In vitro sex-based analysis of T cell proliferation and differentiation

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Research Objective

To investigate sex-based differences in T cell proliferation and differentiation by studying the activation and stimulation of murine CD8⁺ lymphocytes *in vitro* during early cell divisions.

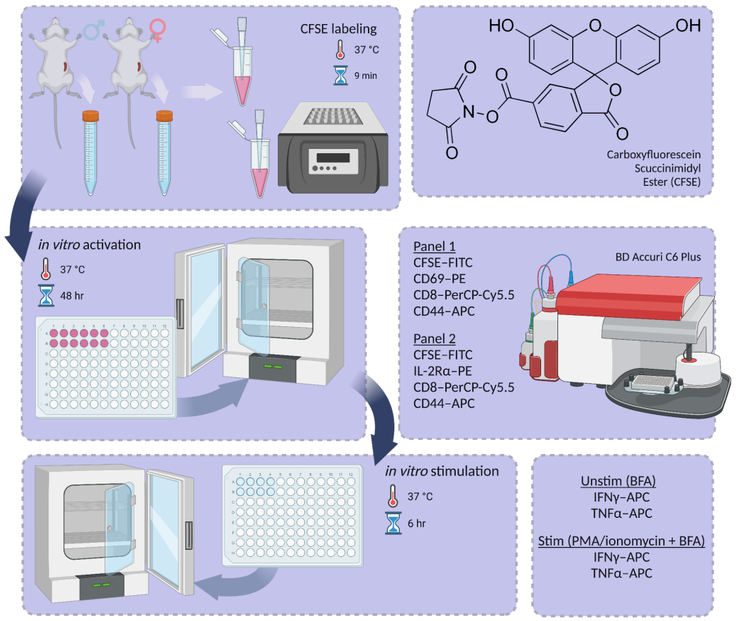
Introduction and Methods

Currently, studies and literature describe:

- Lymphocyte counts are higher in males, while post-activation and post-stimulation counts are greater in females than in males, as shown in PBMC.
- Females have greater rates of activation and inflammation-associated gene expression than their male counterparts.

How does proliferation and differentiation differ intrinsically to the cell's sex and what are their changes over successive divisions?

We hypothesize that in these early events, there will be differences in the activation markers after *in vitro* culturing with IL-2, as well as discrepancies in cytokine production between the male and female T cell proliferation and differentiation due to probable distinct characters in the microenvironments between the two sexes.



Total splenocyte count is higher in males, post-isolation, while CD8⁺ percentage is higher in females, post-activation

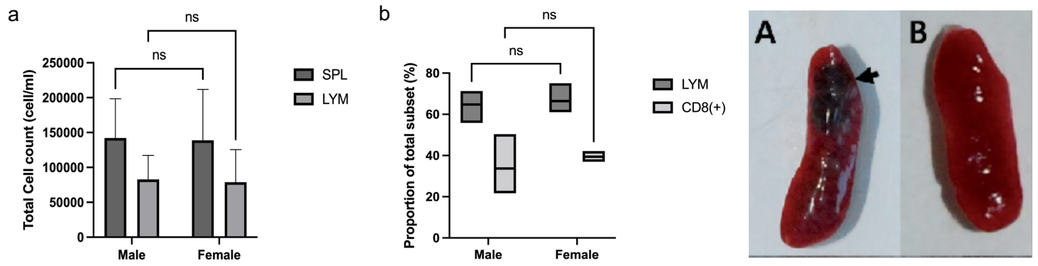


Fig 1. (a) Cell count (cells/ml) post-isolation of splenocytes. (b) Lymphocyte and CD8+ percentage post-activation *in vitro.* (A) Splenic melanosis observed in Trial 2 male spleen, compared with (B) normal male spleen.

Female CD8⁺ T cells have higher CD44 expression after *in vitro* activation than male CD8⁺ T cells

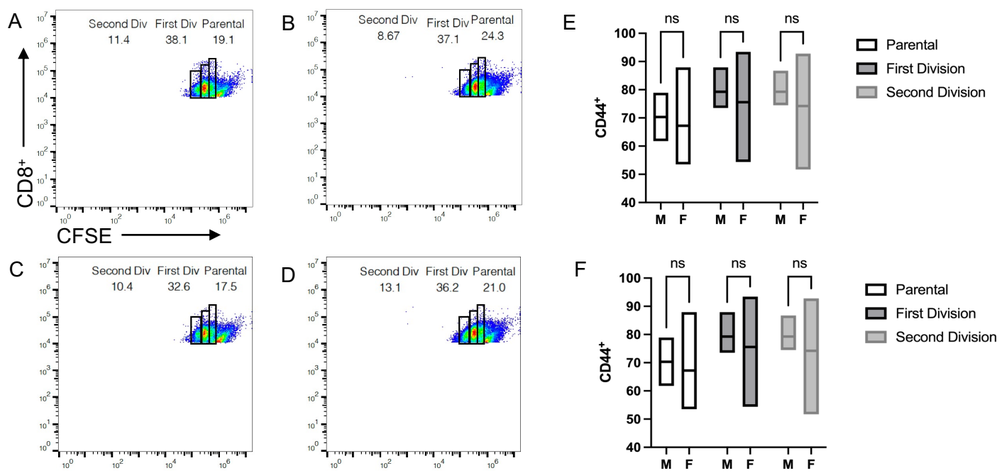


Fig 2. CD44 expression percentage post- activation *in vitro*. (E) Two-way ANOVA effect of sex in Panel 1: $p = 0.9936$, $n = 3$. (F) Two-way ANOVA effect of sex: $p = 0.9936$, $n = 3$. Representative contour plots (A) Panel 1 male, (B) Panel 1 female, (C) Panel 2 male, (D) Panel 2 female.

IFN-γ and TNF-α production is greater in females

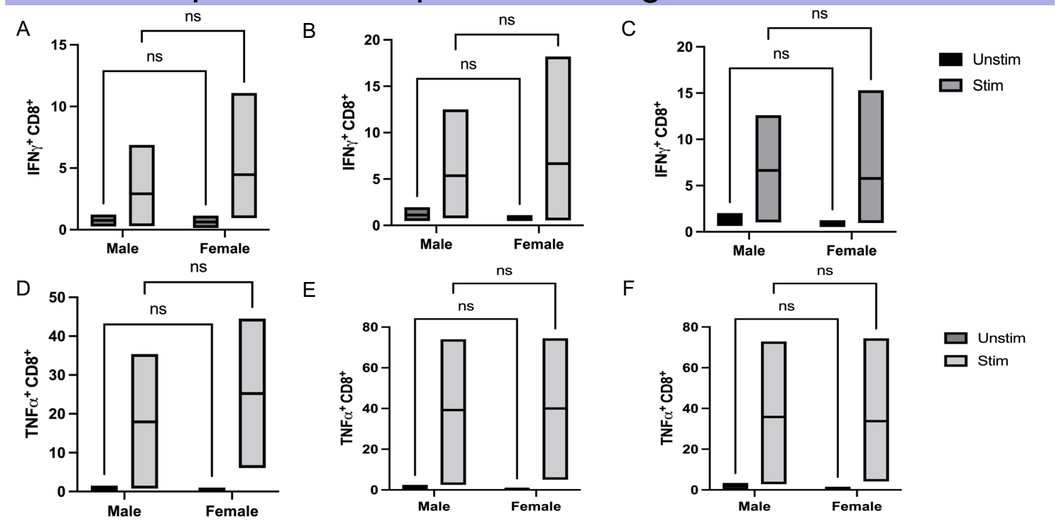


Fig 3. Pro-inflammatory cytokine production percentage post-stimulation *in vitro.* (A) Parental IFN-γ, (B) First division IFN-γ, (C) Second division IFN-γ, (D) Parental TNF-α, (E) First division TNF-α, (F) Second division TNF-α productions.

Conclusions

Altogether, these results were consistent with:

- Current studies on the sex differences in immune responses in adult humans.
- Existing studies claiming that females, in general, have a more vigorous immune system than their male counterparts.
- Also, the low production of pro-inflammatory cytokines such as IFN-γ and TNF-α in males may address the increased prevalence of specific disorders in males than females (e.g., childhood wheeze).

More importantly, these reveal novel trends:

- during the early T cell events, such as activation;
- during the early stages of cell division and proliferation;
- about activation markers and pro-inflammatory cytokine productions that are already occurring prior to when they were investigated in current literature; and
- that can be used as preliminary studies for large-scale investigations on the sex differences in T cell immunity.

Future Directions

Having shown important conclusions, future directions may include:

- large-scale study to explore the specific intrinsic property in female cells that causes their propensity to form a more robust effector T cell army than males
- Adoptive transfer experiments that can explore the following variables:
  - Type of cognate antigen
  - Antigen dosage
  - Sensitivity to regulatory cytokines

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