Assessment of the Time Course of Chronic Inflammation in the Murine House Dust Mite Model

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Introduction

- House dust mite (HDM) allergens are associated with allergic disorders and the use of this clinically relevant antigen is increasing in preclinical animal models.
- We assessed the chronic inflammatory time course and the anti-inflammatory efficacy of a phosphodiesterase 4 (PDE4) inhibitor and a corticosteroid in a five week HDM model.

Methods

- BALB/c mice were challenged intranasally with 25 µg of house dust mite extract (HDM) (Dermatophagoides pteronyssinus) under light isoflurane anaesthesia for 5 days/week for 5 weeks.
- Oral therapeutic intervention with roflumilast (10 mg/kg b.i.d) and prednisolone (10 mg/kg b.i.d) was initiated in Week 3 and continued until the end of the protocol.
- Airway hyperresponsiveness (AHR) to increasing nebulized concentrations of methacholine was assessed in conscious mice longitudinally using Non-Invasive Airway Mechanics (Buxco) 24 h following the last weekly HDM exposure.
- Cohorts of mice were euthanized weekly, 24 h following the last challenge and a bronchoalveolar lavage (BAL) performed. A differential and total cell count was performed using the Sysmex-XT-Vet, BAL levels of allergic Th2 cytokines measured using a Luminex 200 and lymphocyte phenotyping using cell surface markers assessed in BAL using the FC500 MPL.
- The thoraxes of the mice were opened, the lungs removed and insufflated with 10% neutral buffered formalin for between 24 and 48 h and subsequently stained with H&E, Periodic acid-Schiff and Masson's trichome for histological evaluation.
- In Week 5, right lung samples were also collected in RNAlater® and tissue mRNA analysis performed.

Results

- Chronic HDM extract exposure resulted in significant recruitment of eosinophils, lymphocytes and neutrophils as early as Week 1, peaking (1.13±0.32, 0.66±0.10 and 0.31±0.05 x10^6 cells/animal respectively) between Weeks 3 and 5. Within the BAL lymphocyte population, the proportion of B cells increased from 24 to 46% over the five-week exposure period.
- Therapeutic treatment with prednisolone and roflumilast significantly ($p<0.001$) reduced the recruitment of all BAL cell types following 7 and 14 days of treatment.

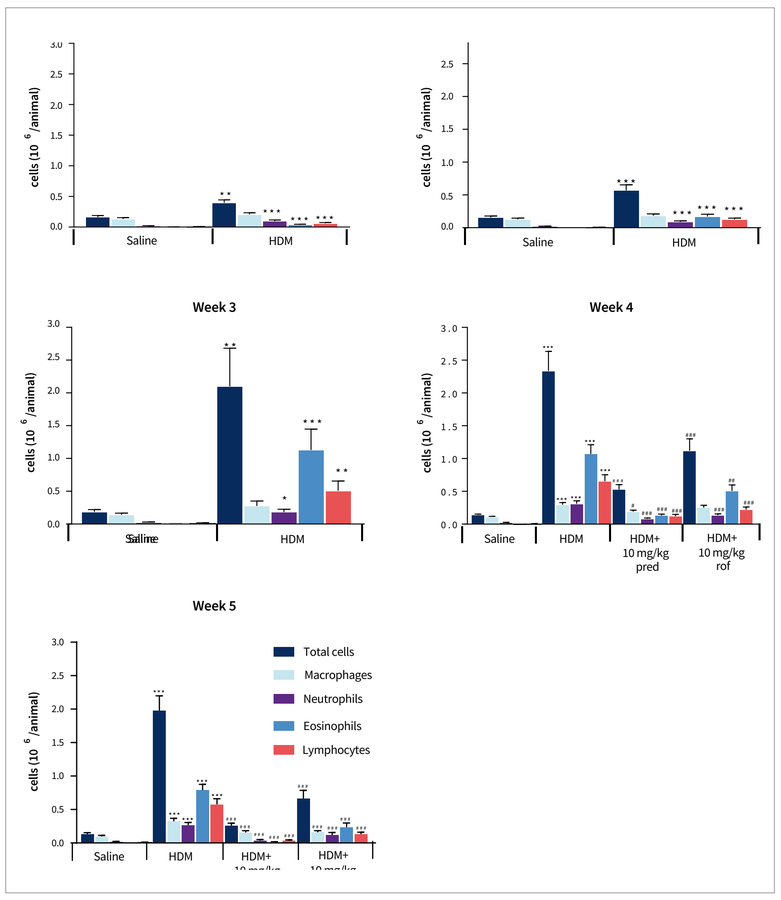

Figure 1. Effects of therapeutic prednisolone and roflumilast treatment on chronic HDM-induced BAL inflammatory cell infiltration. Mean ± SEM (n=8-10 per group). *p<0.05, **p<0.01, ***p<0.001 when compared to the saline/vehicle treated animals. #p<0.05, ##p<0.01, ###p<0.001 when compared to the HDM/vehicle treated animals.

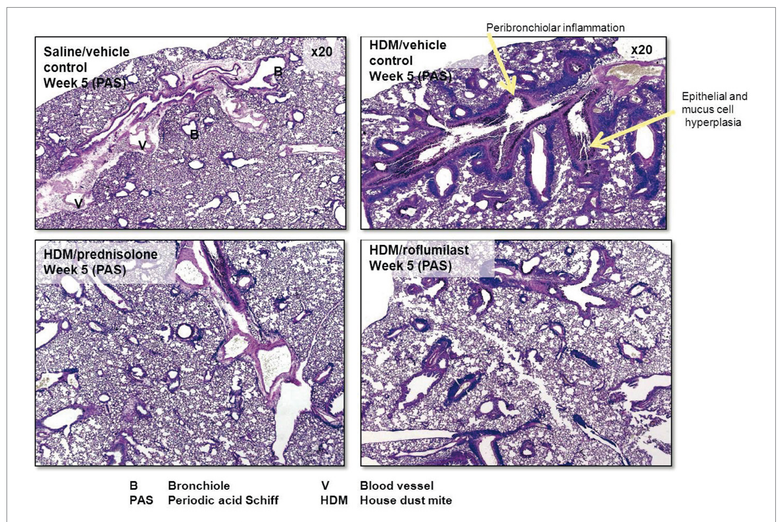

Image Source: Internal Labcorp

Figure 2. Photomicrographs of representative sections of lungs from saline, HDM challenged mice and from HDM challenged mice treated with prednisolone and roflumilast.

- Pathological changes including epithelial and mucous cell hypertrophy/hyperplasia in the bronchi and bronchioles reached maximum severity during Weeks 3 to 5 and was reduced in severity in animals treated with prednisolone but not roflumilast. Perivascular/peribronchiolar inflammation comprising of granulocytes, lymphocytes and plasma cells increased in severity during the five week exposure; alveolar inflammation dominated by macrophages reached maximum severity during Weeks 4 and 5.
- Both findings were reduced in severity by prednisolone or roflumilast treatment.
- Perivascular/peribronchial fibrosis was observed in HDM treated animals from Week 3 onwards, peaking at Week 5 and was absent in those animals treated with prednisolone or roflumilast.

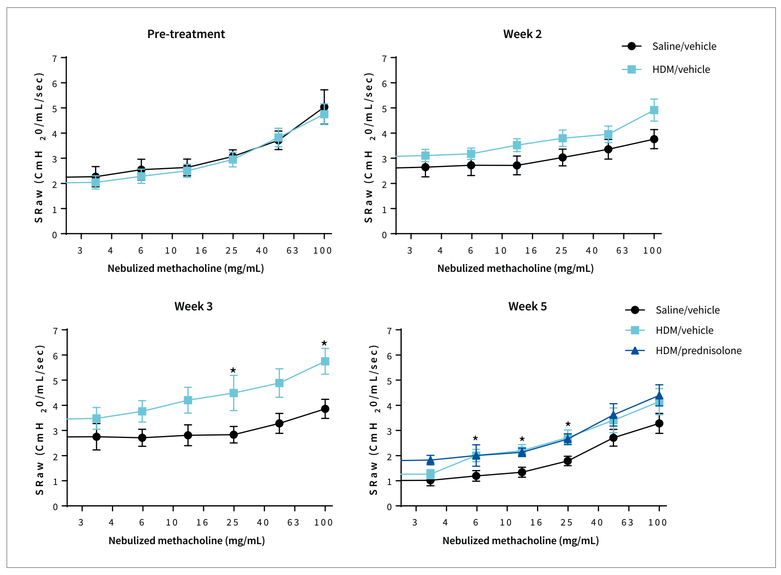

Figure 3. Airway hyperresponsiveness (AHR) to nebulized methacholine following chronic exposure to house dust mite. Mean ± SEM (n=8-10 per group). *p<0.05, when compared to the saline/vehicle treated animals.

- Increases in baseline specific airway resistance (SRaw) and significant ($p<0.05$) AHR to methacholine was recorded in HDM treated animals from Week 3 onwards when compared to the saline challenged animals.
- Therapeutic treatment with prednisolone for 14 days did not significantly reduce HDM-induced AHR in this study.

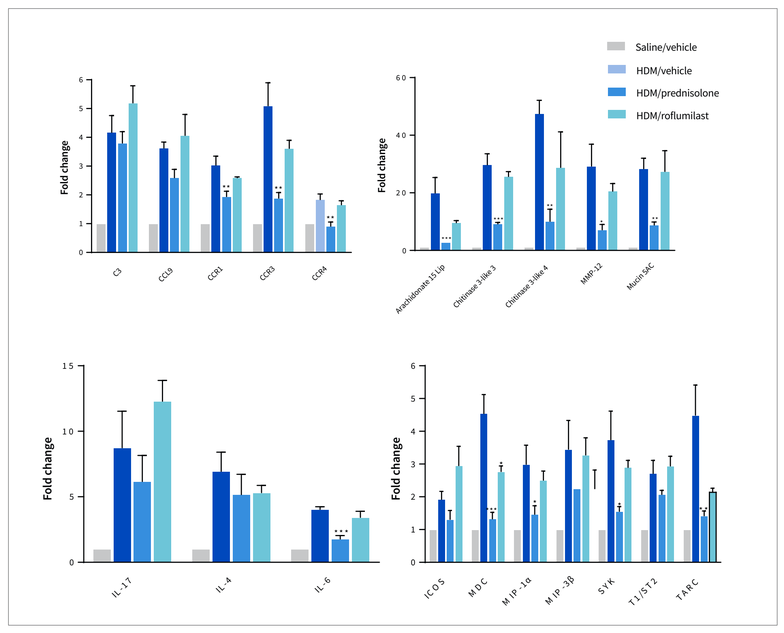

Figure 4. Effects of therapeutic prednisolone and roflumilast treatment on chronic HDM induced upregulation of lung tissue gene expression 24 h following the last exposure in Week 5. Mean ± SEM (n=3-4 per group). *p<0.05, ** p<0.01, ***p<0.001 when compared to the HDM/vehicle treated animals.

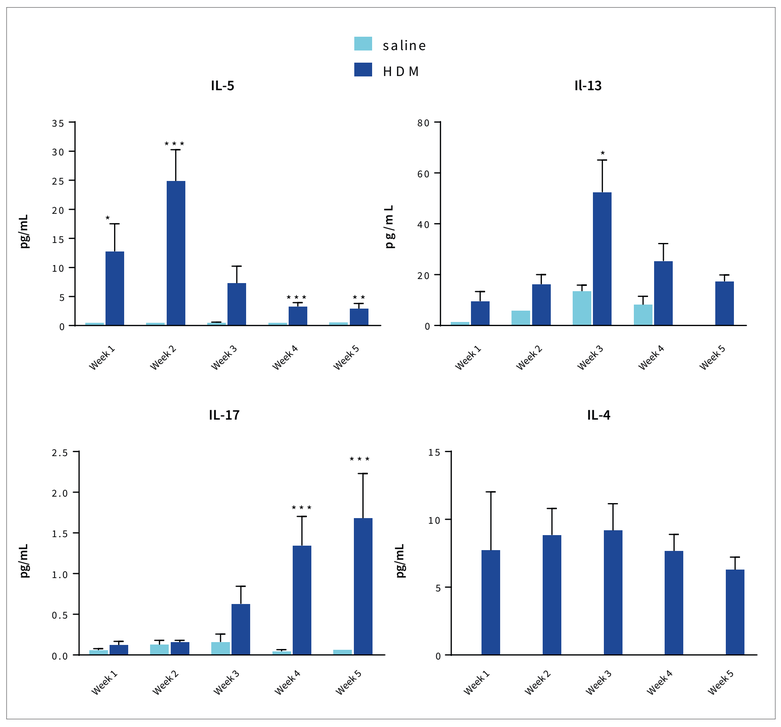

Figure 5. Effects of chronic HDM treatment (25 µg/mouse/ 5 days/week) on BAL Cytokines. Mean ± SEM (n=8-10 per group). *p<0.05, **p<0.01, ***p<0.001 when compared to the saline/vehicle treated animals.

- mRNA analysis of lung tissue from HDM-exposed mice resulted in increased expression of 25 genes including upregulation of chemokine receptor genes CCR4, CCR3 and CCR1, interleukins IL-13 (150-fold) , IL-17, IL-4 and IL-6 and 30-fold increases in MMP-12 and Mucin 5AC genes.
- The upregulation of T helper (Th) 2 cytokine genes in Week 5 was paralleled with small but significant increases in BAL cytokines IL-5, IL-13, IL-17 and IL-4.
- BAL IL-13 levels peaked in Week 3 correlating with the onset of significant AHR.
- Therapeutic treatment with prednisolone and roflumilast significantly ($p<0.05$) reduced the expression of 13 and 2 out of 25 upregulated genes respectively.

Conclusion

It was shown that the HDM model can mimic aspects of chronic allergic asthma including eosinophilic inflammation, airway remodelling and AHR and that this model, in our laboratory, has the potential to test novel compounds for the treatment of allergic disorders.

Acknowledgement

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References

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3. Ulrich K, Hincks JS, Walsh R, Wetterstrand C, Fidock MD, Sreckovic S, Lamb DJ, Douglas GJ, Yeadon M, Perros-Huguet C, Evans SM. Anti-inflammatory modulation of chronic airway inflammation in the murine house dust mite model. Pulmonary Pharmacology & Therapeutics 21 (2008) 637-647.

Animal experiments were conducted according to the Animals (Scientific Procedures) Act, 1986, and 2012 amendments following local ethical approval. Work was conducted in an AAALAC-accredited facility.

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