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Book of Abstracts
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Nobel Day Festivities at Biomedicine

9th of December 2010

Traditionally, on 10th of December, the anniversary of Alfred Nobel's death, is awarded the Nobel Prize in Physiology or Medicine. Biomedicine shows attention to this day by organizing own research activities and festivities.

School of Health and Medical Sciences
Department of Clinical Medicine
Örebro University
9th of December 2010

Program Committee:
Nikolaos Venizelos, Assoc. Professor
Anita Hurtig-Wennlöf, PhD
Torbjörn Bengtsson, Professor

VHL overexpression in smoking mice model: similarities to changes in human COPD

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Objectives: Development of pulmonary cachexia syndrome (PCS) in COPD patients directly correlates with their poor clinical status and increased rate of mortality. Decreased skeletal muscle capillarization has been demonstrated in cachectic muscles of patients with COPD; however the mechanisms mediating this impairment remain largely unknown. The current study aims to examine the effects of cigarette smoke exposure on pivotal angiogenic factors driving skeletal muscle capillarization within an experimental mouse model.

Methods: *129SvJ WT* mice were exposed to cigarette smoke or sterile air during 6 months. Skeletal muscle biopsies collected from the gastrocnemius and soleus muscle were analyzed for expression of the hypoxic-angiogenic signaling components using quantitative RT PCR and western blot. The signals studied included crucial angiogenic factors HIF1, VHL and VEGF.

Results: Exposure of *129SvJ WT* mice to cigarette smoke induced a significant overexpression of HIF-1 mRNA (p*=0.029 vs. controls). Likewise, significant VHL mRNA overexpression was elicited (p***=0.00063 vs. controls) and western blot detected increase in VHL protein level. By contrast, VEGFa *mRNA* expression was not altered (p=0.88).

Conclusion: The current results confirm our recent findings of enhanced VHL expression in skeletal muscles of COPD patients. These findings are further extended by demonstrating that exposure of normal mice to cigarette smoke induces HIF-1 and VHL overexpression without concurrent increase in VEGF expression and hence novel capillary formation. This most likely indicates that disturbed transduction of the hypoxic signal towards angiogenesis occurs already at the early stage of smoking and not secondary to development of COPD in these patients.

References:

1. *Jatta K, Eliason G, Portela-Gomes G, Grimelius L, Caro O, Nilholm L, Sirjsö A, Piehl-Aulin K, Abdel-Halim SM. (2009). Overexpression of von Hippel-Lindau (VHL) in skeletal muscles of patients with chronic obstructive pulmonary disease (COPD). J Clin Patho.362:70-6.*
2. *Michaud SE, Ménard C, Guy LG, Gennaro G, Rivard A. (2003). Inhibition of hypoxia-induced angiogenesis by cigarette smoke exposure: impairment of the HIF-1α/VEGF pathway. FASEB Journal. 17:1150-1152.*

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Expression of suppressor of cytokine signalling (SOCS) transcript in UPEC-infected human bladder epithelial cells

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Objective: Suppressor of cytokine signalling (SOCS) proteins are negative regulators of the innate immune system in immune cells. Different pathogens appear to have the ability to modify the host SOCS proteins to evade the immune response and improve their chances to infect the host. Uropathogenic *Escherichia coli* (UPEC) are able to suppress the pro-inflammatory host response evoked by bladder epithelial cells, but the mechanisms are not fully understood. The purpose of this study was to investigate if SOCS are induced in bladder epithelial cells in response to UPEC.

Methods: Isolated bladder epithelial cells (RT-4) were stimulated with the UPEC strain IA2 and a cytokine mixture of IL-1β, TNF-α and IFN-γ. The fold increase of SOCS1 and 3 in comparison to an untreated control was evaluated by real time PCR.

Results: The results showed that SOCS3 transcript was increased 1, 2, 6 and 12 hours after UPEC infection with a peak 5.1±2.3 fold increase after 6 hours of infection. In contrast, UPEC did not increase SOCS1 mRNA expression. Both SOCS1 and SOCS3 transcripts were increased in bladder epithelial cells upon stimulation with cytokines.

Conclusions: This study shows that the expression of SOCS3, but not SOCS1, increases in bladder epithelial cells in response to UPEC infection *in vitro*. The data also suggest that uroepithelial cells may use SOCS proteins to counterbalance the immune response similar to what has been established for immune cells. However, further studies are needed to clarify the significance of SOCS3 in regulation of the uroepithelial host response.

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Physical activity patterns in patients with different degrees of chronic obstructive pulmonary disease

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Objective: It has previously been suggested that exercise capacity is decreased in COPD patients and that it is associated with degree of disease. The reduced exercise capacity may plausibly be due to low levels of physical activity in this patient group. In the present study we aimed to assess exercise capacity and physical activity in different stages of COPD and to examine the associations between exercise capacity, pulmonary function and degree of physical activity.

Methods: 44 COPD patients and 17 healthy subjects participated in the study. Exercise capacity was assessed using the 6 minute walking test and physical activity was assessed using a uniaxial accelerometer worn all waking hours during seven days.

Results: Mean exercise capacity was significantly lower in COPD patients compared with healthy subjects. Mean physical activity level and time spent at least moderately active were significantly lower in patients with moderate and severe COPD compared with healthy subjects while no differences in time spent sedentary were observed between the study groups. Pulmonary function, mean physical activity level and time spent at least moderately physically active were significantly associated with exercise capacity in the patients.

Conclusions: Patients with moderate and severe COPD are significantly less physically active compared with healthy subjects. Furthermore, mean physical activity level as well as physical activity of at least moderate intensity is positively associated with exercise capacity in COPD patients while time spent sedentary is not which stresses an important role of physical activity on exercise capacity in this patient group.

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A functional polymorphism in the retinoic acid catabolizing CYP26B1 affects lipoprotein levels and atherosclerosis.

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Objective: Retinoids regulate many processes, including proliferation, migration, apoptosis, differentiation, inflammation, coagulation and lipid metabolism. Levels of active retinoids are strictly controlled by retinoid catabolizing enzymes in the CYP26 family. We hypothesized that genetic variants of a key retinoid-regulating enzyme, CYP26B1, would affect its activity and, thereby, retinoid levels, inflammation and atherosclerosis development.

Methods: Real-time reverse-transcriptase-polymerase chain reaction was carried out for determining level of CYP26 in atherosclerotic lesion and after exposed for 6h with atRA or vehicle (DMSO). We searched for SNPs in the human CYP26B1 gene in (www.ncbi.nlm.nih.gov) and we found three nonsysnonymous SNPs in CYP26B1: rs2241057, rs2286965 and rs7568553. Rs2241057 is associated with a Leu to Ser substitution at the 264 amino acid position, rs2286965 with a Glu to Lys substitution at the 380 amino acid positions and rs7568553 with a Gly to Ala substitution at the 420 amino acid position. Plasmid constructs with the major T-allele or the minor C-allele in position 264 in CYP26B1 were created and expressed in COS-1 cells.

Results: We studied CYP26B1 in human vascular biopsies, the effect of a functional genetic polymorphism in vitro and in a human cardiovascular cohort. Levels of CYP26B1 mRNA were significantly higher in arteriosclerotic biopsies compared with normal arteries and treatment with retinoids significantly increased CYP26B1 levels in atherosclerotic tissue. Immunostaining confirmed presence of CYP26B1 in atherosclerotic lesions. Three nonsynonymous SNPs were identified in CYP26B1. In a cohort of 1000 healthy Swedish individuals, variation was detectable only in rs2241057 and this locus was chosen for further studies. The minor allele variant catabolized atRA twice as efficiently as the wild-type CYP26B1 when expressed in COS-1 cells. The effect of rs2241057 was then investigated in the Stockholm Coronary Atherosclerosis Risk Factor Study cohort, which includes 387 survivors of a first myocardial infarction and 387 controls. The atherosclerosis burden was larger among carriers of the minor allele and serum lipid levels were higher among statin treated carriers of the minor allele.

Conclusions: The minor allele of the functional polymorphism rs2241057 strongly enhanced the retinoid catabolizing capacity of CYP26B1. Carriers of the minor allele suffered from a larger atherosclerotic burden. Taken together, the findings suggest that CYP26B1-regulation of retinoid levels affects atherosclerosis development.

References:

1-Gidlof AC, Ocaya P, Krivospitskaya O, Sirsjö A. Vitamin A: a drug for prevention of restenosis/reocclusion after percutaneous coronary intervention? Clin Sci (Lond). 2008 Jan;114(1):19-25

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Magnetic Resonance Imaging (MRI) utilizing patient-controlled breath holding – a new direction for improving patient comfort and image quality?

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Objectives: To present an ongoing project in clinical imaging of patients undergoing MRI- liver examination with sequences that require periods of breath holding. These patients are often elderly and seriously ill, which can influence their ability to hold their breath during image acquisition. The aim of the project is to investigate whether image quality and patient comfort is influenced if the breath hold is initiated by the patient or by the Radiographer.
The results from a previous pilot study of seven healthy volunteers' suggest that both Image quality and patient comfort improve when the patient initiate the breath hold (1).

Methods: Thirty adult consecutive patients referred to the Radiology Department at Örebro University Hospital for an MRI examination of the liver will be included. The MRI-scans are performed on an Achieva 1,5T MRI-scanner (Philips Medical Systems) using a 16 channel SENSE-torso XL-coil.
The examinations will be analyzed from two different perspectives: image quality and patient experience.
Study one; a quantitative study will evaluate image quality with a Visual Grading Scale, looking at different criteria like image noise and motion artifacts.
Study two; a descriptive qualitative study with interviews and qualitative content analysis. Qualitative studies investigate phenomena's, in an in-depth, holistic way.

Current status: The project has been initiated (sep 2010) and data collection is running. The local ethic committee has approved the project.

References

1. *Degrell U, Thunberg P, Törnvall AS, Birgersson T, Widell M (2010) MR Liver examinations with patient controlled breath holding – a direction for improving image quality and patient comfort. Poster at annual SMRT meeting, Stockholm 2010.*

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Association between physical activity and blood pressure in young adolescents

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Objectives: The study aimed at describing the associations between physical activity (PA) and blood pressure (BP) in children and young adolescents, statistically controlling for cardiorespiratory fitness (CRF) and adiposity, variables which have previously been shown to be associated with BP (Ruiz, 2007).

Methods: The study involved children (9 years old) and young adolescent (15 years old) from Sweden and Estonia who participated in the European Youth Heart Study (n=1502). Body mass index (BMI) was calculated and used as a measure of adiposity; BP measures were taken by an automatic oscillometric method; CRF was determined by a maximal cycle ergometer test; and PA were measured by the MTI Actigraph® accelerometer, worn for four consecutive days. PA variables derived were: minutes spent sedentary and moderate-to-vigorous PA (MVPA). Pearson's correlation coefficient and multivariate linear regression were used in the statistical analyses, and α set to 0.05.

Results: Significant correlations were observed between BP and the measured explanatory variables. MVPA showed a negative association with systolic BP in adolescents (β= -0.127, p=0.001) and it also modified the associations BP vs. BMI and BP vs. CRF. Sedentary time showed a positive association with systolic BP in adolescents (β=0.077, p=0.034) and it also modified the associations BP vs. BMI and BP vs. CRF. Neither MVPA, nor sedentary time presented any significant associations with BP in children.

Conclusion: More time spent in moderate physical activity is associated with lower systolic blood pressure in young adolescent.

Reference:

Ruiz JR *et al*, Journal of Hypertension. 2007 Oct;25(10):2027-34.

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Challenge of Airway Epithelium by *Staphylococcus aureus* and *Staphylococcus epidermidis* in vitro

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Objective: Cystic fibrosis (CF) patients have an abnormal composition of the airway mucus and are particularly vulnerable to microbial infection. We determined if *S. aureus* and *S. epidermidis* have any effect on the expression of the epithelial sodium channel (ENaC) and chloride channel (CFTR), and could influence ion/water transport and the hydration of the mucus.

Methods: Monolayers of normal human bronchial epithelial cells were infected with clinical isolates of *S. aureus* and *S. epidermidis* at a concentration of 10^4 cfu/ml for 1h, 4h, 6h, 12h, 24h, 36h, and 48h. The mRNA expression of α-, β-, and γ- ENaC, and of CFTR was measured by quantitative real time - PCR (qRT-PCR).

Results: Infection with *S. epidermidis* caused on average a small increase in the expression of α-ENaC. The expression of β-ENaC showed a marked increase of 6.1±0.03 and 8.5±0.03 fold after 36h, and 48h respectively. The expression of γ-ENaC increased 3.7±0.02 fold after 36 h, and 4.3±0.008 fold after 48h. Infection with *S. aureus* induced an increase in the expression of γ-ENaC of 2.1±0.01 fold after 48h. The expression of CFTR was not modulated as distinctly as that of ENaC. *S. aureus* decreased the expression of CFTR to 60% of the control (0.6±0.003) after 36h.

Conclusions: *S. aureus* and *S. epidermidis* modulate the mRNA expression of ENaC and to some extent that of CFTR. In a mouse model overexpressing β-ENaC, CF-like symptoms (viscous mucus) have been produced (Mall *et al*., 2004). The lung inflammation induced by *S. aureus* and *S. epidermidis* infection seems to result in an upregulation of ENaC expression, which may cause Na^+ hyperabsorption and thick mucus in airways. Furthermore, a small decrease in the expression of CFTR may also contribute to water-deficient airway mucus.

Reference:
*Mall M, et al. (2004) Increased airway epithelial Na^+ absorption produces cystic fibrosis-like lung disease in mice. Nat Med **10**: 487-493.*

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Quantitative determination of the meningococcal vaccine component factor-H binding protein (fHbp) in clinical isolates

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Background: To date there is no broad-spectrum vaccine against *Neisseria meningitidis* (nm). Vaccines against serogroup A, C, W-135 and Y are based on the polysaccharide capsule. However, serogroup B, which is the most common cause of disease in many countries (especially Europe) resembles the human sialic acid and is therefore weakly immunogenic. fHBP is currently being evaluated in clinical trails as a vaccine candidate for a meningococcal group B vaccine. We have previously evaluated the prevalence and sequence variation of fHbp and will now investigate the expression of the antigen on clinical isolates to determine its potential success as a vaccine.

Methods: The material will consists of; all invasive nm isolates collected in Sweden during 2001-2002, all invasive nm isolates from patients with a deadly outcome collected from 1995-2004 and 51 carrier isolates from 1995-2004. The fHbp sequences of all isolates will be examined using the LightCycler PCR system and BioEdit Sequence Alignment Editor program. Protein expression will be examined under *in vivo* like conditions using flow cytometry.

Results: The analyses of the results are currently ongoing. A preliminary indication shows that all strains express fHbp but with a large variation in expression pattern. The isolates can be divided into three groups: low, intermediate and high expression.

Conclusions: fHbp is widely expressed and is therefore a potentially successful vaccine candidate against serogroup B nm.

Reference:

Susanne Jacobsson, Sara Thulin, Paula Mölling, Magnus Unemo, Maurizio Comanducci, Rino Rappuoli,and Per Olcen (2006). Sequence constancies and variations in genes encoding three new meningococcal vaccine candidate antigens. Vaccine 24: 2161-2168

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Disturbed tryptophan and alanine transport in fibroblasts from children with attention-deficit/hyperactivity disorder (ADHD)

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Objective: The catecholaminergic and serotoninergic neurotransmitter systems have been implicated in the pathophysiology of Attention-deficit/hyperactivity disorder (ADHD). The amino acid tyrosine is the precursor for synthesis of the catecholamines and tryptophan is the precursor for serotonin. Amino acids are actively transported across cell membranes, like the endothelial cells that constitute a part of the blood brain barrier (BBB), by different transport systems and a competition between amino acids using the same transporters exists. Tyrosine and tryptophan are mainly transported by the amino acid transport system L, specifically the LAT1 isoform [1]. Since a disturbed transport of tyrosine, as well as other amino acids, previously has been found in neuropsychiatric disorders such as bipolar disorder and autism, the aim of this study was to investigate whether also children with ADHD have changes in amino acid transport mechanisms.

Methods: Fibroblast cells obtained from 14 boys with ADHD and from 13 matching controls were cultured. Transport of tyrosine, tryptophan and alanine across the cell membranes was measured by the cluster tray method. The kinetic parameters, maximal transport velocity (V_{max}) and affinity constant (K_m) were determined by use of the Line Weaver Burke plot equation.

Results: The boys with ADHD had significantly decreased V_{max} (p=0.025) and K_m (p=0.026) of tryptophan transport and also had a significantly higher V_{max} of alanine transport (p=0.031) in comparison to controls, but the K_m of alanine transport did not differ significantly. Regarding tyrosine transport, no differences were found in any of the kinetic parameters.

Conclusions: In fibroblasts the selectivity of the LAT1 isoform for tryptophan is similar to the selectivity of LAT1 isoform for tryptophan in hBME cells [2]. Hence, the present finding of a decreased tryptophan transport in the children with ADHD implies that these children may have a decreased transport of tryptophan to the brain. Decreased levels of tryptophan in the brain could lead disturbances in the serotoninergic system. The physiological relevance of an increased alanine transport, which was found in the children with ADHD in the present study, has not been explored. However, at physiological plasma concentrations there is a competition between amino acids for transport across the blood brain barrier (BBB) [1]. So, although alanine is not involved in the syntheses of neurotransmitters, an elevated transport of alanine might influence the transport of other amino acids that are of vital importance for normal brain activity.

References:

1. *R. Vumma, F.A. Wiesel, L. Flyckt, L. Bjerkenstedt, N. Venizelos, Functional characterization of tyrosine transport in fibroblast cells from healthy controls, Neurosci Lett 434 (2008) 56-60.*
2. *Umeki, N., et al., mRNA expression and amino acid transport characteristics of cultured human brain microvascular endothelial cells (hBME). Drug Metab Pharmacokinet, 2002. 17(4): p. 367-73.*

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Toll like receptor mediated adhesion of platelets on bacterial peptide-mimetic surfaces.

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Background: Besides their role in haemostasis, platelets are nowadays considered to have an important function in inflammatory processes, for instance atherosclerosis, as key players of the innate immune system. Toll like receptors (TLRs), expressed on the platelet surface, recognize foreign pathogens and trigger immune responses when activated. Pam_3CSK_4, a synthetic lipopeptide, acts as a ligand for TLR2/1 and activates platelets. This study aims to investigate how adsorbed Pam_3CSK_4, resembling a biofilm, affects the adhesion and activation of platelets, and whether adenine nucleotides and production of cytokines are involved in this process.

Methods: To examine activation and granule secretion due to Pam_3CSK_4 stimulation, Pam_3CSK_4 was coated onto hydrophobic surfaces at which platelets pre-incubated with the $P2X_1$- (ATP) antagonist MRS2159, the $P2Y_1$- (ADP) antagonist MRS2179 or the phospholipase C- (PLC) inhibitor U73122 were allowed to adhere. Interleukin-1β was measured by immunoassay.

Results: A significant decrease both in cell number and covered area was found when platelets were pre-incubated with MRS2159 before added to Pam_3CSK_4-coated surfaces, indicating that platelets are dependent on ATP-secretion for activation. Treatment with MRS2179 instead led to an increase in cell number and covered area, however, not significantly. Inhibition of the PLC signalling pathway induced a modest increase in number and covered area.

Conclusions: Pam_3CSK_4-induced adhesion of platelets is dependent on ATP-release while inhibition of ADP-receptors and PLC does not affect platelets in the same manner. These results further strengthen the role of the platelets as an active player in sensing bacterial infections.

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Is there an altered fibroblasts phenotype in chronic venous wounds?

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A proportion of chronic venous wounds fail to heal in response to standard therapy. One contributory mechanism may be inhibition of fibroblast proliferation and induction of a stress-induced premature senescence phenotype, due to the continuing inflammation found in chronic wounds.
With this study we want to investigate the impact of environmental-driven cellular aging on wound healing by conducting a comprehensive analysis of chronic wound fibroblast (CWF) behaviour in comparison with patient-matched healthy normal skin fibroblasts (NF): Proliferation, migration and growthfactor response are investigated.
We are currently collecting and isolating fibroblasts from skin biopsies as well as setting up methods. Other studies have showed that the replicative capacity of CWF exhibited a decreased proliferative lifespan compared with NF before becoming senescent. The dysfunctional wound healing potential have further been corroborated using monolayer scratch wound assays, they showed that the rate of wound repopulation by all CWF samples was significantly reduced compared to patient-matched NF.

References:

Wall IB, Moseley R, Baird DM, Kipling D, Giles P, Laffafian I, Price PE, Thomas DW, Stephens P. Fibroblast dysfunction is a key factor in the non-healing of chronic venous leg ulcers. J Invest Dermatol. 2008 Oct;128(10):2526-40.

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Increased frequencies of Ki67$^+$ proliferating and CD45RO$^+$ activated/memory CD8$^+$ and CD4$^+$8$^+$ T lymphocytes in the intestinal mucosa of collagenous colitis patients

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Objectives: Microscopic colitis (MC) is a common cause of chronic diarrhoea, comprising lymphocytic (LC) and collagenous colitis (CC). The goal of this project is to phenotypically characterize mucosal lymphocytes in CC patients.

Methods: Lamina propria lymphocytes (LPLs) and intraepithelial lymphocytes (IELs) were isolated from mucosal biopsies from hitherto 3-5 CC patients and 4-7 healthy individuals, and analyzed by flow cytometry.

Results: In CC patients, the frequencies of CD8$^+$ (28% vs 32%) and CD4$^+$8$^+$ double positive (DP) (2.2% vs 6.5%) LPLs were increased compared to healthy controls. In addition, the frequency of proliferating Ki67$^+$CD8$^+$ T cells in the LP were increased (4.4% vs 14.2%), as well as the frequencies of memory CD45RO$^+$CD8$^+$ (50% vs 79%) and the CD45RO$^+$ DP T cells (42% vs 71%). Similarly, the frequencies of CD8$^+$ IELs (31% vs 49%) as well as DP T cells (1.5% vs 12%) were increased in CC patients. The frequencies of CD45RO$^+$CD8$^+$ (59% vs 88%) as well as CD45RO$^+$ DP IELs (41% vs 84%) were also increased in CC compared to controls. The numbers of Ki67$^+$CD8$^+$ IELs were also increased (7.2% vs 32.1%) in CC. In contrast, the frequency of CD4$^+$ LPLs was decreased (60.6% vs 50.5%). Despite this, the CD4$^+$ population consisted of higher frequencies of CD45RO$^+$ (60% vs 78%) as well as Ki67$^+$ cells (1.5% vs 5%).

Conclusions: Increased numbers of CD8$^+$ and DP T cells with a phenotype characteristic of memory/activated and proliferating cells are found in the intestinal mucosa of CC patients.

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Modulation of histamine 4 receptor mRNA and protein expression in Gαi2-deficient mice during colitis progression.

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Objectives: Many studies on IBD have been performed in different knockout mice as animal models of colitis; mice deficient in the G protein αi2 subunit, Gαi2$^{-/-}$ mice, being one of them. They develop a disease similar to UC including development of colon cancer. The most recently described histamine receptor; the histamine 4 receptor (H4R) is expressed on various immune cells. We have analyzed expression of histamine receptors in Gαi2$^{-/-}$ mice.

Methods: The expression of H1R, H2R, and H4R in mucosal tissues from Gαi2$^{-/-}$ mice in different stages of colitis has been analysed by qRT-PCR and H4R by immunohistochemistry.

Results: We found that in colon, there were 3 fold reductions of both H4R and H2R mRNA expression in precolitic mice compared to wild type mice ($P<0.05$). The H4R levels increased with colitis progression, as they were markedly elevated from pre colitis to late colitis ($P<0.01$). In the small intestine, our preliminary data demonstrate that the H4R levels were slightly increased in early and late colitis mice compared to wild type mice, whereas there were no difference between precolitic and wild type mice. In contrast the H2R and H1R mRNA expression was not changed by the colitis development. Immunohistochemical analysis revealed expression patterns on H4R in colon similar to mRNA results; the H4R protein levels were reduced in precolitic mice compared to wild type mice and they were increased from pre colitis to late colitis.

Conclusions: The H4R levels are altered during colitis development in Gαi2$^{-/-}$ mice.

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Lipoxin A_4 inhibits *Porphyromonas gingivalis*-induced aggregation and ROS production - role of neutrophil/platelet interaction and CD11b expression

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Objective: *Porphyromonas gingivalis* is an etiological agent strongly associated with periodontal disease and correlates with numerous inflammatory disorders, such as cardiovascular disease. Circulating bacteria may contribute to atherogenesis by promoting CD11b/CD18-mediated interactions between neutrophils and platelets, causing reactive oxygen species (ROS) production and aggregation. Lipoxin A_4 (LXA_4) is an endogenous anti-inflammatory and pro-resolving mediator that is protective of inflammatory disorders. The aim of this study was to investigate the effect of LXA_4 on the *P. gingivalis*-induced activation of neutrophils and platelets, and the possible involvement of Rho GTPases and CD11b/CD18 integrins.

Methods: Platelet/leukocyte aggregation and ROS production was examined by lumi-aggregometry and fluorescence microscopy. Integrin activity was studied by flow cytometry, detecting surface expression of CD11b/CD18 as well as integrin exposing its high affinity epitope, whereas activation of Rac2/Cdc42 was examined using a GST-pulldown assay.

Results: The study shows that *P. gingivalis* activates Rac2 and Cdc42 and up-regulates CD11b/CD18 and its high affinity epitope on neutrophils, and that these effects are diminished by LXA_4. Furthermore, we found that LXA_4 significantly inhibits *P. gingivalis*-induced aggregation and ROS generation in whole blood. However, in platelet-depleted blood and in isolated neutrophils and platelets, respectively, LXA_4 was unable to inhibit either aggregation or ROS production.

Conclusions: This study suggests that LXA_4 antagonizes *P. gingivalis*-induced cell activation in a manner that is dependent on leukocyte-platelet interaction, likely via inhibition of Rho GTPase-signalling and down-regulation of CD11b/CD18. These findings may contribute to new strategies in the prevention and treatment of periodontitis-induced inflammatory disorders, such as atherosclerosis.

References:

1. Seymour, G. J., P. J. Ford, M. P. Cullinan, S. Leishman, and K. Yamazaki. 2007. Relationship between periodontal infections and systemic disease. Clin Microbiol Infect 13 Suppl 4:3-10.

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The effects of platelets on fibroblast genes involved in extracellular matrix turnover, myofibroblast differentiation and reepithelialisation evaluated in an *in vitro* model

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Platelets have a significant function in the primary haemostasis and are also important for wound healing and inflammatory processes. Upon activation, the platelets release an arsenal of growth factors and cytokines that stimulates cell proliferation, chemotaxis and differentiation. Platelet rich plasma (PRP) is a promising treatment for impaired healing conditions such as chronic wounds but clinical trials gives contradicting results and the underlying molecular processes are poorly described. This study aims to investigate the effects of isolated platelets on the expression of fibroblast genes involved in extracellular matrix (ECM) turnover and in this context the role of transforming growth factor β1 (TGF-β1) as an extracellular mediator. In a three-dimensional cell-culture model, human skin fibroblasts were cultured in a collagen gel, separated from activated platelets by a semi-permeable membrane. The fibroblast mRNA expression of 14 genes coding for structural proteins, proteases, protease inhibitors and intermediate filaments, as well as keratinocyte growth factor (KGF), was analysed by real-time PCR. The level of transforming growth factor β1 (TGF-β1) in the culture medium was analysed with ELISA. The amount of TGF-β1 released was dependent on platelet number and increased with incubation time. Changes in fibroblast mRNA expression varied with time and the number of platelets. The myofibroblast-specific marker α-smooth muscle actin was significantly upregulated in a time-dependent manner by activated platelets. This was also the case with KGF. The ECM remodelling genes responded more inconsistently and no specific pattern could be discerned when it comes to net ECM accumulation. Since a net increase in ECM accumulation is needed for a proper wound healing, this may, at least in part, explain the inconsistent clinical outcome by the use of PRP. However, the effects of activated platelet on myofibroblast marker and KGF in fibroblasts suggest roles for other aspects of wound healing.

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Association of CARD8 variant rs2043211with Myocardial Infarction

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Objective: Inflammation is a key factor in the development of Myocardial Infarction (MI). Card8 *is* a protein – protein interacting motif, regulating and modulating the expression of genes involved in various inflammatory pathways by predominantly suppressing NF-kB. The polymorphism, rs2043211 in the *CARD8* gene introduces a stop codon at codon 10 due to A >T transversion resulting in the enhancement of chronic inflammation.

Methods: We analyzed the expression of Card 8 in the lesions using Real time PCR and Microarray. We also analyzed rs2043211 using allelic discrimination in MI patients within two Swedish cohorts, the FIA and Intergene cohorts from the Northern and Southern regions of Sweden respectively. IL-1, IL-18, TNF, and MCP-1 were measured in serum from MI patients using ELISA.

Results: We found significantly higher expression of *CARD8* in plaques with respect to normal vessel. Moreover, the rare allele was associated with lower expression of Card8 in the lesions. This association was more prominent in men when compared to women. In serum we found the association of rare allele with lower level of MCP-1 and no association was found with respect to IL-1, IL-18, and TNF levels. A significant association between the *CARD8* rs2043211 polymorphism and risk of MI in men (p=0.02) was evident in the FIA cohort but not in the Intergene cohort.

Conclusion: Overall, our findings provides the association of rs2043211rare allele with the development of MI risk in men, requiring further cohort studies and explores the higher expression of the *CARD8* gene, prominently in plaques with association significantly lower levels of MCP-1.

Reference:

1. *Bagnall R, Roberts R, Mirza M, Torigoe T, Prescott N, Mathew C. 2008. Novel isoforms of the CARD8 (TUCAN) gene evade a nonsense mutation. Eur J Hum Genet.16:619-25.*
2. *Razmara M, Srinivasula S, Wang L, Poyet J, Geddes B, DiStefano P, et al. 2002. CARD-8 protein, a new CARD family member that regulates caspase-1 activation and apoptosis. J Biol Chem. 277:13952-8.*

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S. aureus pore-forming toxins activates the inflammasome

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Objective: *S. aureus* is a renowned/prominent pathogen that gives rise to all from superficial to life-threatening bacteraemia or endocarditis. Knowledge on why some are stricken with infections with high fatality whereas others only show mild disease symptoms is lacking. An understanding of the body's mechanisms during an infection is of importance, to be able to hinder difficult bacterial infections in the future, especially with the elevated threat from antibiotic resistant bacteria. Knowledge about the inflammatory process in infections has increased through the discovery of intracellular receptors, Nod-like receptors (NLR), which at activation of inter alia bacterial components, forms multi-proteincomplex called inflammasomes. The inflammasome activates caspase-1 that later cleaves pro-interleukin-1β (proIL-1 β) to its active form. IL-1 β is the body's most potent pro-inflammatory cytokine and causes fever and leucocytosis.

Material and Methods: Whole blood from blood donors is stimulated with bacteria from different strains with consideration to the strains toxin production, Wood 46 (high α-toxin), Cowan I (low α-toxin), and also bacterial isolates from patients, α-toxin strains (high respectively low producing), PVL (Panton-Valentine leukocidin) strains (+ and -).
Active caspase-1 is detected in human neutrophil granulocytes with the help of FLICA that is a fluorescent labelled inhibitor, which is analyzed by flowcytometry.

Results: Initial results show that bacteria strains, which produce a lower amount α-toxin respectively PVL-toxin leads to an activation of caspase-1, whereas bacteria strains which a produce large amount of toxins results in an apparent activation of caspase-1.

Conclusion: The results indicate that pore-forming toxins from *S. aureus* activates the inflammasome

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From Revolution to Evolution - Digitization of Radiology in Sweden

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Objective: Health care has undergone great changes during the last decade. The decision to introduce digital technology in a radiology department has commonly been made with the intention of meeting demands for increased productivity, rationalization and organizational changes in medical health care.

Aim: The aim of this study was to illustrate the productivity of five Swedish radiology departments before and after digitization, classified by the total number and by specific types of examination in the department.

Methods: Total numbers of examinations for the departments were divided into total numbers and classified in the subgroups CT (computed tomography), MRI (magnetic resonance imaging), ultrasound, gastrointestinal fluoroscopy, urogenital, chest and skeletal radiology. The numbers of completed examinations were analysed for the period two years before and four years after.

Results: This study shows that the number of examinations produced was similar or shows an increase over the period. The numbers of examinations have changed in the direction of more CT and MRI examinations and fewer traditional abdomen examinations such as those involving urology, and gastrointestinal fluoroscopy.

Conclusions: The change to more CT and MRI examinations gives much more information for the radiologist when setting the diagnosis. This will lead to better diagnostics and higher quality within each examination. These types of examinations are much easier for the patient to perform and give more information and possibilities when setting the diagnosis. Much more complicated examinations can be performed in an easy way and the patients don't have to perform other interventions.

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Elevated Celiac disease related antibodies in children with cerebral palsy

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Objective: To study immunomorphology in small bowel biopsies from children with Cerebral Palsy (CP) and elevated levels of celiac disease (CD) related antibodies despite earlier normal routine mucosal histology. Genetic susceptibility for CD (HLA-DQ2 and 8) was also analyzed.

Methods: Immunomorphology including IgA colocalizations with tTG2 were analyzed in small bowel biopsies from 16 children with CP. HLA - typing was performed with PCR-SSOP.

Results: 10/16 children (62%) were HLA-DQ2 and/or HLA-DQ8 positive. Elevated numbers of α/β+ and γ/δ+ lymphocytes, positive staining for CD3 and DR3 as well as IgA co-localized with tTg2 was found in the mucosa from one child with elevated serum levels of IgA-antibodies against tissue transglutaminase. Slightly elevated numbers of mucosal α/β+ or γδ+ lymphocytes and positive DR-staining without IgA-deposits were found in the biopsies from another two children. These three children were HLA DQ2 and/or HLA DQ8 positive.
The biopsies from the 13 remaining children showed normal numbers of intraepithelial lymphocytes and no IgA-deposits were found. However, 7 of these showed slightly positive DR staining indicating an unspecific mucosal inflammation.

Conclusions: Routine histological analyses of small bowel biopsies may not be sufficient to identify CD at an early stage. The majority of children with CP and elevated levels of CD-related seromarkers do not have classical CD, but rather an increased immune reactivity. Could the elevated levels could be due to some extra intestinal manifestation of CD such as the brain?
Further studies are needed to evaluate if this 'gluten sensitivity' has any impact on their nutritional problems or other clinical symptoms.

Key words: CP, CD, tTG2, HLA-DQ 2 and 8

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AUTHORS & PARTICIPANS INDEX, 9 DECEMBER 2010