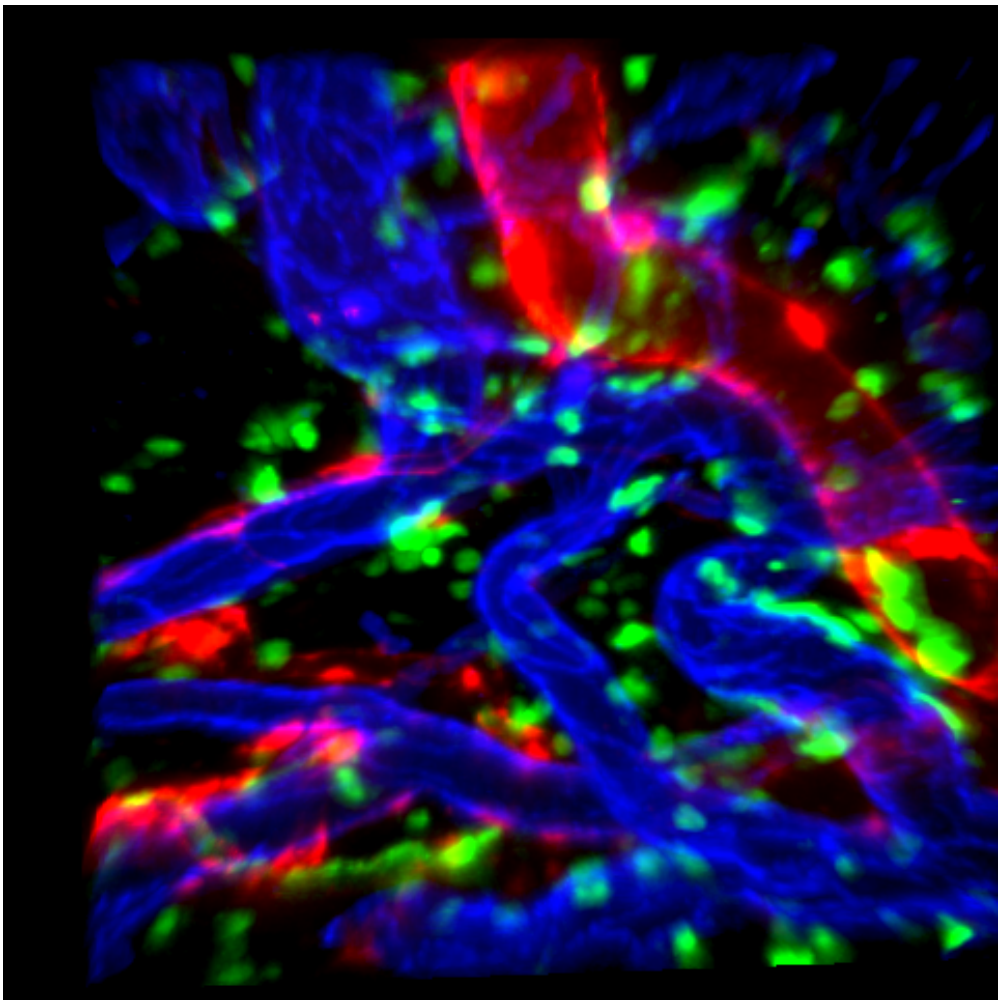
UPPSALA UNIVERSITET

Department of Medical Cell Biology

ANNUAL REPORT

2014



Fastställd av Institutionsstyrelsen 2015

Department of Medical Cell Biology

ANNUAL REPORT

2014

Introduction

The year of 2014 promised a successful future for the department of Medical Cell Biology (MCB). During this year research groups at the department of MCB have obtained several prestigious research grants. For example, young investigators, such as Mia Phillipson, Sebastian Barg, and Fredrik Palm were awarded new grants from the Swedish Research Council. New grants were also obtained from Diabetes Wellness (Sebastian Barg), Barndiabetesfonden (Per-Ola Carlsson and Daniel Espes), NOVO-Nordisk (Anders Tengholm and Sebastian Barg), Cancerfonden (Johan Kreuger), Vinnova (Evelina Vågesjö and Mia Phillipson), SSF (Mia Phillipson) and the European Foundation for the Study of Diabetes (Anders Tengholm and Sebastian Barg). In addition, several young investigators have received awards/prizes/ positions/stipends during 2014; Olof Idevall (the M L Philipson and Göran Gustavsson awards), Per-Ola Carlsson (the DPLU/LUDC Nordic Prize), Nikhil Gandasi (the Young Investigators Award from SSSD), Mia Phillipson (Eric K Fernströms prize), Femke Heindryckx (Post-doc from Cancerfonden), Gustaf Christoffersson (Benzeliusreward from the Royal Academy of Sciences, SFD price for best preclinical PhD in diabetes research, International post-doc from VR). Fredrik Palm has been oppointed American Pysiological Society (APS) Renal Section representative on APS Committee on Committees, member of the American Heart Association/Hypertension Council Professional & Public Education and Publications Committee and to the editorial board of American Journal of Physiology Renal Physiology.

During May 2014, MCB arranged the 49th annual meeting of the Scandinavian Society for the Study of Diabetes (local chairman Per-Ola Carlsson). It is also noteworthy that the MCB investigators have eminently communicated their research achievements to the general public via the news media ("tredje uppdraget"). For example, Per-Ola Carlsson has been featured in TV, radio and newspapers for new treatments in type 1 diabetes, and Gunilla Westermark have been featured in the local newspaper Upsala Nya Tidning for communicated new and exciting findings about islet transplantation and amyloid spreading. In addition, Mia Phillipson has been interviewed on national television news following publishing of one research study, as well as by the nationwide newspaper Dagens Nyheter about her research situation at this department, and she pinpointed strategical strengths and future challenges to adress. Leif Jansson arranged a full day in Uppsala for nurses in diabetic care and a diabetic patient group from northern Sweden (Västernorrland). Several senior MCB scientists lectured about their latest findings in diabetic research. This has become a yearly requested arrangement from the Swedish Diabetic Foundation (Diabetesförbundet), as well as from the diabetes community in association with the World Diabetes Day. Fredrik Palm lectured about stroke and blood pressure for a group of retired citizens on assignment from the Heart and Lung Foundation (Hjärt- Lungfonden).

In view of the recent and strong achievements by many investigators of this department and our recent recruitments of Anders Tengholm (professor in secretion research), Mia Phillipson (professor in physiology), Fredrik Palm (professor in physiology), Johan Kreuger (lectureship in cell biology) and Pär Gerwins (research group), I am confident that the future for the research of this department will turn out increasingly bright.

Although the financial situation of research groups that belong to MCB are in many cases strong, the collective economy of the department has become weaker during the past 1-2 years. Since 2013 the department has recruited three professors (Anders Tengholm, Mia Phillipson, Fredrik Palm), two senior lectureships (Mats Hjortberg, Johan Kreuger), four lectureships (Faranak Azarbayjani, Per Holmfeldt, Sara Bohman, Martin Blixt), and during the same time span only one professorship (Erik Gylfe), one lectureship and three guest teacher positions have ended. Our senior lecturer Håkan Borg retired in 2014, after many years of excellent service to the department, but his activities as an organizer of medical and biomedical student projects were mainly financed by the faculty. Thus, the teaching staff has increased, and at the same time the 2015 faculty allocation to MCB for teaching and research/research education was reduced for the second year in a row. Therefore, from having a large budget surplus in 2013, we now have a budget deficit for 2015. To cover this deficit, the following savings measures have been introduced in late 2014/early 2015:

1. Departmental support of laboratory technicians has been reduced from 40% to 20%
2. Increased teaching for senior teachers
3. Decreased teaching for external teachers
4. Departmental support of PhD-student teaching has been reduced
5. Ended support to the electron microscope facility
6. Decreased support to confocal microscope facility
7. Decreased co-financing of external grants.

In addition to these savings measures there will be two retirements in late 2015 (Ulf Eriksson and Parri Wentzel), which will hopefully lead to a balanced budget in 2016.

To further improve the organization and research environment of the department the following actions have been recently taken or are planned to take place in the near future:

1. In late 2014 we initiated regular PI-meetings, at which our investigators discuss current projects and possible collaborations, to further improve the strength of MCB research.
2. We are planning to re-start the “Frontiers in Diabetes Research” seminar series, which will hopefully involve activities of our PhD-students and attract international diabetes experts as speakers to Uppsala.
3. We plan to instigate a PhD-student prize, to encourage and motivate commendable PhD-student achievements.
4. Laboratory and office space at the department has been inventoried, which has led to a better use of space and to the opening of a new seminar room at the department.
5. Departmental work and tasks by laboratory technicians has been inventoried and reorganized.
6. Teaching costs by different teaching personnel groups have been inventoried.
7. Regular departmental management team meetings, in which departmental heads, directors of studies and administrators participate, have been initiated to improve management and spreading of information between different groups of staff.
8. Departmental computer and IT support is now handled by Uppsala University IT support.

The achievements of this department depend heavily on hard and inspired work performed by our teachers, technicians, researchers, post-docs and PhD-students, but also on our staff of co-heads and administrators. First, I would like to thank Marianne Ljungkvist, who retired as a technician in 2014, for the many years of great work at the department. Peter Hansell, deputy and assistant head of the department, manages undergraduate teaching with great finesse, and Gunilla Westermark, assistant head of the department, takes a firm responsibility for both our graduate student education, as well as safety and working environment issues. Our financial and human resource administration duties are proficiently handled by Shumin Pan and Camilla Sävmarker, and teaching administration is excellently executed by Björn Åkerblom, Lina Thorvaldson and Erik Sandin. Göran Ståhl helps us all with practical matters and Oleg Dyachok keeps our microscopes in great shape. For all this many sincere thanks, and a special thank you to the PhD students that arranged the delightful "julgransplundrings party", and to Erik Gylfe, our former head, who has helped me getting started with the many duties that are associated with this position.

Uppsala 2015-

Nils Welsh

Department Head

List of Contents

Organization

Chairman
Nils Welsh

Deputy chairman
Peter Hansell

Vice chairmen
Peter Hansell (Director of undergraduate studies)
Gunilla Westermark (Director of graduate studies)

Department board
(From mid 2014)
Peter Hansell, teacher representative
Mia Phillipson, teacher representative
Leif Jansson, teacher representative
Per-Ola Carlsson, teacher representative
Mats Hjortberg, teacher representative, deputy
Stellan Sandler, teacher representative, deputy
Anders Tengholm, teacher representative, deputy
Gunilla Westermark, teacher representative, deputy
Björn Åkerblom, representative for technical/administrative personnel
Lisbeth Sagulin, representative for technical/administrative personnel, deputy
Lisa Grapensparr, PhD student representative
Carl Johan Drott, PhD student representative, deputy
Fredrik Lyngfalk, student representative
Shumin Pan, economy administrator, adjunct
Camilla Sävmarker, personell administrator, adjunct

Professors emeriti
Erik Gylfe
Ove Nilsson
Bo Hellman
Erik Persson
Örjan Källskog
Jan Westman
Mats Wolgast
Arne Andersson

Administration
Shumin Pan
Erik Sandin
Göran Ståhl
Camilla Sävmarker
Lina Thorvaldson
Björn Åkerblom

Computers/IT
Peter Öhrt
Magnus Jansson
Tobias Holm (BMC computer department)

Technical staff
Parvin Ahooghalandari
Angelica Fasching
Antoine Giraud
Annika Jägare
My Quach
Lisbeth Sagulin
Monica Sandberg
Jan Saras

Scientific Reports

Islet vascular physiology and cell therapy

Per-Ola Carlsson, Leif Jansson

The research of the group is mainly focused on the vasculature of the pancreatic islets and its relation to islet endocrine function during normal and diabetic conditions and after transplantation. The endothelial cells, which line all blood vessels, are important not only to distribute nutrients and oxygen to the islets, but our findings show that they also produce mediators which are involved in the regulation of hormone release, cell growth and the blood perfusion through the islets. Furthermore, endothelium-derived substances are likely to modulate the pathogenesis of both type 1 and type 2 diabetes. We have in recent years identified a functional reserve of islet endocrine cells. Normally 20-25% of islets are low oxygenated and with low protein biosynthesis, but these cells may be activated upon need during increased functional demands. On the other hand, more islets become downregulated when beta-cell mass is increased. We have also observed that the most blood-perfused islets, having a higher vascular density (Fig. 1), have a superior beta-cell function, proliferation and gene expression. However, these islets are also more prone to cellular death when stressed by hypoxia or cytokines, and they are also more prone to develop amyloid deposits.

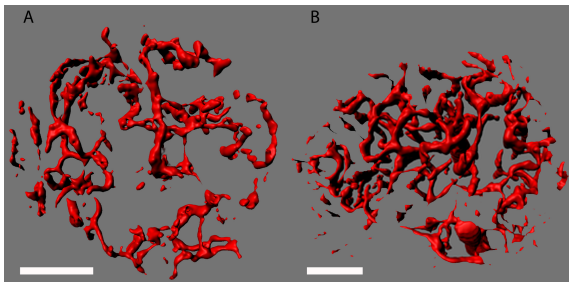


Fig 1. Two-photon confocal images of vascularity in pancreatic islets with low (A) or high (B) blood perfusion (blood perfusion identified by microsphere measurements).

Much of our research within the last years have been devoted to the adaptation of transplanted islets of Langerhans (which contain the insulin-producing beta-cells) to the implantation organ, i.e. the so-called engraftment process, and how this may be affected by different conditions in the recipients. Such transplantations are performed also in humans, but the long-term results are disappointing, probably due to impaired engraftment. Novel strategies to improve engraftment, as well as aspects to prevent cell death and regenerate beta-cells in native and transplanted islets by stem-cell stimuli are based on these findings presently tested by the research group in both experimental and clinical studies (cf. below).

Islet transplantation and beta-cell regenerative medicine (Per-Ola Carlsson)

The overall aim of the research on islet transplantation and beta-cell regenerative medicine is to develop means to intervene with the development of type 1 diabetes mellitus and find treatment strategies to restore glucose homeostasis in patients with type 1 diabetes mellitus using cell therapy. The dual role of the P.I. as experimental and clinical scientist simplifies translational approaches, and the research group is active both at the Department of Medical Cell Biology and the Department of Medical Sciences. Experimental studies are conducted to elucidate the importance of islet endothelial, neural or their progenitor cells for beta-cell regeneration and function, and investigate the concept of islet heterogeneity. Other studies investigate the adaptation of pancreatic islets to the implantation organ, i.e. the so called engraftment process, following transplantation (Fig. 2), and develop bioengineering strategies (coating of islets with

supporting stem cells, oxygen carriers and growth factors, as well as with use of scaffolds) to improve results of pancreatic islet transplantation by enhancement of engraftment e.g. by improved revascularization. Human islets are tested in these experimental systems with a focus to produce clinically applicable protocols. We also perform research to develop safe and effective means to generate new human beta-cells by stimulating adult beta-cell proliferation, e.g. by stem cell stimulation, or by stem cell differentiation in vivo. Clinical studies are performed to prevent development of type 1 diabetes in patients, e.g. by autologous mesenchymal stem cell transplantation, and to develop means for beta-cell imaging by positron emission tomography. We also conduct studies to improve the results of clinical islet transplantation, e.g. by encapsulation in order to avoid immune suppression of the patients. In one of these studies, a newly developed oxygenized macrochamber to harbor the islets is tested in a pilot trial with type 1 diabetes patients. The macrodevice protect the islets from immune rejection, whereas oxygen is supplied daily into a refillable oxygen tank.

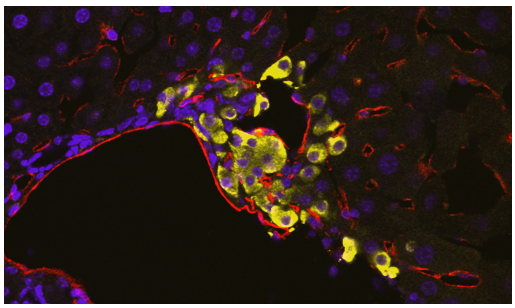
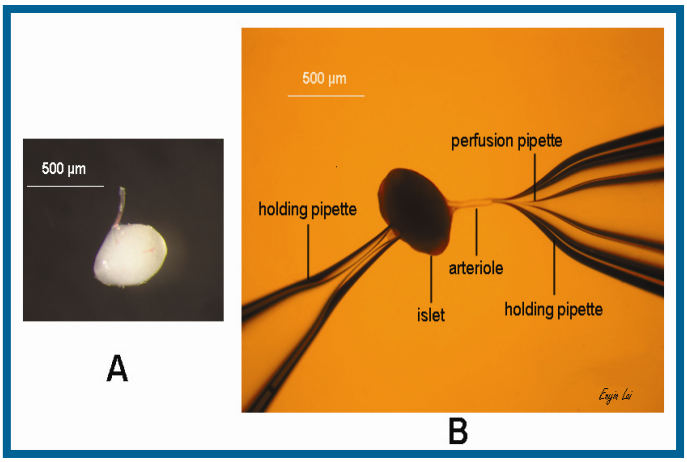


Fig 2. Micrograph showing vascularization of intraportally transplanted islet with disrupted integrity in the wall of a portal vein tributary. Yellow depicts insulin; red CD31 staining for blood vessels and blue DAPI.

Pancreatic islet blood flow and endocrine function (Leif Jansson)

The overall purpose is to functionally evaluate the vascular system and especially map blood flow regulatory mechanisms in the islets of Langerhans during normal conditions and during various degrees of glucose intolerance and overt diabetes**.** An important rational behind these experiments is the diabetes-induced endothelial dysfunction, which also affects the islet vasculature. This detailed knowledge can then be applied to facilitate targeted delivery of substances to the islets by selectively increasing the blood flow to the islets. This is initially performed in experimental animals, but we intend to transfer our results also to humans. Substances of interest for facilitated delivery include immunomodulatory substances, substances stimulating regeneration of beta-cells, contrast agents to increase visibility of islets during imaging procedures for easier quantification of islet mass, substances affecting islet endothelial cells to ameliorate the endothelial dysfunction seen in diabetes, which is generalized but also specifically affects the islets.



In this context we will also study islet endothelial cells (EC) from different animal models and human islets both in vivo and in vitro.

EC are isolated aand cultured under flow with various additions to study the expression of different intracellular and plasma membrane proteins. This will enable us to *e.g.* identify changes in endothelial function caused by hyperglycemia, hyperlipidemia and other conditions associated with

impaired glucose tolerance (IGT). It will also identify endothelial markers, which can be used to further improve selective delivery of substances to the islet vasculature. Changes in endothelial function can then be further evaluated with our palette of in-vivo techniques.
On the basis of our findings on normal islet blood flow regulation we will continue our studies to evaluate disturbances occurring during IGT and type 2 diabetes. We have previously observed that these conditions are invariably associated with an increased islet blood flow. Thus, in this context we would be interested to evaluate mechanisms by which to decrease islet blood flow. However, the mechanisms underlying this are as yet largely unknown. We now aim to further clarify these mechanisms and to evaluate to what extent we can normalize islet blood flow. In relation to this, we also plan to investigate if normalization of islet blood flow can ameliorate IGT. We also aim to study, by imaging techniques, if the results on blood flow regulation obtained in rodents are applicable also to humans.
In summary, the general aim is to advance and use our knowledge on islet blood flow regulation to develop techniques to affect islet endocrine function by modulation of islet blood flow. Thereby we will, in a longer perspective, be able to more selectively target drugs to the islets, and facilitate imaging of the islets to obtain improved determinations of beta-cell mass during T1D and T2D.

Members of the group

Per-Ola Carlsson, MD, professor, Senior Consultant in Endocrinology and Diabetology

Leif Jansson, MD, professor

Arne Andersson, MD, professor em.

Joey Lau, post-doc

Monica Sandberg, post-doc

Sara Bohman, post-doc

Svitlana Vasylovska, post-doc

José Caballero, MD, post-doc

Carl Johan Drott, MD, PhD student

Daniel Espes, MD, PhD student

Liza Grapensparr, PhD student

Sara Ullsten, PhD student

Xiang Gao, post-doc

Hanna Liljebäck, MD/PhD student

Astrid Nordin, laboratory engineer

Ing-Britt Hallgren, laboratory engineer

Zhanchun Li, laboratory engineer

My Quach, laboratory engineer

Lisbeth Sagulin, laboratory engineer

Birgitta Bodin, laboratory technician

Eva Törnelius, laboratory technician

Violeta Armijo Del Valle, research nurse

Rebecca Hilmius, research nurse

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* Shared contribution as last author

Agencies that support the work

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The European Foundation for the Study of Diabetes

The Swedish Research Council

The Swedish Diabetes Association

The Torsten Söderberg Foundation

The Diabetes Wellness Foundation

AFA

The Swedish Juvenile Diabetes Fund

The Novo Nordisk Foundation

The Knut and Alice Wallenberg Foundation

The Olle Engkvist Byggmästare Foundation

The Uppsala-Örebro Regional Research Foundation

The Gunvor & Josef Ane'rs Foundation

The Thuring Foundation

Svenska Sällskapet för Medicinsk Forskning

The Family Ernfors Foundation

Goljes Memorial Fund

Islet function in childhood obesity and type 2 diabetes mellitus

Peter Bergsten

Background

The prevalence of persons with metabolic disease including type 2 diabetes mellitus (T2DM) is expected to rise from 3% in 2000 to almost 5% in 2030. Since obesity is strongly linked with T2DM, the increasing prevalence of over-weight and obesity especially among children, reaching 20% in Sweden, is of particular concern. The rise in obesity has a multi-factorial background, where both genetic and environmental factors contribute. Our research focuses on the role and function of the islet of Langerhans in the early stages of obesity and obesity-related complication including T2DM.

Aim

The overall aim is to find therapeutic approaches to halt the rise in childhood obesity and related metabolic disease including T2DM. This will be attempted by applying a translational approach, where obese and lean patients are examined and characterized and underlying mechanisms investigated in islet cellular systems.

Beta-cell function in juvenile type 2 diabetes and obesity (Beta-JUDO)

The FP7 project "Beta-cell function in JUvenile type 2 diabetes Diabetes and Obesity (Beta-JUDO)" started 2012 and will end 2016 and is coordinated from MCB. In the project the role of the beta-cell in development of obesity is addressed. Beta-JUDO encompasses both *in vitro* work, where isolated human islets and beta-cell lines are used, and *in vivo* work, where obese and lean children are examined.

Elevated palmitate concentrations

When isolated islets are exposed to prolonged elevated palmitate levels, as observed in obese subjects and T2DM, insulin secretion is impaired (Fig 1). However, this impaired insulin sceretion is preceded by islet insulin hypersecretion (Fig 1; Kristinsson et al 2013). Thus, it appears that before palmitate-induced impairment of insulin secretion and loss of beta-cell mass occur, enhanced insulin secretion is observed.

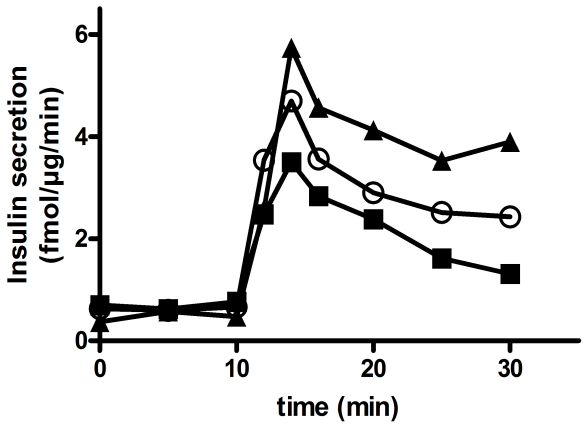

Figure 1: Glucose-stimulated insulin secretion from isolated human islets exposed to 0.5 mM palmitate for 0 (open circles), 2 (closed triangles), or 7 (closed squares) days.

In young obese and lean children belonging to the "Uppsala Longitudinal Study of Childhood Obesity" (ULSCO) (Forslund et al 2014), we have investigated if the observed palmitate-induced alterations in insulin secretory patterns were evident also *in vivo*. Obese children are referred to the Uppsala University Children's Hospital, where they are examined and treated. Both the obese children and lean controls are enrolled in the ULSCO cohort, which together with similar patient cohorts in Salzburg, Leipzig and Cambridge form the Beta-JUDO childhood obesity cohort. Circulating palmitate concentrations were determined in the lean and obese subjects (Ubhayasekera et al, 2013). When their insulin secretory response to glucose was measured by oral glucose tolerance test (OGTT), insulin levels at fasting and 30 min of OGTT were elevated in obese children with elevated palmitate levels but attenuated in obese adolescents with elevated palmitate levels (Fig 2). Indeed, secretory levels in the adolescents were similar to those observed in lean controls. Based on the findings in the isolated islets and the fact that some of these adolescents progressed to overt T2DM, we hypothesized that this "normalization" reflects impaired beta-cell function in the older obese individuals and that insulin hypersecretion observed in isolated human islets (Fig 1) and obese children (Fig 2) is an etiological factor in the development of obesity precipitating overt T2DM in susceptible individuals.

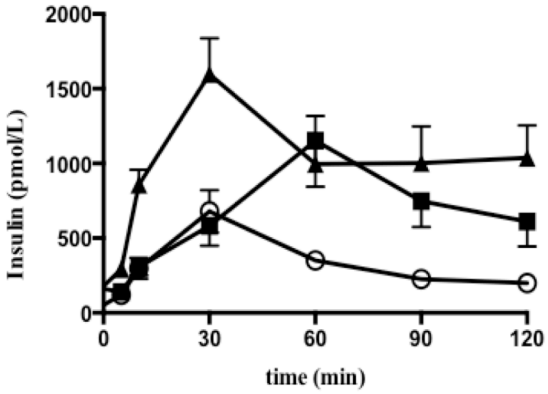

Figure 2: Oral glucose tolerance test in obese pre-pubertal (closed triangles), pubertal (closed squares) children with high palmitate and lean controls (open circles).

Attenuation of insulin hypersecretion

In isolated islets approaches to attenuate beta-cell hypersecretion are conducted to defining underlying causes for the observed accentuated secretory activity in insulin-producing beta-cells using a collaborative, translational approach. Isolated human islets are exposed to compounds known to affect insulin secretion and their effects on insulin hypersecretion determined. These approaches are expected to give information on pharmacological treatment alternatives for the obese children. Among these approaches the antagonists to the free fatty acid receptor (FFAR)1 have been investigated. In the presence of FFAR1 antagonist the negative effects of elevated free fatty acids, which are ligands to FFAR1, were attenuated (Kristinsson et al, 2014).

Insulin processing

Accentuated insulin secretion, as observed in isolated islets after 2 days exposure to elevated palmitate concentrations and in obese children with high circulating palmitate concentrations, puts high demands on the insulin biosynthetic machinery. We have investigated how the amount of fully processed insulin and non-processed proinsulin is affected in obesity. Measurements of insulin and proinsulin were conducted both in isolated islets exposed to palmitate and in obese and lean children. In islets expression of enzymes responsible for cleavage of proinsulin to insulin were also measured.

Sphingolipids

When palmitate concentrations are elevated the formation of the sphingolipid ceramide is increased. Since this sphingolipid has been implicated in apoptosis we have investigated how sphingolipid metabolism is affected in obesity. This was done by measuring multiple shingolipid species by GC-MS both in beta-cell exposed to elevated palmitate concentrations (Manukyan et al, 2015) and in the circulation of obese and lean children.

Islet architecture

The islet of Langerhans is a complex organ containing diffeernt cell types, The interaction between these cell types is essential for proper function. We have investigated the role of coupling between beta-cells for glucose-stimualted insulin secretion (Chowdhury et al 2013a) and also how signalling is altered if such coupling is disrupted (Chowdhury et al 2013b).

Significance

The results of the project are expected to identify novel principles of normalizing hypersecreting beta-cells. These principles will be evaluated in the young obese individuals as intervention strategies, which are critical since the window of opportunity to preventing impaired beta-cell function and apoptosis in juvenile obesity appears to be limited.

Members of the group

Peter Bergsten, professor

Anders Forslund, MD, PhD

Ernest Sargsyan, researcher

Karlfried Groebe, visiting researcher

Levon Manukyan, postdoctoral person

Azazul Chowdhury, postdoctoral person

Anders Alderborn, PhD

Johan Staaf, graduate student (MD/PhD-programme)

Hjalti Kristinsson, graduate student

Hannes Ohlsson, graduate student (MD/PhD-programme)

Jing Cen, graduate student

Rasmus Stenlid, undergraduate student

Iris Ciba, MD

Marie Dahlbom, research nurse

Malte Lidström, research nurse

Helena Vilén, research dietician

Malin Meirik, research psychologist

Emmelie Brandt, research physiotherapeuist

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Selander's Foundation

Collaborations

Uppsala University:

- Fredrik Ahlsson (Womens's and Children's Health)
- Håkan Ahlström (Radiology)
- Jonas Bergquist (Analytical Chemistry)
- Barbro Diderholm (Womens's and Children's Health)
- Jan Gustafsson (Womens's and Children's Health)
- Mats Gustafsson (Medical Sciences)

Other universities:

- Ali Moazzami (Swedish University of Agricultural Sciences)
- Antje Körner (University of Leipzig, Germany)
- Reinhard Schneider (EMBL, Germany)
- Daniel Weghuber (Paracelsus Medical University, Salzburg, Austria)
- Kurt Widhalm, (University of Vienna, Austria)
- Jean-Charles Sanchez (University of Geneva, Switzerland)
- Sadaf Farooqi, (University of Cambridge, Great Britain)
- Dave Smith (AstraZeneca, Great Britain)
- Ulrika Hammarström (Scandnavian CRO, Uppsala)

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Physiology of pancreatic islet hormone secretion

Anders Tengholm

The research in our group aims at clarifying the mechanisms regulating the release of insulin, glucagon and other hormones from the islets of Langerhans. Insufficient secretion of blood-glucose-lowering insulin and dysregulated secretion of blood-glucose-elevating glucagon are hallmarks of diabetes. Elucidation of the mechanisms underlying islet hormone secretion and the malfunctions causing diabetes is expected to provide new strategies for treatment of the disease. By combining biochemical and molecular biological techniques with fluorescent cell signalling biosensors and live cell imaging methods, we study the spatio-temporal dynamics of signalling processes regulating secretion in single cells and intact mouse and human pancreatic islets. At present we are focusing on the following issues.

ATP, Ca^{2+} and cAMP signalling in β-cell stimulus-secretion coupling

Insulin is released from β-cells in response to glucose, other nutrients, hormones and neural factors. The hormone is released in pulses with the kinetics determined by a complex interplay between second messengers and signalling proteins beneath the β-cell plasma membrane. Glucose stimulation of β-cells results in uptake and metabolism of the sugar, elevation of the intracellular ATP/ADP ratio, closure of ATP-sensitive K^+ channels in the plasma membrane, depolarization and voltage-dependent Ca^{2+} influx, which triggers exocytosis of insulin secretory granules. The exocytosis response is amplified by the messenger cAMP, which is generated in β-cells during glucose stimulation as well as upon glucagon and incretin hormone receptor activation.

Our lab has discovered that glucose triggers coordinated oscillations of Ca^{2+} and cAMP in β-cells, and that this response is important for pulsatile insulin secretion. However, the mechanisms underlying the generation of these oscillations are not clear. ATP plays a central role, linking metabolism to electrical activity by blocking the ATP-sensitive K^+ channels, and variations in metabolism may underlie the Ca^{2+} oscillations in glucose-stimulated cells. There are also feedback effects of Ca^{2+}on cell metabolism and we are currently employing various imaging tools to investigate the relationship between ATP and Ca^{2+}in β-cells (Fig 1).

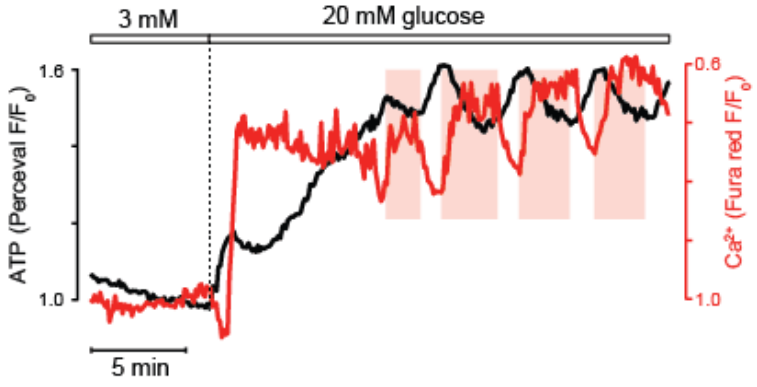

Figure 1. Relationship between the intracellular concentrations of ATP (black trace) and Ca^{2+} (red trace) beneath the plasma membrane of a β-cell within a mouse islet. When the glucose concentration is increased from 3 to 20 mM there is an immediate rise of ATP followed by increase of Ca^{2+} that triggers insulin secretion. After 5-10 minutes there are antiphase oscillations of ATP and Ca^{2+}, which reflect interactions between the two messengers important for generating pulsatile insulin secretion.

We are also using cell signalling biosensors to clarify the mechanisms underlying the generation of cAMP oscillations and how the cAMP effectors protein kinase A and Epac, a guanine nucleotide exchange factor for Rap GTPases, are involved in the regulation of insulin secretion. For example, we have found that protein kinase A, in addition to potentiating exocytosis in response to cAMP-elevating hormones, is important for proper initiation of insulin secretion by glucose. Moreover, recent work from the lab has demonstrated that cAMP

and Ca^{2+} signals trigger translocation of Epac to the β-cell plasma membrane. The downstream effects as well as functional importance of these signalling steps are currently under investigation.

Autocrine feedback signalling in β-cells

Exocytosis of insulin granules not only results in the release of insulin, but also of several other granule constituents, which affect β-cell function in an autocrine manner. Activation of insulin receptors leads to PI3-kinase-mediated formation of the phospholipid PtdIns(3,4,5)P_3. Using fluorescent reporters we have demonstrated that glucose stimulation of β-cells results in pronounced PtdIns(3,4,5)P_3 oscillations in the plasma membrane that reflect pulsatile insulin secretion and the associated autocrine insulin receptor activation. Although insulin has been found to exert positive feedback on insulin biosynthesis and β-cell proliferation, it is less clear whether insulin acutely stimulates or inhibits insulin secretion.

Insulin is stored in a crystalline complex with Zn^{2+} and this ion is co-released with insulin and exerts feedback effects at multiple levels. The granules also contain ATP and we recently discovered that ATP co-released with insulin activates purinergic $P2Y_1$-receptors, which results in phospholipase C activation and short-lived (<10 s), local increases of diacylglycerol (DAG) in the plasma membrane (Fig 2). These DAG spikes results in rapid recruitment and activation of several protein kinase C isoforms. Using various optical single-cell assays we are currently investigating how insulin, Zn^{2+} and ATP affect signalling and secretion in β-cells.

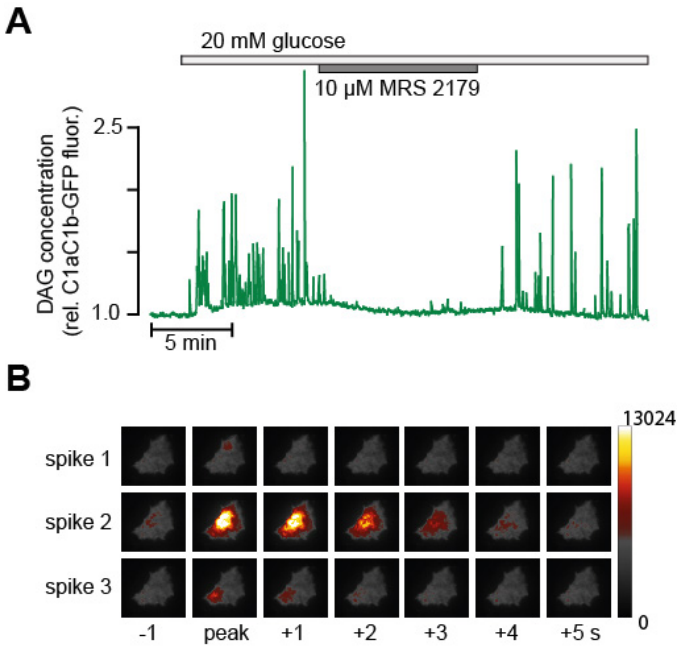

Figure 2. (A) Glucose stimulation of a mouse β-cell triggers pronounced DAG spiking in the plasma membrane that is monitored with a fluorescent DAG reporter. The response is reversibly inhibited when the autocrine action of ATP is blocked with the purinergic receptor antagonist MRS2179.

(B) The DAG spikes are typically spatially confined. Each row shows a sequence of pseudo-colored 14-bit images starting 1 s before the appearance of a DAG spike and displays the DAG reporter fluorescence every second during the following 6 seconds.

Mechanisms controlling the release of glucagon, somatostatin and pancreatic polypeptide

In diabetes there is not only an impaired secretion of insulin, but poor regulation of blood-glucose elevating glucagon contributes to the hyperglycemia underlying diabetes complications. Pancreatic polypeptide is another islet hormone of potential importance for blood glucose regulation by effects on gastric emptying. The fourth islet hormone, somatostatin, is a potent inhibitor of the release of the other hormones and probably has a paracrine function. Other paracrine events in the islets involve insulin-promoted inhibition of glucagon secretion and glucagon-potentiated insulin secretion. Like for insulin, the secretion of glucagon and somatostatin is pulsatile. Our lab has demonstrated that glucagon is released in

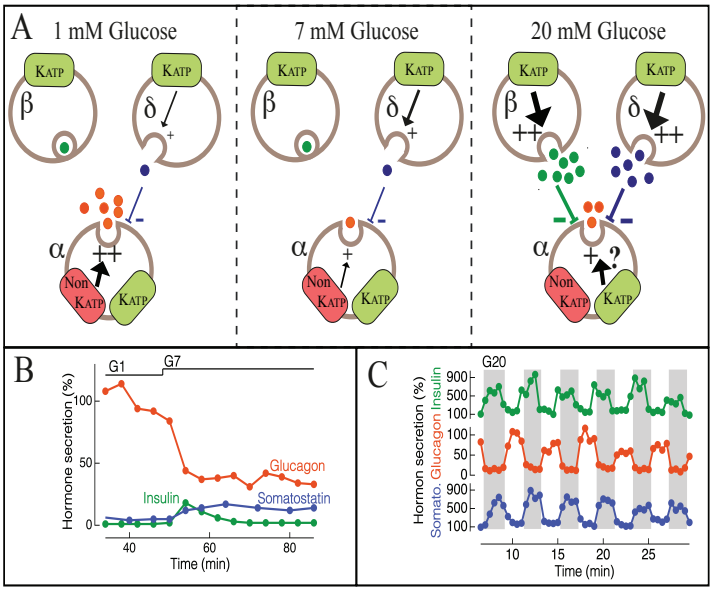

Figure 3. Model for glucose regulation of glucagon release. (A) In the 1-7 mM range (G1, G7), glucose controls glucagon release via an intrinsic non-K_{ATP} channel-dependent mechanism in α-cells and paracrine release of somatostatin from δ-cells has only a tonic inhibitory effect. (B) The graph showing glucose inhibition of glucagon secretion is expressed in percent of stimulated secretion at 1 mM glucose. To get an impression of the relative magnitudes of the corresponding insulin and somatostatin responses, their secretion are expressed in percent of stimulated secretion in response to 0.5 mM tolbutamide. (A, C) At 20 mM glucose (G20) the K_{ATP}-independent mechanism no longer stimulates glucagon secretion and the pulsatility is generated via paracrine release of inhibitory factors from β- and δ-cells. The question mark indicates that a stimulatory effect of high glucose in the α-cell is not necessarily K_{ATP} channel-dependent. Hormone secretion data have been recalculated as percentage of estimated secretion at 1 mM glucose (From Gylfe Diabetes 62:1391-1393, 2013.

opposite phase to insulin and somatostatin, which has important implications for the understanding of the action of insulin and glucagon on glucose production in the liver. Glucose inhibits glucagon secretion and stimulates somatostatin secretion but consensus is lacking regarding the underlying mechanisms. Like in β-cells, glucose metabolism plays a key role and Ca^{2+} is the main trigger of exocytosis in both glucagon-releasing α-cells and somatostatin-releasing δ-cells. We are currently investigating intracellular ATP, Ca^{2+} and cAMP signalling in relation to hormone release from the different islet cells as well as the importance of paracrine intercellular communication for generating the different secretory patterns. Fig 3 illustrates our current working model for glucose regulation of glucagon secretion.

Members of the group

Parvin Ahooghalandari – Research engineer
Ida Alenkvist – Graduate student
Helene Dansk -Research engineer
Oleg Dyachok – Senior research engineer
Eva Grapengiesser - Associate professor
Erik Gylfe - Professor
Bo Hellman - Professor
Olof Idevall-Hagren – Assistant Professor
Lisen Kullman - Researcher
Jia Li – Graduate student
Hongyan Shuai – Graduate student
Anders Tengholm – Professor
Antje Thonig – Laboratory technician
Yunjian Xu - Senior research engineer
Qian Yu – Graduate student

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Jia Li: "ATP dynamics in pancreatic α- and β-cells". December 2014.

Cellular architecture and organelle-organelle communication

Olof Idevall-Hagren

The architecture of the prototypic mammalian cell has been the focus of intense study since the early days of microscopy. With the development of electron microscopy for the biological sciences in the 1950's came the detailed characterization of most cellular organelles, like the endoplasmic reticulum (ER), the Golgi apparatus and secretory vesicles. More recently, using live cell imaging techniques, it has been found that these organelles are highly dynamic structures that constantly reform, reshape and redistribute within the cell. Many organelles also seem to communicate through direct contacts, formed by protein and lipid complexes. At these sites, information flow between the organelles in the form of lipids, ions and proteins help control the specific organelle function. Using high-resolution fluorescence microscopy together with genetically encoded biosensors and molecular tools we study and manipulate these cellular structures in order to better understand their function.

Novel roles of an ancient organelle

The endoplasmic reticulum (ER) is the cellular organelle responsible for lipid and protein synthesis as well as Ca^{2+} homeostasis, and disturbances in one or more of these processes are associated with a plethora of human diseases, including diabetes, neurological disorders, immunodeficiency and cancer. Some of these disease conditions are associated with altered ER morphology, such as redistribution or altered shape, but it is not

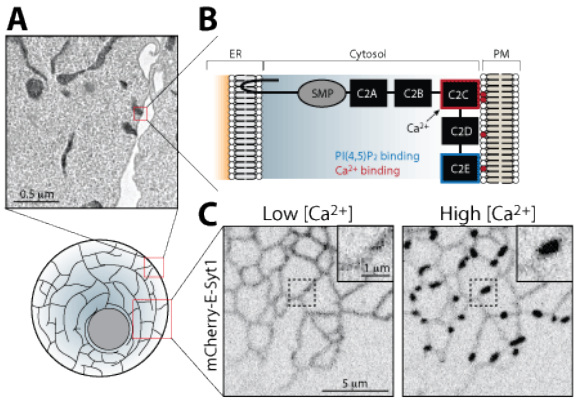

***Figure 1**: **A**. Electron micrograph of a fibroblast where the ER is stained black. Notice how parts of the ER are in close proximity of the cell periphery (red box). **B**. Schematic illustration depicting Extended-Synaptotagmin-1 (E-Syt1), an ER-anchored protein that also binds the plasma membrane by interactions with specific lipids. C. Confocal microscopy images of a very flat cell expressing fluorescence-tagged E-Syt1 under conditions where the cytoplasmic Ca^{2+} concentration is low or high. Notice how the molecules aggregate at the*

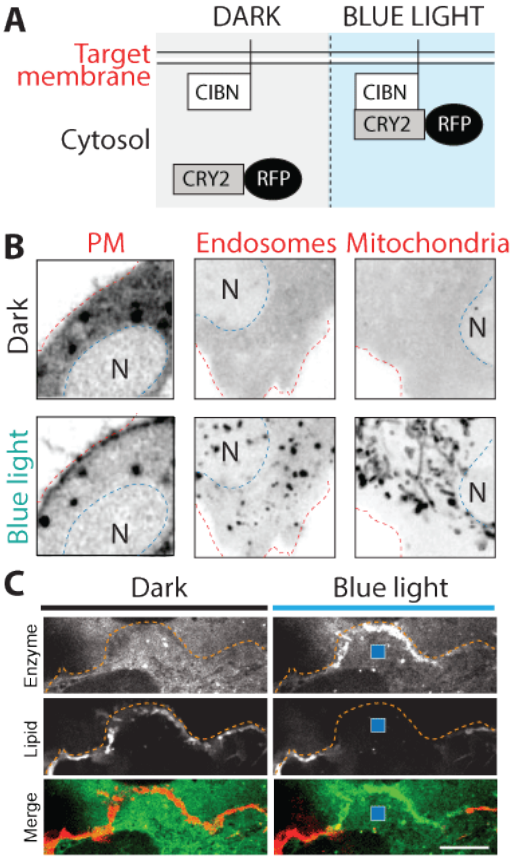

***Figure 2: A,B.** Drawing showing the principle of light-induced hetero-dimerization. One part of the optogenetic module (CIBN) can be anchored to any cellular membrane (target membrane) whereas the other part (CRY2) can be fused to a protein of interest (here a Red Fluorescent Protein). Blue-light illumination promotes the interaction between CIBN and CRY2 and causes redistribution of the protein of interest to the target membrane. **C.** Focal blue-light illumination (blue square) allows recruitment of a lipid-degrading enzyme (green) to a restricted part of*

clear if these changes are a cause or a consequence of disease. In recent work we show that the ER is anchored to the plasma membrane via protein-lipid interactions and that genetic ablation of these contacts results in massive rearrangement of the ER (4). We are currently characterizing how this morphological change affects the function of the ER. Hopefully this can help us better understand how altered ER function contributes to the progression of various diseases. Specifically, we are investigating how ER morphology, distribution and function influence the production and secretion of insulin from the pancreatic beta-cells and also how these characteristics change during pathologic conditions, such as diabetes.

Optogenetic tool development and implementation

Optogenetics is the modification and use of light-regulated proteins, typically isolated from plants or bacteria, to enable control of cellular processes by illumination. Expression of optogenetic tools has for example enabled light-dependent control of neurotransmitter release, insulin release, cell migration and transcription. We have previously used light-dependent protein-protein interactions to recruit lipid synthesizing and degrading enzymes to the plasma membrane, leading to the discovery that rapid changes in lipid levels can polarize cells and is sufficient to induce e.g. directed cell migration (3,4,8). Current work aims at using these optogenetic tools to generate inducible contacts between various cellular organelles and determine how this affects cell function. Since optogenetics is a non-invasive technique, we also work on adapting it to *in vivo* settings.

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Members of the group

Olof Idevall-Hagren – Assistant Professor

Shanshan Li – Project student

Antje Thonig – Laboratory technician

Beichen Xie – Master thesis student

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Mechanisms of regulated exocytosis

Sebastian Barg

Exocytosis is fundamental to every cell and crucial to intracellular transport, protein sorting, and cell-to-cell communication. In both neurons and endocrine cells, exocytosis leads to the release of neurotransmitters and hormones, and defects in this process can underlie disease, such as type-2 diabetes. In our lab we are interested in the cell biology of insulin secretion, with a focus on the life-cycle of insulin-containing secretory granules. We study exocytosis in pancreatic ß-cells using advanced light microscopy (TIRF, super-resolution and single molecule imaging) in combination with electrophysiology. Both methods are sensitive enough to observe single granules and even individual protein molecules in a living cell

Molecular architecture of the insulin granule release site

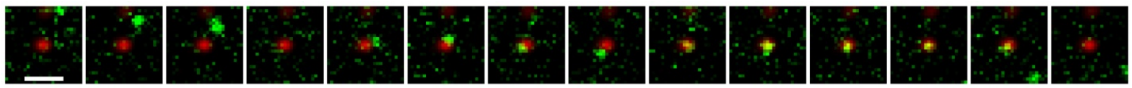
Every ß-cell contains thousands of secretory granules that store insulin. When blood glucose is elevated, these granules undergo regulated exocytosis and release the hormone into the blood stream. Before this can happen, granules have to reach the plasma membrane, where they

"dock" and then assemble the exocytosis machinery. When insulin is released, these steps quickly become limiting for how much insulin is released.

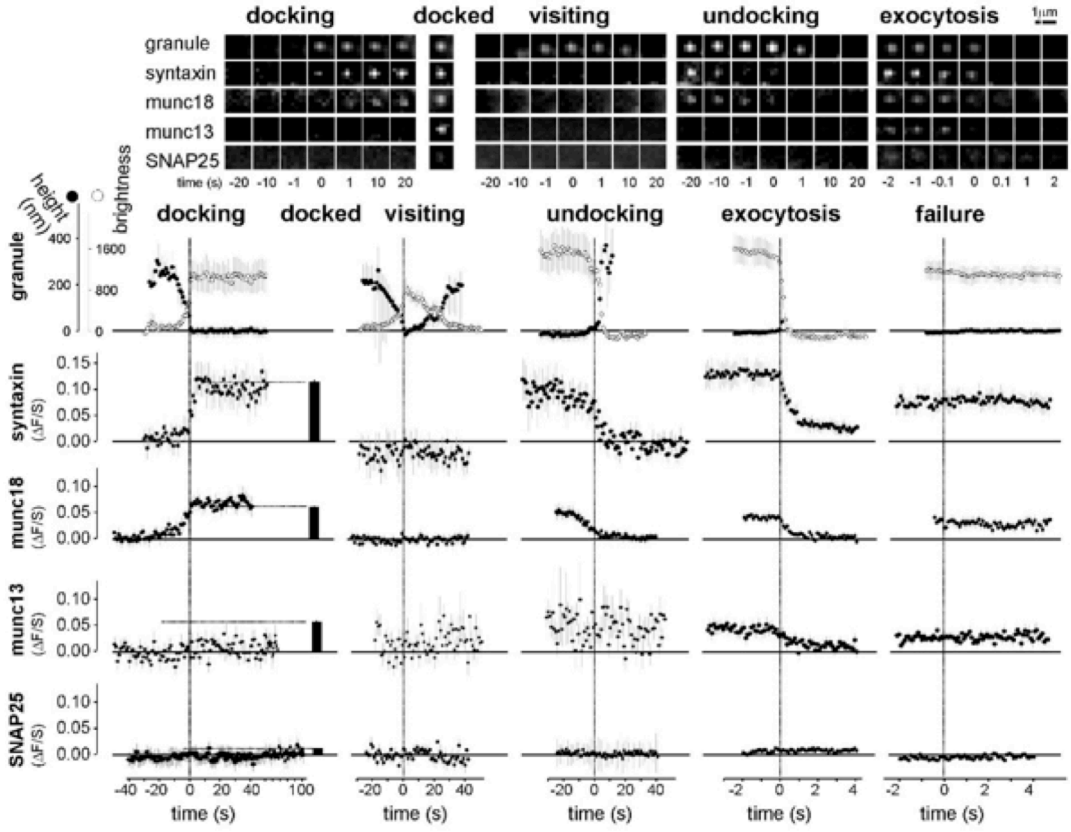
The docking process is not understood in molecular terms, but many of the proteins involved have been identified. One hypothesis that we are currently testing is that some of these proteins (including t-SNAREs) pre-assemble at small hotspots in the plasma membrane. These hotspots, perhaps related to lipid rafts, may then recruit granules and act as "launching pads" for exocytosis. There is evidence that this docking step is impaired in type-2 diabetes, and the most important "diabetes gene" affects expression of a protein involved in granule docking. How do cells compartmentalize their plasma membrane to organize such sites? Which proteins are recruited to these hotspots, when, and at how many copies? And how are docking sites regulated and what distinguishes release-ready granules from those that are merely docked?

The three SNARE proteins syntaxin, SNAP25 and synaptobrevin are central to membrane fusion during exocytosis. Since two of these, the t-SNAREs syntaxin and SNAP-25 inhabit the plasma membrane, one expects them to collect at the exocytic site before a vesicle or granule can fuse there. Indeed, we found t-SNAREs cluster near docked granules and quantitative image analysis shows association of GFP-labeled syntaxin and SNAP25 with granules in live Ins1- or PC12-cells. The interaction depends on the N-terminal Habc domain of syntaxin, rather than formation of a SNARE complex. Up to 70 molecules of syntaxin are recruited to the granule site during docking, and lost during undocking and exocytosis. However, individual molecules of both proteins diffuse rapidly in the plasma membrane and are only occasionally captured beneath a granule, for a short time (<1s). Thus, the protein composition of individual granule-associated nanodomains is remarkably dynamic and correlates with the granules' ability to exocytose. This organization is established during or just after granule docking, which suggests that granules approaching the plasma membrane might induce the formation of their own docking site. Dynamic association of exocytosis proteins with individual granules occurs on a timescale consistent with rapid cellular signaling, and may be important for the short-term regulation of insulin secretion (Barg et al PNAS 2010; Knowles et al PNAS 2010). We have until now quantified to over 20 other exocytosis proteins (syntaxin, SNAP25, munc18, munc13, rab3 etc) , and established the time course of their recruitment to the the insulin granule release site. These measurements show that insulin granule docking coincides with rapid *de novo* formation of syntaxin1/munc18 clusters at the nascent docking site, which stabilizes the docked state. Interfering with this clustering prevents docking. We could also show that the proteins SNAP25 and munc13 are recruited to the docking site with a delay of at least a minute, consistent a role in granule priming rather than docking. We conclude that secretory vesicles dock by inducing syntaxin1/munc18 clustering in the target membrane, and find no evidence for preformed docking receptors.

Our work is unique in that we have correlated release probability or individual docking/undocking events with local protein recruitment. We provide the first quantitative timecourse for the assembly of the release site, which puts constraints on molecular models of docking and exocytosis. For example, we show that Ca^{2+}-channels, SNAP25 and munc13 are not detectable at the release site for at least 30 s after docking, implying that these proteins are recruited as part of secretory vesicle priming, rather than docking. This is not due to limits in sensitivity, as our method can detect specific association of on average less than one labeled molecule with the docking site To our knowledge, this is also the first time that the formation of protein-containing membrane rafts has been directly visualized and quantified in living cells.



A single molecule of Syx-EGFP (green) binds to a secretory granule (NPY-cherry, red). Scalebar 1µm; 20ms per frame.



Quantification of protein affinity during the lifecycle of the docking/release site. (Gandasi and Barg, *Nat Comm* i2014).

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Members of the group

Sebastian Barg - Docent

Nikhil Gandasi- Graduate student

Yin Peng, Graduate student

Omar Hmeadi, Graduate student

Emma Kay, postdoc

Jan Saras, research engineer

Maria Helou, project student SOFOSKO

Kim Vesto, Master thesis student

Agencies that support the work

Diabetes Research Wellness Foundation

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Novo Nordisk Foundation

European Foundation for the Study of Diabetes/MSD

European Foundation for the Study of Diabetes/BI

The Carl Tryggers Foundation

The Göran Gustafsson Foundation

Family Ernfors Foundation

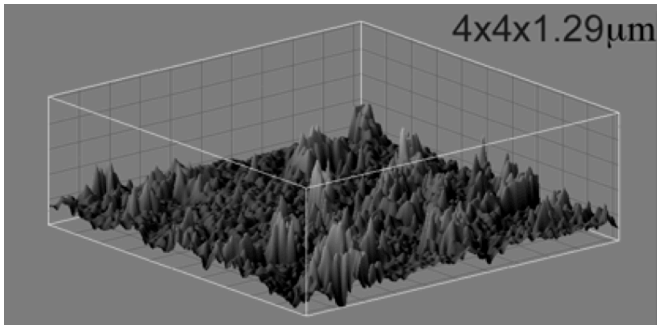
OE och Edla Johanssons stiftelse

PO Zetterlings stiftelse

The functional organisation of the plasma membrane

Ingela Parmryd

The plasma membrane of eukaryotic cells contains ordered nanodomains, commonly referred to as lipid rafts, which are more ordered than the rest of the plasma membrane. The high order has been suggested to be caused by the tight packing of cholesterol and sphingolipids as observed in model membranes. However, we have recently demonstrated that lipid rafts form when actin filaments are attached to the plasma membrane via phosphoinositides (Dinic et al., 2013), suggesting that the mechanism for lipid raft formation is lipid-protein interactions. We have shown that T cell signalling is initiated upon lipid raft aggregation that can be triggered by cold stress and changes in plasma membrane cholesterol content. We have recently shown that the T cell receptor in resting T cells resides in lipid rafts that are brought together upon receptor engagement (Dinic et al., 2015). We are investigating what is triggering the formation of ordered plasma membrane domains and to do so we have carefully characterised two environmentally sensitive probes that can determine the proportion of ordered lipid domains in the membrane. Focus areas are the individual order of the two plasma membrane leaflets and the role of phosphatidylinositol (4,5)-bisphosphate and actin dynamics in the formation of lipid rafts.



High resolution hopping ion conductance microscopy image of part of a live FRSK cell. The figure shows that cell topography is an important factor when determining the diffusion coefficients of membrane molecules.

The cell surface is neither flat nor smooth but surface topography is ignored in current models of the plasma membrane. Using high resolution topographical maps of live cells, we and our collaborators have demonstrated that apparent topographical trapping is easily mistaken for elaborate membrane model features like hop diffusion and transient anchorage. Even binding could be the result of apparent topographical trapping when single particle tracks are interpreted in 2D although the molecules are moving in 3D.

We develop image analysis software to get quantitative and objective answers to our questions. We have developed a method where image noise, which is unavoidable and leads to the underestimation of the underlying correlation, can be eliminated from the correlation measurement. We have performed detailed studies on coefficients currently used in colocalisation analyses revealing that several are not fit for their purpose. We advocate that coloocalisation analysis should be divided into the two subgroups co-occurrence and correlation (Adler & Parmryd, 2013) and that only pixels where both fluorophores are present should be included in correlation analyses (Adlet & Parmryd, 2014).

γ9δ2 is a T cell subset that is activated by phosphoantigens, small organic compounds with phosphate groups. Together with collaborators we have found that media from erythrocytes infected with *P. falciparum* can stimulate γ9δ2 T cell prolifieration (Lindberg et al., 2013) suggesting that phosphoantigens are produced in these cells. We will now address at which parasite stage this production occurs and what metabolic pathway is responsible for the production.

Members of the group

Ingela Parmryd, associate professor

Jeremy Adler, research engineer

Warunika Aluthgedara, project assistant

Love Chrisson, undergraduate student

Parham Ashrafzadeh, graduate student

Chenxiao Liu, graduate student

Jan Saras, research engineer

Lijun Zhao, laboratory assistant

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Licentiate thesis

Parham Ashrafzadeh: The role of actin filaments and phosphatidylinositol (4,5) bisphosphate in the formation of ordered plasma membrane domains. April 2015.

Agencies that support the work

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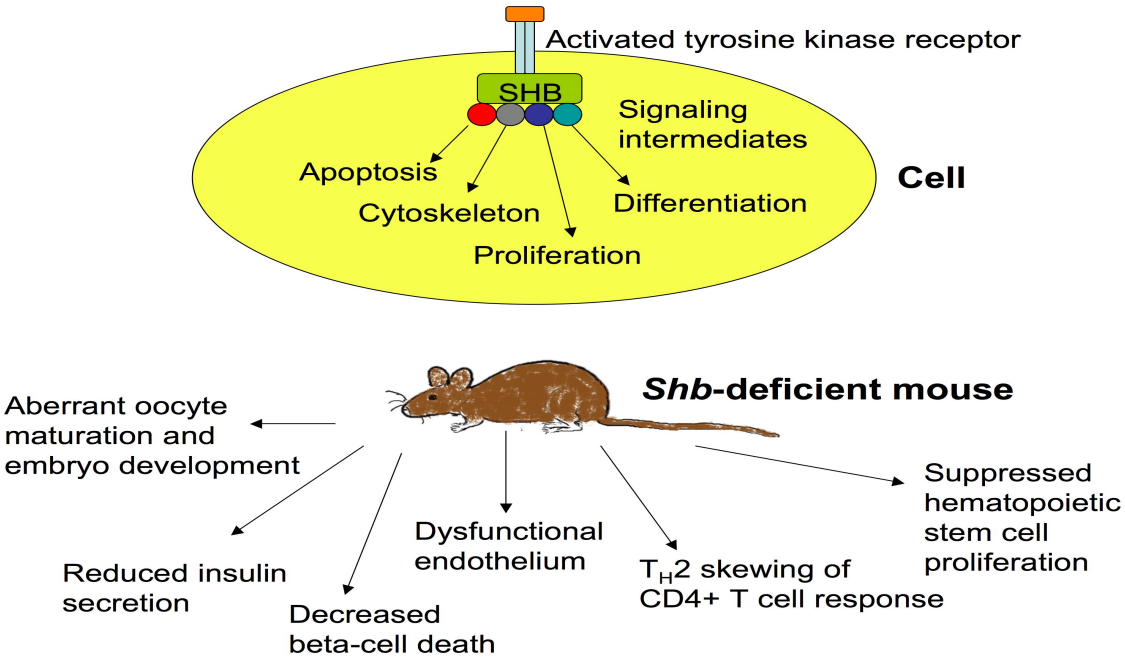
Importance of Shb-dependent signaling for angiogenesis, and hematopoiesis

Michael Welsh

Shb is an SH2-domain adapter protein operating downstream of tyrosine kinase receptors such as VEGFR-2, FGFR-1, PDGF-receptors and the T cell receptor. The effects of Shb are pleiotropic and context dependent. We have recently generated a *Shb*-knockout mouse to assess the physiological relevance of Shb in vivo.



We observe impaired glucose homeostasis due to insufficient insulin secretion in Shb-deficient mice. In addition, the β-cells exhibit reduced stress sensitivity. These effects appear to be a consequence of constitutive FAK activation..

Shb-knockout mice display reproductive abnormalities with a transmission ratio distortion of the knockout allele related to female reproduction. Consequently, oocyte maturation is impaired in the absence of Shb and this relates to abnormal signaling via the ERK-RSK-S6 pathway. In addition to aberrant oocyte maturation, *Shb*-knockout embryos are morphologically abnormal and do not implant well.

Shb-knockout mice also display reduced angiogenesis and this causes diminished tumor expansion (subcutaneously injected tumor cells or inheritable RIP-Tag insulinomas). *Shb* deficient endothelial cells have abnormal cytoskeleton and adherens junctions that may contribute to deficient angiogenesis. In addition, *Shb*-knockout vascular physiology shows signs of compensatory mechanisms (increased blood flow velocity and an increased frequency of intermediately sized arterioles as determined by micro-CT) to counteract the adverse effects

of the endothelial dysfunction. Although vascular performance under normal conditions appears relatively unaffected by the absence of *Shb*, recovery after ischemia was found to be impaired in both the cremaster and hindlimb muscles, which was primarily dependent on *Shb* deficiency in the vasculature and not in myeloid cells. Multiple signalling abnormalities in *Shb* knockout endothelial cells were noted, included elevated basal and reduced VEGF-stimulated FAK, ERK, Akt and Rac1 activities. An important aspect that has not yet been determined is whether tumor metastasis is affected or not by the absence of *Shb* and this will be studied.

The absence of Shb exerts effects on hematopoiesis and peripheral T lymphocyte function. CD4+ T lymphocytes show a Th2 skewing of their response to stimulation in the absence of Shb and this could be of relevance for understanding allergic responses. *Shb* knockout hematopoietic stem cells show lower rates of proliferation due to elevated FAK signalling. Development of BCR-ABL1-induced leukemia was accelerated in the absence of Shb, again due to elevated FAK activity. Further studies will be conducted in order obtain a better understanding of Shb in leukemia.

Our current research effort is mainly focussed on investigating:

A) The relevance of vascular dysfunction as a consequence of Shb deficiency for tumor metastasis

B) The development of leukemia in relation to Shb deficiency

Members of the group

Michael Welsh - Professor

Björn Åkerblom-Post-Doc

Maria Jamalpour – PhD-student

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Agencies that support the work

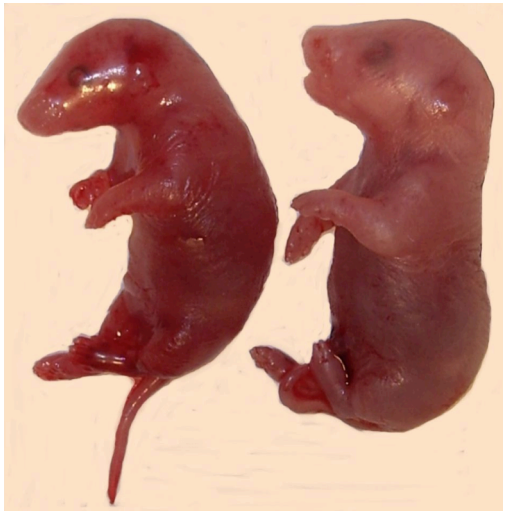
The Swedish Cancer Foundation

Stiftelsen Familjen Ernfors fond

Complications in pregnancy

Ulf Eriksson, Parri Wentzel

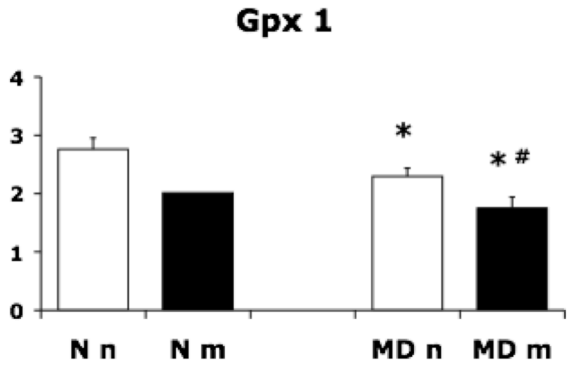
We are studying different types of pregnancy complications, resulting in disturbed embryo-fetal development as a consequence of altered maternal metabolism (caused by diabetes, obesity, or ethanol intake). Our short-term aims are to clarify and understand the mechanisms and patterns of dysmorphogenesis; the long-term aim is to prevent the maternal and fetal damage. We work with animal models *in vivo*, and *in vitro* culture of whole embryos, embryonic tissues and embryonic cells.



Fetuses with facial malformation (left) and normal morphology (right), from diabetic rats.

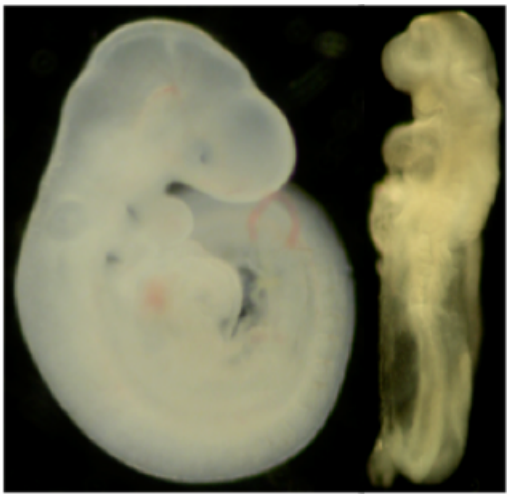
Diabetes in the pregnant women is associated with an increased risk for malformations in the offspring and preeclampsia in the mother. We have studied the mechanisms behind the disturbed development of the offspring in animal models, embryo culture, as well as by *in vitro* culture of embryonic tissues and cells. In earlier work, we reported the occurrence of oxidative stress in embryos exposed to a diabetic environment. We have been able to block the diabetes-induced damage to the embryo and fetus by several agents, such as arachidonic acid, inositol, N-acetylcysteine, BHT, vitamin E and C, and folic acid. We have also started to investigate the importance of genetic predisposition for the development of malformations, a project, which is currently yielding data regarding the importance of the maternal and fetal genomes and epigenomes for the development of fetal dysmorphogenesis in diabetic pregnancy.

We have identified one gene, Glutathione Peroxidase-1, which is underexpressed in malformed offspring of diabetic rats (compared with non-malformed offspring of same litter), and its gene product, the antioxidative enzyme Gpx-1, is less distributed in the embryonic tissues, and its enzymatic activity markedly decreased. These findings can be related to the enhanced oxidative stress involved in the embryo-fetal dysmorphogenesis of diabetic pregnancy.

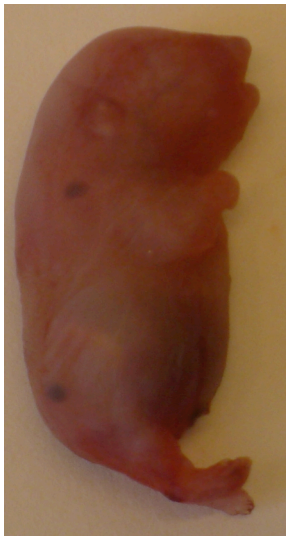


Decreased expression of Gpx-1 in malformed compared to non-malformed offspring of diabetic rats.

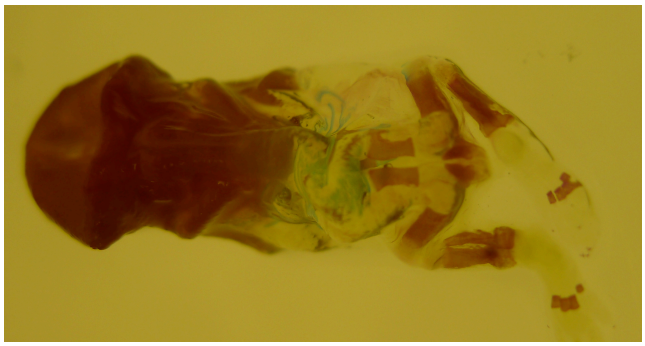
Recently we have found evidence for a new teratological pathway in diabetic pregnancy, activation of the receptor for advanced glycation end products (RAGE). We will persue this line of research by identifying the ligand(s) causing the RAGE activation, and by investigating the possible therapeutical effects of blocking the RAGE response in embryos exposed to a diabetic environment.



Obesity in the pregnant woman is associated with increased risk for congenital malformations, in particular the risk for neural tube defects and cardiac malformations been found to be increased. We are currently involved in creating an animal model for this type of pregnancy, as well as attempting to affect embryonic development *in vitro* by subjecting the embryos from control rats to serum from either control or high-fat diet rats in whole embryo culture for 48 hours. We found increased incidence of growth retardation and malformations in the embryos cultured in serum from hig-fat diet rats.



Rat fetus lacking tail, from obese mother



Intake of ethanol during pregnancy can harm the offspring; the risk increases with increased consumption. We have studied this situation, and attempted to alter the maternal defense against free oxygen radicals *in vivo* and *in vitro*, in order to diminish the ethanol-induced damage. We are studying possible biomarkers for maternal ethanol intake, by investigating embryonic tissues exposed to ethanol.

We are currently conducting a collaborative study on the dietary habits during pregnancy of women who have given birth to a child with Attention-Deficit/Hyperactivity Disorder.

Members of the group

Ulf Eriksson, professor

Parri Wentzel, associate professor

Andreas Ejdesjö, postdoc

Collaborators

Peter Nawroth, professor

Heidelberg, Germany

Emilio Herrera, professor

Madrid, Spain

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3. Post-implantation Diabetic Embyopathy. Eriksson UJ & Wentzel P, Chapter 41. Textbook of Diabetes and Pregnancy, Informa Healthcare, 2015

Agencies that support the work

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Stiftelsen Familjen Ernfors fond

Pathogenesis of type 1 Diabetes Mellitus

Stellan Sandler

The prevailing view is that an autoimmune reaction selectively destroys the insulin-producing β-cells in the pancreas in type 1 diabetes (T1DM). The aim of this project is to investigate cellular and molecular mechanisms involved in pancreatic β-cell damage and repair in this disease. We postulate that after certain types of damage β-cell function can be restored (Fig. 1). Furthermore, we believe that the β-cell is not a passive victim during a situation of potentially harmful exposure, but depending on gene expression and functional activity of the β-cell, the outcome can be affected. The aims of the present research projects are to investigate cellular and molecular mechanisms involved in pancreatic β-cell damage and repair in T1DM.

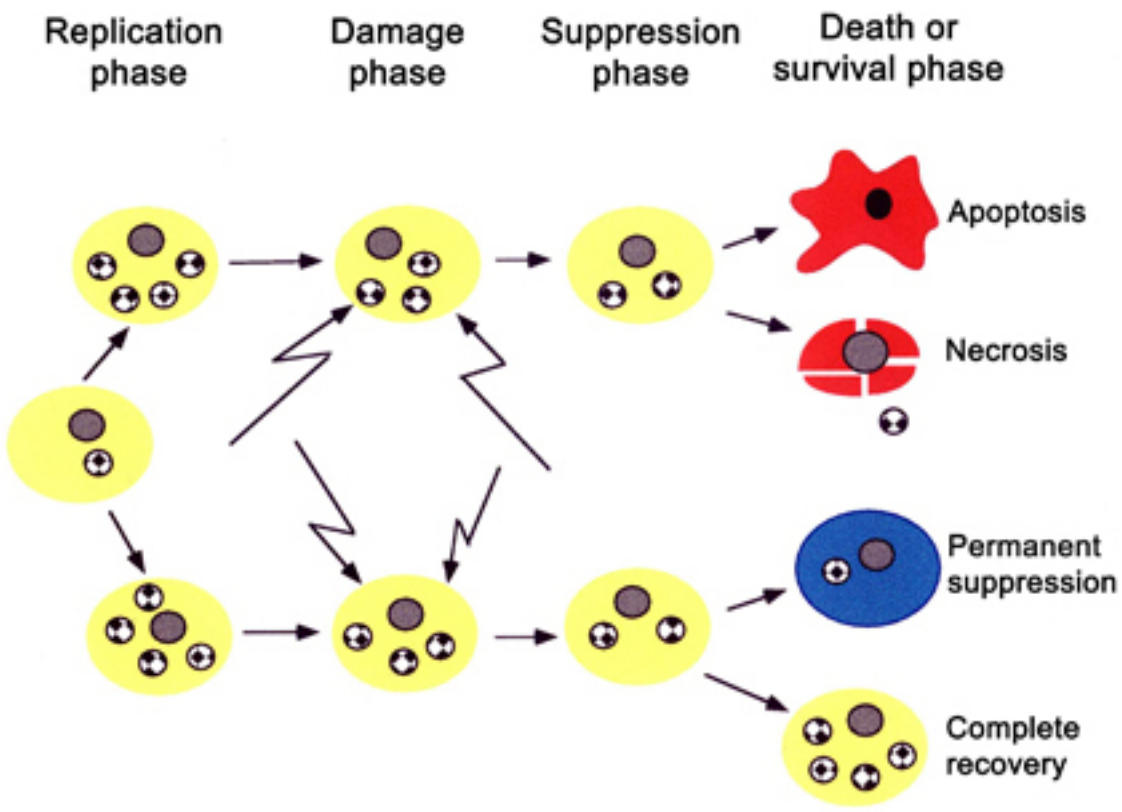

Fig. 1. Schematic view of the β-cell outcome following different immunologic or toxic assaults. In fetal and neonatal life, β-cell replication is increased, but later it becomes restricted. After birth β-cell acquire the full capacity to synthesise and release insulin (speckled symbols) upon appropriate stimuli. At one or several occasions in life, β-cells in some individuals are subject to damage (irregular arrows) which will lead to suppressed β-cell function and a reduction in insulin secretion. Depending on the genetic predisposition an autoimmune reaction will be launched which in certain individuals will cause extensive cell death leading to type 1 diabetes. In other individuals β-cells will survive, but their secretory function is impaired, which may have consequences for the glucose homeostasis. In some other individuals the β-cells may completely recover and the glucose tolerance will only be transiently disturbed. The latter outcome is most likely also dependent on genes regulating β-cell resistance to damage and β-cell repair.

Topics that are currently being investigated

A. Characterization of the regulatory T cell response in diabetic mice

B. Evaluation of cytokine traps in experimental diaetes

C. Mitochondrial targeted preconditioning, using K_{ATP}-channel openers (KCO), to rescue β-cells against acute destruction

D. Exploration of the bank vole as an animal model for human diabetes

E. Antiviral intervention in NOD mice

Example of findings and hypopthesis

Role of regulatory T-cells (T_{reg}) in T1DM (cf. topic A)

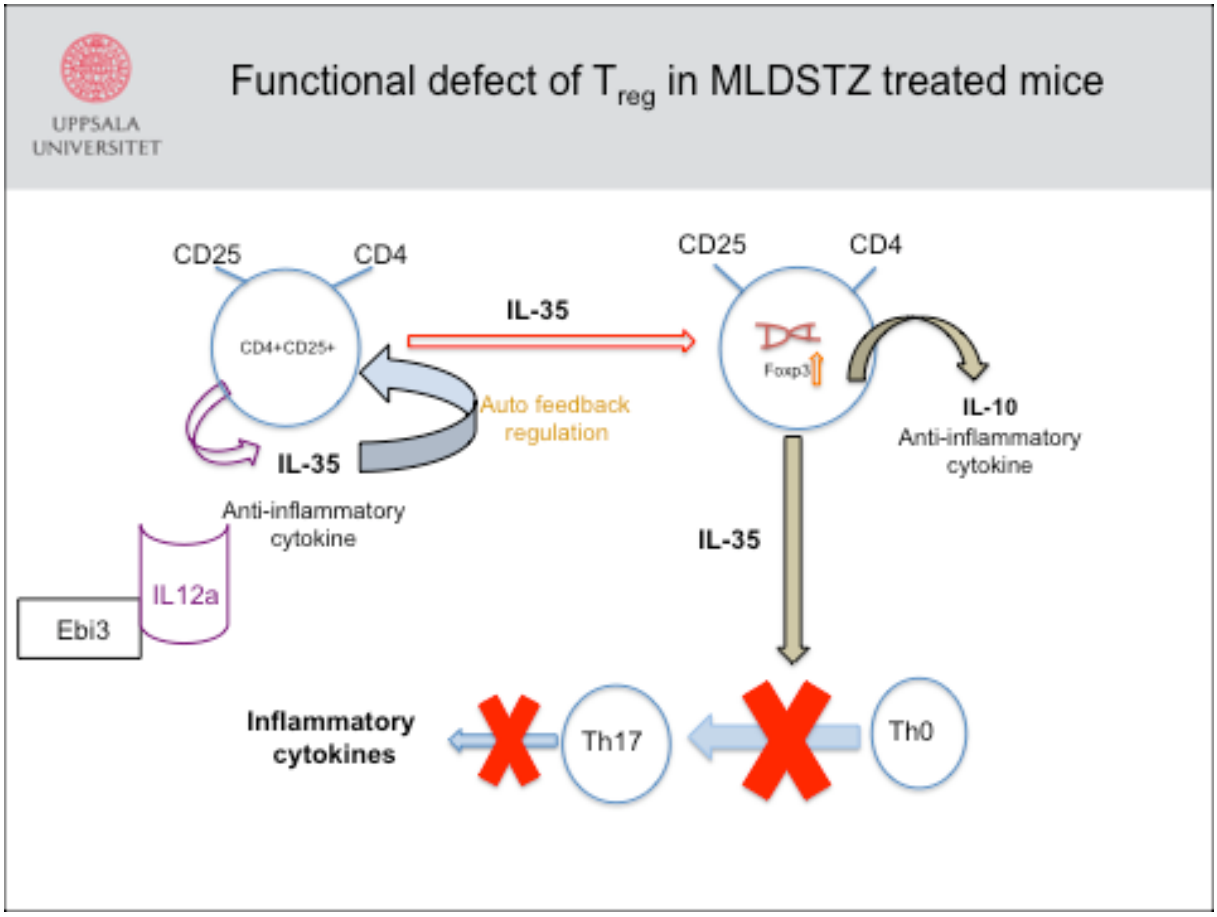

Fig. 2. Based of a number of different experiments we propse that Tregs in the multiple low dose streptozotocin (MLDSTZ) model of T1DM are functionally impaired, since a key cytokine (IL-35) is not being up-regulated in response to the proinflammatory environment induced by MLDSTZ.

Mechanism of mitochondrial K_{ATP} channel opening and β-cell protection, (cf topic B)

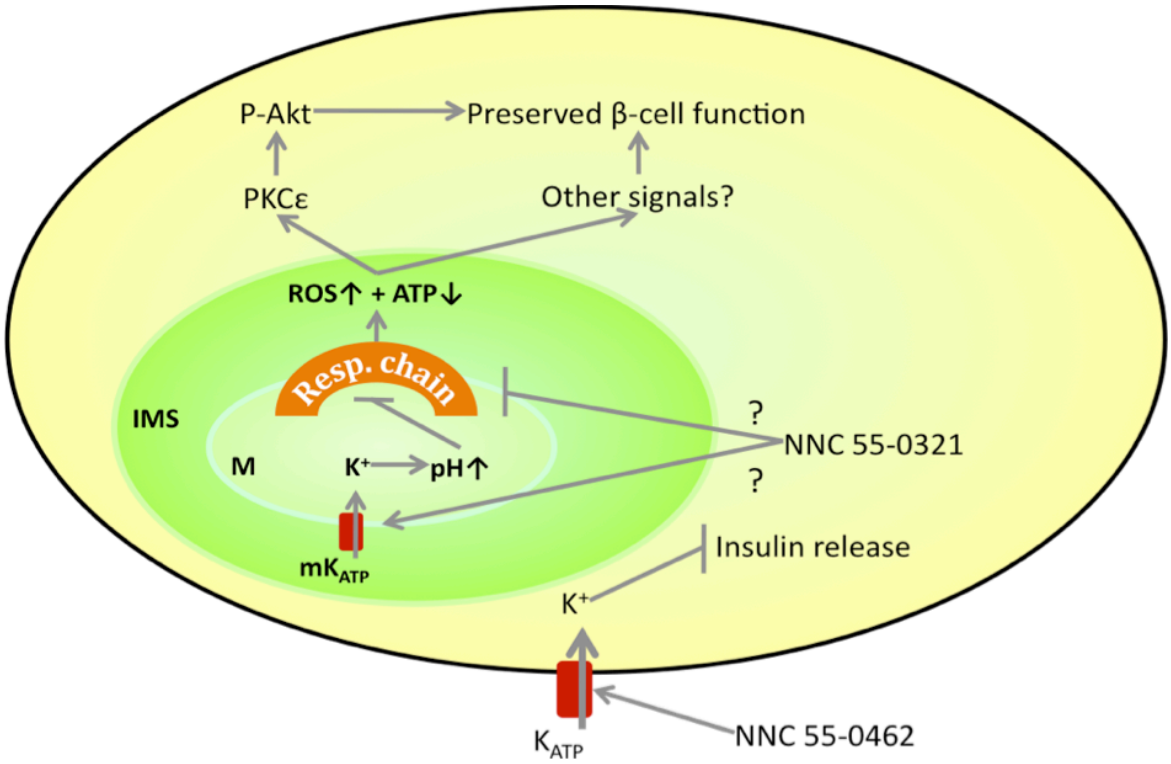

Fig. 3. NNC 55-0321 acutely down-regulates mitochondrial function. A lowered respiratory chain activity is accompanied by increased ROS production, PKCe activation and phosphorylation of the survival promoting kinase Akt. Inhibition of mitochondrial function by NNC 55-0321 may be caused by opening of a mitochondrial potassium channel (mK_{ATP}), which promotes K^+ entry from the intermembrane space (IMS) into the mitochondrial matrix (M), thereby increasing pH and inhibiting the respiratory chain (I). Alternatively, NNC 55-0321 can directly inhibit mitochondrial respiration independently of the presence of and conductance in an mK_{ATP} (II). NNC 55-0462 primarily acts on the plasma membrane bound K_{ATP} channel and causes inhibition of insulin secretion by preventing depolarization of the plasma membrane, but this does not provide protection against b-cell damage (cf aim C above).

Pancreatic islet in a diabetic bank vole

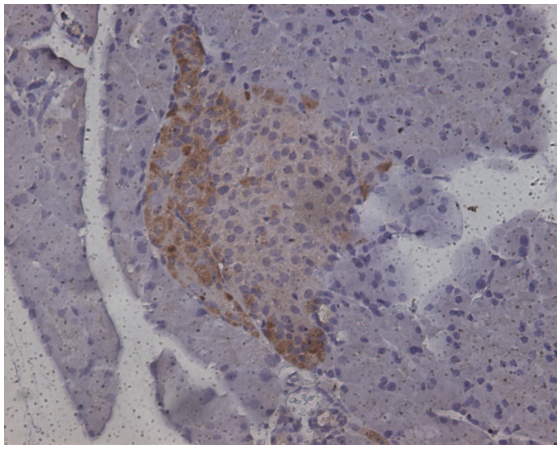

Fig. 4. Pancreatic islet of a female colonized bank vole 18 weeks of age. The bank vole was hyperglycemic (17.4 mM) 120 min after the IPGTT and serum insulin was elevated (2.34 mg/ml). The section was IHC stained with an Ljungan virus-specific antibody (brown colour) showing strong staining in some areas and weaker staining in the remaining area of the islet. Magnification 400X (cf Aim D above).

Significance

The aims of the present research projects are to investigate cellular and molecular mechanisms related to pancreatic β-cell damage and repair in T1DM, and in some cases probably also in T2DM. It is anticipated that a deeper knowledge of these issues will lead to new strategies for intervention in the autoimmune β-cell destructive processes, as well as novel methods to enhance β-cell resistance against direct cytotoxic damage. We hope that by studying cell signaling and the mechanisms leading to β-cell death, it will be able possible to elucidate which factors that are crucial for β-cell survival and possibly indentify candidate genes/proteins conferring β-cell susceptibility or resistance to destruction in T1DM.

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Members of the research group

The following colleagues are engaged in the projects described above:

PhD Martin Blixt (guest lecturer, part-time research)

PhD Tobias Rydgren (post-doc stipend)

PhD (Lina Thorvalson (part time post-doc)

Laoratory technician IngBritt Hallgen (part-time)

Adjunct Prof Bo Niklasson

PhD Student Kailash Singh

PhD student Gutaf Arbrant

Professor Stellan Sandler

Agencies that have supported the work

The Swedish Research Council

The European Foundation for the Study of Diabetes

The Swedish Diabetes Association

Barndiabetesfonden

Stiftelsen Familjen Ernfors fond

Role of tyrosine kinases in β-cell apoptosis and diabetes

Nils Welsh

It has recently been observed that patients suffering from both leukemia and diabetes were cured from not only leukemia, but also diabetes, when treated with the tyrosine kinase inhibitor Imatinib. (Veneri et al., N Engl J Med. 2005 352:1049-1050). An anti-diabetic action of Imatinib in Type 2 diabetes is further supported by our recent observation that Imatinib counteracts high-fat diet induced insulin resistance and hyperglycemia in rats (Hägerkvist et al., Clinical Science, (Lond). 2008 114(1):65-71). Moreover, in a study from 2009, Imatinib was also observed to induce remission of diabetes in db/db mice, possibly via decreasing insulin resistance and increasing the beta-cell mass (Han et al., Diabetes. 2009 58(2):329-3). Thus, in both animal models and in Type 2 diabetes patients Imatinib seems to improve glycemic control, possibly via an insulin sensitizing effect.

Imatinib appears to prevent and reverse not only Type 2 diabetes, but also diabetes of animal models with a Type 1 diabetes resembling disease. We have shown that Imatinib protects against beta-cell death in vitro and prevents diabetes in NOD mice and in streptozotocin-diabetic mice, both models for human beta-cell destruction and Type 1 diabetes (Hagerkvist et al., FASEB J. 2007 Feb;21(2):618-28, Hagerkvist et al., Cell Biol Int. 2006 30(12):1013-7). More recently, it has been observed by others that both Imatinib and Sunitinib not only prevented, but also reversed new-onset diabetes in NOD mice (Louvet et al., Proc Natl Acad Sci U S A. 2008 105(48):18895-900). Thus, there exists proof-of-principle in animal models for an anti-diabetic effect of Imatinib and similar tyrosine kinase inhibitors, and that a limited treatment period will not only reverse diabetes, but also mediate long-term protection against re-precipitation of the disease. This has led us (Mokhtari and Welsh, Clin Sci (Lond). 2009 118(4):241-7) and other investigators to propose clinical trials in which Imatinib is given to new-onset Type 1 diabetes patients.

The work by others and us indicates that Imatinib counteracts diabetes via different molecular mechanisms (Figure 1).

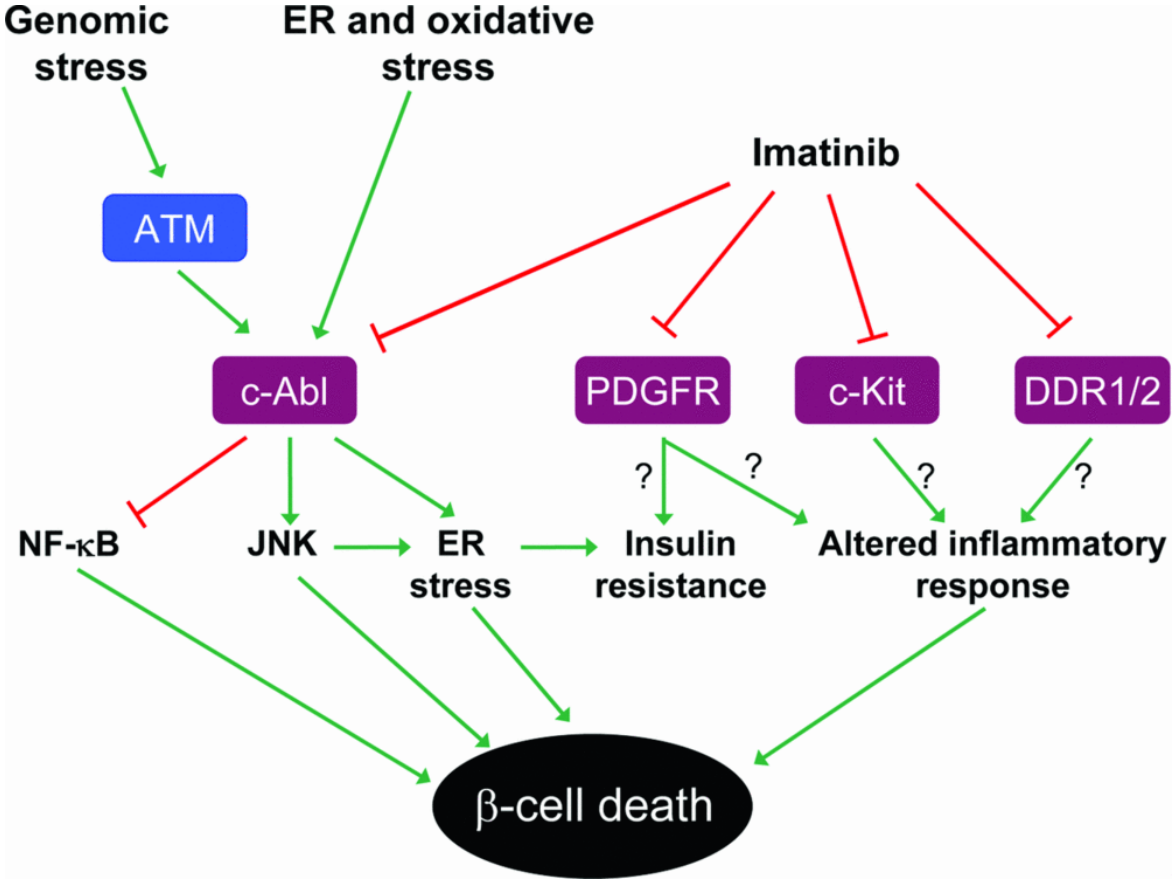

Figure 1 Possible mechanisms for the anti-diabetic effects of imatinib
Imatinib is known to inhibit the tyrosine kinases c-Abl, PDGFR, c-Kit and DDR1/2. Most likely, imatinib-induced protection against diabetes is mediated not by one single pathway, but via different molecular mechanisms. β-Cell survival is promoted by inhibition of c-Abl, which leads to decreased activation of the pro-apoptotic MAPK JNK and increased activation of the anti-apoptotic transcription factor NF-κB. c-Abl inhibition might also lead to a dampened ER-stress response, via JNK or other pathways. Inhibition of PDGFR could contribute to decreasing peripheral insulin resistance and inflammatory processes, thereby promoting β-cell survival. Moreover, inhibition of c-Kit and DDR1/2 might also add to the anti-diabetic effects of imatinib, possibly by interfering with inflammatory responses.

It appears that the four known targets of Imatinib, c-Abl, PDGFR, c-Kit and DDR1/2, may all play a role in the pathogenesis of diabetes. C-Abl is a proapoptotic tyrosine kinase that promotes beta-cell death when activated. Improper activation of the PDGF receptor has also been reported to occur in diabetes, and this may lead to increased insulin resistance of peripheral tissues. Activation of c-Kit and DDR1/2 is known to affect innate immunity, a component of the immune system that promotes inflammation and beta-cell dysfunction. Thus, it is conceivable that Imatinib, by targeting several pathways simultaneously, mediates a stronger antidiabetic effect than other drugs that affect only one particular pathway.

It is the aim of this project to elucidate closer the mechanisms by which tyrosine kinases control beta-cell death and function. We are currently investigating Imatinib-mediated control of NF-kappaB, JNK, p38, PI3-kinase, SHIP2, PTEN, FAK, IRS1/2, beta-catenin, AKT and ERK signaling events. For this purpose insulin producing cells, either at basal conditions or under stress, are analyzed by immunoprecipitation, immunoblotting, confocal microscopy, real-time PCR, microarray analysis, flow cytometry and gel shift analysis. Cells are also genetically manipulated by lentiviral vectors to achieve up-or down-regulation of specific gene products.

Signaling events will be correlated to beta-cell survival and function, as assessed by analysis of insulin production and apoptotic events. This will hopefully lead to a better understanding of the molecular events by which Imatinib protects against diabetes. Such improved knowledge may pave the way for a novel and improved treatment of diabetes.

Members of the group

Camilla Kappe - Post-doc

Kyril Turpaev - Post-doc

Xuan Wang – Post-doc

Chris Hirt – Project student

Andris Elksnis – Project student

Hamid Gavali – Project student

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Wang, X. (2014). Study of the Proliferation, Function and Death of Insulin-Producing Beta-Cells *in vitro*: Role of the Transcription Factor ZBED6. Diss. Uppsala Universitet.

Licentiate dissertation:

Anvari, E. (2014). Histamine H1-receptor-induced signalling in pancreatic beta cells.

Agencies that support the work

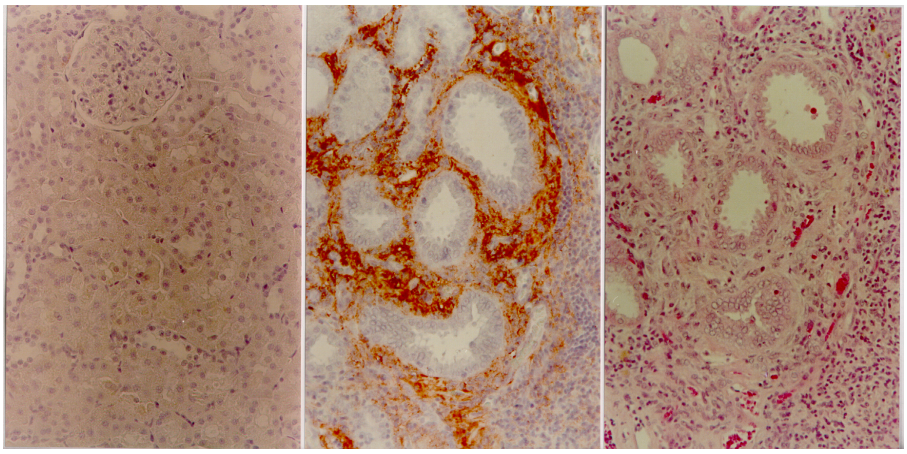
The Swedish Diabetes Association

Stiftelsen Familjen Ernfors Fond

Diabetes Wellness

Intrarenal Hyaluronan in the Regulation of Fluid Balance. Pathophysiological Relevance to Renal Damage during Diabetes and Ischemia-Reperfusion.

Peter Hansell



Histochemical staining for HA demonstrating the absence of HA in the normal renal cortex of rats (left). Patchy accumulation of interstitial HA in the ischemia-reperfusion damaged renal cortex (middle). Accumulated HA is found mainly in the same areas as infltrating immune competent cells, as seen by parallel staining with haematoxylin-eosin (right).

The kidney is a main determinant of fluid/electrolyte balance and of mean arterial blood pressure. Hypertension is often caused by a renal inability to regulate fluid balance. The present research focuses on a matrix component (hyaluronan, HA) with extreme water attracting properties in the regulation of fluid balance. The proinflammatory property of HA is also evaluated in pathophysiological models. In contrast to the renal cortex which is almost void of HA, the interstitium of the renal medulla contains high amounts of HA during normal physiological conditions which changes depending on the body hydration status and, more severely, during pathological conditions.

We have found that HA has an important dynamic role in normal renal water-handling (hydration/dehydration) and that the intrarenal distribution of HA is severely altered during diabetes and after ischemia-reperfusion injury which correlates to renal dysfunction and inflammation. We have also demonstrated that the normal intrarenal distribution of HA is severely altered if angiotensin II tonus is diminished neonatally (during nephrogenesis) in the rat which correlates to renal dysfunction and inflammation. We aim to: a) determine the physiological relevance of the glycosaminoglycan hyaluronan (HA) in the regulation of renal fluid/electrolyte balance; b) determine the pathophysiological relevance of HA in the renal dysfunction during diabetes (diabetic nephropathy) and after ischemia-reperfusion injury; c) determine if hyaluronidase-treatment and siRNA improves renal function during diabetic nephropathy and following renal ischemia-reperfusion; d) elucidate the time frame and mechanisms in the development of the intrarenal heterogenous distribution of HA which occur neonatally in the rat and its angiotensin II dependency.

Both in vivo and in vitro experiments are performed. Diabetes, ischemia, hydration, dehydration, hormones, pharmacological and biomolecular intervention activate/deactivate the systems. Human renal tissue from resections is also analysed. Rats and genetically modified mice are used during in vivo conditions whereafter the renal tissue is analysed using molecular biology to follow HA (amount, size), HA synthases, hyaluronidases and CD44 expression. Renomedullary interstitial cells in culture are used in parallel to follow similar parameters during interventions. In cooperation with the section of diagnostic radiology (assoc prof Per

Liss) the mechanisms underlying diabetic nephropathy is to be validated and the increased sensitivity of the diabetic kidney to radiological contrast agents is elucidated. Cardiovascular disease is a dominant cause for invalidity and mortality. The results of the present projects may give rise to basic understanding of, and new treatment modalities in, fluid balance disorders and cardiovascular diseases.

Members of the group

Peter Hansell – Professor

Angelica Fasching - Laboratory Engineer

Fredrik Palm – Professor

Per Liss – Assoc Professor

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Agencies that support the work

The Swedish Research Council

Renal Physiology

A. Erik Persson

The renal control of excretion is essential for fluid balance and blood pressure. One factor of great importance in regulation of fluid excretion is the tubuloglomerular feedback (TGF) control mechanism in the juxtaglomerular apparatus (Fig1). The macula densa cells in the distal part of the nephron senses the fluid flow rate. This information is used to activate the extraglomerular mesangial cells that modulate the response via influences from both hormones and fluid volume balance factors. Activation of the TGF mechanism finally leads to a contraction of the afferent arteriole. Renal renin release from the granular cells of the juxtaglomerular apparatus is controlled via the same mechanism.

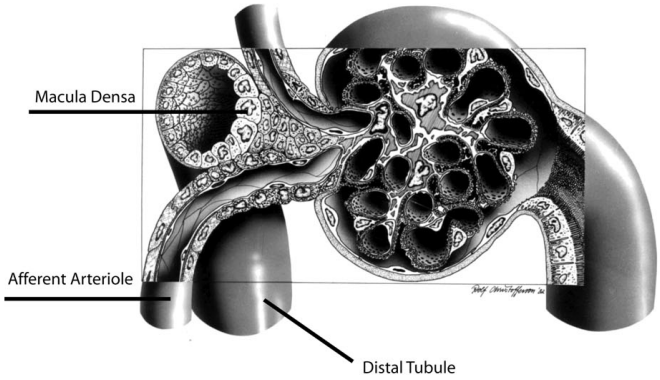

Figure 1. Schematic illustration of the juxtaglomerular apparatus (JGA) with the macula densa cells in wall of the distal tubule and the glomerular arterioles.

Our group studies how hormones and other factors, e.g. nerves and NO, influence the overall function of the TGF mechanism and renin release using micropuncture techniques. We also employ isolated perfused tubule and arteriole techniques using fluorophores and digital imaging methods to determine calcium, chloride and NO in the macula densa cells and in the arteriolar smooth muscle cells. NO is also measured via microelectrodes. These techniques are used to investigate the sensing step in the TGF, the modulation step in the mesangial cells and the calcium release and contractile response of the arterioles. The juxtamedullary nephron preparation is used to visualise afferent arteriolar endothelial cells to measure calcium and NO. Our studies aim at understanding how the TGF mechanism and renin release operates, the effect of renal oxidative stress, NO and nerves on kidney function and to find the mechanism responsible for development of arterial hypertension.

Arterial hypertension is one of the most important health problems in the Western world and an important risk factor for cardio-vascular disease (CVD) and stroke. Unfortunately, these risk

factors are only partly reduced during treatment with the existing drugs. Patients with treatment for hypertension have a reduced risk for stroke of about 50 % but still a 5 times higher risk than those without hypertension. The risk for CVD is only reduced 15 % with treatment and there is a 6-7 times higher risk for CVD compared to individuals without increase in blood pressure. Therefore it is important to further investigate how hypertension develops and find new and effective principles to prevent and treat the disease. Reduction of renal oxidative stress may increase nitric oxide (NO) bioavailability and thereby play an important role in preventing and/or treating CVD. To investigate the potential roles of oxidative stress and NO-deficiency in the development of CVD. Treatment modalities that reduce oxidative stress and/or increase NO-bioavailability will be assessed in both experimental models and clinical trials in order to find new and more efficient ways to treat or prevent CVD.

We have advanced equipment for investigating renal and cardiovascular function, and imaging systems for measuring oxidative stress and NO production (in vivo and in vitro). In collaborations with physicians at different hospitals we have clinical trials to investigate the potential role of oxidative stress and NO-deficiency in CVD. In our experimental and clinical studies we aim to further investigate the link between renal and cardiovascular dysfunction, and to explore the potential benefits from reducing oxidative stress (e.g. antioxidant, nitroxide, low-sodium treatment) or increasing NO production (e.g. L-arginine or nitrate supplementation). The juxtaglomerular apparatus is a critical regulator of glomerular filtration rate, fluid excretion and renin release, factors that determine blood pressure. We believe that treatment strategies aiming to reduce oxidative stress and/or increase NO-bioavailability could be of great value in the future to treat hypertension to prevent stroke and cardio-vascular disease.

Members of the group

A. Erik Persson - Professor emeritus

Mattias Carlström - Researcher

Andreas Patzak - Guest researcher

Suênia Sampaio-Guest resercher

Gau Xian – Post doc

Ammar Farman - Graduate student

Peter Flacker- Graduate student

Zheng Bing Zhuge-Graduate student

Publications 2012-

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Dissertation examination

Xiang Gao: ”Local purinergic control of arteriolear reactivity in pancreatic islets and renal glomeruli” October 2014

Agencies that support the work

The Swedish Research Council

STINT

Gastro-intestinal protection mechanisms studied in vivo

Lena Holm

During homeostasis, the colonic mucus successfully separates the vast luminal microbiota from the single epithelial cell layer and resident immune cells of the mucosa. When this barrier fails, colitis is established. Our research focuses on **the interplay between the commensal microbiota, administered probiotics and the colonic mucosal barrier in health and during colitis**, with special emphasis on the underlying mechanisms of colitis induction and probiotic protection. We have developed an animal model allowing direct access to the colonic mucosa with **intravital microscopy**, and the majority of our experiments include *in vivo* studies of the mucus layers (Fig. 1), epithelium, immune cells and

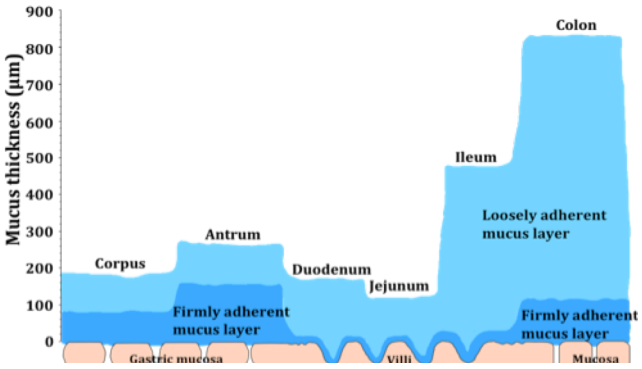
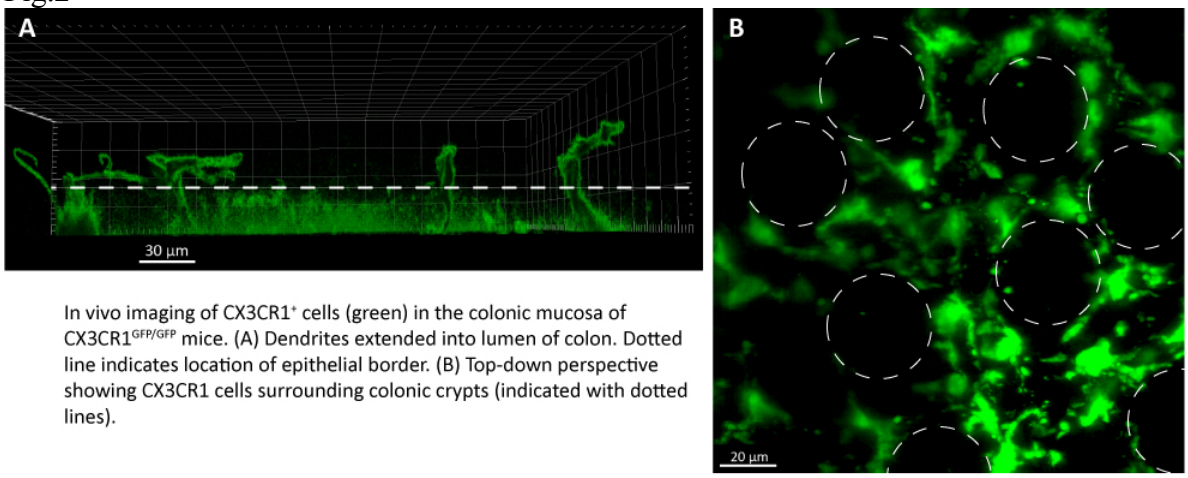


Fig 1. The mucus layers in different parts of the GI tract

blood flow. During last year we have extensively increased our possibilities to perform high-resolution longitudinal *in vivo* studies of interactions of the microbiota/probiotics/mucus with the epithelium and immune cells in real-time by adapting our *in vivo* model to high-speed confocal microscopy available in our lab (Fig.2). Furthermore, with a newly installed Laser Speckle Contrast Analysis setup, blood flow of colitic and healthy parts of the colon will be performed.

Fig.2



In vivo imaging of CX3CR1^{+} cells (green) in the colonic mucosa of CX3CR1$^{GFP/GFP}$ mice. (A) Dendrites extended into lumen of colon. Dotted line indicates location of epithelial border. (B) Top-down perspective showing CX3CR1 cells surrounding colonic crypts (indicated with dotted lines).

The influence of pre- and pro-biotics on mucus dynamics, bacterial composition, inflammatory variables and epithelial tight junctions are studied to elucidate the mechanisms behind their protective effects (Fig. 3). We have shown that pretreatment with **probiotics** (*L. reuteri*) prevent DSS-induced colitis in rats and mice. Up regulation of P-selectin in the colonic venules was prevented by probiotic therapy, and in vivo fluorescence microscopy confirmed these results by showing decreased leukocyte rolling and adhesion to endothelial cells, as well as decreased platelet-endothelial cell interactions. There are no intestinal *in vitro* culture systems that replicate the complexity of the secreted mucus barrier. However, our *in vivo* model uniquely enables reliable measurements of thickness and permeability of the mucus barrier. Using this model we have demonstrated that the adherent gastric and colonic mucus gel in vivo can be divided in two layers, a firmly and a loosely adherent layer (Fig 1). The firmly adherent mucus layer acts as a barrier towards hydrochloric acid in the stomach and luminal bacteria in the colon. In addition to the barrier function of the firm mucus resulting in significantly lower number of bacteria than in the loosely adherent mucus (1/10), we found that the composition of the microbiota differed substantially between the two layers. The difference in bacterial numbers and composition was completely eradicated in DSS-induced colitic rats, where high levels of

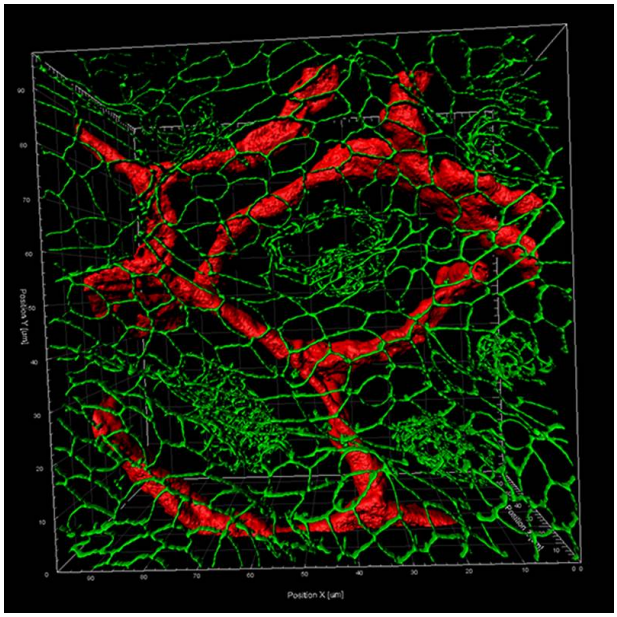


Fig 3. 3D-visualization of capillaries (stained with CD31: red) surrounding colonic crypts just below the luminal epithelial layer (tight junctions stained with ZO-1: green).

translocated bacteria were found in the mesenteric lymph nodes. Interestingly, pretreatment with *L. reuteri* (cocktail of 4 strains) prevented bacterial translocation and colonic inflammation but did not influence on the distorted mucus microbiota.

We have shown that dietary nitrate induces potent protection against NSAID induced upper GI inflammation. Bacteria in the oral cavity reduce nitrate to nitrite, which is further reduced to nitric oxide, NO, in the acidic stomach. NO strengthen the mucosal barrier by increasing mucus thickness and blood flow. We have, however, also shown protection by dietary nitrate even further down in the intestine where luminal NO is not increased. Leukocyte recruitment in response to proinflammatory chemokine and NSAID was decreased. Despite attenuation of the acute immune response, the overall ability to clear a bacterial infection was not suppressed.

Members of the group

Lena Holm, professor

David Ahl, PhD student

Jossan Simran, master student

Annika Jägare, laboratory engineer

Shokoufeh Karimi, PhD student*

Haoyu Liu, PhD, post doc

Richard Shore, PhD student**

Tomas Waldén, PhD, post doc

* Shared affiliation with the Department of Microbiology, SLU, Uppsala

** Shared affiliation with the Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm

Publications 2012-

1. C Jädert, J Petersson, S Massena, D Ahl, L Grapensparr, L Holm, JO Lundberg, M Phillipson. Decreased leukocyte recruitment by inorganic nitrate and nitrite in microvascular inflammation and NSAID-induced intestinal injury. *Free Radic Biol Med*. 2012 Feb 1;52(3):683-92. Epub 2011 Nov 30. PMID: 22178413 [PubMed - in process]
2. J Dicksved*, O Schreiber*, B Willing[a], J Petersson, S Rang, M Phillipson, L Holm, S Roos * These authors contributed equally to this work. Lactobacillus reuteri Maintains a Functional Mucosal Barrier during DSS Treatment Despite Mucus Layer Dysfunction. *PLoS One. 2012;7(9):e46399. doi: 10.1371/journal.pone.0046399. Epub 2012 Sep 27. PMID: 23029509*
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disease. *Redox Biol. 2013 Dec 24;2:73-81. doi: 10.1016/j.redox.2013.12.012. eCollection 2014. PMID: 24494186 [PubMed]*

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Book chapters

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Agencies that support the work

The Swedish Research Council

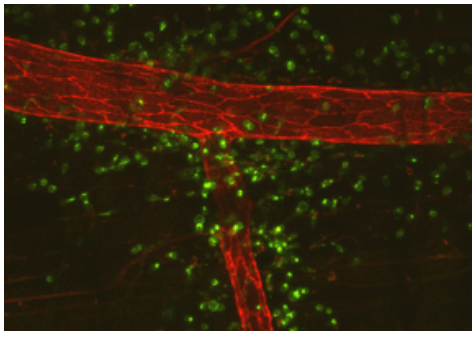
Formas (The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning)

BioGaia AB

Targeting leukocytes in health and disease

Mia Phillipson

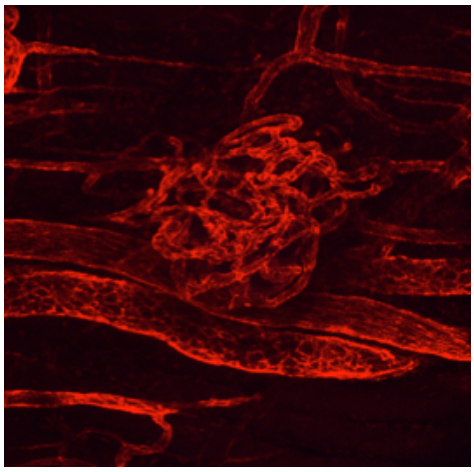
The capacity of circulating leukocytes to arrest on the surface of inflamed endothelium, transmigrate, and penetrate into the underlying tissue are key steps in response to infections as well as other inflammatory processes. The importance of recruited as well as tissue resident leukocytes also during homeostasis, angiogenesis and tumor growth is increasingly being acknowledged. Expanding the knowledge of the mechanisms that regulate the recruitment and actions of leukocytes is very important to be able to control and eventually limit inflammatory response, tumor growth and tissue damage.



A venule (anti-CD31, red) with emigrated neutrophils (anti-Gr1, green).

The overall aim of the research conducted in my laboratory is to uncover novel roles of leukocytes and to find means to regulate their specific functions in settings spanning from organ development to tissue healing, angiogenesis and inflammation. By employing state of the art techniques for studies of leukocyte trafficking and interactions *in vivo* (high speed confocal microscopy), we are delineating how leukocytes are recruited to sites of inflammation or hypoxia as well as their effector functions in tissue. The signals and chemokines initiating leukocyte recruitment as well as the adhesion molecules involved in the different steps of the leukocyte recruitment cascade are being investigated. We have established a new step in the leukocyte recruitment cascade, intravascular crawling, and study how chemokines are transported into the inflamed venules. We recently found that chemokines sequestered on endothelial heparan sulphate direct crawling leukocytes towards optimal sites for transmigration (Massena et al.,

Blood, 2010). We also investigate how the intestine can withstand the constant pressure of the commensal bacterial flora without developing inflammation, and are presently mapping the role, behaviour and interactions of different intestinal leukocytes during homeostasis as well as colitis.



The reestablished glomeruli-like islet vasculature surrounded by muscle blood vessels two weeks after transplantation to striated mouse muscle.

In addition, we recently identified a clinically relevant and attractive approach of curing type 1 diabetes, since islets transplanted to muscle became fully revascularized and therefore functioned better compared to islets implanted in the liver (Christoffersson et al., Diabetes, 2010), the organ traditionally used for islet transplantation. Means to limit the immunosupressing therapies required following allogeneic islet transplantations have also been investigated (Vågesjö et al. Cell Transplatation, 2014). A specific neutrophil subtype with pro-angiogenic features was recently demonstrated in the circulation, and was found to be recruited to sites of hypoxia by Vascular Endothelail Growth Factor A (VEGF-A) (Christoffersson et al., Blood, 2012). We currently aim to accelerate angiogensis and woud healing by affecting the microenvironment to induce specific leukocytes recruitment as well as phenotype shifts of tissue resident leukocytes.

Members of the group

Mia Phillipson - Professor

Gustaf Christoffersson – Post Doc

Antoine Giraud – Research Engineer

Jalal Haft – PhD student

Carmen Herrera Hidalgo – PhD student

Haoyu Liu, PhD, post doc

Cecilia Jädert – PhD student*

Sara Massena Santos – PhD student

John Sedin – Post Doc

Cédric Seigneiz – Post Doc

Evelina Vågesjö – PhD student

Tomas Waldén – Post Doc

* Shared affiliation with the Department of Physiology and Pharmacology, Karolinska Institute, Stockholm

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vascularization in adult mice. * Shared contribution as last author ***Am J Pathol*** 185:1094-1103, 2015

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Dissertations

Gustaf Christoffersson ”Leukocytes in Angiogenesis: Learning from Transplanted Pancreatic Islets” PhD April 2013

Ulrika S Pettersson ”Blood Flow Regulation and Inflammatory Response in Experimental Modles of Diabetes” PhD February 2012

Agencies that support the work

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Swedish Research Council

The Diabetes Wellness Foundation

The Ernfors family foundation

The Novo Nordic Foundation

The Swedish Diabetes Foundation

Vinnova

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Diabetic Nephropathy and Uremic Toxins

Fredrik Palm

Diabetic Nephropathy (core director: Fredrik Palm)

Diabetes mellitus is the most common cause for end-stage renal disease. The exact mechanisms mediating diabetes-induced kidney damage (diabetic nephropathy) are largely unknown despite intense research. The aim of this research program is to study effects of diabetes on renal metabolism and microcirculation in relation to functional changes. The ultimate goal is to find new treatment strategies to avoid the development of kidney dysfunction during diabetes.

We were the first laboratory to report kidney hypoxia in diabetes (Palm et al., Diabetoligia 2003, 46(8):1153-1160) and this finding has recently been confirmed in diabetic patients with established nephropathy (Wang et al., J Magnet Res Imag 2011, 33(3):655-660). Since then, our work has focused on identifying the mechanisms resulting in the diabetes-induced kidney hypoxia. So far, we have identified several contributing mechanisms, including increased oxidative stress, altered red-ox balance, increased renal oxygen consumption and increased tubular electrolyte transport work due to both increased glomerular filtration, but also increased glucose transport in the proximal tubule. Recently we have made a very significant observation in rats treated with the mitochondrial uncoupler dinitrophenol for up to four weeks. These otherwise healthy rats displayed excessive oxygen utilization, due to the uncoupled mitochondria, and developed pronounced kidney hypoxia. Interestingly, these rats also displayed 50% increased urinary protein excretion, tubulointerstitial damage and infiltration of immune cells. Therefrore, we are the first to show that increased oxygen utilization is enough to cause kidney hypoxia and nephropathy. This is a majopr breakthough sine previous studies always have been associated with confounding factors, such as hyperglycemia, increased oxidative stress and altered tubular transport.

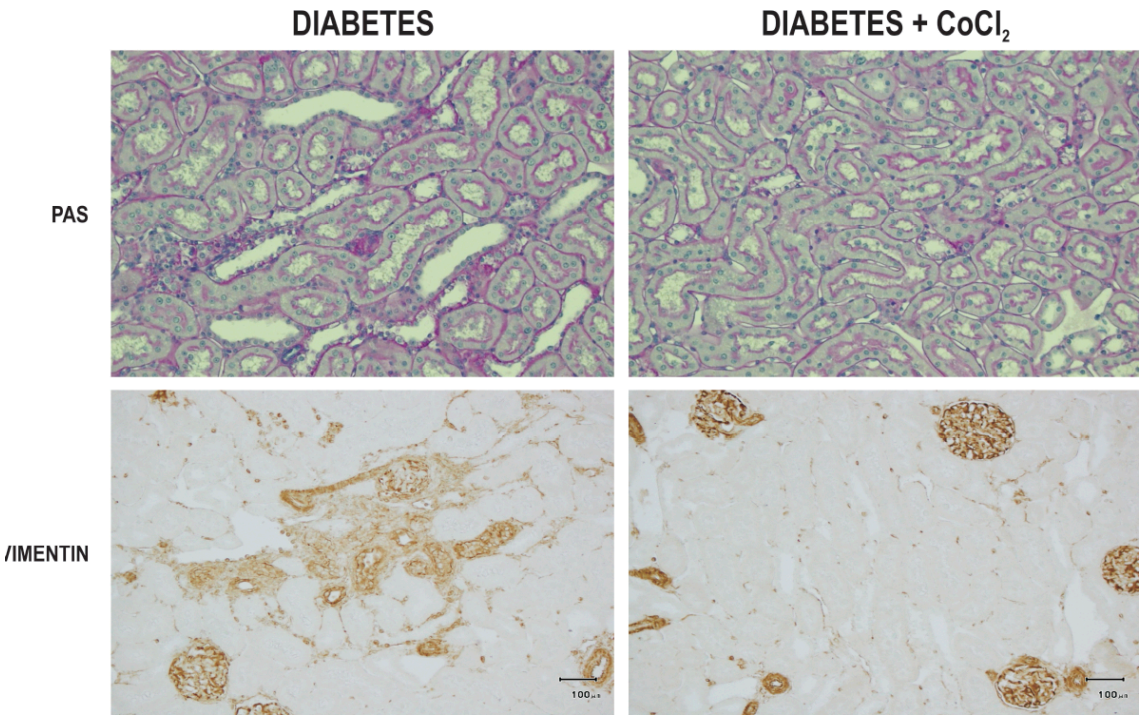

Figure 1. Activation of the hypoxic gene response by chronic $CoCl_2$ treatment prevented the diabetes-inudced kidney hypoxia and the clinical signs of diabetic nephrpathy, such as tubulointerstitial fibrosis, proteinuria and glomerular hyperfiltration.

We have also approached the problem with kidney hypoxia from another angle by chronically treating diabetic animals with $CoCl_2$, which activates the hypoxic gene response (HIF). The results demonstrate that HIF activation prevents the diabetes-induced kidney hypoxia and tubulointerstitial damage (Fig. 1 and 3).

Metabolic and functional alterations occurring in kidneys from diabetic animals (rats and genetically modified mice) are studied using in vivo techniques and molecular biology. Mitochondrial function and internal defence mechanisms are studied in diabetic animals and kidney tissue from diabetic patients. Renal blood flow and oxygen metabolism are studied using Magnetic Resonance Imaging (MRI) in animals as well as in diabetic patients.

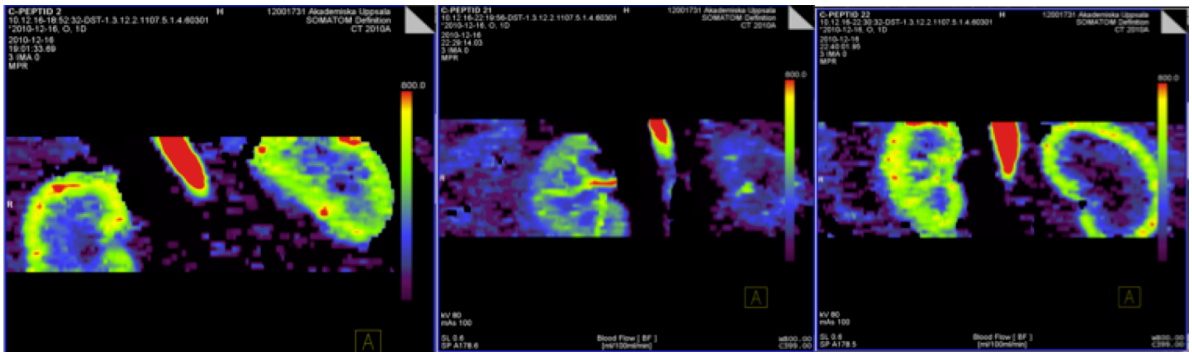

Figure 2. Thirty minuites of warm ischemia to the left kidney (right kidney on the images) did not alter kidney function or the intrarenal blood flow (images abow measured by computed tomography) in control rats four weeks after the ischemic insult. However, the same ischemic insult caused markedly reduced kidney function (glomerular filtration rate about 10% of normal), atrophy and and hypoperfusion of the left kidney. Diabetic rats administered a bolus dose of C-peptide before the ischemic insults were protected against the increased susceptibility to the ischemia-reperfusion injury.

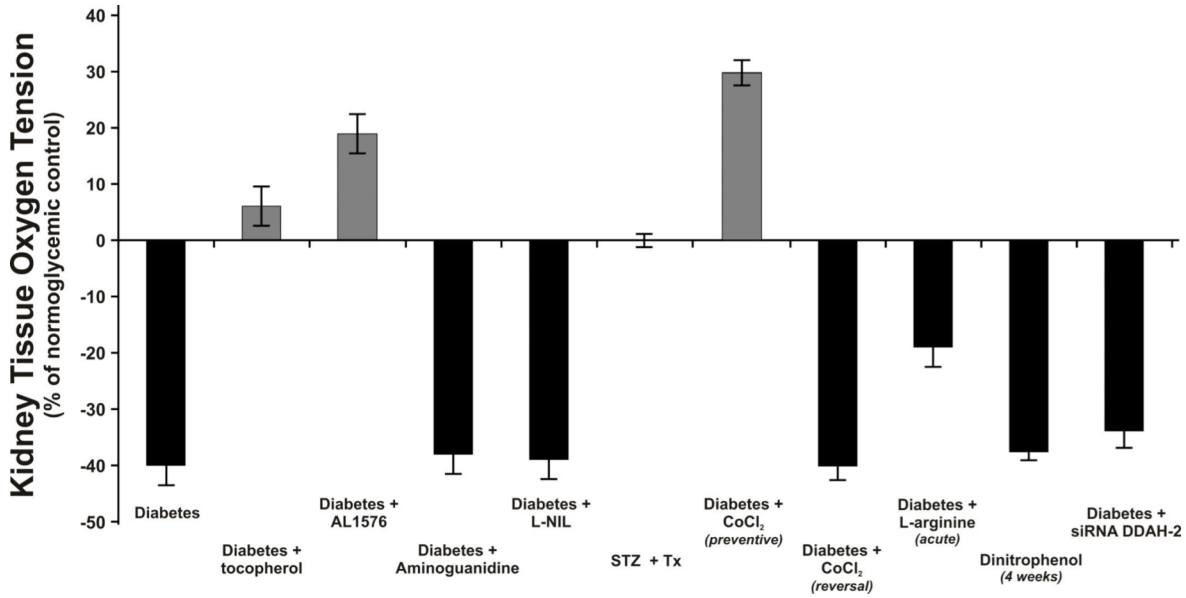

Figure 3. Summary of all investigated mechanisms affecting intrarenal oxygenation in diabetes. Tocopherol (vitamin E) is an antioxidant, AL1576 inhibits aldose reductase and presents activation of the polyol pathway, aminoguanidine inhibits AGE formation, L-NIL inhibits iNOS, STZ + Tx denotes animals administered streptozotocin (to induce diabetes) and 24h thereafter received enough islets of Langerhan's to reverse the hyperglycemia, $CoCl_2$ activates HIF and prevents kidney hypoxia if starting treatment early (preventive) but fails to reverse already established nephropathy (reversal), acute L-arginine administration induces NO release and partly restores kidney oxygenation, chronic dinitrophenol administration results in excessive oxygen utilization causing kidney hypoxia and clinical signs of nephropathy, siRNA directed against DDAH-2 reduced DDAH-2 protein expression by more than 60% but failed to normalize kidney oxygenation.

These non-invasive techniques were used in a recent study, in which we studied the effect of ischemia-reperfusion injury in diabetic kidneys (Fig. 2). It is well-known that diabetic kidneys are increasingly susceptible to an ischemic insult, but we were able to show that administration of a bolus dose of C-peptide had pronounced renoprotective effects in diabetes. Interstingly, we have previously shown that C-peptide reduces oxygen utilization in the diabetic kidney and this

might therefore explain the renoprotective effects against the ischemic insult in these kidneys. This fidning might have important clinical implications since C-peptide is an endogenous substance, which therefore only needs relatively minor administrative work before moving into clinical practice.

By combining basic renal and diabetic research, we believe we can contribute to increase the understanding of the mechanisms involved in diabetic nephropathy, which will facilitate development of novel therapies. Additionally, metabolic alterations always precede histological changes, which potentially can be used as a clinical diagnostic tool when identifying patients at increased risk to develop diabetic nephropathy. This would hopefully enable early treatment modalities before the seemingly irreversible histological changes occur with manifest nephropathy.

Our results so far suggest:

A) Diabetic rats display kidney hypoxia, which is linked to excessive oxygen utilization.
B) Mitochondrial uncoupling results in excessive oxygen utilization and development of nephropathy.
C) C-peptide protects the diabetic kidney against ischemic insults, which may in part be explained by the oxygen utilization-lowering effects of C-peptide in diabetes.
D) By using non-invasive imaging techniques, we may be able to transfer our knowledge from our experimental settings into clinical use.

E) Intrarenal hypoxia per se causes kidney disease.

Agencies that support the work

Swedish Research Council

Swedish Diabetes Association

Swedish Heart and Lung Foundation

Family Ernfors Foundation

Magnus Bergwall Foundation

Åke Wiberg Foundation

ERC Marie Curie IRSES

Uremic Toxins (core director: Lina Nordquist)

In uremic patients, losses of kidney function are accompanied by deteriorating organ function attributable to the accumulation of uremic retention solutes. Compounds that exert an adverse biologic impact are called uremic toxins

Indoxyl sulfate is a representative uremic toxin made by the liver from indole produced by gut bacteria from tryptophan. In addition to causing uremic symptoms, indoxyl sulphate per se accelerates the progression of renal failure. Our recent study for the first time demonstrated that indoxyl sulfate increases oxygen consumption and aggravates local hypoxia in renal tubular cells via enhancement of oxidative stress (Fig. 4). Uremic states per se may accelerate progression of renal dysfunction via aggravation of chronic hypoxia as a final common pathway to end stage renal disease.

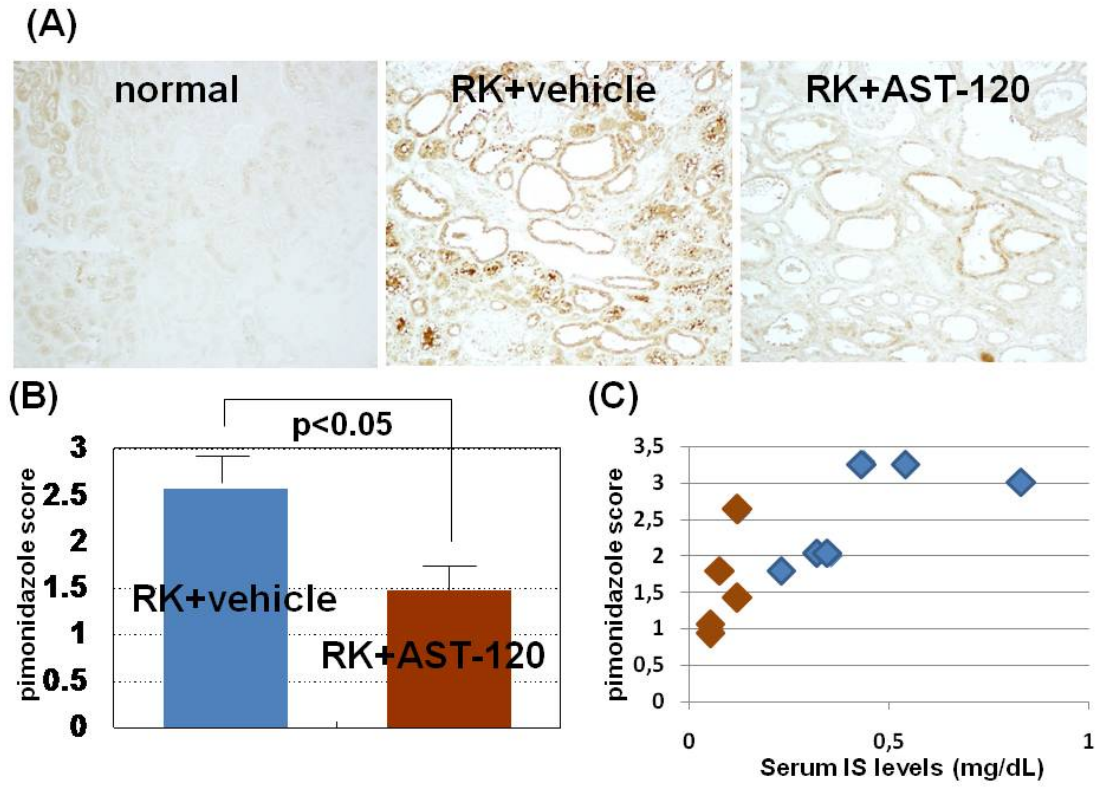

Figure 4. Improvement of oxygenation of the remnant kidney by reduction of uremic toxins. (A) Immunohistochemical staining of pimonidazole accumulation in the cortex showed improvement of oxygenation of the remnant kidney in animals treated with the oral absorbent AST-120 that reduces plasma levels of indoxyl sulfate. No pimonidazole accumulation was observed in cortical tubules of normal animals. (B) Semi-quantitative analysis of pimonidazole accumulation confirmed better oxygenation of the remnant kidney in rats treated with AST-120. (C) Pimonidazole accumulation, an indicator of hypoxia, showed a good correlation with serum IS levels in RK rats.

Agencies that support the work

Swedish Research Council

Swedish Society for Medical Researsh

Lars Hierta Foundation

Members of the group

Fredrik Palm, Ph.D.

Lina Nordquist, Ph.D., core director

Per Liss, MD, Ph.D., Associate professor

Angelica Fasching - research engineer

Malou Friederich – Ph.D. Post Doc

Patrik Persson, Ph.D. Post Doc

Ebba Sivertsson, Ph.D.-student

Per Eckerbom, Ph.D.-student

Publications 2012-

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Dissertations

Malou Friederich-Persson "The Role of Mitochondrial Uncoupling in the Development of Diabetic Nephropathy" PhD March 2012.

Patrik Persson "Aspects of regulation of GFR and tubular function in the diabetic kidney - Roles of adenosine, nitric oxide and oxidative stress" PhD April 2013.

Studies of the pathophysiological mechanisms behind protein aggregation and formation of organ and cell toxic amyloid

Gunilla T Westermark

With our research we aim to pinpoint mechanisms that precede the formation of beta-cell toxic islet amyloid, and also characterize the endogenous mechanism involved in resolution of amyloid. Amyloid defines a fibrilar aggregate where beta strands of protein monomers are assembled perpendicularly to the fibrilar axis. Initiation of amyloid fibrils involves the formation of smaller intermediates, so called protofibrils that has been ascribed the cell toxic activity. Today, 30 different amyloid forming proteins have been isolated from amyloid deposits in man.

Islet amyloid and beta-cell death

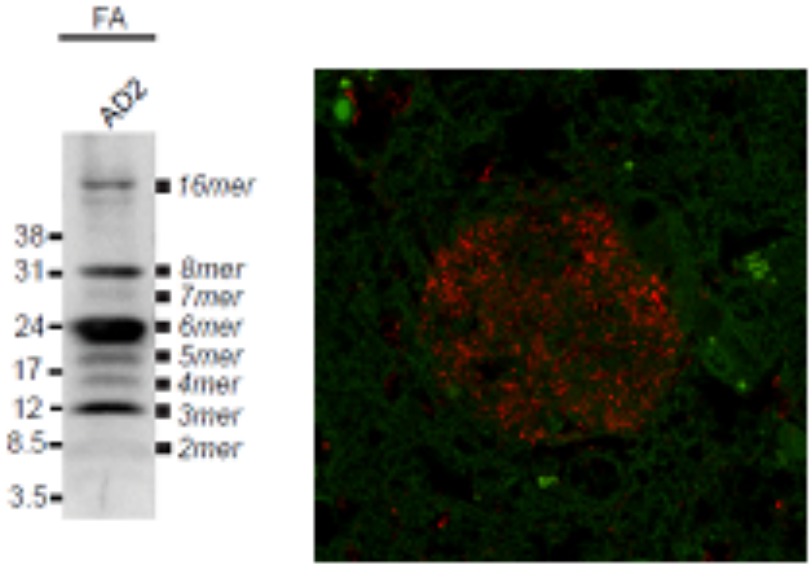
The beta-cell hormone Islet Amyloid Polypeptide (IAPP) is the major amyloid component present in the islets of Langerhans in almost all individuals with type 2 diabetes. IAPP is synthesised as a larger proIAPP and arises after posttranslational processing that comprises the removal of an N-terminal and a C-terminal flanking peptide, formation of a disulfide bond and N-terminal amidation. Processing is performed by the prohormone convertases PC2 and PC1/3 and takes place in the secretory granules. Proinsulin is processed to insulin by the same convertases at the same location. ProIAPP and incomplete processed proIAPP can be detected in amyloid deposits formed in vivo, and at present we investigate how expression of PC2 and PC1/3 is affected by conditions that trigger islet amyloid formation. Mouse and rat do not develop IAPP-amyloid due to sequence variations in the IAPP molecule. Therefore, this work is performed on our human IAPP transgenic mouse strain where islet amyloid develops in male mice fed a diet high in fat for 12 months.

The transgenic hIAPP mouse model is used for studies including prevention or blocking of amyloid propagation. At present we analyse the inhibitory effect that heparin related molecules

exert on amyloid formation. Also, we have established a new mouse strain that over-express heparanase and show that this reduce formation of IAPP amyloid. This work is done in collaboration with Jin-ping Li, IMBIM, UU.

Islet amyloid is also a frequent finding in transplanted islet, and we use isolated islets from the hIAPP transgenic strain and human islets from the *Nordic Network* for clinical islet transplantation to investigate if IAPP amyloidogeneity is influenced by the transplantation local. We have shown that amyloid develops to the same degree in grafts implanted under the kidney or spleen capsule or to the liver. Other locations are under investigation.

Fibrils formed from different amyloid precursor proteins appear to be morphological inseparable. Therefore, it is possible that fibrils formed by one protein can seed amyloid made up by a second amyloid protein. We have seeded islet amyloid in human IAPP transgenic mice through administration of preformed fibrils made up by Aβ protein. Aβ and IAPP exhibits 50% sequence identity and using a high sensitive detection method, proximity ligation assay (PLA) we have identified IAPP in the brain of patients with Alzheimer's disease. The finding is interesting because type 2 diabetes increases the risk of developing Alzheimer's disease. At present, in collaboration with dr Martin Ingelsson, Uppsala and Bradley Hyman, and collegues at Alzheimer Disease Research Center at Harvard Medical School, Massachusetts we compare amyloid plaque composition in AD pateints with and without type 2 diabetes.



Western blot analysis of brain extract from an AD patient with IAPP antiserum shows a ladder like pattern. PLA performed with a combination of IAPP and Aβ antibodies identifies IAPP reactivity throughout the Aβ-paque.

We have established a new model in *Drosophila melanogaster* for studies of proIAPP/IAPP amyloid formation. In transgenic flies expression of human proIAPP or IAPP amyloid is

detected already in 20 days old flies. As expected, amyloid does not develop in control flies expressing non-amyloid-forming mouse IAPP.

The *Drosophila melanogaster* system is used for pinpointing the intracellular events that result in amyloid-linked cell death. We analyse important pathways such as ER-stress, ERAD and autophagy.

Insulin is in vitro a potent inhibitor of IAPP-aggregation and the two peptides co-localize in the secretory granules where they undergo enzymatic processing. A disturbance in cleavage and/or folding in any of the precursors might initiate amyloid aggregation. We use the Drosophila model to investigate if induction of human proinsulin or any of its processing metabolites in flies expressing the amyloidogenic proIAPP or IAPP will prevent amyloid formation.

The *Drosophila melanogaster* will also be used as a tool for analysis of amyloid inhibitors.

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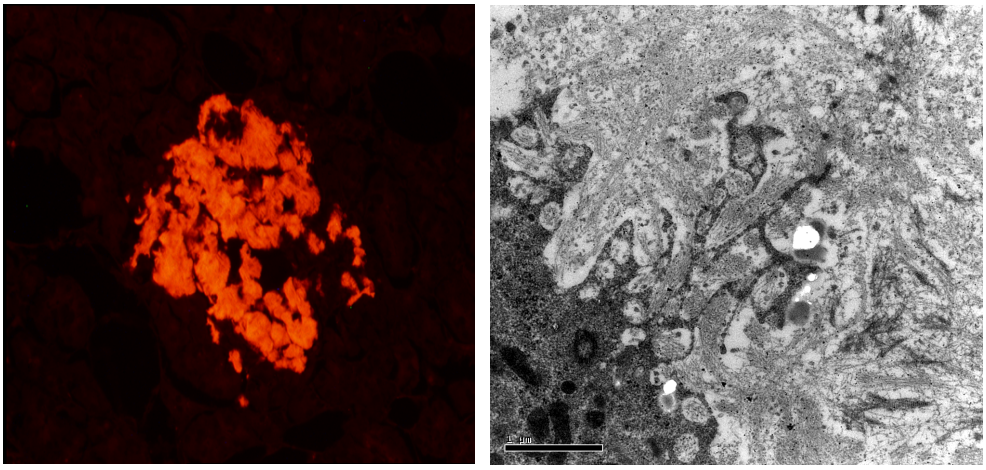


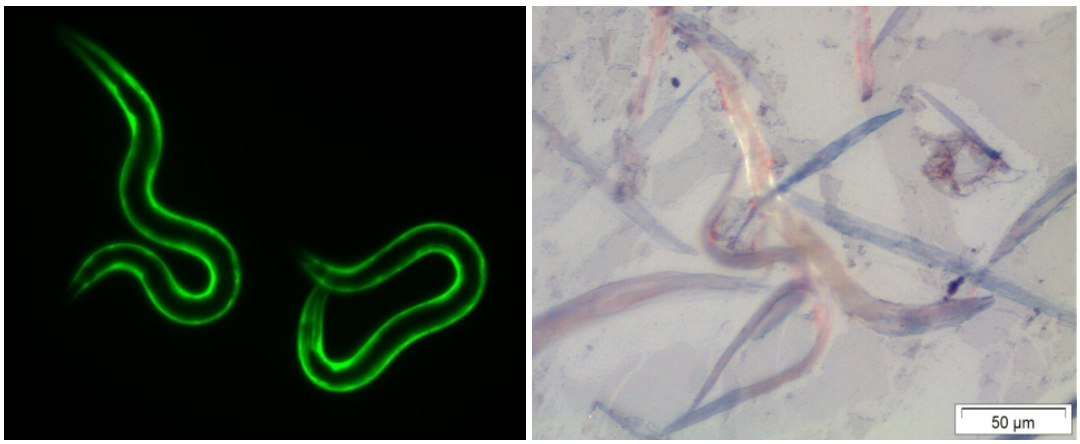
A human islet stained for amyloid by Congo red. The amyloid deposits replace most of the beta-cells. The section is viewed at 546 nm. The electron micrograph shows the border between a beta-cell and extracellular amyloid. Note the close association between the amyloid bundles and the cell membrane.

We have identified autophagy as an important mechanism that link amyloid and cell death. In collaboration with Annica Rönnbeck, KI is autophagy's role in neuronal cell death explored. This work is performed using Aβ-transgenic mice, human brain tissue and Aβ transgenic flies

There is a well-established mouse model for reactive amyloidosis (AA-amyloidosis) where N-terminal fragments (protein AA) of serum amyloid A (SAA) deposit as amyloid. We have used this model and studied resolution of amyloid. This process depends on formation of AA reactive antibodies and activation of macrophages.

We have also used this model to study transmission of amyloid and have recently shown that monocytes from a diseased mouse can prime for the disease in a recipient animal. This result points to a prion-like mechanism for spreading of amyloid. With the model, we have also shown that non-amyloid fibrilar structures can prime for AA-amyloidosis. This finding is interesting and points to a possible environmental component in the pathogenesis of the disease. To reduce the numbers of mice used for our transmission studies we explore the possibility to establish a model for AA amyloidosis in C elegans. This work is ongoing and we have now transgenic worms that express human protein AA, and in these develops amyloid.

Feeding worms on OP50 bacteria mixed with amyloid fibrils leads to disturbance in mobility and is indicative for transmission.



The transgenic C. elegans express GFP and an amyloid protein (e.g. AA 45). The expression is driven to the body wall muscle. The presence of the green GFP allows us to monitor the movements of the worm. Aged worms, for amyloid with Congo red exert green birefringence when viewed in polarised light.

Members of the group

Gunilla T Westermark, PI

Lakshim Kotegala, Post Doc

Sara Bohman, Post Doc

Camilla Krappe, Post Doc

Marie Oskarsson, PhD Student

Gu Xiaohong, PhD student

Ye Wang, Ph.D.

Marianne Ljungkvist, Laboratory engineer

Jan Sara, Laboratory engineer

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The Swedish Diabetes Association

Family Ernfors Foundation

Alzheimer fonden

Diabetes Wellness

Publications 2012-

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Dissertations 2014

- **Chowdhury, A.** (2014). Role of Cell-cell Interactions and Palmitate on β-cells Function. Diss. Uppsala Universitet.
- **Gao, X.** (2014). Local Purinergic Control of Arteriolar Reactivity in Pancreatic Islets and Renal Glomeruli. Diss. Uppsala Universitet.
- **Li, J.** (2014). ATP Dynamics in Pancreatic α- and β-cells. Diss. Uppsala Universitet.
- **Wang, X.** (2014). Study of the Proliferation, Function and Death of Insulin-Producing Beta-Cells *in vitro*: Role of the Transcription Factor ZBED6. Diss. Uppsala Universitet.

Licentiate theses 2014

Staaf, J.

Anvari, E

Sivertsson, E

Vågesjö, E

Gandasi Ravindra, N

Marie E. Oskarsson

Economy

(kSEK)

	2013	**2014**
Undergraduate Education appropriations	33 956	33 510
Faculty appropriations	20 656	20 671
External Grants	35 878	37 620
Contract research	149	1 082
Total	**90 639**	**92 883**

Undergraduate Teaching

The department participates in 7 different study programmes (utbildningsprogram): medicine, pharmacy, biomedicine (Bachelor and Master programmes), nursing, biomedical laboratory science and dieticians and dispensers. In addition, it hosts a number of single subject courses (fristående kurser). Some 1500 students per year are given education at the department.

Medicine

The department contributes teaching in anatomy, cell biology and physiology with both traditional lectures and problem based learning as well as with seminars and laboratory experiments. Most of this teaching is given during terms 1-3 of the programme but extensive parts are also given in the later integrated courses. The overall objective is to provide basic knowledge of the morphology and function of the human body and to create a basis for the following clinical studies. Some 115 students are enrolled every semester.

Biomedicine

This three-year Bachelor programme aims to give students a thorough understanding of normal morphology and function of the human body. The programme is given annually and provides the students training for future activity in research, information and education. The department takes part in the teaching of anatomy, embryology, cell biology and physiology. About 40 students are enrolled each year.

The two-year Master programme in Biomedicine is an international programme that aims to give a deeper knowledge in the subjects taught in the Bachelor's programme, and also offers the students an opportunity to specialize in their field of interest. The department gives the first course in the programme, Major Diseases - Homeostasis and Endocrine Disorders. The programme enrolls approximately 30 students annually.

Pharmacy

The department is responsible for the teaching in anatomy and physiology for the University Diploma of Pharmacy. The courses are in the form of lectures, seminars and laboratory experiments. Some 140 students are enrolled every semester.

Clinical dieticians

The anatomy and physiology course included in the programme for clinical dieticians is given annually by the department, and include lectures, seminars and laboratory experiments for approximately 30 students.

Nursing sciences

The department is responsible for the teaching of anatomy, cell biology and physiology in the form of lectures and seminars. Some laboratory experiments are involved as well. Some 130 students are enrolled in the spring semester and 150 students are enrolled in the autumn semester. Thus approximately 280 students are enrolled every year. A new responsibility since

2014 is the education of nurses on Gotland where MCB through data link lecture for the students in the above subjects just as for our students in Uppsala. Approximately 40 students are enrolled every year.

Biomedical laboratory sciences

The aim of this programme is to produce technicians with appropriate training for a future task in diagnostic and research laboratories. The department is responsible for the teaching in anatomy, histology, cell biology and physiology in the form of lectures, seminars and laboratory experiments. Some 35 students are enrolled each year

Single subject courses (fristående kurser)

Anatomy A (evening course)

Transplantation biology (evening course)

Cell biology I and II (evening course)

Medical cell biology (laboratory project course)

Histology

Physiology

Basic medical physiology

Summer research school

Major Diseases - Homeostasis and endocrine disorders

Graduate Teaching

The department has the responsibility for two of the Mandatory Courses for Graduate Students Introduction to Scientific Research – enrolling 80 Graduate students per year and Scientific Presentation – enrolling 40-50 Graduate students per year.

MD/PhD programme

MCB is responsible for the administration and content of the MD/PhD programme. The Medical Faculty and Upsala Society of Physicians provide grants for three undergraduate medical studies per year to join the MD/PhD programme. These students pursue medical undergraduate students in parallel with a graduate research project. After finishing medical studies the MD/PhD studens have a period of full-time research leading to half-time or Licentiate exam. Students then continue with internship combined with continued graduate research project studies. After obtaining the MD a full-time research period leads to the PhD.

Centres and Facilities

Advanced light microscopic imaging facilities

Within the department there are several advanced setups for fluorescence imaging of living cells and micro-organs. These setups are based on bright field microscopy, conventional fluorescence microscopy, epifluorescence microscopy, total internal reflection fluorescence (TIRF) microscopy and confocal microscopy. We also have a laser capture microscope.

Fluorescence and intra-vital microscopy

Fluorescence imaging using epifluorescence is used for on-line monitoring of the cytoplasmic Ca2+ concentration in superfused islets of Langerhans and dispersed islet cells (Anders Tengholm, 018 471 4481). Similar studies of Ca^{2+}, nitric oxide and oxygen radicals are performed on single perfused kidney glomeruli and the juxtaglomerular apparatus (Erik Persson, 018 471 4180) and interactions between leukocytes and endothelial cells leading to leukocyte transmigration and vessel permeability are studied in the adipose tissue, gastrointestinal tract and skeletal muscle in vivo (Lena Holm, 018 4714325, Mia Phillipson, 018 471 4419). Membrane order is studied in live cells by ratiometric imaging in combination with deconvolution (Ingela Parmryd, 018 471 41 50).

TIRF microscopy

The department possesses 6 TIRF (or evanescent wave) microscopes, two of which are custom-built systems with prism-type configuration and 4 using custom-built or commercial through-the-lens illumination. The systems are differently equipped with gas and diode-pumped solid-state lasers to provide excitation at multiple lines, including 405, 442, 457, 488, 514 and 561 nm. These setups are used for on-line monitoring of cAMP, cytoplasmic Ca^{2+}, IP_3, DAG, PIP_2, PIP_3 and other signalling molecules using indicators based on different spectral variants of green fluorescent protein (Anders Tengholm, 018 471 4481) and imaging of single molecules involved in exocytosis of secretory vesicles (Sebastian Barg, 018 471 4660).

PALM and STORM superresolution microscopy

One of the multicolour TIRF microscopes is equipped for stochastic superresolution microscopy in live and fixed cells. Fluorescently labeled proteins (eg. GFP fusion proteins or antibody labelling) can be localized with a resolution of 20-50 nm. (Sebastian Barg, 018 471 4660).

Confocal microscopy

The laboratory has three inverted confocal microscopes, one fast spinning disc (Nipkow) system used for studies of living islets of Langerhans and dispersed islet cells (Anders Tengholm, 018 471 4481), one scanning confocal system mostly used for structural studies (Nils Welsh, 018 471 4212), one advanced state-of-the-art system suitable for live cell imaging

(Oleg Dyachok, 018 471 4345) and an upright high speed confocal microsope for in vivo studies (Zeiss LSM5 Live, Mia Phillipson, 018 471 4419).

Laser capture microscopy

The department has a laser capture microscope (LMD6000, Leica) that can be used to isolated cells or other regions of interest from sectioned tissues for further analysis. Depending on internal use, this equipment is available for external users on a charged service basis (Per-Ola Carlsson, 018 471 4425, Sara Ullsten, 018 471 4395).

Gel imaging

The department has a Kodak 4000MM gel imaging unit (Nils Welsh, 018 471 4212) and a Bio-Rad Fluor-S MultiImager system for scanning and quantification of proteins in gels and membranes (Peter Bergsten, 018 471 4923).

Digital cameras

Several of the imaging systems are equipped with ultra-sensitive state of the art cameras, some utilizing back-thinned electron multiplying charge coupled device (EMCCD) techniqe.

Other equipment

Real-time PCR (Roche Lightcycler, Nils Welsh, 018 471 4212).

Flow cytometry and cell sorting (BD FacsCalibur, Nils Welsh, 018 471 4212).

Laser Doppler blood flow measurement equipment (Lena Holm, 018 471 4325).

Patch clamp equipment for electrophysiological recordings (Sebastian Barg, 018 471 4660).

Fluoroscan supplied with detectors for luminescence and absorbanse (Gunilla Westermark, 018 471 4169).

Nanodrop for DNA/RNA and protein quantification (Gunilla Westermark, 018 471 4169).

Mesoscale multiplex immunoassays (Erik Gylfe, 018, 471 4428)

EPR (electron paramagnetic resonance) for measuring free radicals (Fredrik Palm, 018 471 4182).

Prizes and awards 2014

Gustaf Christoffersson: Benzelius award from the Royal Academy of Sciences, SFD price for best preclinical PhD in diabetes research

Olof Idevall: the M L Philipson award and the Göran Gustavsson award

Per-Ola Carlsson: the DPLU/LUDC Nordic Prize

Nikhil Gandasi: the Young Investigators Award from Scandinavian Society for the Study of Diabetes

Mia Phillipson: Eric K Fernströms prize

E-mail address list

Department of Medical Cell Biology

www.mcb.uu.se

Address: Uppsala University, Biomedical Center, Box 571, 751 23 Uppsala, Sweden

Office: Fax +46 18 471 4059, Phone +46 18 471 4328, +46 18 471 4431

- ***Adler, Jeremy*** *research engineer*
- ***Ahl, David*** *doctoral student david.ahl@mcb.uu.se*
- ***Ahooghalandari, Parvin*** *research engineer, +46 18 4714924 parvin.ahooghalandari@mcb.uu.se*
- ***Al-Mashhadi, Ammar Nadhom Farman*** *guest doctoral student*
- ***Alenkvist, Ida*** *doctoral student ida.alenkvist@mcb.uu.se*
- ***Andersson, Arne*** *professor emeritus, +46 18 4714397 arne.andersson@mcb.uu.se*
- ***Anvari, Ebrahim*** *doctoral student ebrahim.anvari@mcb.uu.se*
- ***Arbrandt, Gustav*** *doctoral student gustav.arbrandt@mcb.uu.se*
- ***Aresh, Bejan*** *guest doctoral student*
- ***Ashrafzadeh, Parham*** *doctoral student parham.ashrafzadeh@mcb.uu.se*
- ***Azarbayjani, Faranak*** *lecturer, +46 18 4714450 faranak.azarbayjani@mcb.uu.se*
- ***Barbu, Andreea*** *researcher, +46 18 4714316 andreea.barbu@mcb.uu.se*
- ***Barg, Sebastian*** *researcher, +46 18 4714660 sebastian.barg@mcb.uu.se*
- ***Becirovic Agic, Mediha*** *assistant with study grant mediha.agic@mcb.uu.se*
- ***Bengtsson, Johan*** *degree project worker*
- ***Bergsten, Peter*** *professor +46 18 4714923 professor, +46 18 4714923 peter.bergsten@mcb.uu.se*
- ***Blixt, Martin*** *lecturer, +46 18 4715005 martin.blixt@mcb.uu.se*
- ***Bodin, Birgitta*** *research engineer birgitta.bodin@mcb.uu.se*
- ***Bohman, Sara*** *lecturer, +46 18 4715005 sara.bohman@mcb.uu.se*
- ***Borg, Håkan*** *senior lecturer, +46 18 4714353 hakan.borg@mcb.uu.se*
- ***Börjesson, Joey Lau*** *researcher, +46 18 4714395 joey.lau@mcb.uu.se*
- ***Carlsson, Per-Ola*** *professor, +46 18 4714425 per-ola.carlsson@mcb.uu.se*
- ***Carvalho, Carla*** *doctoral student carla.carvalho@mcb.uu.se*
- ***Chahal, Gurdeep*** *degree project worker*
- ***Chowdhury, Azazul Islam*** *doctoral student, +46 18 4714427 azazul.chowdhury@mcb.uu.se*
- ***Christoffersson, Gustaf*** *post doctoral, +46 18 4714324 gustaf.christoffersson@mcb.uu.se*
- ***Ciba, Iris*** *physician*
- ***Dahlbom, Marie*** *research nurse*
- ***Dansk, Heléne*** *research engineer +46 18 4714924 research engineer, +46 18 4714924 helene.dansk@mcb.uu.se*

- **Drott, Carl Johan** doctoral student carljohan.drott@mcb.uu.se
- **Dyachok, Oleg** senior research engineer, +46 18 4714345, +46 76 2374730 oleg.dyachok@mcb.uu.se
- **Eckerbom, Per** consultant
- **Ejdesjö, Andreas** post doctoral, +46 18 4714378 andreas.ejdesjo@mcb.uu.se
- **Elksnis, Andris** degree project worker
- **Eriksson, Ulf** professor, +46 18 4714129 ulf.eriksson@mcb.uu.se
- **Espes, Daniel** doctoral student, +46 18 4714397 daniel.espes@mcb.uu.se
- **Fasching, Angelica** research engineer, +46 18 4714156 angelica.fasching@mcb.uu.se
- **Flacker, Peter** visiting researcher peter.flacker@mcb.uu.se
- **Forslund, Anders** researcher
- **Forslund, Simon** degree project worker
- **Franzén, Petra** laboratory technician petra.franzen@mcb.uu.se
- **Fred, Rikard** post doctoral, +46 18 4714925 rikard.fred@mcb.uu.se
- **Gandasi, Nikhil** doctoral student, +46 18 4714292 nikhil.gandasi@mcb.uu.se
- **Gao, Xiang** doctoral student, +46 18 4714183 gao.xiang@mcb.uu.se
- **Giraud, Antoine** research engineer, +46 18 4714324 antoine.giraud@mcb.uu.se
- **Grapengiesser, Eva** researcher +46 18 4714424 researcher, +46 18 4714424 eva.grapengiesser@mcb.uu.se
- **Grapensparr, Liza** doctoral student, +46 18 4714460 liza.grapensparr@mcb.uu.se
- **Groebe, Karlfried** visiting researcher karlfried.groebe@mcb.uu.se
- **Gu, Xiaohong** doctoral student, +46 18 4714292 xiaohong.gu@mcb.uu.se
- **Gylfe, Erik** professor emeritus i sekretionsforskning , +46 18 4714428 erik.gylfe@mcb.uu.se
- **Hallgren, Ing-Britt** research engineer, +46 18 4714395 ing-britt.hallgren@mcb.uu.se
- **Hansell, Peter** professor, +46 18 4714130 peter.hansell@mcb.uu.se
- **Hellman, Bo** professor emeritus +46 18 4714424 bo.hellman@mcb.uu.se
- **Hermansson, Erik** visiting researcher
- **Herrera Hidalgo, Carmen** assistant with study grant carmen.herrerahidalgo@mcb.uu.se
- **Hirt, Christian** degree project worker
- **Hjort, Marcus** teaching assistant
- **Hjortberg, Mats** senior lecturer, +46 18 4714269 mats.hjortberg@mcb.uu.se
- **Holm, Lena** professor, +46 18 4714325 lena.holm@mcb.uu.se
- **Holmfeldt, Per** lecturer per.holmfeldt@mcb.uu.se
- **Hultström, Michael** researcher, +46 18 4714378 michael.hultstrom@mcb.uu.se
- **Idevall, Olof** postdoctoral research fellow olof.idevall@mcb.uu.se
- **Jamalpournobijari, Maria** doctoral student maria.jamalpour@mcb.uu.se
- **Jansson, Leif** professor, +46 18 4714396 leif.jansson@mcb.uu.se
- **Jägare, Annika** laboratory technician, +46 18 4714324

annika.jagare@mcb.uu.se
- **Jönsson, Sofia** assistant with study grant sofia.jonsson@mcb.uu.se
- **Kappe, Camilla** post doctoral camilla.kappe@mcb.uu.se
- **Kay, Emma** post doctoral emma.kay@mcb.uu.se
- **Kothegala, Lakshmi** post doctoral lakshmi.kothegala@mcb.uu.se
- **Kreuger, Johan** senior lecturer, +46 18 4714079 johan.kreuger@mcb.uu.se
- **Kristinsson, Hjalti** doctoral student hjalti.kristinsson@mcb.uu.se
- **Kullberg, Joel** visiting researcher
- **Kullman, Lisen** researcher (LOA), +46 18 4714345 lisen.kullman@mcb.uu.se
- **Källskog, Örjan** professor emeritus i fysiologi , +46 18 4714184 orjan.kallskog@mcb.uu.se
- **Li, Jia** doctoral student +46 18 4714426 doctoral student, +46 18 4714426 jia.li@mcb.uu.se
- **Li, Zhanchun** senior research engineer zhanchun.li@mcb.uu.se
- **Liljebäck, Hanna** teaching assistant hanna.liljeback@mcb.uu.se
- **Lindfors, Lina** guest doctoral student
- **Liss, Per** docent, consultant
- **Liu, Chenxiao** doctoral student chenxiao.liu@mcb.uu.se
- **Liu, Haoyu** post doctoral haoyu.liu@mcb.uu.se
- **Ljung, Leif** senior research engineer, +46 70 8366755 leif.ljung@mcb.uu.se
- **Ljungkvist, Marianne** research engineer, +46 18 4714967 marianne.ljungkvist@mcb.uu.se
- **Lomei, Jalal** doctoral student jalal.lomei@mcb.uu.se
- **Manukyan, Levon** researcher, +46 18 4714427 levon.manukyan@mcb.uu.se
- **Massena, Sara** doctoral student, +46 18 4714324 sara.massena@mcb.uu.se
- **Melville, Jacqueline Mary** post doctoral jacqueline.melville@mcb.uu.se
- **Müllner, Elisabeth** visiting researcher
- **Nensén, Oskar** teaching assistant
- **Niklasson, Bo** adjunct professor bo.niklasson@mcb.uu.se
- **Nilsson, Ove** professor emeritus i anatomi ove.nilsson@mcb.uu.se
- **Nordin, Astrid** research engineer, +46 18 4714395 astrid.nordin@mcb.uu.se
- **Nordquist, Lina** postdoctoral research fellow, +46 18 4714184 lina.nordquist@mcb.uu.se
- **Ohlsson, Hannes** doctoral student hannes.ohlsson@mcb.uu.se
- **Omar Hmeadi, Muhmmad** doctoral student omar.hmeadi@mcb.uu.se
- **Oskarsson, Marie** doctoral student, +46 18 4714967 marie.oskarsson@mcb.uu.se
- **Palm, Fredrik** professor, +46 18 4714182 fredrik.palm@mcb.uu.se
- **Pan, Shumin** financial administrator, +46 18 4714328 shumin.pan@mcb.uu.se
- **Parmryd, Ingela** visiting teacher, +46 18 4714150 ingela.parmryd@mcb.uu.se
- **Parv, Kristel** degree project worker
- **Persson, Erik** professor, +46 18 4714180 erik.persson@mcb.uu.se
- **Phillipson, Mia** professor, +46 18 4714419 mia.phillipson@mcb.uu.se

- **Quach, My** senior research engineer, +46 18 4714183 my.quach@mcb.uu.se
- **Rojas Vazquez, Ismael** degree project worker
- **Rydgren, Tobias** post doctoral, +46 18 4714411 tobias.rydgren@mcb.uu.se
- **Sagulin, Lisbeth** research engineer, +46 18 4714395 lisbeth.sagulin@mcb.uu.se
- **Sampaio, Suenia** doctoral student
- **Sandberg, Monica** senior research engineer, +46 18 4714440 monica.sandberg@mcb.uu.se
- **Sandin, Erik** course administrator, +46 18 4714414 erik.sandin@mcb.uu.se
- **Sandler, Stellan** professor, +46 18 4714430 stellan.sandler@mcb.uu.se
- **Saras, Jan** research engineer jan.saras@mcb.uu.se
- **Sargsyan, Ernest** researcher, +46 18 4714427 ernest.sargsyan@mcb.uu.se
- **Sedin, John** researcher john.sedin@mcb.uu.se
- **Seignez, Cedric** post doctoral cedric.seignez@mcb.uu.se
- **Shuai, Hongyan** doctoral student hongyan.shuai@mcb.uu.se
- **Singh, Kailash** doctoral student kailash.singh@mcb.uu.se
- **Sivertsson, Ebba** teaching assistant, +46 18 4714378 ebba.sivertsson@mcb.uu.se
- **Skullerud, Andrine** degree project worker
- **Staaf, Johan** doctoral student johan.staaf@mcb.uu.se
- **Stenlid, Rasmus** degree project worker
- **Strömberg, Victoria** degree project worker
- **Ståhl, Göran** department technician, +46 70 8324455, +46 18 4714153 goran.stahl@mcb.uu.se
- **Sävmarker, Camilla** personnel administrator, +46 18 4714431 camilla.savmarker@mcb.uu.se
- **Tengholm, Anders** professor, +46 18 4714481 anders.tengholm@mcb.uu.se
- **Thonig, Antje** laboratory technician antje.thonig@mcb.uu.se
- **Thorvaldson, Lina** course administrator, +46 18 4714216 lina.thorvaldson@mcb.uu.se
- **Tian, Geng** research assistant, +46 18 4714426 geng.tian@mcb.uu.se
- **Torell, Andreas** degree project worker
- **Turpaev, Kirill** visiting researcher
- **Ullsten, Sara** doctoral student sara.ullsten@mcb.uu.se
- **Vera, Rodrigo Hernández** post doctoral rodrigo.hernandez@mcb.uu.se
- **Vågesjö, Evelina** doctoral student, +46 18 4714324 evelina.vagesjo@mcb.uu.se
- **Waldén, Tomas** lecturer tomas.walden@mcb.uu.se
- **Wang, Xuan** post doctoral, +46 18 4714925 xuan.wang@mcb.uu.se
- **Welsh, Michael** professor, +46 18 4714447 michael.welsh@mcb.uu.se
- **Welsh, Nils** professor, +46 18 4714212 nils.welsh@mcb.uu.se
- **Wentzel, Parri** senior lecturer, +46 18 4714033 parri.wentzel@mcb.uu.se
- **Westermark, Gunilla** professor, +46 18 4714169 gunilla.westermark@mcb.uu.se

- ***Wolgast, Mats*** *professor emeritus, +46 18 4714184 mats.wolgast@mcb.uu.se*
- ***Wuttke, Anne*** *researcher, +46 18 4714426 anne.wuttke@mcb.uu.se*
- ***Xie, Beichen*** *degree project worker*
- ***Xu, Yunjian*** *senior research engineer yunjian.xu@mcb.uu.se*
- ***Yin, Peng*** *doctoral student peng.yin@mcb.uu.se*
- ***Yu, Qian*** *doctoral student qian.yu@mcb.uu.se*
- ***wang, ye*** *doctoral student ye.wang@mcb.uu.se*
- ***Åkerblom, Björn*** *course administrator, +46 18 4714412 bjorn.akerblom@mcb.uu.se*
- ***Ögren, Elin*** *degree project worker*