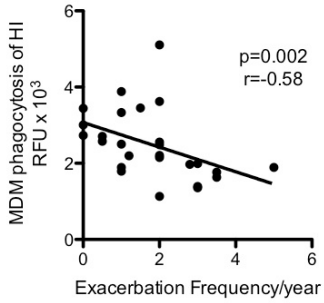

Figure 1. Relationship between MDM phagocytosis of fluorescently-labelled *Haemophilus influenzae* and exacerbation frequency.

Abstract P253 Figure 1

P254 IDENTIFYING MMP-12 SUBSTRATES AS THERAPEUTIC TARGETS IN COPD

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Background Matrix metalloprotease (MMP)-12 is a key protease in COPD which cleaves pulmonary extracellular matrix and non-matrix substrates. Variation in MMP-12 activity affects severity of COPD, yet the mechanism of this, including MMP-12's non-matrix substrates in COPD lungs are unknown. Targeting MMP-12 substrates may lead to the development of drugs for COPD with reduced side effects compared to the broader spectrum MMP inhibitors.

Aims To identify MMP-12 substrates of relevance to COPD and determine how their activity affects disease progression *in vitro* and *in vivo*.

Methods *In vitro* cleavage assays: After literature review the pro-inflammatory mediators osteopontin and tumour necrosis factor (TNF)-α were selected as potential MMP-12 substrates in COPD. Both were incubated with MMP-12 and reaction products analysed by silver stain and western blot. EDTA was used as a metalloprotease inhibitor and thrombin as positive control. COPD cohort: Patients with COPD were recruited during exacerbations at the Nottingham University Hospitals NHS Trust. Sputum, lung function and other data were collected on Day 0 and 1 and 4 weeks later. Sputum was analysed by western blot for proteins of interest. The study was approved by the local research ethics committee and all patients gave informed consent.

Results MMP-12 cleaved osteopontin and pro-TNF-α in a dose and time-dependent manner when visualised by silver staining. Cleavage was dependent on MMP-12 activity as it was inhibited by EDTA. Western blot of cleaved protein fragments gave a characteristic band signature. MMP-12 was present in sputum of patients with COPD as demonstrated by western blotting, ELISA and casein zymography. Western blot analysis of sputum with anti-osteopontin antibodies showed a similar band signature to the *in vitro* cleavage suggesting osteopontin is cleaved in the airways of patients with COPD.

Discussion MMP-12 possesses proteolytic activity against osteopontin and pro-TNF-α *in vitro*. MMP-12, osteopontin and TNFα are present in COPD sputum and our data suggest that MMP-12 may target osteopontin in COPD. Further work is needed to determine the precise mechanisms of such MMP-12 substrate activity in COPD.

P255 THE EFFECTS OF HYPOXIA ON NEUTROPHIL DEGRANULATION

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Tissues such as the skin and intestinal epithelium experience physiological hypoxia whereas pathological hypoxia occurs at inflammatory sites. Neutrophils are recruited to infective/inflamed areas and are thus required to operate under low oxygen tensions. We have shown previously that hypoxia delays neutrophil apoptosis (JEM 2005; 201:105) and impairs bacterial killing (J Immunol 2011; 186:453) and have now studied the effect of hypoxia on the release of histotoxic neutrophil proteases.

Neutrophils isolated from healthy volunteers were subjected to normoxia or hypoxia (3 kPa). Superoxide anion release was measured by the reduction of cytochrome. C Elastase release was quantified by the cleavage of labelled elastin.

Hypoxic incubation for 4 hours resulted in a 3- fold reduction in superoxide anion release from cells stimulated with GM-CSF and fMLP. In contrast, elastase release from the azurophilic granules was augmented almost 3-fold under hypoxia. The release of MMP-9 and lactoferrin was similarly up-regulated, suggesting a more generalised increase in degranulation under hypoxia. In addition to this electron microscopy showed that hypoxia induced a more activated phenotype (e.g. increased membrane ruffling and cell spreading).

We show that hypoxia can induce a more destructive neutrophil phenotype, with enhanced degranulation and release of potentially histotoxic proteases, impaired bacterial killing, and delayed apoptosis. These data suggest that hypoxia aversively affects neutrophil function and may augment neutrophil mediated tissue destruction.

P256 P2X4 AND NNOS EXPRESSION IN HUMAN CILIATED AIRWAY EPITHELIUM

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Background Calcium (Ca^{2+}) and nitric oxide (NO) modulate ciliary beat frequency (CBF). NO synthase (NOS) isoenzymes, responsible for NO production, localise in human airway epithelium and constitutive NOS activity is dependant on Ca^{2+}. Adenosine triphosphate gated purinergic ion channels, comprised of $P2X_{1-7}$ subunits, govern Ca^{2+} influx and $P2X_4$ reportedly localises to rabbit airway cilia. Also, P2X proteins co-localise with neuronal NOS (nNOS) in guinea pig cochlea outer hair cells and rat hypothalamus. We hypothesised that $P2X_4$ and nNOS co-localise in human airway cilia and are involved in modulating CBF.

Objectives To determine $P2X_{1,3-7}$ mRNA expression and $P2X_4$ and nNOS localisation in human nasal epithelium, and assess their possible interaction in CBF modulation.

Methods and Results Relative to *β-actin* expression, consistent and moderate $P2X_{4,6}$ mRNA and variable $P2X_{3,5,7}$ mRNA were detected by RT-PCR in (n=4) human primary airway epithelium cultured at air-liquid interface (ALI). In ALI cultured primary epithelium $P2X_4$ localised (n=3) to cell membranes and cytoplasm and nNOS localised to cilia (n=3) by immunofluorescence. $P2X_4$ localised to the ciliary tips (n=2), whilst nNOS localised to the proximal portion of cilia (specificity confirmed by blocking peptide) in nasal polyp paraffin wax sections by immunohistochemistry (n=6). High speed video microscopy confirmed a 30 minute baseline CBF at 37°C (12.9 Hz SD±0.8, n=5) on nasal epithelium biopsies

(n=2) and 30 minutes of NOS inhibition [with 1 mM: L-N-Ornithine, 1400W and S-Methyl-L-thiocitrulline] reduced baseline CBF by 20% to 9.6 Hz SD±0.9 ($p<0.001$) but the $P2X_4$ inhibitor [10 μM brilliant blue G] had no effect.

Conclusion $P2X_4$ and nNOS are expressed in human airway cilia but do not co-localise. NOS inhibition reduced CBF whilst $P2X_4$ inhibition did not, suggesting that blocking $P2X_4$ activity alone is not sufficient to modify NOS activity or CBF.

P257 HUMAN CYTOMEGALOVIRUS BINDING TO NEUTROPHILS TRIGGERS A PRO-SURVIVAL SECRETOME THAT MODULATES MONOCYTE MIGRATION, ACTIVATION AND PHENOTYPE

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Neutrophils provide the body's first line of defence against invading pathogens. They respond to infection by releasing an array of chemokines, cytokines, and superoxide anions that initiate cascades of other immune mediators and cell types. Although the rapid response and flexibility of neutrophils make them an integral part of the body's immune system, human cytomegalovirus (HCMV), paradoxically, may use neutrophil activation for its own evolutionary advantage. Here we report that human peripheral blood neutrophils exposed to a clinical strain of HCMV display a profound survival phenotype that occurs independent of viral gene expression. The initial HCMV driven survival response was partially dependent on ERK1/2 activation and profoundly inhibited by inhibition of NF-kB. Intriguingly, this initial survival event triggered by virus binding was augmented by a cytokine mediated effect whereby supernatants from infected neutrophils provided uninfected neutrophils with substantial protection against apoptosis – a protection which was PI3K as well as ERK1/2 and NF-kB dependent.

Concomitant with a transferable survival effect the HCMV-neutrophil secretome also markedly manipulated autologous donor monocytes. Enhanced migration and subsequent differentiation to a permissive phenotype for HCMV infection was suggestive of a mechanism for efficient viral dissemination from the site of initial infection. Fascinatingly, although differentiation to a permissive phenotype was observed this was concomitant with down-regulation of a number of key activators of the adaptive immune response. Overall, these data illustrate the manipulation of an anti-viral response by a pathogen to enhance the outcome of infection which, intriguingly, involves a cell type not productively infected by the pathogen itself. These data further illustrate the complexity of pathogen interactions with the host immune system as well as providing new clues into the mechanisms HCMV exploits for efficient viral dissemination which could have implications on our understanding for HCMV pathogenesis.

Improving the care of sleep apnoea

P258 CHANGE IN SLEEP STUDY AND CPAP PROVISION FOLLOWING THE NIHCE CPAP TA

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Introduction We have assessed whether Primary Care Trusts' (PCTs) commissioning of sleep services has improved following the NIHCE 2008 technology appraisal on CPAP.

Methods We wrote to all PCTs in England, (under the Freedom of Information Act, 2000) for information on sleep studies commissioned, and CPAP prescriptions issued, for the years ending 31 March, 2008, 09, and 10. The PCTs were also asked who provided sleep studies or CPAP on their behalf (NHS hospital, GP, private, or other provider). Some PCTs did not reply, or claimed not to hold data, so we contacted appropriate NHS hospitals to obtain further information. Sleep study data was only obtained from approximately 75% of PCTs or associated hospitals. An alternative set of data, on the Department of Health (DH) website, was also used. Limited sales data from CPAP companies was also available for comparison.

Results In almost all PCTs, sleep studies and CPAP provision were from NHS hospitals. The incomplete data from PCTs showed that sleep studies rose from about 30,000 in 2007/8 to 48,000 in 2009/10, a 3-year increase of about 60%. Data on sleep studies published by DH rose from over 61,000 in 2007/8 to over 86,000 in 2009/10, a 3-year increase of 41%. For CPAP prescriptions issued, only 66% of PCTs submitted data for 2007/8, rising to a 75% response by 2009/10. On the basis of this incomplete information and with some assumptions, annual CPAP prescriptions rose from less than 17,000 in 2007/8 to over 37,800 by 2009/10, a three year increase of 126%. There may be some under-reporting in the earlier years, and the industry sources suggested a 3-year lower increase of nearer 80%. In addition there are likely to be errors of coding. However, there was wide variation between PCTs suggesting patchy performance.

Conclusions We believe that the results show a clear improvement in the number of sleep studies and CPAP prescriptions over this period. Thus, following the NIHCE TA there has been an improvement in patient access to the diagnosis and treatment of sleep apnoea, though we are concerned that the wide variation suggests there is a substantial element of post-code lottery.

P259 PREFERENCE OF PATIENTS WITH OBSTRUCTIVE SLEEP APNOEA SYNDROME (OSAS) FOR CONVENTIONAL CONTINUOUS POSITIVE AIRWAY PRESSURE(CPAP) OR BI-LEVEL CPAP (CFLEX) AND CHANGES IN RESISTANCE TO EXPIRATION

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CFlex (Respironics) Bi-Level device lowers expiratory PAP (EPAP) early in expiration returning EPAP to the set inspiratory PAP (IPAP) before the start of the next inspiration. Most patients with OSAS will express a preference for either CPAP or CFlex at the time of treatment initiation. We investigated the possibility that choice of CFlex was related to interaction of breathing pattern and the CFlex device in 43 newly diagnosed patients with OSAS in a single-blind study. Inspiratory and expiratory time (Ti, Te) were measured by rib-cage and abdominal inductance belts. IPAP and EPAP were measured at the mask by pressure transducer. In 23 patients we recorded simultaneous flow in the CPAP circuit and by using mask specific leak rates derived values for inspiratory tidal volume (Vt insp): (Vt insp = (Mean Inspiratory Circuit Flow x Ti) - (Mask leak x Ti)) Patients tried CPAP or CFlex in random order for periods of 10 minutes or until breathing was stable. One minute epochs of stable breathing were used to calculate mean values for Ti, Te, IPAP, EPAP and Vt insp

Results 19 patients stated a preference for CFlex (CFlexpref), 20 for CPAP (CPAPpref). 4 had no preference and received CPAP. We included them in the CPAP. pref group for analysis. For the whole group (n=43) there was a small but significant fall in mean EPAP on CFlex compared with mean EPAP on CPAP (10.4 vs 11.5 cm H_2O).