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H.N. Aguilar, B.F. Mitchell1

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ASSESSMENT OF PARALLEL SIGNALING PATHWAYS IN UTERINE MYOCYTES STIMULATED WITH VARIOUS SMOOTH MUSCLE AGONISTS

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Background: Improved understanding of the regulation of myometrial contractions is essential to develop more successful strategies for prevention of premature birth. Myometrial stimulants activate plasma membrane receptors (GPCRs) responsible for elevating intracellular calcium ($[Ca^{2+}]_i$) and myosin light chain (MLC) phosphorylation via MLC Kinase on Ser 19 (P^{19} MLC). Parallel recruitment of MLCK and the 'calcium sensitization' (CS) enzyme 'ROK' may result in force enhancement beyond that achievable by the rise in $[Ca^{2+}]_i$. Inhibition of MLC Phosphatase by ROK is key in promoting CS. We hypothesized that ROK recruitment is a general feature of GPCR activation by SM contractile agonists in uterine myocytes.

Methods: Primary cultures of human myometrial cells were isolated from biopsies obtained during cesarean section. Phosphorylation was measured by the in-cell-western (ICW) technique.

Results: The SM stimulants tested (oxytocin, prostaglandin F_{2a} , endothelin-1, carbachol, angiotensin II, vasopressin) induced dose-dependant increases in P^{19} MLC in uterine myocytes mediated by MLC Kinase. Pharmacologic inhibition of ROK dramatically reduced both basal and stimulant-induced levels of P^{19} MLC.

Conclusions: In uterine SM, constitutive ROK activity accounts for approximately 50% of the resting level of P^{19} MLC via a constant influence over MLC Phosphatase. During the rising phase of a contractile stimulus, the classical MLC Kinase pathway accounts for the majority of the resultant P^{19} MLC, however the maximum achievable levels of P^{19} MLC are dramatically reduced by ROK inhibition.

TRACTOGRAPHY: A NOVEL TECHNIQUE TO IMAGE FIBER TRACTS OF THE SPINAL CORD

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Background: Traditional magnetic resonance imaging of the spinal cord has very limited clinical correlation. To improve the clinical correlation of MRI, we developed the technique of tractography, a novel MRI technique that utilizes diffusion weighted images to delineate white matter tracts. We utilized tractography to determine whether tractography correlated with patient clinical status and whether it could lead to improved diagnosis compared to routine MRI.

Methods: Healthy volunteers and patients with SC lesions underwent routine MRI (from July 2006 to March 2009) with an added tractography sequence consisting of an axial diffusion-weighted single-shot echo planar imaging sequence using Grappa and 12 noncollinear gradient directions. MEDINRIA version 1.0.31 was used to generate the fiber tracking images. Tractography images were compared with routine MRI and with patient clinical status from the prospectively obtained patient tractography database to determine clinical correlation.

Results: SC tractography in healthy volunteers corresponded to normal anatomical tracts and allowed visualization of tracts as they entered and exited the SC. In patients with SC lesions, tractography enabled the visualization of fiber tracts disruption and displacement by the SC lesion. Tractography with routine MRI was able to determine etiology of neurological deficits and improve diagnosis compared to routine MRI alone.

Conclusions: SC tractography holds promise in improving detection of fiber tract abnormalities to enable improved diagnosis, prognosis and operative management of SC lesions.

MODULATION OF OSTEOCLASTOGENESIS IN INFLAMMATORY JOINT DISEASES

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Background: Bone destruction is of cardinal importance in the pathophysiology of inflammatory arthropathies. Moreover, rheumatoid arthritis has been associated with periarticular bone loss and erosions. Those alterations of bone structure strongly contribute to joint destruction. It has been clearly shown, from animal study, that osteoclasts are essential to joint destruction in rheumatoid arthritis but it is no known how osteoclastogenesis is modulated in this pathology.

Methods: Human osteoclasts were differentiated *in vitro* from PBMC of patients presenting rheumatoid arthritis or healthy controls in the presence of RANKL and M-CSF. The presence of the disease was assessed using the ACR criteria and the activity of the disease using the DAS 28 index. Mature osteoclasts were identified by TRAP staining and apoptosis by TUNNEL assay.

Results: Patients presenting inactive rheumatoid arthritis exhibits an increase osteoclastogenesis and a lower rate of osteoclast's apoptosis compared to healthy controls. Patients presenting inactive rheumatoid arthritis also have an increase osteoclastogenesis compare to patient presenting active rheumatoid arthritis.

Conclusion: This interesting finding suggest that osteoclastogenesis is modulated in rheumatoid arthritis and that the activity of the disease robustly influence osteoclastogenesis. Osteoclastogenesis might therefore be use to help clinicians to assess disease activity in patients suffering from rheumatoid arthritis.

“THE RIGHT THING TO DO? A CRITICAL ANALYSIS OF PUBLIC HEALTH ETHICS, RIGHTS DISCOURSE, AND THE EXPANSION OF ANTIRETROVIRAL THERAPY (ART)”

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Objective: The expansion of HIV treatment in Vancouver's inner city has been discussed in relation to low numbers of individuals accessing ART, changing treatment guidelines, and the reduction of HIV transmission. These justificatory schemas seem to demonstrate convergence of sound ethical, clinical and economic interests. Treatment expansion appears to be an intervention that is coherent, rational and good—an ideal exemplar of the utility of a rights framework for health.

Methods: This paper provides an analysis of ethical considerations surrounding the expansion of ART in a community demarcated by social and economic disadvantage. We draw on 15 months of participant observation, life history and semi-structured, open-ended interviews with individuals in the community clinic, specialist, tertiary care and street-based settings where HIV care is delivered. Participants include women and men living with HIV/AIDS, nurses, physicians and regional health authority programming staff (n= 30).

Results: The subtleties of negotiation, resistance, and acquiescence to the governance of health suggest that “rights” may be insufficiently attentive to the ways in which micronetworks of power shape agency and, by extension, treatment decisions; central issues of poverty and colonialism further trouble a rights framework, given the historical predication of rights on property and citizenship.

Discussion: Although rights-based discourse provides important rhetorical functions in relation to HIV/AIDS, its pervasive and unexamined use may act as a normative “pastoral” power: serving the interests of medical authority, quieting contested decisions, while urging responsibility, self-governance and risk management on the part of ‘citizens.’ Failures in meeting the reciprocal responsibilities attached to rights may further marginalize the most disadvantaged.

COST-EFFECTIVENESS OF IMMEDIATE BASELINE COMPUTED TOMOGRAPHY VS. MAGNETIC RESONANCE IMAGING OF ACUTE ISCHEMIC STROKE IN ONTARIO PATIENTS WHO PRESENT WITH SYMPTOMS SUGGESTIVE OF STROKE.

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Purpose: To evaluate the costs and effectiveness of magnetic resonance (MR) and computed tomography (CT) imaging as the baseline imaging modality for patients with suspected stroke.

Methods: A population-based, retrospective cost-effectiveness analysis was performed, based on data collected by the Registry of the Canadian Stroke Network (RCSN) from a cohort of 34,914 Ontarians with suspected stroke in 2003-2007. Hospital treatment costs were obtained from the Ontario Case Costing Initiative (OCCI) Database, and compared with values in the literature. Costs considered were those accrued in the emergency department and from in-patient stays. For the cost-effectiveness analysis, the study sample was divided into the two imaging groups, and compared in a univariate analysis. Two-tailed *t* tests and chi-squared tests were used to determine differences in case-mix between the patient groups. Outcomes were evaluated on the basis of the difference between: 1) number of in-hospital deaths per group; 2) length of hospital stay per group. The comparison between the imaging modalities was calculated in terms of the incremental cost-effectiveness ratio (ICER).

Results: A total of 14,988 patients met our study eligibility criteria; 97.8% received CT and 2.2% MRI as a baseline imaging modality. Length of stay was estimated at 36.27 vs 9.75 days, while in-hospital deaths were 12.2% and 4.9% for the CT and MRI groups respectively. The resultant ICER was \$538,371/Cdn saved for every in-hospital death avoided.

Conclusion: MRI is the more cost-effective baseline imaging strategy in stroke patients. However, these results should be interpreted with caution, given our study limitations.

CHITOSAN-MEDIATED FGF18 DELIVERY FOR ASSISTED BONE REPAIR

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Rationale: Fracture non-union and allograft failure are significant clinical problems in orthopaedic patients. Current therapeutic options to enhance bone regeneration include bone grafting and protein-based therapy. Although these approaches are adequate for small defects they do not address large nonunion defects that arise from trauma or osteoporosis. Fibroblast growth factor (FGF) 18 was recently identified as an osteogenic factor in vivo.¹ The objective of our research is to target delivery of chitosan/pVax FGF18 plasmid nanoparticles to a segmental defect in the rat femur to expedite bone regeneration and prevent non-union.

Approach: A segmental defect in the rat femur is fixed internally with a polyethylene plate and K-wires to model non-union. Chitosan/pVax FGF18 plasmid nanoparticles will be manufactured as described previously² and delivered to the site of injury. Analytical techniques to quantify bone regeneration and molecular markers of bone cell function will be used as described.³ FGF18 expression and mesenchymal stem cell recruitment and differentiation into osteoblasts at the injury site will be assessed histologically in femurs harvested at 2 and 4 weeks post osteotomy. Bone regeneration repair will be assessed 8 weeks post osteotomy using micro CT to quantify bone architecture and undecalcified histology to evaluate molecular markers of bone cell function.

Significance to human health: A positive outcome will identify chitosan-mediated gene delivery as an effective mechanism for sustained expression of FGF18 to expedite bone repair in critical sized defects or situations of sub-optimal bone quality.

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2. Chevrier A et al. *Osteo Cart* 12007;15: 316-327.

3. Valverde-Franco et al. *Human Mol Genet* 12004;13: 271-284.

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ACTIVE PI3K-AKT SIGNALING PROMOTES THE METASTATIC POTENTIAL OF ASCITES-DERIVED EPITHELIAL OVARIAN CANCER CELLS

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Background: Epithelial ovarian cancer (EOC) possesses a unique mode of metastasis involving direct exfoliation of EOC cells from the primary tumour, aggregation into multicellular spheroids in ascites, and reattachment to form secondary tumours.

Methods: To improve our understanding EOC metastasis, we culture patient ascites-derived EOC cells in suspension where they consistently form 3D spheroids. In addition, we are implementing a novel *ex vivo* assay, where EOC cells and spheroids establish tumour nodules when implanted on the well-vascularized chick chorioallantoic membrane (CAM). These *in vitro* and *ex vivo* systems represent an integrated, physiologically-relevant experimental platform to investigate the underlying molecular mechanisms controlling EOC metastasis. To this end, we have initiated studies of the PI3K-Akt signalling pathway, which is a key mediator of cancer pathogenesis and commonly dysregulated in EOC.

Results: We demonstrate that ascites-derived EOC cells possess active PI3K-Akt signalling; however, this activity is significantly downregulated upon spheroid formation. EOC spheroids have the ability to re-attach and disperse *in vitro*, mimicking the seeding of secondary tumours in patients. Interestingly, EOC cell dispersion from attached spheroids is significantly reduced upon treatment with an Akt inhibitor.

Conclusions: Taken together, these results implicate PI3K-Akt signalling in EOC metastasis. On-going studies involve utilizing our *in vitro* 3D spheroid and *ex vivo* chick CAM systems with many patient samples to evaluate the future therapeutic potential of targeting PI3K-Akt signalling in metastatic EOC.

MECHANISMS OF K65R, D67N, K103N, V106M AND M184V RESISTANCE DEVELOPMENT IN SUBTYPE-B AND C HIV-1

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Background: Understanding how drug resistance development differs in various HIV-1 subtypes is fundamental in tailoring treatment regimens for patients infected with non-subtype B HIV-1. Recent data suggest that a template-based mechanism is responsible for the rapid emergence of the K65R mutation in subtype C HIV-1. Through this study, we wish to further investigate the development of the D67N, K103N, V106M and M184V mutations in subtype B vs. C.

Methods: Recombinant subtype C and B HIV-1 RT enzymes were expressed and purified and gel-based nucleotide extension assays were used to study DNA synthesis from various templates.

Results: The presence of a pause site at a homopolymeric region in the subtype C HIV-1 template at the exact nucleotide position responsible for K65R development is the cause for higher K65R rates in subtype C viruses. On subtype B templates, pausing is seen at the homopolymeric region of codon 67 which is important in thymidine analogue mutation development. A similar homopolymeric region spans codons 103 and 106 of *pol*. The pausing at codons 102 and 105 for both subtype B and C-derived templates confirms previous findings that the rapid emergence of the V106M mutation in subtype C HIV-1 is due to codon usage. Analysis of codon 184 during reverse transcription revealed a strong pausing site that is can only lead to non-viable viruses and has no impact on the rate of M184V development between subtypes.

Conclusions: We have shown that various mechanisms exist and allow for differential drug resistance development in subtype B vs. C HIV-1. These observations warrant further study and reaffirm that drug resistance should be evaluated on a subtype-specific basis.

A MODEL TO DETERMINE FACTORS INVOLVED IN THE INDUCTION OF AN IN VIVO CTL RESPONSE

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Background: Numerous studies have pointed to CD8+ T-lymphocytes as significant players in autoimmunity and anti-tumor immunity. It is therefore important to understand the factors that enable the induction of a cytotoxic T-lymphocyte (CTL) response *in vivo*.

Methods: The RIP-GP mouse ectopically expresses a glycoprotein specifically in insulin-producing pancreatic beta cells. Cytolytic responses towards the glycoprotein result in islet destruction and the induction of diabetes. Because dendritic cells (DC) are crucial to the activation of T-lymphocytes, we have developed a system of DC vaccination, wherein the induction of autoimmune diabetes by CTLs is dependent upon the maturation status of the transferred DC.

Results: We have found that iNOS-deficient DC, unlike their wild-type counterparts, do not require toll-like receptor (TLR) stimulation in order to induce a CTL response upon transfer. Surprisingly, this ability exists in the absence of upregulation of costimulation or pro-inflammatory cytokines. However, unstimulated iNOS-deficient DC display signs of enhanced metabolism, similar to TLR-stimulated wild-type DC.

Conclusion: We hypothesize that iNOS is involved in the negative regulation of a metabolic pathway in unstimulated DC. Furthermore, our work suggests that the metabolic state of transferred DC may be an important determinant in their ability to induce a CTL response upon transfer, and that this occurs independently of cytokines and costimulatory molecules that were previously thought to be essential for the activation of CTLs.

P63 ANTAGONIZES P53 TO PROMOTE THE SURVIVAL OF EMBRYONIC NEURAL PRECURSOR CELLS

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Background: p63, a member of the p53 family of proteins, is involved in the regulation of developmentally-regulated apoptosis in sympathetic neurons of the peripheral nervous system. Our laboratory showed that p63 is expressed in stem cells and neurons within the embryonic brain, and we hypothesized that p63 is involved in regulating the survival of stem cells and developing neurons.

Methods: As neurogenesis is initiated at embryonic day 12, we knocked-down p63 levels in isolated murine cortical precursor cells by using shRNA against p63 or by transfecting floxed-p63 cortical precursors with Cre recombinase. We performed similar experiments *in vivo* using *in utero* electroporation and acutely ablated p63 levels by expressing p63 shRNA or Cre recombinase.

Results: Knockdown of p63, *in vitro* and *in vivo*, resulted in a 2-3 fold increase in apoptosis of cortical precursors and newly-born neurons, without altering cortical precursor cell proliferation. This cortical precursor apoptosis is the consequence of deregulated p53 activity since both basal cortical precursor death and that induced by loss of p63 are rescued by coincident silencing of p53. Finally, we show that the third family member, $\Delta Np73$, does not regulate survival of cortical precursor cells, but collaborates with p63 to regulate survival of newly-born neurons.

Conclusions: This study is the first to identify a role for p63 in the brain, and shows that the balance of $\Delta Np63$ versus p53 determines survival of embryonic cortical precursors, a role that may extend into post-natal and adult life and to other populations of neural precursors and neurons.

SPINAL LOCOMOTOR NETWORK MODULATION BY ENDOGENOUS SEROTONIN IN THE ISOLATED NEONATAL MOUSE SPINAL CORD

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Background: There are networks called central pattern generators (CPGs) that reside in the spinal cord and are sufficient to produce locomotor behaviour in the absence of descending commands.¹ The output of the CPGs can be modulated by monoamines such as serotonin, and monoamine replacement therapies are a therapeutic target in treating spinal cord injury. The focus of our study is to elucidate the role that endogenous serotonin plays in modulating locomotion in a developmental context.

Methods: We record fictive locomotion from the ventral root neurograms of an isolated neonatal mouse spinal cord.² Specific serotonin reuptake inhibitors (SSRIs) are used to increase endogenous serotonin, and their effects on the excitability and rhythm of fictive locomotion are analyzed.

Results: SSRIs produce an overall inhibitory effect. Importantly, motoneuron excitability is significantly decreased, as is left-right and flexor-extensor coordination. There is less of an effect on the rhythm regularity, suggesting that the effects of increased endogenous serotonin are primarily to modulate the excitability of interneurons and not the rhythm-generating kernel.

Conclusions: The modulation of locomotor networks by excess endogenous serotonin is inhibitory. Until now, it had not been demonstrated that endogenous serotonin could modulate locomotor output at such an early developmental stage in mouse. These findings are relevant in cases where development is recapitulated, such as in spinal cord injury.

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THE TUMOR PROMOTING AND REPRESSING EFFECTS OF INTEGRIN-LINKED KINASE ARE DIFFERENTIATED BY JNK1 IN HUMAN CANCER CELLS

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Introduction: While most reports detail an oncogenic function for the protein kinase integrin-linked kinase (ILK) in human cancer, few describe a contradictory growth-suppressive function. Recently we demonstrated that ILK may function as either an oncogene or tumor-suppressor depending on expression of the stress-activated protein kinase *JNK1* in pediatric rhabdomyosarcoma¹. With the advent of bioavailable small molecule inhibitors of ILK kinase activity, defining the role of ILK signaling in different tumors is of critical importance.

Methods: We used murine models and *in vitro* cell culture, in combination with adenoviral overexpression and RNAi-mediated depletion methodologies to interrogate ILK function in a panel of human cancer cell lines.

Results: Screening by proliferation, survival and anchorage-independent growth assays defined two populations of human cancer cell lines, in which ILK signaling suppressed or promoted growth. Consistent with prior reports, kinase-dead ILK (ILK-R211A) could not induce these phenotypes. RNAi-mediated depletion of ILK in xenograft models confirmed these opposing dual functions *in vivo*. Quantitative RT-PCR demonstrated that growth-suppressive cell lines displayed elevated expression of *JNK1*, but not *ILK*, relative to oncogenic cell lines.

Conclusions: These findings indicate that ILK may function as an oncogene or tumor-suppressor protein in multiple human malignancies, in a manner related to expression of JNK1. Further, these results suggest that *JNK1* may represent a biomarker for ILK neoplastic activity, providing a rationale for stratifying patients to receive kinase inhibition therapy based on tumor-specific ILK function.

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INCREASED EXCITATION IN MICE OVER-EXPRESSING NEUROLIGIN-1 IS ASSOCIATED WITH IMPAIRED LONG-TERM POTENTIATION AND LEARNING AND MEMORY

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Purpose: To test the hypothesis that over-expression of the post-synaptic cell adhesion molecule neuroligin-1 (NL1) leads to increase in the ratio of excitation to inhibition. *Materials and*

Methods: Field and whole cell electrophysiology was conducted on pyramidal neurons in the CA1 subfield of the hippocampus to investigate the excitability of this population of neurons as well as individual neurons. Electrophysiology was also employed to conduct synaptic plasticity experiments. Golgi and electron microscopy (EM) were employed to elucidate a morphological basis correlate to the physiological findings at the level of dendritic spines. The Morris water maze (MWM) was used to assess alterations in learning and memory.

Results: Whole cell recordings showed that excitatory post-synaptic currents (EPSCs) are significantly larger in tissue sections obtained from mice over-expressing NL1, while there was no apparent change in the magnitude of inhibitory postsynaptic currents (IPSCs). At the population level, this was associated with a significantly smaller increase in field excitatory post-synaptic potentials (fEPSPs) following blockade of GABA-A inhibition. Correspondingly, Golgi and EM studies revealed more dendritic spines and asymmetrical synapses. Finally, long-term potentiation (LTP) was significantly attenuated in the CA1 subfield and learning and memory was impaired as evaluated using the MWM.

Conclusions: NL1 over-expression leads to an increase in excitatory connections which corresponds to an increase in the ratio of excitation to inhibition measured physiologically. This appears to alter the ability of this neural circuit to show LTP and impairs learning and memory.

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A NOVEL ROLE FOR CDK5/P35 IN MEDULLOBLASTOMA FORMATION

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Background: Medulloblastoma is a highly malignant pediatric brain tumor that kills ~50% of affected patients. It is a heterogeneous cancer that is associated with many abnormalities, including defects in the sonic hedgehog (Shh) signaling pathway and errors in DNA break-repair mechanisms¹. Indeed, mice lacking the tumor suppressor protein, p53, and the DNA break-repair proteins, DNA ligase IV (Lig4^{-/-}p53^{-/-}) or Xrcc2 (Xrcc2^{-/-}p53^{-/-}), possess an inactive Shh receptor (Patched), and form medulloblastoma². p53 is phosphorylated and activated by cyclin-dependent kinase 5 (Cdk5)³, an abundant kinase in brain that depends on p35, or its calpain-cleaved form, p25, for activity. Our objective is to examine the potential involvement of Cdk5 in the Shh signaling pathway that leads to medulloblastoma formation.

Methods: Medulloblastoma from Lig4^{-/-}p53^{-/-} and Xrcc2^{-/-}p53^{-/-} mice, and control cerebellar tissues from corresponding wt mice were isolated, homogenized, and tested for Cdk5 histone kinase activity. Homogenates were also resolved by SDS-PAGE and analyzed by western blotting for Cdk5, p35 and p25 levels, and for calpain activity based on the presence of proteolyzed calpain small subunit.

Results: Compared to wt cerebellum, Cdk5 activity in Lig4^{-/-}p53^{-/-} and Xrcc2^{-/-}p53^{-/-} medulloblastoma is dramatically reduced. Decreased Cdk5 activity in medulloblastoma tissues correlates with the absence of cleaved p25, indicating lack of calpain activity. Calpain activation occurred only in the wt cerebellum.

Conclusion: We propose that lack of Cdk5 kinase activity, resulting from lack of p35 cleavage into p25, is involved in the Shh signaling pathway that promotes medulloblastoma development.

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ALTERED PSYCHOSOCIAL BEHAVIOUR AND STRESS RESPONSE FOLLOWING ‘MINOR’ STROKE IN THE RAT

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Background. Despite vast heterogeneity in size and location, a stroke is considered ‘major’ if it results in lasting motor impairments or aphasia. In contrast, patients that regain a near-normal ability to walk and speak are designated as having a ‘minor’ stroke and discharged despite enduring mental fatigue, emotional lability, or expressed difficulty coping with stress. In the present study we examine behaviour of rodents following chronic mild stress (CMS) following non-motor (‘minor’) stroke.

Methods. Groups of male Sprague-Dawley rats received either sham surgery or focal ischemia of the left cingulate cortex (Cg). After 1 wk subjects were randomized to control handling or CMS for 3 wk. Social observation was used to determine dominance. Behaviour testing followed intermittently for 5 mo.

Results. Cingulate cortex lesions did not cause motor deficits. Hyperarousal and habituation- behaviours sensitive to psychosocial stress- were tested repeatedly using an open field 1 mo post-stroke. Subordinate animals were more hyperactive, but minor stroke induced hyperarousal irrespective of dominance. Minor stroke, but not CMS, significantly attenuated habituation over repeated trials; however, minor stroke combined with CMS caused defecation rates- an index of visceral sensitivity to stress- to be greater in subordinate rats. Additionally, Cg lesions slowed strategy acquisition in the Morris water maze and increased distress vocalizations. Interestingly there were no differences in long-term memory. Assays of hypothalamic-pituitary-adrenal axis function indicate impaired corticosterone signalling.

Conclusions. These findings suggest that ‘minor’ stroke may result in biological vulnerability to stress. Future research should explore mechanisms and treatments for stroke-induced psychosocial disturbance.

TUMOUR PATHOLOGY PREDICTS MICROSATELLITE INSTABILITY IN COLORECTAL CANCER

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Background: Lynch Syndrome is an autosomal dominant trait that accounts for 3% of all cases of colorectal cancer (CRC). It is caused by mutations in DNA mismatch repair genes, causing high levels of microsatellite instability (MSI-H) in the tumours. Approximately 15% of CRCs are MSI-H, and of these about one quarter are due to Lynch Syndrome. MSI testing of all CRCs to identify potential Lynch Syndrome cases is not practical.

Purpose: To create and validate a model for predicting MSI-H tumours, using clinical and pathological features, and to compare the accuracy of our model with existing models

Methods: We collected a population-based cohort of 716 CRC cases diagnosed before age 75 years in Newfoundland. For each case we determined family history, MSI status, and numerous pathological features. Regression analyses were performed to determine the value of the clinical and pathological features at predicting MSI-H tumours. The most powerful predictors were used to create a model. We compared our model with the Revised Bethesda Guidelines (Umar *et. al.* 2004), which is based on clinical features and family history, and the MsPath model (Jenkins *et. al.* 2007), which is based on the histological features described in the 3rd Bethesda criterion.

Results: Our model has a sensitivity of 96% and a specificity of 55% for identifying MSI-H tumours. This is an improvement over the existing models. In our population the MsPath model has a sensitivity of 95% and a specificity of 39%, while the Bethesda Guidelines have a sensitivity of 67% and a specificity of 51%. Our model has been validated in a different cohort, by an independent pathologist.

Conclusions: 1. Predictive models of MSI-H are more accurate, cheaper, and faster when dependent upon histology than when dependent upon family history.

2. Our model demonstrates that histological features other than those used in the Bethesda Guidelines can also be predictive of MSI-H tumours.

PROTEINASE-ACTIVATED RECEPTOR-2 (PAR₂) IS A POTENTIAL TARGET FOR THE ANTI-INFLAMMATORY EFFECTS OF INSULIN

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Background and aims: Trypsin is a potential activator of an important inflammatory mediator, proteinase-activated receptor-2 (PAR₂). Interestingly, trypsin has been known to cause insulin-like effects in tissues by activating the insulin receptor. The aim of this study was to study the possible crosstalk between PAR₂ and insulin receptor signaling pathways in inflammatory settings.

Methods: Synthetic PAR₂-activating peptide (PAR₂-AP) was injected intraplantarly in mice paws and the increase in paw thickness/myeloperoxidase (MPO) activity was measured. PAR₂-AP was also injected intraluminally to induce leukocyte trafficking (visualized by intravital microscopy). In other mice groups, PAR₂-AP was co-injected with insulin. Calcium signaling in endothelial and neuronal cells induced by PAR₂-AP was measured with or without pre-treatment with insulin. Cells were also pre-incubated with phosphoinositide 3-kinase inhibitor (LY294002) or pan-protein kinase C inhibitor (GFX). Lastly, PAR₂-AP was injected into paws of diabetic mice.

Results: Increase in paw oedema and MPO activity induced by PAR₂-AP was reduced in mice co-injected with insulin. PAR₂-AP induced leukocyte rolling and adherence were significantly reduced with insulin co-injection as well. Insulin was able to reduce calcium signaling induced by PAR₂-AP in endothelial and neuronal cells. This effect of insulin was abolished with LY294002 or GFX pre-incubation. Lastly, the degree of paw inflammation (oedema and MPO activity) induced by PAR₂-AP was potentiated in diabetic mice.

Conclusion: Insulin can negatively regulate PAR₂ activity. Thus, the anti-inflammatory effects of insulin may in part be due to inhibiting PAR₂ mediated pathways. This study further highlights the relationship between inflammation and diabetes.

CHEMOSENSITIVE PROPERTIES OF THE VENTRAL MEDULLA IN VITRO

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Background: Respiratory activity in mammals is modulated, in part, by the partial pressure of carbon dioxide (pCO₂) within the brainstem¹. *In vivo* studies have suggested that this effect is mediated by CO₂-sensitive (chemosensitive) neurons within the ventral medulla^{2,3}. The specific location(s) of these cells, however, remains controversial¹. We sought to understand whether chemosensitivity is a general feature of ventral medullary brainstem neurons as has been proposed (distributed theory)², or whether it is the unique property of discrete neuronal populations within the ventral medulla (specialized theory)³.

Methods: Recordings from ventral medullary neurons were obtained in neonatal (P8-18) rat brainstem slices (n=27). Action potential frequency was recorded for the duration of the 35 minute protocol during which the pCO₂ of the superfusate was systematically manipulated (pH 7.18-7.65). Correlation analysis was used to determine whether neuronal activity was associated with extracellular pCO₂.

Results: The majority of sampled neurons (89%; 24/27) were not CO₂-sensitive. A minority (11%; 3/27), all located within the pyramidal tracts, were observed to fire in a CO₂-sensitive manner.

Conclusion: We conclude that the majority of ventral medullary neurons are not chemosensitive *in vitro*. This observation favours the specialized theory of central chemoreception. In addition, our data suggests that a previously unknown population of chemosensitive cells may reside within the pyramidal tracts.

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NOVEL DOPAMINE RECEPTOR-N TYPE CALCIUM CHANNEL INTERACTIONS: POTENTIAL THERAPEUTIC TARGETS FOR DISORDERS ASSOCIATED WITH ABERRANT DOPAMINERGIC SIGNALLING

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Background/Purpose: In the mammalian brain, the major catecholamine neurotransmitter dopamine (DA) is instrumental in regulating numerous physiological functions. Moreover, disturbances in dopaminergic signaling are implicated in several neuropathological disorders. The dorsal striatum, nucleus accumbens, and pre-frontal cortices (PFC) receive dense dopaminergic innervation; both the dopamine type 1 (D1R) and type 2 (D2R) receptor subtypes are highly expressed in these areas. N-type calcium channels are located on dendrites and at pre-synaptic nerve terminals where they are fundamental for neurotransmitter release. It is well established that D2Rs inhibit N-type channel activity. However, the coupling of the D1R to these channels remains poorly characterized.

Conclusion: Collectively, our data provide a novel molecular mechanism to account for dopaminergic regulation of calcium signalling in neurons. Furthermore, they provide a framework for identifying novel therapeutic interventions for disorders associated with aberrant dopaminergic signalling, including schizophrenia, Parkinson's disease and addiction.

TRUNCATION OF THE C-TERMINAL DOMAIN OF CONNEXIN43 INCREASES INFARCT VOLUME DURING STROKE

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Background: Connexin43 is a gap junction protein known to play a role in neuroprotection. Reducing expression of connexin43 in astrocytes enhances injury upon middle cerebral artery occlusion. As the C-terminal region of connexin43 is important for channel activity we have carried out middle cerebral artery occlusion experiments in mice expressing a truncated form of connexin43 (connexin43CT) to determine if this region is important for neuroprotection.

Methods: Middle cerebral artery occlusion was performed on connexin43CT mice, and brain sections were analyzed for infarct volume, astrogliosis and microglial invasion. Adult cortices and astrocyte cultures were examined for connexin43 expression by immunohistochemistry and Western blot. Cultured astrocytes were also examined for susceptibility to cell death, dye coupling, channel conductance, hemichannel activity and Ca²⁺ wave propagation.

Results: Connexin43CT mice exhibit enhanced cerebral injury following stroke. In the peri-infarct region of these mice, astrogliosis was reduced and inflammatory cell invasion was increased. Connexin43 expression was altered in connexin43CT mice. Connexin43CT cells were more susceptible to cell death, were less coupled, and displayed prolonged channel openings reflecting a change in channel gating. Hemichannel activity was increased and Ca²⁺ wave propagation properties were attenuated.

Conclusions: These results suggest that astrocytic connexin43 contributed to the regulation of cell death following stroke and support the view that connexin43CT protects during ischemic insults.

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EVALUATION OF THE DELIVERABILITY AND TOLERABILITY OF INTENSIVE WEEKLY DOUBLET ADJUVANT CHEMOTHERAPY IN NON SMALL CELL LUNG CANCER

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Objectives: Several trials and meta-analyses demonstrate a survival benefit to the use of Cisplatin based adjuvant chemotherapy in completely resected Non Small Cell Lung Cancer (NSCLC). Due to their intensity and a notion that these regimens were not deliverable, many centers in Canada have been reluctant to adopt this practice. We evaluated the deliverability and tolerability of Cisplatin-Vinorelbine chemotherapy in patients with resected stage IB, II and III NSCLC within our center.

Methods: We conducted a retrospective review of patients with NSCLC who received Cisplatin and Vinorelbine adjuvantly following R0 resection at the Juravinski Cancer Center between January 2005 and September 2007. Information abstracted included demographics, toxicities, dose delays, reductions and omissions. Dose intensity (DI) and relative dose intensity (RDI) were calculated.

Results: Forty one patients underwent this treatment. Mean age was 62 yr (SD=8). 52% were female. 81% were ex-smokers. Stage IB, II and III disease were noted in 37%, 39% and 24% of patients respectively. Median DI was 23.5mg/m²/week (range: 13.1 - 27.9mg/m²/week) for Cisplatin and 18.8mg/m²/week (range: 8.4- 25.1mg/m²/week) for Vinorelbine. Median RDI for Cisplatin and Vinorelbine were 94% and 63% respectively. 67% tolerated all cycles of treatment. Toxicities $\geq$ Grade 3 included neutropenia (62%), fatigue(18%), anemia (12%), febrile neutropenia (7%), vomiting (5%), and constipation (2%). Blood transfusions and Erythropoiesis Stimulating Agents were administered to 24% and 18% of patients, respectively.

Conclusion: The deliverability of adjuvant chemotherapy with Cisplatin -Vinorelbine regimen, administered weekly for sixteen weeks, appears as good or better when compared to published data and support the evidence based implementation of this practice.

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A POLICY-ORIENTED SYSTEMATIC REVIEW OF THE SAFETY AND EFFICACY OF ENDOSCOPIC THERAPIES FOR THE TREATMENT OF BARRETT'S ESOPHAGUS

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Background and Methods: Barrett's esophagus (BE) is a metaplastic condition that may progress to high grade dysplasia (HGD) and adenocarcinoma. Endoscopic treatments are increasingly used to eradicate BE +/- HGD. We reviewed the safety and effectiveness of photodynamic therapy (PDT) relative to other treatment options.

A systematic review including studies of any design, treating BE +/- HGD patients using PDT, argon plasma coagulation (APC), cryotherapy, endoscopic mucosal resection (EMR), laser ablation, multipolar electrocoagulation (MPEC), radiofrequency ablation (RFA), thermocoagulation, or esophagectomy, was performed. Data were summarized using tables and simple weighted averages.

Results: 97 studies were included. 6 studies were RCTs. However, the comparisons featured were limited. Quality was low in the remaining studies.

Endoscopic therapies were relatively safe. Photosensitivity and strictures were common with porfimer PDT. One death and a small number of severe complications occurred. In contrast, esophagectomy patients experienced substantial morbidity and several deaths.

Complete response (CR) of BE and HGD occurred in 53% and 78% of porfimer PDT patients. CR of BE $>53\%$ was observed following other treatments modalities, except EMR. CR of HGD $>78\%$ was observed after APC, cryotherapy, EMR, laser, and RFA. However, studies varied highly, especially respecting the number of sessions each patient received. Progression to cancer and recurrence were also reported.

Conclusions: The reviewed therapies were safe and effective to varying extents, and may be viable treatment options. However, the paucity of well-designed studies made it difficult to recommend particular modalities. These findings were fed into an economic decision model (not reported here).

THE SRC-LIKE ADAPTOR PROTEIN, SLAP, PLAYS A ROLE IN MONOCYTE-DERIVED DENDRITIC CELL MATURATION

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Background: The Src-like Adaptor Proteins, SLAP and SLAP2, are hematopoietic proteins involved in the negative regulation of T-cell receptor (TCR) signaling. SLAP and SLAP2 link the TCR and T-cell signaling components with the ubiquitin ligase, c-Cbl, leading to their down regulation and lysosomal degradation. SLAP and SLAP2 contain SH2 domains which allow their interaction with phosphotyrosine motifs on activated receptors. Our recent work revealed that SLAP2 binds to and down regulates the Monocyte Colony Stimulating Factor receptor, M-CSFR. Granulocyte/Monocyte (GM)-CSF, similar to CSF-1, is an important growth factor for the proliferation and differentiation of macrophages that also promotes the *in vitro* generation of Dendritic cells (DC) from monocytic bone marrow progenitors. DC produced with IL-4 and GM-CSF are thought to model the *in vivo* recruitment of monocytes to the DC lineage during inflammatory processes.

Hypothesis: SLAP is expressed in GM-CSF/IL4-derived DC and may be involved in regulating receptors during monocyte-derived DC development.

Methods: Recombinant GM-CSF and IL-4 were used to generate DC from the murine bone marrow. DC were analyzed by flow cytometry 10 days after plating. Stimulations were performed using 100ng/ml of GM-CSF for 5, 10 and 20 minutes at 37°C.

Results: DC from double knock-out and wild-type mice expressed the myeloid markers CD11c and CD11b, however, SLAP1/2-/- DC showed low expression of MHCII, CD86 and CD80 expression in comparison to wild-type cells. These results suggest that DC maturation is dysfunctional in monocyte-derived DC lacking SLAP and SLAP2. We also observed increased levels and hyperactivation of the GM-CSF receptor in these cells, indicating that dysregulated GM-CSF contributes to suppression of DC maturation.

Conclusions: These findings indicate that SLAP negatively regulates GM-CSF in monocyte-derived DC leading to an alteration in DC maturation. This could potentially lead to more tolerance of foreign antigens in SLAP1/2-/- mice and thus less robust immune responses.

SWEET PEE: A NEW MOUSE MODEL FOR GLOMERULOCYSTIC KIDNEY DISEASE AND GLUCOSURIA.

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Background: Cystic kidney diseases represent the major genetic cause of end-stage renal failure in North America. Diabetic nephropathy, on the other hand, is the most common acquired renal disease in developed countries. Recently, there is an increased interest in manipulating renal glucose homeostasis as a potential therapy for diabetes.

Purpose: The aim of this study is to discover genes that are crucial for renal diseases.

Methods: A complementary phenotype-driven approach using N-ethyl-N-nitrosourea (ENU) mouse mutagenesis identified an autosomal dominant heritable renal mutant, SweetPee, which exhibits both glomerulocystic kidney disease and glucosuria. Genome-wide microsatellite is used to generate rough maps and SNPs then further narrows down the critical regions.

Results: To date, over 150 cages have been set up, more than 1500 mice have been born and survived to weaning. Affected mice exhibit dramatic cystic dilation of the Bowman's capsule, and collapse of the glomerular tufts. Although less dramatic, some of these mutants also have cystic changes in other organs. The kidneys are not dysplastic or hypoplastic. Homozygote mutants died within the first 24 hours of birth. They already demonstrated glomerulocystic changes at E17.5 days. Abnormal hepatic vasculature was also observed. The glomerulocystic phenotype maps to a region on the mouse Chromosome 6 syntenic to human Chromosome 7q21-32, not previously been associated with cystic kidney phenotype. Fine mapping identified a 3-MB critical region, consisting of eighteen candidate genes. Sequencing of these genes for point mutations is underway. The glucosuric phenotype is now isolated to a 3.5 Mb region on mouse chromosome 7, within which SLC5A2, the gene encoding for the sodium-glucose cotransporter SGLT2 is identified. To date, no knockout animal models are available for this gene.

Conclusions: In summary, we have identified 2 novel heritable autosomal dominant mutations with reproducible phenotypes of glomerulocystic changes and glucosuria. Further phenotypic and genotypic characterization will elucidate potential pathway(s) for kidney cysts formation, as well as tubular glucosuria. The glucosuric mouse model may be used to investigate if downregulation in expression is protective against end-organ damage in diabetes.

CARDIOGENIC SHOCK IN ASPHYXIATED NEONATE PIGLETS: IS COMBINATION INOTROPE THERAPY BETTER THAN HIGH-DOSE DOPAMINE?

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Purpose: To determine the effect of combination inotrope treatment as an alternative to high-dose dopamine in the resuscitation of asphyxiated newborn piglets.

Hypothesis: In hypoxia-reoxygenated (H-R) neonate piglets, co-administration of dopamine with a second inotropic agent will improve systemic and pulmonary hemodynamics when compared with high-dose dopamine.

Methods: Forty-six newborn piglets (1.5 – 2.5 kg, 1 – 4 days old) were block randomized to one of six groups: sham (SHAM), H-R controls (CONT), high-dose dopamine (DOPA) or combination dopamine and one of dobutamine (DOB), epinephrine (EPI) or milrinone (MIL). Following anesthesia and instrumentation for monitoring of cardiac output, systemic and pulmonary arterial pressures (SAP and PAP, respectively), hypoxia was induced for 2h followed by resuscitation with 100% oxygen for 0.5h, and 21% thereafter. Inotropes were administered for 2h following reoxygenation. Data were analyzed using ANOVA.

Results: Following hypoxia, H-R piglets suffered cardiogenic shock with systemic hypotension and pulmonary hypertension. With infusion of EPI, MIL or DOB, there were no sustained differences in heart rate, SAP, PAP/SAP ratio, cardiac index or systemic or pulmonary vascular resistance, in comparison to DOPA ($p > 0.05$).

Conclusion: With respect to central hemodynamics, no combination treatment studied offers significant advantage over high-dose dopamine alone.

THE RELATIONSHIP BETWEEN FLOW-MEDIATED DILATION, HYPEREMIC SHEAR STRESS, AND VARIOUS ANTHROPOMETRIC INDICES OF OBESITY

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Background: There is controversy over the best anthropometric way to measure 'obesity.' It is unclear whether different measures of obesity are differently correlated with vascular parameters used to assess cardiovascular risk.

Methods: Data from the Firefighters and Their Endothelium study were used. We evaluated the relationship between 3 measures of vascular function: flow mediated dilation (FMD), hyperemic velocity time integral (HVTI), and hyperemic shear stress (HSS), and four measures of adiposity: body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR). Univariate comparisons were made; models adjusted for traditional risk factors were constructed.

Results: Our study included 1462 males (mean age 48.9 years, SD 9.91) without coronary disease. In univariate analysis, the only risk factor associated with FMD was systolic blood pressure (standardized $\beta = -0.0809$, $p = 0.002$). HVTI and HSS were negatively correlated with all measures of adiposity (p -values < 0.0001). In adjusted models, no measure of obesity was associated with FMD. All measures of obesity were associated with HVTI and HSS (p -values < 0.05). A model including traditional risk factors and BMI was most predictive of HVTI ($r^2 = 0.1613$), and a similar model with BMI was most predictive of HSS ($r^2 = 0.1032$). In models including all 4 measures simultaneously, BMI, WC and WHtR were predictive of HSS, and BMI and WHR were predictive of HVTI (all p -values < 0.05).

Conclusions: HVTI and HSS have stronger correlations than FMD to both traditional risk factors and measures of obesity. Anthropometric measures of obesity may help refine our estimations of atherosclerotic burden. Obesity may contribute to cardiovascular disease through its impact on microvascular dysfunction.

RAPID LOCALIZATION OF NEUTROPHILS TO SITES OF CELL DEATH BY MAC1-DEPENDENT ADHESION AND INTRAVASCULAR CRAWLING

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Background: Necrotic cell death is a potent inflammatory stimulus. The pro-inflammatory danger signals released by dead cells have recently begun to be defined, but little is known about how inflammatory cells such as neutrophils sense these danger signals and home to sites of injury *in vivo*. We aimed to elucidate the mechanisms of recruitment that guide neutrophils to sites of cell death using a novel model of local hepatic necrosis.

Methods: A 200-400 µm necrotic burn lesion was induced in the livers of mice. Spinning-disk confocal intravital microscopy was then used to examine the recruitment parameters of fluorescently labelled neutrophils *in vivo* in real-time towards necrotic lesions (labelled with propidium iodide). Various antibodies, inhibitors, and knockout mice were used to study the roles of danger signals and adhesion molecules.

Results: Within the vessels around necrotic lesions, neutrophils promptly (60 mins after burn) adhered to endothelium through Mac1-ICAM1 interactions. Adherent neutrophils then migrated rapidly towards the dead cells via high velocity Mac1-dependent intravascular crawling, thereby infiltrating directly into the lesion and limiting the inflammatory reaction in the surrounding healthy tissue. Preliminary evidence indicates that neutrophil recruitment is activated through a mechanism involving extracellular ATP signalling and NALP3 inflammasome activity.

Conclusion: Neutrophil infiltration at sites of cell death occurs rapidly via Mac1-dependent adhesion and intravascular crawling, enabling neutrophils to quickly localize to the necrotic foci. We believe the rapid and precisely focused recruitment mechanism identified in this study occurs specifically during cell death-induced inflammation as a means of limiting collateral damage to the surrounding healthy tissue.

THE ROLE OF SHIP-1 IN CEACAM1-MEDIATED HOST RESPONSES TO NEISSERIA GONORRHOEAEE INFECTION

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Background: CEACAM1 is expressed on the surface of lymphocytes and acts as a receptor for *Neisseria gonorrhoeae*. Previously, in activated CD4⁺ T cells, our lab has shown CEACAM1 acts as a co-inhibitory receptor via recruitment of the tyrosine phosphatase SHP-1. Inhibition of CD4⁺ T cells is one mechanism by which *N. gonorrhoeae* can evade the adaptive immune response. Recent studies suggest CEACAM1 may act as a dual function co-receptor, able to recruit multiple effector molecules and thereby mediate multiple pathways. SHIP-1 is a 5-inositol phosphatase considered to be a negative regulator of activating pathways such as the PI3K pathway. Negative regulation of phosphoinositide signaling in T cells is not well understood but evidence suggests it is an important mode of signaling. We hypothesize that SHIP-1 is associating with CEACAM1 in response to infection by *N. gonorrhoeae* and negatively regulates phosphoinositide signaling pathways thereby contributing to neisserial inhibition of T cells.

Methods: We assessed the interaction of SHIP-1 and CEACAM1 in response to infection by *N. gonorrhoeae* in a transfected epithelial cell model using immunofluorescent microscopy.

Results: SHIP-1 colocalizes with *N. gonorrhoeae*-bound CEACAM1 but not with bacteria adhering to other receptors. The addition of a phosphatase inhibitor, pervanadate, causes colocalization to persist; suggesting that phosphorylation of the cytoplasmic domain of CEACAM1 is required.

Conclusions: SHIP-1 associates with neisserial-bound CEACAM1 in an epithelial cell model. The CEACAM1-dependent recruitment of SHIP-1 upon *N. gonorrhoeae* binding suggests that SHIP-1 may be negatively regulating phosphoinositide pathways and thereby contributing to neisserial inhibition of T cells.

USING VOLTAGE-SENSITIVE DYES TO RECORD BRAIN ACTIVITY IN NATURALLY MOVING MICE

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Background: A goal of neuroscience research is to understand how neural circuits in the brain control behaviour. Existing techniques generally permit only the study of a small number of neurons. Voltage-sensitive dyes hold excellent promise to circumvent this limitation as they allow recording of brain activity with networks and connections intact, meaning findings are representative of natural functions.

Methods: We have developed the techniques and equipment to record brain activity of mice during whisking and reaching. We adapted a specialized treadmill to work with our existing voltage-sensitive dye recording system (Figure 1). To ensure a stable image of brain activity, we developed a system of small connectors to restrain the mouse being studied without obstructing the brain regions being studied.

Results: This system allows activity over a very large (8 x 8 mm) region of cortex to be recorded. The treadmill can be replaced with a platform to allow reaching to food pellets.

Conclusion: These techniques will allow us to monitor the plasticity in neural circuits that underlies functional recovery following brain injury such as stroke.

Figure 1: Schematic showing equipment developed to record brain activity using voltage-sensitive dyes during reaching and walking.

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POTENTIAL MECHANICAL INFLUENCE IN MICROVASCULAR PATHOLOGY IN THE ACL DEFICIENT RABBIT KNEE

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Background: Rupture of the anterior cruciate ligament (ACL) is a significant soft tissue injury that results in pathological deterioration of the knee joint. Following ACL rupture, the medial collateral ligament (MCL) experiences injurious loads and shows mechanical and anatomic degenerative changes. These changes correspond with development of microvascular pathology in the ligament, both endothelial and vascular dysfunction. Overt MCL injury leads to diminished mechanical and structural properties of the MCL however vascular function has not been evaluated. Differences in vascular function in ACL deficiency as compared to overt MCL injury may highlight diverse inflammatory, healing, or degenerative processes between chronic ligament injury and acute ligament injury.

Methods: 18 New Zealand White Rabbits were assigned to control, ACL transected (ACLt), or MCL transected (MCLt) cohorts. ACL transection was accomplished using anterior lateral capsulotomy approach. MCL transection was performed by surgical exposure and transection of the midsubstance. 100 μ L aliquots of phenylephrine or acetylcholine were applied topically in doses ranging 10^{-10} moles to 10^{-8} moles. Blood flow was measured using laser speckle perfusion imaging and was analyzed using custom software. All values were analyzed for statistical significance using a one-way ANOVA with a Tukey post-hoc.

Results: Phenylephrine decreased blood flow in the MCL of control animals by $-75.4 \pm 7.1\%$ at 10^{-8} moles. Phenylephrine response was significantly decreased in ACL-t rabbits, but was not significantly affected in MCL-t rabbits ($-44.2 \pm 4.3\%$ and $-67.3 \pm 6.1\%$ at 10^{-8} moles respectively). Acetylcholine increased blood flow in the MCL of control animals by $48 \pm 4.5\%$ at 10^{-8} moles. Dilatory responses were not present ACLt rabbits and were diminished in MCL-t rabbits ($-4.0 \pm 0.2\%$ and $15.8 \pm 2.2\%$ respectively).

Conclusion: Vascular pathophysiology is a phenomenon of chronic joint injury and has limited involvement in direct ligament injury. This may reflect differences in inflammatory involvement between chronic joint injury and ligament healing processes which may cause endothelial and vascular dysfunction in the chronically unstable joint, and limited endothelial dysfunction in the healing ligament.

OSTEOBLAST MECHANOSENSITIVITY: THE ROLE OF HYDROSTATIC PRESSURE

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Background: Bone growth and fracture healing are complex processes guided by both chemical and mechanical factors. With regard to the latter, fluid shear stress and hydrostatic pressure are both involved in coordination of tissue growth and remodelling. Of these, hydrostatic pressure (HP) effects are the least well understood, in part due to practical difficulties with experimentation.

Methods: A novel apparatus was developed to allow application of intermittent cyclic HP (ICHP) to *in vitro* cell cultures. This apparatus was used to apply ICHP to either partially differentiated human mesenchymal stem cells (hMSC) or an osteoblast-like cell line, in a variety of experimental situations: 2D monolayer on standard culture dish, 3D collagen matrix, and artificial scaffolds (titanium and polymer) with or without collagen and chondroitin sulphate coatings.

Results: Osteoblast-like cells showed differential responsiveness to ICHP depending on the *in vitro* environment. ICHP loading upregulated matrix metalloproteinases involved in collagen gel contraction, and had a timing-dependent effect on cell proliferation, calcium production and ALP activity on artificial scaffolds.

Conclusion: HP sensitivity and specific responsiveness of osteoblast-like cells appear to be dependent on the differentiation state of the cell. As the mesenchymal stem cell undergoes the transition to osteoblast and then osteocyte, the sensitivity to HP stimulus changes, as does the cell's response to such stimulation. These findings are relevant to clinical practice, as they suggest that properly timed and administered mechanical loading could accelerate and improve *in vivo* fracture healing and osseous integration of joint replacements.

ENDOTHELIAL PROGENITOR CELLS FOR HEALING AND ANGIOGENESIS IN A SEGMENTAL BONE DEFECT MODEL: A COMPARISON WITH MESENCHYMAL STEM CELLS

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Purpose: Fracture healing requires a coordinated coupling between osteogenesis and angiogenesis.¹ The purpose of this study was to compare the effects of two types of stem/progenitor cells on the healing of critical sized bone defects in a rat model. Endothelial progenitor cells (EPCs), a novel cell type with previously demonstrated effects on angiogenesis in animal models of vascular disease², were compared to both a control group of no cell therapy, and a treatment group of mesenchymal stem cells (MSCs).³ The hypothesis was that EPCs would demonstrate both superior bone healing and angiogenesis, when compared to the control group and MSC group.

Methods: EPCs and MSCs were isolated from the bone marrow of syngeneic rats by differential culture and grown *ex vivo* for 10 days. Subsequently the cells were harvested, seeded on a gel foam scaffold, and implanted into a 5mm segmental defect in a rat femur that had been stabilized with a plate and screws. Bone healing was assessed radiographically and by microCT. Angiogenesis was assessed by histology and physiologically, using laser doppler to assess blood flow in the bone and soft tissues.

Results: The EPC group demonstrated radiographic evidence of healing of the bone defect as early as 2 weeks, and all specimens were radiographically healed at 6 weeks. Both the control group and the MSC group showed no radiographic evidence of healing at 10 weeks. MicroCT comparison of the EPC group versus the control group showed significantly greater bone volume and density at the defect site ($p < 0.001$). More blood vessel formation was observed in the EPC group versus the control group on histology at 2 weeks. Laser Doppler assessment showed significantly more soft tissue and bone blood flow at 2 and 3 weeks in the EPC group versus the control group ($p = 0.021$).

Conclusions: EPCs are effective as cell-based therapy for healing critical sized bone defects in a rat model. EPCs are superior to mesenchymal stem cells in this model. EPCs demonstrate superior angiogenesis over controls in a rat model of fracture healing. These results strongly suggest that EPCs are effective for therapeutic angiogenesis and osteogenesis in fracture healing.

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DELAY OF DNA METHYLATION IN PERINATAL MALE GERM CELLS IN THE ABSENCE OF DNMT3L RESULTING IN INFERTILITY.

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Background: DNA methylation is an epigenetic modification involved in gene expression, genome stability, and genomic imprinting that is catalyzed by DNA methyltransferase (DNMT) enzymes. In the germline, methylation patterns are initially erased in primordial germ cells (PGCs) while entering the gonadal ridge and then acquired on CpGs during perinatal gametogenesis. Correct establishment of the germ-cell specific pattern is essential for normal spermatogenesis. Mice lacking DNMT3L, an enzyme involved in germline methylation acquisition, demonstrate spermatogenic failure resulting in infertility. Additionally, germ cell numbers are decreased by Day 6 after birth and methylation is lost at some interspersed repeat elements, all paternally imprinted genes and multiple intergenic sites on chromosomes 4 and X. This study's aim was to investigate DNA methylation in DNMT3L haploinsufficient and deficient perinatal male germ cells.

Methods: *Dnmt3L*^{+/+}, *Dnmt3L*^{+/-} and *Dnmt3L*^{-/-} germ cells were isolated using fluorescence activated cell sorting at 16.5 days post coitum (dpc) and postnatal day 6 from matings between *Dnmt3L*^{+/-} GFP⁺ mice. Primers for 24 intergenic sites spanning chromosome 9 at 5 Mb intervals were used to perform quantitative analysis of DNA methylation using real-time PCR (qAMP).

Results: Results showed a significant loss of 19.4% (p<0.001) and 51.6% (p<0.001) methylation in *Dnmt3L*^{+/-} and *Dnmt3L*^{-/-} prospermatogonia respectively at 16.5 dpc across sites on chromosome 9. By day 6, *Dnmt3L*^{+/-} spermatogonia showed no significant differences from the wild type but in *Dnmt3L*^{-/-} spermatogonia, 11/24 sites were hypomethylated and 9/24 sites had attained complete methylation compared to control. No significant difference was observed in transcripts of *Dnmt1*, *Dnmt3a*, *Dnmt3a2*, and *Dnmt3b* between *Dnmt3L* genotypes suggesting an absence of compensation by other DNMT enzymes.

Conclusions: These results indicate that DNMT3L is critical in the timing of DNA methylation acquisition in the male germ line and that in its absence, methylation is delayed sufficiently to result in infertility.

INVESTIGATING CRMP4 FUNCTION IN CNS NERVE REGENERATION

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Background / Purpose: CNS neurons fail to spontaneously regenerate following injury, in part due to the expression of inhibitory molecules including myelin associated inhibitors (MAIs) and chondroitin sulfate proteoglycans (CSPGs) in the glial scar. The signaling cascades of MAIs and CSPGs converge to activate the cytosolic protein RhoA and influence cytoskeletal dynamics. RhoA antagonists promote neuronal survival and regeneration in animal models of nerve injury. However, the widespread role of RhoA in multiple cellular processes and cell types may limit its potential as a therapeutic target. In an attempt to discover more specific therapeutic targets to promote nerve regeneration, our lab previously identified the cytosolic phosphoprotein CRMP4b (Collapsin Response Mediator Protein 4b) as a protein that interacts with RhoA to mediate neurite outgrowth inhibition. Blockade of the RhoA-CRMP4b interaction with a competitive peptide C4RIP (Crmp4b-RhoA Inhibitory Peptide) attenuates neurite outgrowth inhibition on both myelin and CSPG inhibitory substrates.

Methods: To evaluate the therapeutic potential of C4RIP in vivo, we used an adeno-associated virus (AAV) to express C4RIP in retinal ganglion cells and measured their ability to regenerate after optic nerve injury. We are currently developing a readily deliverable peptide to further investigate the ability of C4RIP to promote nerve regeneration.

Results: Preliminary results demonstrate AAV-mediated C4RIP expression in retinal ganglion cell bodies and their axons. We are currently investigating the ability of C4RIP to modulate regeneration following optic nerve injury.

Conclusion: These studies will continue to evaluate the potential of C4RIP as therapeutic agent to facilitate recovery after CNS injury.

A NOVEL DNA DAMAGE-DEPENDENT REGULATORY PATHWAY FOR AKT IN VIVO

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Background/Purpose: Akt is a serine/threonine kinase that promotes mammalian cell growth and survival as a target of phosphatidylinositol 3-kinase (PI3K) signalling. Using the round worm *Caenorhabditis elegans*, we recently demonstrated that the Akt homologue *akt-1* promotes the survival of germ cells that normally apoptose in response to DNA damage. *akt-1* prevents germ cell death by inhibiting the functions of CEP-1, the sole p53 homologue in worms. In *C. elegans*, a single, conserved PI3K signalling pathway composed of the Insulin-like receptor (InsR/*daf-2*), PI3K (*age-1*), and the 3-phosphoinositide-dependent protein kinase 1 (PDK1/*pdk-1*) functions upstream of *akt-1* to regulate developmental decisions and lifespan. It is not known whether this pathway also regulates the apoptosis-specific function of *akt-1* *in vivo*.

Methods: We used differential interference contrast and epifluorescence microscopy, as well as Real Time PCR to determine the genetic interactions between *akt-1* and other components of the worm PI3K signalling pathway.

Results: We found that loss-of-function mutants in *daf-2/InsR*, *age-1/PI3K*, and *pdk-1/PDK1* were all extremely resistant to ionizing radiation-induced germ cell apoptosis. This demonstrated a potential *in vivo* bifurcation of PI3K signalling, with *akt-1* preserving its anti-apoptotic function and *daf-2/age-1/pdk-1* instead promoting damage-induced cell death. Further experimentation confirmed the genetic independence of *akt-1* from canonical upstream PI3K signalling elements and suggested that *akt-1* is also regulated independently of PDK1-like kinases.

Conclusions: Significant rearrangement of a single PI3K signalling pathway has allowed *C. elegans* to regulate Akt in a stimulus-specific manner *in vivo*. Moreover, DNA damage-dependent regulation of AKT-1 may be accomplished by a novel, PDK1-independent mechanism.

CHOP AS A TARGET FOR PRESERVATION OF TRANSPLANTED ISLET GRAFT MASS

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Background: Pancreatic islet transplantation offers improved glycemic control in type 1 diabetic patients, potentially decreasing macro- and microvascular complications of diabetes mellitus while obviating the need for insulin injections. Long-term success has been limited, however, with fewer than 10% of patients retaining insulin independence at two years post-transplantation. Up to 60% of transplanted graft mass is lost in the first week following transplantation. Excessive metabolic demand upon remaining beta cells greatly increases protein trafficking through the endoplasmic reticulum (ER). Elevated loads of unfolded proteins in the ER lumen activate ER stress responses to restore homeostasis; prolonged activation of these responses may activate the pro-apoptotic CCAAT/-enhancer-binding protein homologous protein (CHOP). It has been shown that CHOP expression is increased in the first week following transplantation.

Methods: To study the role of CHOP in the early post-transplantation period, we performed syngeneic transplants of islets isolated from CHOP-deficient (CHOP^{-/-}) mice or wild-type (WT) C57Bl/6 controls into streptozotocin-diabetic C57Bl/6 recipients.

Results: Upon transplantation of an optimal mass of 300 WT or CHOP^{-/-} islets, normoglycemia was restored immediately. Following transplantation of a marginal mass (100 islets), restoration of normoglycemia was delayed by 2-3 weeks in recipients of WT islet grafts, but was restored much earlier in recipients of CHOP^{-/-} islets. At 1 week post-transplantation, 86% WT islet grafts remained diabetic (blood glucose >15 mM) as compared to only 53% of CHOP^{-/-} islet grafts ($p < 0.05$). At 2 weeks post-transplantation, 57% of recipients of WT islet grafts remained diabetic as compared to 24% of recipients of CHOP^{-/-} islet grafts ($p < 0.05$).

Conclusions: These findings suggest that CHOP plays an important role in islet graft dysfunction in the early post-transplantation period and raise the possibility that ER stress is an important contributor to graft failure.

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TREATMENT OF ACHILLES TENDINOPATHY

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Background/Purpose: The purpose of this research project is to optimize the clinical outcome of patients with Achilles tendinopathy. The incidence of Achilles tendon injuries has increased over the past two decades, with an estimated prevalence of 7-18% in runners.¹ History suggests that somewhere between 24-44% of Achilles tendinopathy patients fail conservative management requiring either significant activity modification or surgery.² Neovascularisation (growth of new blood vessels and nerves into a damaged area of the tendon) appears to be associated with pain and subsequently a decrease in function and use of the affected tendon.³ Sclerotherapy is a procedure whereby the neovascularisation can be removed from the affected tendon. There are no sclerotherapy studies that have administered a dextrose solution to a homogenous Achilles tendinopathy population in a prospective, controlled and randomized manner.

Methods: Triple blinded randomized controlled trial. Patients who fail a standardized physical therapy regiment (eccentric training) will be randomly allocated into two groups: one receiving an injection of hypertonic glucose and Lidocaine (sclerosing solution) and the other receiving an injection of Lidocaine (control) for up to 3 injections over 12 weeks.

Results: The mean difference in VISA-A (composite score assessing pain and function) in the active group was 24.1 (14.1, 34.1), whereas the mean difference in the placebo group was -2.14 (-11.2, 2.9) from baseline to 12 weeks. This difference is considered significant when assessed using ANCOVA ($p=0.0003$).

Conclusion: Sclerotherapy with a solution of 25% dextrose is successful in decreasing pain and increasing function (as determined using the VISA-A) for patients with chronic Achilles tendinopathy.

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PLACENTAL LACTOGEN FUNCTION IN POST-IMPLANTATION MURINE PREGNANCY

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Background/Purpose: In rodents, the placenta produces Placental Lactogen (PL) hormones, detectable in the blood of pregnant mothers from mid-gestation that act through the Prl receptor (PrlR). PL knockout mice have not been reported, but is complicated because there are four PL genes. Prl and PrlR mutant female mice are infertile but in PrlR mutants (129SvJ genetic background) their implantation defect can be rescued by exogenous progesterone (Binart et al 2000). Our aim was to infer PL function by comparing the phenotypes of Prl^{-/-} and PrlR^{-/-} mice, as any differences must be due to effects of PLs.

Methods: Maternal parameters (blood pressure, blood glucose, metabolites, spleen weight), implantation rates and fetal growth were observed in Prl^{-/-} and PrlR^{-/-} mice crossed with wildtype males and supplemented with progesterone.

Results: 1) In the C57/Bl6 background, pregnancy has been established in 13/17 Prl^{-/-} mice versus only 1/7 PrlR^{-/-} mice to date; 2) Pregnant Prl^{-/-} females had normal blood pressure and blood glucose; 3) In Prl^{-/-} mice, ~50% of fetuses detectable at mid-gestation survived to term; 4) All fetuses in Prl^{-/-} mothers had reduced heart rates and crown-rump lengths at mid-gestation and were leaner at term.

Conclusion: Maternal Prl is not required for pregnancy if implantation is rescued with progesterone. The greater severity of the PrlR mutant phenotype implies that PLs, acting through the PrlR, are essential for establishment and/or maintenance of pregnancy. Of interest, embryos in Prl^{-/-} females are developmentally delayed. Whether this is related to Prl deficiency or progesterone supplementation is unclear.

DECODING NEURAL SIGNALS FROM MULTIELECTRODE ARRAYS IN THE PRIMATE DORSOLATERAL PREFRONTAL CORTEX

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Background: Efforts to interface the primate brain with external robotic devices have largely focused on the primary motor area because of its well tuned motor signals, and established somatotopic organisation. However, this area is limited by the musculo-centric reference frame, and its apparent lack of cognitive signals active during states of reward, learning, and conflict between motor plans.

Methods: We surgically implanted microelectrode arrays (100 electrodes) in 2 brain areas, supplementary eye fields (SEF, Brodmann area 6) and dorsolateral prefrontal cortex (dlPFC, Brodmann area 8v) of an adult macacca fascicularis. We recorded neuronal activity during two behavioral tasks. In the first task, the monkey must make delayed saccades to concentric targets. In the second task, the monkey was presented with an arbitrary cue indicating the direction in which to direct gaze, and makes a saccade based on this decision.

Results: Stable recordings have been obtained from > 70% of recording electrodes, in which single unit activity can be isolated. Preliminary analysis of the SEF array showed some neurons exhibiting a response to error trials, but we did not find signals related to motor activity. However, in the dlPFC array, some of the neurons showed saccade related activity.

Conclusion: Neurons in the prefrontal cortex encode complex behavioral signals. Arrays of electrodes can be safely and effectively surgically implanted for chronic use in monkeys. This allows for reliable analysis of neuronal signals to examine network effects, as well as single neuron properties.

THE ROLE OF TRANSFORMING GROWTH FACTOR ALPHA IN A MOUSE MODEL OF OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is the leading cause of physical disability North America. Despite the severity of this disease, its underlying mechanisms are not well understood. Recently, our lab established a rodent model of OA in order to study gene expression changes in degenerating articular cartilage. We found that transforming growth factor alpha (TGF α) gene expression was upregulated in our model, and thus identified TGF α as a novel growth factor involved in OA¹. Further *in vitro* studies showed that TGF α treatment suppressed chondrocyte expression of the anabolic factors aggrecan and type II collagen and increased expression of the catabolic factor matrix metalloproteinase 13 (MMP13)². The purpose of this study is to examine the requirement for TGF α in the progression of osteoarthritis *in vivo*.

Hypothesis: *Tgfa* null mice experience delayed OA progression compared to control littermates in a surgical disease model.

Methods: Ten week old *Tgfa* null mice and their heterozygous littermates underwent meniscotibial transaction (MTX) or sham surgery of the left knee joint. Seven weeks post-surgery, animals were sacrificed and joint histopathology was scored using the Osteoarthritis Research Society International (OARSI) criteria. Tissues were also immunostained for MMP13 expression.

Results: MTX produces mild OA while sham surgery shows no sign of disease at seven weeks post-surgery. Importantly, *Tgfa* null mice have lower OARSI scores and express less MMP13 than their heterozygous littermates.

Conclusions: TGF α appears to play an important role in the progression of osteoarthritis *in vivo*. Future studies will examine the role of TGF α in the cartilage growth plate.

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SKIN-DERIVED STEM CELLS ACT AS FUNCTIONAL SCHWANN CELLS WHEN TRANSPLANTED INTO LESIONED PERIPHERAL NERVE

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Background and Purpose: Peripheral nerve injuries are common and often followed by poor functional recovery. Schwann cells (SCs) distal to the injury are crucial for successful nerve regeneration; however their capacity to support axonal elongation steadily declines with prolonged denervation. Progenitor cells isolated from the dermis (termed Skin-derived precursors or SKPs) can be differentiated into functional SCs that survive within injured nerves. We hypothesized that SKP derived SCs (SKP-SCs) could represent an easily-accessible source of replacement SCs for transplantation into lesioned peripheral nerve.

Methods: Donor SKPs were generated from the dermis of neonatal Lewis rats and differentiated into SCs using established protocols. We assessed the therapeutic potential of SKP-SCs by transplanting them into both acellular nerve graft and chronic nerve injury models and used a combination of electrophysiological and histological measures to evaluate regenerative success. To further characterize SKP-SCs, we carried out various biochemical analyses of cell lysates and SKP-SC-conditioned media.

Results: SKP-SC treatment provided superior axonal regeneration, remyelination, and muscle reinnervation than control media in both surgical paradigms. Analysis of culture supernatant demonstrated that SKP-SCs secrete several bioactive neurotrophic factors that may promote axonal regeneration in injured nerve. Additionally, SKP-SCs actively produce enzymes capable of degrading inhibitory extracellular matrix components, which are upregulated following peripheral nerve injury and normally present a barrier to regeneration.

Conclusions: SKP-SCs elicit a significant beneficial effect on peripheral nerve regeneration via several potential mechanisms acting both at the level of the axon and the surrounding microenvironment. Given this demonstration of utility, SKPs represent an easily accessible, potentially autologous source of stem cell derived Schwann cells for transplantation therapies.

TLR4 MEDIATES SUSCEPTIBILITY TO STREPTOZOTOCIN-INDUCED DIABETES

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Background: Toll-like receptors (TLRs) are a family of germ-line encoded pattern recognition receptors critical to the innate immune response. Recent evidence suggests that TLRs may be involved in the pathogenesis of both type 1 and type 2 diabetes. While functional TLRs are expressed by insulin-producing pancreatic b-cells, their role in b-cell function and islet inflammation remains unclear. In this study, we evaluated the effect of TLR4 on multiple low-dose streptozotocin (STZ)-induced diabetes, a model of islet inflammation in which STZ provides an initial b-cell-specific insult.

Methods: Eight- to ten-week-old male C57BL/10 ScNJ (TLR4^{-/-}), C57BL/6 MyD88^{-/-}, and strain-matched C57BL/6 or C57BL/10 control mice were injected i.p. with 40 mg/kg STZ for five consecutive days. Morning non-fasting tail vein glucose was monitored three times per week. Pancreata were removed three days post-STZ and stained for insulin. Islet viability following *in vitro* culture with STZ was evaluated by Alamar Blue assay.

Results: TLR4^{-/-} mice were significantly more resistant to multiple low-dose STZ-induced hyperglycemia than wild-type controls, while MyD88 deficiency had no effect on diabetes development. Pancreatic islets from TLR4^{-/-} but not MyD88^{-/-} mice exhibited reduced b-cell damage and improved islet integrity compared to wild-type controls three days post-STZ.

Conclusion: Our results suggest that TLR4 may play a previously unappreciated role in mediating b-cell susceptibility to STZ or subsequent islet inflammation, and that this effect may occur independently of the downstream adaptor MyD88. Further studies are underway to characterize TLR signalling pathways in b-cells and to identify endogenous TLR ligands that may affect b-cell function.

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**A FUSION OF GMCSF AND IL-21 (GIFT-21)
POTENTLY INDUCES INFLAMMATION AND
APOPTOSIS THROUGH SIGNALS
DOWNSTREAM OF THE IL-21R ALPHA
CHAIN**

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Background: We have previously demonstrated a therapeutic pro-inflammatory synergy by fusing GMCSF and IL-2 for cancer immunotherapy (Stagg et al. Cancer Research 2004). Thus our previous data serves as a rationale for the generation of GMCSF-based cytokines with potent anti-cancer properties. We hypothesized that GIFT-21 would lead to synergistic anti-cancer effects because of the respective roles of each cytokine in mediating inflammation.

Methods: We manufactured a synthetic transgene coupling GMCSF as the N-terminal domain to full-length IL-21 as the C-terminal domain (GIFT21). Transduced B16 melanoma cells were shown to secrete the fusion and were implanted in immune competent and deficient mice. Immune cells were characterized using flow cytometry and ELISA. We have also transduced mesenchymal stromal cells to deliver GIFT-21 *in vivo*.

Results: Transduced B16 melanoma did not develop in immune competent C57Bl/6 mice and growth was delayed in NOD SCID mice. Further mechanistic analysis demonstrated that GIFT21 promoted the differentiation of M1 macrophages. We have also demonstrated that GIFT-21 could deplete IL-21Ra expressing lymphocytes and that this was dependent upon STAT1. Only GIFT-21 expressing MSCs could significantly delay the appearance and development of IL-21Ra+ EL-4 lymphoma tumors implanted subcutaneously in C57Bl6 mice.

Conclusion: This data indicates that GIFT-21 is promising as a selective killer of IL-21Ra expressing malignancies and as a modulator of inflammation.