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Immune System Innovation:

Ushering in a new era of immunology research by characterizing cell populations most impacted by normal microbial exposure for preclinical research and medical treatment development success

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10-Minute Guided Tour

Two-Minute Overview

PURPOSE

To extend our understanding of human infant immunology to help develop more efficacious medical treatments and increase preclinical trial success rates.

RATIONALE

- Preclinical trial success rates are less than ideal, with pediatric drugs having a 50% success rate on average (Polson et al., 2012).
- A more accurate understanding of the human infant immune system would enable the production of more efficacious medical treatments during preclinical trials and save time and money.
- A source of error in preclinical trials is the sample of mice used to model the human immune system (Hamilton et al., 2021).

MOUSE MODELS

- Laboratory mice are the primary organism used to model and investigate the human immunity. Currently, research mice are raised in Specific Pathogen Free (SPF) conditions where there is no microbial exposure (Hamilton et al., 2020).
- Humans, including infants, are exposed to a variety of microbes from birth and thus develop an immunological memory not reflected in SPF mice (Huggins et al., 2019).
- Mice which undergo Normal Microbial Exposure (NME) from conception were demonstrated to be a more accurate model for adult human immunology (Hamilton et al., 2020).

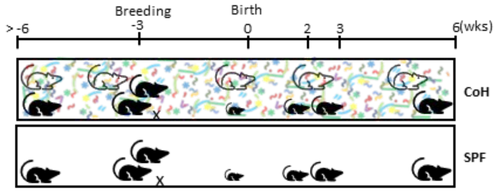


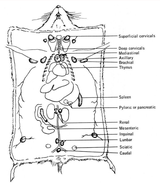
Fig 1 Representation of NME vs SPF mice environments and breeding timeline (development of NME mice from cohousing).

ADULT VS. INFANT IMMUNITY

- Adaptive and innate immune systems are stifled at birth to accept maternal immune conditions during the neonatal phase (Lim et al., 2014).
- Early life immunity is characterized by anti-inflammatory and tolerogenic activity (Dowling and Levy, 2014).
- The vast differences between adult and infant immune systems warrant an independent study on using NME mice to model infant immune systems, the focus of this project (Simon et al., 2021).

MATERIALS & METHODS

Step 1: Spleens & lymph nodes were harvested from 20 NME & 20 SPF mice at two-, three-, and six- week-old mouse tissue for 120 total samples.



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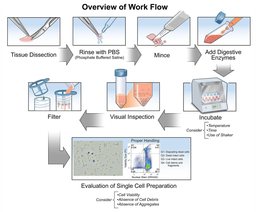
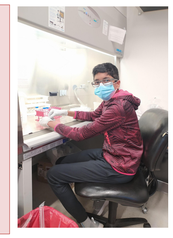
Step 2: Single Cell Suspensions were made from the samples and evaluated for viability.



https://doi.org/10.1002/cyto.a.23690

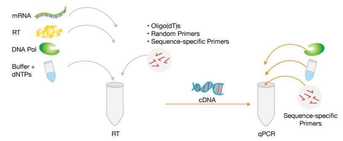
Step 3: Intracellular and cell-surface targets are stained for identification. Super Bright Staining Buffer and compensation beads are used.



Step 4: Flow cytometry was used to analyze samples for the cell counts and frequencies of components of interest.

Step 5: An Enzyme Linked Immunosorbent Assay was used to antibody concentrations in the samples. Cytokine analysis was outsourced.

Step 6: qPCR was used to study immune-gene expression.



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RESULTS & DISCUSSION

LYMPH NODE CELLS

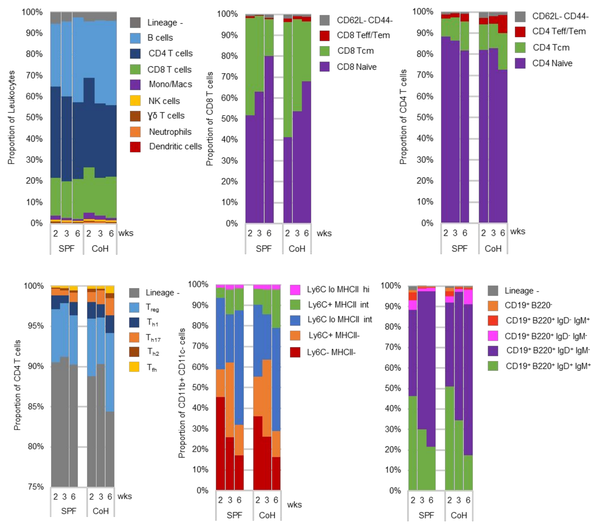

Fig 2 Cell frequencies of lymphocytes, CD8 T cells, CD4 T cells, additional CD4 T cells, Monocytes and Macrophages, and B Cells in SPF vs CoH (NME) mice at the two-, three-, and six-week timepoints in the lymph nodes.

SPLEEN CELLS

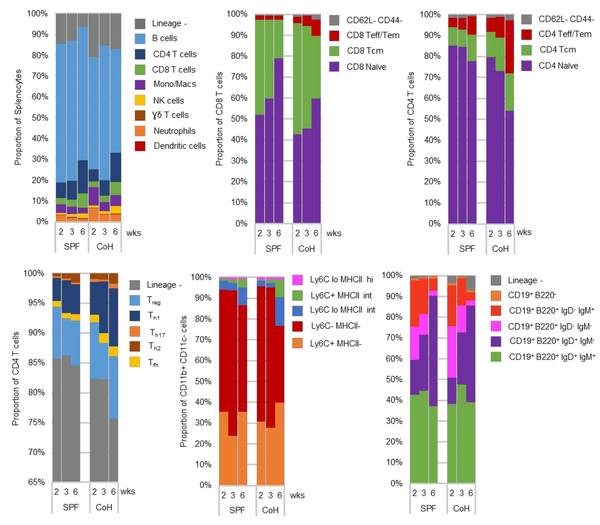

Fig 3 Cell frequencies of splenocytes, CD8 T cells, CD4 T cells, additional CD4 T cells, Monocytes and Macrophages, and B Cells in SPF vs CoH (NME) mice at the two-, three-, and six-week timepoints in the spleen.

INFANT IMMUNITY

- Prioritizes defensive ability.
- The true system is better equipped to identify and defend against pathogens than SPF models hint.
- Adaptive and innate immunity are developed.
- Ready to prevent post-vaccination infection or reinfection.
- Potent inflammatory responses.

ADOLESCENT IMMUNITY

- Increased focus on adaptive immunity development.
- Decreased focuses on B, Dendritic, and T Cell differentiation than described in SPF models.
- Widespread immune system growth.
- Stronger “first-line” defenses.
- Greater focus on inflammation.

PRE-ADULTHOOD IMMUNITY

- Greater defensive capability than described by SPF models.
- More potent antibacterial, antiviral, and anti-toxin antibody production mechanisms and tumor prevention.
- Less potent autoimmune regulation.
- Smaller focus on cytokine release.

CYTOKINE ANALYSIS

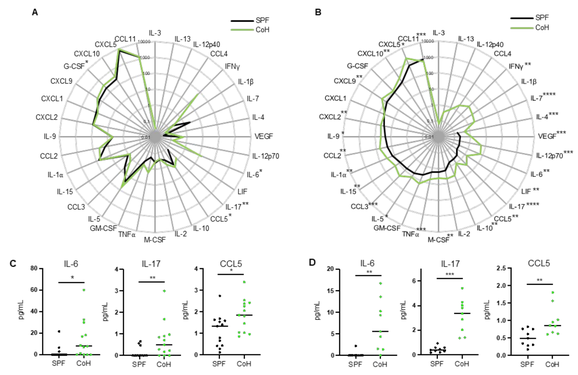

Fig 4 SPF vs NME cytokine counts in three- (A) and six- (B) week samples. Comparisons of densities of representative inflammation-regulation cytokines (IL-6, IL-17, and CCL5) at the three- (C) and six- (D) week checkpoints are also shown.

CONCLUSIONS

- The hypothesis that NME mice would develop elevated profiles of numerous immune system components was supported.
- SPF mice in preclinical trials tend to have underdeveloped immune systems and are different from those of human infants.
- The NME model more closely represents human infant immune systems than SPF mice.
- This NME model will better equip researchers for future projects, enable a greater applicability of immunology research to healthcare settings, and improve the efficacies of healthcare treatments.

FUTURE WORK

- The physiological effects of the differences between NME and SPF models are only theorized.
- *Listeria monocytogenes* and CLP-induced sepsis challenges may be used to identify these effects.
- Preclinical trials that previously failed may be retested with the new mouse model.
- NME models may be used to characterize prenatal immunity and immunity in the maternal-environment.

SELECT REFERENCES

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