The role of macrophages in mammary stem cell development.

Gyorki DE, Lindeman G and Visvader J

Introduction:
Increasing evidence suggests that macrophages play a role in the normal development of certain organs, including the breast\(^1\). Intriguingly, macrophages are often found in the stroma of breast tumours, where they may promote tumour growth and metastasis. The mechanism underpinning the requirement for macrophages during normal development may involve the mammary stem cell. Recently, a population enriched for mammary stem cells has been prospectively identified in mice\(^2\). Our study aims to determine whether macrophages are required for normal mammary stem cell function.

Methods:
The role of macrophages in mammary development was studied in mice with an inactivating mutation in the CSF-1 gene (CSF\(^{op/op}\)). Transplantation assays were performed where a mammary stem cell (MaSC) enriched population was prepared by cell suspension from the epithelial subpopulation using flow cytometry. The MaSC-enriched cells were then transplanted into the mammary fat pads of three-week old mice cleared of endogenous mammary epithelium. Cells were co-injected with liposomes containing either clodronate (which is toxic to macrophages) or a saline control.

Results:
Macrophages are closely apposed to the mammary epithelium throughout the epithelial tree. Mammary outgrowths are significantly impaired in both frequency and morphology when reconstituted in vivo in a macrophage deficient milieu.

Conclusions:
Macrophages play a role in supporting stem cell growth during mammary development. This may relate to a role in the stem cell niche. Understanding the mechanisms by which mammary stem cells interact with macrophages during mammary development will shed light on why macrophage infiltration portends a poor prognosis in breast cancer.


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**Blockade With Soluble ICOS-Ig Prolongs Survival of Cellular Xenografts**

Hodgson R, Christiansen D, Al-Obaidi S, Ierino FL, Sandrin MS

**Introduction:**
Xenografts are one possible solution to the lack of donor organs for diseases such as Diabetes Mellitus. T cell costimulatory pathways are integral to acute cellular rejection against these grafts. Inducible Co-Stimulator (ICOS) pathway blockade has been shown to prolong allograft survival, but there is limited data for xenograft models.

**Methods:**
Porcine Iliac Endothelial cells (PIEC) were transfected with cDNA of the fusion molecule ICOS-Ig. Intracellular and secreted expression was confirmed and quantified using immunoperoxidase staining and Western Blot analysis. *In vitro* testing of supernatant in mixed lymphocyte reactions was performed. *In vivo* survival was examined using a subcutaneous graft model in mice.

**Results:**
ICOS-Ig containing supernatant gave a 99.5% reduction in proliferation of an allograft mixed lymphocyte reaction. Similarly, xenogeneic proliferation was inhibited by 84.3%. In addition an 84.5% reduction in proliferation was observed when PIEC expressing ICOS-Ig were used as stimulators. PIEC-ICOS-Ig xenografts showed prolonged survival compared to wild-type PIEC xenografts (mean survival 34 vs 12 days, p=0.0025) in a subcutaneous graft models in Balb/c mice.

**Conclusion:**
Blockade of T cell co-stimulation by the fusion molecule ICOS-Ig has been demonstrated to decrease proliferation in allograft and xenograft *in vitro* models. Further, there is significant prolongation of survival of PIEC transfected with ICOS-Ig *in vivo*. These data suggest that further investigations for the role of T cell co-stimulatory blockade, through local expression of ICOS-Ig, in xenografts are warranted.

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**Lymphatic connections between the pancreas and intestine: Do they exist?**

Benjamin PT Loveday¹ MBChB, Anubhav Mittal MBChB², Saad Al-Ali PhD³, John A Windsor⁴ MBChB MD FRACS, Anthony Phillips⁵ MBChB PhD

**Introduction:**
Mesenteric lymph (ML) can be toxic following shock or carry infection during bacterial translocation. Toxic lymph exacerbates acute pancreatitis, and translocated bacteria can infect pancreatic necrosis. It is unknown whether ML can reach the pancreas directly via lymphatics. The aims of this study were to identify and characterise lymphatic connections between the pancreas and intestine, and determine if ML can reach the pancreas directly.

**Methods:**
In diabetic (n=30) and non-diabetic (n=15) Wistar rats, four lymphatic preparations were used: retrograde cannulation of the mesenteric duct (MD) or thoracic duct (TD), and antegrade cannulation of the MD with partial or total TD obstruction. Gadolinium, dye, resin, or fluorescent microspheres were perfused into the lymphatics. Lymphatic connections were identified visually (dye/resin), with fluorescent microscopy (microspheres), and with magnetic resonance imaging (MRI, gadolinium). Lymphatic pressures were measured during perfusions.

**Results:**
Median MD pressures at baseline and during partial and total TD obstruction were 2.3, 4.5, and 32.9 cmH₂O respectively. Enhancement of parts of the pancreas was seen on MRI. Efferent lymphatics from pancreatic head lymph nodes drained into the MD. When lymphatic valves were absent or incompetent, dye, resin and microspheres passed retrograde from the MD through pancreatic lymph nodes, afferent lymphatics and into the pancreatic parenchyma.

**Conclusion:**
Functional lymphatic connections were identified between the intestine and pancreas through pancreatic head lymph nodes when valves were absent or incompetent. Further research is required to determine if mesenteric lymph can induce toxicity and infection in the pancreas via these lymphatic connections.

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Trends in the Minimally invasive Management of Necrotizing Pancreatitis: A Survey of Australian and New Zealand Surgeons

Benjamin PT Loveday¹ MBChB, Jeremy Rossaak MBChB PhD FRACS², Anubhav Mittal MBChB³, Anthony Phillips⁴ MBChB PhD, John A Windsor⁵ MBChB MD FRACS

Introduction:
Minimally invasive techniques to manage infected pancreatic necrosis have been recently developed, and changes in their pattern of use are not known. The aims of this survey were to determine, in Australia and New Zealand: 1) the trends in the role of minimally invasive techniques to manage infected complications of necrotizing pancreatitis; and 2) barriers to using minimally invasive necrosectomy.

Methods:
In 2007, members of the Australian and New Zealand Hepatic Pancreatic and Biliary Association were surveyed. Participant demographics and necrotizing pancreatitis caseload were determined. The role of percutaneous catheter drainage and minimally invasive necrosectomy in the management of pancreatic abscess, infected pseudocyst and infected pancreatic necrosis were scored on Likert scales, comparing 2002 with 2007. Barriers to using minimally invasive necrosectomy were scored.

Results:
The response rate was 49% (44/90). Between 2002 and 2007, percutaneous catheter drainage became more important role as primary (p=0.05) and secondary (p<0.01) treatment for pancreatic abscess, and prior to minimally invasive necrosectomy for abscess, pseudocyst and necrosis (p<0.01). Minimally invasive necrosectomy became increasingly important as primary treatment for infected necrosis (p<0.01), and had been used by 47% of respondents. The greatest barriers to using minimally invasive necrosectomy were lack of training and experience in the techniques, and the anatomical position and complexity of the target lesion.

Conclusion:
Minimally invasive techniques are becoming increasingly important to manage pancreatic abscess, infected pseudocyst and infected pancreatic necrosis. Further evidence is required to determine the best techniques for treating each form of infection associated with necrotizing pancreatitis.

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Adhesion Formation and Modulation in the Rat Model

Chris Lauder

Introduction:
This project tests the hypothesis that abdominal adhesions may be reduced by the application of a Chitosan-Dextran (CD) gel to the intraperitoneal cavity. This is a unique synthetic gel form of chitosan, a biocompatible polymer derived from squid exoskeleton.

Methods:
Adhesions were formed in a standardised rat model by two methods:

1. Bowel resection group: A 1cm enterotomy was made in the caecum, closed with a continuous 6/0 prolene suture

2. Abrasion group: Caecal abrasions over 1cm² were formed by rubbing with sterile gauze until sub-serosal haemorrhage developed.

Sixty rats were randomly assigned to four experimental groups as follows:

Group 1 = Bowel resection + CD gel
Group 2 = Bowel resection only
Group 3 = Abrasion + CD gel
Group 4 = Abrasion only

Rats in the treatment groups had 3ml of CD gel administered into the abdominal cavity at the end of the procedure, the control group received no additional treatment.

Animals were euthanized at day 21 by CO₂ inhalation and adhesions graded by an investigator blinded to treatment groups using a standardised adhesion assessment scoring system.

Results:
Twenty six rats have had adhesion grade assessed so far. The mean adhesion score for the control group was 3.1 whilst CD treatment group was 0.1

Conclusions:
Chitosan-Dextran gel appears to significantly reduce the formation of intra abdominal adhesions without adversely affecting wound or anastomotic healing.

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The effect of warmed, humidified insufflation on pain after laparoscopy: a meta-analysis.

Tarik Sammour, Arman Kahokehr, Andrew G Hill

Introduction:
Studies on the use of warmed and humidified insufflation (WHI) in laparoscopic abdominal procedures have been inconclusive due to small sample sizes resulting in significant type II error. The objective of this study was to perform a meta-analysis of randomised controlled trials to assess the effect on post-operative pain, of warming and humidifying the laparoscopic insufflate.

Methods:
An electronic database search identified all randomised controlled trials on adults undergoing elective laparoscopic abdominal surgery under general anaesthetic, where the exposure group had WHI, and the control group had standard cold and dry carbon dioxide insufflate. The outcome measure was pain by visual analogue score, or morphine equivalent usage. Analysis of combined data was performed using RevMan version 4.2 (The Nordic Cochrane Centre, Rigshospitalet, Norway).

Results:
7 RCTs were included in the analysis. Of these 3 were for gastric bypass, 2 for laparoscopic cholecystectomy, 1 for Nissen fundoplication, and 1 for gynaecological procedures. A total of 322 patients were included, with 160 in the HWI group and 162 in the control group. In the WHI group, there was a significant reduction in pain scores at 6 hours ($p < 0.006$), day 1 ($p = 0.010$), and day 3 ($p < 0.0001$); and in morphine equivalent usage on day 2 ($p = 0.040$).

Conclusion:
Humidification and warming of laparoscopic gases reduces postoperative pain after laparoscopy. Further studies are required in procedures where the insufflation time is prolonged and the peritoneal desiccation extensive.

Table 1: Summary of combined results.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Overall effect ($Z$)</th>
<th>p value</th>
<th>Mean Difference</th>
<th>CI</th>
<th>Chi²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain at 6 h</td>
<td>2.75</td>
<td>0.006 *</td>
<td>-0.39</td>
<td>-0.67 to -0.11</td>
<td>5.97</td>
<td>0.200</td>
</tr>
<tr>
<td>Pain on day 1</td>
<td>2.47</td>
<td>0.010 *</td>
<td>-0.34</td>
<td>-0.61 to -0.07</td>
<td>14.02</td>
<td>0.02 c</td>
</tr>
<tr>
<td>Pain on day 2</td>
<td>0.17</td>
<td>0.870</td>
<td>0.03</td>
<td>-0.27 to 0.32</td>
<td>13.07</td>
<td>0.010 c</td>
</tr>
<tr>
<td>Pain on day 3</td>
<td>4.02</td>
<td>&lt;0.0001 *</td>
<td>-0.88</td>
<td>-1.3 to -0.45</td>
<td>3.87</td>
<td>0.140</td>
</tr>
<tr>
<td>Morphine at 6 h</td>
<td>0.46</td>
<td>0.650</td>
<td>-0.07</td>
<td>-0.37 to 0.23</td>
<td>4.84</td>
<td>0.090 c</td>
</tr>
<tr>
<td>Morphine on day 1</td>
<td>1.59</td>
<td>0.110</td>
<td>-0.33</td>
<td>-0.74 to 0.08</td>
<td>1.85</td>
<td>0.400</td>
</tr>
<tr>
<td>Morphine on day 2</td>
<td>2.01</td>
<td>0.040 *</td>
<td>-0.48</td>
<td>-0.95 to -0.01</td>
<td>2.13</td>
<td>0.140</td>
</tr>
</tbody>
</table>

CI: confidence interval
Chi²: Chi squared test of heterogeneity,
* statistically significant ($p < 0.05$) for overall effect
\(c\) statistically significant ($p < 0.1$) for Chi²

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Early Organ Specific Mitochondrial Dysfunction Seen During Experimental Acute Pancreatitis


Introduction:
Multiple organ dysfunction syndrome (MODS) is the main cause of death in severe acute pancreatitis. Primary mitochondrial dysfunction is now thought to play a central role in the development and progression of organ failure in critical illness. The aim of this study was to investigate mitochondrial function in seven tissues during early acute pancreatitis in two experimental rodent models.

Methods:
Twenty-eight male Wistar rats (463 ± 2 g; mean ± SEM) were studied. Group 1 (n = 8), saline control; Group 2 (n = 6), caerulein induced acute pancreatitis; Group 3, (n = 7) sham surgical controls; Group 4 (n = 7), taurocholate induced acute pancreatitis. Animals were euthanased at 6 h from the start of the experimental protocol and mitochondrial function was assessed in the heart, lung, liver, kidney, pancreas, duodenum and jejunum. Mitochondrial function was assed using a substrate and inhibitor protocol on the Oroboros machine.

Results:
Significant mitochondrial dysfunction was present in the pancreas, lung and jejunum in the models of acute pancreatitis compared to control groups. The heart, liver, kidney and duodenal mitochondria were unaffected.

Conclusions:
This study found an early and unexpected inhibition of mitochondrial function present selectively in the lung and jejunum of rats with early pancreatitis. Subsequent failure of these two organ systems is implicated in the morbidity and mortality associated with severe acute pancreatitis and MODS. These findings suggest that early mitochondrial dysfunction occurs selectively in certain organs remote from the pancreas as a key event during early pancreatitis. Further research is now needed to evaluate the use of early mitochondrial specific therapies in early acute pancreatitis.

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Proteomic Changes in Mesenteric Lymph Secondary to Haemorrhagic Shock


Introduction:
Biologically active factors produced by the intestine and transported by the aqueous and protein fraction of mesenteric lymph are now thought to contribute significantly to the development of distant organ failure in haemorrhagic shock. Despite the likely relevance of the protein composition of mesenteric lymph conditioned by haemorrhagic shock, there is no detailed description of its proteome.

Methods:
20 male Wistar rats were randomized to either control or haemorrhagic shock group. Mean arterial pressure was reduced to 30 mmHg in the shock group for 90 minutes followed by resuscitation back to baseline and mesenteric lymph collection for another 90 minutes. Control group had identical surgery to the shock group. Proteomic changes in mesenteric lymph due to haemorrhagic shock were identified using LC-MS/MS.

Results:
245 proteins were identified in mesenteric lymph, including 35 hypothetical proteins. 60 of the 245 proteins had a significant increase in their relative abundance in the haemorrhagic shock group vs. sham group. Potentially toxic pancreatic enzymes were identified in mesenteric lymph and had increased relative abundance post-shock. In addition, a bioinformatics approach allowed for the identification of key pathways relating to defence and metabolic responses. A number of potentially important proteins were also identified as targets for future research in hemorrhagic shock: peroxiredoxins, 14-3-3 zeta/epsilon, calmodulin, cofilin, and profilin.

Conclusions:
In conclusion, this study not only provides the first comprehensive list of protein changes in the haemorrhagic shock conditioned mesenteric lymph, but also provides rational for future research.

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Stereotactic Image-Guided Navigation in Perforator Flap Reconstructive Surgery: A Prospective Trial and Comparison of Techniques

Warren M Rozen, Mark W Ashton, G Ian Taylor AO.

Introduction:
Perforator flap surgery is widely used for oncological and trauma reconstruction, requiring the identification of vessels <1mm in diameter. We recently described computed tomographic angiography (CTA) for preoperatively imaging these perforator vessels, which has since been shown to maximise operative success and minimise operative morbidity. Image-guided stereotactic surgery is a recent technique, used with success in several fields of surgery, but has not been described in reconstructive surgery.

Methods:
A study was undertaken to determine the feasibility of CTA-guided stereotaxy in perforator flap surgery. Through imaging 130 patients with CTA, a standardised protocol was established. Nine consecutive patients undergoing elective surgery were recruited, with each patient imaged with Doppler ultrasound, conventional CTA and CTA-guided stereotactic imaging. ‘Registration’ was performed using three available modalities: fiducial marker, surface-landmark and surface-laser registration. Imaging findings were compared to operative findings.

Results:
All major perforators were accurately localised with stereotactic imaging and with conventional CTA. Both modalities were more accurate than Doppler ultrasound. Stereotactic navigation demonstrated a slightly better (0.2cm) correlation with perforator location than conventional CTA. Fiducial marker registration was successful in all cases, while surface-landmark and surface-laser registration were unable to register in all cases.

Conclusions:
CTA-guided stereotactic imaging is highly accurate for imaging perforators, and can achieve ‘real-time’ virtual surgery. This can provide additional data to conventional CTA. Fiducial marker registration was achieved in all cases, is highly accurate, and was the only reliable registration process. With no additional scanning required, this allows the combined use of both modalities.

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Erythropoietin improves functional recovery of the rat heart after prolonged cold storage in Celsior solution

Alasdair J. Watson, Ling Gao, Jireh Tsun, Jair C Kwan, Andrew Jabbour, Peter S Macdonald, Mark Hicks

Introduction:
Recombinant human erythropoietin (rhEPO) has been shown to protect against myocardial ischaemia-reperfusion (I-R) injury in animal models of regional and global warm ischaemia. We sought to evaluate its potential use as an adjunct to Celsior solution in our ex vivo rat model of global, prolonged cold myocardial ischaemia.

Methods:
Male Wistar rat hearts were rapidly excised, mounted on a modified Langendorff apparatus, and perfused via the left atrium in working mode. Baseline functional indices (aortic flow, coronary flow, cardiac output, heart rate, pulse pressure) were measured prior to arresting the heart. The following groups were assessed (each n = 6).
1. Celsior (CON)
2. Celsior + rhEPO 1U/mL (EPO1)
3. Celsior + rhEPO 5U/mL (EPO5)
Hearts were stored at 4°C for 6 hours. After 30 mins reperfusion functional parameters were recorded and expressed as percentage recovery of baseline.

Results:
Recovery of functional parameters (expressed as mean ± SEM) after 6 hours are expressed in the following table:

<table>
<thead>
<tr>
<th></th>
<th>Aortic Flow</th>
<th>Coronary Flow</th>
<th>Cardiac Output</th>
<th>Heart Rate</th>
<th>Pulse Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>1.7 ± 0.4%</td>
<td>4.7 ± 0.6%</td>
<td>2.6 ± 0.4%</td>
<td>38.3 ± 12.7%</td>
<td>19.4 ± 2.8%</td>
</tr>
<tr>
<td>EPO1</td>
<td>48.5 ± 11.2%</td>
<td>72.9 ± 14.0%</td>
<td>55.3 ± 11.7%</td>
<td>101.3 ± 13.7%</td>
<td>82.7 ± 13.3%</td>
</tr>
<tr>
<td>EPO5</td>
<td>46.0 ± 11.5%</td>
<td>70.0 ± 13.4%</td>
<td>52.5 ± 11.3%</td>
<td>92.8 ± 9.9%</td>
<td>88.5 ± 28.8%</td>
</tr>
</tbody>
</table>

EPO1 and EPO5 hearts demonstrated significantly improved recovery of all functional parameters compared to CON hearts (p < 0.05). There were no differences in recovery between EPO1 and EPO5 hearts.

Conclusions:
rhEPO at a dose of 1U/mL conferred protection against I-R injury in this model of prolonged global cold ischaemia. There was no additional benefit when the dose was increased to 5U/mL. To our knowledge, this is the first demonstration of the potential use of rhEPO for myocardial preservation in the setting of cardiac transplantation. Further studies are underway to determine the optimal dose, timing of administration, and molecular pathways involved in this form of cardioprotection.

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Evidence Based Plastic Surgery: Is Australian Research Keeping Up With The Global Trend?

Dhar, S.C.

Introduction:
In the past, the plastic surgical literature has been criticized for lacking proper study designs. A recent comprehensive review of the literature suggests that the quality of scientific papers has improved globally since 1990. It is unknown if Australian research in particular has shown a similar trend towards best evidence clinical practice.

Methods:
A hand search was conducted identifying original articles published in 1997 and 2007 by Australian research units in three plastic surgery journals, Annals of Plastic Surgery, British Journal of Plastic Surgery, and Plastic and Reconstructive Surgery. All randomised control trials and controlled clinical trials were assessed based on established quality criteria.

Results:
In the past ten years, the proportion of studies using level 1 “best” evidence has increased. Under reporting of sample size calculations continues to exist.

Conclusions:
Plastic surgery research in Australia has responded to the need for well-designed outcome studies thus moving away from empiricism.

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Introduction:
Patient with hand injuries is a common encounter in any Accident & Emergency Department. Spectrum of injuries vary from minor injuries warranting outpatient management to severe injuries requiring multidisciplinary involvement. Aim of this study is to audit the causes and types of hand injuries admitted to a singled surgical unit.

Methods:
A prospective audit involving forty eight consecutive patients admitted with hand injuries to the Accident Service under the care of the University Surgical Unit of Colombo South Teaching Hospital. The study was conducted for a period of six months. Hand injuries sustained beyond the level of the distal anterior wrist crease were included. Patients requiring only reassurance and those with multiple injuries were not included. The initial assessment was made by the first or the second author in all the cases and the data were collected with an interviewer administered questionnaire. The recorded data included patients demographics, the aetiology, type of injury (fracture was confirmed by the X-Ray), anatomical area of involvement, handedness and the duration of hospital stay.

Results:
Fifty percent of the hand injuries occur between 20 – 41 years age group. There was a male preponderance (Male Female ratio is 11:1). Twenty three of forty eight injuries are due to occupational accidents followed by domestic and road traffic accidents. Hand injuries sustained at carpentry were the commonest of occupational injuries (12/48). Commonest types of injury is a fracture (22/48) followed by superficial cuts and lacerations. Most patients showed right hand dominance (47 Right, 1 Left) and the injuries were common in the dominant hand. Index finger involvement is seen in 50% of the cases followed by ring and middle finger. Hospital stay was 2-5 days in the majority (33/48)

Conclusion:
Hand injuries are commonly a problem in the middle aged males and mostly related to the occupational injuries. Adoption of safety measures at work in this population should minimise the occupational hand injuries and economic loss.

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