## 388. Insight into mechanisms of respiratory infections

**P3490**

**LSC 2011 Abstract: The role of IL-25 in rhinovirus-induced asthma exacerbations**

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Rhinoviruses (RV) are the major causative factor of asthma exacerbations (AE). While Th2-mediated inflammation is implicated in asthma, it is unknown how the immune response to RV infection interacts with Th2 immunity causing an AE. Epithelial-derived IL-25 is an important regulator of Th2 immunity and plays a role in asthma pathogenesis. We hypothesized that RV infection of the epithelium induces IL-25 production facilitating immunopathogenesis of AE. We measured IL-25 mRNA in mouse models of RV infection and RV-induced exacerbation of allergic airway inflammation [1]. *In vitro* IL-25 gene induction was also assessed in asthmatic and normal bronchial epithelial cells (BEC) infected with RV and stimulated with IL-4. *In vivo* and *in vitro* results demonstrated that RV induced IL-25 mRNA as measured by qPCR. Airway challenge with ovalbumin (OVA) followed by RV infection in sensitised mice exacerbated allergic airway inflammation and coincided with enhanced IL-25 mRNA expression compared with allergen or infection alone. Similarly, RV and IL-4 treatment of BECs resulted in the highest levels of IL-25 mRNA. The novel finding that RV infection induces IL-25 represents a link between antiviral responses and Th2 inflammation identifying a role for IL-25 in RV-induced AE. Allergen/IL-4 treatment enhanced RV-dependant IL-25 expression thus a Th2 environment and virus may result in exacerbated Th2 inflammation mediated by IL-25.

**Reference:**

[1] Bartlett, N.W., *et al.* Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat Med* 14, 199-204 (2008).

**P3491**

**Pulmonary viral infection in hematologic patients with and without stemcell transplantation**

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Pulmonary complications are frequent in hematologic patients. Regular investigation of BAL fluid includes bacterial/fungal culture, staining and PCR for mycobacteria, immunohistochemistry/Grocott staining for pneumocystis and cell differentiation. The increasing use of PCR allows to search for different viruses. In this study we analysed the diagnostic yield of a recently established multi PCR for detection of 13 viruses. 219 hematologic patients underwent diagnostic bronchoscopy with BAL from september 2009 to january 2011. 28 of patients received high dose chemotherapy, 143 underwent allogeneic and 13 autologous stemcell transplantation. Bacteria were cultured from 43 (20%) BALs: staphylococcus aureus, pseudomonas aeruginosa, streptococcus pneumoniae, enterococcus, moraxella, enterobacteriaceae, klebsiella, corynebacterium, mycoplasma pneumoniae, mycoplasma hominis, chlamydia pneumoniae, bordetella pertussis. There were 7 cases of pneumocystis. In two patients we found mycobacterium gordonae. Aspergillus was cultured in 4 cases. In 81 (37%) patients viruses were documented. Most often we found rhinovirus (n=31; 14%), followed by CMV (n=18; 8%), HSV (n=13; 6%), RSV (n=10; 4.5%), coronavirus (n=8; 3.6%), adenovirus (n= 7; 3.2%), parainfluenza- and metapneumovirus (each n=5; 2.2%), H1N1 (n=4; 1.8%), influenza (n=3; 1.4%), and HHV6 (n=1; 0.4%).
**Summary and conclusion:** The incidence of viral infections is very high in patients with hematologic diseases and pulmonary symptoms. Multiplex PCR in the BAL should be introduced as a routine diagnostic procedure in this specific patient group allowing to withdraw or avoid antibiotic or antifungal therapy in many cases.

**P3492**
**Impaired innate immunity to rhinovirus in severe asthmatic children**
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The pathogenic mechanisms of rhinovirus-induced asthma exacerbations are incompletely understood. Impaired production of innate IFN-β and IFN-λ have been identified in bronchial epithelial cells and bronchoalveloar lavage macrophages from atopic mild moderate asthmatics upon rhinovirus infection *in vitro*. These cells display similar production of pro-inflammatory cytokines when compared to cells cultured from non-asthmatic, non-atopic individuals, and are observable in steroid treated and steroid naive individuals. In the present study, bronchial epithelial cells were cultured from severe asthmatic children (*n*=8, mean age 11yr, range 9-15, 63% male) and non-atopic non-asthmatic controls (*n*=10, mean age 7yr, range 2-15, 70% male). Cells were infected with RV1B, RV16, or medium and mRNA, protein and virus release was measured at 8-48h post infection. Cells from severe asthmatic children displayed significantly reduced IFN-β ($p<0.05$) IFN-λ1 ($p<0.05$) and IFN-λ2/3 mRNA ($p<0.05$), but not IL-8 ($p>0.05$) or ENA-78 ($p>0.05$) compared to controls. Cells cultured from severe asthmatics had significantly higher RV1B ($p<0.01$), RV16 ($p<0.05$) release at 48h, compared to controls. Impaired RV1B induced IFN-β and IFN-λ2/3 also showed strong negative correlations with increased virus load ($r=-0.79$, $p=0.013$ and $r=-0.65$, $p=0.015$ respectively). RV1B induced IFN-λ2/3 from severe asthmatics also showed strong negative correlations with total serum IgE ($r=-0.75$, $p=0.04$) and a trend for a negative correlation with total number of positive RAST tests which was not significant ($r=-0.69$, $p=0.06$). This is the first report of impaired IFN in severe asthma, and support the previous findings of impaired IFN production in asthmatics.

**P3493**
**Tyrosine sulfation in the N-terminal domain of human C5aR is necessary for high-affinity binding of chemotaxis inhibitory protein of staphylococcus aureus**
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**Background:** Staphylococcus aureus evades host defense through releasing several virulence proteins, such as chemotaxis inhibitory protein of staphylococcus aureus (CHIPS). Previous studies have shown that extracellular N terminus of C5a receptor (C5aR) forms the binding domain for CHIPS. Tyrosine sulfation is emerging as a key factor in determining protein-protein interaction. The goal of this study was to evaluate the role of tyrosine sulfation of N-terminal C5aR in binding to CHIPS.
**Methods:** Expression plasmids encoding C5aR and its mutants were made by PCR and site-directed mutagenesis. HEK 293T cells were transfected with plasmids encoding C5aR using calcium phosphate. Recombinant CHIPS protein was purified. Western blotting was used to assay the binding of CHIPS to C5aR or its mutants and p-38 phosphorylation.
**Results:** We report that CHIPS exclusively binds to C5aR, but not to C5L2 or C3aR. A nonspecific sulfation inhibitor, sodium chlorate, diminishes the binding ability of C5aR to CHIPS. Blocking sulfation by mutation of tyrosine to phenylalanine at positions 11 and 14 of C5aR N terminus completely abrogates CHIPS binding. When tyrosine 14 alone was mutated to phenylalanine, the binding affinity of recombinant CHIPS was substantially decreased. CHIPS fails to induce p-38 phosphorylation in cells overexpressing wild-type C5aR or its mutants.
**Conclusion:** This study defines a structural basis of C5aR-CHIPS association, in which tyrosine sulfation of N-terminal C5aR plays an important role in CHIPS binding. Our data would make it possible to develop potent drugs for therapeutic intervention.

**P3494**
**shRNAs significantly reduce the replication of RSV in vitro**
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**Background:** RNA interference (RNAi) is a powerful tool to silence gene expression on the level of mRNA. To knock-down gene expression by using RNAi two major methods of mRNA silencing exist. First method utilizes siRNA (small interfering RNA), a readily processed dsRNA, that enters RISC complex and destroy target mRNA after transfection into the cells. The second method based on the construction of plasmid DNA that expresses shRNA (short harpin RNA) from U6 or CMV promoter. shRNA gets processed by Drosha and Dicer RNAses inside the cell before it translocates to the cell cytoplasm and affects the level of target RNA. In this study we investigated the ability of specific vector constructs coding shRNA molecules to target the expression of respiratory syncytial virus (RSV) phosphoprotein P in vitro and thus inhibit the replication of RSV.
**Methods:** Lentiviral vector pGIPZ expressing tGFP-IRES-Puro-shRNAmir30 cassette was modified by introducing BamH I restriction site downstream of this cassette. This modification makes possible to clone specific shRNA sequences in pGIPZ vector using XhoI/BamHI restriction sites instead of the original recombination.
**Results:** Three shRNAs against phosphoprotein P RSV and shRNA against human CD43 as a control were generated and cloned into modified so-called pGIPD vector. Monkey kidney cells MA-104 were stably transduced with four shRNA constructs. MA-104 cells transduced with shRNA constructs against RSV P protein demonstrated significant inhibition of of RSV replication after the infection than compared to the control and not transduced cells.
**Conclusion:** The generated constructs can be successfully used for efficient gene silencing and virus replication inhibition in vitro.

**P3495**
**Novel narrow spectrum kinase inhibitors inhibit rhinovirus replication via enhancement of interferon expression in nasal epithelial cells of atopic and non-atopic patients**
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**RATIONAL** Rhinovirus infection is one of important causes of asthma exacerbation. Deficient innate immune response was reported in asthma accounting for increased susceptibility of asthmatic patients to respiratory viral infections. The aim of this study is to evaluate the effects of RV568 and RV1088, narrow spectrum kinase inhibitors (NSKIs), on HRV16 replication and interferon expression in nasal brushing epithelial cells (NBEC) from atopic (A) and non-atopic (NA) patients.
**Methods:** NBECs were collected from 9 A- and 9 NA-rhinitis patients. Cells were treated with RV568, RV1088, fluticasone propionate (FP) or tiotropium bromide (TB), and infected with HRV16 (5MOI). After 1 hr absorption, cells were washed with PBS and then treated with compounds again. At 8hrs after infection, cells were collected to determine viral RNA and IFN-λ, IFN-β mRNAs by real time RT-PCR.
**Results:** HRV16 viral load (copy number) was 9 fold higher in NBECs of A-patients than those of NA-patients ($48.6\times10^5$ and $5.4\times10^5$, respectively, $p=0.083$). In addition, IFN-λ expression was significantly lower in A-patients than NA-patients (copy number, $801\pm122$, $1363\pm174$, $p=0.02$). IFN-β expression showed similar trends. Both RV568 (100nM) and RV1088 (10, 100nM) inhibited HRV16 replication, and significantly increased IFN-λ, -β expression, particularly in A-patients. FP (100nM) and TB (100nM) did not inhibit viral load or increase IFN expression.
**Conclusions:** HRV16 infection is more severer in NBECs from A- compared to NA-patients due to deficient HRV16-induced IFN expression, that is restored by RV568 and RV1088.

**P3496**
**Cigarette smoke extract increases the adhesion receptor for S. pneumoniae in vitro**
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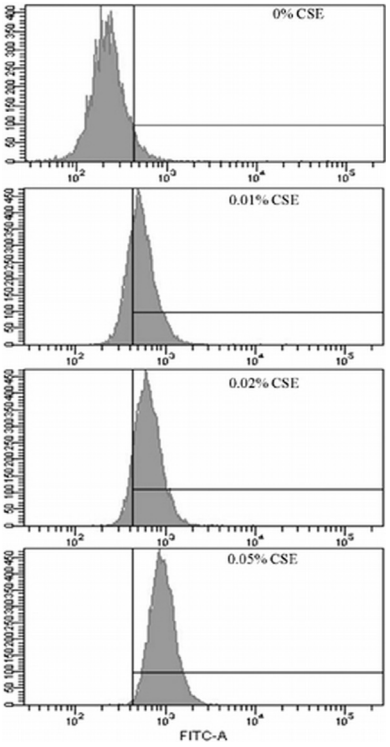
**Background:** Although the mechanism is unknown, cigarette smoke exposure increases the risk of pneumococcal infections in humans1. We have recently shown that fossil fuel derived particulate matter increases adhesion of S. pneumoniae

via upregulation of platelet activating factor receptor (PAFr) expression on airway cells2. We therefore aimed to assess whether cigarette smoke extract (CSE) upregulates the PAFr expression in vitro.
**Methods:** The human lung epithelial (A549) cell line was incubated with very low concentrations of CSE (0.01%, 0.02% and 0.05%) for 4 hr. PAFr expression, adjusted for non-specific staining, was then assessed by flow cytometry using a PAFr human monoclonal antibody.
**Results:** CSE stimulated dose-dependent increase in PAFr expression, with an increase in mean PAFr fluorescence and % positivity (Figure 1 and Table 1).

FACs analysis showing dose (%) of CSE vs mean fluorescence

| Dose (CSE) | % Positivity | Mean Fluorescence (isotype subtracted) |
|---|---|---|
| 0 | 95 | 21 |
| 0.1 | 95.4 | 332 |
| 0.2 | 96.1 | 432 |
| 0.5 | 96.3 | 735 |




**Conclusion:** CSE stimulates PAFr, the receptor for S. pneumoniae adhesion to lower airway cells, and may be the mechanisms underlying the epidemiological association between active and passive cigarette exposure and invasive pneumococcal disease in adults and children.
FACs analysis showing a dose response relationship between CSE and PAFr expression.

**P3497**
**Assessment of tracheal tube biofilm translocation during mechanical ventilation and lung injury**
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**Introduction:** The role of bacterial biofilm, within the endotracheal tube (ETT), in the pathogenesis of respiratory infections is still under debate
**Objectives:** To study effects of length of stay under mechanical ventilation (MV) on bacterial biofilm translocation from within the ETT into health and injured lungs
**Methods:** ETTs colonized by biofilm were obtained from an associated study where pigs underwent oropharyngeal bacterial challenge by *Pseudomonas Aeruginosa* (PA) and 72h MV. Those ETTs were used in 8 healthy pigs (32.1±2.5 kg) on MV ($V_T$ 8ml/kg, PEEP 0, RR adjusted based on pH, $T_I/T_{TOT}$ 0.25). Pigs were randomized into 4 groups (2 pigs/group) to be MV up to 24h (Group1), 48h (Group2), 72h (Group3) and 48h with lung injury caused by oleic acid (OA) (Group4). Upon autopsy, 4 samples from trachea and main bronchi and 7 from segmental bronchi were excised for PA quantification
**Results:** In pigs of group 3, 1 hour after OA instillation, $PaO_2/FiO_2$ decreased to 189.8±27.7. Upon extubation, ETT PA colonization was 5.7±1.6, 6.4±0.5, 6.7±0.5 and 7.6±0.6 log cfu/cm in groups 1-4, respectively (p=0.21). As depicted in figure 1, the airway tissue/ETT PA colonization ratio of trachea and main bronchi was 0.59±0.37. Colonization of segmental bronchi was infrequent.

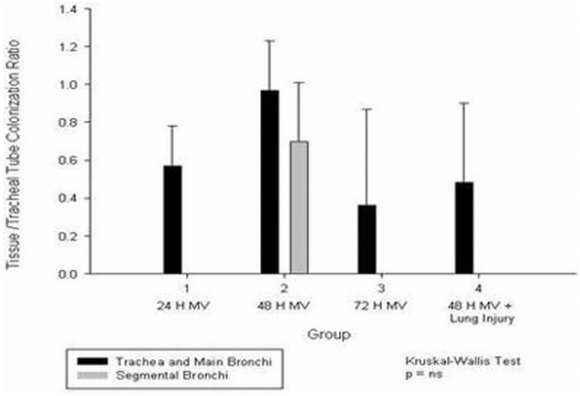


Figure 1

**Conclusions:** Bacteria from within the ETT PA biofilm rapidly translocate up to the main bronchi, however distal colonization is uncommon even when lung are injured.

**P3498**
**Detection of linezolid in endotracheal tube biofilm of ventilated pigs with methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia**
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**Introduction:** Linezolid presents good penetration into respiratory secretions; we hypothesized that secretions could transport linezolid into ETT and limit burden of MRSA in biofilm.
**Aim:** To determine linezolid concentration in biofilm inside the ETT using High-performance liquid chromatography (HPLC).
**Methods:** We analyzed 16 samples of ETT from pigs with MRSA pneumonia, ventilated up to 96 hours and treated with linezolid (10 mg/kg every 12h IV). To determine linezolid concentration by HPLC we disrupted the biofilm matrix with several sonications, enzymes and perchloric acid. We also assessed MRSA count in each ETT sample.
**Results:** We retrieved from the ETT samples 63±54 mg of biofilm. In 4 (25%) samples linezolid was inferior to the sensitiveness of the technique (1.56 μg/mL). In the remaining 12 samples the concentration of linezolid was 38 (11-83) μg/mL of biofilm (median (IQR)). Hence, linezolid concentration in biofilm was 19 (5-41) times above the MRSA MIC for linezolid (2 μg/mL). The concentration of MRSA in the biofilm of pigs treated with linezolid was 1.98±0.84 Log CFU/mL. No significant correlation was found between biofilm concentration of linezolid and MRSA burden (r=0.48, p=0.11).
**Conclusion:** Despite the high concentration of linezolid above the MIC in biofilm inside the ETT, MRSA was found in most samples of pigs treated with this antibiotic, without significant correlation between linezolid levels and MRSA burden.
Funded: FIS 05/0620, 070419, 016077 and 050136, SEPAR 2005, Fundación Lilly, Ciberes (CB06/06/0028), 2009-SGR-911, IDIBAPS, FUCAP 2010.

**P3499**
**Moderate aerobic exercise training attenuates inflammatory response to *streptococus pneumoniae* in mice**
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*Streptococus pneumoniae* is one of the most important causes of morbidity and mortality in respiratory diseases. Aerobic exercise is known to attenuate inflammatory processes in some lung injuries.
**Objectives:** To study if moderate exercise training prior to bacterial infection alters the pulmonary inflammatory profile.

**Methods:** 40 Balb/C mice (14-16 weeks) were divided into 4 groups: Control (C), Aerobic Exercise (AE), *S. pneumonia* infection (P), *S. pneumonia* + Aerobic Exercise (P+AE). Moderate intensity treadmill training was performed over 4 weeks, 5 time/wk, 60 min/session in the AE and P+AE groups. After 72 hs of the last exercise training session, P and P+AE groups were challenged intranasally with pneumococcal strains M10 (type 11A) through the inoculation of 50 μl of the suspension of the bacteria in 0.9% saline. C group did not receive intranasal instillation or exercise training. Bronchoalveolar lavage (BAL) was performed 10 days after the intranasal challenge to quantify the number of total cells, macrophages, neutrophils and lymphocytes.
**Results:** *S. pneumonia* inoculation resulted in increase number of total cells ($18.63\times10^4$cels/mL ± 9.02; $p<0.001$), macrophages ($6.49\times10^4$cels/mL ± 1.94; $p<0.001$) and neutrophils ($8.19\times10^4$cels/mL ± 6.84; $p<0.001$) while moderate exercise training in *S. pneumonia* inoculated animals resulted in significantly decrease in total number of cells ($10.15\times10^4$cels/mL ± 1.10; $p<0.001$) and neutrophils ($1.53\times10^4$cels/mL ± 0.84; $p=0.003$) in BAL.
**Conclusion:** These results suggest that moderate aerobic exercise training attenuated the neutrophilic inflammation in an animal model of bacterial infection.Supported by FAPESP, LIMHC-FMUSP, CNPq, Brazil.

**P3500**
**Different modes of allergen – Rhinovirus interaction control chemokine production**
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Interacting immune responses to rhinovirus (RV) and allergen in asthma are thought to increase the risk of asthma exacerbations. To investigate this we used a mouse model of RV-induced asthma exacerbation and assessed expression of eosinophil, Th1 and Th2 recruiting chemokine protein in BAL fluid. Experimental groups: allergen (OVA)-challenged, RV-infected (RV-OVA); OVA challenged, mock infected with UV-inactivated RV (UV-OVA), RV-infected mock allergen challenged with PBS (RV-PBS) and double negative control (UV-PBS).
**Results:** CCL11 and CCL24 (eotaxin 1 and 2) in RV-OVA were significantly increased ($P<0.001$) vs UV-OVA. RV infection alone did not induce either eotaxin but synergistically augmented eotaxin production in allergic mice. For Th1 chemokines CXCL11 (I-TAC) and CCL5 (RANTES) the opposite was true. RV infection drove expression and this was synergistically increased by allergen (RV-OVA compared to RV-PBS for CXCL11 $P<0.001$ and CCL5 $P<0.01$). Next we examined Th2 cell recruiting chemokines CCL17 (TARC) and CCL22 (MDC). Both OVA- and RV-alone stimulated increased expression (UV-OVA and RV-PBS $P<0.001$ compared to UV-PBS for CCL17 and CCL22). Thus the increased levels observed in RV-OVA ($P<0.05$ and $P<0.001$ for UV-OVA and RV-PBS respectively) were consistent with an additive interaction between OVA and RV. Associated with increased chemokine production we observed greater lung recruitment of activated (CD69+) CD4+ T cells ($P<0.01$ compared to UV-OVA) and expression of IL-5 and IL-13. Thus for the chemokines investigated we observed 3 different modes of allergen-virus interaction 1) allergen-induced, augmented by virus, 2) virus-induced, augmented by allergen, 3) allergen and virus additive.

**P3501**
**An anti-human ICAM-1 antibody inhibits human rhinovirus infection in the mouse model of human major group rhinovirus infection**
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**Introduction:** Human rhinoviruses (HRV) cause the common cold and the majority of acute exacerbations of asthma and COPD. 90% of HRV belong to the major group and use human ICAM-1 (intercellular adhesion molecule 1) for cell attachment and entry. We previously established a human/mouse chimeric ICAM-1 transgenic mouse model of human major group rhinovirus infection. However, antiviral approaches have not yet been studied in this model.
**Aim:** To evaluate the inhibition of HRV-induced airway inflammation by a mouse anti-human ICAM-1 antibody.
**Methods:** Transgenic mice were treated i.n. or i.v. with an ICAM-1 antibody before HRV16 or HRV14 challenge. To exclude possible effects of an ICAM-1 antibody on cell trafficking we evaluated an ICAM-1 antibody in the transgenic mice by infecting them with minor group HRV1B or in an LPS challenge model.
**Results:** Both i.n. and i.v. dosed ICAM-1 antibody inhibited HRV16 induced bronchoalveolar lavage (BAL) cells ($p<0.001$ and $p<0.001$, respectively), lymphocytes ($p<0.001$ and $p<0.01$), neutrophils ($p<0.001$ and $p<0.001$) and macrophages ($p<0.01$ and p=ns). Intranasal administered ICAM-1 antibody reduced HRV16 induced IL-1β ($p<0.001$), IL-6 ($p<0.001$) and IFNλ2/3 ($p<0.01$) as well as ITAC ($p<0.001$), IP-10 ($p<0.001$) and KC ($p<0.001$) in BAL. Similar data were observed with ICAM-1 antibody and HRV14 infection. Control experiments with minor group HRV1B as well as in an LPS challenge model showed no effect of ICAM-1 antibody on either HRV1B- or LPS-induced airway inflammation (p=ns for all outcomes).
**Conclusion:** We have shown for the first time that an anti-human ICAM-1 specific antibody can be used to prevent human rhinovirus entry and replication and induction of airway inflammation in vivo.

**P3502**
**Rhinovirus infection upregulates SLPI in macrophages**
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**Background:** Antimicrobial peptides (AMPs) such as Secretory Leukoprotease Inhibitor (SLPI) and Elafin are an important component of host innate immune responses that protect against infections at mucosal surfaces. Their role in bacterial infection is well decribed but little is known regarding their role in respiratory viral infections.
**Aims:** We investigated the effects of rhinovirus infection on production of SLPI and elafin by monocyte derived macrophages (MDM).
**Methods:** MDM derived from healthy donors were infected with rhinovirus-16 (RV16). Cells and supernatants were harvested and SLPI and elafin mRNA and protein were measured by qPCR and ELISA respectively.
**Results:** Compared to cells infected with UV-inactivated virus RV16 induced expression of SLPI mRNA (11.7-fold induction, P=0.005) and protein (53.04±9.76pg/mL vs 95.23±2.63, P=0.014) in MDM at 24 hours.

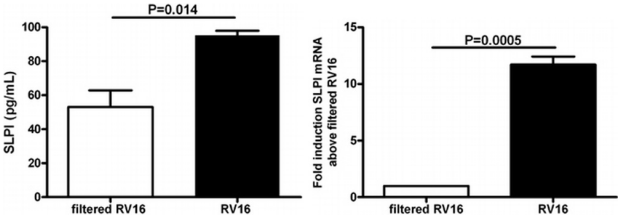

Figure 1

There was a trend towards upregulation of elafin at 48 hours for both mRNA (2.17-fold induction, P=0.055) and protein (13.33±13.33pg/mL vs 107.8±37.45,P=0.14) but this was not statistically significant.
**Conclusions:** Rhinovirus infection of MDM upregulates production of SLPI and elafin and this is dependent on virus replication. These molecules may play an important role in the host defence response to respiratory virus infections.

**P3503**
**Potential synergism of drugs with anti-viral and pulmonary oedema clearance activity may be advantageous for influenza patients**
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Influenza is a serious public health problem that causes severe illnesses and deaths for higher risk populations. Large numbers of people need hospital treatment and many die from the disease every year. 30% of hospitalised patients with Influenza infection require intensive care and despite medical treatment 10% die. The most common cause of death is viral pneumonia and ARDS. Antiviral drugs like oseltamivir and zanamivir block increase and spreading of the virus in the body. Already existing virus particles will not be affected. In case of complications, 2/3 of patients show infiltrates on chest radiographs, giving strong evidence for ARF and pulmonary oedema that can deteriorate to ALI/ARDS. However, it has been shown that the AP301 peptide, representing the TIP domain of TNFa, has alveolar liquid clearance activity. It was proposed that a combination of anti-viral and anti-oedema substances would act synergistically. This was assessed in mice having been sublethally infected with Influenza strain A/PR8/34 per nasal. On day of infection, mice were treated with oseltamivir. AP301 peptide was applied intratracheally on days 0, 2 and 4 post infection. On day 9 post infection the effect on wet-dry weight ratio and pulmonary microvascular permeability was assessed. AP301 peptide alone had a beneficial effect compared to untreated mice. The additive effect of the combination of AP301/oseltamivir combination was greater for reduction of lung oedema, which was in the range of control-treated but uninfected mice. In conclusion, these preliminary experiments suggest a benefit of combining anti-viral drugs and substances with alveolar liquid clearing activity.

**P3504**

**Toll-like receptor (TLR)-signaling mediates enhanced inflammatory effects in coinfections of viral and bacterial respiratory pathogens in human lung tissue – A new approach to the pathogenesis of COPD exacerbations?**

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Acute exacerbations of COPD are mainly mediated by respiratory viruses or bacteria like *nontypeable* Haemophilus influenzae (NTHi) and Chlamydia pneumoniae (Cpn). To elucidate inflammatory responses to coinfections, we investigated the interaction of viral/atypical pathogens and NTHi in respiratory cells and human lung tissue with the TLR3-agonist Poly(I:C) as a model of viral infection and Cpn as an atypical, TLR2-inducing agent.

Alveolar Epithelial Cells (AEC) and human lung tissue (HLT) were pre-incubated with Poly(I:C) or Cpn (strain CWL-029) and costimulated with NTHi $10^6$cfu/ml (strain Rd KW20). After 24h cells and supernatants were harvested for ELISA and Western Blot analysis and tissue was used for *in situ*-Hybridisation (ISH).

NTHi led to significant induction of Interleukin-8 (IL-8) production (HLT: Med 134848±22483pg/ml vs. NTHi 341926±34390pg/ml, n=12, $p<0.01$). NTHi and Poly(I:C) increased IL-8-production and had significant costimulatory effects as well as Cpn/NTHi-coinfection (AEC: NTHi+Poly(I:C) 18.48±1.93ng/ml vs. NTHi 8.70±2.22ng/ml, $p< 0.05$, n=4; HLT: Cpn 199395±32882pg/ml vs. Cpn+NTHi 364777±53085pg/ml, $p<0.05$, n=6). TLR3- and TLR2-stimulation were mediated via MAP-kinases. ISH showed increased IL-8-and TLR2-expression in HLT after Cpn/NTHi-coinfection. Blocking TLR2 with a specific antibody led to partial reduction of IL-8.

These data indicate that TLR2- and TLR3-stimulation are necessary for inflammatory responses in NTHi-infection and that TLR-signaling might be important for enhanced inflammation in viral/NTHi and atypical/NTHi coinfections.

**P3505**

**Gamma/delta T cells regulate airways disease during rhinovirus-induced asthma exacerbations**

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Human rhinovirus (RV) infections are associated with asthma exacerbations but the underlying mechanisms are poorly understood. In a human asthma exacerbation model we found increased γδT cell numbers in the airways of atopic asthmatics following RV infection and that greater bronchoalveolar lavage (BAL) γδT cell numbers were associated with greater airway hyperresponsiveness (AHR) and obstruction.

**Aim:** To define the role of γδT cells in RV-induced asthma exacerbations using a mouse model.

**Methods:** The mouse RV-induced asthma exacerbation model comprised sensitisation and challenge of BALB/c mice with ovalbumin and infection with RV1B (RV-OVA) or UV-inactivated RV1B control (UV-OVA) concomitant with final allergen challenge. γδT cells were depleted by intraperitoneal injection of anti-γδTCR antibody.

**Results:** RV infection increased total and activated lung γδT cell numbers in OVA sensitised and challenged mice. Anti-γδTCR vs isotype control antibody treatment in RV-OVA mice enhanced AHR and BAL neutrophilia at 48 hrs post-infection ($p<0.05$) and lung Th2 cell numbers on day 7 ($p<0.001$), with enhancements in BAL mucus protein and serum IgE levels reaching near significance. BAL IL-10 levels were conversely reduced by 50% ($p<0.01$) 24 hrs after infection. Intracellular cytokine staining showed lung γδT cells to be potent producers of IL-17a.

**Conclusions:** Consistent with the human model, RV infection in the mouse asthma exacerbation model enhances γδT cell numbers in the airways. Depletion studies support a disease suppressing function for γδT cells in RV-induced asthma exacerbations, possibly via production of Th2 regulating cytokines IL-10 and IL-17a.

**P3506**

**Comparison of respiratory virus infection between human nasal epithelial cell monolayer and air-liquid interface 3D culture**

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**RATIONAL** Viral infection has been implicated in exacerbations of COPD and asthma, and appropriate in vitro model is required to evaluate infectious mechanism and the efficacies of new therapy. In this study, we compared the replication of virus, such as human rhinovirus (HRV16), respiratory syncytial virus (RSV A2) and influenza virus (WSN33, H1N1) in 3D culture air-liquid interface human nasal epithelial cells (NEC-3D) and NEC monolayer culture (NEC-M).

**Methods:** HRV16 (1MOI) and RSVA2 (0.1 MOI) (both from ATCC) and WSN33 (1 MOI) (HPA, UK) were infected to normal NEC-3D (Epithelix Sárl, MuciAir®) and NEC-M (Epithelix), and washed out with PBS after 1hr virus absorption. The cells were incubated further up to 10 days. The virus titre in culture supernatant was measured by serial dilutions CPE assay in Hela cells for HRV16, in Hep2 cells for RSV and MDCK cells for H1N1. The results were shown as Log, $TCID_{50}$ value/20uL. IL-8 was determined by ELISA.

**Results:** HRV16 replicated in NEC-M and the $TCID_{50}$ was 3 at maximum. In contrast, HRV16 replicated well in NEC-3D, and $TCID_{50}$ reached to 6.3 at Day 6 post-infection. RSV A2 replicated well in both cells with peak $TCID_{50}$ of 4 in NEC-M and 5.3 in NEC-3D. WSN33 only weakly replicated in NEC-M with peak $TCID_{50}$ of 2, but it replicated well in NEC-3D with peak $TCID_{50}$ of 6.8 at Day 5. All virus induced IL-8 production by less than 10 fold in NEC-M and by 400 fold in NEC-3D over baseline.

**Conclusions:** All respiratory virus used in this study replicated more in NEC-3D than NEC-M. This unique and robust 3D culture cell system (MuciAir®) will be a better in vitro model for evaluation of respiratory virus infection.

**P3507**

**Enhanced platelet activating factor receptor (PAFR) expression in smoker airways**

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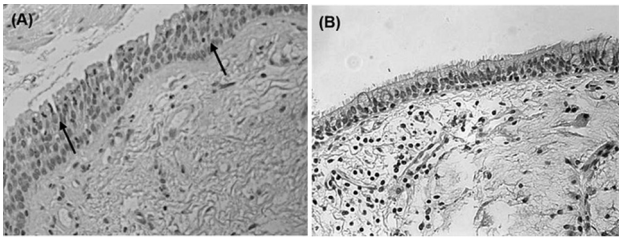
**Introduction:** Cigarette smoking increases the risk of pneumococcal disease, and epidemiologic studies suggest that air pollution from cigarette smoke increases vulnerability to bacterial pneumonia. We have shown that inhalable particulate matter (PM) stimulates pneumococcal adhesion to human lower airway cells. Increased pneumococcal adhesion is associated with increased invasiveness of bacteria. Oxidative stress mediates the adhesive response of airway epithelial cells, and platelet activating factor receptor (PAFR) is a receptor co-opted by pneumococci to facilitate PM-stimulated adhesion.

**Objective:** To investigate the expression of PAFR in the airways of normal lung function smokers (NS) in vivo.

**Methods:** Endobronchial biopsies from 16 NS and 11 normal controls (NC) were stained for anti-PAFR monoclonal antibody. PAFR expression was assessed as percentage of epithelium stained for PAFR over total basement membrane length by using computer-assisted image analysis.

**Results:** Percentage of epithelial staining for PAFR was increased significantly in NS (*A*) compared to NC (*B*) (Chi-Square=30.3, 0 (0–4.8), $p<0.001$).




**Conclusions:** This is the first description of in-vivo expression of PAFR in the epithelium of NS compared to NC. Our data suggest that enhanced PAFR expression may be the mechanism of increased vulnerability of smokers to pneumococcal infection.