S125 POTENTIAL INTERACTIONS OF CRACM ION CHANNELS WITH THE CALCIUM ACTIVATED POTASSIUM CHANNEL KCa3.1 IN HUMAN LUNG MAST CELLS

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Introduction and Objectives The influx of extracellular Ca^{2+} is essential for the IgE-dependent release of both preformed mediators and newly-generated autacoids and cytokines from human lung mast cells (HLMC). Members of the recently discovered CRACM (also known as Orai) ion channel family may be responsible for this influx. These channels carry Ca^{2+} selective currents (I_{CRAC}) that are activated when endoplasmic reticulum Ca^{2+} stores are emptied. The major K^+ selective conductance in HLMC is generated by the Ca^{2+}-activated K^+ channel $K_{Ca}3.1$. There appears to be a close functional relationship between CRACM and $K_{Ca}3.1$ channels. $K_{Ca}3.1$ channels activated by the influx of extracellular Ca^{2+} act to hyperpolarise the cell membrane, thus maintaining the driving force on CRACM channels. Here we investigate whether CRACM and $K_{Ca}3.1$ channels physically interact.
Methods Vectors were assembled directing the expression of either CRACM1 or CRACM2 channels tagged at their N-terminus with the c-Myc epitope and human $K_{Ca}3.1$ tagged at its C-terminus with the FLAG epitope. HEK293 cells were transiently transfected to express either a single epitope tagged channel or both a CRACM-myc channel and $K_{Ca}3.1$-FLAG. Cell lysates were prepared and potential interactions between CRACM and $K_{Ca}3.1$ proteins tested by immunoprecipitation and immunoblotting.
Results Expression of CRACM1-myc, CRACM2-myc and $K_{Ca}3.1$-FLAG proteins was confirmed by immunoblotting of lysates of HEK293 cells transiently transfected with the appropriate vector(s). Using an anti c-myc antibody to immunoprecipitate CRACM1-myc protein, $K_{Ca}3.1$-FLAG was found to be co-immunoprecipitated. Co-immunoprecipitation of $K_{Ca}3.1$-FLAG was observed only when it was co-expressed with CRACM1-myc. Similarly, using an anti-FLAG antibody to immunoprecipitate $K_{Ca}3.1$-FLAG protein, CRACM1-myc was co-immunoprecipitated. Again co-immunoprecipitation was dependent on both the CRACM1-myc and $K_{Ca}3.1$-FLAG proteins being expressed together. In contrast, under identical reaction conditions, no co-immunoprecipitation of $K_{Ca}3.1$-FLAG was observed when co-expressed with CRACM2-myc.
Conclusions The co-immunoprecipitation of $K_{Ca}3.1$-FLAG with CRACM1-myc and vice versa provides evidence that these channels do physically interact. Such an interaction in HLMC would make both channels a potential therapeutic target in the treatment of asthma and would allow $K_{Ca}3.1$ to be added to the growing list of CRACM1 protein binding partners.

S126 THE CELL ADHESION RECEPTOR CADM1 REGULATES OTHER ADHESION RECEPTORS IN HUMAN MAST CELLS

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Introduction and Objectives Cell adhesion molecule 1 (CADM1) contributes to adhesion, viability and adhesion-induced degranulation in mast cells. Severity of asthma correlates with accumulation of human lung mast cells in specific compartments, including airway smooth muscle (ASM). human lung mast cells adhesion to ASM is known to be mediated by CADM1, integrins and other receptors. Here we investigated the role of CADM1 in the cell-cell and extracellular matrix (ECM) adhesion of mast cells to primary ASM.
Methods The mast cell line HMC-1 was used in adhesion assays to primary ASM and ECM produced by these cells. ECM was prepared by hypotonic extraction with detergents to remove cell content. Modulation of CADM1 expression by adenoviral delivery in HMC-1 cells was verified by FACS and Western blotting. Cells with modulated CADM1 were examined in adhesion assays.
Results HMC-1 cells adhered to ASM in a time-dependent manner, reaching 92% and 70% adhesion at 1 h to ASM and ECM, respectively. Addition of EDTA resulted in a reduction of adhesion to ASM and ECM to 55% and 22%, respectively, indicating a major role of cation^{2+}-independent receptors in cell-cell and integrins in ECM adhesion of mast cells. CADM1-overexpression did not change adhesion to ASM (98%), but decreased adhesion to ECM to 55%. CADM1 RNA interference in HMC-1 resulted in 60% reduction of surface CADM1 and complete loss of CADM1 determined by FACS and Western blotting, respectively, 6 days after transduction. CADM1 downregulation resulted in drastically reduced adhesion to both ASM and ECM down to 37 and 17%, respectively. Addition of EDTA further decreased adhesion to ASM and ECM to 13% and 6%, respectively. Thus, CADM1 downregulation drastically decreased net adhesion of mast cells.
Conclusions Mast cell adhesion receptor CADM1 plays a regulatory role in mast cell adhesion by affecting not only cell-cell adhesion, but also ECM adhesion.

Lung infections: mechanisms of disease

S127 ANTI-PROTEIN SEROLOGICAL RESPONSES TO *STREPTOCOCCUS PNEUMONIAE*, IN DIVERSE POPULATIONS

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Although vaccine-induced immunity to *Streptococcus pneumoniae* depends on anti-capsule antibody responses, naturally acquired adaptive immunity may also depend on antibody against surface protein antigens. Unlike capsular antigen, protein antigens are often highly conserved between *S pneumoniae* strains and thus could be effective vaccines against all *S pneumoniae* capsular serotypes. We have investigated the pattern of naturally acquired antibodies to *S pneumoniae* protein antigens in diverse populations and assessed whether they could be protective. Immunoblots against lysates of wild-type and mutant *S pneumoniae* with sera from adult humans demonstrated antibody responses to a large number of protein antigens including PspC, PspA and PhtD. Using a multiplex assay of 18 recombinant pneumococcal antigens conjugated to fluorescent beads (xMAP) we made semi-quantitative assessments of serological responses in sera obtained from individuals from the UK and Malawi, as well as in commercial immunoglobulin (IVIG) preparations, pooled from donors in either Europe or USA. All individuals, in both geographical populations had significant levels of antibody to a number of pneumococcal proteins. Individuals varied in their response to specific pneumococcal antigens, with absent responses to some antigens that were dominant in other subjects. However, pooled sera from Malawi and IVIG products from both Europe and the USA had remarkably similar patterns of antigen dominance, with consistently high levels of antibody responses to the antigens PhtD, PspC, PspA and PsaA and weak responses to PilusA, Eno, NanA and SlrA. To investigate the functional importance of these protein antigens we used in vitro flow cytometry assays of complement deposition and neutrophil phagocytosis using bacteria

which had been incubated in serum pre-treated with the enzyme IdeS (Immunoglobulin-G degrading enzyme of *S. pyogenes*, which selectively cleaves IgG). Complement deposition and neutrophil phagocytosis of unencapsulated *S pneumoniae* TIGR4 strain was reduced in IdeS treated serum compared to untreated serum. These data demonstrate that there are naturally acquired functionally significant antibody responses to a range of conserved *S pneumoniae* protein antigens. The same antigens induce responses in diverse populations, suggesting that these protein antigens would be useful components for a polyvalent protein vaccine that is broadly protective against *S pneumoniae* infections.

S128 HIGHLY INVASIVE CAPSULAR SEROTYPES OF *STREPTOCOCCUS PNEUMONIAE* BIND HIGH LEVELS OF FACTOR H AND ARE RESISTANT TO COMPLEMENT AND PHAGOCYTOSIS

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The *Streptococcus pneumoniae* polysaccharide capsule is an essential virulence factor that varies in structure between serotypes. While certain serotypes are highly invasive, it is unknown why these serotypes frequently cause infection yet others generally cause nasopharyngeal colonisation. Complement is vital in immunity to pneumococcus, and the capsule is known to affect complement deposition. Activation of the alternative complement pathway is promoted by factor B (Bf) binding, but inhibited by factor H (FH) activation. We hypothesised that capsule effects on FH and Bf interactions could alter *S pneumoniae* complement sensitivity, partially explaining serotype-dependent differences in invasiveness. C3b/iC3b deposition, FH and Bf binding to *S pneumoniae* were measured using flow cytometry assays on 20 distinct capsule-switch variants constructed on TIGR4 genetic background. These strains were therefore identical in protein structure, differing only in capsular serotype. Phagocytosis was investigated using an established flow cytometry assay, fresh human neutrophils and FAMSE labelled *S pneumoniae*. FH binding showed wide variations between TIGR4-capsular switched strains, with a 7.5-fold difference between the highest (serotype2) and lowest (serotype11A) results. Differences in FH binding between strains did not correlate with capsular thickness, or with capsule structural motifs such as numbers of carbon atoms per repeating unit. FH binding negatively correlated with Bf binding and C3b/iC3b deposition, demonstrating that increased FH binding was associated with reduced alternative pathway activity and increased resistance to complement. IgG binding did not correlate with C3b/iC3b results, suggesting C3b deposition was independent of antibody-mediated complement activity. Neutrophil association correlated with C3b/iC3b deposition (R^2=0.47, p=0.0008) but negatively with FH binding (R^2=0.74, p<0.0001), confirming that high FH binding by pneumococcus was associated with reduced neutrophil phagocytosis. Weakly- and highly-invasive serotypes showed large significant differences between median C3b deposition (p=0.007), neutrophil association (p=0.0002) and FH binding (p=0.0005). Weakly-invasive serotypes had reduced FH binding, increased C3b/iC3b deposition and increased neutrophil association. Our novel finding that the degree of FH binding to *S pneumoniae* capsular serotypes is associated with large variations in virulence offers a mechanistic explanation as to why certain serotypes are more invasive than others. It also provides a potential in vitro method for identifying highly invasive strains.

S129 NEUTROPHIL FUNCTION AND ADVANCING AGE: THE EFFECTS OF SIMVASTATIN IN HEALTH AND DURING PNEUMONIA

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Background Age is associated with a decline in immunity, including neutrophil function. This may partially explain the worsening clinical outcomes seen following pneumonia in the elderly. Statins may improve outcomes from pneumonia although it is unknown whether they influence neutrophil function at conventional therapeutic concentrations. This is crucial, as statins may be beneficial adjuvants during infections.

Methods We studied the effect of 5 ng/ml Simvastatin (equivalent to 80 mg orally) on key neutrophil functions: speed and accuracy of migration using time-lapse imaging (μm/min), Neutrophil Extracellular Trap formation using cell-impermeable DNA-binding dye (in AFU) and generation of reactive oxygen species (ROS, in RLU).

Results We studied 70 healthy subjects (aged 20–90 years, 10 in each decennial) and 6 young (<35 years) and 6 older (>65 years) patients during an admission with pneumonia. All studied neutrophil functions declined with age (eg, neutrophil chemotaxis, r =−0.7, p<0.001). Specifically, average neutrophil chemotaxis for subjects >65 yrs was 0.72 μm/min (SD ± 0.28, p<0.001) slower than subjects <35. These neutrophils produced less NETS (average difference =−1725AFU±283, p=0.007) and peak ROS (average difference −117RLU±31; p=0.04). Neutrophils from young patients with pneumonia displayed an up-regulation of function that was not seen in older pneumonia patients. There were no age-associated differences in the surface expression of chemo-attractant receptors, but there appears to be differential intracellular signalling with reduced expression of adhesion molecules. Incubating neutrophils from older subjects with Simvastatin improved all functions back to that seen in young subjects (chemotactic speed + 0.92 μm/min ±0.27, p=0.001: NET + 1386AFU±273, p=0.04: ROS +223RLU±39, p=0.005). Similar improvements were seen with neutrophils from older subjects with pneumonia. This was a dose-dependent phenomenon; not seen at higher concentrations of Simvastatin.

Conclusion With age, there is a global deterioration in neutrophil function and no up-regulation when pneumonia is present, which may partially explain the age-associated mortality. Simvastatin up-regulates neutrophil function in the elderly, even during pneumonia. This up-regulation may explain the beneficial effects seen clinically. In vivo studies are warranted, to determine if simvastatin should be utilised during episodes of acute infection.

S130 HIV-1 INFECTION OF MACROPHAGES DYSREGULATES PRO-INFLAMMATORY HOST RESPONSES TO MYCOBACTERIUM TUBERCULOSIS THROUGH INHIBITION OF INTERLEUKIN 10

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Introduction Human immunodeficiency virus (HIV)-1 greatly increases the risk of active *Mycobacterium tuberculosis* (Mtb)