Preface

This annual report gives a comprehensive overview on activities in the Institute for Surgical Research leading the institute into its 45th year of successful research.

The institute was founded back in 1966 based on generous donations given by the Norwegian Society for Fighting Cancer. Egil Amundsen, who was trained as a surgeon and in physiology, was appointed leader of the new institute. Several competent researchers quickly became connected to the institute and Amundsen's open-minded strategy provided for excellent research. Sten Sander was the first who completed his doctoral theses at the Institute for Surgical Research in 1969.

Today the institute can look back on an impressive high number of doctorial dissertations altogether 143 at the end of 2010.

This year the orthopedic surgeon Sigbjørn Dimmen and the cardiologist Kristina Hermann Haugaa defended their doctorial theses from the institute.

• SIGBJØRN DIMMEN: "Effects of cox inhibitors on bone and tendon healing".

• KRISTINA HERMANN HAUGAA: "Prediction of cardiac ventricular arrhythmias by echocardiography in patients at risk".

In 2010, 31 original research publications have been released from the institute with an average impact factor of 5,06.

The institute recognizes with great appreciation that professor Thor Edvardsen of Research group Integrated Cardiovascular Function was awarded a great research donation from the Norwegian Research Council this year. He has now become leader of a new prestigious research center "Center for Cardiovascular Innovation" where he and his colleagues can enjoy a research budget of NOK 80 million for the coming 8 years period.

Egil Amundsen lecture 2010 was held by professor Henrik Kehlet from Rigshospitalet, University of Copenhagen, and was a great success and very much appreciated both by clinicians and researcher at our hospital.

Our progress in the year 2010 would not be possible without the enthusiastic and hard work of all our staff. However the following members of the staff deserve to be particularly acknowledged for taking care of important in-house administration responsibilities: Jorunn H. Larsen (Secretariat), Per-Stian Støle (Administration), Signe Flood Kjeldsen (Laboratories), Vivi Bull Stubberud, Sera Sebastian, Aurora Pamplona, Roger Ødegård and Kristine Kloster-Jensen (Operating theatres), Myrna Pacil (Housekeeping), Kirsten Strømme, Shakil Ahmed, Biljana Stangeland and Håvard K. Skjellegrind (Seminars).

During 2010 several groups from different departments in the hospital have performed their research activities in the institute. We are very thankful for their important cooperation, which are important links for the institute to the clinical departments.

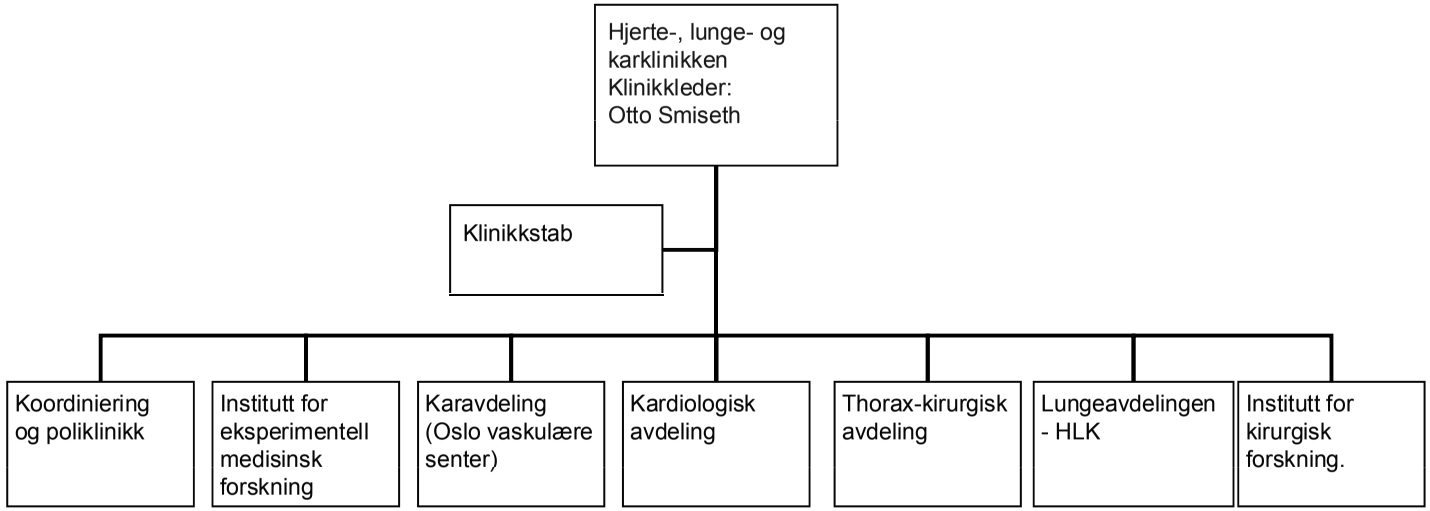
Institute for Surgical Research sincerely thanks Oslo University Hospital, University of Oslo and external institutions including the Norwegian Research Council, the Norwegian Advisory Committee for Cardiovascular Disease and the Health Authorities of South/Eastern Norway for important financial support.

This report was edited by Jorunn H. Larsen, Per-Stian Støle, Håvard Attramadal and Ansgar O. Aasen.

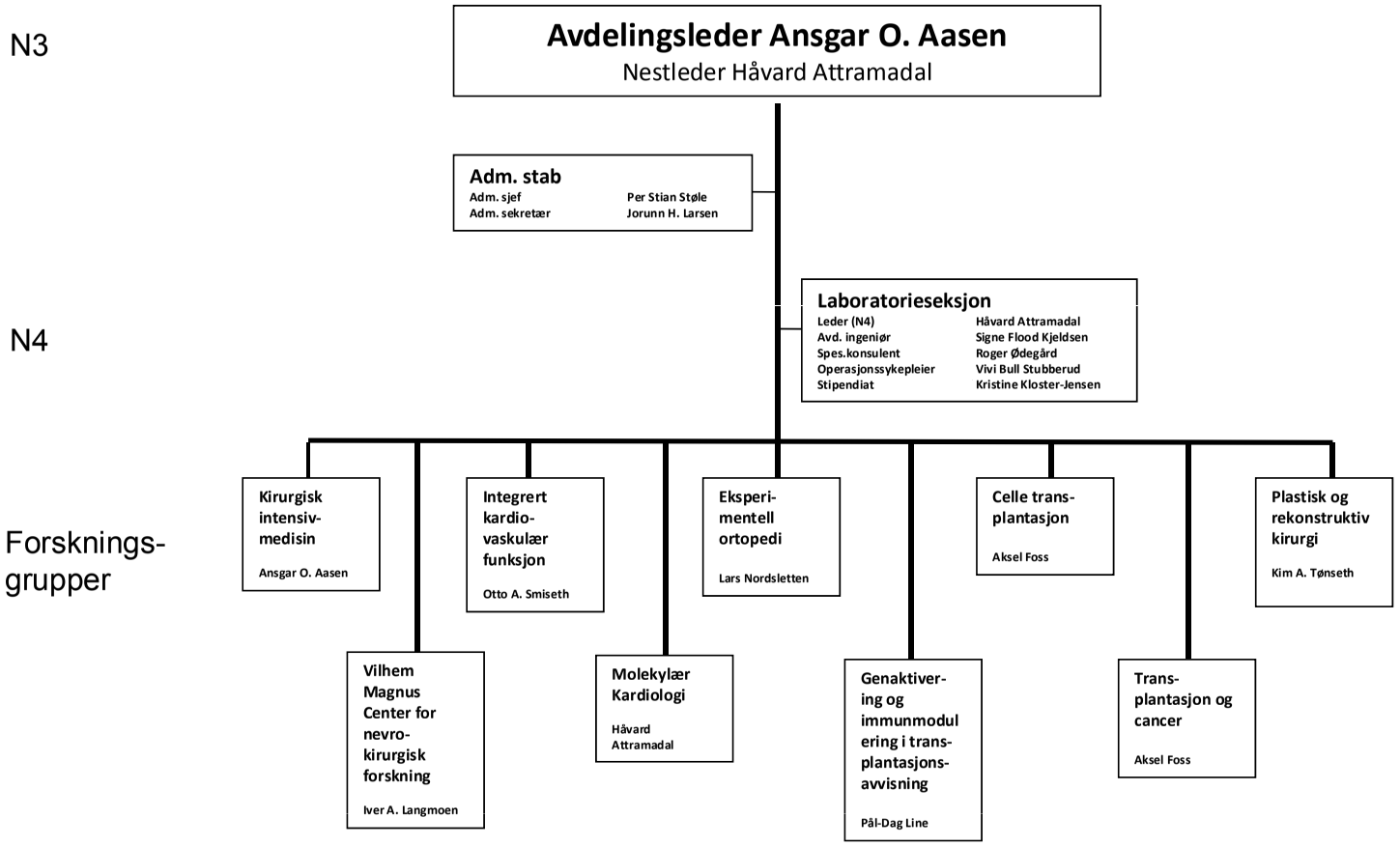
Institute for Surgical Research,
March 2011

Ansgar O. Aasen
Professor/Head of Institute

Hjerte-, lunge- og karklinikken



Institutt for kirurgisk forskning



Abbreviations

AHUS	Akershus University Hospital
CAST	Cancer, stem cell innovatin center
CIT	Clinical Islet Transplantation Consortium
FP 7, EU	Seventh Framework Programme, European Union
LO	Lovisenberg Diaconical Hospital
NCCD	The Norwegian Council on Cardiovascular Diseases
NRC	Norwegian Research Council
OC	Orthopaedic Centre, Oslo University Hospital
OUH	Oslo University Hospital
SENRHA	South-Eastern Norway Regional Health Authority
UIO	University of Oslo
VUSP	Valencia University School of Pharmacy

Research Groups

Surgical Intensive Care Medicine

Leader:

Ansgar O. Aasen, Professor, MD, PhD (UiO/OUH)

Professor Ansgar O. Aasen

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Yngvar Gundersen, Senior scientist, MD, PhD (OUH)
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Kristin Bjørnland, Consultant, MD, PhD (OUH)
Yun Yong Wang, MD, PhD (OUH)
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Signe Flood Kjeldsen, Research engineer, MSc (UiO)

Research area

Infections following surgery or trauma continue to be a major clinical problem. Due to the immunological consequences of surgery, infections frequently develop into severe septic complications and multiple organ injuries. The problem in severe sepsis is a paradoxical and self-destructing inflammation leading to dysfunctional host defense and lethal injury to vital organ systems.

More than one million patients are expected to die annually from severe sepsis world wide.

Aims

Our aim is to develop novel means to prevent or ameliorate the self-destructive inflammation in patients with infection. A major focus of our work is research into the cellular mechanisms involved.

Ongoing Projects in 2010:

Molecular mechanisms in the development of sepsis-induced Multiple organ dysfunction

Sepsis remains a challenging problem in intensive care medicine. Much is known about the immune-pathophysiological mechanisms in sepsis, but the mechanisms behind sepsis-induced organ failure are still a matter of debate.
The clinical outcome of sepsis is directly correlated to the number of organs failing, as the mortality inclines from 25% in single organ failure to above 72% with failure of three or more organs.

To address this critical aspect of sepsis two novel projects to investigate molecular mechanisms involved in the development of sepsis-induced organ failure, have been initiated.

CCN proteins in the development of sepsis-induced organ failure
The CCN proteins belong to a group of extracellular matrix (ECM)-associated proteins that have emerged as potential candidates in the regulation of parenchyma function.
The protein family has a regulatory rather than structural role in the ECM and includes the six proteins CCN1 (Cyr61), CCN2 (CTGF), CCN3 (NOV), CCN4 (Wisp-1), CCN5 (Wisp-2) and CCN6 (Wisp-3). Their synthesis is induced by multiple factors including serum growth factors, cytokines, steroids, hypoxia, and mechanical and environmental stressors. The proteins function primarily through interaction with cell adhesion receptor integrins, which allows for variation in the response due to integrin subtype specific effects in different cell types. The role of CCN proteins in the development of sepsis-induced organ failure has not been investigated.
Research has previously worked with the role of CCN2 in development of liver fibrosis as well as the roles of several CCN proteins in the development of cardiovascular failure. However, possible roles of these proteins have also been indicated during acute lung and kidney injury. Interestingly, the CCN proteins also affect inflammatory responses by modulating the production of cytokines and reactive oxygen species, and by stimulating adhesion and migration of lymphocytes and monocytes/macrophages.
Initial studies have been conducted in two different rodent models of abdominal sepsis induced by cecal ligation and puncture (CLP). In a short-term model using male wistar rats the animals were divided in three groups; Baseline,

CLP, and Sham. Animals were sacrificed 18 hours after surgery. Studies in a unique long-term mouse model were conducted in collaboration with professors Heniz Redl and Soheyl Bahrami from the Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna. Mice were divided in four groups and sacrificed at baseline or 18, 48, or 96 hours following CLP.

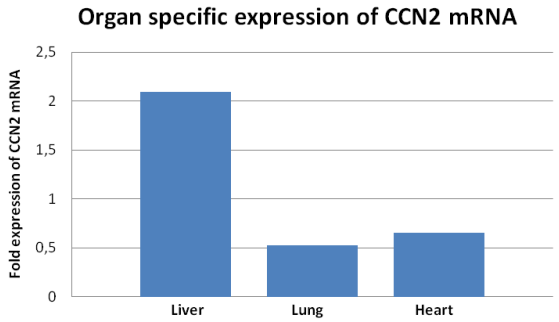

Figure 1. *Tissue specific regulation of CCN2 mRNA expression in the livers, lungs and hearts of rats subjected to 18 hours of CLP. Expression levels of sham animals set to one. Relative ecpression levels in CLP animals displayed.*

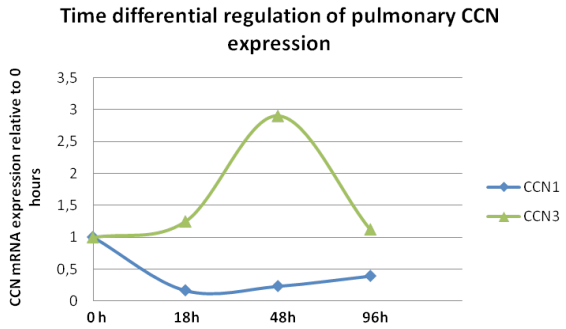

Figure 2. *Time differential regulation of CCN1 and CCN3 mRNA expression in mice subjected to CLP for 0, 18, 48 or 96 hours. Expression levels standardized to 0 hour.*

Preliminary data shows both a tissue-specific (Figure 1) and time-differential regulation of CCN gene expression in the models (Figure 2).
Furthermore, a specific gene expression profile during sepsis for single CCN genes in different organs has been documented.
This provides novel indications of involvement of the ECM associated CCN proteins in sepsis-induced organ failure.

Endoplasmic reticulum (ER) stress and apoptosis – links to development of organ failure in sepsis
The ER is the main site of protein modification and folding and is highly sensitive to stresses that alter the cellular energy level, the redox state or the Ca2+ concentration.

Such stresses cause accumulation of unfolded proteins in the ER leading to a condition called ER stress. Protein aggregation is toxic for the cells and several pathological conditions are thus associated with ER stress. Adaption to altered environment and restoration of cell homeostasis involve cell protective mechanisms, Figure 3. Cellular responses to ER stress degrade misfolded proteins by the ubiquitin proteasome system in the cytoplasm, and regulate mRNA translation and assist protein folding by a pathway called unfolded protein response (UPR). The adaptive mechanisms during UPR are mediated through the three ER transmembrane receptors; pancreatic ER kinase (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1). Initial signalling through these receptors induces protein folding and degradation of ER-associated proteins and inhibits mRNA translation thereby reducing the influx of proteins to the ER. However, if ER stress is prolonged and the adaption fails, the signalling will become pro-apoptotic and the cell will go into programmed cell death, apoptosis.

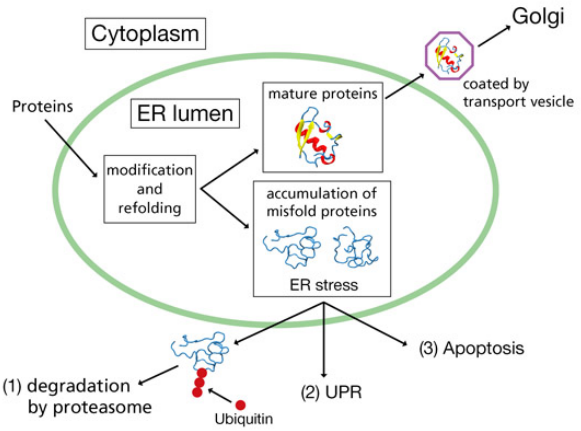

Figure 3.: *Cellular responses to Endoplasmatic Reticulum (ER) stress.*

Cellular responses to ER stress involve degradation of misfolded proteins by the ubiquitin proteasome system (1), induction of protein folding and inhibition of mRNA translation by the UPR, unfolded protein response (2) or can lead to apoptosis (3) if the stress is prolonged.

Two previous studies report a possible role of prolonged ER stress in the development of organ dysfunction in septic animals. These studies show an increased expression of known markers of ER stress in spleen of CLP-operated mice and in liver of LPS-treated rats. Initial data generated in our group show similar results in liver of CLP-operated rats

supporting the presence of ER stress in failing organs during sepsis. Further functional studies are needed to elucidate the importance of these findings in the development of organ dysfunction in sepsis.

A new approach to monitoring inflammation: Thrombus, Thrombin Generation and Thrombolysis.

Assessment of inflammation is usually carried out by the measurement of known individual inflammatory markers. While often showing changes of up-regulation or down-regulation in individual components of the inflammatory process they rarely provide an insight into the overall effects of inflammation on other defence mechanisms such as those involved in haemostasis. In addition to this the cost consequences of carrying out such a large battery of test assays is now considerable.

In previous reports we have outlined parameters that link inflammation to changes in both the coagulation and fibrinolytic systems as well as describing some of the newer technologies that can be used to monitor such changes. These assays revolve around the changes that occur in blood using thromboelastography and the measurement of thrombus/thrombin generation.

Inflammatory changes have been shown to be associated with the shedding of microparticles in the blood. Microparticles are released in a number of disease processes with considerable numbers having been found to be associated with tissue factor (TF) release in the blood. The production of TF bearing microparticles will markedly influence thrombin production and coagulation in conditions such as septicaemia. The assessment of the influence on thrombin production and the global stimulation of the haemostatic processes are best effected by thromboelastography/thrombus/thrombin generation.

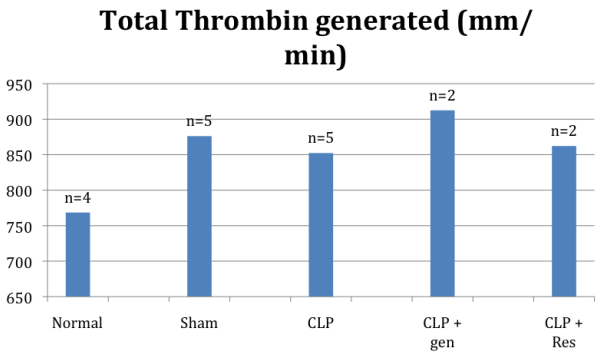

Figure 4.: *Changes in total thrombin generation mm/min assayed by thrombo elastography in rat CLP sepsis model.*

In the last annual report we described preliminary studies using thromboelastography in a rat model of septic shock (Fig. 4). Although only a small number of animals could be studied we observed that thrombus generation was increased in rats following surgical intervention and there was a correlation between this and a number of inflammatory markers.

This preliminary study suggested that the use of a global assay using thromboelastography/thrombus/thrombin generation could be a more cost effective way of monitoring inflammatory changes in a variety of models.

The previous preliminary study is to be extended with a larger cohort of animals allowing for a more detailed analysis of the results.

Comparison between laparoscopic and open antireflux surgery on cellular immune responses in children

Laparoscopic procedures are increasingly being performed in children. In adults, several randomized studies have shown that laparoscopic surgery is safe, have at least as good results as open surgery, and cosmesis, influence of the immune system, need of postoperative analgesics, and recovery are superior as compared to open surgery. In children, there are very few randomized studies comparing open and laparoscopic surgery. Because of the rapid introduction of and the enthusiasm for laparoscopic procedures, it has been difficult to arrange randomized trials to validate laparoscopy in children.
Antireflux surgery (fundoplication) is one of the most commonly performed gastrointestinal procedures performed by pediatric surgeons. In contrast to adult patients operated with fundoplications, most children referred for antireflux surgery have several co-morbidities. As compared to results from adult series, recurrence rates after fundoplication seem to be higher in children than in adults. Particularly children with severe gastrointestinal dysmotility (neurologically impaired children) have the highest recurrence rates. Furthermore, children seem to have longer convalescence time than adults.
To compare results of open and laparoscopic fundoplication, a prospective, randomized study has been performed in 88 children. Various results such as complication rates, recurrence rates, hospital stay and convalescence will be compared between the methods. In addition, we will assess if the influence on cellular immune responses vary between laparoscopic and open operations, and if clinical parameters are related to immune response.
For studies on cellular immune responses, blood was taken preoperatively and on the first and second day postopera-

tively. Plasma levels of several cytokines will be measured by ELISA (multiplex). Furthermore, blood was incubated in a whole blood model. The blood was first anticoagulated with heparin (25 U/mL) and incubated at 37°C with slow rotation in the presence of either Lipopolysaccaride (10 ng/mL blood), Peptidoglycan isolated form Staphylococcus aureus (1 µg/mL blood) or saline, respectively. At 0,1,4,6,12 and 24 h, plasma was obtained by centrifugation and stored at -70°C. Levels of cytokines will be measured to further assess inflammatory responses after laparoscopic and open surgery.
Clinical data are recorded prospectively during hospital stay and at follow-ups 6, 12 and 24 months postoperatively.

Determination of P.aeruginosa virulence .

Pseudomonas aeruginosa (PAER) is an opportunistic bacterium which seldom cause disease in healthy individuals. But, the increasing number of immunocompromised individuals has provoked a rise in PAER infections. In vitro determination of PAER virulence is complicated, and the current gold standard is a C. elegans killing assay. The killing assay measures relative virulence of PAER serotypes, but a major drawback is that it's time consuming and resource demanding.

In collaboration with Department of Infection Prevention headed by Egil Lingaas, MD, we investigate markers of the innate immune-response as an improved alternative to the established assay.

Clinical isolates of PAER from patients with bacteremia were subjected to comparative evaluation in both the established killing assay and a well-established human whole blood model.

The *C. elegans* killing assays optimized by Reza Assalkhou, PhD, (Dep. of Infection Prevention) showed that this assay is highly reproducible and can differentiate between strains of PAER with high, moderate and low virulence respectively. The ex vivo whole blood model to study the inflammatory response by measurment of the cytokine production of leukocytes was used. Testing blood from 3 different persons clearly indicated the variation in regulation of cytokines production between these persons in response to bacterial infection.(IL (Beta, Ra, 2, 2R, 4, 6, 7, 8, 10, 12, 15), TNF-Alpha, INF-Alpha, INF-Y, GM-CSF, MIP (1Alpha & 1Beta), IP-10, MIG, Eotaxin, RANTES, MCP-1, G-CSF, FGF basic, HGF, VEGF).

Due to variation in individual response in human whole blood model we decided to use a cellular model: THP-1 cell line. The viability assay confirmed the differences in virulence of the strains tested on C. elegans, whereas the highly virulent strains for C. elegans reduced significantly the viability of the cells during the assay. The expression of host factors of innate immunological response is currently being investigated to establish a better understanding of host/pathogen interactions and to identify host factors mediating susceptibility or resistance to virulent PAER isolates

Studies on possible effects of a newly developed hemapheresis filter (TM100) on inflammatory mediators and hemodynamic parameters during experimental endotoxinemia in pigs.

Therapy in sepsis is still greatly discussed due to a high mortality. Lipopolysaccaride (LPS) is a main initiator of cascade chain reactions during gram negative sepsis that may lead to multi organ failure, circulatory collapse and eventually death. There are some few publications concerning possible positive effects using hemofiltration in humans during severe infections. We have started to test a newly developed hemofiltration filter (TM 100) in anaesthetized pigs during ongoing LPS infusion. Twelve heparinised animals have been used during 2010 to look for an optimal filter content and experimental model. The filter device was placed in the inferior caval vein. We have searched for criteria for possible effects on different parameters.
Results so far have revealed a systemic reduction in the TNF-α concentration by 30%. During ongoing hemofiltration an initial drop in systemic blood pressure was detected but could be reversed after intravenous saline. Further studies comparing effects of different hemofiltration systems are planned.

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Molecular Cardiology

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Else Marie Valbjørn Hagelin, MSc, Senior Engineer (SENRHA)

Professor Håvard Attramadal

Research Area

Heart failure, the common end-point in cardiac disease of diverse etiologies, is a major cause of morbidity and mortality in affluent societies. Indeed, the incidence and prevalence of heart failure in these countries are increasing due to altered demographics with increased proportion of the elderly, as well as increased survival of myocardial infarction. Despite implementation of several new treatment modalities during the last 20 years, heart failure is still a progressive and ominous disease indicating that important pathogenic mechanisms remain unmodified by the most current treatment modalities.

In evolving heart failure multiple compensatory actions are triggered in order to maintain cardiac output, among which is activation of the sympathetic nervous system, the renin-angiotensin system, as well as a number of autocrine/paracrine factors synthesized in myocardial tissue. The compensatory actions also reflect alterations in of cardiac structure, collectively called cardiac remodeling. These structural alterations comprise dilatation of the ventricular chambers, myocardial hypertrophy and fibrosis. Although cardiac remodeling may initially balance loss of contractile force, the continuum of these structural alterations often feeds into vicious circles leading to progression of cardiac dysfunction and overt heart failure. Increasing evidence points to myocardial hypertrophy, fibrosis, and dilatation of the ventricular cavities as independent risk factors of heart failure. Indeed, recognition of these structural alterations of the heart is implemented in the new recommendations for the evaluation and management of chronic heart failure recently published by the American College of Cardiology/American Heart Association Task Force. According to these recommendations, which are meant to complement the New York Heart Association (NYHA) functional classification, patients are to be stratified according to risk factors for developing heart failure, including the absence or presence of structural alterations of the heart.

Despite substantial new insights into the mechanisms of myocardial hypertrophy and fibrosis, many of the nodal points that orchestrate these structural alterations still remain to be identified. Furthermore, current knowledge largely precludes from deciphering of adaptive versus maladaptive cellular responses to insufficient cardiac output. Thus, the focus of our research group is to unravel the signal transduction mechanisms leading to dysfunctional signaling responses and pathologic remodeling of the heart. The purpose of these investigations is to provide new knowledge of disease mechanisms enabling development of novel pharmacological interventions for heart failure.

Our research group is a multidisciplinary team of experts in gene technology, molecular and cellular biology, as well as experimental and clinical medicine. The research efforts comprise studies of isolated cardiac myocytes, integrated physiology in transgenic animal models, as well as clinical investigations. Our research group is member of Center for Heart Failure Research, University of Oslo (www.heartfailure.no), a thematic research initiative and focus area of research selected by the Faculty of Medicine. Center for Heart Failure Research has also become a regional research network sponsored by Helse Sør-Øst Regional Health Authority. The Institute for Surgical Research provides infrastructure with state-of-the-art equipment for high-resolution echocardiography and integrated physiologic assessment of cardiac function in transgenic mice.

Major Aim

Dysfunctional cardiac signaling mechanisms and signals astray are considered major causes of pathologic myocardial hypertrophy and predisposition to heart failure. Increasingly, dysfunctional signaling mechanisms are implicated in increased production of free oxygen radicals, mitochondrial dysfunction and reduced tolerance to hypoxia and/or free oxygen radicals per se. Thus, the major goal of our research group is to dissect the function of myocardial autocrine/paracrine factors, their cognate receptors, and intracellular pathways in cardiac myocytes and fibroblasts. New knowledge on the function and mechanisms of signaling pathways in the heart may provide basis for development of new and more effective therapeutic intervention in acute coronary syndromes and heart failure.

Current specific aims of the research group:

1) Providing novel insights into the function of myocardial G protein-coupled receptor kinases, i.e. a family of kinases that are important proximal modulators of many receptor-controlled signal transduction pathways involved in regulation of myocardial function in health and disease.

2) Uncovering the function of myocardial autocrine/paracrine factors or cytokines that are activated or induced in heart failure. Current focus is on the secreted matricellular proteins CCN2/CTGF (connective tissue growth factor) and CCN5/WISP-2 (Wnt-inducible secreted protein-2), as well as the TGF-β superfamily cytokine GDF-15 in heart failure of various etiologies.

Report from 2010:

1) Investigation of substrate specificities and function of cardiac G protein-coupled receptor kinases (GRKs)
We have previously investigated distribution of GRK2, GRK3, and GRK5 in myocardial tissue. These studies revealed that GRK2 was enriched in endothelial cells, whereas GRK3 was confined to cardiac myocytes. GRK5, on the other hand, was ubiquitously expressed among the cellular elements of myocardial tissue. The restricted distribution of GRK3 in cardiac myocytes clearly points to a role for this GRK isoform in regulation of G protein-coupled receptors on cardiac myocytes. However, since both GRK2 and GRK3 could be demonstrated in cardiac myocytes, studies of the substrate specificity of these kinases were an imminent issue. In isolated fully differentiated cardiac myocytes we investigated the substrate specificities of the GRK isoforms GRK2 and GRK3. These studies revealed that GRK2 and GRK3 display striking specificity on G protein-coupled receptors controlling different aspects of cardiac function. Overall, the present data have uncovered the novel findings that GRK3 has substantially higher potency and efficacy than GRK2 at endogenous endothelin receptors and α1-adrenergic receptors. This did not seem to be the case for the β1-adrenergic receptor as GRK3 potency at this receptor appeared much weaker than for the ET-R, and was equipotent with GRK2. Thus, GRK3 emerges as a primary regulator of ET-R- and α1-AR-signaling, which may have important implications in cardiac function. The studies provide biochemical evidence of widely different functional roles of GRK2 and GRK3 in cardiac myocytes. These functional differences are currently subject of investigations in transgenic and gene-targeted mice.

A recent novel finding from our laboratory is that myocardial GRK5 is upregulated in transgenic CCN2/CTGF mice and causes reduced sensitivity of cardiac β-adrenergic receptors to endogenous agonists. Furthermore, increased GRK5 initiates G protein-independent signaling by recruitment of β-arrestin to the receptor allowing ß-arrestin to act as a scaffolding protein for signaling complexes at the plasma membrane. Indeed, the altered signaling specificity of ERK1/2 initiated by GRK5 elicits cardioprotective actions. These findings have been recapitulated in cardiac myocytes pretreated with recombinant human CTGF. Yet, the signaling pathway(s) implicated in CTGF-induced GRK5 is yet to be characterized. Furthermore, the relative contribution of GRK5 to the cardioprotective actions afforded by CCN2/CTGF remains to resolved

2) Role of CCN2 - connective tissue growth factor - in regulation of tolerance towards ischemia-reperfusion injury and in resisting maladaptive cardiac remodeling during chronic pressure overload.
Myocardial CCN2 is highly expressed in the developing heart in fetal life and apparently plays crucial role in cardiac development. However, myocardial expression of CCN2 is repressed in the postnatal heart under physiologic conditions. Interestingly, myocardial expression of CCN2 is reactivated or induced during evolving heart failure. Previous findings from our laboratory demonstrate that induction of myocardial CCN2 appears to be a general response to evolving heart failure, i.e. induction of myocardial CCN2 occurs in heart failure of diverse etiologies. Induction of tissue expression or increased plasma levels of CCN2 is often associated with diseases in which fibrosis is an important morphologic characteristic. However, to what extent CCN2/CTGF actually elicits fibrosis is yet to be demonstrated. Indeed, the physiologic and/or patho-physiologic functions of CCN2 in myocardial tissue have not yet been resolved. Thus, a major focus of our research effort has been to elucidate the function of CCN2 in the heart. Does CCN2 exert salutary actions in heart failure or does CCN2 contribute to progres-

sion of heart failure? Does CCN2 cause myocardial fibrosis? In order to elucidate to the physiologic actions of CCN2 in the heart and to investigate how the actions of CCN2 may contribute in the pathophysiology of heart failure, we are currently investigating various transgenic models with constitutive or conditional overexpression of CCN2 in the heart generated in our laboratory. The transgenic mice with cardiac-restricted, constitutive overexpression of CCN2/CTGF displayed marginal increase of myocardial collagen contents despite 70-fold overexpression of CCN2/CTGF (Ahmed, MS et al. Am J Physiol Heart Circ Physiol; Dec 24, 2010 [epub ahead of print]). This finding appears to be consistent with overexpressors of CCN2/CTGF in other tissues or organs. Thus, the interpretation of the available data both from our and other research groups is that additional factors are required for CCN2 to induce fibrosis. A surprising, novel finding in our laboratory was that CCN2 exerts striking cardioprotective actions, increasing tolerance towards ischemia-reperfusion injury both in Langendorff-perfused hearts ex vivo and in mice subjected to transient ligation of the left anterior descending coronary artery in situ. These findings have led to filing of patents for protection of the potential commercial development of CCN2/CTGF as a pharmacologic treatment in acute coronary syndromes with the objective of minimizing myocardial necrosis. Verification of the data in large animal models, commercial development plans, including plans for early clinical testing, are currently being pursued in collaboration with Birkeland Innovation/Inven2 AS, the TTO of University of Oslo and Oslo University Hospital.

A cognate receptor for CCN2 or any of the other CCN proteins has not yet been characterized. Despite several reported interactions between CCN proteins and extracellular matrix-associated protein, data from our laboratory indicate that CCN2 also acts directly on cells by binding to ligands at the surface of the plasma membrane. Furthermore, analysis of the phosphoproteome of cardiac myocytes stimulated in the absence of presence of recombinant CCN2 revealed that the PI3 kinase/AKT/GSK-3ß pathway is major intracellular signaling pathway of CCN2 (Fig. 1). Indeed, our data also demonstrate that this pathway is crucial for CCN2-dependent cytoprotection towards hypoxia. The mechanisms of the cytoprotective actions of CCN2 are currently a major endeavor in our research group.

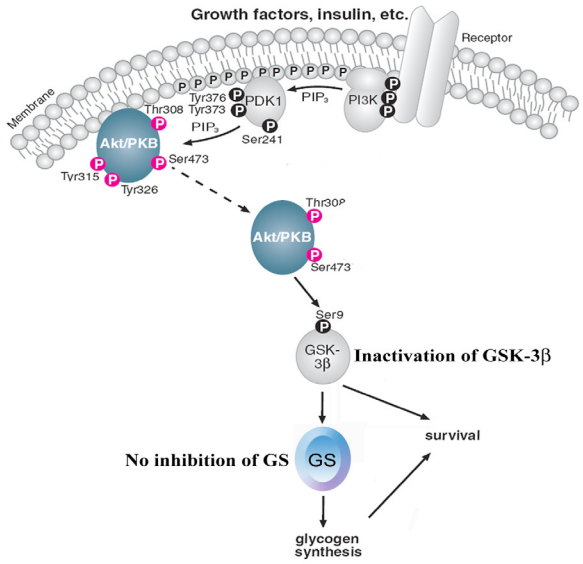

Figure 1. *Schematic illustrating the intracellular signaling pathways of CTGF/CCN2 in cardiac myocytes. A cognate receptor for CTGF upstream of PI3 kinase has not yet been characterized.*

Collaborators

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Integrated Cardiovascular Function

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General objectives:

The Integrated Cardiovascular Function group studies cardiac mechanics in experimental studies and studies in patients. The idea is to develop better diagnostic understanding and solutions into clinical practice.

Specific objectives:

1) To investigate mechanisms of LV dyssynchrony and develop better methods for selecting patients for cardiac resynchronization therapy.
2) To investigate LV mechanical-electrical interactions and improve risk stratification for ventricular arrhythmias.
3) To investigate mechanisms of LV (left ventricular) diastolic dysfunction.
4) To develop better diagnostic methods to identify viable myocardium
5) To investigate hemodynamic effects of Cardiac resynchronization therapy (CRP) in patients with heart failure and narrow QRS.
6) To develop better diagnostic tools to identify optimal timing of surgery in valvular heart disease.

1. LV dyssynchrony: Cardiac resynchronisation therapy (CRT) has been documented to be a powerful treatment in patients with severe congestive heart failure, causing reverse LV remodelling, improvement of symptoms and reduction of mortality (Cleland 2005). In left bundle branch block (LBBB) the LV wall contraction is dyssynchronous due to electrical conduction delay to the free LV wall, and CRT resynchronizes the contractions by bi-ventricular pacing and thereby improves the contractile function. Currently, patients are selected for CRT on basis of the presence of wide QRS in the ECG. However, in about 30 % of these patients, there is no improvement in symptoms, and in some cases aggravation of symptoms by CRT (Jarcho, 2006). The high number of non-responders represents a major problem with CRT, and better criteria for selection of candidates for this treatment modality are therefore needed.
Because ECG has limited ability to identify candidates for CRT, echocardiography with tissue Doppler imaging has been proposed as a more sensitive and more specific method to identify dyssynchrony. However, so far echocardiography has no proven clinical value in selection of candidates for bi-ventricular pacing. We suggest that a better understanding of the underlying mechanism of dyssynchrony is important to interpret the echocardiographic findings, and thereby to improve patient-selection for CRT. To achieve this we have investigated mechanisms of dyssynchrony and have established a new method to differentiate between mechanical and electrical etiologies of dyssynchrony.
In LBBB, the pathological motion of the inter-ventricular septum during early systole has been proposed as a good predictor of response to CRT. The mechanisms responsible for this motion are, however, poorly understood. We challenge existing theories on the timing of active contraction in the septum, and have proven that the early-systolic abnormal septal motion is due to active contraction and not merely a passive consequence of pressure differences across the septum. This phase has not previously been considered active when assessing dyssynchrony, and this might explain the failure of most echocardiographic indices of dyssynchrony to predict CRT-response.

Furthermore, we have identified a new regional marker for mechanical activation based on a segment's first sign of active force generation (onset AFG). Using onset AFG we have been able to discriminate between electrical and mechanical dyssynchrony because onset AFG accurately reflect regional electrical activation (Fig1). This is important when assessing patients for CRT since primary electrical dyssynchrony is more likely to respond to the therapy. Our experimental findings have been very promising and we have now started patient based studies. Electrical activation sequence also influences regional work distribution in the LV. Using strain data (% myocardial deformation) by echocardiography in combination with LV pressure allows for assessment of regional work. We are currently working on a new method to detect wasted work done during

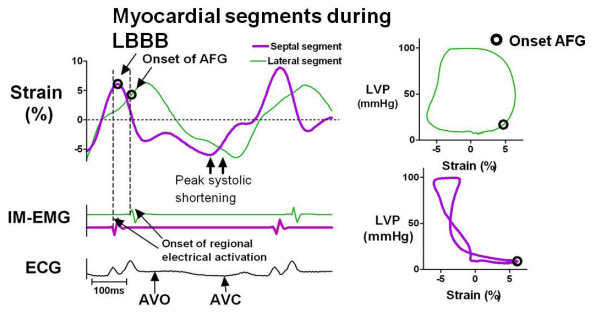
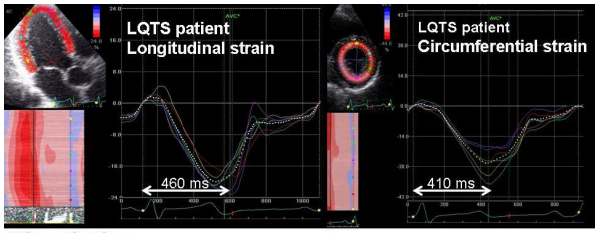


Figure 1. *Assessment of onset active force generation (AFG) by LV pressure and strain by speckle tracking echocardiography. Representative traces from septal (thick lilac trace) and lateral (thin green trace) segments during left bundle branch block (LBBB). Strain measurements are performed in short axis view. Vertical dotted line represents regional electrical activation by intramyocardial electromyogram (IM-EMG). Aortic valve opening (AVO) and closing (AVC) indicated by arrow.*

LBBB, which can subsequently be reversed by bi-ventricular pacing.

2. LV mechanical-electrical interactions: Evaluating patients with susceptibility for cardiac arrhythmias and sudden cardiac death is a major challenge in daily cardiology practice. Electrophysiological studies have demonstrated that damaged myocardium (e.g. infarcted or genetically altered) provides the substrate for malignant arrhythmias. Echocardiographic techniques can accurately quantify regional myocardial function. There is limited insight into how regional mechanical dysfunction may predict risk for ventricular arrhythmias. We have recently demonstrated how mechanical dispersion can predict ventricular arrhythmias in patients with LQT syndrome and in myocardial infarction. One study about mechanisms for ventricular arrhythmias in LQT syndrome was published in Circulation (Fig 2). We will



Circulation KH Haugaa et al, Circulation 2010;122:1355-1363.

Figure 2. Transmural mechanical dispersion in a symptomatic LQTS patient. Shown are the longitudinal (left) and circumferential (right) strain curves from a symptomatic LQTS patient. The anterior basal septal segment from longitudinal strain (left, red curve) shows a contraction duration of 460 milliseconds. From circumferential strain, the contraction duration from the anterior basal septal segment (right, yellow curve) is 360 milliseconds. Subendocardial contraction duration (longitudinal strain) therefore is markedly prolonged com- pared with midmyocardial contraction duration (circumferential strain), indicating transmural mechanical dispersion.

continue to investigate how LV myocardial mechanical dispersion relates to risk for ventricular arrhythmias in ischemic heart disease and in different types of cardiomyopathies.

3. Diastolic dysfunction: Left ventricular function has traditionally been evaluated non-invasively in terms of ejection fraction (LVEF). The assessment of LVEF is important for diagnostics, prognosis and selection of treatment. Many patients with symptoms of heart failure have, however, normal ejection fraction and appear to have diastolic heart failure. In this patient group there is need for other measures than ejection fraction. We are studying LV diastolic lengthening rate and LV untwisting rate and how these indices may be used as markers of LV diastolic dysfunction. In addition to experimental models we utilize mathematical heart modeling to explore our ideas. This includes finite element simulation to interpret measurements of myocardial wall deformation under normal and diseased conditions. The recently introduced method speckle tracking echocardiography represents a simplified, objective and angle-independent modality for quantification of regional myocardial deformation. The software utilizes conventional grayscale B-mode recordings, and tracks myocardial speckles which serve as natural acoustic markers. Radial and longitudinal myocardial deformation can be measured simultaneously from long-axis recordings, radial and circumferential deformation from short-axis recordings and LV torsion from assessment of apical and basal short-axis rotation.

Patients with acute decompensated heart failure (ADHF) suffer from increased morbidity and mortality. The hemodynamic assessment of ADHF offers the potential of tailored therapy. However, the invasive gold standard is not without risks. Accordingly the non-invasive assessment by Doppler

echocardiography can play an important role in this population. We have collaborated with Methodist DeBakey Heart and Vascular Center in Houston in a prospective multicenter study to examine the application of Doppler echocardiography for the hemodynamic assessment of patients with ADHF.

4. Coronary artery occlusion: Although acute myocardial infarction is treated preferably by early percutaneous coronary intervention (PCI), there is limited access to this treatment, and a large fraction of patients receive intravenous thrombolytics as primary treatment. These patients are referred for "rescue PCI" only when there is no reperfusion after thrombolytic treatment. The main problems with the latter strategy are that all myocardium at risk may have undergone necrosis and therefore PCI is unnecessary. Furthermore, we lack reliable methods to determine if reperfusion has been achieved by the thrombolytic. One of our main objectives is to develop better functional imaging in order to differentiate between viable and necrotic myocardium and to determine when reperfusion has been achieved.

5. Narrow QRS: Cardiac resynchronisation therapy (CRT) has been documented to be a powerful treatment in patients with severe congestive heart failure and left bundle branch block (LBBB). Interestingly, there has also been demonstrated clinical effect of CRT in patients with narrow QRS. As more than two thirds of heart failure patients do not have electrical conduction disturbances extending the indications for CRT into this patient group is going to have considerate implications.

The mechanisms of possible effects of this treatment in heart failure patients with narrow QRS have not been discovered properly. Two possible mechanisms have been suggested: CRT may correct electrical dyssynchrony not seen on ECG or CRT induces changes the interventricular interaction. The change in ventricular interaction can be obtained by pacing in the left ventricular lateral wall. The left ventricle is then activated earlier than the right ventricle and a concomitant phase shift in the ventricular filling appears. This phase shift reduces the left ventricular external constraint. External constraint is determined by the pericardial pressure and the right ventricular pressure and constitutes the external resistance to left ventricular filling. The hemodynamically result of reduced external constraint is improvement of left ventricular filling and increase in cardiac output.

An experimental dog model is used to explore these electromechanical and hemodynamic consequences of CRT. The study is ongoing, but some preliminary results have already been presented at international congresses.

6. Optimal timing of cardiac surgery for chronic valvular regurgitations has been a challenge for years. Development of systolic dysfunction precedes the onset of symptoms in more than one fourth of the patients with this condition. Traditional echo-cardiographic methods like preoperative left ventricular ejection fraction (LVEF) and cavity dimensions are the most important determinants of survival and left ventricular (LV) function after valve replacement for regurgitations. However, volume derived measures of LV function have important limitations in assessing myocardial contractile function where a series of compensatory mechanisms, including an increase in end-diastolic volume (EDV) and hypertrophy, can mask underlying changes in myocardial force development. Therefore, the purpose of these studies are to investigate whether global systolic strain measured by 2-dimensional speckle tracking echocardiography (2D-STE) could detect early onset of myocardial dysfunction in patients with chronic regurgitations and preserved LVEF.

Collaborators

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Research area and aims

Vilhelm Magnus Laboratory for Neurosurgical Research is a research group of the Oslo University Hospital and encompasses the Neurosurgical Departments at Ullevål University Hospital (UUS), Rikshospitalet-Radiumhospitalet Medical Center (RR), and the University of Oslo. The goal of the laboratory is to build a bridge between the basic biological sciences and clinical neurosurgery, to explore the biology underlying neurosurgical conditions, and to facilitate translation of new knowledge from the basic research disciplines into the clinic. Research efforts therefore encompass both normal brain cell development and disease states such as tumors. Investigations aim to understand these processes and develop methods to treat disease as well as promote cell replacement to heal damaged brain tissue.

Stem cells from the adult human brain

A central dogma in neuroscience has been that the mature brain is unable to produce new neurons. Towards the end of the 20th century, studies in birds and rodents came to question this doctrine as new markers for labeling neurons combined with techniques for identifying cells that had been born in adult life, suggested that new neurons sometimes may develop later in life in some species.

At the turn of the century, these findings were to some degree extended to the human brain, as a few research groups had been able to culture immature cells from the human ventricular wall and hippocampus. It was still not known, however, whether it would be possible to differentiate these cells into functional neurons, i.e. cells with typical neuronal action potentials with the ability to communicate via synapses.

The putative existence of an adult human brain stem cell type with the ability to proliferate and differentiate into mature neurons created huge interest as one may now envisage treatment of neurological diseases with either transplantation of stem cells that have been expanded *in vitro* or by mobilization of endogenous progenitor cells. The work on developing functional neurons from cells from the human ventricular wall was started in Professor Langmoen's laboratory at the Karolinska Institute in Stockholm with Morten Moe and Mercy Varghese. Human tissue was harvested from the wall of the lateral ventricle in temporal lobe specimens resected due to epilepsy. In keeping with earlier results from other groups they were able to expand stem cells from the ventricular wall as cell clusters (neurospheres) in vitro. Following dissociation

and exposure to differentiation cues (mainly withdrawal of growth factors and addition of serum) these cells went through characteristic steps of morphological and electrophysiological development and developed into the three principal building blocks of the brain:
1. Astrocytes
2. Oligodendrocytes
3. Neurons

Our group was first to demonstrate that it is possible to transform immature progenitor cells from the adult human ventricular zone into functional neurons, i.e. cells with typical neuronal action potentials and the ability to communicate through synapses (Fig. 1). Essentially, our group was able to develop a small nervous system from a single human stem cell in vitro. This "nervous system", consisting of glial cells as well as a large number of neurons, communicated via synapses. These results were selected from more than 15000 abstracts for presentation at the press conference of

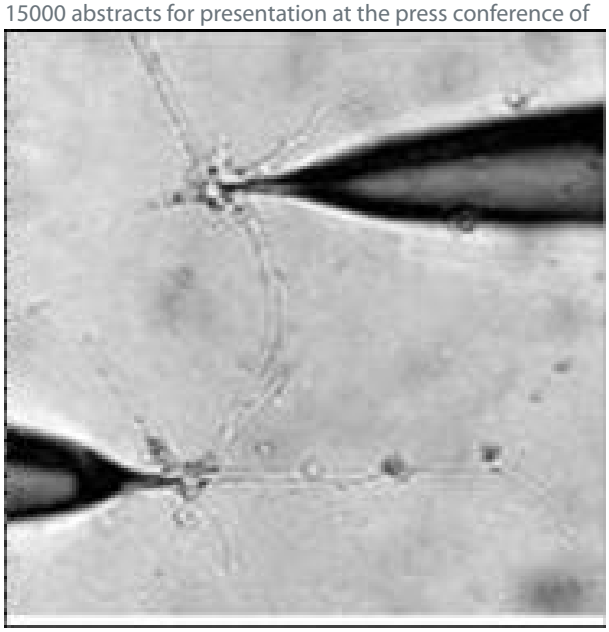

Fig.1
Dual patch-clamp recording from neighboring neuron-like cells, demonstrating synaptic communication between the cells

the Society for Neuroscience six years ago. See Westerlund U et al Exp Cell Res 2003, Moe MC et al Brain, 2005 and Neurosurgery 2005.

For stem cells to be useful in the clinical situation, it must be demonstrable that after transplanting them to another adult brain they can survive and integrate into the recipient neuronal circuitry. Using rats with a selective lesion of the hippocampal CA1-region (a small part of cerebral gray matter), Håvard Ølstørn and Morten Moe demonstrated that stem cells from the adult human brain are not only able to survive in the rat brain, but also selectively target and migrate to the area with the lesion (Olstorn H et al Neurosurgery, 2007).

Use of specific antibodies against human nuclei (HuN) demonstrated survival of the transplanted cells and showed that the grafted cells frequently express the immature marker human nestin. Less frequently, the cells expressed the immature neuronal marker doublecortin and the glial marker GFAP. Ølstørn and Moe further showed that by using 'predifferentiation', i.e. "pushing" the cells in a neuronal direction prior to transplant, it was possible to significantly enhance the development of neurons following transplantation. This study for the first time showed that stem cells from the adult human brain are able to survive and differentiate in another adult brain. The first part of the study secured Ølstørn the first prize at the Scandinavian Neurosurgical Society's Annual Congress in 2005. See Olstorn H et al Neurosurgery, 2011.

Stem cells isolated from adult human filum terminale.

Mercy Varghese and coworkers isolated neural progenitors from the adult human filum terminale (FTNPs). This terminal end of the spinal cord has been referred to as a fibrovascular tag without neurogenic potential and of no clinical significance. Similar to brain stem cells mentioned above these cells from the filum terminale generated functional neurons capable of firing action potentials. When transplanted into the adult central nervous system, FTNPs survived, differentiated and showed targeted migration to site of injury. See Varghese M et al Stem Cells and Development, 2008.

Stem cells and brain cancer

Brain cancers in principle always recur despite apparent complete removal under the operating microscopic and subsequent adjuvant therapy. This is particularly true for the most common intracranial tumor type, the glioblastoma (GBM), where 50 percent of treated patients die within one year from diagnosis. In parallel with results emerging from other research institutions, our group has shown that only a subpopulation of cells in brain cancers have the ability to proliferate and initiate new tumors following transplantation to immunodeficient mice. This cell population infiltrates surrounding brain tissue, appears resistant to both irradiation and chemotherapy, and is the likely explanation for recurrence.

In a leading study, Mercy Varghese and Morten Moe showed that these cells share a number of the properties of normal

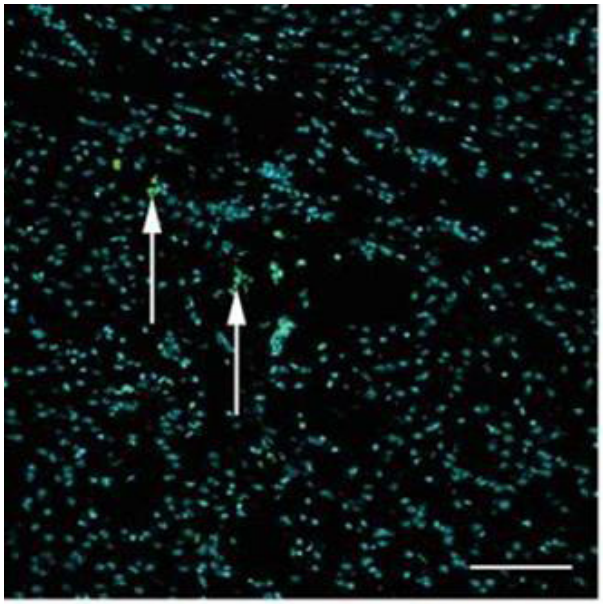

Fig.2a
Transplantation of normal progenitor cells from the adult human brain (top panel, green) did not result in tumor formation, whereas stem cells isolated from brain tumors (green, bottom panel) reproduced a highly invasive tumor in the mouse brain. Blue nuclear staining in both panels.

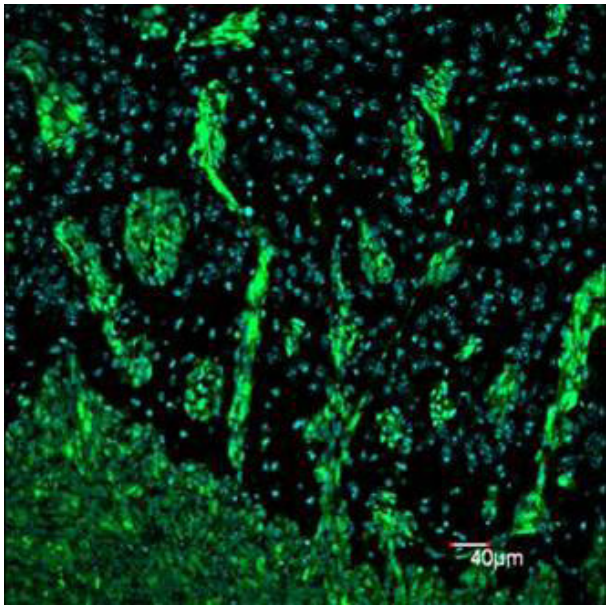

Fig.2b

2010 2010). He has also explored the cellular organization of neuro- and tumorspheres, looking at the cellular heterogeneity of such spheres (Vik-Mo et al, Exp Cell Res. 2011 2011). By sorting tumor cells based on surface antigens, we hope to establish methods for better identification of the progenitor population.

We have also used this technology and experience to establish a clinical protocol. This protocol is designed to harness the patients' own immunity. The inclusion of patients into the "Phase I/II trial of vaccine therapy with hTERT, survivin and tumor stem cell derived mRNA- transfected dendritic cells in patients receiving standard therapy for glioblastoma" started in February 2009. So far 16 patients have been recruited to the study. This clinical trial is backed up from the collaboration through the Cancer Stem Cell Innovation Center (SFI CAST) and is a collaboration with the Neurosurgical department, Avd. for klinisk kreftforskning ,Avd. for celleterapi, and Avd. for immunologi, Institutt for kreftforskning, Radiumhospitalet and the Oncological department at Oslo University Hospital.

Cecilie Sandberg has compared the global gene expression in normal stem cells and tumor stem cells, in order to identify possible targets for treatment and to better understand the biology of the cell population that escapes current treatment and causes recurrences. The results of this comparison study show a significant upregulation in tumour stem cells of genes connected to regulation of focal adhesion, actin cytoskeleton, axon guidance as well as the Wnt pathway. Putative target genes have been confirmed at the protein level using immunohistochemistry and Western blot. Currently, their role in gliomas is investigated using siRNA-knockdown based technology and its effect on proliferation, apoptosis and sphere-forming capacity. The roles of the possible targets in the Wnt pathway are investigated by Kirsten Strømme. Cecilie Sandberg is also, in collaboration with Morten Moe, exploring the transcriptome of single cells in neuro- and tumorspheres, looking at the cellular hetereogeneity of such spheres.

Biljana Stangeland, identified a set of 20 genes that were up-regulated in GBM tumour cultures using C. Sandberg's micro-array data. These results were confirmed by quantitative RT-PCR (qPCR) comparing human adult neural stem cells and tumours. In order to measure the relative expression of genes, tumour and aNSCs cultures have to be grown under identical conditions. B. Stangeland and Awais Mughal tested different growth conditions and modified the current procedure so that it could be used for qPCR. For some candidates where the corresponding antibodies were available immuno-labelling and Western-blot were applied. Genes that are up-regulated in tumours are involved in cell-cycle/division, epigenetic regulation, signalling or have unknown functions. A few candidates seem to be inhibiting known tumour-suppressors. The next goal is to down-regulate (knock-down) the expression of the candidate genes using

shRNA and siRNA technology in the tumor cell-cultures. The growth of the tumor cells will be monitored by different assays (proliferation, sphere formation, apoptosis etc). In addition to B. Stangeland and C. Sandberg, two PhD students Mrinal Joel and Awais Mughal are currently working on this project.

Awais Mughal started as a PhD student in the group in 2009. His project is generated by Cecilie's microarray data focusing on upregulated gene candidates confirmed by Biljana Stangeland. A selection of gene candidates that may serve as clinical targets is being investigated with immunohistochemistry and Western Blots to evaluate the protein expression. Lentiviral based shRNA-technology is applied to silence genes of interest and establish stable Knock-Down cultures. The gene silencing efficiency is measured on mRNA- and protein level and the cultures are subjected to functional studies. In vitro functional studies investigate effects on proliferation, apoptosis and sphere-formation. The biological importance of these findings can be further investigated using our xenotransplantation model in immunodeficient mice. This functional screening will hopefully enable us to identify novel clinical targets in GBM.

Aquaporins or water-channels are targets indicated by Cecilie's work. Former medical student, Guri Fossdal has investigated the expression of these in tumor stem cells and their differentiated counterparts. This work is currently being submitted for publication.

Mrinal Joel is studying transplantation of GFP-transduced brain tumour stem cells to an embryonic environment using the chick embryo model. She investigates the behaviour and differentiation potential of tumor cells when placed in this environment. So far, interestingly, tumor cells display a more restricted activity in an embryonic environment compared to adult. Further studies will be based on the analyses of proliferation, cell death and differentiation ability of these cells into other cell types. Co-culture studies of the tumor cells with the cells from the central nervous system of chick embryo are also under investigation to examine these effects.

Proteomic studies to find surface markers in adult neural and cancer stem cells have been carried out by Post-Doc Linda Paulson. Human adult neural stem cells could be one avenue to repair brain damage. In this project we are going to characterize cultured human brain stem cells by proteomics. Transplantation of well characterized cells could in the future be a treatment for a range of patients with different brain disorders such as Parkinson's Disease (PD), Alzheimer's Disease, and also patients with trauma. The underlying conditions affecting a patient may well demand different types of transplanted cells, for example, PD patients need cells that are potentially dopaminergic. It is desirable to characterize these cells before and after induction to differentiate.

In this study we have also compared human normal stem cells with tumor stem cells. Hopefully this may reveal why the two types of cells behave differently in the brain. This information may help in designing treatments and could have a major impact on clinical practice.

We have adapted a method called SILAC proteomics. SILAC is a straightforward approach for in vivo incorporation of a label into proteins for mass spectrometry-based quantitation. SILAC relies on metabolic incorporation of a given 'light' or 'heavy' form of an amino acid into the proteins; amino acids with substituted stable isotopic nuclei (e.g. containing deuterium, 13C, or 15N). Thus in an experiment, two cell populations are grown in culture media that are identical except that one of them contains a 'light' and the other a 'heavy' form of a particular amino acid. When the labeled analogue of an amino acid is supplied to cells in culture instead of the natural amino acid, it is incorporated into all newly synthesized proteins. The process is efficient and reproducible as the incorporation of the isotope label is 100% and does not affect protein behaviour. By adding stable, non-radioactive isotopic forms of amino acids to media when growing cells, it is possible to get a mass difference of 6 kDa in the same protein from different samples. Thus we can quantify relative protein abundance as cellular and metabolic processes occur. Experimental results achieve high fidelity with minimal bias, allowing relative quantitation of even small changes in specific protein abundance.

Characterization of adult human retinal stem cells¬

During embryogenesis, the neural retina, ciliary body (CB) and distal iris of the human eye are also formed from the multipotent stem cells of the neuroectoderm. In adults, the retina has limited regenerative potential, and severe injuries will lead to permanent damage. However, recent studies have shown that the adult CB harbours a small population of progenitors with characteristics of neural stem cells that are quiescent in vivo but can be expanded in culture. In a collaboration project with the Centre for Eye Research, Department of Ophthalmology, Oslo University Hospital, Morten Moe and colleagues are currently studying the isolation, characterization and differentiation of retinal stem cells from the adult mammalian eye.

Moe and coworkers (Exp Eye Res 2009) published the first

direct comparison of human CB spheres with neurospheres derived from the human subventricular zone (SVZ). They found that both CB and SVZ spheres contained a population of neural progenitors, but that CB spheres, as opposed to SVZ-spheres, also contained cells with an epithelial phenotype, as judged by morphology, ultrastructural characteristics, expression of epithelial markers, and downregulation of neural progenitor markers.

Recently the team has shown that cells from the human iris pigmented epithelium (IPE) form spheres in culture with similar properties to those derived from the CB. They have also shown that such cells can be isolated with a minimal invasive surgery, the peripheral iridectomy, thus suggesting the putative scenario of autotransplantation. After induced differentiation, cells from both CB and IPE spheres acquire a mature neuronal morphology and strongly express more mature neuronal markers such as beta-III-tubulin and Map-2. A subpopulation of the beta-III-tubulin-positive cells also shows robust expression of rhodopsin, a marker of mature photoreceptors. Thus, spheres derived from the human CB and IPE have the ability to differentiate into neuronal-like cells, including photoreceptors.

Håvard K. Skjellegrind started as a PhD student in 2010. He is doing live imaging of single cells, aiming to identify the "true stem cells" in heterogeneous normal stem cell and tumour stem cell cultures. To identify expression of specific genes in live cells, the cells are labelled with genetically driven reporter constructs. Fluorescent protein coding genes linked to the promoter sequence for cell type-specific genes were transferred to the cells using lentiviral vectors. These vectors were created by Yasu Watanabe whilst working at the Australian National Adult Stem Cell Centre. By live fluorescence microscopy, expression of the genes can be assessed. We expect these experiments will reveal insights into developmental events in both normal and tumour development. Our lab possesses reporters for neural stem cells, glia, neurons, Schwann cells, and dopaminergic neurons. Mitochondrial membrane potential is also examined in single cells as a potential marker for "stemness".

Towards tissue repair

An avenue where adult human neural stem cells offer tremendous promise is in the treatment of degenerative diseases such as Parkinson's disease. Parkinson's disease is characterized by loss of pigmented dopamine-secreting cells in the substantia nigra. Though transplantation of embryonic stem cell- or fetal stem cell-derived dopaminergic cells has shown promising results, the results have not been consistent. Furthermore, the ethical issues regarding the use of the aforementioned cell types, limit their clinical use. Dopaminergic cells derived from adult human brain stem cells have obvious benefits: firstly, they pose no ethical challenges and secondly, they make autologous transplantation possible. We are investigating the potential of adult human brain stem cells to develop into dopaminergic neurons. PhD graduate Mercy Varghese obtained preliminary results that in vitro, adult human brain stem cells can develop into neurons expressing tyrosine hydroxylase, a rate-limiting enzyme in dopamine synthesis.

Wayne Murrell joined the Vilhelm Magnus lab as Senior Scientist at the end of 2007. Wayne is from Australia and for the previous seven years has led laboratory research at Griffith University, Brisbane, on neural stem cells derived from a region of the peripheral nervous system, the olfactory mucosa. Wayne is first author of a paper Multipotent stem cells from adult olfactory mucosa (Murrell et al, Dev Dyn 2005) which demonstrates the potential to generate neurospheres, direct their differentiation towards neuronal and non-neuronal lineages and suggests autologous tissue repair is a real possibility. This paper was pivotal in gaining support from the Australian Federal Government for the establishment of an Australian National Adult Stem Cell Centre in Brisbane working on human-derived neural stem cells for potential to treat human disease. Wayne has broad experience in the fields of molecular, developmental and cell biology. In recent times he has investigated stem cell transplant in various animal models of disease including Parkinson's, heart attack, disc degeneration and motor neuron diseases (Murrell et al, Stem Cells 2008, Spine J 2009, Dis Model Mech 2010, N Engl J Med 2010). Now he helps guide the efforts of the researchers of the Vilhelm Magnus lab. Any effective tissue replacement therapy will require capability to produce authentic stem/progenitor cells in bulk quantities whilst maintaining cell integrity.

We have now developed culture methods for the rapid isolation, maintenance and proliferation of undifferentiated adult stem cells. Many suggestions for improvement made by former Post-Doc John Bianco have been quantitated systematically by Wayne Murrell and Emily Telmo. Today, a robust technique for the culture of these cells in billions is now established. At present, studies are being performed to assess the fate of these stem cells and their differentiation potential. Directed differentiation of these cells towards a specific fate such as down the dopaminergic pathway to obtain dopaminergic neurons is being examined.

What are the cellular processes of healing? What is involved in tissue repair? Surgical ablation of vital brain tissue or the damage accidents cause to the spine and subsequent

paraplegia are both relevant scenarios for cell replacement therapy. As well treatments of neurological disorders such as Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's are desirable. We have shown that stem cells exist throughout the adult human nervous system. Human neural stem cells can be manipulated to differentiate to defined phenotypes in vitro. Thus we believe that human neural stem cells can repair damaged neural tissue. Are neural stem cells from different regions equivalent? Or can they be induced to be interchangeable? This is a fundamental question pertaining to any attempt at human neural tissue repair. For instance could cells from the filum terminale or the olfactory mucosa be used to repair the human brain.

Olfactory mucosa has been shown a potential source for autologous stem cell therapy for Parkinson's disease (Murrell et al, Stem Cells, 2008). Parkinson's disease is a complex disorder characterised by degeneration of dopaminergic neurons in the substantia nigra in the brain. Stem cell transplantation is aimed at replacing dopaminergic neurons because the most successful drug therapies affect these neurons and their synaptic targets. Murrell et al. have shown that neural progenitors can be grown from the olfactory organ of persons even with Parkinson's disease. These neural progenitors proliferated and generated dopaminergic cells in vitro. They also generated dopaminergic cells when transplanted into the brain and reduced the behavioural asymmetry induced by ablation of the dopaminergic neurons in the rat model of Parkinson's disease. These results indicate that Parkinson's patients could provide their own source of neuronal progenitors for cell transplantation therapies.

Artem Fayzullin joined the Vilhelm Magnus Lab in 2010. The aim of his project "The transplantation of adult human neural stem cells in rat model of Parkison's disease" is to evaluate the possibility of autologous neural stem cell transplantation-based treatment of patients with Parkinson's disease. The specific aims of the study: 1.To investigate the ability of transplanted neural stem cells to reduce the signs of disease in Parkinsonian rats. 2. To estimate and compare the therapeutical efficacy of neural stem cells transplants from different sources (olfactory mucosa, subventricular zone and filum terminale). 3. To test the hypothesis that neural stem cell transplants will produce dopaminergic neurons in the striatum and/or will promote increased innervation of grafted striatum by surviving dopaminergic afferents.

This report therefore summarizes the Vilhelm Magnus lab's current work on the cell biology of the human brain and nervous system. We seek to broaden knowledge of cell development encompassing the biological concepts of stem cells, cancer stem cells and the development of tumors, cell physiology of anesthesia, and as well the future development of cell replacement therapy.

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Experimental Orthopaedic Research

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Research area

Joint injuries, diseases and fractures are main reasons for disability in the community and are often subjected in younger age groups. It certainly involved large costs for the society and improved health care in this area would be a significant improvement both for the individual and the society. The Experimental Orthopaedic Research group applies experimental methods on different aspects of orthopaedics, including research on clinical material (biopsies, joint fluid, and retrievals), animal experiments and cell culture. Mechanical testing of structures, including live anaesthetized animals, and materials has been one of the main parts of our research methods. Several of the projects worked on during the last years were fulfilled with publication or PhD dissertations during 2010. The experimental work in the laboratory is close connected to ongoing or clinical studies under planning for improvement of orthopaedic care of these patients in the community and the involvments of the clinicians is one of the strength in the group.

Aims

Develop a novel treatment of focal cartilage defects
Reduce the numbers of deficient fracture healing
Improve healing of tendon grafts in orthopaedic surgery
Delineate the best biomaterial surface for prothesis surgery

CARTILAGE RESEARCH

Malfunction of the knee joint is often associated with cartilage injury. Whether healing or restoration of lost or wounded portions of articular cartilage with newly formed fully functional cartilage is possible remains one of the unsolved problems in orthopaedic practice. These knee patients have more problems than patients with rupture of anterior cruciate ligament rupture and experience severe limitations in their daily life. Despite this the knowledge about the best treatment and if surgical treatment do offer a better outcome than the natural history is still not documented. A better understanding of articular cartilage biology, pathophysiology and biomechanics are definitely warranted. Focused research questions in the group, have been to improve the understanding of the cartilage repair process and to figure out if mesenchymal stem cells should be used instead of chondrocytes for cartilage repair. The ongoing work of the cartilage research group can be divided in three main areas

Experimental cell-culturing cartilage research

The group has worked intensively providing the best cell source for cartilage repair. Mesenchymal stem cells harvested from the bone marrow implanted in hyaluronan-based scaffold have been intensively studied in the laboratory for production for production of articular hyaline cartilage specific markers. Theoretical these cells have a promising potential for repair of normal hyaline cartilage. The collaboration with the Ex Vivo Laboratory (RH) headed by Jan Brinchmann has improved our ability to be supplied with improved cells for cell based cartilage repair and to test and develop new scaffolds (Fig. 1).

Experimental cartilage research in an animal model

An important issue in cartilage treatment is the location of

defect in the knee and the consequence this might have for the treatment of defect and risk of degenerative changes. One experimental study has been performed to specifically investigate this issue and the location is essential as shown in the current study. The difference in the outcome between microfracture and mosaicplasty has been difficult to evaluate clinically and an experimental study has been conducted to evaluate these clinically techniques. Changes are observed in the subchondral bone that is of concern for the long-term risk of degenerative changes in the joint. Mesenchymal stem cells implanted under a commercially available scaffold for repair of articular defect have been evaluated experimentally to look at feasibility of this technique and the amount of cartilage obtained in the defect.

Clinical projects

The experimental work is close connected to the clinical studies of the group and there is an ongoing study on stem cell treatment versus chondrocyte implantation. Patients with cartilage defects of the knees treated with chondrocyte implantation have been evaluated with electron microscopy biopsies to look at ultra organization of the tissue and deaths of chondrocytes in a recent publication.

Deficient fracture healing

Delayed fracture healing or non-union may occur in patients with systemic diseases, with hormonal or nutritional deficiencies, or in patients taking specific medications like non-steroidal anti-inflammatory drugs (NSAIDs and COXIBS). NSAIDs have been reported in general to affect bone metabolism, and indometacin has been shown to reduce fracture healing experimentally, and to inhibit ectopic bone formation clinically.

In these studies we have investigate fracture healing in rats on a short time medication with an injectible COX-2-inhibitors, and in rats made severely osteoporotic by estrogen and vitaminD_3 depletion. Parecoxib is a new and selective NSAID targeting cyclooxygenase-2 (COX-2). Through their selective action these drugs are supposed to lack many of the main side effects of other NSAIDs and thereby, expected to be drugs of choice in near future for several patient groups. The effects on bone metabolism and healing have, however, not been elucidated. Furthermore, it is demonstrated that COX-2 is required for both intramembranous and endochondral bone formation. Thus there are reasons to concern regarding the potential negative effects of these drugs on bone metabolism and bone repair. Concern has been expressed about its potential negative effects on bone. The present study was designed to investigate the effects of short-term administration of parecoxib on bone healing. Based on the current knowledge our hypothesis is that the drug will delay the healing process and this is verified and published.

Another project on intramedullary nailing and external fixation in lower leg fractures evaluates bone healing by quantitative micro computer tomography (qmCT), in addition to mechanical testing and densitometric measurements. This includes development of a segmentation technique applied to qmCT images and an investigation of correlation between the segmented qmCT data and mechanical properties. The group documents the efforts and results made in these novel projects in a recent publication This work is soon to be finalize in the PhD defense scheduled to the 11th March 2011, by one of the group members Ulf W Sigurdsen. Allograft is important both in bony defects as well as in arthroscopic surgery and experimental work on the biological events of allograft is recently published.

Tendon healing to bone surfaces/tunnels
Healing of tendon material to bony surfaces is of major importance in both shoulder and knee surgery where understanding of the biology and biomechanics in this process is a key element. A recent experimental paper from the group demonstrates that this is significantly affected by COX-2-inhibitors.

PhD-dissertations
There was two PhD-dissertations in 2010 based on the work from the recent years by consultant Sverre Løken concerning cartilage injuries and Sigbjørn Dimmen on the effects on NSAIDS in fracture and tendon healing. In May 2011 another of the group members (Stig Heir) will fullfill his PhD dissertation. Furthermore the fracture research soon will be fulfilled with the PhD disseratation March 2011 of Ulf W. Sigurdsen.

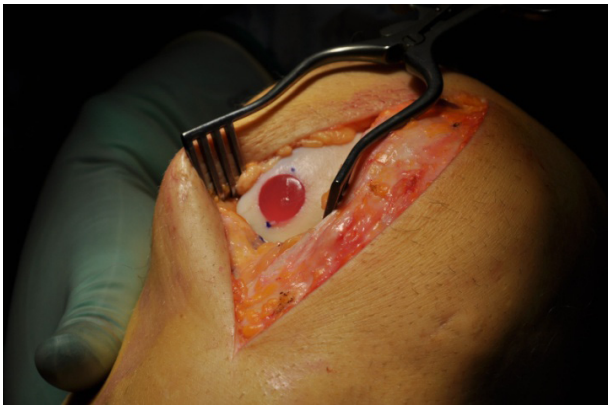
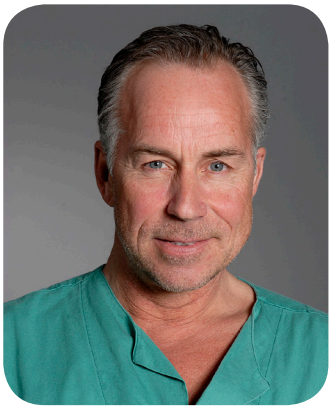


Figure 1. *Cartilage defect treated with a chondrocytes loaded scaffold*

Cell Transplantation

Leader:

Aksel Foss, Professor, MD, PhD, MHA, FEBS (OUH/UiO)



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Introduction

Cell transplantation is a fast growing field in medicine. In front of a broad area of potential therapeutic options for cell transplantation is transplantation of insulin producing pancreatic islets as a feasible alternative for treatment of unstabile type 1 diabetes. As such the treatment is highly successful, but several hurdles have to be overcome before islet transplantation can be a routine treatment for patients with diabetes.
The first clinical islet transplantation in Norway was performed at Rikshospitalet in 2001 by Foss and co-workers. The Research Group for Cell Transplantation has been located at the Institute for Surgical Research, Rikshospitalet since 2005. From that time on, several articles of experimental islet transplantation have been published. In 2009 the first doctoral thesis from the work of the research group was published (Tormod Lund). A master degree was published in 2008 (Ingrid Aursnes Stølen). Currently, two PhD-students and one post doc is working full time with experimental islet transplantation. The Research Group for Cell transplantation is part of the Nordic Network for Clinical Islet Transplantation and works in close collaboration with Uppsala University.
During the last years extensive work has been carried out to establish facilities for isolation and culture of other cell types such as hepatocytes, neural cells and even xeno-cells, with the aim of creating a 'Center for Cell Transplantation' at Rikshospitalet. A working group of key individuals from IKF, the University of Uppsala and the Karolinska Institute have joined to bring forward a robust inter-Scandinavian collaboration to address new potential cell therapies and a defined research program has been established. The strength in this organization is that the people involved are active clinicians who rapidly are able to transfer new knowledge from the laboratory into the clinic which is essential in modern medical science.

For further advance in the field of cell transplantation, knowledge obtained from islet transplantation research is important. Inflammatory reactions are key elements in islet isolation, culture and transplantation as it is in many other biological processes. Functional and experimental studies suggest that that the inflammatory status of cells and tissues as well as at the implantation site is important determinants for the outcome of cell transplantation. To understand and control the processes of inflammation is therefore necessary for further advances in the field of cell transplantation.

Pancreatic islets

General Objectives

- To utilize the present knowledge on clinical islet transplantation and to induce C-peptide production in unstable type 1 diabetics, thus improving quality of life and reducing long term complications of type 1 diabetes.
- To develop strategies to suppress inflammatory reactions elicited by islet transplantation and to establish non-toxic immunosuppressive protocols in islet transplantation, able to induce immune tolerance.
- To explore and develop striated musculature as an alternative site for islet transplantation.
- To obtain methods for in vivo surveillance of islet cell engraftment, distribution and function by bioluminescent- and PET imaging.

Background

Type 1 diabetes is an autoimmune disease with destruction of insulin producing β cells, leading to dysregulated glucose homeostasis. Despite effective insulin therapy, allowing these patients to survive, imperfect blood sugar control may result in micro-vascular complications. These complications can to some extent, be prevented by maintaining near-normal glucose control by multiple daily insulin injections or insulin-pump therapy. However, stable metabolic control is difficult to achieve in some patients despite the use of modern insulin analogues and delivery systems, resulting in life threatening episodes of insulin-induced hypoglycemia. Transplantation of whole organ pancreas is an optional treatment for type 1 diabetes. A major drawback of the procedure is the complexity of the surgery and postoperative complications caused especially by the exocrine pancreatic tissue. On the opposite, islet cell transplantation is a simple and gentle procedure with few complications. It is performed in local anesthesia. A catheter is inserted trans-hepatically into the main stem of the portal vein. Purified islets flow by gravity from the infusion bag through the catheter and are dispersed in the sinusoids throughout the liver (Fig. 1). Subsequently, a neo-angiogenesis occurs (engraftment) and the islets begin to produce and release insulin within two weeks after the transplantation. The patient remains awake during the entire procedure which lasts for less than one hour.

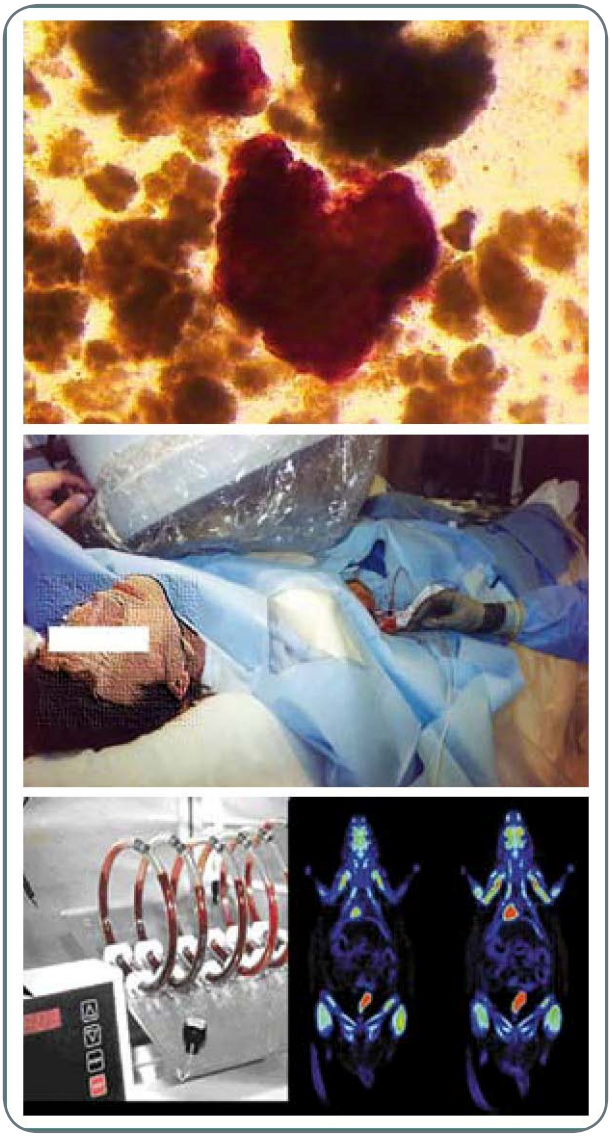

Figure 1. *Aspects of islet cell transplantation.*

Several reports have shown that up to 80% of the patients transplanted are off insulin one year after islet transplantation, but only a few remain free from insulin requirement long term. However, the majority of patients have significant C-peptide production five years after the transplantation and thus, many patients who previously were suffering from life threatening hypoglycemia exhibit well-regulated metabolic control and improved quality of life.

Multiple deleterious events affect the amount and quality of islets from the time of ICU-treatment of the donor, through the processes of brain death, organ retrieval, islet isolation, infusion and engraftment. Following successful engraftment islets are influenced by allo- and autoimmunity and

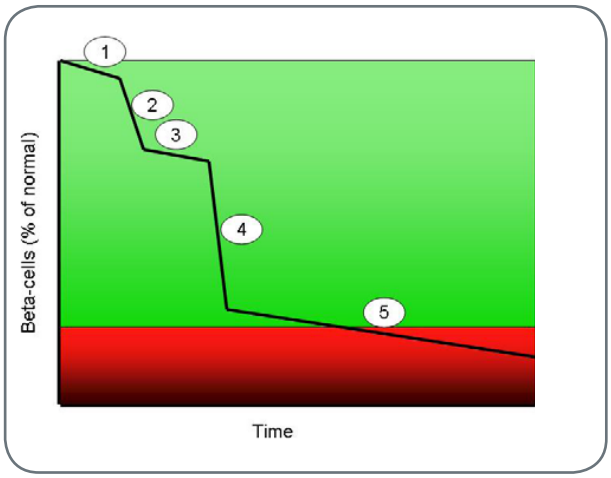

Figure 2.
Schematic presentation of loss of islets from the time of organ retrieval to revascularization in the recipient.
1. Loss of islets during brain death and organ harvest. 2. Loss of islets during islet isolation. 3. Loss of islets during pre-transplant culture. 4. Loss of islets during the islet transplant procedure 5. Loss of islets during post-transplant period prior to revascularization. Green area represents insulin-independence, red area insulin dependence for the patient.

toxic effects of immunosuppressive drugs (Fig. 2). Important stress factors for islets include hyperglycemia and islet hypoxia. Hypoxia is a well-known trigger of inflammation, of which TF, MCP-1, IL-8 and MIF probably are the most important factors. We have discovered and reported that the amount of TF expressed on in islets varies considerably in organ donors. Recent, we have found a correlation between the expression of TF and MCP-1 in pancreas biopsies obtained prior to islet isolation and the clinical outcome of islet transplantation indicate. Others and we have demonstrated that a thrombotic/inflammatory reaction is elicited when islets come in direct contact with blood. This reaction is termed the instant blood-mediated inflammatory reaction (IBMIR). In clinical islet transplantation, IBMIR is triggered within minutes after islet infusion. The detrimental effects of IBMIR provide an explanation for the need for islets from 2-4 donors in order to obtain normoglycemia in many transplantation series. The Nordic Network for Clinical Islet Transplantation recently obtained an NIH grant of $ 10 million to conduct a multi center clinical trial on 72 patients, with the aim of improving results in islet transplantation by reducing IBMIR.

Research Plan

The research group is focusing on improvements in organ harvesting, islet isolation techniques, ex vivo manipulation of islets, and development of large-scale techniques for islets isolation from other species for potential use in humans. The overall aim of our experimental and clinical work is to identify strategies to counteract detrimental effects on islets prior to engraftment and to improve islet growth and function post transplantation.

Project 1. Clinical trials

1.1. Open, prospective, controlled study to evaluate the effect of islet transplantation on quality of life in selected patients with unstable type 1 diabetes.
It is well established that islet transplantation regularly induces sustained C-peptide production in type 1 diabetes, resulting in reduced insulin need and improved metabolic control. Furthermore, C-peptide production corrects peripheral insulin insensitivity and the fatty acid profile of the patients. It has been shown that endogenous C-peptide improves quality of life of the patients and reduces long term complications of the disease.
Despite optimal insulin therapy, more than one thousand type 1 diabetes patients in Norway suffer from unawareness of hypoglycemia with recurrent episodes of unconsciousness (Brittle diabetes). These patients have neuroglycopenia without adrenergic warnings making them completely disabled by the disease. The incidence of sudden death is significantly higher than the normal population. The research group is conducting clinical trial where the goal is to induce endogenous C-peptide (and not insulin independence). Patients transplanted with allogeneic islets require life-long immunosuppression (IS). Several of these drugs may induce diabetes, accelerate atherosclerosis and some are nephrotoxic. Therefore, the risk of islet transplantation (and immunosuppression) must be balanced to the risk of having the disease. Sample size (n= 15) is calculated by improvements in QOL of 30 % with a power of 80 %. Primary end point is QOL at 12 months. The complete study protocol is available at http://www.oslo-universitetssykehus.no

1.2 Open randomized multi-center study to evaluate safety and efficacy of low molecular weight sulfated dextran in islet transplantation.
Low molecular weight dextran sulfate (LMW-DS) is a strong candidate to prevent early islet graft destruction caused by the instant blood-mediated inflammatory reaction. In addition, LMW-DS has shown to increase endogenous release of islet protective hepatocyte growth factor which could be an additional beneficial effect of LMW-DS during the first critical hours after transplantation. 72 patients will be recruited in the study, and 6 patients in Oslo are so far enrolled in this study.

Project 2. Establishing improved non-diabetogenic immunosuppressive protocols in islet transplantation, able to induce immune tolerance.

A key factor for success in islet transplantation is said to be avoidance of steroids as shown by the Edmonton group in 2000. The IS regimen in the Edmonton protocol consists of tacrolimus, rapamycin and IL-2 antagonist induction therapy. Following the publication of the Edmonton group almost all clinical trials have utilized this type of IS after the transplantation (although it is well documented that tacrolimus induces de novo diabetes in up to 25 % of allotransplanted patients). The aim of project 2 is to identify an immunosuppressive maintenance regimen that inhibits IBMIR, has minimal islet toxicity and the ability to induce tolerance. In a step-wise methodological approach different immunosuppressive compounds are screened for diabetogenic properties and effect on proinflammatory cytokines (TF, MCP-1).

2.1 Screening of immunosuppressants and anti-inflammatory agents

A selection of compounds is tested regarding their relevance in clinical and experimental transplantation with special

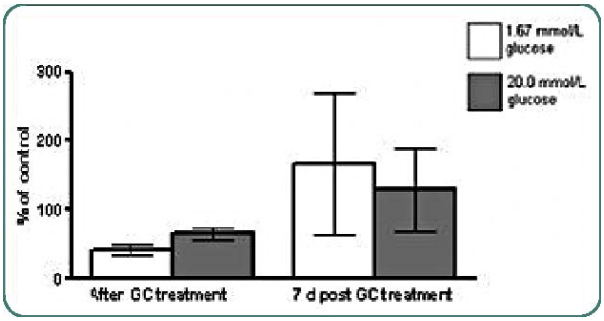

Figure 3. *Effects of glucocorticoids (GC) on insulin secretion.*

emphasis on their influence on insulin metabolism in human islets. The agents that are tested are cyclosporine A, tacrolimus, rapamycin, daclizumab, mycophenolate mofetil, FTY 720, LEA 29 and the glucocorticoid Solu-Medrol (Fig. 3). Each drug is added to human islets in culture medium at four different concentrations in order to establish the dose-response relationship. The effect on islets is analysed regarding insulin secretion, expression of TF, MCP-1 and TNF-alfa, mRNA content, and apoptotic genes (Siva (CD27BP), TNFR 1, TNFR2) at different time points after incubation. Specifically, our data have shown that glucocorticoids induce a marked reduction of TF mRNA levels which correlates to the strength of IBMIR and subsequently, the success rate of islet transplantation. Furthermore, the data show that a negative influence of glucocorticoids on insulin secretion early after incubation of human islets is completely reversed on day 7 (see fig.). This suggests that brief exposure of glucocorticoids to islets pretransplant suppresses IBMIR and aid engraftment of transplanted islets.

Several other anti-inflammatory compounds have been tested such as resolvin E1 and the LXR-agonist GW3965. Currently, the effects of the IL-1β receptor antagonist Anakinra and the anti-IL-6 receptor monoclonal antibody Tocilizumab on islets are investigated. These studies are defined in a PhD-project for dr. Afaf Sahraoui.

2.2 Whole-blood tubing loop system

Many of the immunosuppressive agents (e.g. calcineurine inhibitors) are largely bound to erythrocytes in vivo. Due to the IBMIR effect it has been shown that a large number of transplanted islets rapidly are trapped in clots of erythrocytes containing high concentrations of the immunosuppressive drug. We have postulated that a slow release of the drug to the islets will ensue. To test this slow-release effect, islets (4000 IEQ) are suspended in 6 whole-blood tubing loops (Fig. 4) and drugs are added to the loops at different time points i.e. 0, 30, 60, 120 and 240 minutes

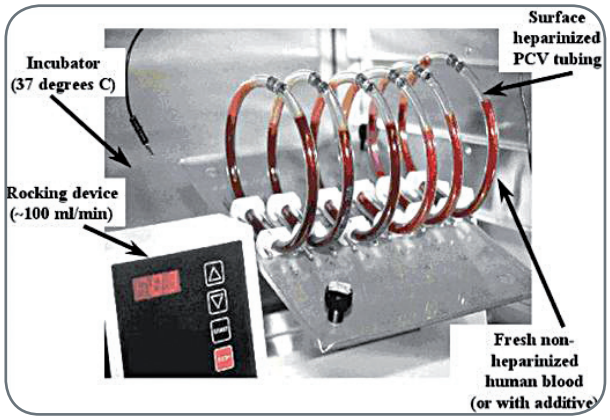

Figure 4. *The whole-blood tubing loop system.*

2.3. Intracellular regulation of immunosuppressive drugs in human islets

Together with the i2mc research group, headed by Prof. Stein Bergan we are investigate the pharmacokinetics, pharmacodynamics and pharmacogenomics of the immunosuppressive drugs in human islets.

Project 3. To explore and develop striated musculature as an alternative site for islet transplantation

Intraportal transplantation of islets is the most common method for islet allo- and auto transplantation. However, functional and experimental studies suggest that a large part of the intraportal transplanted islets are destroyed shortly after infusion, due to islet-blood interactions. In addition, it has been suggested that intrahepatic islets are exposed to high concentrations of diabetogenic immunosuppressive drugs, nutrients and gut hormones. This may lead to hyper secretion of undiluted insulin into surrounding hepatocytes and focal steatosis. Steatosis following islet transplantation may cause islet lipotoxicity and eventually be a risk factor for the development of adenomas in the liver.

Finally, due to procedural risks, it has been difficult to obtain the serial biopsies necessary for characterization of islet engraftment or rejection in the liver. For these reasons, development of an alternative site for islet transplantation has been suggested to be an important factor for further progress in the field. Although porcine islets could be an unlimi-

Figure 5. *The musculature islet transplantation site.*

ted source for xenotransplantation, still, IBMIR and other factors that hamper the success of allo-islet transplantation will remain. The intramuscular site allows manipulation of the graft and the implantation site prior to transplantation and could easily be excised if necessary (Fig. 5). We have shown that islet transplantation into striated musculature is feasible, however, in its present form the intramuscular site is less efficient compared to the liver. Ex vivo manipulation of islets is discussed, especially coating of the islets with mesenchymal and endothelial cells from the recipient and thus reducing the antigen presentation of islets after transplantation. Explorative studies on striated musculature as a potential site for islet transplantation is defined in a PhD program (Kristine Kloster-Jensen).

Project 4. In vivo surveillance of transplanted islets

Transplanted islet cell mass is not static but changes in response to the environment, e.g. inflammation at the transplant site, toxicity of immunosuppressants and influence by the immune system. Non-invasively monitoring of changes in islet mass following islet transplantation is important to provide new insight into factors regulating post transplant function. The research group is exploring two different approaches for post transplant islet mass surveillance.

4.1 Bioluminescent in vivo imaging

Recently, in collaboration with the Transplantation Research Laboratory, UCSF (Dr. Hanne Scholz) the transgenic mouse stock, insulin I promoter (MIP-luc) has been imported to our laboratory. In these mice, luciferase-expressed beta cells is visualized by bioluminescent imaging and has been shown to correlate with beta cell mass (Fig. 6). Thus, this non-invasive model can be utilized to monitor changes in beta-cell mass over time in individual animals. The bioluminescent imaging technique is an important tool for the research in islet transplantation, and is implicated in many of the ongoing studies.

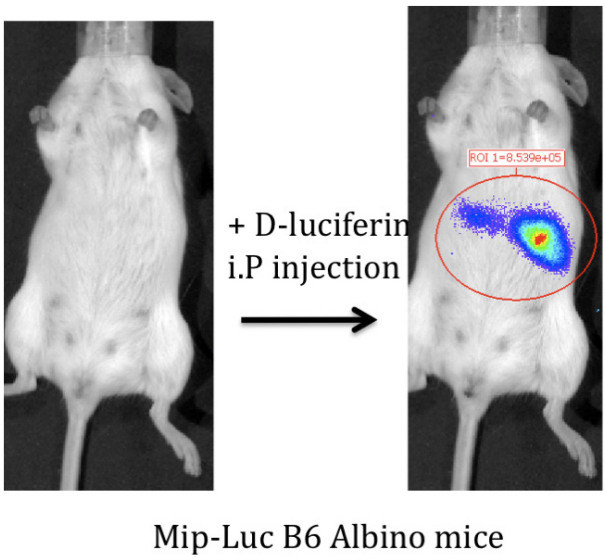

Figure 6. *Bioluminescent in vivo imaging.*

4.2 PET in vivo imaging

Pancreatic islet cell engraftment, distribution, function and survival can be visualized by PET in vivo imaging. The application of the PET technology in clinical islet transplantation takes advantage of the recent rapid development of non-invasive imaging technology in a new area of medicine. The aim of this project is to obtain a new unique method to acquire detailed knowledge of the engraftment process of intraportal implanted islets in man.

Hepatocytes

General objectives

Uppsala university/Karolinska institute/Oslo University (Institute for Surgical Research)

- Approaches to overcome the Instant Blood-Mediated Inflammatory Reaction (IBMIR) induced by hepatocytes at the time of transplantation.
- Enhancement of hepatocyte engraftment by LMW-DS induced HGF
- Development of a clinically applicable imaging technique for the evaluation of hepatocyte engraftment.

Background

Whole organ liver transplantation (Ltx) is standard clinical treatment for patients with acute and chronic liver diseases and even for selected patients with primary and secondary liver tumors. However, due to organ shortage a significant number of patients are dying on the waiting list. Several initiatives such as split-liver (one liver for two recipients) and live donor transplantation have been introduced to reduce the waiting lists for Ltx. However, additional approaches are necessary to overcome pretransplant mortality. Experimental studies and some clinical trials have shown that hepatocyte transplantation may be useful in patients as a bridge to Ltx or while waiting for the liver to regenerate (acute toxic liver failure). In selected patients with single enzymatic defects, hepatocyte transplantation may even heal the disease. More than 200 patients with acute and chronic liver failure

have been treated with hepatocyte transplantation.

In principle, the procedures for islet isolation, culture and transplantation may also be applied for hepatocytes. The liver tissue is digested with a two-step collagenase perfusion, followed by continuous-density Ficoll gradient purification in a refrigerated COBE 2991 centrifuge. Hepatocyte preparations are maintained in culture medium supplemented with ABO-identical serum. Data confirm that isolation of hepatocytes from the liver tissue is easier to perform than islet isolation and the isolation technique produces large quantities of viable hepatocytes. Thus, islet isolation facilities can be used also for hepatocyte isolation.

Routes of hepatocyte transplantation

As in islet transplantation, hepatocytes may be transplanted into the liver via the portal vein and it has been demonstrated that hepatocytes then translocate from the portal pedicle into the space of Disse by disrupting the sinusoidal endothelium and then join with adjacent host hepatocytes. Hepatocytes are regularly well engrafted in the liver (better than islets). Other potential implantation sites are striated musculature, peritoneal cavity, mesenteric leaves, the spleen, lung parenchyma, under the kidney capsule, and in the subcutaneous space.

Therapeutic hepatocyte mass

Hepatocytes occupy almost 80% of the total liver volume. The cell mass required to restore adequate liver function in a patient is dependent on the underlying disease. The cell mass needed to correct a single enzymatic defect e.g. familial hypercholesterolemia, is significantly less than for treatment of either acute or chronic liver failure. Thus, it has been suggested that 1- 5% of normal liver mass may be sufficient to restore adequate function of a single enzymatic liver defect, while a considerably larger hepatocyte mass is required for the treatment end stage liver failure. 10% of the liver mass (150 g) can be transplanted safely as hepatocytes via the intraportal route into the diseased liver. Since both fulminant and chronic liver failure require replacement of more than 10% of functional hepatocytes into the liver, additional ectopic sites such as the peritoneal cavity or musculature, may provide alternative sites for hepatocyte implantation. To correct enzymatic defects or to bridge a patient to Ltx or liver recovery 10 % functional hepatocytes from a normal liver may sufficient.

An important distinction from islets is that there exists a wide range of endogenous hepatic growth stimulators which increase the mitotic activity of transplanted hepatocytes and thus increases total hepatic mass. Several growth factors such as insulin and hepatocyte growth factor (HGF) can be injected and thereby could speed up the mitotic activity further. Interestingly, it has been shown that low molecular weight dextran sulfate (LMW-DS), a newly developed drug (within the Nordic Network for Islet Transplantation) used to counteract islet-blood interactions in clinical islet transplantation, induces a100-fold increase in the patient's level of HGF. Interaction between foreign material and blood is a universal reaction following infusion, thus the inflammatory response elicited by hepatocyte-blood interactions may probably be diminished by LMW-DS, as it does in islet transplantation. Thus, the potential of LMW-DS in hepatocyte transplantation is promising. These hypotheses need further investigation.

Sources of hepatocytes

A limiting factor of clinical hepatocyte transplantation is shortage of mature functioning human hepatocytes, which currently are obtained from livers rejected for Ltx. The most common cause for rejecting a donor liver for Ltx is excessive steatosis. Other potential sources of hepatocytes are part of livers after reduced sized Ltx, fetal livers and livers from patients transplanted for other causes than disordered hepatocytes such as polycystic livers and livers explanted due to hemangiomas. An other important source of hepatocytes could be livers from PSC patients (without cirrhosis) transplanted due to risk of developing malignancy in the liver. PSC is the most common cause of Ltx in Scandinavia, comprising more than 20% of all Ltxs. The suitability of such livers for hepatocyte transplantation should be explored, and if suitable, a significant number of livers will be available for hepatocyte transplantation in the Nordic countries.

Furthermore, hepatocytes derived from bone marrow stem cells, immortalized hepatocyte cell lines and xenogenic hepatocytes should be investigated further.

Banking of hepatocytes

The most important indications for hepatocyte transplantation beyond enzymatic defects in children, are acute fulminant hepatic failure and liver failure following too extensive liver resections in patients with liver malignancy. In these acute situations, stored hepatocytes should be available. Therefore, in order to achieve this goal, a functional freezing protocol needs to be developed for maintenance of hepatocyte viability and metabolic capacity for a long period of time. In this aspect several cryopreservation techniques are developed but need further refinement.

Conclusions

The major factor limiting hepatocyte transplantation research at present is the lack of availability of good quality human hepatocytes. However, PSC livers without cirrhosis and malignancy may represent an important source. Ultimately, as in islet transplantation, an unlimited supply of cells may be established through the creation of immortalized hepatocyte banks or the use of stem cells.

Experimental microsurgery and Transplantation

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Vivi Bull Stubberud, RN, OR Department (OUH)

Introduction

The research group is a collaborative project between the Section for Transplant surgery at the Division of specialiced Medicine and Surgery and the Institute of pathology. A subset of various experimental transplantation models and other surgical models have been established in order to form a solid platform that allow us to explore our main fields of interest within experimental transplantation, transplantation immunology and hepatic regeneration and cancer.

Surgical models

In the cuff-based cervical heterotopic transplantation technique we transplant the donor heart to the neck of the recipient. Anastomoses between the graft aorta and the carotid artery, and between the pulmonary artery and the external jugular vein are cuffed as reported by Heron. A transplanted heart is shown in figure 1.

Compared to conventional techniques of anastomosis the cuff method reduces graft cold ischemia and bleeding from the anastomoses. Another advantage with the method is the superficial localization of the graft in the recipient's neck which simplifies registration of graft function and enables exact judgement of endpoint for the rejection process. Graft ischemia is typically 5-10 min, and the technical success rate above 90%.

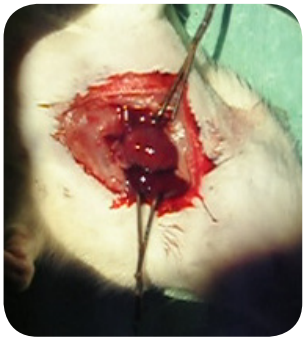


***Figure 1**. Heart transplanted to the neck of the recipient animal.*

Head of Department Pål-Dag Line

We have also established orthotopic liver transplantation models in the rat. This is a non arterialized, model, where the portal vein and the infrahepatic cava is anastomosed by cuff technique whereas the proximal, suprahepatic cava is sutured. The model is performed partly as a whole graft transplant, but when indicated partial liver transplantation of various size is achieved by performing lobar resections on the graft.. The model can also be combined with portocaval shunting, utilizing a carotid artery graft as a shunt.

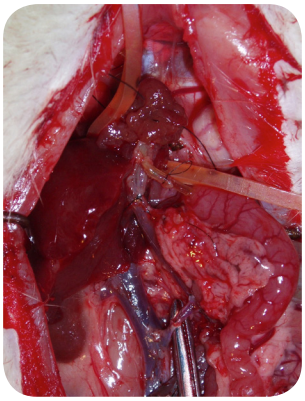
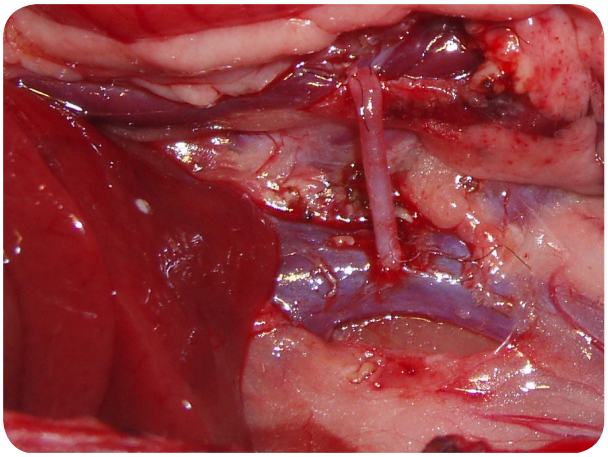


***Figure 2** (left) shows a transplanted rat Liver*

***Firure 3** shows a portocaval shunt where a carotid artery graft is sutured end-to-side to the vena porta and cava respectively.*



Research topics

Genome-wide transcription profiles of endothelial cells during cardiac allograft rejection

In this project, we have performed studies of the interaction between the graft endothelial cells and the recipients immune cells by characterization of the gene activation profile in the vascular endothelium of allografts in acute rejection, with the specific aim to investigate wether there are genetic transcript profiles that can be linked to allograft rejection.

Heart transplantation have been performed in Lewis rats with DA animals as donors., and Lewis isotransplants as controls. In the allo-tx model, acute rejection develops in the course of 4-6 days postoperatively. Grafts have been harvested at day 0,2,3,4,5 and 6.

Transcriptome analyses of organ transplants have until now usually focused on whole tissue samples containing activation profiles from different cell populations. Here, we enriched endothelial cells from rat cardiac allografts and isografts, establishing their activation profile at baseline and on days 2, 3 and 4 after transplantation. Modulated transcripts were assigned to three categories based on their regulation profile in allografts and isografts. Categories A and B contained the majority of transcripts and showed similar regulation in both graft types, appearing to represent responses to surgical trauma. By contrast, category C contained transcripts that were partly allograft-specific and to a large extent associated with interferon-gamma-responsiveness. Several transcripts were verified by immunohistochemical analysis of graft lesions, among them the matricellular protein periostin, which was one of the most highly upregulated transcripts but has not been associated with transplantation previously. In conclusion, the majority of the differentially expressed genes in graft endothelial cells are affected by the transplantation procedure whereas relatively few are associated with allograft rejection.

Growth of hepatocellular carcinoma in the regenerating liver

Hepatocellular carcinoma (HCC) ranks fifth in frequency worldwide among all malignancies and is the third most common cause of cancer mortality, with about 600000 cancer related deaths annually [1]. Liver resection or transplantation represents the only potential curative treatment modalities for most HCC patients. Despite successful surgery achieving R0 resection, tumour recurrence is a major problem and might be as high as 75 % at five years of observation.

Recurrence after resection is presumably caused by undetected, disseminated micrometastasis or de novo cancer in the liver remnant. The cellular and molecular changes

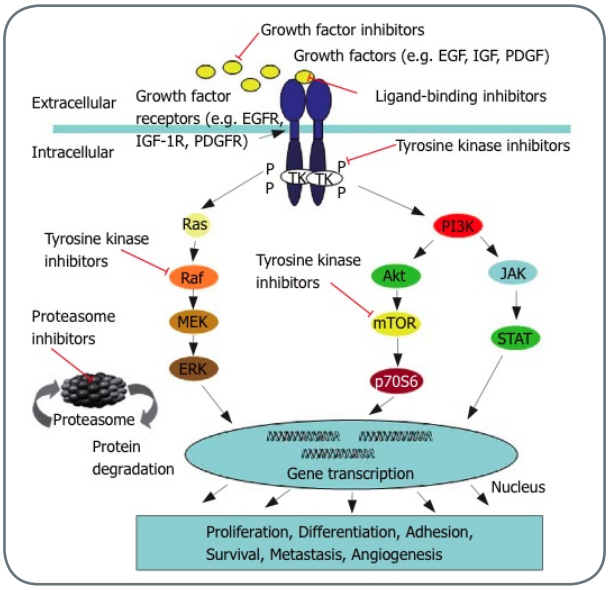


Figure 4. *Major growth factor receptor signallling pathways in HCC:*

resulting from hepatectomy, and the subsequent liver regeneration process may influence the kinetics of tumor growth and contribute to recurrence. Accumulating clinical and experimental evidence suggests that factors involved in liver regeneration may also stimulate the growth of occult tumours and the reactivation of dormant micrometastasis. Some of the most prominent growth factors are oulined in Figure 1 (adapted from Höpfner M et al. World J Gastroenterol 2008 January 7; 14(1): 1-14)

The aim of this project was to test the hypothesis that a microscopic HCC tumor in the setting of partial hepatectomy will show enhanced growth and signs of increased invasiveness corresponding to the size of the liver resection. Varying grades of hepatectomy were performed in groups of Buffalo rats with concomitant implantation of a fixed number of hepatoma cells in the remnant liver. The following groups were compared:

- Group T0: Sham operated animals (n=6), laparotomy and no hepatectomy with 1.0 ×106 tumor cells injec-tion.
- Group T30: 30% hepatectomy group (n=6), resection of left lateral lobe with 1.0 ×106 tumor cells injection;
- Group C70: 70% hepatectomy without hepatoma group (n=6), resection of left lateral and median lobes with no hepatoma cell injection;
- Group T70: 70% hepatectomy group (n=6), resection of left lateral and median lobes with 1.0 ×106 tumor cells injection;

- Group T80: 80% hepatectomy group (n=6), resection of caudate, left lateral and median lobes with 1.0 ×106 tumor cells injection.

After 21 days, tumor size and number as well as the expression of alpha fetoprotein (αFP), cyclin D1, calpain-small subunit 1 (CAPNS1), microvessel density marker CD34, Vascular Endothelial Growth Factor (VEGF) and its receptor 2 (VEGFR-2) were evaluated and compared (Fig. 5).
Tumor volume and number increased significantly with the size of the partial hepatectomy (P<0.05). The largest resections were also associated with increased hepatoma cell infiltration in the lungs and significant upregulation of Cyclin D1, αFP, CAPNS1, CD34, VEGF and VEGFR-2.

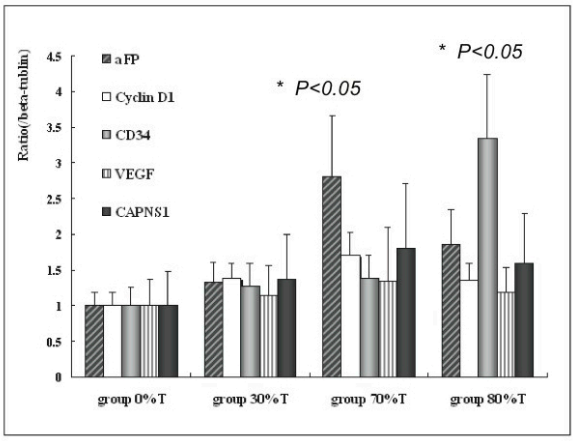


Figure 5. *Comparison of tumor proliferation and invasiveness with tumor biochemical cellular markers after 0%, 30%, 70% and 80% hepatectomy (group T0, T30, T70 and T80) by western blotting quantification.*

The levels of tumor specific marker αFP, Cyclin D1, VEGF, CD34 and CAPNS1 were significantly higher in tumors from group T70 and T80 compared with group T0 and T30 (F=4.685, 11.647, 5.041, 4.685 and 3.047, P<0.05).

The results suggest that liver regeneration after partial hepatectomy facilitates growth and malignant transformation of microscopic HCC. This could be of significance for liver resection and partial liver transplantation strategies for HCC.

The current work was presented at the annual meeting of the International Liver Transplantation Society (ILTS) and was given a young investigator award.

The role of adjunct therapy for Hepatocellular Carcinoma in the regenerating liver following surgical resection.

Tumour recurrence after surgery is a major problem partly because liver regeneration may stimulate the growth of occult tumours. Hence, there is a need for adjuvant therapy that can suppress tumor recurrence and simultaneously promote postoperative liver regeneration. Novel agents that inhibit specific protein kinases involved in intracellular signal transduction pathways could possibly be used in this context.
The objective of this project was to evaluate the effect of the Raf inhibitor Sorafenib on cell viability and proliferation of hepatoma cells and hepatocytes in vitro and an in vivo model of hepatocellular carcinoma growth.
Sorafenib was dissolved in DMSO and diluted in cell culture medium to the desired concentrations with a final DMSO concentration of 0.1% for in vitro studies. Sorafenib was dissolved in Cremophor EL/ethanol for in vivo experiments. Sorafenib was applied to rat hepatocyte and hepatoma cell lines, human HCC cell lines and the cell viability was accessed with MTT assay. DNA synthesis was measured with [3H]-thymidine incorporation assay and Raf/MAPK kinase signalling pathways were investigated by Western Blotting. In the in-vivo experiment, rats with and without hepatoma cell implantation after 70% hepatectomy received daily oral gavages of Sorafenib at a dose of 2.5/10 mg/kg. The following six groups were investigated:

- Group C, n=6, 70% hepatectomy of median and left lobes.
- Group HS, n=6, 70% hepatectomy group with 2.5 mg/kg Sorafenib treatment.
- Group T, n=6, 70% hepatectomy and 1.0 ×106 McA-RH 7777 cells intrahepatic implantation.
- Group TS1, n=6, 70% hepatectomy and 1.0 ×106 McA-RH 7777 cells intrahepatic implantation with 2.5 mg/kg Sorafenib treatment.
- Group TS2, n=6, 70% hepatectomy and 1.0 ×106 McA-RH 7777 cells intrahepatic implantation with 10 mg/kg Sorafenib treatment.

At Day 21, rats in 70% hepatectomy groups with tumor cell implantation were euthanatized, tumours with and with-out Sorafenib incubation groups were compared with tumor size, number and tumor makers (Cyclin D1, αFP and CD34). Liver regeneration was recorded by liver function tests and hepatocyte proliferation markers (Cyclin D1 and Ki-67) by Western Blotting and immunohistocheministry respectively. (Fig. 6)

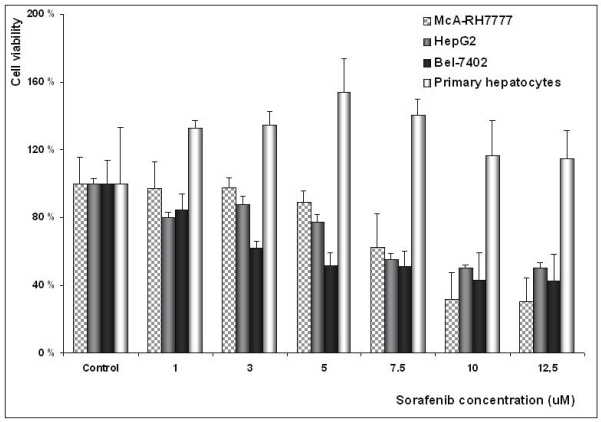


Figure 6. *Cell viability of hepatoma cell lines and primary hepatocytes with Sorafenib and EGF for 2 days.Primary hepatocytes showed higher cell viability compared with hepatoma cell lines after being cultured for 48 h Sorafenib (0-12.5μM) induction and EGF (10 μM) stimulation.*

Results:

After Sorafenib exposure, primary hepatocytes showed higher cell viability, proliferation and stronger Raf/MAPK kinase activity compared with all hepatoma cell lines. In the in vivo model, the tumor volumes and number of intrahepatic and distant metastases were significantly decreased ($P<0.05$) whereas no significant change in liver regeneration was found ($P>0.05$).

The current project indicates that Raf targeted inhibitor Sorafenib can reduce tumor proliferation and metastasis without retarding liver regeneration. This is a result of the different responses of primary hepatocyte and hepatoma cells to Raf/MAPK inhibition.

Transplantation and Malignancy

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Background

Organ transplantation requires lifelong immunosuppression. A side effect is increased post-transplant de novo malignancy. During the last decades, the effectiveness of standard immunosuppressants in allograft transplantation has improved and so has the incidence of *de novo* cancer. A recent study of 905 recipients of transplanted hearts, lungs, or both has shown a 7.1 times increase in de novo cancers compared to the general population. Cancer-related death following transplantation is increasing and accounts for 13% of post transplant mortality.
Regulatory T cells (T regs) maintain self-tolerance to autoantigens and are involved in the pathogenesis of various clinical conditions such as autoimmune diseases, chronic viral infections and cancer. T regs appear more frequently in peripheral blood lymphocytes of cancer patients than healthy controls and interestingly, it seems that high levels of T-regs are a prerequisite for allograft tolerance following transplantation. By manipulation of the interaction between CD4+ CD25+ T regs and dendritic cells, it may become possible to influence host offence and defense in cancer and organ transplantation. In these aspects it is of particular interest that immunosuppressive drugs used in transplantation have both an anti-rejection and anti-neoplastic activity. Rapamycin (Sirolimus, Rapamune®) is an established drug for prevention allograft rejection by blocking the intracellular pathway complex mTOR. It also appears that a Sirolimus based immunosuppression protocol has beneficial effects on tumor recurrence and survival with an acceptable rate of rejection and toxicity in liver transplanted HCC patients. Rapamycin is a potent VEGF antagonist showing significant anti angiogeneic effects in addition to a direct inhibitory effect on tumor growth and proliferation. The drug has shown clinical effect and objective x-ray responses and stabilization of disease in different types of cancer, such as advanced breast and renal cancer that has previously progressed on other treatments. Accordingly, rapamycin is an effective anticancer drug in addition to its immunosuppressive effects.

This supports the use of the drug for patients transplanted for cancer and in patients with de novo post transplant malignancy.

In 2006 we acquired an ethical approval (S-05409 Regional Ethics Committee, Helse Sor-Ost) for a clinical pilot study (SECA-study) to investigate liver transplantation (Ltx) as treatment option for selected patients with non-resectable liver metastases after colo-rectal carcinoma (CRC), using the mTOR inhibitor Rapamycin as standard immunosuppression from postoperative day 1. So far 19 patients have been enrolled in the study. All of the patients had advanced metastatic disease solely to the liver not eligible to resection at the time of Ltx. 14 of the 19 patients (are alive at 2 to 50 months follow-up. The preliminary 2 years survival is 87.4%The burden of the surgery and convalescence thereafter is minimal compared to regular chemotherapy (the patients represent their own controls). Health related quality of life (HRQOL) of the patients, measured with EORTC QLQ-C29/30 is excellent As expected, many patients have experienced recurrence of the disease, mainly to the lungs.

Previous studies have shown that aggressive treatment of CRC lung metastases with resection and/or radiofrequency ablation resulted in a 5 year overall survival of 34-58% (15,16). Thus, some patients included in the SECA study who develop lung metastases have been resected or treated with radiofrequency ablation for recurrent disease in lungs, and are currently disease free.

The pilot data show that Ltx is feasible in selcted patients with liver metastases from CRC and QOL is excellent after Ltx. The burden of the surgery and convalescence thereafter is minimal compared to regular chemotherapy (the patients represent their own controls).

In order to get valid effect measures for Ltx for colorectal cancer the SECA2 study developed. This is a randomized trial where Ltx is compared to best available oncological treatment. The study got the approval from the Regional Ethics Committee, Helse Sor-Ost in December 2010 and it was initated in January 2011.

The study objectives for SECA2:

- In a randomized controlled trial to explore whether liver transplantation in selected patients with liver metastases from CRC can obtain significant life extension and better health related quality of life compared to patients receiving chemotherapy.
- To explore if patient selection according to nomo-grams for outcome of colorectal cancer can define a subgroup of patients with a 5 year survival of at least 50% or even cure from the disease

Primary endpoint

- Overall survival after randomization

Secondary endpoints

- Survival related to Memorial Sloan-Kettering nomograms for recurrence after liver resection of metastatic CRC.
- Disease free survival (RECIST criteria and time to lung lesions of ≥10 mm)
- Time to start of new treatment (change in strategy)
- Quality of life (EORTC QLQ-C30), time to decrease in physical function and global health score
- Registration of symptoms (NCI-CTCAE version 4.0)
- Diagnosis of other malignancies
- Determine nomo-gram scores of patients who survive for 5 years or more
- Survival in relation to biological markers (plasma TIMP-1 and TPA , circulating tumor cells and micro-metastatic disease in bone marrow biopsies at time of Ltx, different micro-arrays of liver biopsies, regulatory T-cells, infiltrating T-lymphocytes in liver biopsies and KRAS and BRAF mutations.
- Correlation between recurrence/metastases and other biological parameters (proteomics, microarray, micro

Based on pilot data from the SECA-study the assumptions are; 3-year survival of 70% in the Ltx- and 30% in the chemotherapy group (allocation ratio 1). To detect differences in survival, sample size is calculated to n=39 patients in each study arm, based on a two-sided type I error or 5% and a power of 80%. Accrual period is 30-36 months. A group-sequential trial analysis with a triangular test designed for sequential inspection for every sixth deaths and early termination as soon as results are conclusive will be performed

Project 1. Characterization of the primary tumor, liver metastases and temporal T-cell immunity in the SECA-patients

1.1 Recent studies have addressed the importance of regulatory T-cells (T regs) in suppressing T-cell immunity to tumor associated antigens. There is evidence that T regs may be a main obstacle of successful tumor immunotherapy and that patients who exhibit allograft tolerance have increased levels of T regs. Temporal levels of T regs are measured at both preoperatively and at fixed intervals postoperatively. Lymphoid infiltration in tumors will be assessed, as this has shown to be an independent prognostic factor in CRC.

1.2 Cytokine profiles will be assessed at Ltx and at defined time points at follow up. The cytokine profiles will be evaluated in regard to immunosuppressive treatment, to the cancer disease and in recognition of the liver as an active immunological organ participating in local and systemic host defense. Relevant cytokines will be quantified by Bioplex assays.

1.3 T-cell immunity will be assessed by mRNA from the tumor cells at time of Ltx (RNA-later). The RNA will be used to transfect dendritic cells and monocytes in order to measure T-cells ability to recognize this material post transplant.

1.4 T-cell receptor clonotype mapping will be performed. CD4+ and CD8+ T cells will be isolated and used for blocking experiments. Results will be correlated to the clinical course of the patients.

1.5 Samples of tissue from the metastases as well as normal liver tissue are being collected and frozen (-70ºC) for later investigations. The properties of this tissue and the possible relation to the clinical course of the patients will be explored. The exact modalities of analysis are not yet fully established as de-freezing is a nonreversible event that needs meticulous planning. The analyses will be performed after all patients have been included in the trial. There are several possible paths to follow:

1.6 Tumor gene expression profiles will be determined in metastases and in normal liver tissue (oligonucleotide micro array analysis)

1.7 Proteome profiles will be assessed. Clinical proteomics is a field which has been reported to have high sensitivity and specificities for early detection of several solid tumors, and is promising in the search for novel biomarkers in CRC. Analyses of the proteomic profiles will be performed by MALDI-TOF mass spectrometry and peptide sequencing using linear ion trap mass spectrometry (q-Trap).

1.8 DNA-Ploidy pattern and chromosomal instability will be assessed by flow cytomtery complemented by image analytic techniques. Abnormalities in DNA content (aneuploidy) have been independently associated with adverse prognosis in CRC. Analyses of microsatelite stability and chromosomal instability will be performed as these factors also have shown to be of prognostic value.

1.9 The role of disseminated tumor cells ((DTC, micro metastases). The prognostic value of disseminated tumor cells in bone marrow (BM) has been investigated in several studies comprising patients with epithelial cancers such as breast-, colorectal- and gastric cancer. The presence of DTC in BM seems to be of predictive value for the development of metastases not only in bones but also in other distant organs even in tumors that rarely develop skeletal metastases such CRC. Concerning breast cancer it has been shown that the detection of disseminated tumors cells in bone marrow by immunocythochemical methods is a strong and independent prognostic factor. Although not completely unequivocal, possibly because of a certain degree of methodological concern and lack of standardization, identification of DTC in BM also seems to predict disease free survival for patients with CRC. The incidence of DTC in BM in patients with isolated liver metastases from CRC is low when compared to patients with primary CRC or disseminated CRC disease, but their presence is associated with a poor prognosis. There is strong evidence, at least from breast cancer that DTC in the peripheral blood from patients with N0 disease is correlated with a high incidence of systemic relapse and mortality. In CRC the inconsistency of detection is larger and a more standardized methodology and sets of markers are called for. Nevertheless, it is probable that such a correlation will be found using a combination of markers. Little is known about the molecular characteristics of isolated DTC cells. Recent technological advances involving enrichment of the relevant cell population, amplification procedures for DNA and RNA as well as high-throughput molecular screening tools now render such characterization feasible. Samples containing from DTCs in BM, systemic- and portal blood give the possibility to compare molecular characteristics of cells from these compartments with each other and with the hepatic metastatic tissue. A novel aspect is that peripheral blood is collected for DTCs at Ltx, both at induction of anaesthesia and after manipulation of the liver in order to assess the potential effect of manipulation of the liver. Samples from BM, portal- and systemic blood will be processed at the Department of Tumor Biology immediately after sampling, using standard procedures for mononuclear cell isolation i.e. immunobead rosetting and preparation of cytospins for immunocythochemical analysis. Tumor cells isolated by immunobead rosetting will be further enriched using the CellPick system followed by RNA/DNA isolation and molecular profiling.

Project 2 Mechanisms of regulatory T cell immune suppression and characterization of the regulatory T cell – effector T cell interaction in vitro, with special emphasis on the potential of cytostatic drugs as immunosuppressants in SECA-patients and in transplanted patients with de novo malignancy.

The concept of transplantation of cancer patients raises several immunologic considerations. 1) Immunosuppressive treatment to prevent rejection may promote cancer recurrence. 2) High tumorload in the liver may lead to a relative immune suppression that possibly involves regulatory T cells (TR cells), thus the patients may need less intense immunosuppressive treatment than liver transplant recipients treated on other indications. 3) Cytostatic drugs with immunosuppressive effects may be an alternative to standard immunosuppressive treatment for the SECA patients.

1.1 The role of regulatory T cells in cancer immunity in SECA patients. Regulatory T cells have been shown to inhibit anti-tumor immune responses in various studies in patients with malignant diseases such as colorectal cancer, ovarian cancer, breast cancer and melanoma. In this project we aim to investigate the suppressive role of regulatory T cells in anti-tumor immune function in the SECA patients. This cohort of patients will be compared to patients with lower tumor burden and who are eligible for local liver resection (the Ullevål patient cohort). Anti-tumor immune function will be evaluated i) prior to transplantation; ii) four months after; iii) one year after transplantation and; iv) at the time of recurrence.

1.2 Identification of down-stream cell-signaling pathways involved in TR cell function. As a part of a broader strategy to test known anti-cancer drugs with immunosuppressive effects to prevent rejection and reduce risk of recurrence in the SECA-patients, we wish to assess the effects of known

anti-cancer drugs, immunosuppressive drugs and small molecular compounds (Src-, PI3-, MEK and p38-kinase inhibitors) on TR cell function and effector T cell function. Compounds with a preferential, different or selective effect on either T cell subset, may point to important physiological differences in the intracellular signal transduction machinery with potential clinical value. Cell proliferation and intracellular cytokine production will be assessed by flow cytometry in CD4+ and CD8+ T cells.

1.3 The physiological role of regulatory T cells and the mechanisms of action in interaction with effector T cells. In this in vitro project, we will aim to elucidate the physiologic role of regulatory T cells and the mechanisms of action in interaction with effector T cells. Regulatory T cells have been extensively studied for the last ten years. However, several fundamental questions remain unanswered. The molecular interactions underlying the immunosuppressive activity of regulatory T cells are not known. Furthermore, the temporal and cellular requirements of the immunosuppressive activity of the regulatory T cells during the cell-cell interaction with the effector T cells during an ongoing immune response, have not been thoroughly dissected.

We have been addressing several basal l properties of Treg function by seris of experiments studying the activation Requirements and Kinetic Properties of Human Regulatory T cells :

Naturally occurring regulatory T (TR) cells are crucial in maintaining self tolerance by dominant suppression of potentially self-reactive T cells in peripheral tissues. The activation requirements, the temporal aspects of the suppressive activity and mode of action of human naturally occurring TR cells are subjects of great controversy. Ex vivo, TR cells display great variability in the suppressive activity dependent on donor and experimental conditions. Here we show that by ex-vivo anti-CD3/anti-CD28 stimulation and subsequent fixation in paraformaldehyde, TR cells acquire full suppressive activity after 6 hours of stimulation. However, the activation requirements are heterogenous as 70% of healthy blood donors have fully suppressive TR cells without the need of ex-vivo stimulation. The suppressive activity of TR cells operates in a contact-dependent manner that is not dependent on dendrittic cells or other antigen-presenting cells. The suppressive activity of paraformaldehyde fixated TR cells can be fully obliterated by trypsin treatment indicating that cytokine secretion is not required for suppressive activity, but that a cell-surface protein is directly involved. By using inhibitors for downstream signaling pathways involved in T cell activation, we were not able to inhibit the upregulation of TR cell mediated suppressive activity, indicating that this mechanism may be dependent on several pathways that operate in concert.

These findings establishes a method for further studying a core suppressive mechanism in TR cells wich in turn could lead to therapeutically exploitable targets.

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Plastic and reconstructive surgery

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Introduction

Plastic and reconstructive surgery is performed to restore normal anatomy and function in patients with congenital and acquired disorders, and in patients with tissue defects after trauma or cancer surgery. During the last decades research in plastic and reconstructive surgery has led to development of a large number of treatment options for patients with different kinds of disorders and defects. These methods are often based on experimental research which has been refined through clinical procedures. The main outcome is improved quality of life and patient satisfaction based on restoration of anomalies and dysfunction.

Research areas

Free tissue transfer is a relatively new technique which has revolutionized the field of reconstructive surgery over the past three decades. During the 1970s, reconstructive surgeons started to use the microscope to perform anastomosis of small vessels (±1mm). Tissue, based on these small vessels, could be transposed from a distant part of the body (donor site) to the location where reconstruction was needed and the vessels anastomosed to a recipient artery and vein. In 1989 a new area of free flap surgery was initiated with the introduction of flaps based on perforator vessels. This technique improved reconstruction by reducing donor site morbidity and by allowing new alternative flap designs. There is a constant need for optimising the reconstruction techniques to give the best possible result with minimal disadvantages at the donor site. Our research group has focused on the following areas:

1. *Microcirculation in random flaps on rats*

In order to investigate the distribution of blood and microcirculation in random flaps we have designed a rat model that enables us to perform multiple measurements with laser Doppler perfusion imaging (LDPI; see below). A random flap is raised with width-length proportions of 1:5. The flap is monitored in 5 equally sized squares on which a LDPI measurement is performed every hour for 6 hours. The circulation is then evaluated with regards to the blooddistribution within the flap.

2. *The effect of prostaglandin E1 on microcirculation*

Several studies suggest a positive effect of prostaglandin E1 (PGE1) on the circulation of flaps. In the same rat model as described above we compare the circulation of random flaps with i.v. infusion of PGE1 alternative saline and perform LDPI measurement. The flap is monitored in 5 equally sized squares on which a LDPI measurement is performed every hour for 6 hours (fig 1). The circulation is then evaluated for every square and comparison between the control and intervention groups is performed and verified statistically.

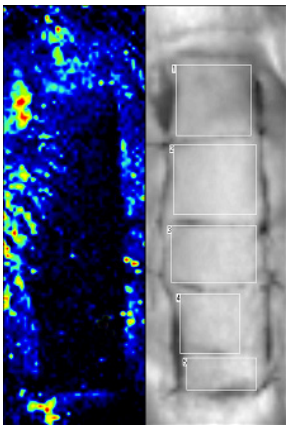

Figure 1. A LDPI scan after raising the random flap based cranially. Perfusion is measured in the five zones.

3. Microcirculation and wound healing:
To resemble a clinical situation, we are using animals with skin structure and function similar to the human skin. Pig skin has many similarities to human skin, including histological appearance and wound healing ability. We are using Norwegian pigs (Norsk landsvin) with weight between 25 and 30 kg in our studies. Microcirculation and histological measurements are performed to evaluate the effect of different reconstructive procedures or other interventions on wound healing. To investigate microcirculation and wound healing in an isolated setting, we use rat models as described below.

4. Experimental perforator flaps and other rat models
Dissection of the perforator flaps preserves the muscle and minimizes the donor site morbidity. Nevertheless, the method may have undesirable effects on the muscle because of damage of its innervation, blood supply or by direct injury when dissecting the perforator. This damage can be reduced by including a minimum number of perforators. However, a reduced number of perforators may be detrimental to the flap viability and wound strength.
To study the effect of different numbers of perforators in a lipocutaneous flap we are using Wistar rats where two symmetrical abdominal lipocutaneous flaps are raised around the midline. On one side all major perforators of the flap are left intact and on the other side only the largest perforator is retained within the flap. After dissection, the flaps are fixed to the original position by a continuous suture. Microcirculation, flap viability, wound strength and histological changes are measured preoperatively and during the first week after the operation.

To continue improvements in both a clinical and scientific setting research using animal models is important. The groin flap based on the superficial inferior epigastric artery (SIEA) is well described. However, as shown in our previous research there are problems with autocannibalism. In addition, postoperative flap monitoring is difficult when the flap is translocated to the abdominal side of the animal. Some studies have addressed these problems by transferring the flap to the dorsum of the rat. However, in these experiments the femoral vessels were ligated, with danger of an ischemic limb and possible tissue necrosis. The ischemic tissue may release factors that affect the microcirculation of the free flap. We have established a new SIEA flap model in the rat with good conditions for flap monitoring, without danger of flap autocannibalisation and with preserved limb circulation (fig 2). This model is used when performing studies on microcirculation and histological changes where we want to compare different interventions on the flap or the animal over a longer period of time.

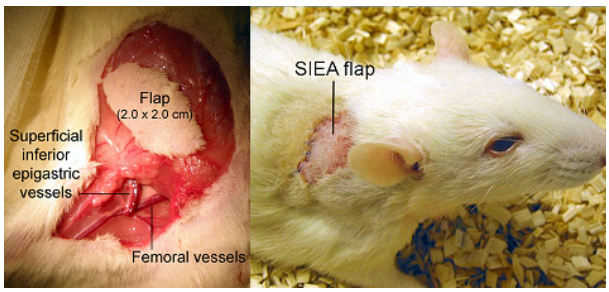

Figure 2. *Dissection of the groin flap (left). Transposistion of the flap to the dorsum of the rat (right)*

5. Microcirculation and reinnervation in human perforator flaps.
The deep inferior epigastric artery perforator (DIEAP) flap from the abdomen is one of the most suitable perforator flaps used for breast reconstruction (fig 3 and 4). This procedure has had a significant impact on the field of plastic and reconstructive surgery, because of the high number of women requiring breast reconstruction after cancer surgery. Based on the experimental research and clinical experi-

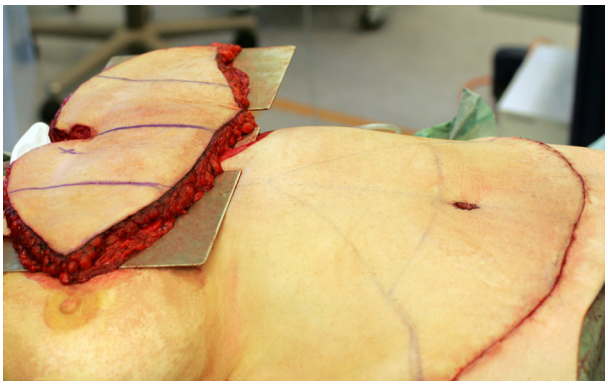

Figure 3. *The abdominal flap is transposed to the thorax and is ready for revascularization.*

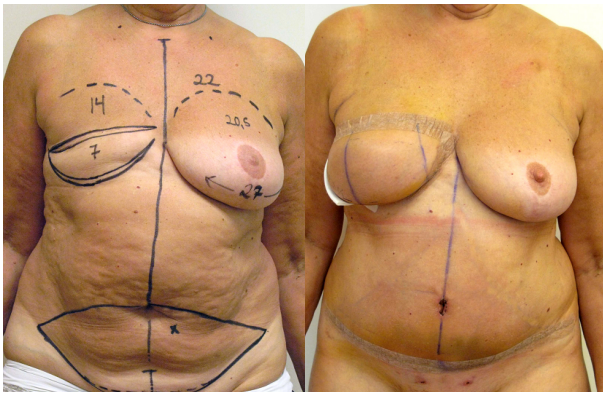

Figure 4. *Preoperative markings on the patient (left), and after breast reconstruction with DEIAP flap (right).*

ence, our group is performing investigations to optimize the reconstruction technique and to minimize the donor morbidity.

Until now little attention has been paid to reinnervation of the flap. We have investigated the spontaneous reinnervation of the DIEAP flap after breast reconstruction and at the donor site at the abdomen. Pressure thresholds have been analysed on the skin using Semmes-Weinstein monofilaments. Histological studies to evaluate the reinnervation in skin are planned both for the perforator flaps and for the donor site.

Through better understanding of flap anatomy, physiology and better surgical technique the complication rate has decreased and the cosmetic outcome has improved. However, partial flap necrosis is still a recurrent complication that can affect the final cosmetic result and the patient satisfaction. In most cases this can be avoided by discarding parts with unreliable capillary refilling after transferring the flap to the recipient site. The abdominal flap is divided into four equal vertical perfusion zones based on clinical observations. The zone with the best perfusion was designated zone I represented by the quarter part of the flap where the vascular pedicle entered. Zone II was represented by the kontralaterale neighbor zone and zone III the ipsilaterale neighbor zone. Zone IV was the remaining part most distant from zone I. Since the introduction of this perfusion model it has been widely accepted. Today it is common clinical practice to discard zone IV to avoid partial flap necrosis using the DIEAP flap for unilateral breast reconstruction. Occasionally further trimming is necessary to obtain an optimal cosmetic result because the flap is still too full. In order to preserve tissue with better vascularity the next zone to be sacrificed would be zone III. However, we have little scientific data that prove the validity of these perfusion zones. In other words trimming zone III before zone II could be wrong. We are performing quantitative evaluation of the perfusion zones with laser Doppler perfusion imaging (LDPI) in order to get a more exact picture of the microcirculatory differences in the DIEAP flap (fig 5). Our results showed that the perfusion of zone II was significantly lower than zone III in the period between 2 hours and 3 days after surgery. This suggest that it may be right to convert zone II to III and zone III to II, which will have major clinical impact on all surgical procedures involving DIEAP-flaps.

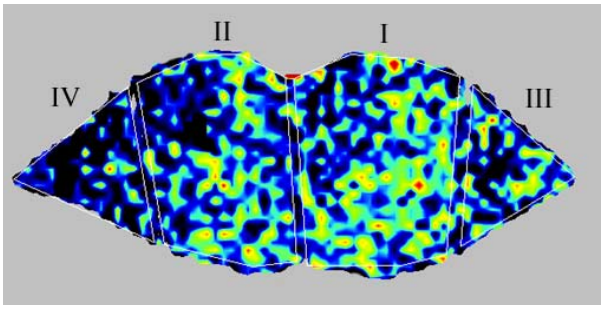

Figure 5.
Colour map of blood flow for the DIEAP flap processed by laser Doppler perfusion imaging. The flap has been divided into perfusion zones. The colour scale red-yellow-green-blue-black represents perfusion values where red is the highest and black the lowest value

Measurements of microcirculation with laser Doppler perfusion imaging (LDPI)

Measurements of microcirculation are a central part of all our animal and human experiments. It is performed with a PIM 3.0 LDPI from Perimed, Stocholm, Sweden. The LDPI generates, processes and displays colour-coded images of tissue perfusion. An optical scanner guides a low power laser beam stepwise to the tissue surface. The LDPI measures microcirculation to a depth of a few hundred micrometers. When the laser beam hit moving erytrocytes in the subepidermal plexus the light is backscattered and detected by a photodetector, this convert the light intensity to electrical signals and colour-coded images.

Projects guided by external project leaders

The effect of a synthetic surfactant CHF 5833 versus poractant alfa in a newborn pig model of meconium aspiration syndrome

Department of Pediatric Research, Oslo University Hospital, Rikshospitalet

Investigators
Ola Didrik Saugstad, Professor, MD, PhD, (UiO/OUH)
Bodil Salvesen, MD, PhD, (OUH)

Poractant alfa is an animal derived surfactant important in the treatment of respiratory distress syndrome (RDS) in premature infants. Surfactant therapy significantly reduces mortality and morbidity. Shortly after administration, the surfactant rapidly coats the alveoli to stabilize against collapse. By reducing surface tension, it helps facilitate lung expansion and gas exchange in premature children. Naturally derived surfactants are expensive to produce. The development of clinically active synthetic surfactants has been complicated especially to synthesize the hydrophobic surfactant proteins SP-B and SP-C. Meconium displaces surfactant from the alveolar surface and inhibits surfactant function. Bronchial lavage fluid from infants contains increased levels of albumin, total protein, and membrane derived phospholipids which also may inhibit surfactant activity.
CHF5633 is a new syntethic surfactant designed to act similar to poractant alfa.

OBJECTIVE
The aim of the study was to compare the effect of poractant alfa versus CHF5633 in an experimental model of meconium aspiration syndrome mainly focusing on surfactant inhibition.

EXPERIMENTAL SET UP
26 newborn pigs age 12 to 36 hours, weight 1.4 to 2 kg were randomized into two groups.
The pigs were anesthetized according to a well known model of MAS. Ten minutes after meconium was instilled endotracheally, either poractant alfa or CHF5633 was instilled.
Bronchial lavage fluid was obtained every second hour to measure the phospholipid concentration reflecting surfactant activity.
Respiratory data were registered every hour. Blood was drawn every hour to investigate complement activation and cytokine formation.

RESULTS
Oxygenation index increased to 22 in the poractant alfa group versus 19 in the CHF5633 group.
Ventilation index increased to 85 in the poractant alfa group versus 84 in the CHF5633 group.
Bronchial lavage fluid will be analyzed in Stockholm.
The terminal complement complex reflecting complement activation and proinflammatory cytokines will be analyzed in Oslo.

Focal hydrothermal ablation

Principal investigator:
Sumit Roy, Dept. of Radiology, Stavanger University Hospital

Thermal destruction of tissue with the help of alternating electric current, radiofrequency ablation, has been steadily gaining acceptance as treatment for hepatic metastases that cannot be resected. The growth of the tumours can be halted, and an appreciable prolongation
of survival can be achieved (that are not amenable to surgery).
However due to limitations imposed by the electrothermal processes underlying the procedure, the utility of radiofrequency ablation is largely restricted to relatively small tumours. The goal of the study is therefore to investigate whether interstitial instillation of steam in the target lesion, focal hydrothermal ablation, can be a more effective alternative. As the use of steam as energy vector avoids the intermediary step of converting electrical energy to thermal energy, more efficient transfer of energy to the lesion can be anticipated.
The exclusion of dielectric heterogeneity as a confounding factor, could translate into more homogenous and predictable heating of tumours being treated. Furthermore, steam's high latent heat of condensation would permit very rapid deposition of large amounts of thermal energy at the target site, thereby considerably shortening the time required for treatment. Given that the thermal ablation with steam represents an entirely new concept, the study willaddress the fundamental questions of feasibility and efficacy, in an animal model.

In-vivo evaluation of an implant for non-sutured anastomosis

Principal investigator
Sumit Roy, Dept. of Radiology, Stavanger University Hospital

A structurally sound, geometrically optimal anastomosis is the key to therapeutic success in peripheral vascular surgery. A simple technique for achieving this goal without suturing has for long been the focus of research. One product is currently commercially available for mechanically join peripheral blood vessels without sutures. However, it is unsuitable for use when the if the arterial wall is not pliable, as is not uncommon in elderly patients. Further, based on published literature, it is unlikely that the product can be incorporated into synthetic blood vessel substitutes commonly used in clinical practice.

To satisfy the unmet need for a versatile method for anastomosing tubular organs without sutures, a simple, inexpensive implant has been developed. Based on the results of assessment of the first iterations of the implant in porcine coronary arteries, the design has been refined, and scaled up for use in larger arteries. The goal of the study is to determine whether the alterations in design serve their purpose.

Cyclic AMP signalling in inflammation and sepsis

Principal Investigator
Petter Kirkeby Risøe, Cand.med. (UiO)

Supervisor
Maria K. Dahle, MSc, PhD (UiO)
Guro Valen, Professor, MD, PhD (UiO)
Håvard Attramadal, Professor, MD, PhD (OUH/UiO)

Aim
Investigate the role and regulation of the intracellular cyclic AMP signalling system in innate immune cells, with a particular focus on cAMP-producing adenylyl cyclases.

Background
As a former member of the Group for Surgical Intensive Care Medicine, these projects are rooted in the field of innate immune responses and sepsis. The intracellular second messenger cyclic AMP (cAMP) acts as a negative regulator of proinflammatory cytokine release from macrophages, leukocyte adhesion, T-cell signaling, cytotoxic responses, endothelial disruption and cytokine-mediated signaling events. By utilizing ten different isoforms of cAMP synthesizing adenylyl cyclases (ACs) and an even larger family of cAMP hydrolyzing phosphodiesterases (PDEs) organized in subcellular microdomains, the cAMP signaling system can mediate a multitude of cellular effects. Circulating levels of cAMP have been found to be diminished in septic patients, and in animal models of systemic inflammation and endotoxic shock, elevating cAMP by inhibition of PDE4-isoforms has been reported to protect organ function and reduce fatalities.

Projects 2010
Previous studies initiated by Maria K. Dahle have demonstrated an isoform-specific AC attenuation in organs from endotoxemic rats, as well as in rat Kupffer cells and alveolar macrophage populations. In 2010, we continued work on a study of lipopolysaccharide-mediated AC regulation in human blood, which revealed differential regulation of the isozymes in a gender-specific manner. Preliminary results were presented at the 5th International Congress on Gender Medicine in December. We also collected blood samples for further molecular study of innate immune function in chronic inflammation in cooperation with Diakonhjemmet Sykehus, as part of a larger clinical trial (the RORA Study). Lastly, we finalized a manuscript on AC regulation in rats undergoing the cecal ligation and puncture (CLP) procedure, which support previous published data on AC attenuation in the context of cataclysmic systemic inflammation. The manuscript also demonstrates an increase in the micro-RNA miR-142-3p among CLP rats, which correlated with the downregulation of a specific AC-isoform (AC9) suspected to be key player in inflammation:

Cecal ligation and puncture sepsis is associated with attenuated expression of adenylyl cyclase 9 and increases in miR-142-3p
Risøe PK*, Ryg U*, Wang YY, Smedsrød B, Christoffersen T, Dahle MK.

External support
These projects are greatly indebted to the gratuitous financial support of Norske Kvinners Sanitetsforening, as well as the benefaction of the Institute for Basic Medical Sciences by the provision of laboratory facilities and first-rate scientific assistance.

Role of the Liver X receptors (LXRs) in inflammatory modulation

Principal investigator:
Joanna Ågren, MD, PhD student (OUH/UiO)

Supervisors
Maria K. Dahle, PhD (UiO)
Michael R. Daws, PhD (UiO)
Håvard Attramadal, Professor, MD, PhD (OUH/UiO)

Research area
Sepsis is a clinical syndrome where an infection (e.g. pneumonia or urinary tract infection) causes systemic illness. Delayed initiation of treatment may lead to organ failure (severe sepsis) and/or septic shock. Severe sepsis has an overall incidence of 2.8/1000 population and a mortality rate of 30%. It is believed that sepsis, and the following organ injury, develops when the body's own regulation of the inflammatory responses to an infection is out of control and defense mechanisms overshoot, rather than from the infectious agent alone. Thus, the complex pathogenesis of sepsis involves several factors, including dysregulation of the immune and endocrine systems, disseminated intravascular coagulation (DIC), genetic susceptibility, and derangement of energy metabolism.

Aims
Our major aim is to study factors that may modulate the overshooting inflammatory responses in sepsis and promote a better balanced immune defense. We believe that the liver X receptor (LXR) may have such properties and intend to elucidate the potential of LXR as an inflammatory modulator in sepsis.

Liver X Receptor and Inflammation
Liver X receptor (LXR) is a ligand binding transcription factor and belong to the family of nuclear hormone receptors. The two subtypes of LXR, LXRα and LXRβ, sense intracellular metabolites of cholesterol and are important regulators of cholesterol, fatty acid and glucose metabolism. Studies have also indicated that the LXRs are involved in regulation of both acute and chronic inflammation. We have previously demonstrated that activation of LXR by a synthetic agonist (GW3965) reduces liver injury and modulates systemic inflammation in rats subjected to acute endotoxemia or polymicrobial sepsis, and that liver injury is increased in LXR-deficient mice in the same models. Our in vitro studies have also shown that the release of pro-inflammatory mediators from white blood cells (Kupffer cells (liver macrophages) and human monocytes) is reduced when the cells are pretreated with the synthetic LXR agonist before stimulation with the gram negative cell wall component lipopolysaccharide (LPS).
To study the impact of LXR on inflammation, we use models that closely resembles the endogenous responses during an infection. For example, we use a model of human whole blood as well as cultures of freshly isolated human white blood cells (monocytes, macrophages, neutrophils and T cells). We also use murine models to study the systemic effect of LXR on inflammation. The cecal ligation and puncture model resembles acute polymicrobial sepsis, and the oil induced arthritis model is used to study immune responses in chronic inflammatory disease.
In 2010, the focus has mainly been on the different subtypes of LXR and their individual impact on inflammatory responses. In particular, we have studied cytokine responses and markers of macrophage polarization and apoptosis following CLP in organs from mice deficient in either LXRα or LXRβ. In parallel, we have studied the effects of LXRα or LXRβ knockdown by siRNA on cytokine responses in human macrophages. Furthermore, at the immunobiological laboratory at the Department of Anatomy, Institute of Basic Medical Sciences, UiO, we have studied the effect of LXR activation on the development of oil induced arthritis and on the migration of dendritic cells to lymph nodes in this model.

Modulation of monocyte inflammatory responses: Effects of 9cisRA and the impact of surgical trauma

Principal Investigator:
Ingrid B. Moss Kolseth, medical student (OUH/UiO)

Supervisor:
Maria K. Dahle PhD (UiO)

Aims
Elucidate the influence of 9cisRA on the inflammatory responses of human monocytes
Evaluate the state of inflammatory responses and inflammatory modulation mechanisms in whole blood from colon cancer patients before and after laporascopic surgery.

Background
Vitamin A or retinol is crucial for the body homeostasis. Two nuclear receptors mainly convey the activity of retinoic acid (RA). The retinoid acid receptors (RARs) bind all-trans-(atRA) and 9-cis retinoic acid (9cisRA). The retinoid X receptors (RXRs) bind 9cisRA only. In previous studies by Kolseth, 9-cis retinoic acid (9cisRA) was shown to modulate inflammatory responses in human adherent monocytes.
Surgery has a profound effect on innate immune responses. The whole blood model allows ex vivo monitoring of human inflammatory responses and inflammatory modulation pathways in circulating immune cells. In a collaborative study with Professor Egil Johnson, MD and PhD-student Dag T. Førland and co-workers at Oslo University Hospital Ullevaal we investigate the status of immune modulatory mechanisms in patients having elective colon resections performed with minimal invasive techniques. In total, 20 patients with respectable colon cancer (left and right) (T1-3 N0-1) will be included.

Projects 2010
Experiments has been performed showing that treatment of adherent monocytes by 9cisRA increases the ability to induce further recruitment of monocytes. Possibly through the production of Monocyte Chemotactic Protein (MCP) -1.
In the study on surgical patients, samples from the first 13 patients were analysed. Results show strong inflammatory responses to LPS in samples taken prior to colon surgery, whereas directly after surgery, most inflammatory mediator responses were abolished. Several inflammatory cytokine responses remained decreased for up to 3 days, whereas anti-inflammatory mediators were increasingly induced at day 1 and 2 postoperatively. The appearent shift towards anti-inflammatory responses corresponded with an increase in suppressor of cytokine signaling (SOCS) 3 mRNA in patient monocytes. Preliminary results from this study was presented at the joint FEBS/EFIS Workshop "Inflammatory Diseases & Immune Response: Basic Aspects, Novel Approaches & Experimental Models", in Vienna october 2010. In 2011, all 20 patients are planned to be included, analysed and final results reported.

Kolseth finished the Medical Student Research Program in november 2010, and results were presented in a thesis entitled "Modulation of monocyte inflammatory responses: Effects of 9cisRA and the impact of surgical trauma".

Training Courses and Seminars (Norwegian)

Introduksjon til nyfødtmedisinske teknikker og prosedyrer

08.03.2010 – 12.03.2010
Kurs O-24386

11.10.2010 – 15.10.2010
Kurs O-24387

Kurskomité:
Thor Willy Ruud Hansen (kursleder), Morten Grønn, Arild Rønnestad, Terje Rootwelt, Inger Elisabeth Silberg, Per Arne Tølløfsrud, alle Nyfødtseksjonen, Rikshospitalet. Dag Sørensen, Avdeling for komparativ medisin, Rikshospitalet. Vivi Bull Stubberud, Insitutt for kirurgisk forskning, Rikshospitalet. Thoams Rajka og Vigdis Skaug, Barneklinikken og Undervisningssenteret ved Ullevål Universitetssykehus.

Målgruppe:
Leger under utdanning i barnesykdommer med særlig fokus på de helt ferske og uerfarne. Kurset kan også ha interesse for andre kolleger i det barnemedisinske faget som arbeider ved avdelinger der visse typer nyfødtmedisinske prosedyrer utføres svært sjelden. Videre vil kurset være relevant for anestesileger som arbeider ved avdelinger der akuttbehandling av nyfødte ivaretas av anestesiolog.

Læringsmål:
Deltakerne skal i løpet av kurset få demonstrert teknikker og selv få utføre praktiske øvelser. Målsettingen er å gi deltakerne grunnleggende kunnskaper og ferdigheter slik at de med større trygghet kan utføre prosedyrer på syke nyfødte.

Temaoversikt:
Følgende teknikker/prosedyrer vil bli undervist:
- Innleggelse av perifer venenål
- Innleggelse av perifer arterienål/-"kran", inkludert etablering av trykkmåling
- Kapillær blodprøvetagning
- Innleggelse av navlevenekateter
- Innleggelse av navlearteriekateter
- Innleggelse av perkutant sentralvenekateter
    1. "Peel away" teknikk
    2. Seldinger teknikk
- Innleggelse av thoraxdren
    1. Vanlig rett dren med mandreng
    2. "Pig-tail" dren med Seldingers teknikk
    3. Bruk av drenasjekammeret
- Spinalpunksjon
- Endotrakeal intubering
- Blærepunksjon (suprapubisk)
- Lokalbehandling av ekstravasering
- Innleggelse av intraossøs nål for infusjon.

Kursleder Thor Willy Ruud Hansen instruerer kursdeltakere.

Program

Mandag 8/3	Tirsdag 9/3	Onsdag 10/3	Torsdag 11/3
09:00 Teknikk: Perifer venenål. Perifer arterie + etablering av trykkmålingModell:Rotte (føtter/hale)	09:00 Teknikk: Perkutan CVK. Modell: Rotte.	09:00 Teknikk: Intubering. Modell: 1) Katt. 2) Gris.	09.00 Teknikk: Spinalpunksjon. Intraossøs infusjon. Modell:Gris.
12:00 Pause	12:00 Pause	12:00 Pause	12:00 Pause
13:00 Teknikk: Venøs bl.prøve Art. bl.prøve Kap. bl.prøve. Modell:Rotte	13:00 Teknikk: Navlekateter. Modell: Human navlesnor.	13:00 Teknikk:Thoraxdren. Modell:Gris.	13:00 Teknikk:Blærepunksjon. Blærekateterisering. Kursevaluering. Modell:Gris.
16:00 Slutt	16:00 Slutt	16:00 Slutt	16:00 Slutt

Thorako-/laparoskopisk kirurgi

22.09.2010 – 24.09.2010
Kurs nr. O-24400

Kurskomité:
Trond Buanes og Arne R. Rosseland (kursledere), Erik Trondsen, Bjørn Edwin, Vivi Bull Stubberud og Stefan Kutzsche.

Målgruppe:
Leger under utdanning i generell kirurgi og gastroenterologisk kirurgi.

Læringsmål:
Indikasjoner, operasjonsmetoder, resultater og kvalitetssikring innen laparoskopisk kirurgi. Kurset vil omhandle teoretisk undervisning og praktiske øvelser på simulatorer samt demonstrasjonsoperasjoner.

Temaoversikt:
Laparoskopets oppbygging og vedlikehold.
Fysiologiske effekter av pneumoperitoneum.
Anestesi ved laparoskopi.
Laparoskopi ved akutt abdomen, cholecystecomi, antirefluxkirurgi, laparoskopisk cancerkirurgi.
Laparoskopi i urologien.
Thoracoskopisk kirurgi.
Praktiske øvelser under supervisjon, på modeller og anestesert gris vil være en vesentlig del av kurset.

Kursleder Arne Rosseland instruerer deltagerne.

Thorako-/laparoskopisk kirurgi

Onsdag 22/9 **Møteledere: Bjørn Edwin & Trond Buanes**	**Torsdag 23/9** **Møteledere: Ole Christian Olsen & Arne Rosseland**	**Fredag 24/9** **Møteleder: Olaug Villanger**
08:30 Registrering/kaffe 09:00 Åpning av kurset. Arne R. Rosseland, Kirurgisk avd. RH 09:05 Hvordan trene laparoskopi? Fredrik Halvorsen, Sørlandet Sykehus. 09:20 Simulatortrening. Fredrik Halvorsen, Sørlandet Sykehus. 09:40 Gynekologisk akutt abdomen. Anton Langebrekke, Gyn. avd. UUS. 10:10 Anestesiteknikker, Kirsti Myre, Anesteseiavd, UUS. Hva er den ideelle anestesiform ved laparoskopiske prosedyrer. 10:25 Pause 10:40 Hvordan etablere pneumoperitoneum. Buanes (åpen/lukket tilgang – videoillustert) 11:00 Thoracoskopisk kirurgi. Steinar Solberg, Thoraxkirurgisk avd. RR 11:20 Urologi. Bjørn Brennhovd, Kir.avd. RR 11:45 Lunsj 12:45 Teoretisk gjennomgang av diatermi ved representant fra Covidien 13:05 Teoretisk gjennomgang av ultralydsaks (vibrasjon) ved representant fra Ethicon. 13:30 Praktiske øvelser ved Rikshospitalet, Inst for kirurgisk forskning 19:30 Kursmiddag	07:45 Kaffe 08:00 Laparoscopi ved akutt abdomen/ akutt appendicitt. Ole Christian Olsen 08:45 Utredning før laparoskopisk cholecystectomi. Choledoccussteiner. Erik Trondsen 09:00 Galleveiskirurgi. Laparoskopisk kolecystectomi. "How to do it" - prinsipper. Trond Buanes 09:20 "The easy gallbladder"/intraoperativ cholangiografi & "difficult gallbladder" (video). Trond Buanes 09:40 Pause 10:00 Gallegangsskader. (video). Arne Rosseland 10:20 Gallevideoer 10:45 Laparoskopisk brokk-kirurgi: I lyskene. Erik Trondsen, Overlege UUS 11:00 Ventralhernier. Bård Røsok, Overlege RH 11:15 Teknikk ved laparoskopisk anti-reflukskirurgi. Erik Trondsen 11:35 Komplikasjoner etter laparoskopisk anti-refluks kirurgi. Ronald Mårvik, Overlege, St. Olavs Hospital, Trondheim 11:50 Diagnostisk laparoskopi. Kroniske smerter, stageing, biopsi. Erik Trondsen 12:05 Lunsj 13:00 Praktiske øvelser ved Rikshospitalet, Institutt for Kirurgisk Forskning 16:30 Slutt	07:45 Kaffe 08:00 Laparoskopisk colonkirurgi. Olsen 08:20 Avansert laparoskopisk kirurgi (milt, pancreas, binyre, lever). Bjørn Edwin 09:00 Praktiske øvelser ved Rikshospitalet, Institutt for Kirurgisk Forskning, Intervensjonssenteret 12:00 Lunsj 14:45 Evaluering med kursprøve 15:45 Slutt

Trening i bokser

Trening i bokser

Bakvaktkurs i akuttpediatri

25.-26.11.2010

Kurskomité:

Kursledere: Overlege, professor dr.med. Thor Willy Ruud Hansen, Kvinne- & Barneklinikken, Oslo Universitetssykehus Rikshospitalet, og Overlege Thomas Rajka, Kvinne- & Barneklinikken og Utdanningssenteret, Oslo Universitetssykehus Kirkeveien.
Operasjonssykepleier Vivi Bull Stubberud, Institutt for Kirurgisk Forskning, Oslo Universitetssykehus Rikshospitalet.
Overlege Tor Einar Calisch; sykepleier/instruktør Kari Hoved og Vigdis Skaug (alle: Kvinne- & Barneklinikken og Utdanningssenteret, Oslo Universitetssykehus Ullevål).
Overveterinær Gro Flatekval Furset, Avd. for Komparativ Medisin Oslo Universitetssykehus, Rikshospitalet.

Målgruppe:

Vaktgående overleger i barnesykdommer med særlig fokus på de har behov for oppfrisking av ferdigheter i akuutpediatriske prosedyrer.

Læringsmål:

Deltagerne skal i løpet av kurset få demonstrert teknikker og selv få utføre praktiske øvelser og trene i teamarbeid. Målsettingen er å styrke deltagernes kunnskaper og ferdigheter slik at de med større trygghet kan ivareta sine oppgaver i akutt pediatri som bakvakt.

Temaoversikt:

Følgende teknikker/prosedyrer vil bli undervist:
Innleggelse av perifer arterienål/-Akran@,
Innleggelse av navlekateter
Innleggelse av navlearteriekateter
Innleggelse av thoraxdren
i) Vanlig rett dren med mandreng
ii) @Pig-tail@ dren med Seldinger teknikk
Endotrakeal intubering
Innleggelse av intraossøs nål for infusjon
Trening på akuttsituasjoner i simuleringslaboratorium

Kurssted:

Institutt for Kirurgisk Forskning, Oslo Universitetssykehus Rikshospitalet og Utdanningssenteret, Oslo Universitetssykehus Ullevål.

Dagsprogram for bakvaktskurset i akuttpediatri 25.-26.november 2010

Torsdag 25/11-2010	**Fredag 26/11-2010**
Institutt for Kirurgisk Forskning, OUS-Rikshospitalet	**Utdanningssenteret, OUS-Ullevål**
0800-1200 Navlekateter, Arteriekran, Intubering, Thoraxdren, Intraossøs nål Modell: Human navlesnor, spedgris	0800 – 1015 Workshops 1015 - 1030 Pause 1030 – 1050 Briefing dukke 1030 –1115 Scenario I 1115 - 1200 Scenario II 1200 - 1230 Pause 1230 -1315 Scenario III 1315 - 1400 Scenario IV 1400 - 1415 Pause 1400 - 1500 Scenario V 1500 – 1545 Scenario VI 1545 – 1600 Evaluering
Utdanningssenteret, OUS- Ullevål	
1315 – 1330 Demo BHLR 1330 – 1415 Luftveier/ Maske/ Kompresjoner 1415 – 1430 Demo intubering 1430 - 1515 Intubering i grupper 1515 – 1530 Pause 1530 – 1545 Demo defib 1545 – 1615 Defib i grupper 1615 - 1700 AHLR praktisk	

Seminars

Dato	Name	Title	Department
07.01.2010	Arne Klungland	Mouse models reveal detailed biochemical pathways for hydroxylases with unique activities for DNA, Histones and tRNA.	Professor, Laboratory for Genome repair and regulation, RH
21.01.2010	Akhtar Hussain	Differential risk factors for DM in Asian population	Professor, Inst of General Practice and Community Medicine, Dept. International Health
29.01.2010	Petter Strømme	Neurological deficits caused by Na+/H+ exchanger 6 (NHE6) loss of function: studies in humans and knockout mice	Professor, Child Neurology, OUS, Ullevål
18.02.2010	Thomas von Lueder	Emerging therapies for chronic heart failure (Morgendagens hjertesviktbehandling)	M.D., PhD, Department of Cardiology, OUS Aker
04.03.2010	Magne Nylenna	Ny helseforskningslov	Professor dr.med., Nasjonalt kunnskapssenter for helsetjenesten
11.03.2010	Ragnar Stien	Fridtjof Nansen; polarforsker, fredsprisvinner og nevrobiologisk pioner.	Overlege
18.03.2010	Kirsten Bjørklund Holven	Inflammation in children with Familiar hypercholesterolemia	Professor i klinisk ernæring ved Avdeling for Ernæringsvitenskap, Institutt for medisinske basalfag, UiO
03.06.2010	Frode Tuvnes	The pach clamp technique- and after hyperpolarization in hippocampal neurons.	PhD in neurophysiology, Kavli Institute for Systems Neuroscience and Centre for the Biology of Mamory in Trondheim.
10.06.2010	Øyvind Svendsen	Mikrovaskulær væske- og proteintransport ved inflammasjon og lymfødem	Overlege Kirurgisk Serviceklinikk, Haukeland Universitetssjukehus
21.10.2010	Ida Leren	Katekolaminerg polymorf ventrikkeltakykardi	Legestudent UiO, 6. året
28.10.2010	Johannes Lagethon Bjørnstad	"The TGF-β/SMAD signaling system and extracellular matrix changes in myocardial remodeling and reverse remodeling following correction of pressure overload"	Lege. OUS, Ullevål, Thoraxkirurgisk avd.
04.11.2010	Ingvild Paur	"Coffee,- the most important source of antioxidants in the Norwegian diet?"	Ernæringsfysiolog, Post-doc, Centre d'Immunologi Marseille Luminy, Frankrike
25.11.2010	Yvonne Andersson	The immunotoxin story, from laboratory bench to cancer patients".	Department of Tumor Biology, Institute of Cancer Research, The Norwegian Radium Hospital
02.12.2010	Prof. Per Brandzæg	Homeostatic impact of indigenous microbiota and secretory immunity	Lab. For Immunohistochemistry and Immunopathology, Dept. of Pathology
09.12.2010	Jo C. Bruusgaard	"Memories; are they made of this? - Myonuclei acquired by overload are not lost on detraining"	IMBV
16.12.2010	Morten Eriksen	Segmental cardiac work, what does it tell us?	Lege, doktorgrad i sirkulasjonsfysiologi

Publications and PhD-Theses

Publications 2005 – 2010

2010

1. Odland HH, Kro GA, Edvardsen T, Thaulow E, Saugstad OD. Atrioventricular valve annulus velocity and acceleration during global hypoxia in newborn pigs - assessment of myocardial function. Neonatology 97(2) 100-7, 2010

2. Ueland T, Fougner SL, Godang K, Lekva T, Schurgers LJ, Scholz H, Halvorsen B, Schreiner T, Aukrust P, Bollerslev J. Associations between body composition, circulating interleukin-1 receptor antagonist, osteocalcin, and insulin metabolism in active acromegaly. J Clin Endocrinol Metab 95(1) 361-8, 2010

3. Øie E, Ahmed MS, Ueland T, Sikkeland LI, Dahl CP, Hagelin EM, von Lueder T, Edvardsen T, Andreassen AK, Gullestad L, Aukrust P, Yndestad A, Vinge LE, Attramadal H. Adrenomedullin is increased in alveolar macrophages and released from the lungs into the circulation in severe heart failure. Basic Res Cardiol 105(1) 89-98, 2010

4. Bergestuen DS, Gravning J, Haugaa KH, Sahakyan LG, Aakhus S, Thiis-Evensen E, Øie E, Aukrust P, Attramadal H, Edvardsen T. Plasma CCN2/connective tissue growth factor is associated with right ventricular dysfunction in patients with neuroendocrine tumors. BMC Cancer 10 6, 2010

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7. Haugaa KH, Leren IS, Berge KE, Bathen J, Loennechen JP, Anfinsen OG, Früh A, Edvardsen T, Kongsgård E, Leren TP, Amlie JP. High prevalence of exercise-induced arrhythmias in catecholaminergic polymorphic ventricular tachycardia mutation-positive family members diagnosed by cascade genetic screening. Europace 12(3) 417-23, 2010

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12. Lund T, Korsgren O, Aursnes IA, Scholz H, Foss A. Sustained reversal of diabetes following islet transplantation to striated musculature in the rat. J Surg Res 160(1) 145-54, 2010

13. Paulson L, Persson A, Vonck K, Van Dycke A, Kuhn HG, Boon P, Ben-Menachem E, Raedt R. Effect of levetiracetam on hippocampal protein expression and cell proliferation in rats. Epilepsy Res 90(1-2) 110-20, 2010

14. Sigurdsen U, Reikeras O, Utvag SE. Conversion of external fixation to definitive intramedullary nailing in experimental tibial fractures. J Invest Surg 23(3) 142-8, 2010

15. Solevåg AL, Dannevig I, Nakstad B, Saugstad OD. Resuscitation of severely asphyctic newborn pigs with cardiac arrest by using 21% or 100% oxygen. Neonatology 98(1) 64-72, 2010

16. Bjørnstad JL, Sjaastad I, Nygård S, Hasic A, Ahmed MS, Attramadal H, Finsen AV, Christensen G, Tønnessen T. Collagen isoform shift during the early phase of reverse left ventricular remodeling after relief of pressure overload. Eur Heart J 32(2) 236-45, 2010

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B. Delayed Onset of Cardiac Compressions in Cardiopulmonary Resuscitation of Newborn Pigs with Asphyctic Cardiac Arrest. Neonatology 99(22) 153-62, 2010

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28. Solberg R, Løberg EM, Andresen JH, Wright MS, Charrat E, Khrestchatisky M, Rivera S, Saugstad OD. Resuscitation of newborn piglets. short-term influence of FiO2 on matrix metalloproteinases, caspase-3 and BDNF. PLoS One 5(12) e14261, 2010

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8. Dimmen S, Nordsletten L, Madsen JE. Parecoxib and in-

domethacin delay early fracture healing: a study in rats. Clin Orthop Relat Res 467(8) 1992-9, 2009

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12. Hergum T, Langeland S, Remme EW, Torp H. Fast ultrasound imaging simulation in K-space. IEEE Trans Ultrason Ferroelectr Freq Control 56(6) 1159-67, 2009

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14. Moe MC, Kolberg RS, Sandberg C, Vik-Mo E, Olstorn H, Varghese M, Langmoen IA, Nicolaissen B. A comparison of epithelial and neural properties in progenitor cells derived from the adult human ciliary body and brain. Exp Eye Res 88(1) 30-38, 2009

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system and brain tumors: properties in vivo following xenotransplantation. ISBN: 978-82-8072-570-7, h. Thesis, University of Oslo, 2009

28. Bains R. Isoflurane and sevoflurane: effects on mitochondrial function in the rat and human brain. ISBN: 978-82-8072-365-9, h. Thesis, University of Oslo, 2009

29. von Lueder T. Molecular mechanisms of myocardical hypotrophy and heart failure: Experimental studies on cardiac G protein-coupled receptor signaling with emphasis on endothelin-1. ISBN: 978-82-8072-799-2, h. Thesis, University of Oslo, 2009

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2008

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Nitric oxide administration restores the hepatic artery buffer response during porcine endotoxemia.
Journal of Investigative Surgery 21(4):183-94, 2008

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Isolation of Human Multipotent Neural Progenitors from Adult Filum Terminale
Stem Cells and Development. Epub, 2008

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PhD-Theses

Sigbjørn Dimmen

Kristina Hermann Haugaa

2010

DIMMEN, Sigbjørn: Effects of cox inhibitors on bone and tendon healing.

HAUGAA, Kristina Hermann: Prediction of cardiac ventricular arrhythmias by echocardiography in patients at risk.

2009

VON LUEDER, Thomas G: Molecular Mechanisms of Myocardial Hypertrophy and Heart Failure
Experimental Studies on Cardiac G Protein-Coupled Receptor Signaling with Emphasis on Endothelin-1.

LUND, Tormod: Strategies to counteract β-cell loss in pancreatic islet transplantation.

BAINS, Ravi: Isoflurane and sevoflurane: Effects on mitochondrial function in the rat and human brain.

ØLSTØRN, Håvard: Neural stem, cells from the adult human central nervous system and brain tumors. Properties in vivo following xenotransplantation.

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ANDRESEN, Jannicke: Neuroprotective effects of intravenous nicotine administration. An experimental study in newborn piglets.

WANG, Yun Young: Novel targets in modulation of inflammatory responses in experimental endotoxemia and sepsis: focus on the liver X receptor.

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ØVERLAND, Gunhild: Kupffer cell activation in systemic inflammation.

MYHRE, Anders E: Gram-positive endotoxemia and modulation of the innate immune response.

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BØRKE, Wenche Bakken: Myocardial injury and performance in hypoxaemic neonates: Effects of oxygen and carbon dioxide during reoxygenation. An experimental study in newborn pigs.

LARSEN, Geir Arne: Early mechanisms of brain ischemia. An experimental study in isolated rat neurons.

LYSEGGEN, Erik: Assessment of myocardial viability and reperfusion by tissue Doppler echocardiography.

LINDENSKOV, Paal HH: Inflammation in experimental meconium aspiration syndrome.

VINGE, Leif Erik: Myocardial G protein-coupled receptor kinases – Distinct isoform specificities at receptors as basis for diverse roles in regulation of cardiac function.

PAASCHE, Joachim Dericq: Molecular Mechanisms of Regulation and Intracellular Trafficking of the Endothelin Receptors.

AHMED, Muhammad Shakil: Autocrine / Paracrine Regulators of Myocardial and Pulmonary Remodeling in Heart Failure.

SKULSTAD, Helge: New insights into the function of normal and ischaemic myocardium.

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LYNG, Kristin: Experimental induction of fetal brain damage in pigs as a model of infection-induced brain damage in humans.

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MUNKEBY, Berit Holthe: Resuscitation of the newborn with 100% O2 - detrimental effects on the brain, lungs and heart.

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KUTZSCHE, Stefan P: Effects of hypoxemia and reoxygenation on cerebral microcirculation, nitric oxide, hydrogen peroxide and excitatory amino acid concentrations. An experimental study in newborn pigs.

SCHOLZ, Tim: Studies on activation of the humoral defence system and inflammatory cells in liver transplantation.

JØRGENSEN, Pål Foyn: Immunomodulation and innate immune responses in experimental sepsis. A study with emphasis on the monocytemacrophage system.

QI, Wei: A-and B-type cardiac natriuretic peptides-markers of atrial pressure and left ventricular hypertrophy.
FRØEN, Fredrik: Effects on inflammation and nicotine on apnea, respiratory response and hypoxic-ischemic brain damage in the newborn piglet.

2001

WANG, Jacob: Cellular responses involved in sepsis caused by gram-positive bacteria and fungal pathogens.

BORTHNE, Kjell: The role of 1- og -adenoceptors in sympathetic inotropic myocardial control.

MEDBØ, Sverre: Pulmonary hemodynamics during neonatal hypoxemia and reoxygenation. Role of oxygen, endothelin-1 and nitric oxide in piglets.

HYSTAD, Marit: Origins of increased plasma concentrations of Atrial –and Brain natriuretic peptid in heart failure.

2000

ROY, Sumit: Interventional radiologic management of deep vein thrombosis.

ØIE, Erik: Autocrine/Paracrine Regulatory mechanisms and myocardial remodeling during ischemic heart failure. An experimental study in rats with special emphasis on the role of myocardial endothelin and adrenomedulin.

KJEKSHUS, Harald: Vasoactive mediators and vascular function.

KLINGE, Randi: Cardiac natriuretic peptides as endocrine markers of cardiac function. A study of changes in plasma concentrations during development and treatment of heart failure and angina pectoris

1999

BJØRNLAND, Kristin : Importance of proteolytic enzymes in tumor cell invasion.

MADSEN, Jan Erik: Neural regulation of bone repair. Experimental studies of fracture healing, posttraumatic osteopenia and bone graft incorporation in the rat.

PhD-Theses

GUNDERSEN, Yngvar: Modulation of nitric oxide values in endotoxaemia. An experimental study with special emphasis on hepatic circulation and function.

HOSAKA, Junro: Preclinical evaluation of the temporary venous "spring filter" for prevention of pulmonary embolism.

ODDEN, Jan Petter: Cerebral and Ocular Blood Flow responses in the posthypoxemic newborn piglet.

FIANE, Arnt: Pig-to-human xenotransplantation. Inhibition of hyperacute rejection.

LEHNE, Gustav : P-glycoprotein- a target for circumvention of multidrug resistance in cancer.

HVAAL, Kjetil: Attenuation of ischaemic skeletal muscle necrosis.

KAASTAD, Trine Sand: Osteoporotic fractures relative to strength of bone and muscle. Experimental rat models for studying means of prevention.

ÅSBERG, Anders: Effects of cyclosporine A on microvascular function and endothelin-1 in renal transplant recipients.

YU, Xiang-Qing: Hemodynamic and metabolic effects of nitric oxide. An experimental study in newborn piglets with acute lung injury.

SATAS, Saulias: Pharmacokinetics, posthypoxic hypothermia and cerebral microdialysis in the newborn pig.

KROHN, Claus D: Pathophysiological aspects on postoperatively, drained, untreated blood for autologous transfusion.

STEINE, Kjetil: Mechanisms of early diastolic left ventricular intracavitary flow: a study of flow patterns by colour M-mode doppler echocardiography and measurements of intraventricular driving pressures.

ANFINSEN, Ole-Gunnar: Radiofrequency catheter ablation: a study concerning electrode configuration, lesion size and potential complications.

HAALAND, Kirsti: Hypothermia as treatment of asphyxia in a newborn piglet model.

RISE, Ingunn: Cerebral blood flow during high intracranial pressure and blood loss.

1998

FEET, Bjørn: Resucitation of hypoxic newborn piglets with different oxygen concentration

MOEN, Atle: Circulatory effects of surfactant replacement. An experiental study in depleted newborn piglets.

GRØNDAHL, Tor Ø.: Ion fluxes during cerebral ischemia with special emphasis on measurements of intracellular calcium. An experimental study in vitro.

1997

KARLSRUD, Tove Sigstad: Human kininogens as multifunctional proteins. Studies focusing on their function as cysteine protease inhibitors.

TRONDSEN, Erik: Treatment of gallstone disease. Aspects of Surgical Treatment in the early laparoscopic era.

LARSEN, Morten: Isoflurane: Effects on synaptic release and uptake of glutamate and GABA.

1996

BENTDAL, Øystein H.: Immune responses in protein-energy-malnutrition. A study in patients with anorexia nervosa, carcinoma of cardia or oesophagus.

STORM, Hanne: Beta-endorphin in victims of sudden infant death syndrome.

KONGSGÅRD, Erik: Charateristics of radiofrequency current catheter ablation. An experimental study *in vitro* and *in vivo*.

BUØ, Laila: Proteolytic mechanisms in tumor spread. Studies on the role of serine proteinases in human gastrointestinal cancer.

HAUGSTAD, Tor: Mechanisms of amino acid release during cerebral ischemia.

AUNE, Arne Kristian: The muscle stabilized knee. Significance for anterior cruciate ligament injury and reconstruction.

LINE, Pål-Dag: Laser Doppler assessment of limb perfusion. Physiological and methodological aspects.

PhD-Theses

1995

STUGAARD, Marie: Intraventricular filling pattern and diastolic function. Clinical and experimental studies by color M-mode doppler echocardiography.

STEEN, Torkel: Noninvasive evaluation of left ventricular diastolic function. A critical investigation of two proposed methods.
SANDERUD, Jon: Oxygen radical induced pulmonary hypertension.

ROOTWELT, Terje: Resuscitation with 21% or 100% oxygen.

HEGSTAD, Elisabeth: Amino acids in the human brain. A study of possible roles in synaptic transmission and ischemia with special reference to glutamate and GABA.

1994

VEDDENG, Odd J.: Cardiac mechanical effects of selective positive end-expiratory pressure ventilation.

SKJELDAL, Sigmund: Acute Skeletal Muscle Ischemia. Microcirculation and histological changes in rat hindlimbs.

NORDSLETTEN, Lars: Muscle protection against fracture. A biomechanical study in the rat tibia.

SAMDAL, Frode: Suction-assisted lipectomy. A clinical and experimental study.

RISØE, Cecilie: Changes in splanchnic vascular capacitance during experimental and clinical heart failure.

STOLTENBERG, Lauritz: Biochemical, immunological and experimental studies of sudden infant death syndrome (SIDS).

1993

SOLHEIM, Erik: Effects of Bioerodible Polyorthoester on Heterotopic and Orthopic Bone Induction in rats.

KIRKEBY, Ole Jørgen: Incorporation of Bone Grafts. An experimental study in rats.

POULSEN, Jan Petter: Extracelluar oxypurines during hypoxia and reoxygenation.

KROHG-SØRENSEN, Kirsten: Laser Doppler Flowmentry in Evaluation of Colonic Perfusion.

QIAO, Zhi-Gui: Measurement of electroderman and micro-circulatory activities and responses in the human palm.

1992

KONGSGAARD, Ulf E: Changes in the plasma protease systems during open heart surgery.

PINHOLT, Else Marie: Experimental alveolar ridge augmentation studies.

STIRIS, Tom Arne: Ocular blood flow in the newborn. Effects of lights and blood gas variations on retinal and choroidal blood flow in the new born piglet.

1991

RIDDERVOLD, Fridtjov: Atrial mechanical and endocrine function during changes in heart rate and loading. Clinical and experimental studies.

BØRSUM, Tone: Investigations on cultured human endothelial cells. Cytotoxic effects of low density lipoprotein. Protein composition and surface structure.

KARLSEN, Steinar Johan: Acute effects of extracorporeal shock waves on kidney function and morphology.

1990

EMBLEM, Ragnhild: Straight ileonal anastomosis in patients with familial poliposis and ulcerative colitis.

MYRENG, Yngvar: Relaxation and filling of the left ventricle assessed by doppler echocardiography. A clinical and experimental investigation.

1989

HALL, Christian: Experimentally induced acute ischemic heart failure. A study in dogs of some endocrine and tissue blood

flow responses, with special reference to effects of angiotensin converting enzyme inhibition.

STORDAHL, Arvid: Water-soluble contrast media in obstructed and in ischemic small intestine. A clinical and experimental study.

1988

ROSSELAND, Arne R: Endoscopic papillotomy. A clinical and experimental study.

1987

BRØNDBO, Kjell: Reinnervation of laryngeal muscles. An experimental study in dogs.

SOLHEIM, Ludvig Fjeld: Influence of nonsteroidal anti-inflammatory drugs on bone. A mechanical and biochemical study with acetylsalicylic acid and naproxen in rats.

MATHISEN, Svein R: Healing of synthetic and biological protheses in the cardiovascular system. An experimental study.

1986

RØNNINGEN, Helge: Bone ingrowth in porous fiber titanium Experimental investigation of ingrowth for the purpose of fixation of weight-bearing skeletal implants.

GLENNÅS, Anne: Experimental studies on resistance to certain cytotoxic effects of three gold-compounds, with special reference to metallothionein.

BARTH, Elin: Bioactive, non-porous and bioinert, porous implant materials: comparison of two principles for cementless prosthesis fixation.

1985

ENDRESEN, Liv: Metallothionein. Experimental studies on its protective function against certain anticancer agents.

ENDRESEN, Gerhard KM: Immunological studies on human platelet proteins. Findings in relation to auto-immune diseases.

SMITH-ERICHSEN, Nils: Septicemia evaluated by means of chromogenic peptide substrate assays. A retrospective study in man.

SCHRADER, Harald: Dynamics of intracranial expanding masses, An experimental study with particular reference to the Cushing response.

1983

LÆRUM, Frode: In vivo and in vitro studies of contrast media effects in lower limb phlebography.

BAKKA, Arne: Studies on possible functions of metallothionein.

1982

BREKKE, Inge B: Transplantation of the duct-occluded rat pancreas. Long-term endocrine function and metabolic effects.

1981

AMLIE, Jan P: Mode of actions of digitoxin and digoxin on cardiac electrophysiology and inotropy.

GULDVOG, Ivar: Gastric acid and pepsin secretion in dogs. The role of vagal innervation.

FRENG, Atle: Transversal growth of the maxillary base following resections of the mid-palatial suture - a biometrical and morphological study in the man and the cat.

EKELAND, Arne: Influence of calcitonin on healing and intact bone and skin. A biomechanical and biochemical study in rats.

1980

WIE, Henrik: Effects of cyclophosphamide on connective tissues. An experimental study in rats.

HJORT, Erling F: An experimental study on hematogenous pyelonephritis in the rat and its possible bearing on human pathology.

PhD-Theses

1979

HENRIKSEN, Tore: Interactions of serum lipoproteins with human endothelial cells in culture.

HOLEN, Jarle: The quantification of flow obstructions in the mitral flow channel with doppler echo-cardiography.

ENGESÆTER, Lars B: Biomechanical and biochemical effects of antibiotics on bone and skin.
LILLEAASEN, Per: Hemodilution in open-heart surgery.

WILLE, Sven Øivind: Numerical models of arterial blood flow.

1978

AASEN, Ansgar O: Activities and inhibition of proteases found in plasma during endotoxin shock. An experimental study in dogs.

BERG, Knut Joachim: Effects of acetylsalicylic acid on renal function.

SLAATTELID, Olav: Studies in hypothermic kidney perfusion.

1977

SAUGSTAD, Ola Didrik: Hypoxanthine as an indicator of tissue hypoxia. A study of plasma, cerebro-spinal fluid and brain tissue concentrations.

KVEIM, Morten: The acetate ion as a source of base. Experimental and clinical studies.

ENGE, Ivar: Angiography in regeneration of the liver. An experimental and clinical study.

1976

BERGAN, Anstein: Aspects of bilirubin metabolism in normal and cholestatic dogs.

1975

BLIX, Arnoldus Schytte: The elicitation and regulation of the cardiovascular responses to diving.

SUDMANN, Einar: Vital microscopy of bone remodelling in rabbit ear chambers.

HØIE, Johan: Hemodynamic changes following acute thrombin-induced intravascular coagulation. An experimental study in dogs.

LANGELAND, Norvald: Effects of oestradiol on bone collagen metabolism. An experimental study in female rats.

1974

NUSTAD, Kjell: The relationship between urinary and kidney kallikrein in the rat.

BENUM, Pål: Autogenous transplantation of apophyseal cartilage to osteochondral defects of joints. An experimental study in dogs.

1973

JAKOBSEN, Arnt: Rabbit anti-rat lymphocyte serum. A study on the influence of the antigen dose and immunization schedule on some in vitro characteristics and in vivo immunosuppressive potency.

NESBAKKEN, Ragnar: Aspects of free fatty acid metabolism during induced hypothermia.

1972

SEMB, Bjarne KH: Cardiac transplantation. An experimental study in dogs.

1970

OFSTAD, Egil: Formation and destruction of plasma kinins during experimental acute hemorrhagic pancreatitis in dogs.

TVETER, Kjell: Studies on selective uptake and metabolism of testerone-3H in the prostate and the seminal vesicles of the rat.

TRIPPESTAD, Arne: Aspects of host defence reactions during experimental intestinal strangulation obstruction in rats.

UNHJEM, Olav: Studies on the interaction between androgen and macromolecular components of the rat ventral prostate.

1969

SANDER, Sten: The uptake of oestradiol in normal breast tissue and in induced breast cancer of the rat.

2009:

RISØE, Petter: Cyclic AMP - A promising approach for immunomodulation in sepsis?

RYGH, Una: The role of liver X receptors and adenylyl cylcases in experimental sepsis.

2007

FOSSDAL; Guri: The presence of aquaporins in brain tumours and brain tumour stem cells.

SKJELLEGRIND, Håvard: The role of Ca2+ stores and mitochondria in neuronal ischemia.

ÅGREN, Joanna: Modulation of endotoxemia by CpG DNA and the liver X receptor.

2006

MYHRE, Anders E.: Pathophysiology of endotoxemia

STUESTØL, Jon Fredrik: Systemic inflammation caused by emerging pathogens

Egil Amundsen Lecture

22.09.06	**Professor Iver A. Langmoen** "Stem cells in the Adult Human Brain"
27.04.09	**Professor Olle Korsgren** " Clinical Islet Transplantation: an emerging therapy for patients with type I diabetes"
18.10.10	**Professor Henrik Kehlet** "Fast-track surgery – what is in it and why should we do it?"

Professor Henrik Kehlet has by professor Otto A. Smiseth been awarded the Amundsen medal and diploma.