388. Insight into mechanisms of respiratory infections

P3490
LSC 2011 Abstract: The role of IL-25 in rhinovirus-induced asthma exacerbations
Janine Beale, Nathan Bartlett, Sebastian L. Johnston, Respiratory Medicine, Imperial College London, National Heart and Lung Institute, UK

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-depndant IL-25 expression thus a Th2 environment and virus may result in exacerbated Th2 inflammation mediated by IL-25.

Reference:

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Pulmonary viral infection in hematologic patients with and without stemcell transplantation
Lilian Junker1, Jörg Halter2, Alexis Dumoulin1, Daiana Stolz1, Dominik Heim2, Martin Stern2, Christoph Bucher2, JakobPasweg2, Hans Hirsch2, Michael Tamm1, 1Clinic of Pulmonary Medicine and Respiratory Cell Research, University Hospital Basel, Basel, Switzerland. 2Clinic of Hematology, University

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Tyrosine sulfation in the N-terminal domain of human C5aR is necessary for
P3493
activation of interferon expression in nasal epithelial cells of atopic and
non-atopic patients

Marco Contoli1 , Kazuhiro Ito2 , Donatella Poletti1 , Antonio Pastro2 , Alberto Papi1 .
1Asthma and COPD Centre, Ferrara University, Ferrara, Italy; 2Biology, RespiVert Ltd, London, United Kingdom; 3Ear, Throat and Nose Clinic, Ferrara University, Ferrara, Italy

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 (NBECs) from atopic (A) and non-atopic (NA) patients. Materials and Methods: NBECs were isolated from 9 A-patients and 9 NA-thalassemia patients. Cells were treated with RV568, RV1088, fluticasone propionate (FP) or tiotropium bromide (TB), and infected with HRV16 (SM01). 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±10 and 5±4 respectively, p=0.038). In addition, IFN-λ expression was significantly lower in A-patients than NA-patients (copy number, 80±e1222, 1363±e174, 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 severe in NBECs of A-compared to NA-patients due to deficient HRV16-induced IFN expression, that is restored by RV568 and RV1088.

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Cigarette smoke extract increases the adhesion receptor for S. pneumoniae in vitro

Naseem Mushaq1 , Iain Dickson1 , Cang-Bao Xu1 , Lars Edvinsson2 , Jonathan Grigg1 .
1Academic Unit of Paediatrics, QMUL, London, United Kingdom; 2Medicine, Lund University, Lund, Sweden.

Background: Although the mechanism is unknown, cigarette smoke exposure increases the risk of pneumococcal infections in humans. We have recently shown that fossil fuel derived particulate matter increases adhesion of S. pneumoniae to lymphocytes at positions 11 and 14 of C5αR 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 C5αR or its mutants.

Conclusion: This study defines a structural basis of C5αR-CHIPS association, in which tyrosine sulfation of N-terminal C5αR 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

Musa Khaitas, Igor Shilovski, Dmitry Mazurov, Evgeny Fairuzalev. Biomedical Technologies, NRC Institute of Immunology, FMBIA, Moscow, Russian Federation

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 downregulates the mRNA of a specific target. The second method is based on the construction of plasmid DNA that expresses shRNA (short hairpin RNA) from U6 or CMV promoter. shRNA gets processed by Dicer 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 GFP-IRE-PURO-shRNAHuman30 cassette was modified by introducing BamHI restriction site downstream of this cassette. This modification makes possible to clone specific shRNA sequences in the reverse GFP gene using XhoI/BamHI restriction sites instead of the original recombinant.

Results: Three shRNAs against phosphoprotein P RSV and shRNA against human CDA3 as a control were generated and cloned into modified so-called pGIPZ vector. Monkey kidney cells MA-104 cells stably transduced with four shRNA constructs. MA-104 cells transduced with shRNA constructs against RSV P Protein demonstrated significant inhibition of RSV replication after the infection 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.
via upregulation of platelet activating factor receptor (PAFR) expression on airway cells. 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%L, 0.02%L 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

<table>
<thead>
<tr>
<th>Dose (CSE)</th>
<th>% Positivity</th>
<th>Mean Fluorescence (isotype subtracted)</th>
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<tr>
<td>0</td>
<td>95</td>
<td>21</td>
</tr>
<tr>
<td>0.1</td>
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<td>96.3</td>
<td>735</td>
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**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.

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**Assessment of tracheal tube biofilm translocation during mechanical ventilation and lung injury**

Gianluigi Li Bassi1, Laia Fernández-Baral1, Joan Daniel Martí1, Montserrat Rigo1,2, Laura Muñoz1, Miquel Ferrer1, Jordi Vila1, Francesco Blasi4, Antoni Torres1, Pneumology Department, Thorax Clinic Institute, Hospital Clinic-IDIBAPS, Cibers, Barcelona, Spain; 1Cardiology Department, Thorax Clinic Institute, Hospital Clinic, Barcelona, Spain; 2Microbiology Department, Hospital Clinic, CSERB, Barcelona, Spain; 1Pneumology Department, Thorax Clinic Institute, Hospital Clinic-IDIBAPS, Cibers, Barcelona, Spain; 1Cardiology Department, Thorax Clinic Institute, Hospital Clinic-IDIBAPS, Cibers, Barcelona, Spain; 1Microbiology Department, Hospital Clinic, CRESIB, Barcelona, Spain; 1Pneumology Department, Thorax Clinic Institute, Hospital Clinic-IDIBAPS, Cibers, Barcelona, Spain

**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 (VT 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, PaO2/FiO2 decreased to 199.8±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.

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**Detection of linezolid in endotracheal tube biofilm of ventilated pigs with methicillin-resistant Staphylococcus aureus (MRSA) pneumonia**

Laia Fernández-Baral1, Miquel Ferrer1, Laura Guerrero2, Delors Soy2, Jordi Vila1, Gianluigi Li Bassi1, Josep Maria Sierra1, Lina Maria Saucedo2, Pilar Martínez-Olóndriz1, Montserrat Rigo1, Mariano Esperatti1, Néstor Luque1, Joan Daniel Martí1, Antoni Torres1, Pneumology Department, Thorax Clinic Institute, Hospital Clinic-IDIBAPS, Cibers, Barcelona, Spain; 2Pharmacy Service, Hospital Clinic-IDIBAPS, Cibers, Barcelona, Spain; 3Microbiology Department, Hospital Clinic, CRESIB, Barcelona, Spain; 4Microbiology Department, Hospital de Bellvitge, Barcelona, Spain; 5Cardiology Service, Thorax Clinic Institute, Hospital Clinic-IDIBAPS, Cibers, Barcelona, Spain

**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.


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**Moderate aerobic exercise training attenuates inflammatory response to Streptococcus pneumoniae in mice**

Clarice Olivera1, Maria Leonor Oliveira1, Eliane Miyagi1, Francine Almeida1, Petra Araújo1, Paulo He2, Fernanda Lopes1, Milton Martins1, 1Department of Medicine, Universidade de Sao Paulo, Sao Paulo, Brazil; 2Biotechnology Center, Butantan Institute, Sao Paulo, Brazil

**Streptococcus 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. pneumoniae* infection (P), *S. pneumoniae* + Aerobic Exercise (P-AE). Moderate intensity treadmill training was performed over 4 weeks. 5 tons/mw, 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. pneumoniae* inoculation resulted in increase in number of total cells (18.63 × 106/μl ± 9.02 vs. P < 0.001), macrophages (6.49 × 106/μl ± 1.94; P < 0.001) and neutrophils (8.19 × 106/μl ± 6.84; P < 0.001) while moderate exercise training in S. pneumoniae inoculated animals resulted in significantly decreased in total number of cells (10.15 × 106/μl ± 1.10; P < 0.001) and neutrophils (1.53 × 106/μl ± 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, LIM-HC-FMUSP, CNPq, Brazil.

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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 mouse model of human major group rhinovirus infection. However, antiviral approaches have not yet been studied in this 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 < 0.001), neutrophils (P < 0.001 and P < 0.001) and macrophages (P < 0.01 and pns). Intranasally administered ICAM-1 antibody reduced HRV16 induced IL-β (P < 0.001), IL-6 (P < 0.001 and IFN-γ) (P < 0.001) as well as iNOS (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 HRV18 as well as in an LPS challenge model showed no effect of ICAM-1 antibody on either HRV18- 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.

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**P3502**

**Rhinovirus infection upregulates SLPI in macrophages**

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**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 (53.0±4.976pg/mL vs 95.2±2.63, P <0.014) in MDM at 24 hours.

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**P3503**

**Potential synergism of drugs with anti-viral and pulmonary oedema clearance activity may be advantageous for influenza patients**

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**P3504**

**Rhinovirus infection upregulates SLPI in macrophages**

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**P3505**

**An anti-human ICAM-1 antibody inhibits human rhinovirus infection in the mouse model of human major group rhinovirus infection**

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**P3506**

**Rhinovirus infection upregulates SLPI in macrophages**

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Acute exacerbations of COPD are mainly mediated by respiratory viruses or bacteria like non-typeable 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(IC) 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 10^4cfu/ml (strain Rd KW20). After 24 hrs and supernatants were harvested for ELISA and Western Blot analysis and tissue were used for in situ Hybridisation (ISH).

NTHi led to significant induction of Interleukin-8 (IL-8) production (HLT: Med 34390pg/ml vs. NTHi 341926pg/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±3.93pg/ml vs. NTHi 7.80±1.22ng/ml, p<0.05; n=4; HLT: Cpn 19935±328pg/ml vs. Cpn+NTHi 8057±7750pg/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 Poly(I:C) or Cpn treatment and that TLR-signaling might be important for enhanced inflammation in viral/NTHi and atypical/NTHi coinfections.

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.

**Conclusions:**

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