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Synchrotron-Fourier transform infrared maps of ovalbumin-induced murine chronic allergic airways disease: Correlation with conventional histology

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ABSTRACT

Asthma is a chronic respiratory disease characterised by airway inflammation, remodeling and hyperresponsiveness. The ability to replicate these asthma traits in the well-established ovalbumin induced chronic model of allergic airways disease is an important tool for asthma research and preclinical drug development. Here, spectra derived from focal plane array and Synchrotron-Fourier transform infrared maps were used to analyse biochemical changes in lung tissue from an ovalbumin-induced murine chronic allergic airways disease model. Analysis of the chemical maps resulted in distinct clusters and significant changes in the lipid and proteins regions of the spectra between the saline control and diseased lung tissue samples. Overall, the utilisation of conventional histological methodologies and Synchrotron infrared microspectroscopy has the ability to expand the characterisation of murine models of asthma.

INTRODUCTION

Asthma is a chronic inflammatory airway disease. It affects 300 million people and results in approximately 250,000 deaths annually worldwide (GINA, 2012). The hallmarks of asthma comprise of airway inflammation, remodeling, and airway hyperresponsiveness (AHR) (Porter et al., 2011, Endo et al., 2014). Phenotypically, asthma is classified into various types including; allergic, non-allergic, aspirin-exacerbated, infection or exercise-induced, or alternatively by inflammatory profiles; eosinophilic, neutrophilic, or a combination of eosinophilic and neutrophilic or paucigranulocytic (Bergquist et al., 2014).

Effective management involves combination therapies of inhaled β -agonists and corticosteroids (Pruitt and Lawson, 2011). However, there are subsets of people with severe asthma that are steroid insensitive (Adcock et al., 2010). New potential therapeutics are consistently being developed to alleviate some of the limitations of current asthma therapies. Such avenues include relaxin to target airway remodeling (Bani et al., 1997), statins (Cheng et al., 2012) and stem cells (Sun et al., 2012) which are aimed to reduce inflammation of the airways without significant side effects in the lungs, and trefoil factors aimed at targeting epithelial cell repair and anti-airway remodeling factors (Royce et al., 2014, Hoffmann, 2007).

Efficacies of these drugs are tested in animal models of asthma which attempt to model specific features of the disease. Typically these models are developed based on their inflammatory cell profiles; eosinophilic, neutrophilic or paucigranulocytic (Moore et al., 2014). One of the most widely explored animal models of asthma is the ovalbumin (OVA)-induced chronic allergic airways disease model in BALB/c mice. This established model (Kumar et al., 2008, Temelkovski et al., 1998) replicates the gross morphometric changes of airway eosinophilic inflammation, remodeling including epithelial thickening, goblet cell metaplasia, fibrosis and AHR (figure 1, A) (Agache et al., 2012, Chung, 2014, Gibson, 2009). A standard timeline of this process, shown in figure 1 (B), highlights the key time points of OVA sensitisation (days 0 and 14) and challenge (six weeks), to induce a chronic allergic T-helper 2 response. BALB/c mice represent an important strain for immunoglobulin E and T-helper 2 cytokines that include interleukin (IL)-4 and IL-5 production (Rückert et al., 1998, Kumar et al., 2008).

The use of the OVA-induced model of allergic airways disease has been of particular importance in aiding our understanding of asthma. Basic tools used in this identification process include lung function tests, such as spirometry and peak flow analysis, as well as the addition of current β -agonists in the immediate measurement of air flow reversibility. Furthermore bronchial alveolar fluid (BALF) is used for inflammatory cell counts, and lung biopsies for histological analysis (Apter, 2015). A novel avenue of disease characterisation is the identification of molecular fingerprints through focal plane array (FPA) and Fourier-transform infrared (FTIR) microspectroscopy.

Utilisation of FTIR tissue mapping as a diagnostic tool has been explored in numerous conditions including, cervical cancer (Wood et al., 2004), lung adenomatoid malformation (Krafft et al., 2008) and colorectal cancer (Lasch et al., 2004). FTIR microspectroscopy mapping is an optical technique used to identify the structure and biochemical fingerprint of diseased tissue (Krafft et al., 2008). Similarly, FPA-FTIR microspectroscopy is used to identify larger areas of tissue, for a broader spectrum of biochemical maps. This study is to our knowledge first to utilise FPA-FTIR and Synchrotron-FTIR microspectroscopy to map tissues of an OVA induced chronic model of allergic airways disease. We examined lung tissue samples as a preliminary measure of the biochemical signatures of asthma. Overall, our aim was to investigate the potential novel biochemical markers associated between saline and OVA induced mice in a chronic model of allergic airway disease with the aid of Synchrotron-FTIR microspectroscopy tissue mapping.

METHODS

Animal model

The OVA-induced chronic allergic airway response model was implemented in six-week old female BALB/c mice at Monash University, Clayton, Australia. Ethics approval was granted by Monash University Animal Ethics Committee which complies with the Australian Guidelines for the Care and Use of laboratory Animal for Scientific Purposes.

Saline mice ($n=6$) were interperitoneally (IP) sensitised with 0.9% (500 μ L) saline solution (Baxter Health Care, NSW, Australia), and the OVA treated mice ($n=6$) were sensitised with 10 μ g Grade-V OVA (Sigma-Aldrich, MO, United States of America) and 400 μ g aluminium potassium sulphate adjuvant (AJAX Chemicals, Kotara, NSW, Australia) in 0.9% saline solution (Baxter Health care), on days 0 and 14. Starting at day 21, mice were nebulised, using an ultrasonic nebuliser (Omron NE-U07; Omron, Kyoto, Japan) three times a week for six weeks with 0.9% saline alone, or in combination with 2.5% (w/v) OVA (AJAX Chemicals, Kotara, NSW, Australia) for 30 minutes, depending on their treatment groups, as previously described (Royce et al., 2015, Temelkovski et al., 1998).

Methacholine-induced Airway hyperresponsiveness

Three days following the last nebulisation mice were anaesthetised with ketamine (IP: 200 μ g/g) and xylazine (IP: 10 μ g/g), prior to inducing airway resistance with methacholine. Measurements were recorded using Fine Pointe (Buxco Electronics, NC, USA) as a change of airway hyperresponsiveness (AHR) from baseline to corresponding increasing doses of methacholine (Sigma-Aldrich) every three minutes using an invasive plethysmograph nebulizer (Elan, Buxco Electronics, Troy, NY, United states of Amercia).

Tissue collection

Following invasive plethysmography lung tissue was fixed in 10% neutral buffered formalin overnight and embedded in paraffin ready for histological methodologies.

Histological assessment

Tissues (3 μ m) were sectioned with a Leica RM 2135 microtome (Leica Biosystems, Wetzlar, Germany) onto microscope slides (Mikro-Glass, Australia). Tissue sections were stained for haematoxylin and eosin (H&E), Masson trichrome, Gomori aldehyde fuchsin and silver impregnation for histological assessment. For each stain, five airways per mouse between 150-300 μ m in diameter, were imaged using an FSX100 widefield microscope (Olympus, Tokyo, Japan). The degree of inflammation amongst the different treatment groups from the H&E stain were identified using a scoring method, such that a score of 0

indicated no presence of inflammatory cells, 1 for 3 layers, 2 for 6 layers, 3 for 10 layers and 4 for more than 10 layers of inflammatory cells. Morphometric analysis obtained from the Masson trichrome stain, of epithelial and sub-epithelial thickness was determined amongst the different treatment groups using Image-Pro Plus 6.0 software (Media Cybernetics, Maryland, United States of America) and shown as a measurement per 100 μm of basement membrane length.

Immunohistochemistry

The OVA-induced chronic model of allergic airways disease sections were cut and stained for α -SMA (1:150) (DAKO) by immunohistochemistry. Five airways per mouse were imaged on a brightfield setting with an FSX100 microscope (Olympus). Quantification of α -SMA was performed using Image-Pro Plus 6.0 Software (Media Cybernetics), by a count of surrounding fibroblasts per 100 μm of basement membrane length.

Focal Plane Array (FPA)-Fourier Transform Infrared (FTIR) microspectroscopy

Paraffin-embedded tissue sections of the OVA-induced chronic allergic airway response model ($n=3$ per group) were cut ($4\mu\text{m}$) onto calcium fluoride (CaF_2) windows (Crystran, Dorset, UK), and deparaffinised by two consecutive washes of xylene (Sigma-Aldrich, St Louis, USA). The samples were stored in a desiccator prior to imaging (4×4 grid maps) with a FPA-FTIR microscope (Bruker Optik GmbH, Ettlingen, Germany), at the Australian Synchrotron, equipped with a liquid- N_2 cooled 64×64 element FPA detector and a $15\times$ objective lens, coupled to a Vertex 70/70v FTIR spectrometer (Bruker). FPA-FTIR spectra, collected in the transmission mode within a $4000\text{-}800\text{cm}^{-1}$ spectral region using 8-cm^{-1} resolution, 64 co-added scans, Blackman-Harris 3-Term apodization, Power-Spectrum phase correction and a zero-filling factor of 2, operated through OPUS 7.2 imaging software (Bruker, Billerica, Massachusetts, USA). Spectra were first subjected to an atmospheric compensation water correction prior to band area integration of the lipid composition ($3000\text{-}2830\text{cm}^{-1}$) and protein ($1695\text{-}1600\text{cm}^{-1}$) regions.

Synchrotron-Fourier Transform Infrared (FTIR) microspectroscopy and analysis

Saline ($n=2$) and OVA ($n=2$) tissue sections were cut ($4\mu\text{m}$) using a microtome (Leica Biosystems) onto CaF_2 windows (Crystran), and deparaffinised by two consecutive washes in xylene (Sigma-Aldrich) before being air dried and stored in a desiccator. Tissue maps of samples were collected on the Fourier transform infrared (FTIR) beamline at the Australian Synchrotron, Clayton. A rectangular grid (25×25) area of $15,625\mu\text{m}^2$ (aperture of $5\mu\text{m}$), on a $36\times\text{IR}$ objective were utilised for all tissue samples.

The hypercube of each tissue map was accessed using CytoSpec™ (Berlin, Germany), to manually separate the raw spectra into selected tissue sections; respiratory tract epithelium, subepithelium, and

endothelium. The overall spectra were cut (3600-1000cm⁻¹), outliers were removed and data were exported into the Unscrambler® 10.1 software package (CAMO Software AS, Oslo, Norway) for spectral processing. Prior to multivariate principal component analysis (PCA), each tissue section data was converted to a 2nd derivative (25 smoothing points) using the Savitzky-Golay algorithm and corrected by extended multiplicative scatter correction (EMSC) at the spectral regions of 3033-2792cm⁻¹ and 1789-1008cm⁻¹. The average absorbance spectrums of each data for each tissue regions were also derived.

Statistics

Statistical analyses were performed with GraphPad Software Prism 6.02 (San Diego, California, United States of America). Results were analysed by a two-way analysis of variance (ANOVA), with a post-hoc Bonferroni test. Statistical significance was determined at *p<0.05, **p<0.01, and ***p<0.001. All results were displayed as means ± standard error of mean (SEM).

RESULTS

Airway inflammation, remodeling and collagen deposition

Morphological changes were found in the histological stains for inflammation, collagen deposition, and reticular fibrosis, as seen in figure 2 (A). An inflammatory cell score (B, i) derived from the H&E stain was quantified in the airways, with a significant increase ($p=0.0004$) of inflammation in the OVA sensitised group (2.97 ± 0.35) in comparison to the saline control (0.00 ± 0.0). Repeated OVA nebulisation induced a significant increase ($p<0.0001$) in mean epithelial (B, ii, OVA: $19.0\text{ }\mu\text{m}\pm0.44$, saline: $13.06\mu\text{m}\pm0.71$) and subepithelial (B, iii, OVA: $27.81\mu\text{m}\pm1.18$, saline: $13.53\mu\text{m}\pm1.26$) thicknesses in Masson's trichrome stained sections. Both the Gomori aldehyde fuchsin and silver impregnation (figure 2, A) stains were performed for the presence of reticular fibres, whereby minimal reticular fibres were present in the saline group in comparison to the OVA group. α -SMA was quantitated as a representation of myofibroblasts per $100\mu\text{m}$ of basement membrane length (exclusion of the myofibroblasts found in the epithelium smooth muscle tissue). As the results in figure 2 (B, iv) illustrate, there was a significant increase ($p=0.0021$) in myofibroblasts per $100\mu\text{m}$ basement membrane length in the OVA model, in comparison to the saline control group (OVA: 1.10 ± 0.16 , saline: 0.20 ± 0.05).

Airway hyperresponsiveness

The OVA challenged mice displayed increased airway resistance in response to methacholine, as seen in figure 2 (B). In particular, AHR was significantly increased in OVA challenged mice compared to saline-challenged mice at the methacholine dose of 12.5mg/ml ($p<0.01$) and greater significance was identified with the respective higher doses ($p<0.001$).

FPA-FTIR and Synchrotron-FTIR microspectroscopy

Biochemical maps of the integrated lipid composition ($3000\text{-}2800\text{cm}^{-1}$) and protein ($1695\text{-}1600\text{cm}^{-1}$) regions obtained from the FPA-FTIR microspectroscopy are shown in figure 3. These results are indicative of higher expression levels of both the lipid composition and proteins within these regions in the OVA induced mice as compared to the saline mice. Furthermore figure 4 illustrates the PCA analysis separation in the scores plot (iv) amongst both saline and OVA treated mice in the epithelium, subepithelium and endothelial tissue. According to the loadings (iii) and second derivative (ii) the separation of the epithelial cells amongst the two groups of mice is largely attributed to the amide I ($\sim 1654\text{cm}^{-1}$) and amide II band ($\sim 1542\text{-}50\text{cm}^{-1}$). Other heavily loaded peaks were found in the C-H stretching region included 2912 , 2842 , 2935 and 2867cm^{-1} . Respiratory subepithelium cells displayed strong clusters, primarily attributed to the amide I ($\sim 1656\text{cm}^{-1}$) band from the PC-1 loadings. Stretching in C-H lipid region of the spectrum at point 2933 , 2965 and 2840cm^{-1} also contributed to this separation of

the subepithelial cells. Lastly, endothelial cells which made up the smallest number of spectra collected, displayed separation of along the PC-1 loadings at the amide I band (1656cm^{-1}). Other major loaded peaks were found at 2935 , 2869 , 2912 and 2842cm^{-1} , in the lipid region in the saline and OVA tissue groups.

DISCUSSION

Conventional histology and immunohistochemistry highlighted the hallmark characteristics of human asthma (Royce et al., 2012a) in the OVA-induced chronic allergic airway model, consistent with previous studies (Mac Sharry et al., 2014, Royce et al., 2011). Of note are the significant increases in inflammation, mean epithelial and sub-epithelial thicknesses (figure 2 B: i, ii, iii), of OVA-allergen exposed mice as compared to the saline group, essential features in the establishment of airway remodeling in asthma (Mauad, 2007, Yang, 2013). Additionally, airway resistance was measured with increasing doses of methacholine (mg/ml). Figure 2 (B: v) demonstrates that the OVA group responded with increasing resistance statistically different from the saline model, particularly at the final doses of methacholine. This is of particular importance as it has been suggested that airway remodeling, collagen deposition and inflammation all contribute to AHR (Karagiannis et al., 2012).

The sensitivity and accuracy of FPA-FTIR and Synchrotron-FTIR microspectroscopy was utilised to identify molecular information about the chronic allergic airways model. FPA-FTIR microscopy has the advantage of imaging larger map areas such that the overall identification of changes within the proteins and lipids can be determined following absorption corrections. As seen in figure 3, the grid (4x4) maps allowed imaging of the entire airway and surrounding tissue areas. The results indicate that the OVA-induced tissue contains higher expression levels of C-H stretching associated with lipid composition and proteins within the respective $3000\text{-}2830\text{cm}^{-1}$ and $1695\text{-}1600\text{cm}^{-1}$ spectral integrated region. This highlights the vast differences seen in the lung airway between diseased and non-diseased tissue, particularly within the epithelial cells.

Synchrotron-FTIR infrared biochemical maps provided more detail in the absorption spectrum in conjunction with enabling PCA separation of the diseased and non-diseased tissue. Manual selection of spectra segregated in the airway epithelium, subepithelium and endothelium aids in the determination of the key biochemical markers within these regions. Figure 4 presents the airway epithelium average absorbance spectrum of approximately 400 spectra from each group in the OVA-induced chronic allergic airways model. Clear clusters of the saline and the OVA group can be seen in the scores plot (figure 4: iv), however a large variability amongst the data, specifically in the saline mice is apparent. Strong peak loadings in the PC-1 plot (iii) were evident, indicating the key differences in the molecular composition within the saline and OVA groups. Of note is the negative loadings peaks of amide I band at 1656cm^{-1} (Bocker et al., 2007) and amide II band at 1552cm^{-1} (Miyazawa and Blout, 1961). The assignment of the amide I band in combination with the positive loadings peak at 1625cm^{-1} , is indicative of the formation of proteins into the secondary structure of α -helices (Bellisola et al., 2010). Other heavily loaded peaks were found within the C-H stretching lipid region (Dovbeshko et al., 2000, Wu et al., 2001) of the

spectrum at bands 2935, 28967, 2912 and 2842cm⁻¹. Although not identified as a significant peak in the PC-1 loadings, a higher absorption unit was found at the band 1404cm⁻¹ in the OVA exposed mice in comparison to the saline group. This band in particular has been found to be associated with the deformation of methyl group bending modes of proteins (Agarwal et al., 2006).

Airway subepithelium is comprised of inflammatory cells, smooth muscles and fibrotic tissue. According to the scores plot of the airway subepithelium and endothelial tissue (figure 4; iv) there was an apparent separation across the PC-1 plane of the saline and OVA groups with approximately 400 and 100 spectra selected in each group, respectively. Significant peaks were assigned to amide I band, with α -helix conformation due to the negatively loaded 1623cm⁻¹ band, which predominates in the proteins associated with OVA sensitisation. Strong loaded peaks in the lipid region where C-H stretching occurs was also a contributor to the separation of the groups. However, limited detail in the biochemical fingerprint is apparent as compared to the gross morphometric changes in inflammation and fibrosis characteristic to that of asthma, as seen in our histological analyses (figure 2).

FTIR tissue mapping has provided some insights into the biochemical fingerprint of an OVA induced chronic allergic airways model. However, there are some shortcomings with FTIR spectroscopy. Firstly, the DNA region (less than 1000cm⁻¹) of the IR spectrum is not reliable with the CaF₂ windows used in our experiment. These windows are optimal for FTIR imaging, however lack the sensitivity to lipid heads bands found below 1000cm⁻¹ (Krafft et al., 2008). Considering this is the first study to investigate a chronic murine model of airways disease, our results have assisted in preliminary understanding of biochemical peaks associated with diseased and non-diseased tissues. Ultimately once these biochemical fingerprints are confirmed for lung tissue samples, analogous fingerprints may be associated with blood serum samples. This has already been investigated with cancer patients (Ricciardolo et al., 2015, Barnes and Adcock, 2003, Wang et al., 2014). Overall, we have extended analysis of the widely used OVA induced murine chronic allergic airways disease model to encompass analysis of spectra derived from FPA-FTIR and Synchrotron-FTIR maps. Analysis of chemical mapping identified significant changes in the lipid and protein regions which can be correlated with structural and biological changes observed with conventional histological and immunofluorescence methodologies. Although prolonged mapping times are necessary, infrared chemical mapping is an automated process and data analysis yields a plethora of biochemical information. Future studies will be aimed at extended the size and resolution of mapping to improve clustering and to include detailed analyses of the inflammatory process which is prevalent in the OVA induced murine chronic allergic airways disease model. An interesting approach would involve potentially developing predictive models based on biochemical markers in circulating blood cells in control compared to disease states. Finally, additional investigations will be aimed at exploring the

biochemical basis accounting for the biological effects of compounds that can mitigate the allergic responses observed in this model of allergic airways disease.

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REFERENCES

- ADCOCK, I. M., MARWICK, J., CASOLARI, P., CONTOLI, M., CHUNG, K. F., KIRKHAM, P., PAPI, A. & CARAMORI, G. 2010. Mechanisms of corticosteroid resistance in severe asthma and chronic obstructive pulmonary disease (COPD). *Current Pharmaceutical Design*, 16, 3554-3573.
- AGACHE, I., AKDIS, C., JUTEL, M. & VIRCHOW, J. C. 2012. Untangling asthma phenotypes and endotypes. *Allergy*, 67, 835-846.
- AGARWAL, R., TANDON, P. & GUPTA, V. D. 2006. Phonon dispersion in poly(dimethylsilane). *Journal of Organometallic Chemistry*, 691, 2902-2908.
- APTER, A. J. 2015. Advances in adult asthma diagnosis and treatment in 2014. *The Journal of Allergy and Clinical Immunology*, 46.
- BANI, D., BALLATI, L., MASINI, E., BIGAZZI, M. & SACCHI, T. B. 1997. Relaxin counteracts asthma-like reaction induced by inhaled antigen in sensitized guinea pigs. *Endocrinology*, 138, 1909-1915.
- BARNES, P. J. & ADCOCK, I. M. 2003. How Do Corticosteroids Work in Asthma? *Annals of Internal Medicine*, 139, 359.
- BELLISOLA, G., DELLA PERUTA, M., VEZZALINI, M., MORATTI, E., VACCARI, L., BIRARDA, G., PICCININI, M., CINQUE, G. & SORIO, C. 2010. Tracking infrared signatures of drugs in cancer cells by Fourier transform microspectroscopy. *The Analyst*, 135, 3077-3086.
- BERGQUIST, M., JONASSON, S., HJOBERG, J., HEDENSTIERNA, G. & HANRIEDER, J. 2014. Comprehensive multiplexed protein quantitation delineates eosinophilic and neutrophilic experimental asthma. *BMC Pulmonary Medicine*, 14, 110-110.
- BOCKER, U., OFSTAD, R., WU, Z., BERTRAM, H. C., SOCKALINGUM, G. D., MANFAIT, M., EGELANDSDAL, B. & KOHLER, A. 2007. Revealing covariance structures in fourier transform infrared and Raman microspectroscopy spectra: a study on pork muscle fiber tissue subjected to different processing parameters. *Appl Spectrosc*, 61, 1032-9.
- CHENG, Y., LIN, Z., JIYUN, C., JINGYING, Z., YUE, T., MAO, H., ADCOCK, I. M., BARNES, P. J. & XIN, Y. 2012. Statins as potential therapeutic drug for asthma? *Respiratory Research*, 13, 1-7.
- CHUNG, K. 2014. Defining Phenotypes in Asthma: A Step Towards Personalized Medicine. *Drugs*, 74, 719-728.
- DOVBESHKO, G. I., GRIDINA, N. Y., KRUGLOVA, E. B. & PASHCHUK, O. P. 2000. FTIR spectroscopy studies of nucleic acid damage. *Talanta*, 53, 233-46.
- ENDO, Y., HIRAHARA, K., YAGI, R., TUMES, D. J. & NAKAYAMA, T. 2014. Review: Pathogenic memory type Th2 cells in allergic inflammation. *Trends in Immunology*, 35, 69-78.
- GIBSON, P. G. 2009. Inflammatory phenotypes in adult asthma: clinical applications. *Clinical Respiratory Journal*, 3, 198-206.
- HOFFMANN, W. 2007. TFF (trefoil factor family) peptides and their potential roles for differentiation processes during airway remodeling. *Current Medicinal Chemistry*, 14, 2716-2719.
- KARAGIANNIS, T. C., LI, X., TANG, M. M., ORLOWSKI, C., EL-OSTA, A., TANG, M. L. K. & ROYCE, S. G. 2012. Molecular model of naphthalene-induced DNA damage in the murine lung. *Human & Experimental Toxicology*, 31, 42-50.
- KRAFFT, C., CODRICH, D., PELIZZO, G. & SERGO, V. 2008. Raman mapping and FTIR imaging of lung tissue: congenital cystic adenomatoid malformation. *The Analyst*, 133, 361-371.
- KUMAR, R. K., HERBERT, C. & FOSTER, P. S. 2008. The "classical" ovalbumin challenge model of asthma in mice. *Current Drug Targets*, 9, 485-494.
- LASCH, P., HAENSCH, W., NAUMANN, D. & DIEM, M. 2004. Imaging of colorectal adenocarcinoma using FT-IR microspectroscopy and cluster analysis. *BBA - Molecular Basis of Disease*, 1688, 176.
- MAC SHARRY, J., SHALABY, K. H., MARCHICA, C., FARAHNAK, S., CHIEH-LI, T., LAPTHORNE, S., QURESHI, S. T., SHANAHAN, F. & MARTIN, J. G. 2014. Concomitant Exposure to Ovalbumin and Endotoxin

- Augments Airway Inflammation but Not Airway Hyperresponsiveness in a Murine Model of Asthma. *PLoS ONE*, 9, 1-12.
- MIYAZAWA, T. & BLOUT, E. R. 1961. The Infrared Spectra of Polypeptides in Various Conformations: Amide I and II Bands. *Journal of the American Chemical Society*, 83, 712-719.
- MOORE, W. C., HASTIE, A. T., LI, X., LI, H., BUSSE, W. W., JARJOUR, N. N., WENZEL, S. E., PETERS, S. P., MEYERS, D. A. & BLEECKER, E. R. 2014. Asthma and lower airway disease: Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *The Journal of Allergy and Clinical Immunology*, 133, 1557-1563.e5.
- PORTER, P. C., ONGERI, V., LUONG, A., KHERADMAND, F. & CORRY, D. B. 2011. Seeking common pathophysiology in asthma, atopy and sinusitis. *Trends in Immunology*, 43.
- PRUITT, B. & LAWSON, R. 2011. Assessing and managing asthma. *Nursing*, 41, 46-53 8p.
- RICCIARDOLO, F. L. M., BLASI, F., CENTANNI, S. & ROGLIANI, P. 2015. Therapeutic novelties of inhaled corticosteroids and bronchodilators in asthma. *Pulmonary Pharmacology & Therapeutics*, 1.
- ROYCE, S. G., DANG, W., YUAN, G., TRAN, J., EL OSTA, A., KARAGIANNIS, T. C. & TANG, M. L. K. 2011. Resveratrol has protective effects against airway remodeling and airway hyperreactivity in a murine model of allergic airways disease. *Pathobiology Of Aging & Age Related Diseases*, 1.
- ROYCE, S. G., LI, X., TORTORELLA, S., GOODINGS, L., CHOW, B. S. M., GIRAUD, A. S., TANG, M. L. K. & SAMUEL, C. S. 2014. Mechanistic insights into the contribution of epithelial damage to airway remodeling. Novel therapeutic targets for asthma. *American Journal Of Respiratory Cell And Molecular Biology*, 50, 180-192.
- ROYCE, S. G., SHEN, M., PATEL, K. P., HUUSKES, B. M., RICARDO, S. D. & SAMUEL, C. S. 2015. Mesenchymal stem cells and serelaxin synergistically abrogate established airway fibrosis in an experimental model of chronic allergic airways disease. *Stem Cell Research*, 15, 495-505.
- RÜCKERT, R., HERZ, U., PAUS, R., UNGUREANU, D., POHL, T., RENZ, H. & BULFONE-PAUS, S. 1998. IL-15-IgG2b fusion protein accelerates and enhances a Th2 but not a Th1 immune response in vivo, while IL-2-IgG2b fusion protein inhibits both. *European Journal Of Immunology*, 28, 3312-3320.
- SUN, Y.-Q., DENG, M.-X., HE, J., ZENG, Q.-X., WEN, W., WONG, D. S. H., TSE, H.-F., XU, G., LIAN, Q., SHI, J. & FU, Q.-L. 2012. Human pluripotent stem cell-derived mesenchymal stem cells prevent allergic airway inflammation in mice. *Stem Cells (Dayton, Ohio)*, 30, 2692-2699.
- TEMELKOVSKI, J., HOGAN, S. P., SHEPHERD, D. P., FOSTER, P. S. & KUMAR, R. K. 1998. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax*, 53, 849-56.
- WANG, X., SHEN, X., SHENG, D., CHEN, X. & LIU, X. 2014. FTIR spectroscopic comparison of serum from lung cancer patients and healthy persons. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 122, 193-197.
- WOOD, B. R., CHIRIBOGA, L., YEE, H., QUINN, M. A., MCNAUGHTON, D. & DIEM, M. 2004. Fourier transform infrared (FTIR) spectral mapping of the cervical transformation zone, and dysplastic squamous epithelium. *Gynecologic Oncology*, 93, 59-68.
- WU, J. G., XU, Y. Z., SUN, C. W., SOLOWAY, R. D., XU, D. F., WU, Q. G., SUN, K. H., WENG, S. F. & XU, G. X. 2001. Distinguishing malignant from normal oral tissues using FTIR fiber-optic techniques. *Biopolymers*, 62, 185-92.

FIGURE LEGENDS

Figure 1. Typical histological analysis and timeline for establishing the ovalbumin-induced chronic allergic airways disease model in mice. A haematoxylin and eosin (H&E) stain of OVA-sensitised mice displays the gross morphological changes that occur in the airways to replicate some of the key hallmarks of asthma (A). These include smooth muscle thickening (SM), epithelial thickening (E) and inflammation (I). This is typically established with mice first sensitised to OVA via an intraperitoneal (IP) injection on days 0 and 14, followed by nebulisation of OVA three times a week for six weeks, allowing for the development of the chronic allergic airway model to establish. Mice are commonly culled within 72 hours of last nebulisation treatment (B).

Figure 2. Histological analysis and methacholine induce airway hyperresponsiveness (AHR) of the murine chronic allergic airways model. The histological stains; haematoxylin and eosin (H&E), Masson trichrome, Gormori aldehyde fuschin, silver impregnation and immunohistochemically stained α -smooth muscle actin (SMA) are represented for the saline ($n=6$) and OVA ($n=6$) control groups (A), acquired on the brightfield Olympus FSX100 microscope. Scale bars= 100 μ m (x20 objective lens). Quantification of these stains (B) for an inflammatory cells score (i), mean epithelial (ii) and sub-epithelial thickness (iii) and myofibroblasts per 100 μ m of basement membrane (BM) length (iv) were analysed with Image-Pro Plus 6.0 Software (Media Cybernetics). AHR was induced by methacholine via invasive plethysmography and measure from baseline cmH₂O/ml/sec (v). A two-way ANOVA with a post-hoc Bonferroni test statistical analyses (** $p<0.001$) with a mean $\pm$ standard error of mean (SEM) was performed.

Figure 3. FPA-FTIR chemical mapping of the murine chronic allergic airways model. Integrated chemical tissue maps of saline ($n=3$) and OVA ($n=3$) challenged mice in the lipid composition (3000-2830cm⁻¹) and protein (1695-1600cm⁻¹) regions.

Figure 4. Synchrotron-FTIR tissue mapping of the murine chronic allergic airways model. Infrared spectral analyses of the epithelium (A), subepithelium (B) and endothelium (C) regions, within each of the saline ($n=2$) and OVA ($n=2$) groups, as displayed in the absorption spectrum (i), second derivative spectrum (ii), PC-1 loadings plot (iii) and PCA-scores plot (iv).

FIGURE 1

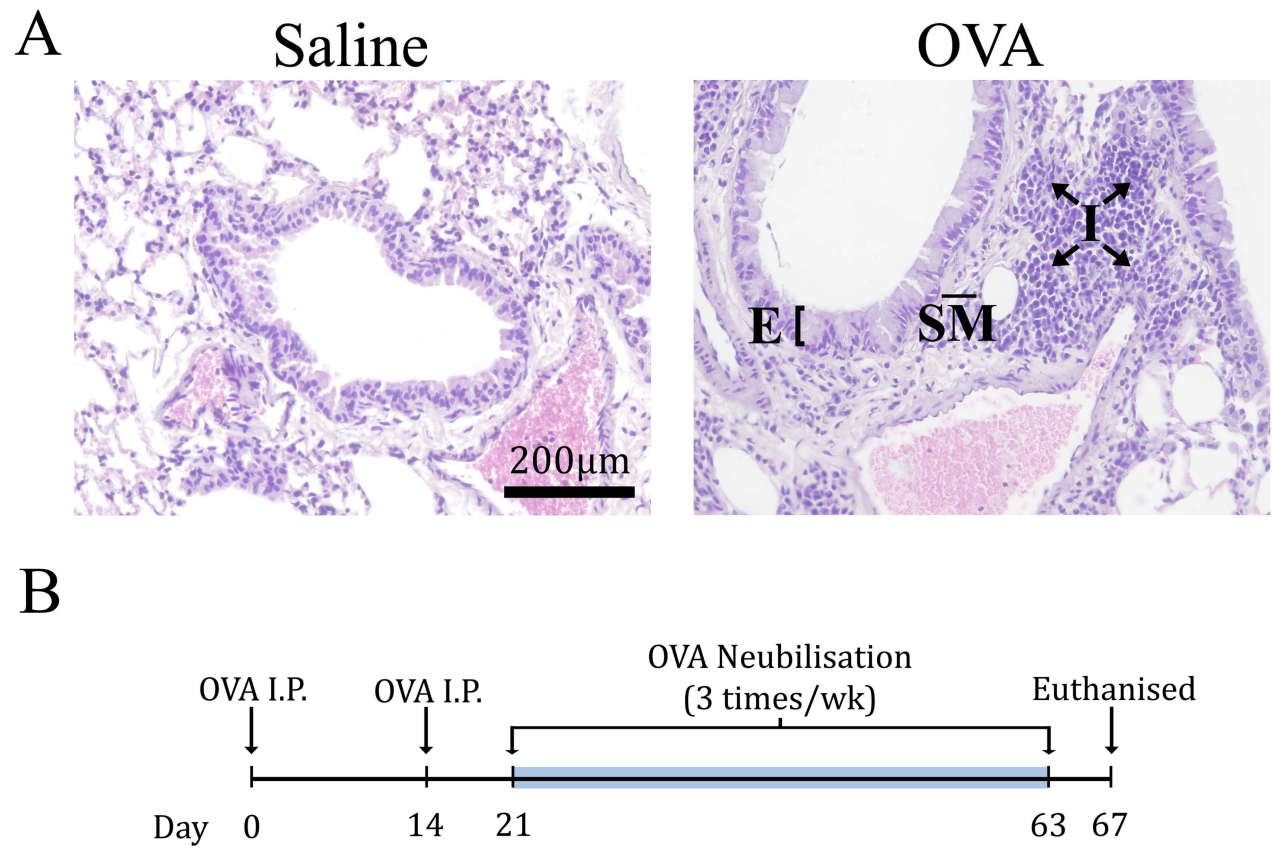


FIGURE 2

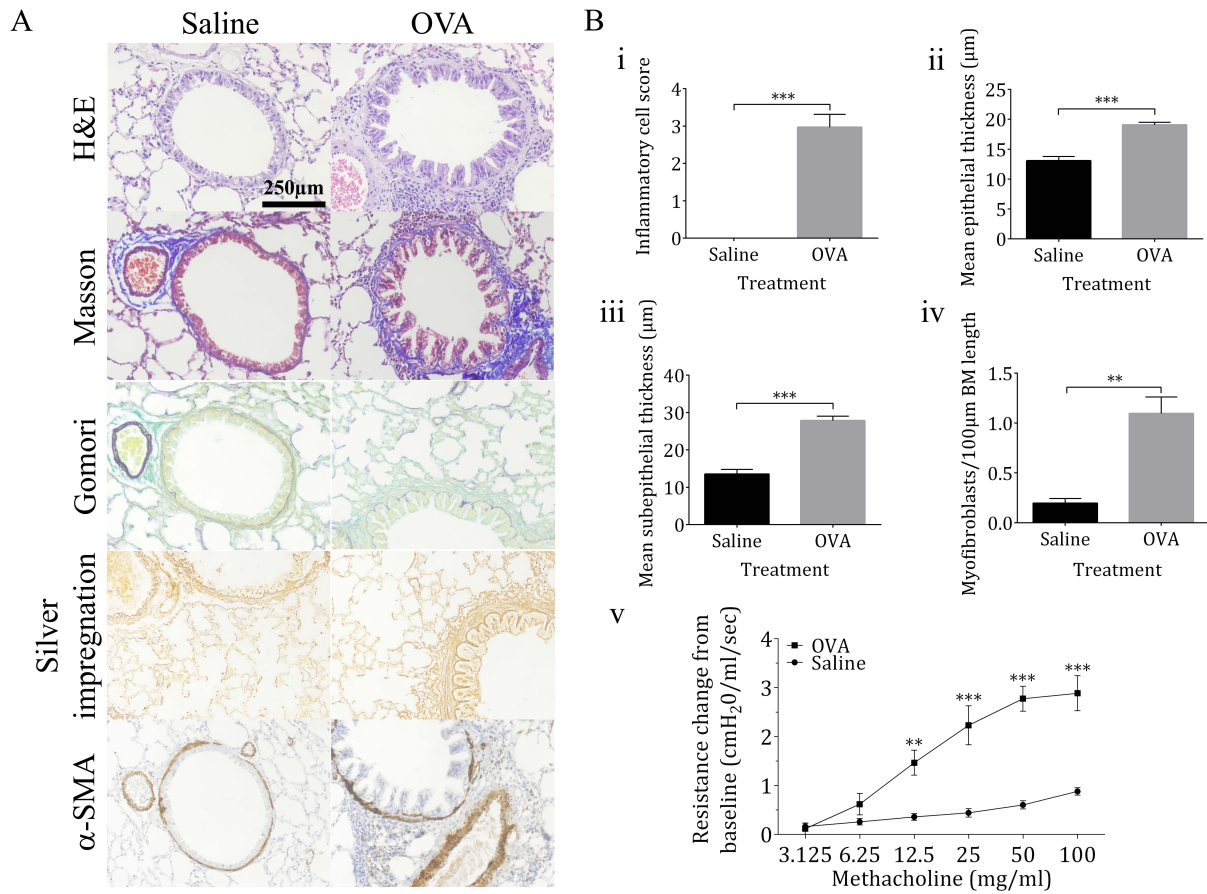


FIGURE 3

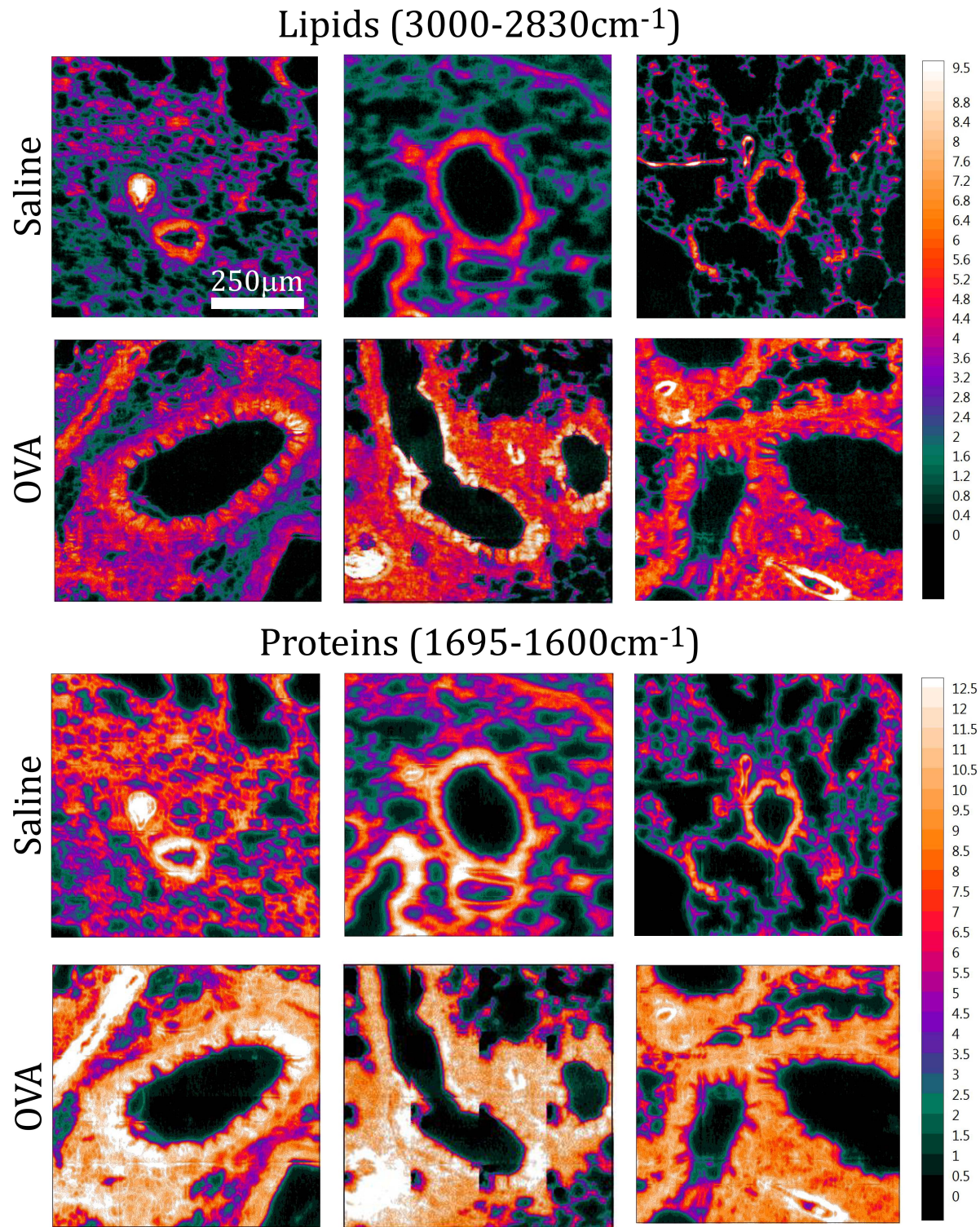


FIGURE 4

