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Features of Environmental Dust Exposure Induced Lung Disease Regulated by MyD88 Result in a Prolonged Adaptation Response

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INTRODUCTION

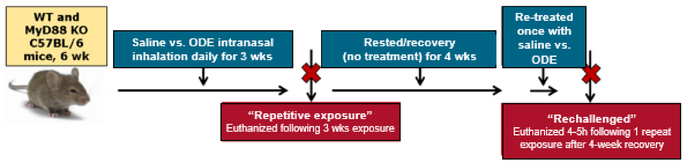
- Agricultural organic dust exposures that are rich in Toll-like receptor (TLR) agonists are associated with occupational asthma and chronic bronchitis.
- The TLR adaptor protein MyD88 is fundamental in regulating the acute inflammatory response to organic dust extract (ODE).
- The role of MyD88 in mediating airway inflammatory response to repetitive, prolonged exposure is unknown and could inform future targeted approaches.
- We sought to 1) determine the role of MyD88 in repetitive exposures and 2) whether prolonged recovery following repetitive exposure would result in heightened or refractory response to subsequent ODE rechallenge.

HYPOTHESIS

The MyD88 signaling pathway is central to governing lung responses to repetitive organic dust exposures.

METHODS

Organic dust extract (ODE) from swine confinement operations was utilized in an established *in vivo* model. Wild-type (WT) and MyD88 knockout (KO) mice on C57BL/6 background were intranasally treated with ODE 12.5% or saline daily for three weeks and euthanized (repetitive exposure) or rested with no treatment for four weeks (recovery phase) followed by challenge once with saline or ODE. Bronchoalveolar fluid (BALF), lung tissues, and serum were collected. Data are presented as the mean ± SEM. Statistics were performed using ANOVA with Mann-Whitney test. All animal procedures were in accordance with the NIH guidelines for the use of rodents and were approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center.



Key Abbreviations of rechallenge after recovery from repeated exposures:
S-S: saline repeated exposure - saline rechallenge
O-S: ODE repeated exposure - saline rechallenge
S-O: saline repeated exposure - ODE rechallenge
O-O: ODE repeated exposure - ODE rechallenge

Key for Lung Sections:
b= bronchiolar airway; a = alveolar parenchyma; v = blood vessel; asterisk = ectopic lymphoid aggregates; arrow = Ly-6B.2+ neutrophils (Fig 2 A,C) or PAS+ mucus cells (Fig 3A) or tryptase+ cellular aggregates (Fig 3C). Line scale bar = 200 µm (Figs 2A, 2C, 3A) or 100 µm (3C)

RESULTS

MyD88-knockout (KO) mice demonstrate a significantly reduced airway inflammatory response following repetitive ODE exposure.

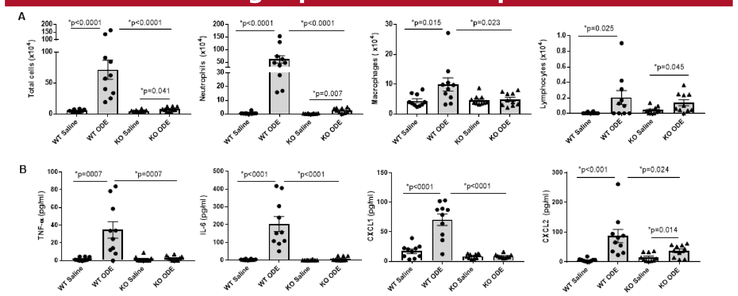


Figure 1. Bronchoalveolar lavage fluid (BALF) cell differential **(A).** Pro-inflammatory cytokines implicated in ODE-induced disease quantified in cell-free BALF **(B)**. Statistical differences denoted as #p<0.05; ##p<0.01; ###p<0.001 vs. saline or denoted by line.

Repetitive ODE exposure-induced alveolitis, but not B cell lymphoid aggregates, is dependent on MyD88.

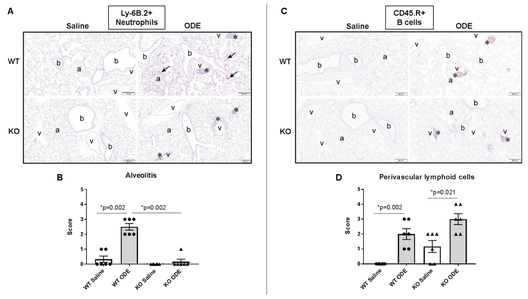


Figure 2. Lung tissues stained for neutrophils (**A**, Ly-6B.2⁺) and B cells (**C**, CD45.R⁺) with representative images for each treatment group shown. Lung sections were semi-quantitatively scored from 0-5 for alveolitis **(B)** and perivascular lymphoid cells **(D)**. Scatter plots (**B, D**) depict mean with SEM; N=6 mice/group.

Mucus cell metaplasia is increased in MyD88 KO mice repetitively exposed to ODE. Tryptase⁺ lung mast cells, serum IgE, and lung IL-33 are increased following repetitive ODE and variably modulated by MyD88.

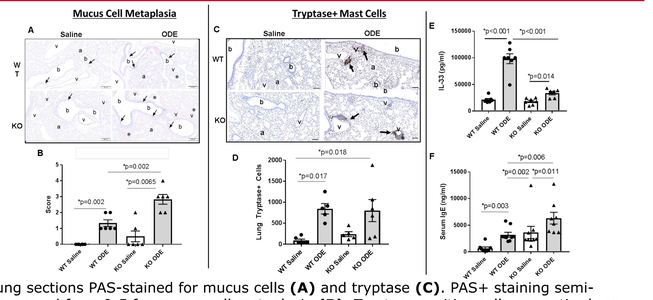


Figure 3. Lung sections PAS-stained for mucus cells **(A)** and tryptase **(C)**. PAS+ staining semi-quantitatively scored from 0-5 for mucus cell metaplasia **(B)**. Tryptase positive cells per entire lung section were quantified by Definiens software **(D).** ODE-induced lung homogenate IL-33 levels quantified by ELISA **(E)**. Serum IgE quantified by ELISA **(F)**. All graphs are scatter plots depicting mean with SEM bars. Sample size ranged from 6-9 per treatment group based upon sample availability.

Prolonged adaptation response following rechallenge with ODE after a 4 week recovery period following repetitive ODE exposure. MyD88 is required for eliciting immune responses upon rechallenge.

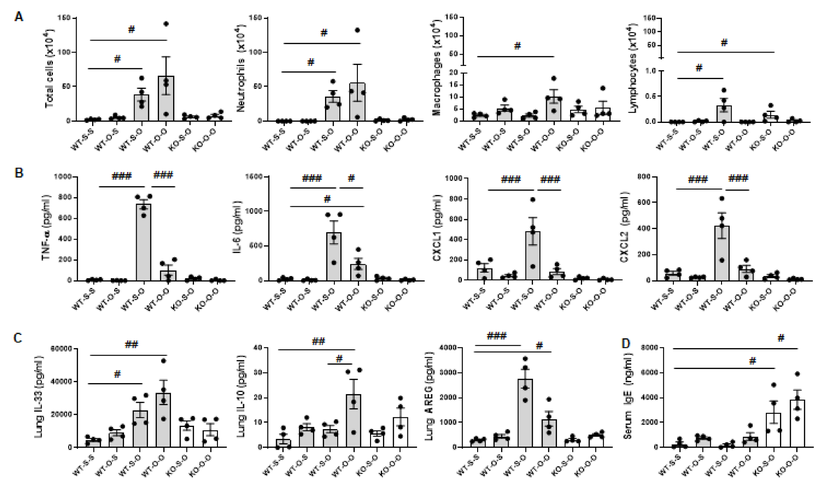


Figure 4. Bronchoalveolar lavage fluid (BALF) cell differential across treatment groups **(A)**. Pro-inflammatory cytokines quantified in cell-free BALF by ELISA **(B)**. Mediator levels of IL-33, IL-10, amphiregulin (AREG) quantified in cell-free lung homogenates **(C)**. Serum IgE levels **(D)**. Scatter plots depict mean with standard error bars. N=4 mice per treatment group run in side-by-side experiments. Statistical significance (#p<0.05; ##p<0.01; ###p<0.001) denoted by line.

DISCUSSION

- MyD88-dependent signaling is essential in mediating the classic airway inflammatory response to not only acute, but repetitive organic dust exposures.
- However, targeting MyD88 does not reduce mucous cell metaplasia, lymphocyte influx, or generalized IgE responsiveness. In fact, mucous cell metaplasia and serum IgE levels were augmented in MyD88 KO mice.
- TLR-enriched dust exposures induce a prolonged adaptation response as evident by a blunted inflammatory mediator response following rechallenge after a 4 week rest.
- Exploiting the MyD88 signaling pathway in the lung could represent future preventative or therapeutic targets, relevant to both acute and repetitive exposure settings.

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