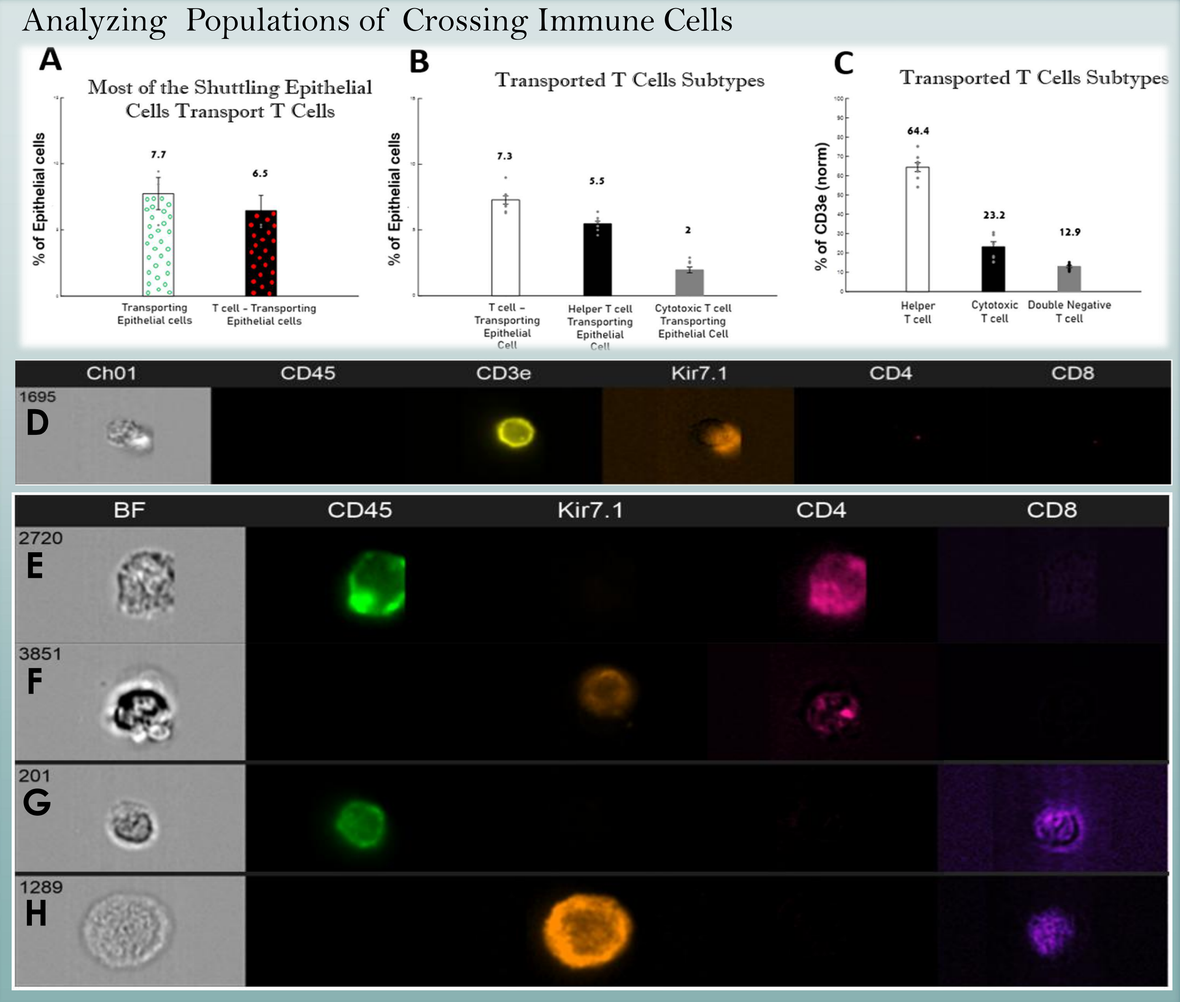
Immune Cells captured inside ChP Epithelial Cells in flow cytometry. A. Schematic pipeline of the experimental approach: ChP was extracted from LPR mice, dissociated into single cells, and live cells were stained for epithelial and immune cell markers. In the next step, cells went through fix/perm protocol to allow staining of immune cells located inside epithelial cells, followed by image stream analysis. **B.** Examples of free immune cells versus crossing events: cell number 99 shows an example of an epithelial cell (Kir7.1$^+$), labeled only with the immune marker of the fix/perm staining (CD45-FITC$^+$) but not with the same immune marker of the live staining (CD45-PE$^-$), suggesting process of immune cells transportation. Cell number 44803 represents non epithelial, free immune cell stained with both live and post-fix/perm staining (CD45-FITC$^+$/CD45-PE$^+$).

Immune Cell Crossing and Shuttling in LPR ChP Epithelium



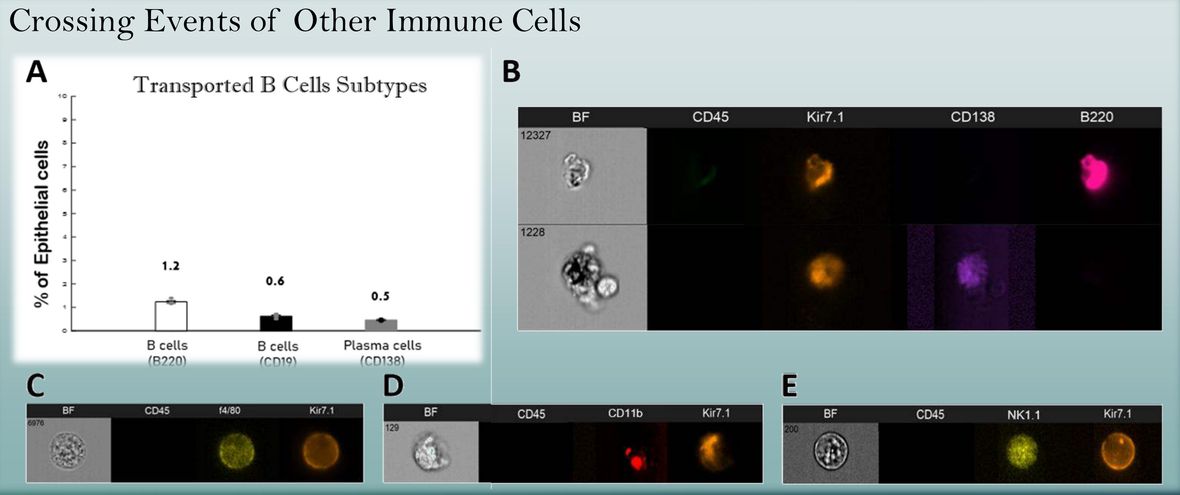
A. In the choroid plexus of sick 16-week-old female LPR mice, 30.9% of cells were epithelial (Kir7.1$^+$); another 35.3% of cells were CD45$^+$ immune cells. **B.** ImageStream analysis showed that of all immune cells, 6.6% were inside an epithelial cell (stained only internally). **C.** In healthy BALB/c mice, much fewer CD45$^+$ cells were found (2.6%) and internalized cells were barely detectable (0.1%, not shown).). **D.** Further analysis shows that 6.1% of epithelial all cells were transporting (Kir7.1$^+$ CD45 perm$^+$/non-perm$^-$), 93.9 were non transporting. 6.7% (Kir7.1$^+$ CD45 perm$^+$/non-perm$^+$) were possibly reflecting attached immune cells (not shown).

Analyzing Populations of Crossing Immune Cells



A. Among the epithelial cells internalizing immune cells (CD45$^+$, 7.7%), most were T cells (CD3e$^+$, 6.5%). **B.** Further analysis showed that of these internalized T cells, the majority were T helper cells (5.5% of 7.3%), and a smaller fraction were cytotoxic T cells (2% of 7.3%). All were CD45$^-$ (non-perm stain), confirming true internalization rather than attached. **C.** Most shuttled T cells (CD3e$^+$) were single positive (exclusive CD4$^+$ or CD8$^+$; 87.6%), yet a meaningful subset were double negative T cells, highlighting their disease relevance. **D.** ImageStream reveal transported T cell (non-perm stain CD45$^-$/Kir7.1$^+$) double negative to both CD4 and CD8. **E.** Free CD4$^+$ T cells, identified by non-perm CD45 staining, and negativity to Kir7.1; **F.** CD4$^+$ T cells internalized within epithelial cells, lacking non-perm CD45 staining; **G.** Free CD8$^+$ T cells, positive for non-perm CD45 staining, and negative to Kir7.1; and **H.** CD8$^+$ T cells internalized within epithelial cells, negative for non-perm CD45 staining. This visualization demonstrates how combining CD4/CD8 labeling with non-perm versus perm CD45 staining enables differentiation between parenchymal (free) T cells and those engulfed by epithelial cells.

Crossing Events of Other Immune Cells



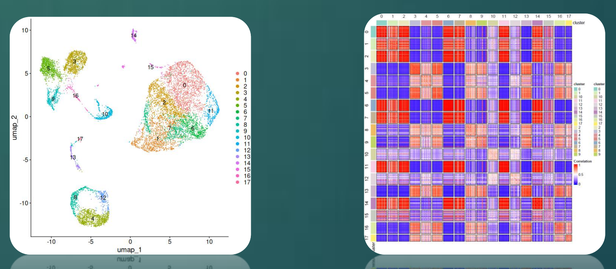
A. Additional experiments revealed that 1.2% of epithelial cells contained intracellular B220+ B cells, and 0.6% had CD19+ B cells. Plasma cells (CD138+) were rare, at 0.5%, yet interesting for their potential of antibody production in the CNS compartment. **B.** Epithelial cell presents B220 and CD138, indicating B cell and plasma cell shuttling. **C-E.** Negligible but detectable epithelial cells internalized macrophages (F4/80$^+$), CD11b$^+$ myeloid cells, and NK cells (NK1.1$^+$), suggesting limited involvement of these subsets.

CONCLUSIONS

- ✓ *In the ChP of LPR mice, epithelial cells actively internalize immune cells, especially T cells.*
- ✓ *Combined surface and intracellular CD45 staining identified a distinct population of internalized immune cells.*
- ✓ *Most internalized immune cells are T helper, with smaller fractions of cytotoxic and double-negative T cells.*
- ✓ *Healthy mice showed significantly fewer immune cells and negligible internalization, underscoring the disease - specific nature of this process.*
- ✓ *Lower abundance immune crossing populations include B cells, NK and macrophages.*
- ✓ *These findings suggest choroid plexus epithelial cells may contribute to immune cell trafficking and modulation in the CNS.*

Ongoing experiments include

Developing a single cell RNA-Seq approach for unbiased profiling –



Future plans include *identifying, targeting, and manipulating the molecular mechanisms driving immune cell transport to potentially reduce the neuropsychiatric pathology of lupus.*

[1]Gelb, S. *et al.* Mechanisms of neuropsychiatric lupus: The relative roles of the blood–CSF barrier vs blood–brain barrier. *J. Autoimmun*, 2018.