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Leukocytes as mediators of gut-brain communication

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Background

Food allergies

- Reactions range from mild/delayed to severe/rapid.
- People with mild allergic reactions have increased re-exposure risks.
- Cow's milk allergy tends to manifest with milder allergic reactions.

Cow's milk allergy (CMA)

- CMA has been associated with behavioral and neurological disorders.
- How allergic inflammatory signals from the gut reach the brain is unclear.

Hypothesis

Repeated allergen consumption by individuals with mild food hypersensitivities promotes leukocytes migration to the central nervous system (CNS), leading to neuroinflammatory pathologies and subsequent behavioral changes

Objective

- Compare the number of leukocytes in the brains of naïve, sham, and β-lactoglobulin (BLG)-sensitized mice by flow cytometry
- Determine the immunophenotypes of the leukocytes found in the brain.

Method

Animals: Four-week-old male C57BL/6J mice were used. Procedures involving animals were approved by UND IACUC.

Sensitization and allergen exposure: Sensitized to either vehicle or BLG for 5 weeks and exposed to whey containing diet.

Sample collections: Collected brains end of week 7.

Week 1-5	Week 6-7
Naïve: no treatment; CTL diet	CTL diet
Naïve: no treatment; CTL diet	WP diet
Sham: CT only x 5; CTL diet	WP diet
BLG: 1 mg+CT x 5; CTL diet	WP diet

Fig 1: Experimental groups and timeline. CTL diet: whey-protein-free rodent chow (Envigo Teklad 8640); **WP diet:** whey-protein-containing rodent chow (Envigo Teklad 2018). **Week 1-5:** sensitization phase; **Week 6-7:** allergen exposure phase. **CT:** cholera toxin. **CTL:** control diet. **WP:** whey protein diet.

Method (Cont'd)

Flow cytometry: Prepared single-cell suspensions using Miltenyi Biotec adult brain dissociation kit for mice and rats (cat# 130-107-677) and incubated with different cell surface markers and performed flow cytometry using BD FACSymphony.

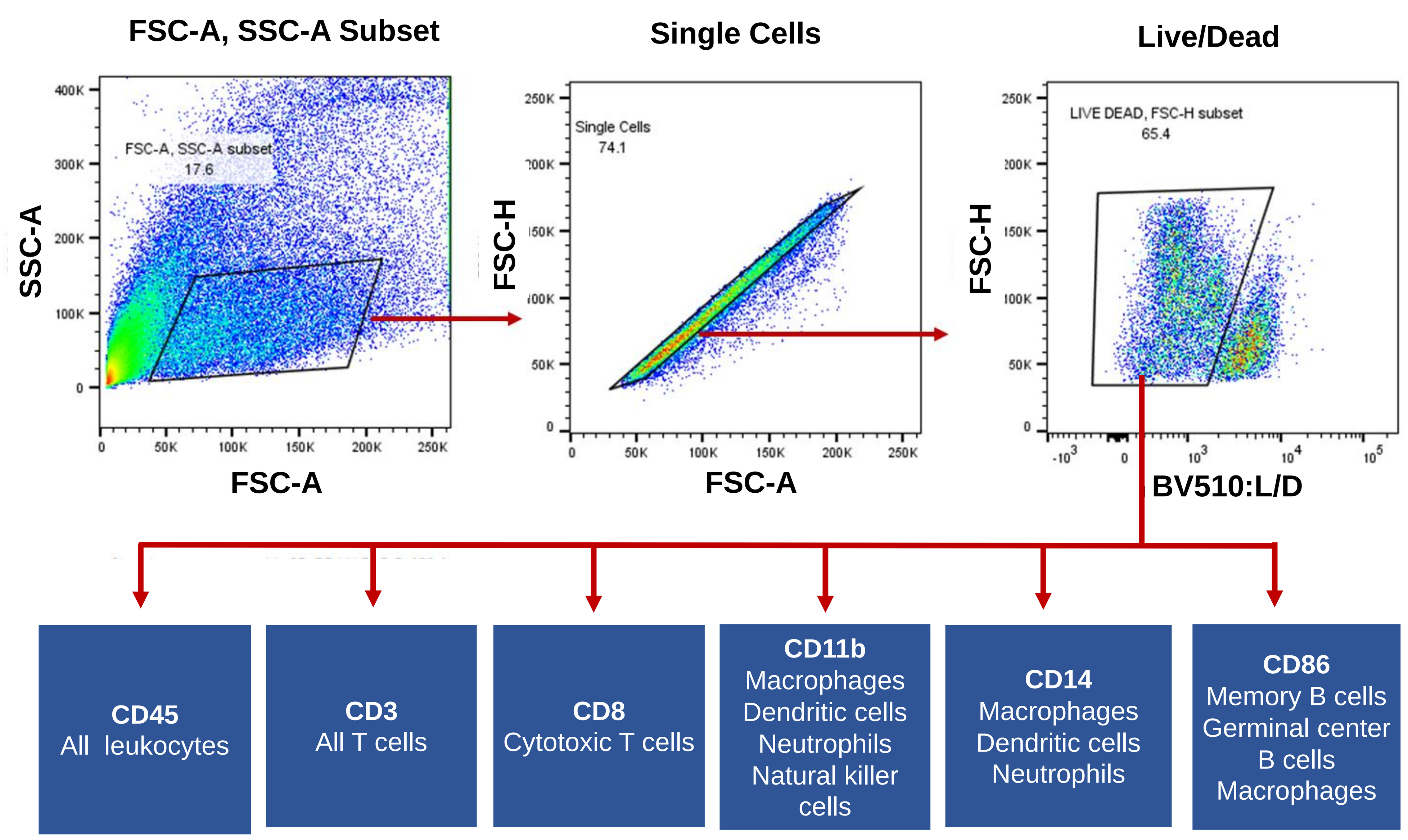
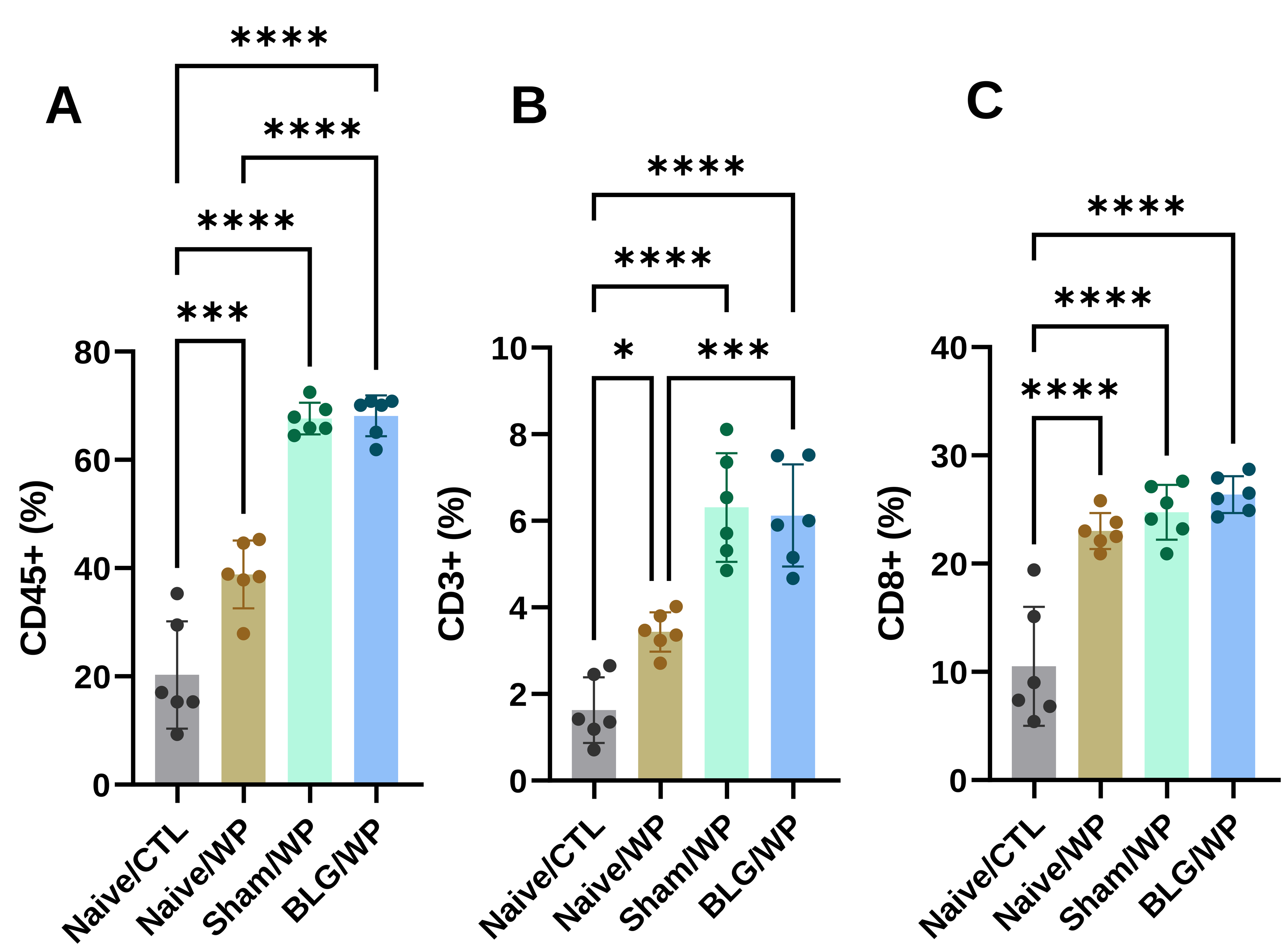


Fig 2: Gating strategy of flow cytometric analysis. SSC: side scatter, FSC: forward scatter. Live cell population further gated to the different surface cell markers to identify the immunophenotypes of cells (Blue boxes).

Results



Results (Cont'd)

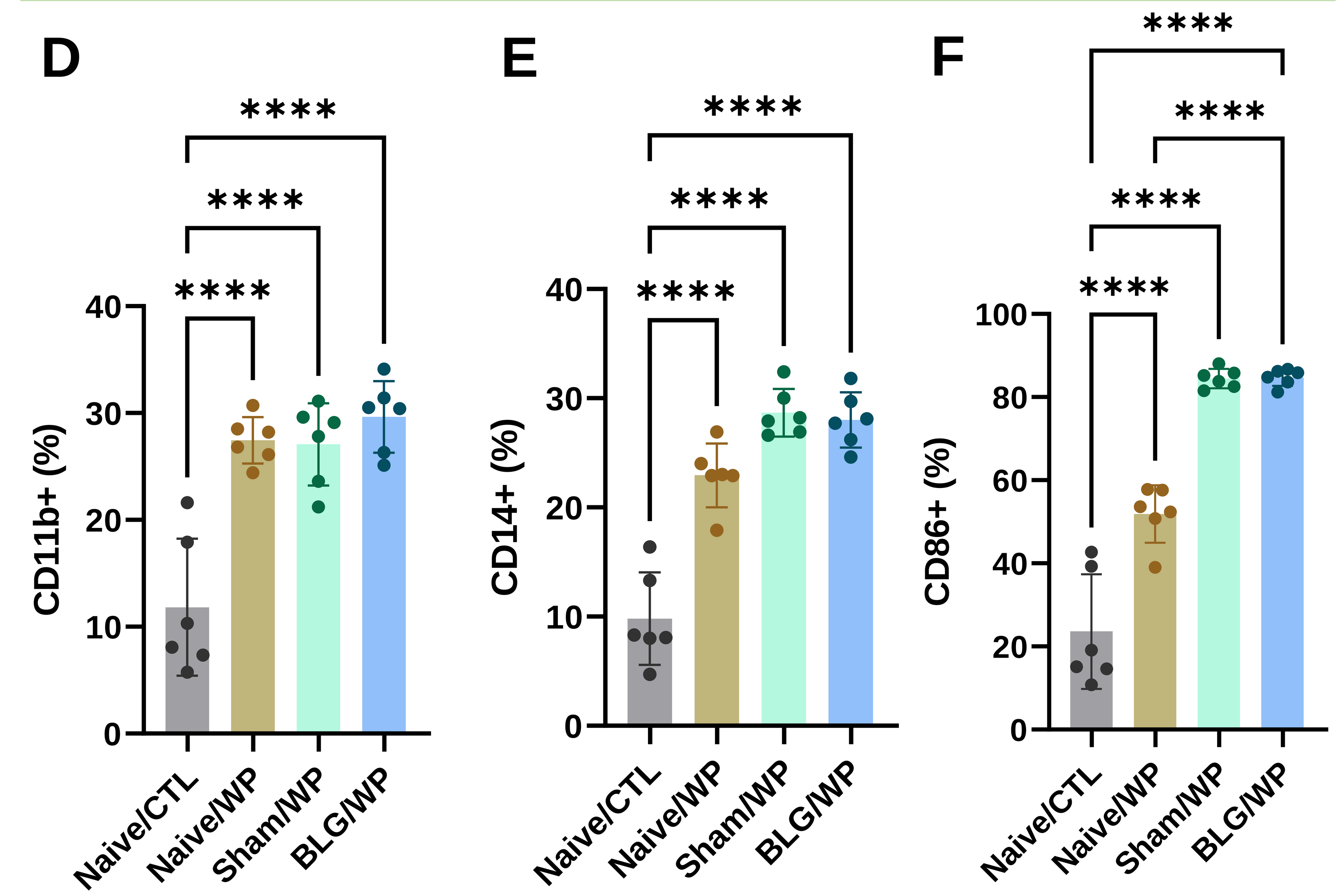


Fig 3: Leukocytes in the brains of naïve, sham, and BLG mice with or without exposure to the dietary allergen. (A) CD45: pan leukocyte marker, **(B)** CD3: pan T cell marker, **(C)** CD8: cytotoxic T cell marker, **(D)** CD11b: surface marker express in macrophages, dendritic cells, neutrophils and natural killer cells **(E)** CD14: surface marker express in macrophages, dendritic cells and neutrophils; **(F)** CD86: surface marker express in memory B cells, germinal center B cells ad macrophages. **CTL:** control diet, **WP:** whey protein diet. Bars indicate group average ± SEM. One way ANOVA.. *p<0.05. N=6 per group.

Conclusion

Consumption of the allergenic dietary protein alone increased number of cells expressing CD45 (total leukocyte), CD3 (pan T cells), CD8 (cytotoxic cells), CD11b (marker express in macrophages, dendritic cells, neutrophils and natural killer cells), CD14 (marker express in macrophages, dendritic cells and neutrophils) and CD86 marker express in memory B cells, germinal center B cells and macrophages) regardless of the sensitization status of the mice.

Discussion

Examining the role of immune cells in the gut-brain axis may provide insight into CMA-associated neuroinflammation and behavioral changes. Further investigation needed to find the activation/differentiation states of these leukocytes.

References

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Acknowledgment

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