408. Phagocytes and dendritic cells

P3859

Characterisation of a new Daisy cell line representative of human alveolar macrophage (hAM)

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Introduction: Techniques used to obtain primary hAM are invasive, often providing low yields which cannot be expanded *in vitro.* No human cell line exists as an alternative. We characterised a new human cell line capable of expansion and spontaneous differentiation.
Methods: Cells were cultured in RPMI1640 with 10% foetal bovine serum and penicillin/streptomycin (100U/ml;0.1mg/ml) in a humidified 5% CO_2 atmosphere, passaged every 2 days. Light and transmission electron microscopy comparing Daisy cells with THP-1 cells stimulated with phorbol myristate acetate (PMA;50μg/ml;24h) was performed. Flow cytometric immunophenotype studies were performed analysing CD 11b, 14, 16, 23, 24, 32, 36, 64, 163 and 206 expression. Results were compared with both THP-1 cells and primary hAM obtained by bronchoalveolar lavage. Phagocytic capability by zymosan uptake (mg/ml;1h), mycoplasma screening by fluorescence microscopy and opsonised antigen binding by flow cytometry were also assessed.
Results: Microscopy showed Daisy cells to be similar in size, shape and granularity to PMA stimulated THP-1 cells with a higher proportion of heterochromatin, pseudopodia and vesicular inclusions. Daisy cells were shown to express lower levels of CD11b, 14 and 32 compared with PMA stimulated THP-1 cells yet higher levels of CD36, 80, 163 and 206. CD marker expression of Daisy cells more closely resembled that of primary hAM. Zymosan was readily phagocytosed, mycoplasma was not detected and high levels of opsonised antigen binding were seen in Daisy but not THP-1 cells.
Conclusions: The new Daisy cell line shows characteristics of mature hAM yet can be maintained and cultured providing a useful tool in respiratory research.

P3860

Sirtuins, the anti-ageing molecules, regulate anti-oxidant capacity via FoxO3 activity in monocytic cell line

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Chronic Obstructive Pulmonary Disease (COPD) is characterized by high levels of oxidative stress due to reduced anti-oxidative stress capacity, leading to an increase of endogenous oxidative stress. Sirtuins, type III histone deacetylases,and FoxO3 are reported to participate of the oxidative stress defence and also the levels of SIRT1 and 6 are reduced in COPD samples (Nakamaru Y, FASEB J, 2009).
Aims: To evaluate the effects of sirtuin inhibition and oxidative stress on the expression of several FoxO3-responsive genes, such as catalase, superoxide dismutase (MnSOD) and thioredoxin (TXN) and also on FoxO3 binding activity.
Methods: THP-1 monocytic cells were treated with a sirtuin inhibitor (sirtinol) and hydrogen peroxide (H202) as oxidative stress reagent. SIRT1, 2 and 6 were knocked down using RNA interference. The mRNA levels of catalase, MnSOD and TXN were measured by RT-PCR and normalized against GNB2L1. FoxO3 binding capacity was measured by ELISA.
Results: FoxO3 nuclear localization was not affected by H202 or sirtinol, but the activity binding was increased with oxidative stress. This activation was abrogated with a pretreatment of sirtinol. H202 increased mRNA levels of MnSOD (25%) and TNX (70%), and again sirtinol pretreatment led to the abrogation of this activation. SIRT1, 2 and 6 were knocked down by 50% at protein level. TXN activation by H202 was blocked in every sirtuin knock down.
Discussion: Defect of sirtuin expression may cause a reduction of the anti-oxidative stress capacity by regulating mRNA levels of TXN through FoxO3 in COPD. These results highlight the important role of sirtuins and its possible use as therapeutic target for treating COPD.

P3861

YKL-40: Novel marker for pro-inflammatory M1 macrophages

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Macrophages play a major role in the pathogenesis of COPD and comprise a heterogeneous cell population with pro- (M1) and anti-inflammatory (M2) cells. CD163 has been identified as a M2 marker, however, many M1 markers are not suitable for analysis. Cells positive for YKL-40, a chitinase-like-protein, are elevated in the lungs of smoking than non-smoking COPD patients. Dexamethasone strongly reduces YKL-40 expression in peripheral blood mononuclear cells *in vitro*, suggesting that inhaled steroids in COPD may decrease YKL-40 expression. We evaluated YKL-40 expression in cultured M1 and M2, and the effect of dexamethasone on its expression.
Monocytes were cultured *in vitro* for 7 days with GM-CSF or M-CSF (for M1 and M2, resp.) and stimulated for 24h with LPS, TNFα, oncostatin M or IL-6. Dexamethasone was added in various concentrations. IL-12 and IL-10 ELISA were used to check differentiation into M1 and M2, resp. YKL-40 ELISA was performed on supernatants; quantitative PCR (qPCR) was used to analyze YKL-40 mRNA. Differences between conditions were calculated with Wilcoxon signed rank tests.
M1 cells secreted more YKL-40 than M2, independent of stimulation (261ng/ml vs 65ng/ml, p=0.03). Compared to medium, LPS, TNFα, oncostatin M or IL-6 stimulation did not affect YKL-40 release in M1 and M2 ($p>0.05$). qPCR confirmed these results. Dexamethasone dose-dependently and strongly inhibited YKL-40 in both M1 and M2 ($p<0.05$).
In conclusion, M1 have a higher expression of YKL-40 than M2, which is unchanged by pro-inflammatory stimuli. In addition, YKL-40 release is strongly inhibited by dexamethasone in both M1 and M2. These results indicate that YKL-40 expression can be used as a marker for M1 macrophages *in vitro*, and possibly *in vivo*.

P3862

Lipid laden alveolar macrophages arise from airway reflux and aspiration

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Introduction: Lipid laden macrophages have previously been reported to be present in the diseased human airway, secondary to gastro-oesophageal reflux. We investigated the possibility that the lipid index (LI) system of scoring cellular lipid content by oil red o (ORO) staining could correlate with disease status and the Hull Airway Reflux Questionnaire (HARQ). We also investigated the hypothesis that lipid could be ingested directly from gastric contents by alveolar macrophages.
Methods: Primary alveolar macrophages were obtained from bronchoalveolar lavage (BAL) fluid of patients undergoing diagnostic bronchoscopy. Patients were asked to fill in the HARQ prior to treatment. Lavage fluid was filtered and centrifuged (350xg;10min.) and cells transferred to glass slides using cytospin equipment. Cells were stained with ORO, counterstained with haematoxylin and macrophages scored according to the LI system. Meanwhile THP-1 cells stimulated to differentiate with phorbol myristate acetate (PMA;50μg/ml;24h) were incubated with varying concentrations of a high fat liquid meal and a fat free liquid meal as a control, at 37°C. The high fat meal contained a combination of canola and sunflower oil.
Results: 18 patients with a range of respiratory diseases had LI scores ranging from 4 to 309. LI score correlates ($r = 0.86$) with the HARQ but not to a particular disease group. PMA stimulated THP-1 cells showed maximal lipid accumulation by ORO staining with 10% v/v high fat liquid meal for 24h. No lipid accumulation was seen with the control feed.
Conclusion: Macrophages were shown to be capable of lipid uptake directly from liquid meal and airway reflux correlates with the presence of lipid laden macrophages in the airway.

P3863

Reprogramming of alveolar macrophages – Prospect of successful treatment in COPD

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Reprogramming of macrophages can be one of new promising approaches in nosotropic therapy influencing initial branch of inflammatory reaction and allowing to reach the optimal Th1/Th2 balance in early pathologic process.
Objective: To testify that serum concentration change with TGF-β as macrophages reprogramming factor in culture medium can purposefully reprogram macrophages phenotype either to M1 pro-inflammatory or to M2 anti-inflammatory and therefore influence Th1/Th2 balance.
Methods: Alveolar macrophages of COPD patients with initial M1 phenotype were cultured and reprogrammed in vitro in RPMI-1640 with different serum concentrations – 5%, 10%, 15% for 48 hours. Reprogramming of macrophages was measured by morphological characteristic of cells with morphological index, expression level of cell markers CD80, CD25 (M1), CD206, CD163 (M2) and cytokine production by flow cytometry.
Results: Significant changes of morphological characteristic and functional activity were educed. Serum concentration change from 5% to 15% led to increasing of fibroblast-like cells (M2) from 24 to 58%, morphological index varied from 0.26 to 1.02; Th2 cytokines prevailed on Th1; CD206 and CD163 expression level significantly changed – 57% and 64% vs 21% and 29% respectively. These data confirm successful reprogramming of macrophages to M2 phenotype. In view of obtained data decreasing of serum concentration from 15 to 5% led to M1 phenotype reprogramming.
Conclusions: Serum concentration in cell culture promotes reprogramming of morphological and functional phenotype of alveolar macrophages, which can be used for reaching Th1/Th2 balance and regulation of lung immunity in COPD patients.

P3864

Lipid laden macrophages in patients with chronic cough

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Background: Gastroesophageal reflux disease (GER) has been described as common cause of chronic cough by either esophageal acid-induced bronchoconstriction or recurrent microaspirations of gastric content. Lipid laden macrophages (LLM) in sputum can appear in these patients as a marker of aspiration. This finding could be interesting mainly when symptoms are absent (up to 75% percent or cases, "silent" GER). However, it is not clear what the presence of LLM in the airways could imply in terms of lung function, radiological lesions or symptoms. We aim to compare a group of patients with chronic cough and LLM in sputum with another group without LLM.
Methods: Twenty five patients with chronic cough that could not be attibuted to any known etiology were included in the study group. Subjects were questioned for pulmonary and GER symptoms, Rhinitis, drugs and underwent physical examination, chest X-rays, bronchial challenge test with methacholine and sputum induction. Prepared cytospins were stained with oil red-O to detect lipid laden macrophages.
Results: 68% were lipid laden positive but only 40% had reflux symptoms. Patients with LLM had similar dyspnea, phlegm's but not rhinitis and 25% were methacholine positive and diagnosed of having asthma. Patients with LLM had radiological findings (mild lesions) in 60% of them and they had less FEV1 240 ml (11% of ref val) and FVC 364 ml (7% ref. val) when adjusted by asthma and anthrometric characteristics.
Conclusions: The presence of LLM in sputum could represent worse pulmonary disease in patients with chronic cough even in absence of GER symptoms.

P3865

Effects of *Bu-Zhong-Yi-Qi-Tang* (*Hochuekkito:* TJ-41) on the inflammatory responses in alveolar macrophages of hyperglycemic mice

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This study examines the influence of *Bu-Zhong-Yi-Qi-Tang* (*Hochuekkito*; TJ-41) on the inflammatory response in alveolar macrophage in hyperglycemic mice. BALB/c mice (males, six to eight weeks old) were divided into three groups: A, B, and C. From the outset, group A and B were fed an ordinary diet, whereas TJ-41 was given to group C. After the initiation of these diets, intraperitoneal injection of streptozotocin (STZ), 250mg/g in mice of groups B and C. Four weeks after the start of these diets, blood-glucose levels were measured, and bronchoalveolar lavage (BAL) was performed. Alveolar macrophages were sampled, and those demonstrating blood-glucose levels of more than 200 mg/dL were selected for use. Toll-like receptor (TLR) ligands (TLR2: peptidoglycan [PGN], TLR4: LPS, TLR5: flagellin [FLG]) were employed to stimulate pulmonary-alveolar macrophages, and ELISA was used to measure TNF-a production. TLR expression on pulmonary-alveoli surface was evaluated on the basis of the emergence of each TLR mRNA in alveolar macrophages. In mice given STZ, stimulus with TLR2, 5 ligands significantly inhibited TNF-a production. But in mice given a diet including TJ-41 inhibition of TNF-a production improved significantly. No differences were observed in the expression of TLR mRNA in pulmonary-alveolar macrophages and on cell surfaces. These results suggests the possibility that TJ-41 may enhance inhibition of pulmonary-alveolar macrophage inflammation response to TLR-ligands stimulus in conditions of high blood glucose.

P3866

LSC 2011 Abstract: Differential effects of diesel exhaust particules and endotoxin on phagocytosis and cytokine release by monocyte-derived macrophages

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Atmospheric pollution is associated with COPD exacerbations. Diesel exhaust particles (DEP) are major contributors to pollution and when inhaled become targets for lung macrophages. The interaction between DEP and bacteria/endotoxin is unclear. Monocyte-derived macrophages (MDM) from non-smokers (NS), smokers (S) or patients with COPD were exposed to DEP (1-300μg/ml) with or without bacterial endotoxin (LPS; 10ng/ml) for 24h. Release of TNFα, IL-6 and CXCL8 was analysed by ELISA, and phagocytosis of fluorescent beads was assessed by fluorimetry. MDM viability was determined by MTT assay.
Exposure of MDM to DEP did not stimulate release of IL-6 or TNFα but increased CXCL8 release (EC_{50}: NS 8±4 μg/ml, n=7; S 4±1 μg/ml, n=4 and COPD 10±4 μg/ml, n=7). MDM from COPD patients were 3.3 times more responsive to DEP (30μg/ml; $p<0.05$) than cells from smokers. When MDM were stimulated with LPS, DEP did not alter TNFα or CXCL8 release. However, DEP inhibited LPS-stimulated release of IL-6 in cells from all subject groups (EC_{50}: NS: 33±14 μg/ml, n=10; smokers: 25±15 μg/ml, n=4; COPD: 18±14 μg/ml, n=10). Exposure of MDM to DEP in both the absence and presence of LPS inhibited phagocytosis of beads in a concentration-dependent manner with 300μg/ml showing ~80% inhibition (control: 31±3 X 10^3 *vs* 300μg/ml DEP: 6±1 X 10^3 RFU, n=29), with MDM being 70% viable at this concentration, with no difference in response between the different subject groups. These data show that DEP impair macrophage phagocytosis and promote chemokine release. These data suggest that DEP may lead to reduced clearance of debris and pathogens from airways and promote COPD exacerbations.

P3867

Changed phagocytic activity and pattern of Fcγ and complement receptors on blood monocytes in sarcoidosis

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We have recently revealed that mycobacterial heat shock proteins (Mtb-hsp), involved in forming of immune complexes (CIs), can induce immune response in sarcoidosis (SA). The complexemia may result from inappropriate phagocytosis and clearance of CIs by monocytes, which results in persistent antigenemia and granuloma formation. It is also possible that aberrant expression of receptors for Fc fragment of immunoglobulin G (FcγR) and complement receptors (CR) on monocytes can be involved in this process.
To test this hypothesis, we have evaluated the expression of FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16) and CR1 (CD35), CR3 (CD11b), CR4 (CD11c) receptors on blood CD14+ monocytes and phagocytic activity of mononuclear phagocytes in 24 patients with SA and 20 healthy volunteers using flow cytometry.
As compared to healthy controls, we found significantly increased expression of all examined FcγR (CD32, CD16, CD64) and decreased expression of CD35 and CD11c on CD14+ monocytes in SA patients. Analysis of the combined phenotypes of monocytes revealed significantly increased percentage of CD14+CD16+CD35-, CD14+CD64+CD35+, CD14+CD64+CD11b+, CD14+CD64+CD11c+ monocytes and decreased presence of CD14+CD32-CD35+, CD14+CD32-CD11b+ and CD14+CD32-CD11c+ monocytes in SA vs controls. The total number and percentage of phagocyting blood monocytes was significantly increased in SA as compared with healthy individuals.
In summary, altered expression of Fcγ and complement receptors on CD14+ monocytes and increased phagocytic activity of monocytes may be responsible for high antigen load, persistent antigenemia and complexemia in SA patients.

P3868

Immune complex binding by Fcγ receptor IIA and regulation of inflammation

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Immune complexes, mainly associated with autoimmune diseases, may also be

present in the blood and lung during respiratory diseases including bacterial pneumonia and ARDS. However, the precise pathogenic role of immune complexes in these conditions is unclear.
Binding of immune complexes to the uniquely human stimulatory IgG receptor FcγRIIA is constitutively suppressed on neutrophils and macrophages. Elucidating the mechanism behind this suppression will help us understand how the body regulates its response immune complexes.
The erythroleukaemia cell line K562 that expresses the FcγIIA inherently and exhibits FcγRIIA suppression was used as a model. The effects of microbial neuraminidases on immune complex binding by the FcγRIIA were tested by flow cytometry. We also investigated whether there is a protein in close proximity to FcγRIIA that could potentially block its IgG binding site by protein cross-linking. Lastly, the stimulatory character of the receptor was explored by calcium signalling.
Although the receptor exhibits suppressed immune complex binding, microbial neuraminidases can significantly augment the ability of the FcγRIIA to bind immune complexes. The cross-linking experiments reveal that there is a protein in close proximity with FcγRIIA that could be blocking its IgG binding site under basal conditions. Finally, while FcγRIIA exhibits limited binding of immune complexes in its native state, the stimulatory signals produced by the receptor on contact with immune complexes are significantly strong.
Understanding how the body regulates immune complexes could lead to identification of novel methods for both activating and inhibiting the progression of immune complex mediated inflammation.

P3869
Anti-inflammatory activity of macrolides in peripheral blood mononuclear cells and the identification of potential biomarkers of azithromycin administration
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Many studies in the recent past have determined that macrolide antibiotics have anti-inflammatory and immunomodulatory activity in addition to their efficacy in treating bacterial infection. Macrolides have been successfully used in the treatment of diverse chronic inflammatory respiratory disorders, including diffuse panbronchiolitis (DPB), cystic fibrosis (CF) and bronchiolitis obliterans (BOS). We investigated the ability of azithromycin to attenuate the effects of a lymphocyte directed pro-inflammatory stimulus in PBMCs from healthy volunteers. Our results demonstrate that azithromycin significantly inhibited the induction of proliferation and the release of IL17 in these cells. Treatment with azithromycin in the absence of a pro-inflammatory stimulus in PBMCs induced the release of IL10 in a dose dependent manner 24 h after challenge. In the same model, azithromycin caused a dose dependent, bell-shaped release of GMCSF 24 h after treatment. In conclusion, azithromycin exhibits significant anti-inflammatory and immunomodulatory properties and IL10 and GMCSF may prove to be useful biomarkers following macrolide therapy in respiratory disorders.

P3870
A novel macrolide/fluoroketolide, solithromycin (CEM-101), reverses corticosteroid insensitivity via activation of protein phosphatase PP2A
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Introduction: Activation of PI3 kinase causes oxidative stress-induced corticosteroid (CS) insensitivity via HDAC2 reduction. We have recently demonstrated that a novel macrolide/fluoroketolide, solithromycin (Soli, CEM-101) restores CS sensitivity via HDAC up-regulation due to PI3K signaling inhibition (ATS2010). However, the mechanism of this effect has not been elucidated.
Aims: To investigate the role of a serine/threonine phosphatase PP2A on regulation of the PI3K pathway as the target of Soli.
Methods: CS sensitivity was determined by IC_{50} of dexamethasone (Dex) on TNFα-induced IL-8 production in U937 monocytic cell. Activities of HDAC2 and PP2A were measured by fluorescence-based activity assay. Phosphorylation levels of Akt as a marker of PI3K activation were determined by Western blotting. Okadaic acid (OA) was used to inhibit PP2A as needed.
Results: OA enhanced H_2O_2-induced Akt phosphorylation and HDAC2 reduction in U937 cells, and recombinant PP2A reduced Akt phosphorylation levels. Soli restored Dex sensitivity under H_2O_2 exposure, but pretreatment with OA abrogated Soli-mediated restoration of Dex sensitivity, inhibition of Akt phosphorylation, and HDAC2 activation. In addition, PP2A immunoprecipitates from the membrane fraction and recombinant PP2A were directly activated by Soli.
Conclusions: PP2A might be a negative regulator of PI3K signalling. Soli activates PP2A directly, inhibits Akt phosphorylation, then restores HDAC2 activity, resulting in the restoration of CS sensitivity under oxidative stress. Thus, Soli may be a potential treatment for steroid insensitive diseases such as COPD and severe asthma.

P3871
Exposure to cigarette smoke affects the response of dendritic cells to pneumococcus
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Development of chronic obstructive pulmonary disease (COPD) is linked to tabagism. Acute exacerbation potentially related to infection by streptococcus pneumoniae is responsible for the progression of the disease. Innate immunity associated with dendritic cells (DC) mobilization is involved in the pathophysiology of the disease. We hypothesize that cigarette smoke impairs the response of DC to pathogens, a mechanism that may be involved in COPD progression.
Monocyte-derived DC of healthy donors were exposed to cigarette smoke extract (CSE) and next to pneumococcus (serotype 1). First, endocytosis and bactericidy of S. pneumoniae was analyzed. DC phenotype (costimulatory molecules and endocytosis receptors) and production of immunoregulatory cytokines was measured as well as the capacity of DC to activate autologous T-cells.
Our data showed that CSE exposure inhibited the pneumococcus-dependent expression of CD40, CD80 and CD86 costimulatory molecules. Similarly, proinflammatory cytokine production (IL-12 and TNF-α) was inhibited by CSE exposure. In DC/T coculture, this was associated with a decrease secretion of IL-17 and IFN-γ by T cells. Unexpectedly, this exposure to CSE increased the endocytosis of pneumococcus, whereas CD206 and CD36 expression was affected in an opposite manner. The effect of CSE was mostly related to oxidative stress since it was inhibited by addition of N-acetyl cystein.
In summary, CSE exposure impairs the capacity of DC to activate antigen-specific T-cells against pneumococcus, although it does not alter their endocytosis. These data will be conforted by the ex vivo analysis of DC phenotype in COPD patients with exacerbation.

P3872
OM-85 shapes dendritic cell activation into a "pre-alert" phenotype
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Background: OM-85 (Broncho-Vaxom®, Broncho-Munal®, Ommunal®) has proven efficacy in the prevention of recurrent infections of the airways and acute exacerbations in COPD. OM-85 is a lyophilized bacterial extract comprising fractions of 21 different inactivated bacterial strains. Previous work has shown that OM-85 acts as an immunostimulant, and that it may act on human dendritic cells (hDC). However, the molecular mechanisms through which OM-85 activates hDC remained largely unknown.
Aims: Investigate the impact of OM-85 stimulation on hDC and identify the immune receptors implicated in OM-85 response.
Methods: Primary and *in vitro* derived hDC were used to determine the precise OM-85 effects on DC biology. Stably transfected cell lines expressing human receptors were used to characterize OM-85 receptor-dependent activity.
Results: In hDC, OM-85 induced the secretion of IL-6 and of several chemokines (i.e. CXCL8, CXCL6, CCL3, CCL20, CCL22) with a potency comparable to the prototypical activating stimulus LPS. OM-85 potentiated the effect of IFNγ in terms of IL-6, IL-12 and IL-10 release. The induction of selected chemokines by suboptimal doses of classical pro-inflammatory stimuli were boosted by the presence of OM-85. In addition, it was identified that OM-85 activates TLR2, NOD1 and NOD2 receptors in a significant, dose-response manner.
Conclusions: OM-85 induces a mild and well shaped hDC activation through selected pattern recognition receptors. This activation may contribute to the generation of a "pre-alert state" resulting in an early protection towards incoming infection. These results provide new insights in the understanding of the mode of action of OM-85, opening new venues for further investigation.

P3873
Altered phenotype of blood dendritic cells in patients with acute pneumonia
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Background: Dendritic cells (DCs) play a key role in the host defense against inhaled pathogens. However, the phenotype of blood DCs in patients with acute respiratory infections is unknown.
Objective: To investigate the number and the expression of function-associated molecules of blood DCs in patients with acute infectious pneumonia.
Methods: Sixteen patients with an acute pneumonia and 19 controls without pneumonia were included in the study. The number as well as the expression of function-associated molecules of myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) were analysed in peripheral blood using four-colour flow cytometry.
Results: Elevated concentrations of procalcitonin (median: 0.55 ng/ml) and the

rapid response to antibiotic treatment suggested a bacterial origin of the pneumonia in the patients. Total mDC (median: 27% of the controls) and pDC counts (median: 53% of the controls) were markedly reduced in patients with pneumonia, as compared to controls. Percentages of blood mDCs, but not pDCs, were negatively correlated with serum concentrations of c-reactive protein (CRP). Patients with pneumonia were characterised by a significantly increased expression of Fc gamma receptors (CD32 and CD64) on mDCs and the Toll-like receptor 9 (TLR9) in pDCs.
Conclusions: Circulating DCs are markedly reduced in patients with pneumonia, and characterised by an upregulation of molecules recognising pathogen-associated molecular patterns (PAMPs) and opsonised antigens.

P3874
Humanized S.typhi flagellin (FliC) transfected non-small cell lung cancer (NSCLC) cells induce local inflammatory and necrotic changes in an experimental model
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Background: Tumors, including NSCLC, develope numerous local mechanisms which impair maturation of dendritic cells (DCs). The delivery of stimulatory signals to DCs in cancer microenvironment is believed to be an effective means to break tumor-induced tolerance.
Aim: Evaluation of immunostimulatory properties of TLR5 ligand, FliC, cell membrane-displayed in experimental model of Lewis Lung Cancer (LLC) transfected with a novel plasmid vector system.
Methods: Cell membrane-expressed humanized FliC transgene was inserted to the pCDNA3.1Zeo(+) plasmid-based construct. Tumor specific two-step transcriptional activation system was additionally used. A549 cells, the model of NSCLC, transfected with FliC-coding plasmid, induced the maturation of human monocyte-derived DCs in co-cultures. Two groups of C57BL6 mice bearing a subcutaneous LLC tumor were injected intratumoraly with in vivo-JetPEI and FliC plasmids or in vivo-JetPEI and phosphate-buffered saline as a control. Four administrations were performed within 1 week. Tumor volumes and animal survival were monitored for 6 weeks.
Results: Plasmid injections delayed the growth of implanted LLC tumors and significantly improved animal survival rates. Histological examination of specific plasmid-injected tumors revealed lymphocyte infiltrates and remarkable necrosis.

P3875
LSC 2011 Abstract: Thymic stromal lymphopoietin is a central regulator of anti-viral CD8+ T cell response
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TSLP is an IL-7 like cytokine that is primarily produced by epithelial cells at mucosal surfaces. It has been widely studied in Th2-driven inflammatory disorders such as atopic dermatitis and asthma. Although both viral nucleic acid analogues and pro-inflammatory cytokines associated with active viral infections are potent stimulators of TSLP expression in vitro, it's role in antiviral immunity is unknown. To elucidate the role of TSLP in viral infections, we investigated the immune response against influenza A in mice deficient in TSLPR. We found that TSLPR-deficient mice exhibited impaired clearance of the virus at late time points post infection, indicative of a defect in the adaptive immune response. Although priming in the lymph node was unaffected, the virus-specific CD8+ T cell response in the lung was compromised in the absence of TSLPR. This defect was characterized by the reduced frequency of virus-specific CD8 T cells as well as reduced effector functions such as granzyme B expression and interferon-γ production. Using mixed bone marrow chimeras and adoptive transfer studies, our data suggests that TSLP affects influenza-specific responses by modulating the function of recruited inflammatory dendritic cells (DCs). Pulmonary DCs isolated from infected lungs of TSLPR deficient mice were defective in inducing proliferation of antigen specific naïve T cells in vitro. Furthermore in the absence of TSLPR the production and trans presentation of IL-15 by CD11b+ inflammatory DC was impaired. We propose that TSLP regulates activation of antigen specific cytotoxic T cells at the site of infection by modulation of DC function and suggest a crucial link between TSLP and IL-15 production during infection.