The Effects of Maternal Stress on TLR Expression in neonatal *Mus musculus* Alveolar Epithelial Cells

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Abstract

Prior research has confirmed an association between maternal stress and offspring asthma. Recent studies suggest a role for epithelial expressed toll-like receptors (TLRs) in allergic asthma development. In this study, we were interested in isolating alveolar epithelial cells (AECs) from neonatal mice and analyzing how maternal stress potentially changes the expression of TLRs in the AECs.

C57bl/6J strain mice were mated and pregnant dams started treatment at midgestation (E12.5) with free access to Vehicle or CORT-treated drinking water until the litter was born. The litter was sacrificed on postnatal day 7 (7 PND). The lungs were harvested and both AECs (EpCAM+) and immune cells (CD45+) were isolated based on staining with epithelial cell adhesion molecule (EpCAM), CD45, and CD31 fluorescent antibodies.

Here we present our optimized protocol for isolating EpCAM+ and CD45+ cells in 7 PND neonates through fluorescence activated cell sorting (FACS). Further experiments to investigate TLR expression in 7 PND AECs will be conducted. Also, we plan to further explore alveolar immune cell populations in the neonates.

Background

Role of Epithelium in Asthma

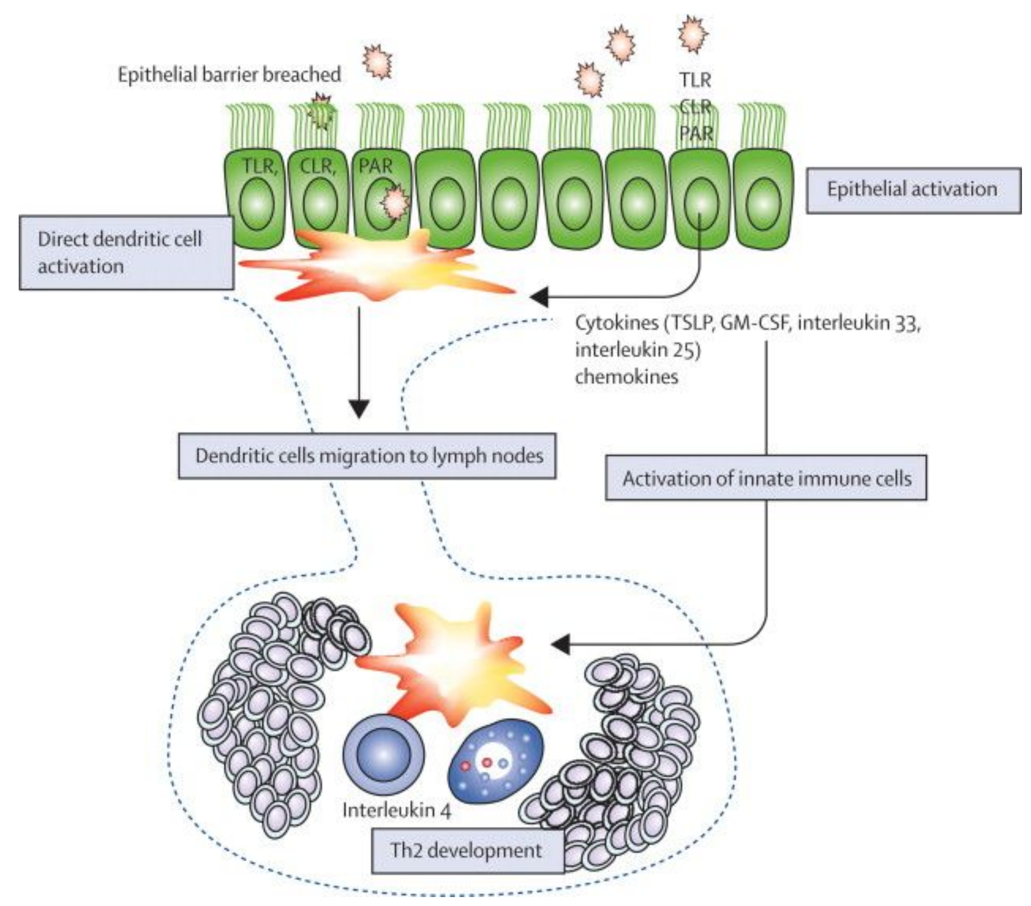

Figure 3 Schematic of alveolar epithelium role in asthma (Lambrecht and Hammad 2010).

Approach

Mouse Model

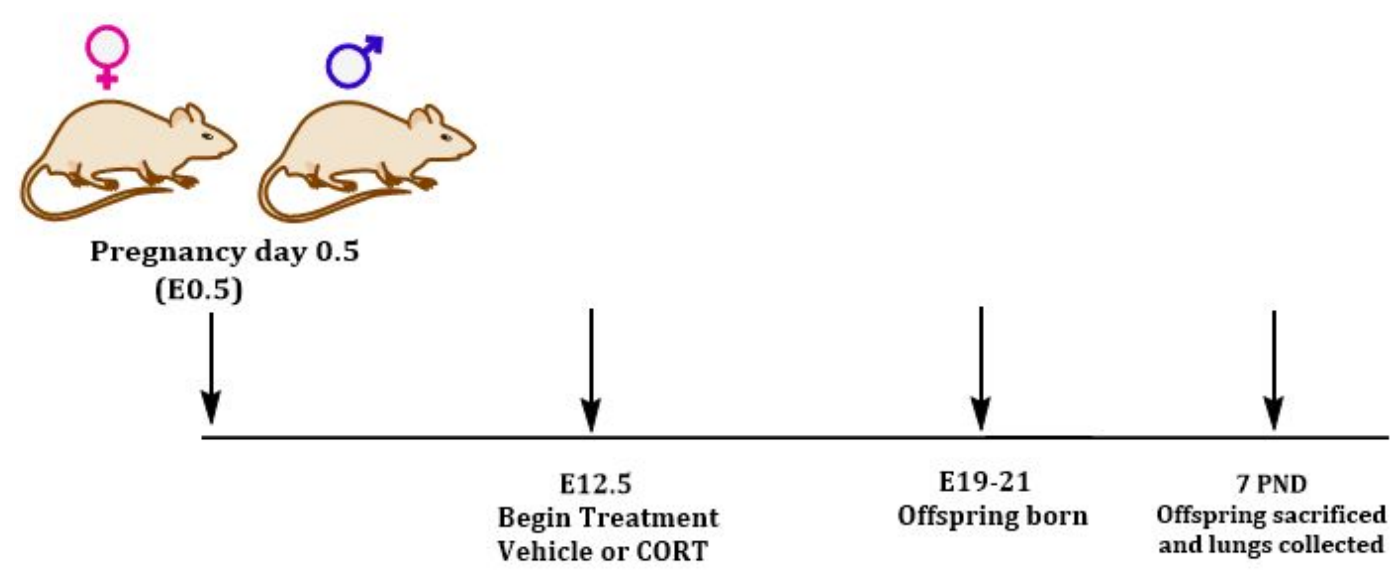

Figure 1 Schematic of maternal stress treatment during pregnancy (ChemDraw).

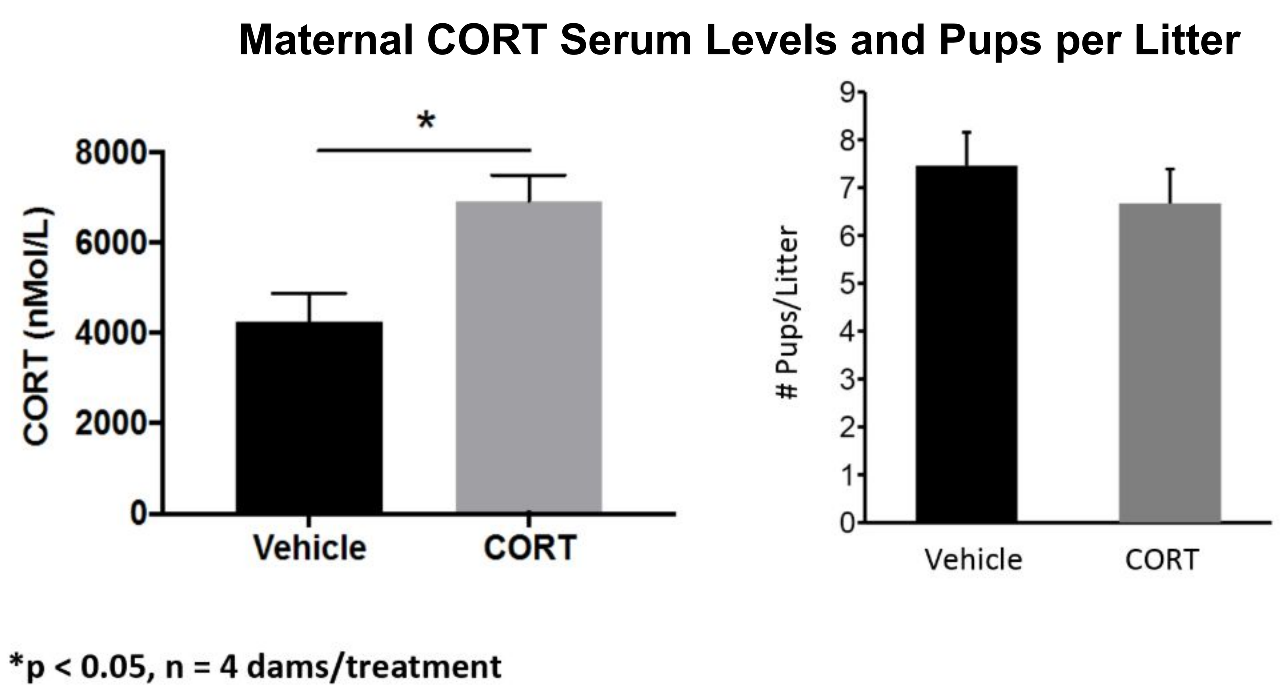

Figure 2 Maternal serum CORT levels and viability of pregnancy (Smith).

Sorting alveolar cell populations

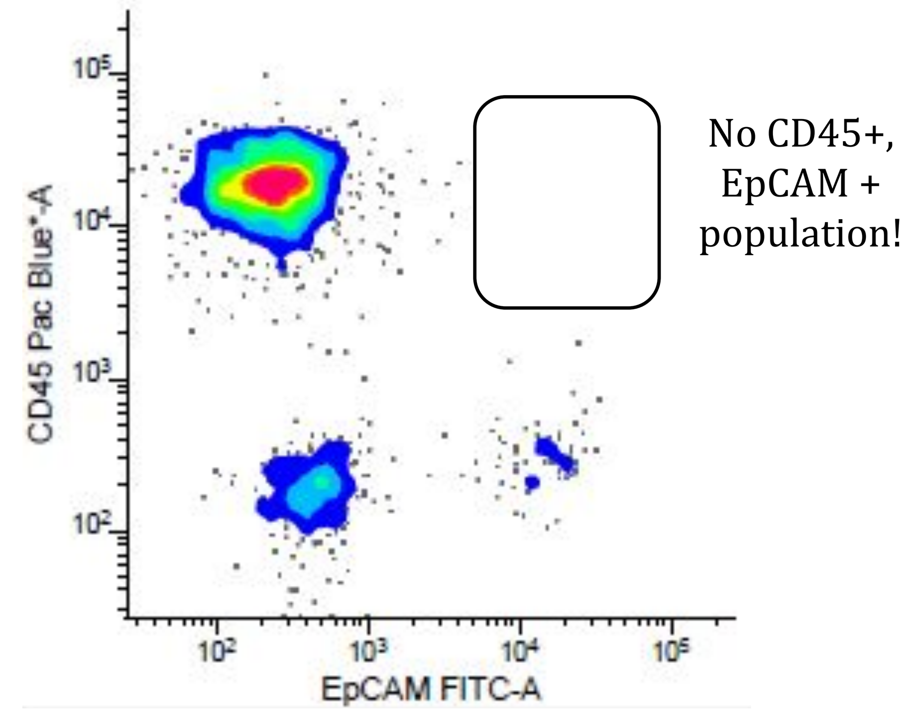

Figure 4 Optimization of fluorescence-activated cell sorting (FACS) protocol for isolating alveolar epithelial and immune cells. Flow cytometry analysis of single-cell suspensions from 7 PND mouse lungs. Flow analysis was performed on a BD FACSMelody using EpCAM (CD326)-fluorescein isothiocyanate (FITC), CD45-Pacific Blue, and CD31-Streptavidin (PE Cy 7) antibodies.

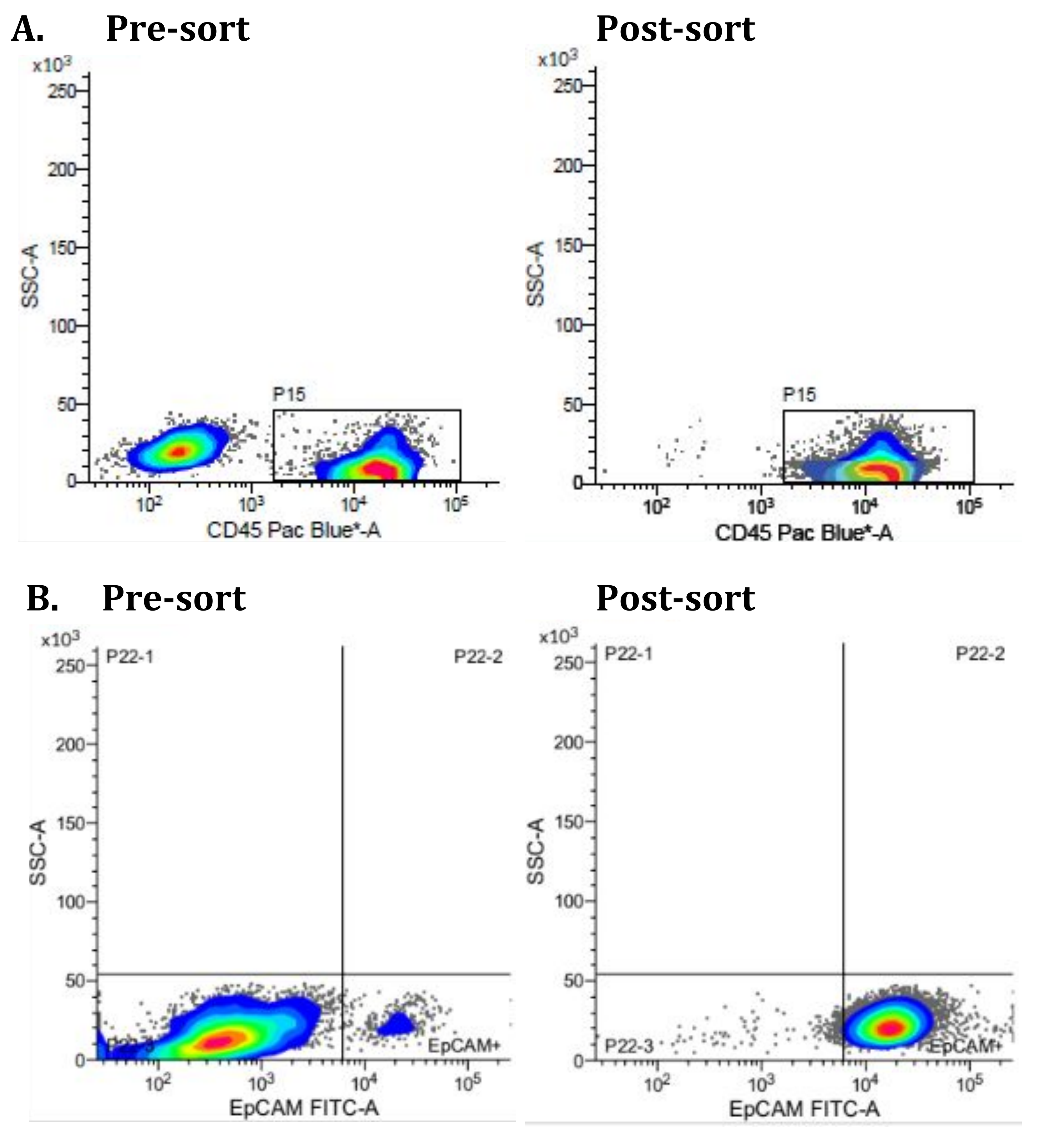

Figure 5 **A.** Pre- and post-sort CD45+ population flow cytometry data. **B.** Pre- and post-sort EpCAM+ population flow cytometry data. Lung cells were collected from day 7 PND strain C57bl/6J mice and data was acquired from a BD FACSMelody. CD45+ and EpCAM+ sorted populations were pooled from 2-4 animals using EpCAM (CD326)-fluorescein isothiocyanate (FITC), CD45-Pacific Blue, and CD31-Streptavidin (PE Cy 7) antibodies.

Table 1 Comparison of EpCAM+ percentages of total cell populations from a similar isolation experiment done by Bantikassegn et al. 2015.

Study	FACS Isolation	
	EpCAM+ (% total cells)	CD45+ (% total cells)
Bantikassegn et al. 2015	3.2 ± 0.2	84.0 ± 0.9
Our Study	7.56 ± 2.05	64.39 ± 9.75

Alveolar cell population proportions

Table 2 Percentages of EpCAM+ and CD45+ cells in total population of 4-6 animals. n=2

Treatment	Cell Populations	
	CD45+ (% of total cells)	EpCAM+ (% of total cells)
Vehicle	64.39 ± 9.75	7.56 ± 2.05
CORT	77.14 ± 5.22	4.06 ± 0.96

Summary

- We optimized our method of isolation as it compares well to a similar study.
- Cort treatment altered the cell population proportions to have a lower overall percentage of EpCAM+ cells in the lungs compared to Vehicle treatment.
    - We did not collect data, but CORT pups were typically smaller than Vehicle pups which could mean smaller lungs, therefore fewer cells and fewer EpCAM+ cells.

Future Directions

TLR expression in AECs

- Our main goal moving forward is to isolate more AECs to extract RNA and look at TLR mRNA expression in AECs.
    - This will most likely take an entire litter (~6 animals) to pool AECs together to acquire enough mRNA for meaningful data

Differences in proportions of immune cell and AEC populations

- We are interested in looking into the types of immune cells present in stressed and non-stressed neonatal mice.
- We are also interested in trying to explain the altered cell proportions with respect to maternal treatment.

References

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Acknowledgments

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