INTESTINAL TRANSPLANTATION

Experimental and clinical studies

Mihai Oltean

UNIVERSITY OF GOTHENBURG

Departments of Surgery and Transplantation

Institute of Clinical Sciences

Sahlgrenska Academy, University of Gothenburg

Gothenburg, Sweden

2010

A Doctoral Thesis at a university in Sweden is produced either as a monograph or as a collection of papers. In the latter case, the introductory part constitutes the formal thesis which summarizes the accompanying papers. These have either already been published or are manuscripts at various stages (in press, submitted, or in manuscript)

''Make everything as simple as possible but not simpler''

Albert Einstein

Intestinal transplantation. Experimental and clinical studies
Mihai Oltean
Departments of Surgery and Transplantation, Institute of Clinical Sciences
at Sahlgrenska Academy, University of Gothenburg
Gothenburg, Sweden, 2010

ABSTRACT

Intestinal preservation-reperfusion injury may result in various degrees of mucosal injury. Interestingly, the preservation injury is similar when using the current preservation solutions, which are given as a intravascular flush. An extensive mucosal injury may ultimately preclude the use of organs that require longer preservation time. The intestine lacks a noninvasive rejection marker, as in the case of liver or kidney transplantation. Several bio-molecules have been suggested as biomarkers, yet their specificity is only partial.

Methods: Using a rat intestinal transplant model we studied the pharmacologic donor preconditioning and the intraluminal preservation with two different macromolecular solutions as means to decrease the intestinal preservation-reperfusion injury. We also investigated the impact of donor preconditioning on the ensuing systemic inflammatory response after transplantation. We analyzed resistin levels after clinical intestinal transplantation and seek to establish its significance and potential as rejection marker.

Results: Intraluminal introduction of low-sodium macromolecular solutions resulted in improved morphology after 8h and 14h of preservation compared with controls receiving only vascular flush with UW-solution. Moreover, intraluminal high-sodium solutions appear detrimental. These solutions also seem to influence differently the TJ conformation during preservation and de-localization of claudin-3 and ZO-1 was more prominent in intraluminal high-sodium solutions.
Following transplantation, pretreated grafts showed accelerated repair and improved morphology. Pretreated grafts revealed reduced NF-kappaB activation after reperfusion and subsequently blunted ICAM-1 expression and PMN sequestration. Pretreated graft recipients had milder liver injury and lower levels of the pro-inflammatory cytokines TNF-alpha, IL-1beta and IL-6 than recipients of untreated grafts.
Resistin levels were studied in seven patients receiving intestinal grafts. Resistin increased in all patients compared with controls and remained increased even during uneventful course. Resistin did not correlate with CRP, BMI, procalcitonin or WBC and it varied greatly between patients.

Conclusions: Preservation-reperfusion injury may be mitigated by the intraluminal introduction of macromolecular solutions or by donor pretreatment with Tacrolimus before graft harvesting. Tacrolimus-pretreated grafts trigger a lower remote organ injury and lower systemic inflammatory response. Plasma resistin levels greatly and were increased in all patients. However, the increase was unspecific and varied between individuals. Resistin appears unsuitable as rejection marker after intestinal transplantation.

Keywords: intestinal preservation, ischemia-reperfusion injury, tight junction, tacrolimus, resistin.

LIST OF PUBLICATIONS

The thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Improved intestinal preservation using an intraluminal macromolecular solution: evidence from a rat model.** Oltean M, Joshi M, Herlenius G, Olausson M. Transplantation. *In press*

II. **Donor pretreatment with FK506 reduces reperfusion injury and accelerates intestinal graft recovery in rats.** Oltean M, Pullerits R, Zhu C, Blomgren K, Hallberg EC, Olausson M. Surgery. 141:667-677, 2007

III. **Reduced liver injury and cytokine release after transplantation of preconditioned intestines.** Oltean M, Zhu C, Mera S, Pullerits R, Mattsby-Baltzer I, Mölne J, Hallberg EC, Blomgren K, Olausson M. J Surg Res. 154:30-37, 2009

IV. **Sustained elevations in plasma resistin, an inflammatory adipokine, after clinical intestinal transplantation.** Oltean M, Herlenius G, Gäbel M, Karlsson-Parra, A, Olausson M. *Manuscript*

POPULÄRVETENSKAPLIG SAMMANFATTNING

Tarmen är det organ som transplanteras mest sällan, delvis pga ett välfungerande alternativ i form av näringsdropp delvis pga allvariga komplikationer såsom avstötning eller infektioner som inte sällan leder till döden. Många av dessa komplikationer uppkommer som följd av en skadad tarmslemhinna. Slemhinnans integritet är viktigt för de två huvudfunktionerna. Dessa är upptagning av näringsämne från tarmen och barriärfunktionen, dvs att förhindra bakterierna som normalt finns i tarmen att ta sig till blodet och ge upphov till livshotande infektioner. En av de viktigaste moment när signifikant slemhinneskada förekommer är under och strax efter preservationstiden, dvs mellan uttaget från organdonatorn tills blodet släpps på genom transplantatet i mottagaren. Under preservationen förvaras organet i ett speciellt isbad efter har blivit genomspolat med speciella lösningar. Den låga temperaturen minskar ämnesomsättningen och fördröjer, men hindrar inte utvecklingen av skador under tiden organet måste förvaras fram till transplantationen.

I denna avhandlig har jag studerat två metoder att minska preservationskadan och dess effekter efter tarmtransplantation. Den ena metoden var att fylla tarmen med två olika lösningar som huvudsakligen skiljer sig endast genom Natrium innehållet. Lösningarna är kommersiellt tillgängliga och består egentligen av de tarmrengöringspreparat som används rutinmässig idag. Vi har upptäckt att införseln av lösningar med lågt natrium innehåll kan fördröja utvecklingen av slemhinneskada. Däremot kan högt Natrium innehåll kan innebära vätskeansamlingen i vävnaden och därmed vara skadlig.

Den andra metoden som jag har beskrivit var att framkalla skyddande proteiner i transplantatet genom en enkel behandling av tarmdonatorn. Läkemedlet som jag har studerat redan används inom transplantation, men ges vanligtvis till mottagaren.

Behandlingen visade en starkt skyddande effekt, förbättrad vävnadsarkitektur och även stimulerande effekter på vävnadsreparationen. Samtidigt upptäckte jag att behandlingen blockerar utsöndringen av vissa molekyler och faktorer som i ett senare skede även kan stimulera inflammation, som i sin tur kan framkalla organsvikt.

Efter tarmtransplantation är man idag tvungen att ta vävnadsprover för att diagnosticera förekomsten av avstötning, eftersom det inte finns blodprov som kan hjälpa ställa diagnosen, såsom vid njur- eller levertransplantation. Jag har studerat om ett visst protein (resistin), som visade sig öka i blodet på patienter med skov av inflammatorisk tarmsjukdom, kan användas som avstötningsmarkör. Jag har analyserat patientprover och har upptäckt att proteinet resistin ökar som förväntat, men detta sker även vid andra sammanhang såsom allvarliga infektioner. Detta begränsar det diagnostiska värdet av resistin, men fyndet är mycket intressant och kan leda till en bättre förståelse av detta proteins egentliga roll i samband med andra sjukdomar.

TABLE OF CONTENTS

INTRODUCTION

The ischemic injury remains a major hurdle for a broader clinical application of the intestinal transplantation, since it may give rise to life-threatening infectious complications and triggers an intense systemic inflammatory response. The thesis explores two different experimental approaches intended to mitigate the preservation/reperfusion injury. Finally, the time-course and the significance of resistin after clinical intestinal transplantation is analysed.

HISTORY OF INTESTINAL TRANSPLANTATION – A WORK IN PROGRESS

Although not described in detail, the first transplantation of intestinal segments in dogs has been reported by Alexis Carrel in the early 1900s. Experimental work was re-initiated in the mid-1950s by Richard Lillehei from the University of Minnesota, particularly focusing on intestinal preservation and the surgical technique [1, 2]. The confidence regarding the technical feasibility, together with the wave of transplantation optimism in the early '60s, owing to several newly available immunosuppressants (steroids, azathioprine) led to the first clinical attempt in 1967 by Lillehei [3]. Alas, this was followed by fierce rejection, sepsis and patient death within 3 weeks. Other sporadic cases were followed by similar catastrophic results and intestinal transplantation virtually ceased over 1970s and '80s. Fortunately, the introduction of total parenteral nutrition (TPN) provided a salutary alternative for the patients with short bowel syndrome (SBS), representing the main patient population candidate for intestinal transplantation [4].

The advent of cyclosporine in the early 1980 renewed the interest for intestinal transplantation. The efficient immunosuppression and accumulating experience from transplanting other organs allowed the teams of Eberhard Deltz from Kiel, David Grant from London (Canada) and Olivier Goulet in Paris to achieve long term survival following combined or isolated intestinal transplantation (1989) [5-7]. These initial successes were later taken over, expanded and consolidated at the University of Pittsburgh during the 1990s [8, 9]. Refined surgical techniques and immunosuppressive regimens as well as protocols for patient selection and management were implemented, finally leading to continuously improving results and increasing frequency of the procedure [10-12]. As of 2009, intestinal transplantation has been performed in about eighty transplant units worldwide.

However, fewer than thirty centers currently perform intestinal transplantations routinely (David Grant, Intestinal Transplant Registry report, Bologna 2009).

The first two transplantations including a substantial part of the intestine in Sweden and in the Nordic countries were performed as cluster procedures in Gothenburg already in 1990. One patient survived for 11 months, while the other patient died at the time of surgery. A year later, the first transplantation of an isolated intestinal segment was attempted in a child by Staffan Meurling in Uppsala, but unfortunately, the recipient succumbed due to rejection and sepsis fifty days later. A second unsuccessful attempt was performed in Stockholm shortly thereafter. The first successful transplantation of an isolated intestine has been performed in Gothenburg by a team led by Gustaf Herlenius in 2007. The first combined liver-intestinal transplantation was performed in 2001 by Michael Olausson and Gustaf Herlenius while the first successful multivisceral transplantation has been performed already in the year 1998 by Michael Olausson, at the transplant unit in Gothenburg, Sweden [13].

INDICATIONS, SURGICAL TECHNIQUES AND RESULTS

Parenteral nutrition has achieved extended success for the majority of patients requiring prolonged treatment, however, complications leading to failure of TPN increase with the duration of therapy. These complications range from frequent infectious complications originating from the catheter or progressive thrombocytopenia to loss of venous access due to repeated thrombosis and cholestatic liver disease with or without portal hypertension [14, 15]. Intestinal transplantation is therefore considered in irreversible intestinal failure (IIF) patients with impending liver failure and frequent catheter-related septic complications, including vanishing central venous access [16-18].

Irreversible intestinal failure, the permanent reduction in the functional intestinal mass has different etiology in adult and pediatric population. Short bowel syndrome (SBS), whether congenital, functional or surgical is the main cause for IIF. In children, the main indication for intestinal transplantation is SBS after neonatal abdominal catastrophes, leading to massive intestinal loss (gastroschisis, necrotizing enterocolitis, volvulus)[19, 20]. Another group of indications are the intestinal functional diseases (microvillus inclusion disease, malabsorbtion, secretory diarrhea) or motility disorders (Hirschprung disease, pseudoobstruction).

In adults, massive intestinal loss is consecutive mainly to Crohn's disease, ischemia and trauma. Certain gastrointestinal tumors requiring extensive intestinal resections as well as radiation enteritis are also among the causes behind IIF/SBS in adults [19, 21].

As briefly mentioned above, the intestine may be transplanted using three different approaches, namely as an isolated intestinal segment, together with the liver in the presence of liver failure (combined liver-intestinal transplantation) or as part of a composite graft that may contain other viscera (multivisceral transplantation) [22, 23]. When performing an isolated intestinal transplant the arterial inflow is achieved by anastomosing the graft's superior mesenteric artery (either directly or through arterial grafts) to the recipient aorta. The venous drainage is performed either directly into the inferior vena cava (systemic drainage) or by anastomosing the graft's portal vein to a branch of the recipient portal vein, usually the superior mesenteric vein (portal drainage), with similar metabolic results. During combined liver-intestine and multivisceral transplantations, the continuity of the portal axis is maintained and the hepato-intestinal graft is drained through the liver veins [22, 24, 25]. At the end of the transplantation, an ileostomy (terminal or lateral) is always performed to allow for the intestinal endoscopies, essential for rejection monitoring.

According to the latest Small Bowel Transplant Registry report (Bologna, September 2009), 2291 intestinal transplants have been performed in 2061 patients at 86 centers worldwide. Results are continuously improving due to increased expertise as well as refined patient selection and posttransplant management. Several analysis performed on this material found that results are dependent on pretransplant patient status (hospitalized vs. waiting at home), the inclusion of the liver into the graft and center volume (at least ten cases performed in total)[19].

Currently, one year survival rate is around 75% and five year survival rate is just above 50%, with the longest surviving patient now twenty years after transplantation. The main causes of patient loss are the infectious complications (≈50%), followed by rejection (≈ 10%) [26, 27].

OBSTACLES, COMPLICATIONS AND SPECIAL ISSUES IN INTESTINAL TRANSPLANTATION

The limited number of intestinal transplants performed to date compared with other transplantable organs explains the complexity of the procedure. The transplant candidates are often malnourished, with frequent hospitalizations, sometimes presenting with growth retardation. The very long and technically challenging surgical procedure frequently takes place in a scarred abdomen with extensive adhesions and fistulae following previous abdominal surgery. Several unique issues further complicate the management of intestinal graft recipients.

HIGH INTESTINAL SUSCEPTIBILITY TO ISCHEMIA-REPERFUSION INJURY

The intestine safely tolerates only up to 8-10 hours of cold ischemia, as compared with the 14-16h affordable in the case of the liver graft or the 24h or more in the case of the kidney [12, 28, 29]. The intestinal graft is unique among the transplantable organs due to its contaminated content. Bacteria and bacterial products may spread out in the recipient in case of mucosal barrier damage secondary to preservation-reperfusion injury, leading to early sepsis outbursts [30]. Therefore, the critical issue of alleviating ischemia-reperfusion injury, including by shortening the cold ischemia time puts considerable pressure on the procurement team, transplant coordinators and the transplantation team [12].

HIGH INTESTINAL IMMUNOGENICITY

Due to the expression of major histocompatibility complex (MHC) class 2 molecules on the enterocytes, the intestine has a very high immunogenicity [31-33]. The immunogenicity is further augmented by the great lymphocyte load, present within the mucosa, mesenteric lymph nodes and in the Peyer patches (gut-associated lymph tissue –GALT) [34, 35]. As a first consequence, acute rejection (AR) occurs and is directed against the epithelial layer of the intestinal mucosa and may lead very rapidly to mucosal barrier breakdown, mucosal ulcerations with subsequent bacterial translocation and ensuing sepsis [30]. The critical vascularization of the intestine, in particular at the villus level makes that any impending endothelial damage during AR may result in ischemia-like changes, further jeopardizing the mucosal barrier integrity [36]. In addition, a massive gut-associated lymphoid tissue (GALT) transfer may, at least theoretically, increase the risk of graft versus host disease (GVHD)[37, 38].

IMMUNOSUPPRESSION AND ITS COMPLICATIONS

The increased immunogenicity requires a more intensive immunosuppressive regimen compared with other organs [39, 40]. This most often includes antibody induction and relatively high Tacrolimus trough levels [41]. This intense immunosuppressive treatment renders the patient susceptible for bacterial, viral and fungal infections [42, 43]. Infections have a high incidence and their range is very broad [42]. The differential diagnosis with AR, based on the clinical findings and routine investigations is often impossible, particularly during enteritis [44]. Thus, it is often necessary to obtain endoscopic biopsies to exclude AR, since a wrong or delayed therapy may bear catastrophic consequences.

Besides increasing the risk of infections, the intense immunosuppressive treatment greatly increases the risk of malignancy, particularly post-transplant lymphoproliferative disease (PTLD) [45]. It also carries the cumulative risk, as well as nephrotoxicity that often leads to chronic kidney disease and even renal failure [46].

HIGH INCIDENCE OF ACUTE REJECTION

Despite intensive immunosuppression, AR is a greater hazard in intestinal than in any other organ transplantation, occurring in about 40-60 % of the cases [47, 48]. The early clinical signs of AR are unspecific (fever, nausea, increased stomal output) and there is no universally accepted serum marker of intestinal rejection, as in the case of kidney and liver. The diagnosis of AR is made on biopsies obtained through invasive and frequent endoscopies, requiring trained personnel, involving supplementary costs and time. Furthermore, it submits the patient to discomfort and potential complications [49]. Delay in the diagnosis and treatment by just hours to days may result in rapid progression from mild to severe exfoliative rejection, which is associated with a substantial risk of graft loss (up to 93%) and high mortality (50–70%)[50].

Several non-invasive markers have been suggested to be useful in diagnosing or predicting the development of AR [51-54]. Unfortunately, despite high sensitivity, many of these markers have low specificity or kinetics that limits their use during the early post-transplant period, when most of AR episodes are occurring [48, 55, 56].

THE ABDOMINAL CAVITY

Contracted abdominal cavity due to the content loss, previous stomas and fistula formation. This "loss of domain" is a major challenge when closing the operative wound over the transplanted intestine without the risk of ,"abdominal compartment syndrome" [57, 58]. Several approaches to overcome this complication have been described to date, this including the use of prosthetic materials (Alloderm), vacuum-assisted abdominal closure (VAC), staged closure or transplantation of the abdominal wall [59, 60].

ISCHEMIA-REPERFUSION INJURY

Ischemia and reperfusion (IR) are unavoidable events in organ transplantation. This will result in complex metabolic and structural changes, whose extent mainly depends on the length of ischemia, surrounding temperature and type of tissue. Paradoxically, the reperfusion and subsequent reoxygenation initiate a cascade of biochemical and molecular changes that may lead to additional injury.

BIOCHEMICAL AND MOLECULAR EVENTS DURING ISCHEMIA AND REPERFUSION

Oxidative stress

At the core of the reperfusion injury lays an event occurring during ischemia, namely the adenosine triphosphate (ATP) degradation [61]. Upon reoxygenation, hypoxantine, an end-product of the anaerobic ATP degradation will be metabolized by xantine oxidase and generate oxygen free radicals (OFR) including the hydroxyl radical, superoxide and nitroperoxide ions [62-65] The oxidative stress occurs early (i.e., minutes) after reoxygenation and will inflict further damage to numerous cellular elements such as mitochondria, proteins, nucleic acids and cellular membranes [65]. The stress may lead either to immediate cell death (necrosis), controlled cell death (apoptosis) or trigger changes of the cell phenotype in response to the injury (activation).

Besides local tissue injury, the oxidative stress may also act on the red blood cells increasing cell stiffness, reducing deformabiliry and creating conditions for lysis. When lysis occurs, released free heme exacerbates the oxidative process, further generating OFR and a harmful iron chelate, which may promote deleterious cellular processes such as oxidative membrane damage.
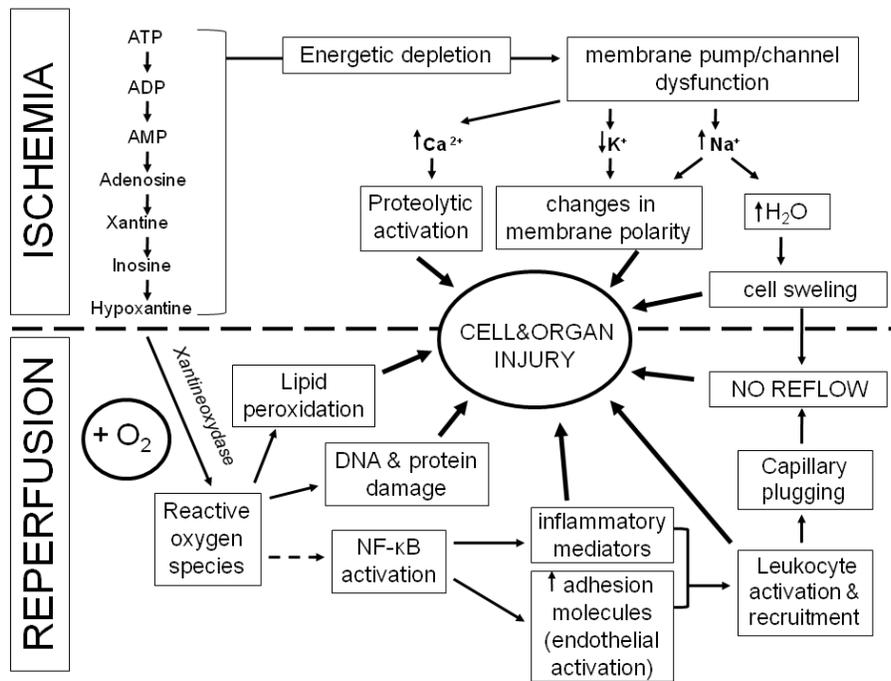
Figure 1. Overview of the main molecular events during ischemia and after reperfusion

These events occur in all cell types of the reperfused organ, yet the vascular endothelium is particularly important in this setting because of its role of interface between the intravascular and the extravascular compartments (Figure 1).

Transcription

The oxidative stress, either directly by OFR or through lipoperoxidation products, may initiate gene transcription as adaptation in response to the injury. Several transcription factors are activated by the oxidative stress, with nuclear factor- κB (NF-κB) as one of the most prominent [66, 67]. NF-κB has a central role in inflammation and innate immunity. NF-κB exists mainly as heterodimer, retained inactive in the cytoplasm by an inhibitory unit (IκB) and requires a signalling pathway for activation [68, 69]. Following IκB phosphorylation by IκB kinases (IKK), the IκB releases the heterodimer, which translocates into the nucleus, binds to specific DNA promoter sequences and initiates gene transcription . Among the numerous genes whose expression is regulated by NF-κB, there are adhesion molecules (vascular cell adhesion molecule (VCAM)-1, intercellular adhesion

molecule (ICAM)-1), matrix metalloproteinases (MMP-2, MMP-9) and other genes that regulate endothelial cell survival, vasodilatation and angiogenesis. In addition to that, NF-κB promotes the expression of various genes essential in the inflammatory response such as TNF-α, IL-1, IL-6, IL-8, and GM-CSF [70] (Figure 2).
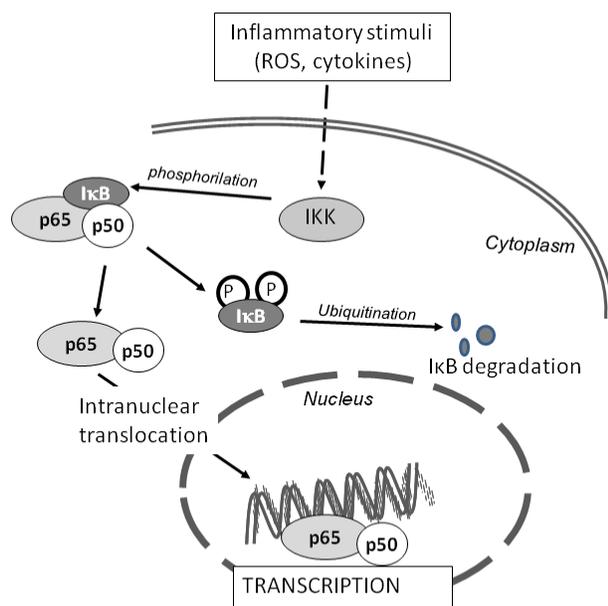
Figure 2. The key molecular events leading to the activation of NF-κB

Apoptosis

Apoptosis is an organized, energy-dependent cascade of events leading to controlled DNA fragmentation and cell death, followed by removal of the debris without inflammatory response. Apoptosis may be triggered by signals from within the cell (the intrinsic pathway) or by extracellular signals (the extrinsic pathway)[71, 72].

Although the initial stages of these two pathways are completely different, both pathways are mainly driven by a similar family of cysteine proteases (i.e., caspases) activated in cascade [71]. Moreover, the two apoptotic pathways converge at one point (caspase-3 activation) to follow a common route leading to the activation of endonucleases and DNA fragmentation [73]. Long time in the development of apoptosis the cellular membrane is maintained intact. In the final stages it fragments and surrounds the nuclear debris and the organelles to form the apoptotic bodies [74]. Recent evidence indicates that apoptotic cell death can signal neutrophil emigration in endotoxin shock and that blocking apoptosis with an

inhibitor of caspases can prevent neutrophil extravasation and hepatocyte necrosis [75, 76].

LEUKOCYTE-ENDOTHELIAL INTERACTIONS

The transcriptional changes triggered by IR injury generate complex changes towards an inflammatory phenotype in the surviving cells of the injured tissue, a process called activation [77, 78]. In addition, the various cell types in the injured tissue release numerous biomolecules (cytokines, chemokines) that may act as pro-inflammatory mediators [79, 80]. Secondary to their local or systemic release, these mediators both activate the endothelium and act upon circulating cells (mainly leukocytes) and promote leukocyte recruitment and enhanced leukocyte-endothelial interactions [81-84].

The endothelial interface

The leukocyte-endothelial interaction is initiated by rolling along the endothelial cell surface mediated by glycoproteins belonging to the selectin family, which are found on the surface of both leukocytes (L-selectin), platelets (P-selectin) and endothelial cells (P- and E-selectins) [85]. This weak leukocyte tethering to the vessel wall brings the leukocytes closer to chemoattractants that include "classical" chemoattractants (eg, leukotriene B4, C5a, platelet activating factor (PAF) and the chemoattractant chemokines [86]. The tethering continues to firm adhesion, followed by transmigration into the tissue to remove the debris at the site of injury [87, 88]. The transmigration requires the expression of selectins and integrins (CD11a, CD11b, and CD11c/CD18 expressed on the leukocytes) and endothelial adhesion molecules (members of the immunoglobulin superfamily, eg, ICAM-1, -2, -3, or VCAM-1) [89-92].

An essential factor for leukocyte extravasation is the chemotactic stimulus that attracts the leukocyte towards the site of injury. Chemokines are potent chemotactic factors and members of this class of mediators can have selective chemotactic properties for neutrophils, lymphocytes, monocytes, or eosinophils [93]. In addition, general cell injury may produce other chemotactic factors (i.e., leukotrienes)[94].

Tissue inflammation and phagocytosis

Leukocyte accumulation in the tissue, i.e., the inflammation, is the final phase of the response to injury [95, 96]. Temporally distinct patterns of expression of adhesion molecules and chemokines offer some specificity to the inflammatory infiltrate.

Although its primary role is the removal of tissue debris through phagocytosis, leukocyte infiltration paradoxically worsens the local injury [97]. It has long been thought that the oxidative stress upon reperfusion leads to cell death by lipid peroxidation. However, the early lipid peroxidation cannot entirely explain the prolonged and severe cell injury observed during reperfusion. The ability of antiproteases to reduce the late reperfusion injury revealed that polymorphonuclear neutrophils (PMN) themselves can cause tissue damage by releasing several proteolytic enzymes such as the elastase from cytoplasmic granules and by producing free radicals via the respiratory burst [98-101].

Another possible mechanism through which leukocytes can aggravate the reperfusion injury is by plugging the capillaries and thus impairing the microcirculation (the "no-reflow" phenomenon) [102, 103].

As a consequence and proof of these hypotheses, reduced leukocyte infiltration has been repeatedly associated with improved structure and function after IR injury [104-106].

THE STRESS RESPONSE

The heat shock proteins- HSP

Oxidative stress and temperature increase are major stimuli for heat shock protein (HSP) induction and graft reperfusion with warm, oxygenated blood leads to massive upregulation of various HSP [107-110].

HSPs are constitutive or inducible proteins mainly named according to their molecular weight. HPSs are primarily involved in protein homeostasis (chaperoning, folding and translocating proteins intracellularly). However, numerous studies signaled beneficial effects of preemptive HSPs overexpression in reducing IR injury as well as several interesting immunomodulatory roles [111, 112].

The molecular mechanisms behind the HSP-mediated cytoprotection certainly involve more than one pathway [113-116]. One mechanism suggested has been the HSP-mediated modulation of NF-κB activation [117-121]. HSP 60 may stimulate the innate immune response by acting as a ligand for several Toll-like receptors (TLR),

particularly TLR 4 [118, 122]. On the other hand, in macrophages submitted to heat stress, NF-κB DNA binding following lipopolysaccharide (LPS) exposure was effectively reduced, supposedly by preventing IκB degradation following its phosphorylation [123, 124].

The inhibition of NF-κB activation has been recorded when upregulating both members of the HSP70 and HSP32 (also known as HO-1) families. HO-1 has important roles in heme degradation, but previous studies also demonstrated that HO-1 may play many other vital functions in cellular homeostasis . It has also been suggested that the initial antioxidative protection conferred by the HO-1 and its byproducts will reset the molecular machinery of the injured cells [121, 125]. Hence, the response to stress leads to a protective phenotype. NF- κB pathway is redox sensitive and may be influenced by the antioxidant effects of HO-1 and its byproducts, while billiverdin may act as kinase by itself [121, 126]. Given its extreme pleyotropism, NF-κB inhibition by HSPs may explain many molecular events following heat-shock preconditioning [127-129]. Thus, HSP induction successfully reduced neutrophil sequestration both in models of warm ischemia reperfusion [129] and transplantation probably through a combination of cytoprotective effects, reducing the initial tissue damage as well as by interfering with various intracellular signaling pathways [121, 130, 131]. Several studies revealed that different HSP can activate dendritic cells and macrophages, making the HSP a large family of endogenous danger signals [132-135]. In nontransplant setting, this would have limited consequences; however in a transplantation setting these signals can prime and precipitate the alloimmune response [111, 136, 137].

Danger activated molecular patterns – DAMP

Recently, a new group of molecules has been described and grouped under the terms 'alarmins' or 'endokines'. These are either released by necrotic cells or actively secreted by activated cells after injury or stress response [118]. The molecules have been collectively suggested as danger-associated molecular patterns (DAMPs), in close similarity with PAMPs (pathogen-associated molecular patterns), a system within the innate immunity that alerts the organism to intruding pathogens and activates several signaling pathways [138]. Conversely, the extracellular presence of normal cell constituents released into the extracellular milieu during states of cellular stress or damage and may also signal cell injury and subsequently activate the immune system [139].

The 'danger signal' model proposed by Matzinger suggests that the immune system recognizes and reacts to damage signals in the body regardless of their origin [140]. The signaling cascade is initiated following the recognition of the danger molecule by the toll-like receptor (TLR) family, present on the membrane of numerous cell types [141]. After first being funneled by the myeloid differentiation primary response gene 88 (MyD88), the intracellular signal transduction pathways will ultimately lead to the activation of transcriptional factors such as NF-κB, or the expression of genes related to inflammatory and immune responses [142, 143]. A growing list of biomolecules has been proposed as DAMPs, including High mobility group box 1 (HMGB1), the S100 proteins, defensins, fibrinogen, soluble hyaluronan, hepatoma-derived growth factor (HGDF), uric acid, several HSPs, and IL-1α [144-149].

THE SYSTEMIC CONSEQUENCES OF ISCHEMIA-REPERFUSION

Apart of the organ-specific systemic effects observed after advanced ischemic injury (renal failure after severe renal IR, liver failure after liver IR), injury and dysfunction may occur in organs remote from the site of IR. Hepatic IR injury may lead to various degrees of acute cardiac, lung or kidney injury while lower body ischemia encountered during abdominal aorta aneurysm repair generates a strong inflammatory response and frequently, multiple organ dysfunction [150-155]. Moreover, intestinal IR is frequently associated with signs of liver, respiratory or kidney failure and significant associated mortality [156-158]. The exact mechanism behind the impairment of distant organs is unclear, though an excessive, sepsis-like inflammatory response is thought to underlie this phenomenon [159-161].

The cytokine release

Upon reperfusion, the oxidative stress stimulates several intracellular signaling pathways, ultimately leading to the transcriptional activation of numerous genes, including various pro-inflammatory cytokines [80, 162]. The transcription products depend on the degree of injury and cell type and their biologic effects are pleiotropic, often redundant, following the interaction with the equivalent structures (receptors). Most of the cytokines and chemokines have pro-inflammatory effects, but there are also molecules with anti-inflammatory role (i.e IL-4, IL-10, IL-13) [163, 164]. Pro-inflammatory cytokines act on mononuclear phagocytes or lymphocytes, modulating innate or acquired immunity [165].

Extensive evidence signals increases in tumor necrosis factor-alpha (TNF-α) in

various models of ischemia-reperfusion [166-168]. TNF-α is the prototype pro-inflammatory cytokine and it is released by monocytes/macrophages, T lymphocytes, Natural killer cells (NK cells) and many other cell types found throughout the body. TNF-α activates the NF-κB transcription pathway, IL-6 and tissue factor secretion, has direct cytotoxicity, up-regulates adhesion molecules, stimulates nitric oxide (NO) synthesis and release, activates neutrophils and induces fever [169]. TNF-α may trigger apoptosis after binding to the death receptors and may also promote synthesis and release of other cytokines, most notably IL-1 and IL-6 [168, 170, 171].

Interleukin-1 (IL-1) is synthesized in precursor form and secreted under two forms (IL-1α and IL-1β) with similar actions. IL-1 mediates its biological effects after binding a membrane receptor that engages signal transduction pathways activating NF-kB and activator protein -1 (AP-1) transcription factors. At low concentrations, IL-1 is a local inflammation mediator (stimulates leukocyte adhesion on endothelium) but at high concentrations it acts as endogenous pyrogen and induces the synthesis of acute phase proteins in the liver [172].

Interleukin 6 (IL-6) is released by monocytes/macrophages, T lymphocytes, endothelial cells, fibroblasts, keratinocytes following TNF-α stimulation [173]. It is a major inductor of the acute phase response, stimulates B cell growth and increases NK activity as well as T cell differentiation [172, 174]. It increases in disease states such as malignancy, burn injury, pancreatitis or sepsis, and it is believed to have the potential of a prognostic indicator for mortality [175]. This is supported by several studies that revealed that deceased septic patients had higher levels of IL-6.

PATHOPHYSIOLOGY OF INTESTINAL ISCHEMIA-REPERFUSION INJURY

The pathophysiology of ischemia-reperfusion injury generally follows the steps mentioned earlier, irrespective of organ. However, due to structural and functional characteristics, each organ has several specific patterns of developing the injury. The following briefly outline the main features and consequences of the intestinal ischemia and reperfusion.

RELEVANT MICROSCOPIC ANATOMY

The intestinal lumen and the intestinal mucosa

An essential difference compared to other transplantable organs is that the intestine is a hollow organ, containing an epithelium with selective permeability. The different cell types making up the epithelium have a rapid turnover. From their origin in the crypts, where a self-renewing population of stem cells normally balances loss of effete enterocytes, the enterocytes continuously move upward towards the villus tip [176]. In the steady state in normal animals, around 10 new cells are produced in each crypt per hour. Thereafter, the enterocytes migrate from the crypt to the villus tip, a process that takes 2 to 3 days. At the tip, the enterocytes enter apoptosis and finally detach into the lumen [177].

The capillary 'hairpin' and the countercurrent exchange

Another morphologic feature relevant for the development of ischemic injury, particularly during hypotension and shock, is the hairpin shape of the capillaries inside the villus. Although capillaries run along the entire length of the villus, the proximity of the afferent and efferent capillary loop at the villus base may allow a short-circuit of the oxygen between these two. Consequently, a countercurrent exchange, oxygen shunting and a subsequent oxygen gradient between base and tip of the villus may occur, generating a relative hypoxia towards the villus tip.

The polarity of the intestinal epithelial cell

The enterocyte is a polarized cell, with the apical membrane biochemically and functionally different from the basolateral membrane [35]. Thus, the apical membrane is involved in digestion, absorption and secretion while basolateral membrane has basically only transport roles [178]. Cells are held together by junctional complexes, structures with selective permeability and rest on the basal membrane, an acellular structure covering the connective matrix that builds up the villus axis (lamina propria) [179]. Besides the matrix, lamina propria contains various cell types (e.g, lymphocytes), capillaries and lacteals [180, 181].

The junctional complex and the tight junctions

As mentioned above, all polarized epithelial cells are held together by an intricate structure called the junctional complex. Besides its mechanical role the junctional complex fulfills a dual function: a fence function, preventing the mixing of membrane proteins between the apical and basolateral membranes and a gate function which controls the intercellular passage of ions and solutes between the cells, preventing them to diffuse back into the lumen through the intercellular space. In certain physiological circumstances, but also in disease, the tight junctions' (TJ) permeability may increase, allowing an increased flow of water and solutes, through the paracellular pathway [182-184].

Cell-to-cell adhesion is also provided by the desmosomes, button-like intercellular contacts that attach the cells together. Desmosomes are formed by a dense cytoplasmic plaque, connected by intracellular filaments to the cytoskeleton. The firm adhesion between plaques is provided by transmembrane proteins belonging to the Cadherin family [185, 186].
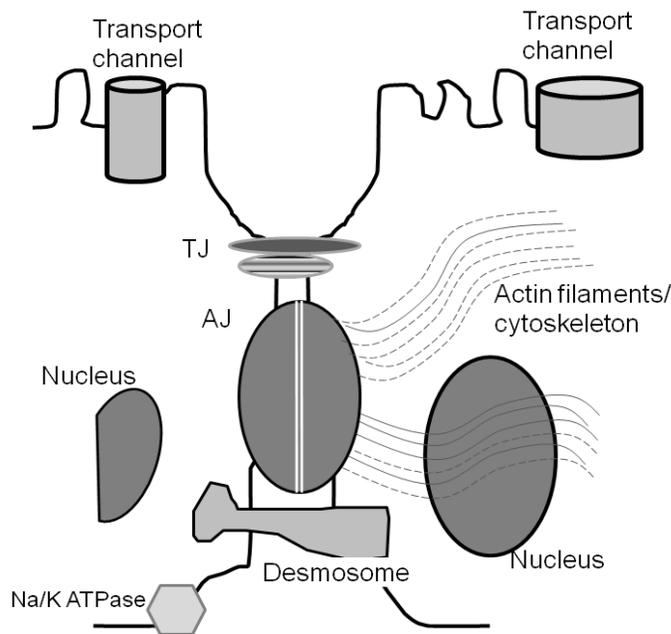
**Figure 3.The intercellular space in a polarized epithelium (adapted after [186])**

The TJ is the most apical component of the junctional complex and functions as the "fence" separating apical from basolateral domains. Several tight junction-associated proteins have been evidenced, such as occludin, zonula occludens-1 (ZO-1) and claudins. The protein content of this complex regulates the gate function

of the paracellular space [187, 188]. Thus, the majority of channels established by claudin interactions regulate paracellular permeability and allow the passage of cations.

The junctional complex also contains the gap junctions. These mediate communication between cells by allowing small molecules to pass directly from cytoplasm to cytoplasm of adjacent cells [186].

MAIN FEATURES DURING INTESTINAL ISCHEMIA AND REPERFUSION

The intestinal ischemic injury presents as progressive subepithelial edema that will ultimately lead to the breakdown of the mucosal barrier. A subepithelial cleft (the Gruenhagen space) appears within one hour at the villus tip and extends towards the villus base, cleaving the epithelium from the lamina propria [189].

The continuity of the epithelium is ultimately lost and lamina propria will be exposed to the lumen, sustaining further structural damage. Extended ischemia leads to changes and structural alterations in the deeper intestinal layers. This sequence of events has been signaled both during normothermic and hypothermic ischemia and forms the basis of the Park score for grading of the intestinal ischemic injury [190].

At ultrastructural level, electron microscopy revealed structural mitochondrial alterations, an apparent redistribution of the intracellular organelles (lisosomes, endoplasmic reticulum), intracytoplasmic protein accumulations as well as widening of the intercellular space including proteinaceous deposits [191]. Upon reperfusion and the initial worsening, the repair process starts with flattening of the villi and increased lateral migration of the enterocytes to seal the denuded areas [192, 193]. Villus contraction has also been reported [194]. This is followed by intense proliferation in the crypts and, depending on the degree of injury, complete structural restoration within 24-48 hours. However, if advanced crypt damage (grade 6 or more on the Park scale) the complete restoration is unlikely and if the subject survives the reperfusion, chronic changes are likely to occur [195].

Microcirculation is also significantly impaired following intestinal IR and intestinal transplantation. This is due to leukocyte adhesion, direct impairment of the endothelium [103, 196, 197] or vasoactive mediators released at reperfusion [198].

REDUCING ISCHEMIA-REPERFUSION INJURY IN TRANSPLANTATION

Experimental and clinical studies indicated that IR injury can be successfully mitigated using a myriad of chemical or biological compounds as well as several maneuvers eliciting a biologic protective response (hyperthermia, ischemic preconditioning) [199-201]. However, most of these studies were performed by clamping and unclamping vascular pedicles (so-called warm ischemia/reperfusion) whereas the transplant setting implies several particularities such as the removal of the organ from the initial milieu, a period of hypothermia, additional warm ischemia as well as denervation. All these features generate several essential differences at cellular and metabolic level and thus only several of these observations are applicable in transplant setting [121, 202, 203]. Below the relevant approaches mitigating IR injury in transplantation will be briefly reviewed.

MINIMIZING THE PRESERVATION INJURY

Shortening cold ischemia time is by far the most significant measure to alleviate IR injury in transplantation [28, 204]. Slowing ATP depletion and maintaining viable ATP stores is very important during the energetic and metabolic recovery at reperfusion [205]. In addition, hypothermia reduces the anaerobic cell metabolism and slower catabolite formation in the ischemic organ. Perfusion with special solutions antagonizes the electrolyte shifts secondary to membrane pump dysfunction (mainly the Na/K ATPase). Moreover, the preservation solutions aim to prevent the osmotic water shifts and subsequent cell swelling, acidosis as well as attenuating the oxidative stress. All preservation solutions currently used generally follow these principles while sharing common characteristics and having particular traits [206].

Belzer-University of Wisconsin (UW) cold storage solution (Viaspan ®) revolutionized organ preservation in late 1980s and it is still considered the gold standard for abdominal organ preservation . It has an 'intracellular composition' containing a high potassium concentration (130 mmol/l) as well as high-molecular-weight impermeants (lactobionic acid and raffinose preventing intracellular edema secondary to ischemia), oncotic support (hydroxyethyl starch, HES), and redox agents (glutathione and allopurinol) mitigating the oxidative stress during reperfusion [207, 208].

An alternative preservation solution increasingly used is Histidine-triptophane-ketoglutarate (HTK, Custodiol ®). Similar to Belzer-UW solution, it has a powerful

buffer system (histidine) and impermeants (ketoglutarate) but its electrolyte contents resemble the extracellular milieu (high sodium and low potassium). Increasing evidence suggests that HTK solution yields comparable short term results with Belzer-UW solution for the preservation of livers and kidneys and also proved safe for intestinal preservation [209, 210]. Recent United Network for Organ Sharing (UNOS) registry analyses question its long term efficacy [211-213].

Celsior is another preservation solution with an 'extracellular' composition. It appears like a hybrid of the previous two solutions and combines reduced glutathione as well as high-molecular-weight impermeants (mannitol and lactobionic acid) like UW solution and high sodium concentration (100 mmol/l), low potassium and histidine as in HTK solution. Celsior solution proved to be suitable for preserving livers [214], kidneys and pancreas [215].

IGL-1 is a newly designed preservation solution intended for the preservation of abdominal organs developed by the French Institute George Lopez (IGL). It has similar key ingredients as Belzer-UW solution, but an 'inverse' composition (low potassium, high sodium), while using a new osmotic agent (polyethylene glycol, PEG 35) instead of HES. A recent clinical trial found IGL-1 at least comparable with Belzer-UW solution in kidney transplantation while an experimental study reveals similar results in liver transplantation [216, 217].

DONOR PRETREATMENT

The rationale for the interventions in the organ donor is the preemptive initiation of endogenous protective mechanisms within the graft that will be transferred with the organ and ultimately alleviate IR injury after reperfusion in the recipient. Two main types of interventions can be recognized, namely the ischemic and the pharmacologic preconditioning.

Ischemic preconditioning (IP) is defined as a brief period of ischemia followed by reperfusion prior to a sustained ischemic episode. IP has been reported to attenuate the tissue damage observed after IR in the heart, liver, kidney and intestine [218-220]. Moreover, the protective effect of IP has been demonstrated for normothermic ischemia and in transplantation setting [199, 221-223]. The mechanisms are only partly unraveled and appear exquisitely intricate and complex. The protective mechanisms behind IP include the involvement of HSP, nitric oxide, adenosine, inhibition of apoptosis, as well as the modulation of several kinases and signaling pathways [223-227].

Pharmacologic preconditioning (PP) aims at triggering one or several endogenous protective mechanisms following the administration of a drug or chemical compound. Several main mechanisms are operational, encountered either alone or in combination, so that virtually all the cellular and molecular events occurring during IR may be influenced by PP. Thus, the pharmacologic induction of a heat shock response, either through up-regulation of HSP32 (heme oxygenase-1, HO-1) or HSP70 has been shown to greatly reduce both the early damage due to IR and the chronic changes triggered by an advanced initial ischemic insult [228-230]. Brief treatments with immunosuppressive drugs or receptor blockade reduced the IR presumably through the modulation of tissue inflammation after reperfusion [231, 232].

Tacrolimus/FK506

Tacrolimus (TAC) is an immunosuppressive drug with a macrolide structure, currently used in liver, kidney, heart, pancreas and intestinal transplantation. Its immunosuppressive action is due to the inhibition of the phosphatase activity of calcineurin, that ultimately results in the inhibition of the nuclear factor of the activated T cells (NF-AT) and suppressed production of IL-2, essential for the clonal expansion of the T cells [233].

However, besides its immunosuppressive properties, a number of other biological actions were signaled [234-237]. Among these, numerous reports indicated a protective effect of TAC against ischemia reperfusion in various settings and suggested a multitude of putative mechanisms [238-242]. Common mechanisms identified by many studies were the modulation of the early inflammation and the decreased oxidative stress.

A single study explored the pretreatment the organ donor with TAC to reduce the ischemia/reperfusion-related changes after kidney transplantation [232]. Although the early reperfusion injury was only briefly analyzed, the study found improved morphology and long term function in animals receiving TAC pretreated renal grafts.

Intestinal preservation

Several standard preservation solutions have been tested for the intestinal preservation. Although superior to crystalloid solutions, such as Ringer or saline,

none provided consistently satisfactory results beyond 8-10 hours. The unsatisfactory results triggered intensive search for alternative solutions or approaches for the preservation of intestine. These included the experimental addition of chemicals (including gaseous compounds) mainly to the Belzer-UW solution that frequently resulted in some molecular and physiologic improvements. However, no notable prolongation of the cold storage time, while being able to achieve only mild ischemic injury, was reported [243, 244].

Another concept was represented by the intraluminal delivery of preservation solution or different tailored combinations [245-247]. This repeatedly resulted in superior morphology after extended preservation time and in some cases improved bioenergetics, suggesting that addressing the luminal compartment may prove beneficial in terms both of maintaining acceptable graft morphology and allowing the safe prolongation of the cold ischemia time [244, 248]. Since the cellular interactions maintaining the mucosal barrier, including the functioning of tight junction are energy-dependent processes, these approaches may definitely represent a particularly promising solution. A modification of the later alternative was represented by the intraluminal introduction of UW solution, followed by the immersion in continuously oxygenated perfluorodecaline (PFD) , a variation of the "two layer method" (TLM) previously described in pancreas transplantation [249]. This strategy allowed a 75% recipient survival after 24 hours of cold storage, otherwise leading to 100% mortality. However, the approach is cumbersome and demands elaborated logistics.

ACUTE REJECTION

A detailed description of the rejection mechanisms is beyond the scope of this chapter. Below a short outline of several circulating markers and mediators during rejection, including cytokines is given.

MARKERS OF INTESTINAL ACUTE REJECTION

Unlike other transplantable organs, the intestine lacks a noninvasive rejection marker. Several tests or bio-molecules have been suggested throughout the years, but they have all ultimately been refuted [52, 250]. In the recent years, the nonessential aminoacid citrulline has been evaluated clinically [55, 251]. Citrulline level is a good indicator of the enterocyte mass and low levels of citrulline have

correlated with rejection [252]. Despite several drawbacks, such as the low levels during the first month after intestinal transplantation, a period when most rejection episodes occur, and dependence on the renal function, citrulline is considered a promising candidate for a noninvasive marker of intestinal acute rejection [56].

An equally interesting candidate-marker is fecal calprotectin. Calprotectin is present in several leukocyte subsets as well as constitutively in the cytoplasm of enterocytes. It is usually released as a result of cell disruption and death or during increased enterocyte shedding and consequently, calprotectin is considered an endogenous danger signal [253].

Increased fecal calprotectin has been reported during active inflammatory bowel disease [254]. A recent study identified increased calprotectin levels in the stomal effluent of patients with intestinal acute rejection, a finding that was later confirmed independently [53, 255]. However, the increase seems rather unspecific, hence independent investigators suggest its use as noninvasive screening marker to document the absence of rejection and thus reduce the need for blank endoscopies. Both these two markers are imperfect, and the search for a more accurate rejection marker continues.

LYMPHOCYTES, CYTOKINES AND REJECTION
The CD8+ and CD4+ cells and the cellular rejection

Cellular rejection starts with the recognition of the foreign antigens by the recipient immune cells during the process of allorecognition [256]. After antigen presentation to naïve T cells, some of these become activated, proliferate by clonal expansion, turn into antigen-specific cytotoxic T lymphocytes (CTLs, CD8+), attack the allograft and destroy it. This process usually takes days to weeks [257]. T cell proliferation requires several soluble molecules (cytokines) such as IL-2 and interferon-gamma (IFN-γ). These are provided either by the CD8+ cells themselves (autocrine) or by the CD4+ T-helper 1 cells.

The CD4+ T-helper 1 (Th1) cells constitute the other major effector limb of the T-cell response to an organ graft. After antigen stimulation, Th1 cells secrete cytokines, such as interferon-gamma (IFN-γ) and TNF-α. These cytokines have important pro-inflammatory actions and further stimulate the synthesis of various cytokines, stimulate B cell growth as well as antibody production. CD4+ T-cells may also

activate monocytes and macrophages, cell types that feature prominently in the cellular infiltrate during allograft rejection.

T-helper cells are also able to manifest anti-inflammatory effects; the subgroup is designated as Th2. The shift towards a pro-inflammatory or anti-inflammatory profile seems to depend on the cytokine environment and the original stimulus, but the exact circumstances that govern the shift are incompletely known [172, 257, 258]. The cytokine network is redundant and one cytokine may sometimes exert often opposite effects on the same cell type, depending on the presence of other factors in the microenvironment.

Two subsets of activated CD4+ T cells were primarily identified, i.e. the Th1 and Th2 cells. Th1 cells had IFNγ as the typical cytokine while Th2 cells produced IL-4 [259]. This straightforward, yet oversimplified classification has been repeatedly challenged and new functional subsets, such as the regulatory T cells (Tregs) or the Th-17 cells have been recognized [260]. However, for the sake of simplicity the literature still refers to a group of several pro-inflammatory cytokines as to Th1 cytokines and to some anti-inflammatory cytokines as Th2 cytokines [172].

The pro-inflammatory cytokines (Th1

IL-1β and **TNF-α** share a multitude of pro-inflammatory properties and appear to be critical to the amplification of mucosal inflammation in inflammatory bowel disease (IBD). Both cytokines are primarily secreted by monocytes and macrophages upon activation, and induce intestinal macrophages, neutrophils, fibroblasts, and smooth-muscle cells to elaborate prostaglandins, proteases, and other soluble mediators of inflammation and injury. Among the effects of TNF-α in the intestine are the disruption of the epithelial barrier and induction of apoptosis of the villous epithelial cells. TNF-α activates the endothelium and induces the expression of cytokines and chemokines. TNF-α also activates neutrophils and macrophages and stimulates the production of IFN-γ by mucosal T cells.

IL-2 is produced by naïve T cells after activation, simultaneously with the IL-2 receptor, in a classical autocrine and paracrine loop, promoting clonal expansion. IL-2 has important effects on other cells, including B cells, monocytes, and NK cells. **IL-8** is produced and released by endothelial cells and epithelial cells and is chiefly involved in neutrophil chemotaxis to the site of injury (chemokine).

IL-12 seems important for driving Th1 responses and IFN-γ production in the initial phases of an immune response, but conversely IL-12 may play a subsequent immunoregulatory role in late-stage inflammation.

IFN-γ has critical roles in both the innate and adaptive immunity. Among its many roles in the adaptive immunity are the upregulation of MHC class I and II expression, the endothelial activation and T cell adhesion and extravasation, as well as the differentiation of naïve CD4+ T cells towards a Th1 phenotype are directly instrumental in the development of rejection [172, 258].

The anti-inflammatory cytokines (Th2)

Th2 cytokines are thought to reduce the severity of allograft rejection by inhibiting the Th1-mediated cytotoxic lymphocyte (CTL) and delayed-type hypersensitivity (DTH) responses. These cytokines are IL-4, IL-5, IL-10, and IL-13.

IL-4 is the prototypic Th2 cytokine, polarizing activated CD4+ T cells to a Th2 phenotype. Systemic treatment with IL-4 of rat heart recipients significantly delayed rejection and inhibited Th1 responses within the graft, regional lymph nodes and spleen [261]. IL-4 also promotes the development of **T-regs** (CD4+CD25+Foxp3+) and thus the induction of transplantation tolerance [262].

IL-10 is produced by macrophages and inhibits macrophages and dendritic cells, thus creating a negative feedback loop. It inhibits the expression of MHC class II and diminishes Th1 cell activity by suppressing secretion of IL-2 and IFN-γ. IL-10 also inhibits the secretion of the pro-inflammatory cytokines IL-6, IL-8, IL-12, TNF-α, thereby attenuating mucosal inflammation [172]. The pivotal role played by IL-10 within the mucosal immune system has been extensively studied in mice lacking IL-10, that develop colitis due to an uncontrolled macrophage activation reacting to the intestinal bacteria [263] . Interestingly, IL-10 reduced the severity of both local and systemic inflammation in a murine model of intestinal ischemia-reperfusion when given either before or after reperfusion, allegedly by blocking the local cytokine production and remote organ inflammation [167].

Recent evidence indicates that **IL-13** can prolong allograft survival and modify graft rejection by inhibition of dendritic cell and/or macrophage function [264, 265].

RESISTIN

Resistin is a recently described polypeptide, first identified in the adipose tissue (but not the leukocytes) of obese mice. Experimental evidence suggested that

resistin, together with adiponectin and leptin is involved in diet-induced insulin resistance, lipoprotein metabolism, obesity and atherosclerosis [266-268].

However, the clear cut conclusions from the animal studies seem only partly valid in humans. The protein sequences of murine and human resistin are only approximately 60% identical while the main resistin source in humans seems to be represented by the leukocytes. While a relationship between resistin and obesity and vascular disese is less clearly defined in humans [269-273], increasing evidence links resistin with inflammation and innate immunity. Thus, resistin increased after endotoxin challenge in healthy volunteers [274] and it was found increased in critically ill patients, proportional with the severity of the disease [275, 276]. Furthermore, resistin was normally found in the amniotic fluid and increased levels were reported during amniotic infection [277] and in the synovial fluid of patients with rheumatoid arthritis [278].

Two studies found increased plasma resistin in patients with inflammatory bowel disease [279, 280]. Resistin correlated with the activity of the disease as well as with C reactive protein (CRP) and white blood count (WBC). Subsequently, resistin has been suggested as a marker of an active intestinal inflammation.

The pathways through which resistin manifest its pro-inflammatory effects are quite unclear, yet it seems resistin can bind to TLR2 and TLR4 and the downstream signaling may involve p38, JNK MAP and NF-κB [281, 282].

There are only a few studies of the adipokines in transplanted patients. All reported elevated resistin levels after kidney transplantation [283, 284]. Although the information is scarce, it seems that that resistin levels were higher in kidney graft recipients than in normal individuals even in the absence of complications (rejection, infection).

CONCLUDING INTRODUCTORY REMARKS

Considering all the issues briefly summarized herein and the actual level of knowledge in intestinal transplantation, it is fair to conclude that we need to considerably increase the available relevant information on both preservation/reperfusion injury and rejection to improve both short and long term graft and patient survival.

This thesis explores two different experimental approaches intended to mitigate the preservation/reperfusion injury. Finally, the time-course and the significance of resistin after clinical intestinal transplantation are analyzed.

AIMS OF THE THESIS

The specific aims of this thesis were:

- to study if intraluminal introduction of different macromolecular solutions has beneficial effects on the preservation injury of the rat intestine

- to determine if donor pretreatment with a single dose of tacrolimus improves the preservation-reperfusion injury after rat intestinal transplantation

- to investigate if transplantation of intestines from donors pretreated with tacrolimus results in a different pattern of postoperative inflammatory response and remote organ injury after rat intestinal transplantation

- to analyze the adipokine resistin after clinical intestinal transplantation and assess its putative roles

MATERIALS AND METHODS

The main methods used in this thesis are shortly described below. More detailed descriptions are found in the 'Materials and methods' sections of papers I-IV

PATIENTS

The patient material is represented by seven adult patients receiving intestinal allografts at the Transplant Institute/Sahlgrenska University Hospital in Gothenburg. Patient characteristics (age, diagnosis, type of graft, pretransplant body mass index) are summarized in Table 1. Informed consent was obtained at the time of the pretransplant evaluation.

Patient	Gender	Age	BMI	Diagnosis	Graft type
#1	F	67	22,3	IPO	MV
#2	M	44	24,3	NEPT	MV
#3	F	37	14,8	Crohn's disease, SBS	MV+S
#4	F	20	20,8	IPO	MV
#5	F	22	17,8	SBS	IT
#6	M	48	25,6	Portomesenteric thrombosis	MV
#7	F	32	21	NEPT	MV

Table1 . Patient demographics, diagnosis, pretransplant body mass index and type of graft received: Abbreviations: M/F- male/female, BMI – body mass index, IPO – intestinal preusoobstruction, NEPT – neuroendocrine pancreas tumor with liver metastasis, SBS – short bowel syndrome, MV – multivisceral graft, MV+S- multivisceral and spleen, IT – isolate intestinal graft.

The intestine was transplanted either alone (one patient) or as part of a multivisceral graft (six patients) or using previously described surgical techniques [22]. The multivisceral grafts consisted in the stomach, duodenum, liver, pancreas and the small intestine.

The immunosuppression regimen consisted of antithymocyte globulin (Fresenius) induction with Tacrolimus (TAC) monotherapy in a steroid-free protocol described previously by the Pittsburgh group [39]. Target levels of TAC were 10 ng/mL during the first 6 months after transplantation and tapered thereafter to approximately 5 ng/mL by the end of the first year posttransplantation. Rejection was treated with a steroid bolus and thereafter the steroids were quickly tapered. Steroid resistant rejection episodes were treated with OKT-3 (Orthoclone).

PATIENT MANAGEMENT AND SAMPLING

Tacrolimus blood through levels, white cell blood count and CRP were obtained daily. Procalcitonin (PCT) was analyzed whenever clinically indicated. Rejection surveillance was achieved by the means of protocol ileoscopies performed twice a week during the first month starting at the end of the first posttransplant week, then weekly after the first month,

After informed consent, heparinized blood samples were obtained weekly for different time intervals after transplantation. Most of the samples (40/46) were taken during the first 8 weeks after transplantation. Samples were immediately centrifuged and plasma was collected, aliquoted and stored at -70 °C until analyzed.

ANIMALS

Male SD rats (190-250 grams) were purchased from B&K Universal (Sollentuna, Sweden) and housed in the Experimental Biomedicine department of the University of Gothenburg, in 12 hours light-dark cycles. The studies followed the rules and regulations outlined by NIH and the European Union and had the approval of the local committee of the Swedish Animal Welfare Agency.

In papers I and II, TAC (Astellas, Osaka, Japan) at a dose of 0,3 mg/kg or equivalent volumes of saline were given intravenously to the donors six hours before graft harvesting (n = 30/group). Five untreated non-operated animals served as reference for histological and biochemical analyses

EXPERIMENTAL SURGICAL PROCEDURES AND SAMPLING

Intestinal transplantation in the rat (II, III)

Graft procurement and transplantation were performed as previously described by Kellersmann et al [285]. In brief, the first half of the intestine was transplanted heterotopically using microvascular anastomoses and microsurgical techniques. The arterial inflow was supplied from recipients' aorta while the venous drainage was systemic, into the inferior vena cava. Graft perfusion and preservation were performed using iced saline (Papers II and III) or Belzer-UW solution (paper I).

Intestinal preservation (I)

Immediately after in-situ perfusion, the grafts were weighed and two different solutions having different compositions were introduced intraluminally. The compositions of the solutions are given in Table 2. In the control group, intraluminal solutions were omitted. Each graft end was ligated and the grafts were stored in 80 ml ice-chilled perfusion solution, without supernatant air. After eight, fourteen or twenty hours of cold storage the intraluminal solution was evacuated and recovered and the grafts were blotted dry and weighed , then tissue pieces were either placed in 4% buffered formalin or snap-frozen (n=10/time-point).

	UW	HSS	LSS
Potassium (*mmol/L*)	120	10	5,4
Sodium (*mmol/L*)	30	125	65
Magnesium (*mmol/L*)	5	-	-
Chloride (*mmol/L*)	-	35	53
Sulphate (*mmol/L*)	5	40	-
Phosphate (*mmol/L*)	25	-	-
Bicarbonate (*mmol/L*)	-	20	17
Glutathione (*mmol/L*)	3	-	-
HES (*mmol/L*)	50	-	-
Raffinose (*mmol/L*)	30	-	-
Lactobionate (*mmol/L*)	100	-	-
PEG-3350 (*g/L*)	-	11,38	13,125
Allopurinol (*mmol/L*)	1	-	-
Adenosine (*mmol/L*)	5	-	-
pH	7,4	7,94	8,11

Table 2. Composition of the solutions used; UW-University of Wisconsin solution, HSS - high-sodium solution, LSS - low-sodium solution (Paper I)

Tissue and blood sampling (I, II, III)

Twenty minutes after reperfusion a three-cm-long graft segment was sampled from the distal graft end to study the early reperfusion injury and pieces were either placed in formalin or snap-frozen. In Paper II the recipients of either pretreated and control grafts assigned for the 12 or 24h endpoints underwent blood sampling from the tail after one or three hours of reperfusion.

After six, twelve or twenty-four hours, animals were reanesthetized and various measurements performed. Thereafter blood was collected through terminal heart puncture using endotoxin-free materials , spun and plasma was then stored at -76°C until analysed. Graft and liver tissue were obtained and stored in formalin or snap-frozen using the same technique.

Mean arterial pressure, heart rate and microcirculation (III)

Mean arterial pressure was recorded six, twelve and twenty-four hours after reperfusion, after cannulating the right femoral artery. After allowing animal to stabilize for 10 minutes, blood pressure and heart rate were recorded for further 10 minutes using a BioPac system (Harvard Apparatus, Cambridge, MA). Superficial liver microcirculation was studied using laser Doppler Flowmetry (LDF). We used an adhesive probe connected to a Periflux 4001 base unit (Perimed, Järfälla, Sweden) gently applied on the liver surface. At least seven measurements were performed at different sites. The median of the measurements was calculated for each animal and the results were expressed as arbitrary Perfusion Units (PU).

HISTOLOGY AND OTHER TISSUE ANALYSES AND TESTS

Paraffin sections were cut and stained with hematoxylin-eosin. In Paper I and Paper III, intestinal grafts were examined blindly and the ischemia-reperfusion injury was graded according to the Park scoring system (Table 3)[190].

Morphometrical analyses were also performed (mucosal thickness, villus height). In Paper III paraffin liver sections were evaluated blindly regarding overall liver architecture, leukocyte adherence, inflammation and necrosis.

Grade	Description
0	- normal mucosa
1	- subepithelial space(bleb) at the villus tip
2	- more extended subepithelial space (upper half of the villi)
3	- epithelial lifting down the sides of the villi
4	- epithelial breakdown
5	- denuded villi
6	- crypt layer infarction
7	- transmucosal infarction
8	- transmural infarction

Table 3 Park scoring system of the intestinal ischemic damage (Paper I, II).

HISTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY

Neutrophils

Intragraft or liver PMNs were stained using the Naphtol AS-D chloroacetate esterase kit (Sigma Chemicals, St.Louis, MO) on paraffinized sections. All samples were coded and positively stained PMNs were identified and counted in a blinded fashion at intermediate magnification (x200).

Tight junctions (I)

Immunofluorescence was used to study the co-localization of the two tight junction proteins zonula occludens-1 (ZO-1) and claudin-3 (double staining). Briefly, the slides were incubated overnight at 4°C with anti-ZO-1 (1:100, Invitrogen, Stockholm, Sweden) and anti-claudin-3 (1:100, Abcam, UK). Slides were then incubated with secondary antibody conjugated with Alexa 488 and Alexa 594 (1:100, Invitrogen), counterstained with DAPI and examined under the fluorescence microscope.

Cell proliferation.(II)

Deparaffinized slides were incubated with Ki67 antibody (Novocastra, Newcastle/Tyne, UK) according to manufacturers' instructions. Ki67 positive nuclei were blindly counted in fifteen randomly selected crypts sectioned transversally in three different sections under high magnification (x400).

DISSACHARIDASE ASSAY (I, III)

We measured the activity of sucrase and maltase, two dissacharidases found on the enterocyte brush border. Whole tissue homogenates were incubated with a substrate solution (2% maltose in 0.1 mol/L sodium maleate buffer, pH 6.0) for 1 h at 37°C according to the method of Dahlqvist [286]. The reaction was stopped by submerging the samples in boiling water for 5 min. One unit of maltase was defined as the quantity of enzyme hydrolyzing 1 μmol/L maltose to glucose in 1 minute. The resulting glucose was measured using the hexokinase assay and results were expressed as activity units/gram protein.

WESTERN BLOT PROTEIN ANALYSIS (II, III)

Frozen intestinal or liver tissue was homogenised in ice-cold lysis buffer (0,2 M Tris-HCl, 0,1M NaCl, 0,1M EDTA, 0,5M DMSF, 1% Triton X-100, pH-7,4). Protein concentration in the supernatant was measured, and then proteins were separated by sodium dodecyl sulphate -polyacrylamide gel electrophoresis (SDS-PAGE). Following SDS-PAGE, proteins were transferred onto polyvinylidene fluoride (PVDF membranes) (Bio-Rad, Hercules, CA). The membranes were blocked in 5% skim milk and incubated for one hour with anti-HSP-72 antibody (1:3000, SPA-812, StressGen). Secondary antibody was added, and then blots were developed by the use of Advanced ECL kit (Amersham Biosciences Ltd, Buckinghamshire, UK).

For ICAM-1 determination, protein extraction, concentration measurements and blotting were performed as described above. Membranes were incubated with a mouse monoclonal antibody against ICAM-1 (1:3000, MCA773 Serotec, Oxford, UK) and an adequate secondary antibody. After development, the bands from immunoblots were quantified using computerized densitometry (Quantity One, BioRad). Results were normalized to β-tubullin and reported as optical density units (OD).

ELECTROPHORETIC MOBILITY SHIFT ASSAY (EMSA) (II)

Frozen intestinal tissue was gently homogenized in 2 ml of hypotonic buffer at 4°C. Following centrifugation at 4°C 14000 g for 10 minutes, the supernatant was

carefully removed and the pellet was resuspended in ice-cold extraction buffer and extracted 2 hours at 4°C on a rotator. Cell particles were sedimented at 14000g at 4°C for 1 hour and the supernatant was collected, aliquoted and stored –80°C until the analysis of NF-κB was performed. EMSA was performed as described elsewhere [287]. Briefly, the double stranded consensus oligonucleotide (sc-2505, Santa Cruz Biotechnology, San Diego CA) was labeled with 32P (Amersham Pharmacia Biotech, Uppsala, Sweden) using T4-polynucleotide kinase (New England Biolabs, Ipswich, MA) and used in the binding reaction with the nuclear extracts. In some binding reactions 1 µl of an antibody against the p65 subunit of NF-κB (Santa Cruz Biotechnology, CA) was added and incubated for another 20 minutes (the supershift). The same mixture using unlabelled oligonucleotides were used as negative control. The protein-DNA complexes were resolved on a native 5 % polyacrylamide gel, then the gel was vacuum-dried and exposed to x-ray film for 24-36 hours at -80°C. Band intensity was analyzed using computerized densitometry.

CASPASE ACTIVITY ASSAYS (II, III)

The activity of caspase-3 and caspase-9 was measured in whole tissue homogenates. The tissue homogenates were incubated at 37°C on a microtiter plate with caspase-specific substrates: Ac-DEVD-AMC for caspase-3 (Peptide Institute, Osaka, Japan) and Ac-LEHD-AFC (for caspase-9, Enzyme System Products, Livemore, CA). Caspase activity was measured using a Spectramax Gemini microplate fluorometer (excitation wavelength / emission wavelenght 380/ 460 nm for caspase-3 and 400/505 nm for caspase-9) over 2 hours and expressed as pmol released AMC or AFC/µg protein/min. Rat testis homogenate was used as negative control.

CYTOKINES, RESISTIN AND ENDOTOXIN IN PLASMA

Several techniques were used to measure the circulating cytokines. These are described in detail in the respective papers. In short, the following methods were used.

ASSESSMENT OF PLASMA CYTOKINES USING ELISA (III)

Plasma levels of TNF-alpha, IL-1 beta and IL-6 at 1h, 3h, 6h, 12h and 24h postreperfusion were measured using commercially available, rat specific ELISA kits (R&D, Minneapolis, MN). The assay sensitivity for TNF-alfa, IL-1 beta and IL-6 were 5pg/mL, 5 pg/mL and 21pg/mL respectively.

ASSESSMENT OF PLASMA CYTOKINES USING MULTIPLEX ASSAY (IV)

Human plasma samples were analyzed for a panel of pro-inflammatory (Th1) and anti-inflammatory (Th2) cytokines. Plasma concentration of IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13 and TNF-α was determined by the electro-chemiluminescence multiplex system Sector 2400 imager from Meso Scale Discovery (K15010A-4, Gaithersburg, MD, USA).

ASSESSMENT OF PLASMA RESISTIN (IV)

Plasma resistin concentrations were determined in 46 plasma samples (n=5-11 /patient using a commercial enzyme-linked immunosorbent assay (ELISA, DRSN00, RND systems, Minneapolis, MN) . This assay employs the quantitative sandwich enzyme immunoassay technique and a monoclonal antibody specific for human resistin. Measurements were performed using manufacturers' instructions. Plasma samples from seven healthy individuals (live kidney donors) were used as controls. The results were correlated with CRP, WBC, immunosupression (TAC blood through levels), procalcitonin and BMI.

ASSESSMENT OF PLASMA ENDOTOXIN (III)

The Chromogenic Limulus Amoebocyte Lysate Assay test kit was used for duplicate determination of endotoxin plasma concentrations (Chromogenix, Mölndal, Sweden), according to manufacturers' instruction. *Escherichia coli* O11B4 LPS was included in the test kit as standard LPS (100 pg of LPS corresponding to 1.2 endotoxin units). To remove inhibitors, the plasma samples were diluted 1/10 in pyrogen-free water and heat treated for 10 min at 75°C. The interassay coefficient of variation was 8%.

STATISTICAL ANALYSES

Nonparametric methods were used throughout the study, either due to small sample size or because Kolmogorov-Smirnov test showed non Normal data distribution. Differences between independent groups were calculated using the Kruskal-Wallis test followed by the Mann-Whitney U test (both non-parametric). In paper IV, correlations between different variables were assessed using the Spearman rank correlation test. P<0,05 was considered significant. Data were analyzed using GraphPrism 5 software.

RESULTS

PAPER I

The intestines which had low-sodium solution intraluminally had significantly improved preservation injury after both eight hours (median grade 2, range 1-3) and fourteen hours of preservation (median grade 3, range 2-5) compared both with grafts receiving intraluminal high-sodium solution (p<0,01) or undergoing vascular flush alone (p<0,05). After 20 h of cold storage, grafts in all groups had advanced ischemic injury consisting of complete loss of villi and injury to the deeper intestinal layers. After fourteen hours of cold preservation, grafts receiving intraluminal high-sodium solution had a tendency to generate a worse average preservation injury (4,5 range 3-6) compared with grafts receiving only vascular flush (4, range 3-5).

Tissue edema and water retention increased during preservation. However, there was always a tendency that tissue water content was highest in grafts receiving intraluminal high-sodium solutions. After 8 h of, the beneficial role of intraluminal preservation was most apparent in the group receiving intraluminal low-sodium macromolecular solutions, resulting in similar water retention compared with controls grafts. With more extended storage time, the water content gradually increased in all groups and all differences subsided.

The intraluminal solutions did not affect negatively the brush border enzymes. The sodium content into the intraluminal solution decreased in time, irrespective of the intraluminal solutions, indicating sodium absorbtion. The most significant absorbtion occurred during the first eight hours but continued thereafter, albeit at a slower rate.

In normal intestines, ZO-1 and claudin-3 co-localized in the crypts and in the villus epithelium mostly along the lateral surface of the enterocytes. The distribution of tight junction proteins changed in all three groups after cold preservation. After eight hours, control grafts showed signs of de-localization along the intercellular membrane owing to a decrease in claudin-3 expression. Co-localization was maintained in the region closer to the enterocyte base. Grafts receiving intraluminal high-sodium solution had marked de-localization on the enterocyte basolateral membrane and reduced claudin-3 expression. ZO-1 expression was maintained. Intestines receiving intraluminal low-sodium solution had maintained strong co-localization along the lateral membrane but reduced ZO-1 expression in the basal

region. After fourteen hours of preservation, the changes in tight junction structure progressed. The staining in the intercellular area appeared broadened and ZO-1 expression displayed a decreased, disrupted and reticular pattern.Claudin-3 was found greatly reduced and its pattern of expression changed from intercellular reticular to granular intracytoplasmic. These changes were recorded in all grafts, regardless of intraluminal treatment. Twenty hours of preservation generated advanced mucosal damage with broad areas of denuded submucosa. Despite frequent morphologically intact crypts, almost complete de-localization of ZO-1 and claudin-3 was also found in the crypts.

PAPER II

Grafts from donors pretreated with TAC had increased HSP72 expression at the time of harvesting. However, this did not influence preservation injury, which was similar between the pretreated and the control group. Pretreated grafts showed however a milder early reperfusion injury compared with controls. Thus, pretreated grafts maintained connective tissue (lamina propria) - (4,1 ± 0,1 vs. 5 ± 0,1 p<0,01). The significant improvement in graft morphology persisted after six and twelve hours post-reperfusion. Thus, pretreated grafts had improved morphological parameters (villus length, mucosal thickness) and milder neutrophil inflammation. Pretreated grafts also revealed an accelerated cell proliferation and enterocyte maturation, reflected by a higher proliferation index (Ki67 positive cells) in the crypts and by the superior dissacharidase levels.

At the same time, we found reduced NF-κB early after reperfusion and a uniform and persistent inhibition of the second wave of NF-κB activation. This inhibition was recorded at least during the first twenty-four hours following graft reperfusion. The effective and enduring abrogation of the NF-κB activation was also reflected by the lower ICAM-1 levels found in the pretreated grafts.

PAPER III

Biochemical evidence of liver dysfunction, revealed by increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), was present in all animals receiving intestinal grafts. The dysfunction seem to be directly related to the presence of the graft, since non-transplanted animals had virtually no

variations in plasma transaminase levels compared with normal animals. However, recipients of pretreated grafts had a milder dysfunction compared with recipients of control grafts. The hepatocellular injury seems to have occurred early but appeared to be short lived.

We found no morphological alterations that could have determined the transient liver injury, such as necrosis, or inflammation. However, we found evidence of ongoing apoptosis in the livers of graft recipients, revealing that the liver had been submitted to sublethal stress and pro-apoptotic stimuli, more intense in the recipients of control, untreated grafts.

Conversely, in the recipients of control grafts we identified a biphasic pattern of TNF-α release and an increasing trend of two pro-inflammatory cytokines (IL-1β and IL-6) throughout the first twelve hours after graft reperfusion. These interesting and characteristic patterns were absent or less obvious in recipients of pretreated grafts.
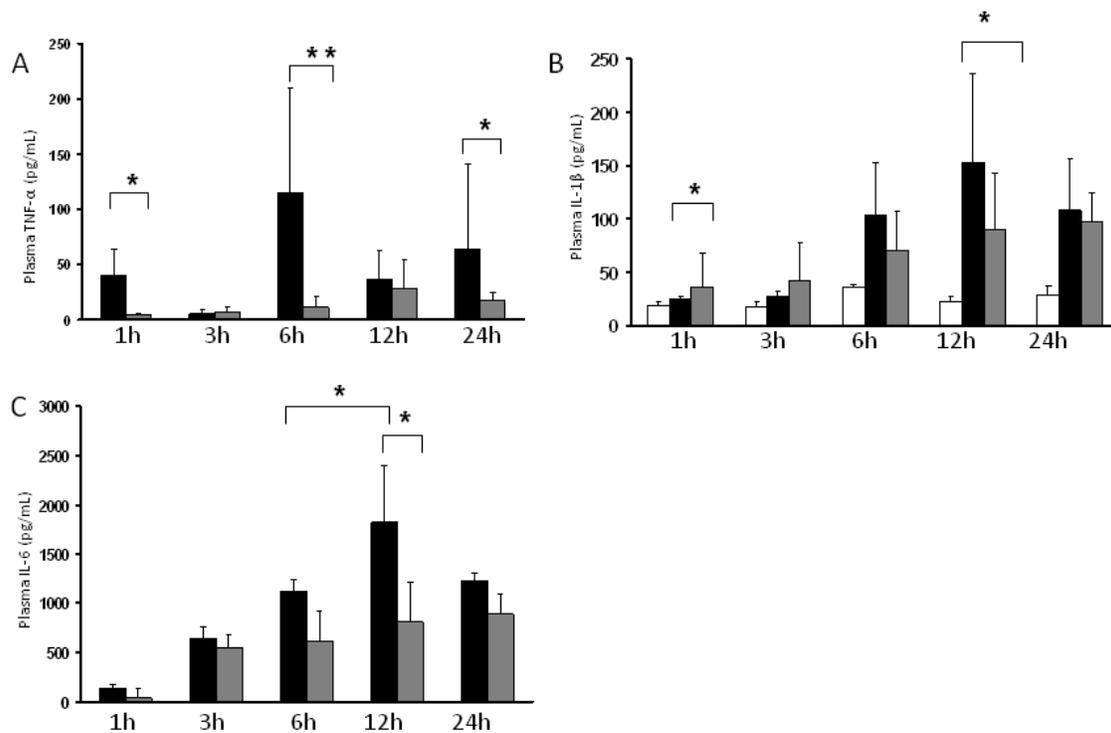

Figure 4. The proinflamatory cytokine response TNF-α (A), IL-1β (B) and IL-6 (C)

Looking at the local ischemia or hypoperfusion as a potential mechanism behind the transient liver dysfunction, microvascular blood-flow measurements at the surface of the liver indicated that microvascular perfusion was similar in all the studied groups at all times..

Circulating LPS had very low levels both after six and twelve hours after reperfusion and was found significantly increased only twenty-four hours after graft reperfusion, similar between the two groups.

PAPER IV

The six month and one year patient survival in the series presented herein was 100% and 85% respectively. Five out of seven patients (75%) have had early acute rejection episodes and the same proportion had severe bacterial infections or sepsis during the first two months after transplantation.

Resistin was detected in all healthy controls. Significantly increased resistin levels were found in all patients, already one week after transplantation. Resistin levels varied considerably between patients and remained increased throughout the first eight weeks after transplantation (Fig.5).
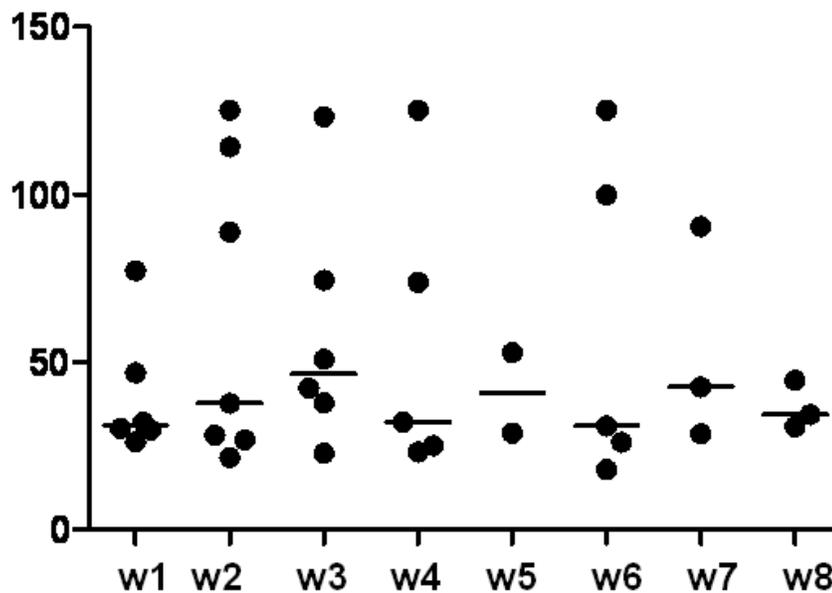
Figure5. Plotted resistin levels over the first eight weeks posttransplantation

Resistin continued to remain increased compared with controls after two and five years respectively, in the absence of infection or rejection.

Resistin levels were found increased in samples taken during acute rejection compared with samples taken during rejection-free course. Sepsis was also associated with increases in plasma resistin.

No significant positive correlation was found between plasma resistin and six different Th1 (pro-inflammatory) cytokines. In one patient we identified a significant negative correlation between IL-1β and IL-8 and resistin.

Several positive correlations were identified between resistin and some anti-inflammatory Th2 cytokines. Thus, IL-4 a regulatory cytokine promoting the Th2 phenotype positively correlated with resistin in two patients, of which one was rejection-free.

The analysis of WBC, BMI, TAC trough levels, CRP and PCT in relationship with plasma resistin revealed only one negative correlation with WBC in patient #7 (r_s = -0,9, p<0,05) and one positive correlation with TAC trough levels in patient #6 (r_s = 0,68, p<0,05). No other significant correlations whatsoever were identified.

GENERAL DISCUSSIONS

In contrast to previous clinical observations of harmful effects of the intraluminal bowel preparation solutions, the experimental results in this thesis suggest that a low sodium content solution may reduce the edema in the intestinal wall during preservation, which in its turn may have favorable effects after reperfusion. Furthermore, pretreatment of the donor with tacrolimus appears both to reduce the graft reperfusion injury and accelerate mucosal morphologic recovery after rat intestinal transplantation, as well as causing a milder systemic inflammatory response in the recipient

The clinical study investigated the potential of resistin as an intestinal rejection marker. As the results in this small group indicate, despite uniformly increasing after intestinal transplantation, resistin is unsuitable for monitoring the acute rejection since the increase is present even in the absence of acute rejection.

Ischemia reperfusion is an extremely relevant issue in intestinal transplantation, because of the potentially contaminated intestinal content. Mucosal barrier breakdown may lead to bacterial translocation and endotoxemia, similarly with other conditions evolving with intestinal ischemia or hypoperfusion [288-290]. The ability of the ischemic intestine to promote sepsis and sepsis-like systemic inflammatory response has long been recognized [291, 292]. Thus, an intact mucosal barrier in these heavily immunosuppressed patients is paramount.

Throughout the 1990s procurement teams routinely performed donor bowel preparations using oral antibiotics and macromolecular solutions (i.e., Golytely) as well as luminal flush with Ringer solution (130 mmol/L NaCl) in an attempt to reduce the intestinal load [24, 293]. However, this approach is currently abandoned and most centers limit the donor preparation to intravenous antibiotics [12, 294].

The lumen has been recognized for a long time as a potential route to deliver nutrients to the mucosa during intestinal preservation. Oxygen, glucose, glutamine, perfluorodecaline and several customized solutions have been introduced intraluminally in an attempt to reduce the ischemic injury and most studies reported improvements [247, 248, 295, 296]. However, we believe that more 'unspecific' causes such as a better control over the composition and osmolarity of the intraluminal content may have contributed equally as the more specific research interventions [297, 298]. We argue that that the low metabolic activity at 4

degrees (either the glutamine metabolism or ATP synthesis) is too modest to decisively contribute to the improvements reported. Few studies were followed by transplantation, further raising doubt about the relevance of the methods.

As mentioned above, our current findings suggest that bowel preparation solutions may successfully be used, not only for cleansing the donor intestine but also as an intraluminal preservation solution, provided that a low-sodium solution is used. It is unknown if using HTK solution will yield similar results due to the different electrolyte composition UW solution. In addition, the optimal volume of solution to be introduced intraluminally should be further studied in large animal models or in the human intestine.

The morphological improvements described herein are not dramatic but rather modest. However, as Paper II and other previous reports show, reperfusion leads to an injury advancement corresponding to one grade on the Park scale, namely from grade 3 (i.e. subepithelial edema) to grade 4 (villus denudation, mucosal breakdown) [193]. In the transplantation setting Park et al showed that increasing reperfusion injury from grade 3 to grade 4 increases mortality from 0% to 70% while Haglind et al report similar results using a warm intestinal ischemia model [193, 299]. Thus, minor morphological improvements may translate into significant differences in distant organ injury, systemic inflammatory response and ultimately improved clinical course in the early post-transplant period.

Furthermore, besides the early benefits of an alleviated reperfusion injury may be reflected in less late changes, as chronic rejection is the new old foe the intestinal transplant community battles today [300]. Advanced preservation injury may increase graft immunogenicity and may precipitate rejection [79, 301]. Some reports demonstrate that alleviated reperfusion injury after donor preconditioning has also been followed by less chronic changes resembling rejection and improved long term graft function [232, 302-304].

The distant organ injury ensuing after intestinal ischemia has been the subject of extensive research [290]. Lung and liver injury have been reported after experimental and clinical intestinal ischemia including after surgery for abdominal aortic aneurysms [153, 305, 306]. The mediators of injury, initially described as "toxic factors from the intestine" seem to circulate both through the portal vein and the lymphatic drainage [160, 306-308] and interrupting the mesenteric lymph drainage resulted in a lower remote organ injury [309, 310]. Considering that the liver dysfunction seen in our model was mild and transient and the inflammatory response was quite modest, despite the rather advanced reperfusion injury, we hint

that the absence of the mesenteric lymph, or perhaps the systemic drainage, may have been beneficial. Furthermore, since the microvascular perfusion of the liver surface was similar in all groups, this virtually rules out local perfusion failure or ischemia as a cause of liver injury. Secondly, we do not believe that endotoxins from the bowel play a major role in our experiments, since circulating LPS had very low levels both after six and twelve hours after reperfusion and was found significantly increased only twenty-four hours after graft reperfusion, similar between the two groups. Thus, LPS did not seem responsible for the systemic inflammatory response, since maximal pro-inflammatory cytokine levels were attained in the presence of very low LPS levels.

The dysfunction seems driven by soluble mediators since we found no evidence of gross morphological abnormalities or neutrophil infiltration, despite earlier reports suggesting a causal role for the neutrophils [311]. Moreover, when transplanting intestines in neutrophil depleted rats we observed a pattern liver dysfunction which was similar with the control group of neutrophil-sufficient animals (own unpublished data). Extrapolating in a clinical perspective that may imply that induction with polyclonal antibodies may reduce the local reperfusion injury but it will not directly influence the remote organ injury.

Graft preconditioning through various pretreatment regimens applied to the organ donor has been increasingly explored and suggested to improve graft quality. Among the numerous approaches tested, pharmacological induction/upregulation of heme oxygenase-1 (HSP32) expression has probably been the most extensively investigated. The reason behind the choice of this inducible protein is the multitude of biological pathways and mechanisms that are presumably influenced by the enzyme and its byproducts [312, 313]. Some of the most interesting effects are the reduction of the oxidative stress and the inhibition of several transcription factors as well as the modulation of apoptosis. The biological potential of heme oxygenase-1 and its byproducts is however counterweighted by the reluctance towards the clinical use of carbon monoxide, a highly toxic gas and the large variability of the biological response in humans [312, 314].

Herein, we showed that donor preconditioning with an accepted drug (TAC) may significantly improve intestinal graft morphology, possibly through the upregulation of the cytoprotective HSP72. Whether the upregulation of heat shock proteins is a safe approach in an allogeneic setting, on both short and long run, remains to be further explored, since numerous studies confirmed the immunostimulatory properties of various heat shock proteins [315, 316]. Moreover, as mentioned

earlier, anti-heat shock protein reactivity has been repeatedly demonstrated in transplant and non-transplant setting (i.e., atherosclerosis) and suggested as a causal factor of undesirable events such as lymphocyte and dendritic cell activation [136, 317, 318].

We also recorded a significant and long lasting NF-κB inhibition in the pretreated grafts. Although NF-κB inhibition by TAC is not entirely new, this feature has not been reported earlier in this setting. Given the multifaceted effects of NF-κB inhibition, it is likely this contributed to the modulation of both the local and systemic inflammation, due to the assumed downregulation of adhesion molecules and reduced cytokine release by the graft.

The microenvironment is an essential factor in regulating cell proliferation [319, 320] In the present study the favorable microenvironmental conditions could have been represented by the lower inflammatory activation and the reduced tissue inflammation observed in the pretreated grafts. Conversely, cells remain quiescent in the presence of unfavorable circumstances (ie, cellular stress) and proliferation may be blocked or delayed [321].

On the other hand, TAC has been shown to stimulate cell proliferation regeneration after liver resection or neuroraphy [322, 323] and several mechanisms have been suggested, including growth factors and the FK-binding proteins. The latter proteins are responsible for the intracytoplasmic TAC transport and are also called immunophilins [324]. Whether the drug itself stimulated the crypt proliferation, seen in our experiments, or if it was the result of a chain of biological events resulting in a microenvironment more favorable for proliferation remains unclear.

As previously mentioned, TAC has been proved useful in reducing ischemia-reperfusion injury and its long term consequences in kidney transplantation in the rat The same study revealed very similar results when prednisolone, a potent synthetic steroid able to alter numerous signaling pathways, was given to the organ donors [232].

Donor pretreatment with steroids for the reduction of the alloindependent graft injury is probably the strategy with the highest chance to become a clinical routine in the future. Experimental studies revealed improved renal graft function after transplantation [325] and donor pretreatment with steroids blocked the organ activation and increased immunogenicity induced by the brain death [326, 327]. Moreover, a recent clinical trial demonstrated improved results using livers from donors systematically receiving methylprednisolone [328]. Lastly, the fact that

56

hormonal resuscitation is already currently used in thoracic organ donors with no adverse effects observed in other organs may advocate for the future routine use of steroids in pretreating the organ donors [329, 330].

At the moment resistin is considered a pro-inflammatory adipo(cyto)kine. While its association with inflammation is supported by extensive evidence [271, 276], a contributory role of resistin in inflammation is less clear. Resistin increases in a broad array of diseases evolving with tissue injury and inflammation, such as active inflammatory bowel disease, cancer, asthma, type 2 diabetes, chronic kidney disease and infections, but has little in common regarding the pathogenesis [277, 279, 331-333]. Hence, based on our observations showing an early rise in plasma resistin in the absence of rejection, in the presence of infection or even during uneventful course we are skeptical towards the hypothesis of a major pathogenetic role of resistin in inflammation as a 'pro-inflammatory cytokine'. Instead for a role of 'pro-inflammatory cytokine' we suggest that resistin is a marker of inflammation or tissue injury. In the present study the increase was triggered by ischemia-reperfusion injury and/or the alloimmune injury.

In brief, arguments for a cytokine role of resistin are its production by leukocytes and its actions over several cell types, leading to changes in their phenotype. Arguments against a cytokine role for resistin are the very high levels, nanograms compared to picograms, compared with other acknowledged cytokines and that no specific stimulus or receptor for resistin has been identified. In addition, no counter-regulatory molecule or loop is yet recognized.

One of our aims was to investigate the potential of resistin as an intestinal rejection marker. This hypothesis was based on previous studies reporting specific increases during active inflammatory bowel disease [279]. We found a large inter-individual variation after transplantation as well as an unspecific increase during several complications, of which one was rejection. Moreover, we found resistin uniformly increased compared to healthy controls. We did not identify correlations with several key Th1 and Th2 cytokines, that could have allow us to speculate on the factors inducing the release of resistin. In contrast with other studies we could not identify any correlation between resistin or CRP or WBC either. This fact may be due to the immunosuppression, a feature absent in studies reporting such correlations [280, 334]. Thus, we believe that further studies on resistin in immunosuppressed subjects could shed more light on the biology of this intriguing molecule.

CONCLUSIONS

With support of the studies presented in this thesis I conclude that:

- the intraluminal introduction of macromolecular solutions having low sodium content may have beneficial effects on the preservation injury of the rat intestine

- donor pretreatment with a single dose of tacrolimus reduces graft reperfusion injury and accelerates mucosal morphologic recovery after rat intestinal transplantation

- transplantation of intestines from donors pretreated with tacrolimus is followed by a lesser liver dysfunction and a milder systemic inflammatory response compared with animals receiving untreated intestinal grafts

- resistin increases early after clinical intestinal transplantation irrespective of the presence of complications and has large individual variations, making it unsuitable as a diagnostic marker for acute rejection

REFLECTIVE STATEMENTS

- The lumen is an easy and obvious route to get direct access to the most sensitive part of the intestinal graft, i.e. the mucosa. Numerous interventions have been attempted over the time with various results but without a decisive breakthrough. Our results identified some morphological features underlying the permeability changes during ischemia. Moreover, the study suggests that intraluminal high-sodium solutions should be avoided when designing future intraluminal preservation solutions. Conversely, previous observations might have missed the importance of the right sodium content in the right compartment and a clinical reassessment in the setting of a multicenter randomized controlled trial may be beneficial

- This study showed once again that targeted interventions in the organ donor may successfully reduce the initial ischemia/reperfusion injury. Whether the present methodology and the conclusions can be directly translated clinically is unknown, since TAC metabolism differs between rats and humans. However, considering the fact that:

  i   TAC is a major immunosuppresant already in clinical use

  ii the graft will be submitted to the same drug shortly after reperfusion

  iii similar beneficial results following TAC pretreatment have been described in other transplantable organs and

  iv the intervention is feasible and straightforward

  may open the perspective for a future clinical trial.

- Although resistin does not seem a reliable acute rejection marker in clinical intestinal transplantation, the need for such a tool is still a high priority. This first report on resistin in a field quite remote from obesity, atherosclerosis, chronic inflammation suggest that resistin is a versatile molecule that is probably involved in many biological processes.

.

ACKNOWLEDGEMENTS

The work included in this thesis stretched over almost eight years. Before and during this time I was very fortunate to meet and get support from many individuals, to whom I express my sincere gratitude and appreciation.

First and foremost **Michael Olausson**, main supervisor, Professor of Transplantation Surgery, Chairman of the Sahlgrenska Transplant Institute and friend - I am enormously grateful for your unceasing support, freedom, personal example and inspiring energy. One of the many things I learned from you is that 'when the going gets tough, the tough gets going'!

My co-authors and friends **Klas Blomgren**, **Changlian Zhu, Eva Hallberg, Inger Mattsby-Baltzer, Johan Mölne** for availability, stimulating discussions and collaboration. Special thanks to
Alex Karlsson-Parra, for his emergency input and bright, surrealistic ideas, whenever needed.

Very special thanks to **Rille Pullerits** and **Meghnad Joshi** who helped me turn results into science**.**

Gustaf Herlenius for his personal example of taking the standards of dedication, professionalism and compassion 'where no man has gone before'

Michael Breimer, for scientific advices and friendship, for revealing me fundamental blue-yellow principles, allowing me the privilege to contribute to the reforestation of Sweden as well as first taking me to IKEA

Professor Emeritus Ove Lundgren, an amazing model of enthusiasm and academic curiosity as well as constant source of inspiration for everyone around, for the privilege of our discussions and for laying seeds of knowledge that have borne many fruits throughout the years.

The late Professor **Andrej Tarkowski**, for his inspirational model as scientist, mentor and a very good person: he is deeply missed.

The members of the Renal Center, **Börje Haraldsson**, **Madeleine Ingelsten** and **Jenny Nyström**, for fruitful and pleasant collaboration. Two packed freezers await!

Göran Kurlberg, for past, present and hopefully future collaboration related to various sorts of intestinal diseases, intestinal transplantation and intestinal failure.

The colleagues at Sahlgrenska Transplant Institute, a true "band of brothers" for sharing skills, commitment and great moments either in the operating room or at various Welcome Receptions, Gala Dinners, farewell parties, special evenings or at various airports throughout the world.

The members of the brand new **Transplant Biology Research Group**, working hard to spice up the research life (sometimes making it really hot)! **Margareta Filipson**, for taking pains to improve my Swedish, cleaning up the countless coffee cups and enthusiastically purchasing anything from 11/0 sutures to -70°C freezers (the large model!)

Professor **Thomas Kraus**, **Markus Golling** and **Octavian Bud,** all formerly at the Surgical Clinic in Heidelberg Germany, for introducing me into the universe of liver transplantation back in 1999

All people at Surgical Research Laboratory in Groeningen, The Netherlands for a truly inspirational time there! **Professor Rutger Ploeg**, **Henri Leuvenink**, **Theo Schuurs**, **Cyril Moers**, **Hugo Maathuis and Vincent Nieuwenhuijs:** Bedankt!

Wholehearted thanks to **George Dindelegan**, **Razvan Scurtu**, **Constantin Ciuce**, **Calin Ionescu** and **Radu Motocu**, former colleagues at The First Surgical Clinic in Cluj-Napoca, Romania, who patiently guided my first steps in surgery and microsurgery

The people at Wallenberg Laboratory, present and past, for making W-lab such a creative and inspiring place. No one mentioned, no one forgotten. Well, I should hint at Lab 1, Lab 5 and The Party Patrol

Heimir Snorasson, the omnipresent 'genie in the bottle' coming out instantly to save the day every time the computer crashed, when updates were badly needed and all that kind of stuff.

All **the nursing staff at the Sahlgrenska Transplant Institute**, particularly "the hard core formerly on, Avdelning 20/33"! YOU ALL set the standards of team spirit, patient care, professionalism and empathy to levels I never met elsewhere.

The team of **transplant coordinators** for practical and psychological support, endless enthusiasm, stimulating feedback and a really good time during very late nights or early mornings

Elisabet Forsell for all the good care and great help throughout the years

Marie Sverkersdotter for constant and generous support, friendship and so many great moments in different locations

My friends from the early days in Sweden: **Nico Heins**, **Ann Lindgård**, **Randa Akouri** and her wonderful family, **Sorin Maruster, Violeta Piculescu** and **Florin Maican** for warmth, friendship and for sometimes succeeding in dragging me out from the lab

My parents, **Gheorghe and Maria Oltean**, for teaching me to never give up once started and to lend a hand whenever others need it. Multumesc pentru toate eforturile, sacrificiile si increderea neconditionata! My 'little' sister **Mihaiela** for doing her best in capturing our parents´ thoughts and attention so that they don't have time to worry about me …

Save the best for last. **Simona**, thank you for being so close while we were so far away. You are my better half and your love and trust gave me mountains of strength and motivation. We did this journey together from its very first day and your contribution to this thesis is immense - despite being on only one paper! Thank you for accepting my dreams as if they were yours. It's hard to find right words to express my absolute gratitude, yet I'll give it a try anyway: TE IUBESC FUARTE FUARTE TARE!

REFERENCES

1. Manax, W.G., et al., *Experimental Preservation of the Small Bowel.* Am J Surg, 1965. **109**: p. 26-31.
2. Eyal, Z., et al., *Successful in Vitro Preservation of the Small Bowel, Including Maintenance of Mucosal Integrity with Chlorpromazine, Hypothermia, and Hyperbaric Oxygenation.* Surgery, 1965. **57**: p. 259-68.
3. Lillehei, R.C., et al., *Transplantation of stomach, intestine, and pancreas: experimental and clinical observations.* Surgery, 1967. **62**(4): p. 721-41.
4. Buchman, A.L., *Complications of long-term home total parenteral nutrition: their identification, prevention and treatment.* Dig Dis Sci, 2001. **46**(1): p. 1-18.
5. Grant, D., et al., *Successful small-bowel/liver transplantation.* Lancet, 1990. **335**(8683): p. 181-4.
6. Goulet, O.J., et al., *Small intestinal transplantation in a child using cyclosporine.* Transplant Proc, 1988. **20**(3 Suppl 3): p. 288-96.
7. Hansmann, M.L., et al., *Small bowel transplantation in a child. Morphologic, immunohistochemical, and clinical results.* Am J Clin Pathol, 1989. **92**(5): p. 686-92.
8. Abu-Elmagd, K., et al., *Three years clinical experience with intestinal transplantation.* J Am Coll Surg, 1994. **179**(4): p. 385-400.
9. Todo, S., et al., *Outcome analysis of 71 clinical intestinal transplantations.* Ann Surg, 1995. **222**(3): p. 270-80; discussion 280-2.
10. Abu-Elmagd, K., et al., *Clinical intestinal transplantation: a decade of experience at a single center.* Ann Surg, 2001. **234**(3): p. 404-16; discussion 416-7.
11. Tzakis, A.G., et al., *Piggyback orthotopic intestinal transplantation.* Surg Gynecol Obstet, 1993. **176**(3): p. 297-8.
12. Abu-Elmagd, K., et al., *Logistics and technique for procurement of intestinal, pancreatic, and hepatic grafts from the same donor.* Ann Surg, 2000. **232**(5): p. 680-7.
13. Olausson, M., et al., *Experiences from the first five-organ multivisceral transplantation in Scandinavia.* Transplant Proc, 2000. **32**(6): p. 1229-30.
14. Roy, C.C. and D.C. Belli, *Hepatobiliary complications associated with TPN: an enigma.* J Am Coll Nutr, 1985. **4**(6): p. 651-60.
15. Buchman, A., *Total parenteral nutrition-associated liver disease.* JPEN J Parenter Enteral Nutr, 2002. **26**(5 Suppl): p. S43-8.
16. Pironi, L., et al., *Candidates for intestinal transplantation: a multicenter survey in Europe.* Am J Gastroenterol, 2006. **101**(7): p. 1633-43; quiz 1679.
17. *American Gastroenterological Association medical position statement: short bowel syndrome and intestinal transplantation.* Gastroenterology, 2003. **124**(4): p. 1105-10.
18. Beath, S., et al., *Collaborative strategies to reduce mortality and morbidity in patients with chronic intestinal failure including those who are referred for small bowel transplantation.* Transplantation, 2008. **85**(10): p. 1378-84.
19. Grant, D., et al., *2003 report of the intestine transplant registry: a new era has dawned.* Ann Surg, 2005. **241**(4): p. 607-13.
20. Reyes, J., et al., *Pediatric intestinal transplantation: historical notes, principles and controversies.* Pediatr Transplant, 2002. **6**(3): p. 193-207.
21. Fishbein, T.M., *Intestinal transplantation.* N Engl J Med, 2009. **361**(10): p. 998-1008.
22. Kato, T., et al., *Intestinal and multivisceral transplantation.* World J Surg, 2002. **26**(2): p. 226-37.
23. Todo, S., et al., *Intestinal transplantation in composite visceral grafts or alone.* Ann Surg, 1992. **216**(3): p. 223-33; discussion 233-4.
24. Todo, S., et al., *Abdominal multivisceral transplantation.* Transplantation, 1995. **59**(2): p. 234-40.
25. Bueno, J., et al., *Composite liver--small bowel allografts with preservation of donor duodenum and hepatic biliary system in children.* J Pediatr Surg, 2000. **35**(2): p. 291-5; discussion 295-6.
26. Hanto, D.W., et al., *Liver and intestine transplantation: summary analysis, 1994-2003.* Am J Transplant, 2005. **5**(4 Pt 2): p. 916-33.
27. Tzakis, A.G., et al., *100 multivisceral transplants at a single center.* Ann Surg, 2005. **242**(4): p. 480-90; discussion 491-3.

28.   Giblin, L., et al., *A comparison of long-term graft survival rates between the first and second donor kidney transplanted--the effect of a longer cold ischaemic time for the second kidney.* Am J Transplant, 2005. **5**(5): p. 1071-5.

29.   Todo, S., et al., *Extended preservation of human liver grafts with UW solution.* JAMA, 1989. **261**(5): p. 711-4.

30.   Cicalese, L., et al., *Bacterial translocation in clinical intestinal transplantation.* Transplantation, 2001. **71**(10): p. 1414-7.

31.   Fromont, G., et al., *Small bowel transplantation in children: an immunohistochemical study of intestinal grafts.* Gut, 1995. **37**(6): p. 783-90.

32.   Jones, S.C., et al., *Expression of interleukin-6 by intestinal enterocytes.* J Clin Pathol, 1993. **46**(12): p. 1097-100.

33.   Mayrhofer, G. and L.D. Spargo, *Subcellular distribution of class II major histocompatibility antigens in enterocytes of the human and rat small intestine.* Immunol Cell Biol, 1989. **67 (Pt 4)**: p. 251-60.

34.   Nagler-Anderson, C., *Man the barrier! Strategic defences in the intestinal mucosa.* Nat Rev Immunol, 2001. **1**(1): p. 59-67.

35.   Turner, J.R., *Intestinal mucosal barrier function in health and disease.* Nat Rev Immunol, 2009. **9**(11): p. 799-809.

36.   Sigurdsson, L., et al., *Bacteremia after intestinal transplantation in children correlates temporally with rejection or gastrointestinal lymphoproliferative disease.* Transplantation, 2000. **70**(2): p. 302-5.

37.   Pomposelli, F., et al., *Induction of graft-versus-host disease by small intestinal allotransplantation in rats.* Transplantation, 1985. **40**(4): p. 343-7.

38.   Kimura, K., S.R. Money, and B.M. Jaffe, *The effects of size and site of origin of intestinal grafts on small-bowel transplantation in the rat.* Surgery, 1987. **101**(5): p. 618-22.

39.   Reyes, J., et al., *Intestinal transplantation under tacrolimus monotherapy after perioperative lymphoid depletion with rabbit anti-thymocyte globulin (thymoglobulin).* Am J Transplant, 2005. **5**(6): p. 1430-6.

40.   Bond, G.J., et al., *Evolutionary experience with immunosuppression in pediatric intestinal transplantation.* J Pediatr Surg, 2005. **40**(1): p. 274-9; discussion 279-80.

41.   Abu-Elmagd, K.M., et al., *Evolution of the immunosuppressive strategies for the intestinal and multivisceral recipients with special reference to allograft immunity and achievement of partial tolerance.* Transpl Int, 2009. **22**(1): p. 96-109.

42.   Guaraldi, G., et al., *Outcome, incidence, and timing of infectious complications in small bowel and multivisceral organ transplantation patients.* Transplantation, 2005. **80**(12): p. 1742-8.

43.   Kimura, T., et al., *Impact of induction therapy on bacterial infections and long-term outcome in adult intestinal and multivisceral transplantation: a comparison of two different induction protocols: daclizumab vs. alemtuzumab.* Clin Transplant, 2009. **23**(3): p. 420-5.

44.   Kaufman, S.S., et al., *Discrimination between acute rejection and adenoviral enteritis in intestinal transplant recipients.* Transplant Proc, 2002. **34**(3): p. 943-5.

45.   Berney, T., et al., *Successful treatment of posttransplant lymphoproliferative disease with prolonged rituximab treatment in intestinal transplant recipients.* Transplantation, 2002. **74**(7): p. 1000-6.

46.   Herlenius, G., et al., *Chronic kidney disease--a common and serious complication after intestinal transplantation.* Transplantation, 2008. **86**(1): p. 108-13.

47.   Selvaggi, G., et al., *Analysis of acute cellular rejection episodes in recipients of primary intestinal transplantation: a single center, 11-year experience.* Am J Transplant, 2007. **7**(5): p. 1249-57.

48.   Sudan, D.L., et al., *Incidence, timing, and histologic grade of acute rejection in small bowel transplant recipients.* Transplant Proc, 2000. **32**(6): p. 1199.

49.   Sigurdsson, L., et al., *Endoscopies in pediatric small intestinal transplant recipients: five years experience.* Am J Gastroenterol, 1998. **93**(2): p. 207-11.

50.   Ishii, T., et al., *Exfoliative rejection after intestinal transplantation in children.* Pediatr Transplant, 2003. **7**(3): p. 185-91.

51.   Gondolesi, G., et al., *Serum citrulline is a potential marker for rejection of intestinal allografts.* Transplant Proc, 2002. **34**(3): p. 918-20.

52. Kaufman, S.S., et al., *Lack of utility of intestinal fatty acid binding protein levels in predicting intestinal allograft rejection.* Transplantation, 2001. **71**(8): p. 1058-60.
53. Sudan, D., et al., *Calprotectin: a novel noninvasive marker for intestinal allograft monitoring.* Ann Surg, 2007. **246**(2): p. 311-5.
54. Yu, H.C., et al., *Utilization of dried blood spot citrulline level as a noninvasive method for monitoring graft function following intestinal transplantation.* Transplantation, 2005. **80**(12): p. 1729-33.
55. Gondolesi, G., et al., *The value of plasma citrulline to predict mucosal injury in intestinal allografts.* Am J Transplant, 2006. **6**(11): p. 2786-90.
56. David, A.I., et al., *Blood citrulline level is an exclusionary marker for significant acute rejection after intestinal transplantation.* Transplantation, 2007. **84**(9): p. 1077-81.
57. Alexandrides, I.J., et al., *Abdominal wall closure after intestinal transplantation.* Plast Reconstr Surg, 2000. **106**(4): p. 805-12.
58. Zanfi, C., et al., *Incidence and management of abdominal closure-related complications in adult intestinal transplantation.* Transplantation, 2008. **85**(11): p. 1607-9.
59. Gondolesi, G., et al., *Use of the abdominal rectus fascia as a nonvascularized allograft for abdominal wall closure after liver, intestinal, and multivisceral transplantation.* Transplantation, 2009. **87**(12): p. 1884-8.
60. Levi, D.M., et al., *Transplantation of the abdominal wall.* Lancet, 2003. **361**(9376): p. 2173-6.
61. Sato, A., et al., *Intestinal energy metabolism during ischemia and reperfusion.* J Surg Res, 1999. **82**(2): p. 261-7.
62. Amersi, F., et al., *Bucillamine, a thiol antioxidant, prevents transplantation-associated reperfusion injury.* Proc Natl Acad Sci U S A, 2002. **99**(13): p. 8915-20.
63. Parks, D.A., et al., *Ischemic injury in the cat small intestine: role of superoxide radicals.* Gastroenterology, 1982. **82**(1): p. 9-15.
64. Adkison, D., et al., *Role of free radicals in ischemia-reperfusion injury to the liver.* Acta Physiol Scand Suppl, 1986. **548**: p. 101-7.
65. Horton, J.W. and P.B. Walker, *Oxygen radicals, lipid peroxidation, and permeability changes after intestinal ischemia and reperfusion.* J Appl Physiol, 1993. **74**(4): p. 1515-20.
66. Meneshian, A. and G.B. Bulkley, *The physiology of endothelial xanthine oxidase: from urate catabolism to reperfusion injury to inflammatory signal transduction.* Microcirculation, 2002. **9**(3): p. 161-75.
67. Fuh, K.C., et al., *Signal transduction by reactive oxygen species: alternative paradigms for signaling specificity.* Surgery, 2002. **131**(6): p. 601-12.
68. Karin, M. and F.R. Greten, *NF-kappaB: linking inflammation and immunity to cancer development and progression.* Nat Rev Immunol, 2005. **5**(10): p. 749-59.
69. Viatour, P., et al., *Phosphorylation of NF-kappaB and IkappaB proteins: implications in cancer and inflammation.* Trends Biochem Sci, 2005. **30**(1): p. 43-52.
70. Lentsch, A.B. and P.A. Ward, *Activation and regulation of NFkappaB during acute inflammation.* Clin Chem Lab Med, 1999. **37**(3): p. 205-8.
71. An, S., Y. Hishikawa, and T. Koji, *Induction of cell death in rat small intestine by ischemia reperfusion: differential roles of Fas/Fas ligand and Bcl-2/Bax systems depending upon cell types.* Histochem Cell Biol, 2005. **123**(3): p. 249-61.
72. Soeda, J., et al., *Cytochrome c release into cytosol with subsequent caspase activation during warm ischemia in rat liver.* Am J Physiol Gastrointest Liver Physiol, 2001. **281**(4): p. G1115-23.
73. Kaufmann, S.H., et al., *Analysis of caspase activation during apoptosis.* Curr Protoc Cell Biol, 2001. **Chapter 18**: p. Unit 18 2.
74. Li, M.O., et al., *Phosphatidylserine receptor is required for clearance of apoptotic cells.* Science, 2003. **302**(5650): p. 1560-3.
75. Jaeschke, H., et al., *Activation of caspase 3 (CPP32)-like proteases is essential for TNF-alpha-induced hepatic parenchymal cell apoptosis and neutrophil-mediated necrosis in a murine endotoxin shock model.* J Immunol, 1998. **160**(7): p. 3480-6.
76. Lawson, J.A., et al., *Parenchymal cell apoptosis as a signal for sinusoidal sequestration and transendothelial migration of neutrophils in murine models of endotoxin and Fas-antibody-induced liver injury.* Hepatology, 1998. **28**(3): p. 761-7.

77.   Yeh, K.Y., et al., *Rapid activation of NF-kappaB and AP-1 and target gene expression in postischemic rat intestine.* Gastroenterology, 2000. **118**(3): p. 525-34.

78.   Kukreja, R.C., *NFkappaB activation during ischemia/reperfusion in heart: friend or foe?* J Mol Cell Cardiol, 2002. **34**(10): p. 1301-4.

79.   Goes, N., et al., *Ischemic acute tubular necrosis induces an extensive local cytokine response. Evidence for induction of interferon-gamma, transforming growth factor-beta 1, granulocyte-macrophage colony-stimulating factor, interleukin-2, and interleukin-10.* Transplantation, 1995. **59**(4): p. 565-72.

80.   Grotz, M.R., et al., *Intestinal cytokine response after gut ischemia: role of gut barrier failure.* Ann Surg, 1999. **229**(4): p. 478-86.

81.   Russell, J., et al., *Regulation of E-selectin expression in postischemic intestinal microvasculature.* Am J Physiol Gastrointest Liver Physiol, 2000. **278**(6): p. G878-85.

82.   Granger, D.N. and P. Kubes, *The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion.* J Leukoc Biol, 1994. **55**(5): p. 662-75.

83.   Kalia, N., et al., *Effects of intestinal ischemia-reperfusion injury on rat peripheral blood neutrophil activation.* Dig Dis Sci, 2003. **48**(9): p. 1677-84.

84.   Rentsch, M., et al., *Intravital studies on beneficial effects of warm Ringer's lactate rinse in liver transplantation.* Transpl Int, 1996. **9**(5): p. 461-7.

85.   Lawrence, M.B. and T.A. Springer, *Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins.* Cell, 1991. **65**(5): p. 859-73.

86.   Mulligan, M.S., et al., *Inhibition of lung inflammatory reactions in rats by an anti-human IL-8 antibody.* J Immunol, 1993. **150**(12): p. 5585-95.

87.   Butcher, E.C., *Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity.* Cell, 1991. **67**(6): p. 1033-6.

88.   Springer, T.A., *Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration.* Annu Rev Physiol, 1995. **57**: p. 827-72.

89.   Kelly, K.J., et al., *Intercellular adhesion molecule-1-deficient mice are protected against ischemic renal injury.* J Clin Invest, 1996. **97**(4): p. 1056-63.

90.   Diacovo, T.G., et al., *Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18.* Blood, 1996. **88**(1): p. 146-57.

91.   Keck, T., et al., *Characterization of ischemia/reperfusion injury after pancreas transplantation and reduction by application of monoclonal antibodies against ICAM-1 in the rat.* Surgery, 2003. **134**(1): p. 63-71.

92.   von Andrian, U.H., et al., *Two-step model of leukocyte-endothelial cell interaction in inflammation: distinct roles for LECAM-1 and the leukocyte beta 2 integrins in vivo.* Proc Natl Acad Sci U S A, 1991. **88**(17): p. 7538-42.

93.   Le, Y., et al., *Chemokines and chemokine receptors: their manifold roles in homeostasis and disease.* Cell Mol Immunol, 2004. **1**(2): p. 95-104.

94.   Cuzzocrea, S., et al., *5-lipoxygenase knockout mice exhibit a resistance to splanchnic artery occlusion shock.* Shock, 2003. **20**(3): p. 230-6.

95.   Heinzelmann, M., M.A. Mercer-Jones, and J.C. Passmore, *Neutrophils and renal failure.* Am J Kidney Dis, 1999. **34**(2): p. 384-99.

96.   Jaeschke, H., *Mechanisms of Liver Injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions.* Am J Physiol Gastrointest Liver Physiol, 2006. **290**(6): p. G1083-8.

97.   Cicalese, L., et al., *Oxygen free radical content and neutrophil infiltration are important determinants in mucosal injury after rat small bowel transplantation.* Transplantation, 1996. **62**(2): p. 161-6.

98.   Schoenberg, M.H., et al., *Involvement of neutrophils in postischaemic damage to the small intestine.* Gut, 1991. **32**(8): p. 905-12.

99.   Phan, S.H., et al., *Mechanism of neutrophil-induced xanthine dehydrogenase to xanthine oxidase conversion in endothelial cells: evidence of a role for elastase.* Am J Respir Cell Mol Biol, 1992. **6**(3): p. 270-8.

100. Kushimoto, S., et al., *Role of granulocyte elastase in ischemia/reperfusion injury of rat liver.* Crit Care Med, 1996. **24**(11): p. 1908-12.

101. Uchida, Y., et al., *The inhibition of neutrophil elastase ameliorates mouse liver damage due to ischemia and reperfusion.* Liver Transpl, 2009. **15**(8): p. 939-47.

102.   Beuk, R.J., et al., *Total warm ischemia and reperfusion impairs flow in all rat gut layers but increases leukocyte-vessel wall interactions in the submucosa only.* Ann Surg, 2000. **231**(1): p. 96-104.

103.   Massberg, S., et al., *In vivo assessment of the influence of cold preservation time on microvascular reperfusion injury after experimental small bowel transplantation.* Br J Surg, 1998. **85**(1): p. 127-33.

104.   Dragun, D., et al., *ICAM-1 antisense oligodesoxynucleotides prevent reperfusion injury and enhance immediate graft function in renal transplantation.* Kidney Int, 1998. **54**(2): p. 590-602.

105.   Farmer, D.G., et al., *Disruption of P-selectin signaling modulates cell trafficking and results in improved outcomes after mouse warm intestinal ischemia and reperfusion injury.* Transplantation, 2005. **80**(6): p. 828-35.

106.   Zou, L., B. Attuwaybi, and B.C. Kone, *Effects of NF-kappa B inhibition on mesenteric ischemia-reperfusion injury.* Am J Physiol Gastrointest Liver Physiol, 2003. **284**(4): p. G713-21.

107.   Nishizawa, J., et al., *Reactive oxygen species play an important role in the activation of heat shock factor 1 in ischemic-reperfused heart.* Circulation, 1999. **99**(7): p. 934-41.

108.   Van Why, S.K., et al., *Induction and intracellular localization of HSP-72 after renal ischemia.* Am J Physiol, 1992. **263**(5 Pt 2): p. F769-75.

109.   Sakai, T., et al., *Evaluation of warm ischemia-reperfusion injury using heat shock protein in the rat liver.* Transpl Int, 2003. **16**(2): p. 88-99.

110.   Cornelussen, R.N., et al., *Heat stress protects aged hypertrophied and nonhypertrophied rat hearts against ischemic damage.* Am J Physiol, 1997. **273**(3 Pt 2): p. H1333-41.

111.   Pockley, A.G., *Heat shock proteins as regulators of the immune response.* Lancet, 2003. **362**(9382): p. 469-76.

112.   Pockley, A.G., *Heat shock proteins, anti-heat shock protein reactivity and allograft rejection.* Transplantation, 2001. **71**(11): p. 1503-7.

113.   Jayakumar, J., et al., *Heat shock protein 70 gene transfection protects mitochondrial and ventricular function against ischemia-reperfusion injury.* Circulation, 2001. **104**(12 Suppl 1): p. I303-7.

114.   Elbirt, K.K., et al., *Mechanism of sodium arsenite-mediated induction of heme oxygenase-1 in hepatoma cells. Role of mitogen-activated protein kinases.* J Biol Chem, 1998. **273**(15): p. 8922-31.

115.   Graves, J.D., J.S. Campbell, and E.G. Krebs, *Protein serine/threonine kinases of the MAPK cascade.* Ann N Y Acad Sci, 1995. **766**: p. 320-43.

116.   Beere, H.M. and D.R. Green, *Stress management - heat shock protein-70 and the regulation of apoptosis.* Trends Cell Biol, 2001. **11**(1): p. 6-10.

117.   Yoo, C.G., et al., *Anti-inflammatory effect of heat shock protein induction is related to stabilization of I kappa B alpha through preventing I kappa B kinase activation in respiratory epithelial cells.* J Immunol, 2000. **164**(10): p. 5416-23.

118.   Basu, S., et al., *Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway.* Int Immunol, 2000. **12**(11): p. 1539-46.

119.   Pritts, T.A., et al., *Induction of the stress response in vivo decreases nuclear factor-kappa B activity in jejunal mucosa of endotoxemic mice.* Arch Surg, 2000. **135**(7): p. 860-6.

120.   Pritts, T.A., et al., *The stress response decreases NF-kappaB activation in liver of endotoxemic mice.* Shock, 2002. **18**(1): p. 33-7.

121.   Nakao, A., et al., *Biliverdin protects the functional integrity of a transplanted syngeneic small bowel.* Gastroenterology, 2004. **127**(2): p. 595-606.

122.   Ohashi, K., et al., *Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex.* J Immunol, 2000. **164**(2): p. 558-61.

123.   Shanley, T.P., et al., *Heat shock inhibits phosphorylation of I-kappaBalpha.* Shock, 2000. **14**(4): p. 447-50.

124.   Schell, M., et al., *Heat shock inhibits NF-kB activation in a dose- and time-dependent manner.* J Surg Res, 2005. **120**: p. 90-93.

125. Wagner, M., et al., *Heme oxygenase-1 attenuates ischemia/reperfusion-induced apoptosis and improves survival in rat renal allografts.* Kidney Int, 2003. **63**(4): p. 1564-73.

126. Salim, M., B. Brown-Kipphut, and M. Maines, *Human biliverdin reductase is autophosphorylated, and phosphorylation is required for bilirubin formation.* J Biol Chem, 2001. **276**: p. 10929-10934.

127. Yonezawa, K., et al., *Suppression of tumor necrosis factor-alpha production and neutrophil infiltration during ischemia-reperfusion injury of the liver after heat shock preconditioning.* J Hepatol, 2001. **35**: p. 619-627.

128. Oltean, M., et al., *Transplantation of preconditioned intestinal grafts is associated with lower inflammatory activation and remote organ injury in rats.* Transplant Proc, 2006. **38**: p. 1175-1178.

129. Kelly, K.J., N.R. Baird, and A.L. Greene, *Induction of stress response proteins and experimental renal ischemia/reperfusion.* Kidney Int, 2001. **59**(5): p. 1798-802.

130. Oltean, M., et al., *Donor pretreatment with FK506 reduces reperfusion injury and accelerates intestinal graft recovery in rats.* Surgery, 2007. **141**: p. 667-677.

131. Hiratsuka, M., et al., *Heat shock pretreatment protects pulmonary isografts from subsequent ischemia-reperfusion injury.* J Heart Lung Transplant, 1998. **17**: p. 1238-1246.

132. Vabulas, R.M., et al., *HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway.* J Biol Chem, 2002. **277**(17): p. 15107-12.

133. Vabulas, R.M., et al., *The endoplasmic reticulum-resident heat shock protein Gp96 activates dendritic cells via the Toll-like receptor 2/4 pathway.* J Biol Chem, 2002. **277**(23): p. 20847-53.

134. Chen, W., et al., *Human 60-kDa heat-shock protein: a danger signal to the innate immune system.* J Immunol, 1999. **162**(6): p. 3212-9.

135. Kol, A., et al., *Cutting edge: heat shock protein (HSP) 60 activates the innate immune response: CD14 is an essential receptor for HSP60 activation of mononuclear cells.* J Immunol, 2000. **164**(1): p. 13-7.

136. Flohe, S.B., et al., *Human heat shock protein 60 induces maturation of dendritic cells versus a Th1-promoting phenotype.* J Immunol, 2003. **170**(5): p. 2340-8.

137. Breloer, M., et al., *Heat shock proteins as "danger signals": eukaryotic Hsp60 enhances and accelerates antigen-specific IFN-gamma production in T cells.* Eur J Immunol, 2001. **31**(7): p. 2051-9.

138. Harris, H.E. and A. Raucci, *Alarmin(g) news about danger: workshop on innate danger signals and HMGB1.* EMBO Rep, 2006. **7**(8): p. 774-8.

139. Foell, D., H. Wittkowski, and J. Roth, *Mechanisms of disease: a 'DAMP' view of inflammatory arthritis.* Nat Clin Pract Rheumatol, 2007. **3**(7): p. 382-90.

140. Matzinger, P., *Tolerance, danger, and the extended family.* Annu Rev Immunol, 1994. **12**: p. 991-1045.

141. Arumugam, T.V., et al., *Toll-like receptors in ischemia-reperfusion injury.* Shock, 2009. **32**(1): p. 4-16.

142. Schnare, M., et al., *Toll-like receptors control activation of adaptive immune responses.* Nat Immunol, 2001. **2**(10): p. 947-50.

143. Goldstein, D.R., et al., *Critical role of the Toll-like receptor signal adaptor protein MyD88 in acute allograft rejection.* J Clin Invest, 2003. **111**(10): p. 1571-8.

144. Tesar, B.M., et al., *The role of hyaluronan degradation products as innate alloimmune agonists.* Am J Transplant, 2006. **6**(11): p. 2622-35.

145. Tsan, M.F. and B. Gao, *Endogenous ligands of Toll-like receptors.* J Leukoc Biol, 2004. **76**(3): p. 514-9.

146. Hermani, A., et al., *S100A8 and S100A9 activate MAP kinase and NF-kappaB signaling pathways and trigger translocation of RAGE in human prostate cancer cells.* Exp Cell Res, 2006. **312**(2): p. 184-97.

147. Kaczorowski, D.J., et al., *Mechanisms of Toll-like receptor 4 (TLR4)-mediated inflammation after cold ischemia/reperfusion in the heart.* Transplantation, 2009. **87**(10): p. 1455-63.

148. Scheibner, K.A., et al., *Hyaluronan fragments act as an endogenous danger signal by engaging TLR2.* J Immunol, 2006. **177**(2): p. 1272-81.

149. Foell, D., et al., *S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules.* J Leukoc Biol, 2007. **81**(1): p. 28-37.

150. Colletti, L.M., et al., *Tumor necrosis factor up-regulates intercellular adhesion molecule 1, which is important in the neutrophil-dependent lung and liver injury associated with hepatic ischemia and reperfusion in the rat.* Shock, 1998. **10**(3): p. 182-91.

151. Swank, G.M. and E.A. Deitch, *Role of the gut in multiple organ failure: bacterial translocation and permeability changes.* World J Surg, 1996. **20**(4): p. 411-7.

152. Hassoun, H.T., et al., *Post-injury multiple organ failure: the role of the gut.* Shock, 2001. **15**(1): p. 1-10.

153. Welborn, M.B., et al., *The relationship between visceral ischemia, pro-inflammatory cytokines, and organ injury in patients undergoing thoracoabdominal aortic aneurysm repair.* Crit Care Med, 2000. **28**(9): p. 3191-7.

154. Behrends, M., et al., *Remote renal injury following partial hepatic ischemia/reperfusion injury in rats.* J Gastrointest Surg, 2008. **12**(3): p. 490-5.

155. Lawlor, D.K., et al., *Cytokines contribute to early hepatic parenchymal injury and microvascular dysfunction after bilateral hindlimb ischemia.* J Vasc Surg, 1999. **30**(3): p. 533-41.

156. Mura, M., et al., *Intestinal ischemia-reperfusion-induced acute lung injury and oncotic cell death in multiple organs.* Shock, 2007. **28**(2): p. 227-38.

157. Kalia, N., et al., *FK409 inhibits both local and remote organ damage after intestinal ischaemia.* J Pathol, 2002. **197**(5): p. 595-602.

158. Koksoy, C., et al., *Intestinal ischemia and reperfusion impairs vasomotor functions of pulmonary vascular bed.* Ann Surg, 2000. **231**(1): p. 105-11.

159. Colletti, L.M., et al., *The production of tumor necrosis factor alpha and the development of a pulmonary capillary injury following hepatic ischemia/reperfusion.* Transplantation, 1990. **49**(2): p. 268-72.

160. Caty, M.G., et al., *Evidence for tumor necrosis factor-induced pulmonary microvascular injury after intestinal ischemia-reperfusion injury.* Ann Surg, 1990. **212**(6): p. 694-700.

161. Welborn, M.B., 3rd, et al., *Visceral ischemia-reperfusion injury promotes tumor necrosis factor (TNF) and interleukin-1 (IL-1) dependent organ injury in the mouse.* Shock, 1996. **6**(3): p. 171-6.

162. McCurry, K.R., et al., *Tumor necrosis factor, interleukin 6, and the acute phase response following hepatic ischemia/reperfusion.* J Surg Res, 1993. **55**(1): p. 49-54.

163. Opal, S.M. and V.A. DePalo, *Anti-inflammatory cytokines.* Chest, 2000. **117**(4): p. 1162-72.

164. Murray, P.J., *The primary mechanism of the IL-10-regulated anti-inflammatory response is to selectively inhibit transcription.* Proc Natl Acad Sci U S A, 2005. **102**(24): p. 8686-91.

165. Takala, A., et al., *Markers of inflammation in sepsis.* Ann Med, 2002. **34**(7-8): p. 614-23.

166. Wada, K., M.C. Montalto, and G.L. Stahl, *Inhibition of complement C5 reduces local and remote organ injury after intestinal ischemia/reperfusion in the rat.* Gastroenterology, 2001. **120**(1): p. 126-33.

167. Lane, J.S., et al., *Interleukin-10 reduces the systemic inflammatory response in a murine model of intestinal ischemia/reperfusion.* Surgery, 1997. **122**(2): p. 288-94.

168. Yao, Y.M., et al., *IL-6 release after intestinal ischemia/reperfusion in rats is under partial control of TNF.* J Surg Res, 1997. **70**(1): p. 21-6.

169. Philip, R. and L.B. Epstein, *Tumour necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, gamma-interferon and interleukin-1.* Nature, 1986. **323**(6083): p. 86-9.

170. Esposito, E., et al., *Splanchnic ischemia and reperfusion injury is reduced by genetic or pharmacological inhibition of TNF-alpha.* J Leukoc Biol, 2007. **81**(4): p. 1032-43.

171. Zhang, Y.H., J.X. Lin, and J. Vilcek, *Interleukin-6 induction by tumor necrosis factor and interleukin-1 in human fibroblasts involves activation of a nuclear factor binding to a kappa B-like sequence.* Mol Cell Biol, 1990. **10**(7): p. 3818-23.

172. *Cytokines*, in *Cellular and mollecular immunology.p. 267-301.*, A.K. Abbas, A.H. Lichtman, and P. Pillay, Editors. 2007: Philadelphia. p. 267-301.

173. Zoja, C., et al., *Interleukin-1 beta and tumor necrosis factor-alpha induce gene expression and production of leukocyte chemotactic factors, colony-stimulating factors, and interleukin-6 in human mesangial cells.* Am J Pathol, 1991. **138**(4): p. 991-1003.

174. Cooper, A.L., et al., *Tumor necrosis factor-alpha and fever after peripheral inflammation in the rat.* Am J Physiol, 1994. **267**(6 Pt 2): p. R1431-6.

175. Cuzzocrea, S., et al., *IL-6 knock-out mice exhibit resistance to splanchnic artery occlusion shock.* J Leukoc Biol, 1999. **66**(3): p. 471-80.

176. Potten, C.S., J.W. Wilson, and C. Booth, *Regulation and significance of apoptosis in the stem cells of the gastrointestinal epithelium.* Stem Cells, 1997. **15**(2): p. 82-93.

177. Shibahara, T., et al., *The fate of effete epithelial cells at the villus tips of the human small intestine.* Arch Histol Cytol, 1995. **58**(2): p. 205-19.

178. Turner, J.R., *Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application.* Am J Pathol, 2006. **169**(6): p. 1901-9.

179. Turner, J.R., et al., *Physiological regulation of epithelial tight junctions is associated with myosin light-chain phosphorylation.* Am J Physiol, 1997. **273**(4 Pt 1): p. C1378-85.

180. Mowat, A., *Intestinal graft versus host disease* in *Graft-vs.-host Disease: Immunology, Pathophysiology and Treatment*, J. Ferrara, J. Deeg, and S. Burakoff, Editors. 1996, Marcel Dekker, Inc: New York. p. 337-383

181. Lloyd, D.A.J. and S.M. Gabe, *Intestinal morphology, intestinal regeneration and the proise of tissue engineering*, in *Intestinal failure: diagnosis, management and transplantation*, A. Langnas, et al., Editors. 2008, Blackwell publishing. p. 13-20.

182. Madara, J.L., *Loosening tight junctions. Lessons from the intestine.* J Clin Invest, 1989. **83**(4): p. 1089-94.

183. Al-Sadi, R.M. and T.Y. Ma, *IL-1beta causes an increase in intestinal epithelial tight junction permeability.* J Immunol, 2007. **178**(7): p. 4641-9.

184. Bruewer, M., et al., *Pro-inflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms.* J Immunol, 2003. **171**(11): p. 6164-72.

185. Lewis, J.E., et al., *Cross-talk between adherens junctions and desmosomes depends on plakoglobin.* J Cell Biol, 1997. **136**(4): p. 919-34.

186. *Cell junctions, cell adhesion and the extracellular matrix*, in *Molecular biology of the cell*, B. Alberts, et al., Editors, Garland Publishing Inc: New York London. p. 949-1009.

187. Colegio, O.R., et al., *Claudins create charge-selective channels in the paracellular pathway between epithelial cells.* Am J Physiol Cell Physiol, 2002. **283**(1): p. C142-7.

188. Hartsock, A. and W.J. Nelson, *Adherens and tight junctions: structure, function and connections to the actin cytoskeleton.* Biochim Biophys Acta, 2008. **1778**(3): p. 660-9.

189. Chiu, C.J., et al., *Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal.* Arch Surg, 1970. **101**(4): p. 478-83.

190. Park, P.O., et al., *The sequence of development of intestinal tissue injury after strangulation ischemia and reperfusion.* Surgery, 1990. **107**(5): p. 574-80.

191. Brown, R.A., et al., *Ultrastructural changes in the canine ileal mucosal cell after mesenteric arterial occlusion.* Arch Surg, 1970. **101**(2): p. 290-7.

192. Park, P.O. and U. Haglund, *Regeneration of small bowel mucosa after intestinal ischemia.* Crit Care Med, 1992. **20**(1): p. 135-9.

193. Park, P.O., et al., *Cold ischemic and reperfusion injury in a model of small bowel transplantation in the rat.* Eur Surg Res, 1991. **23**(1): p. 1-8.

194. Moore, R., S. Carlson, and J.L. Madara, *Villus contraction aids repair of intestinal epithelium after injury.* Am J Physiol, 1989. **257**(2 Pt 1): p. G274-83.

195. Cicalese, L., et al., *Ischemia/reperfusion injury induces chronic changes in the small bowel.* Transplant Proc, 2000. **32**(6): p. 1315.

196. Kalia, N., et al., *Effects of hypothermia and rewarming on the mucosal villus microcirculation and survival after rat intestinal ischemia-reperfusion injury.* Ann Surg, 2002. **236**(1): p. 67-74.

197. Kalia, N., et al., *Effects of FK409 on intestinal ischemia-reperfusion injury and ischemia-induced changes in the rat mucosal villus microcirculation.* Transplantation, 2001. **72**(12): p. 1875-80.

198. Soda, Y., et al., *Suppressed endothelin-1 production by FK506 and cyclosporin A in ischemia/reperfusion of rat small intestine.* Surgery, 1999. **125**(1): p. 23-32.

199.	Clavien, P.A., et al., *Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans.* Ann Surg, 2000. **232**(2): p. 155-62.

200.	Azoulay, D., et al., *Effects of 10 minutes of ischemic preconditioning of the cadaveric liver on the graft's preservation and function: the ying and the yang.* Ann Surg, 2005. **242**(1): p. 133-9.

201.	Pell, T.J., et al., *Renal ischemia preconditions myocardium: role of adenosine receptors and ATP-sensitive potassium channels.* Am J Physiol, 1998. **275**(5 Pt 2): p. H1542-7.

202.	Gao, W., et al., *Apoptosis of sinusoidal endothelial cells is a critical mechanism of preservation injury in rat liver transplantation.* Hepatology, 1998. **27**(6): p. 1652-60.

203.	Mueller, A.R., et al., *The extracellular matrix: an early target of preservation/reperfusion injury and acute rejection after small bowel transplantation.* Transplantation, 1998. **65**(6): p. 770-6.

204.	Ploeg, R.J., et al., *Risk factors for primary dysfunction after liver transplantation--a multivariate analysis.* Transplantation, 1993. **55**(4): p. 807-13.

205.	Lindgard, A., et al., *Preservation of rat skeletal muscle energy metabolism by illumination.* Life Sci, 2003. **72**(23): p. 2649-58.

206.	Belzer, F.O. and J.H. Southard, *Principles of solid-organ preservation by cold storage.* Transplantation, 1988. **45**(4): p. 673-6.

207.	Moen, J., et al., *Preservation of dog liver, kidney, and pancreas using the Belzer-UW solution with a high-sodium and low-potassium content.* Transplantation, 1989. **47**(6): p. 940-5.

208.	Ploeg, R.J., et al., *The importance of a colloid in canine pancreas preservation.* Transplantation, 1992. **53**(4): p. 735-41.

209.	Mangus, R.S., et al., *Comparison of histidine-tryptophan-ketoglutarate solution and University of Wisconsin solution in intestinal and multivisceral transplantation.* Transplantation, 2008. **86**(2): p. 298-302.

210.	Feng, L., et al., *Histidine-tryptophan-ketoglutarate solution vs. University of Wisconsin solution for liver transplantation: a systematic review.* Liver Transpl, 2007. **13**(8): p. 1125-36.

211.	Stewart, Z.A., et al., *Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival in pancreas transplantation.* Am J Transplant, 2009. **9**(1): p. 217-21.

212.	Stewart, Z.A., et al., *Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival of deceased donor kidney transplants.* Am J Transplant, 2009. **9**(5): p. 1048-54.

213.	Stewart, Z.A., et al., *Histidine-Tryptophan-Ketoglutarate (HTK) is associated with reduced graft survival in deceased donor livers, especially those donated after cardiac death.* Am J Transplant, 2009. **9**(2): p. 286-93.

214.	Audet, M., et al., *Comparative evaluation of Celsior solution versus Viaspan in a pig liver transplantation model.* Transplantation, 2001. **71**(12): p. 1731-5.

215.	Karam, G., et al., *A single solution for multiple organ procurement and preservation.* Transpl Int, 2005. **18**(6): p. 657-63.

216.	Codas, R., et al., *IGL-1 solution in kidney transplantation: first multi-center study.* Clin Transplant, 2009. **23**(3): p. 337-42.

217.	Ben Abdennebi, H., et al., *Evaluation of IGL-1 preservation solution using an orthotopic liver transplantation model.* World J Gastroenterol, 2006. **12**(33): p. 5326-30.

218.	Murry, C.E., R.B. Jennings, and K.A. Reimer, *Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium.* Circulation, 1986. **74**(5): p. 1124-36.

219.	Lee, H.T. and C.W. Emala, *Protective effects of renal ischemic preconditioning and adenosine pretreatment: role of A(1) and A(3) receptors.* Am J Physiol Renal Physiol, 2000. **278**(3): p. F380-7.

220.	McCallion, K., et al., *Ischemic preconditioning ameliorates ischemia- and reperfusion-induced intestinal epithelial hyperpermeability in rats.* Shock, 2000. **14**(4): p. 429-34.

221.	Azoulay, D., et al., *Ischemic preconditioning for major liver resection under vascular exclusion of the liver preserving the caval flow: a randomized prospective study.* J Am Coll Surg, 2006. **202**(2): p. 203-11.

222.	Yin, D.P., et al., *Protective effect of ischemic preconditioning on liver preservation-reperfusion injury in rats.* Transplantation, 1998. **66**(2): p. 152-7.

223.   Sola, A., et al., *Protective effect of ischemic preconditioning on cold preservation and reperfusion injury associated with rat intestinal transplantation.* Ann Surg, 2001. **234**(1): p. 98-106.
224.   Wang, Y.P., et al., *Intestinal ischemia preconditions myocardium: role of protein kinase C and mitochondrial K(ATP) channel.* Cardiovasc Res, 2002. **55**(3): p. 576-82.
225.   Tamion, F., et al., *Intestinal preconditioning prevents systemic inflammatory response in hemorrhagic shock. Role of HO-1.* Am J Physiol Gastrointest Liver Physiol, 2002. **283**(2): p. G408-14.
226.   Weinbrenner, C., et al., *Phosphorylation of tyrosine 182 of p38 mitogen-activated protein kinase correlates with the protection of preconditioning in the rabbit heart.* J Mol Cell Cardiol, 1997. **29**(9): p. 2383-91.
227.   Barrier, A., et al., *Ischemic preconditioning modulates the expression of several genes, leading to the overproduction of IL-1Ra, iNOS, and Bcl-2 in a human model of liver ischemia-reperfusion.* FASEB J, 2005. **19**(12): p. 1617-26.
228.   Redaelli, C.A., et al., *Hyperthermia-induced HSP expression correlates with improved rat renal isograft viability and survival in kidneys harvested from non-heart-beating donors.* Transpl Int, 2001. **14**(6): p. 351-60.
229.   Tullius, S.G., et al., *Inhibition of ischemia/reperfusion injury and chronic graft deterioration by a single-donor treatment with cobalt-protoporphyrin for the induction of heme oxygenase-1.* Transplantation, 2002. **74**(5): p. 591-8.
230.   Neto, J.S., et al., *Protection of transplant-induced renal ischemia-reperfusion injury with carbon monoxide.* Am J Physiol Renal Physiol, 2004. **287**(5): p. F979-89.
231.   Farmer, D.G., et al., *CD62 blockade with P-Selectin glycoprotein ligand-immunoglobulin fusion protein reduces ischemia-reperfusion injury after rat intestinal transplantation.* Transplantation, 2005. **79**(1): p. 44-51.
232.   Reutzel-Selke, A., et al., *Short-term immunosuppressive treatment of the donor ameliorates consequences of ischemia/ reperfusion injury and long-term graft function in renal allografts from older donors.* Transplantation, 2003. **75**(11): p. 1786-92.
233.   Danovitch, G.M., *Immunosuppressive medications and protocols*, in *Handbook of kidney transplantation*
G.M. Danovitch, Editor. 2005, Lippincott Williams & Wilkins. p. 72-134.
234.   Cook, L.G., et al., *Tacrolimus reduces nitric oxide synthase function by binding to FKBP rather than by its calcineurin effect.* Kidney Int, 2009. **75**(7): p. 719-26.
235.   Brunn, G.J., S. Saadi, and J.L. Platt, *Differential regulation of endothelial cell activation by complement and interleukin 1alpha.* Circ Res, 2006. **98**(6): p. 793-800.
236.   Du, S., et al., *Suppression of NF-kappaB by cyclosporin a and tacrolimus (FK506) via induction of the C/EBP family: implication for unfolded protein response.* J Immunol, 2009. **182**(11): p. 7201-11.
237.   Emmel, E.A., et al., *Cyclosporin A specifically inhibits function of nuclear proteins involved in T cell activation.* Science, 1989. **246**(4937): p. 1617-20.
238.   Squadrito, F., et al., *Tacrolimus limits polymorphonuclear leucocyte accumulation and protects against myocardial ischaemia- reperfusion injury.* J Mol Cell Cardiol, 2000. **32**(3): p. 429-40.
239.   Yang, C.W., et al., *Pharmacological preconditioning with low-dose cyclosporine or FK506 reduces subsequent ischemia/reperfusion injury in rat kidney.* Transplantation, 2001. **72**(11): p. 1753-9.
240.   Garcia-Criado, F.J., et al., *Tacrolimus (FK506) down-regulates free radical tissue levels, serum cytokines, and neutrophil infiltration after severe liver ischemia.* Transplantation, 1997. **64**(4): p. 594-8.
241.   St Peter, S.D., A.A. Moss, and D.C. Mulligan, *Effects of tacrolimus on ischemia-reperfusion injury.* Liver Transpl, 2003. **9**(2): p. 105-16.
242.   Klettner, A. and T. Herdegen, *The immunophilin-ligands FK506 and V-10,367 mediate neuroprotection by the heat shock response.* Br J Pharmacol, 2003. **138**(5): p. 1004-12.
243.   Olson, D.W., et al., *Potentiating the benefit of vascular-supplied glutamine during small bowel storage: importance of buffering agent.* Transplantation, 2002. **73**(2): p. 178-85.
244.   Zhu, J.Z., et al., *A novel technique of hypothermic luminal perfusion for small bowel preservation.* Transplantation, 2003. **76**(1): p. 71-6.

245. DeRoover, A., et al., *Luminal contact with University of Wisconsin solution improves human small bowel preservation.* Transplant Proc, 2004. **36**(2): p. 273-5.
246. Leuvenink, H.G., et al., *Luminal preservation of rat small intestine with University of Wisconsin or Celsior solution.* Transplant Proc, 2005. **37**(1): p. 445-7.
247. Fujimoto, Y., et al., *Defining the role of a tailored luminal solution for small bowel preservation.* Am J Transplant, 2002. **2**(3): p. 229-36.
248. Olson, D.W., et al., *Human small bowel storage: the role for luminal preservation solutions.* Transplantation, 2003. **76**(4): p. 709-14.
249. Fujino, Y., et al., *Successful 24-hour preservation of ischemically damaged canine small intestine by the cavitary two-layer method.* Transplantation, 2003. **76**(5): p. 777-80.
250. Grant, D., et al., *51Cr-EDTA: a marker of early intestinal rejection in the rat.* J Surg Res, 1989. **46**(5): p. 507-14.
251. Pappas, P.A., et al., *An analysis of the association between serum citrulline and acute rejection among 26 recipients of intestinal transplant.* Am J Transplant, 2004. **4**(7): p. 1124-32.
252. David, A.I., et al., *An association of lower serum citrulline levels within 30 days of acute rejection in patients following small intestine transplantation.* Transplant Proc, 2006. **38**(6): p. 1731-2.
253. Ehrchen, J.M., et al., *The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer.* J Leukoc Biol, 2009. **86**(3): p. 557-66.
254. Aadland, E. and M.K. Fagerhol, *Faecal calprotectin: a marker of inflammation throughout the intestinal tract.* Eur J Gastroenterol Hepatol, 2002. **14**(8): p. 823-5.
255. Akpinar, E., et al., *Fecal calprotectin level measurements in small bowel allograft monitoring: a pilot study.* Transplantation, 2008. **85**(9): p. 1281-6.
256. Rogers, N.J. and R.I. Lechler, *Allorecognition.* Am J Transplant, 2001. **1**(2): p. 97-102.
257. Rocha, P.N., et al., *Effector mechanisms in transplant rejection.* Immunol Rev, 2003. **196**: p. 51-64.
258. Amsen, D., C.G. Spilianakis, and R.A. Flavell, *How are T(H)1 and T(H)2 effector cells made?* Curr Opin Immunol, 2009. **21**(2): p. 153-60.
259. Zhu, J. and W.E. Paul, *CD4 T cells: fates, functions, and faults.* Blood, 2008. **112**(5): p. 1557-69.
260. Harrington, L.E., P.R. Mangan, and C.T. Weaver, *Expanding the effector CD4 T-cell repertoire: the Th17 lineage.* Curr Opin Immunol, 2006. **18**(3): p. 349-56.
261. He, X.Y., et al., *Treatment with interleukin-4 prolongs allogeneic neonatal heart graft survival by inducing T helper 2 responses.* Transplantation, 1998. **65**(9): p. 1145-52.
262. Skapenko, A., et al., *The IL-4 receptor alpha-chain-binding cytokines, IL-4 and IL-13, induce forkhead box P3-expressing CD25+CD4+ regulatory T cells from CD25-CD4+ precursors.* J Immunol, 2005. **175**(9): p. 6107-16.
263. Kuhn, R., et al., *Interleukin-10-deficient mice develop chronic enterocolitis.* Cell, 1993. **75**(2): p. 263-74.
264. Sandovici, M., et al., *Adenovirus-mediated interleukin-13 gene therapy attenuates acute kidney allograft injury.* J Gene Med, 2007. **9**(12): p. 1024-32.
265. Davidson, C., et al., *IL-13 prolongs allograft survival: association with inhibition of macrophage cytokine activation.* Transpl Immunol, 2007. **17**(3): p. 178-86.
266. McTernan, P.G., C.M. Kusminski, and S. Kumar, *Resistin.* Curr Opin Lipidol, 2006. **17**(2): p. 170-5.
267. Steppan, C.M., et al., *The hormone resistin links obesity to diabetes.* Nature, 2001. **409**(6818): p. 307-12.
268. Singhal, N.S., et al., *Loss of resistin ameliorates hyperlipidemia and hepatic steatosis in leptin-deficient mice.* Am J Physiol Endocrinol Metab, 2008. **295**(2): p. E331-8.
269. Janke, J., et al., *Resistin gene expression in human adipocytes is not related to insulin resistance.* Obes Res, 2002. **10**(1): p. 1-5.
270. Nagaev, I. and U. Smith, *Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle.* Biochem Biophys Res Commun, 2001. **285**(2): p. 561-4.

271. Luc, G., et al., *Adipocytokines and the risk of coronary heart disease in healthy middle aged men: the PRIME Study.* Int J Obes (Lond), 2009.
272. Reilly, M.P., et al., *Resistin is an inflammatory marker of atherosclerosis in humans.* Circulation, 2005. **111**(7): p. 932-9.
273. Hamirani, Y.S., et al., *Markers of inflammation and coronary artery calcification: a systematic review.* Atherosclerosis, 2008. **201**(1): p. 1-7.
274. Anderson, P.D., et al., *Innate immunity modulates adipokines in humans.* J Clin Endocrinol Metab, 2007. **92**(6): p. 2272-9.
275. Sunden-Cullberg, J., et al., *Pronounced elevation of resistin correlates with severity of disease in severe sepsis and septic shock.* Crit Care Med, 2007. **35**(6): p. 1536-42.
276. Koch, A., et al., *Serum resistin levels in critically ill patients are associated with inflammation, organ dysfunction and metabolism and may predict survival of non-septic patients.* Crit Care, 2009. **13**(3): p. R95.
277. Kusanovic, J.P., et al., *Resistin in amniotic fluid and its association with intra-amniotic infection and inflammation.* J Matern Fetal Neonatal Med, 2008. **21**(12): p. 902-16.
278. Bokarewa, M., et al., *Resistin, an adipokine with potent pro-inflammatory properties.* J Immunol, 2005. **174**(9): p. 5789-95.
279. Karmiris, K., et al., *Circulating levels of leptin, adiponectin, resistin, and ghrelin in inflammatory bowel disease.* Inflamm Bowel Dis, 2006. **12**(2): p. 100-5.
280. Konrad, A., et al., *Resistin is an inflammatory marker of inflammatory bowel disease in humans.* Eur J Gastroenterol Hepatol, 2007. **19**(12): p. 1070-4.
281. Tarkowski, A., et al., *Resistin competes with lipopolysaccharide for binding to Toll-like receptor 4.* J Cell Mol Med, 2009.
282. Manduteanu, I., et al., *Resistin up-regulates fractalkine expression in human endothelial cells: lack of additive effect with TNF-alpha.* Biochem Biophys Res Commun, 2009. **381**(1): p. 96-101.
283. Malyszko, J., et al., *Resistin, a new adipokine, is related to inflammation and renal function in kidney allograft recipients.* Transplant Proc, 2006. **38**(10): p. 3434-6.
284. Teplan, V., et al., *Asymmetric dimethylarginine and adiponectin after renal transplantation: role of obesity.* J Ren Nutr, 2008. **18**(1): p. 154-7.
285. Kellersmann, R., et al., *Microsurgical techniques for small bowel transplantation in rats . In: Timmermann W, Gassel HJ, Ulrichs K, Zhong R, Thiede A, eds. Organtransplantation in rats and mice. Berlin Heidelberg: Springer, 1998:83*, in *Organtransplantation in rats and mice*, W. Timmermann, et al., Editors. 1998, Springer: Berlin Heidelberg. p. 83-96.
286. Dahlqvist, A., *Assay of intestinal disaccharidases.* Scand J Clin Lab Invest, 1984. **44**(2): p. 169-72.
287. Bergquist, J., B. Ohlsson, and A. Tarkowski, *Nuclear factor-kappa B is involved in the catecholaminergic suppression of immunocompetent cells.* Ann N Y Acad Sci, 2000. **917**: p. 281-9.
288. Baker, J.W., et al., *Hemorrhagic shock induces bacterial translocation from the gut.* J Trauma, 1988. **28**(7): p. 896-906.
289. Van Leeuwen, P.A., et al., *Clinical significance of translocation.* Gut, 1994. **35**(1 Suppl): p. S28-34.
290. Deitch, E.A., D. Xu, and V.L. Kaise, *Role of the gut in the development of injury- and shock induced SIRS and MODS: the gut-lymph hypothesis, a review.* Front Biosci, 2006. **11**: p. 520-8.
291. Caridis, D.T., et al., *Endotoxaemia in man.* Lancet, 1972. **1**(7765): p. 1381-5.
292. Hammer-Hodges, D., et al., *Role of the intraintestinal gram-negative bacterial flora in response to major injury.* Surg Gynecol Obstet, 1974. **138**(4): p. 599-603.
293. Casavilla, A., et al., *Logistics and technique for combined hepatic-intestinal retrieval.* Ann Surg, 1992. **216**(5): p. 605-9.
294. Yersiz, H., et al., *Multivisceral and isolated intestinal procurement techniques.* Liver Transpl, 2003. **9**(8): p. 881-6.
295. Sasaki, K., et al., *Optimum small bowel preservation solutions and conditions: comparison of UW solution and saline with or without glutamine.* Transplant Proc, 1996. **28**(5): p. 2620-1.

296.   Kokudo, Y., et al., *Effect of glucose introduction to the lumen during cold ischemia in rat small bowel.* Transplant Proc, 1996. **28**(5): p. 2626-7.

297.   Salehi, P., et al., *Preserving the mucosal barrier during small bowel storage.* Transplantation, 2003. **76**(6): p. 911-7.

298.   Sakai, T., et al., *Adenine nucleotide metabolism of the small bowel during preservation by the cavitary two-layer method.* Transplant Proc, 1996. **28**(5): p. 2615-9.

299.   Haglind, E., et al., *Graded intestinal vascular obstruction: I. Description of an experimental shock model in the rat.* Circ Shock, 1980. **7**(1): p. 83-91.

300.   Abu-Elmagd, K.M., et al., *Five Hundred Intestinal and Multivisceral Transplantations at a Single Center: Major Advances With New Challenges.* Ann Surg, 2009.

301.   Hoeger, S., et al., *The additional detrimental effects of cold preservation on transplantation-associated injury in kidneys from living and brain-dead donor rats.* Transplantation, 2009. **87**(1): p. 52-8.

302.   Martins, P.N., et al., *Induction of carbon monoxide in donor animals prior to organ procurement reduces graft immunogenicity and inhibits chronic allograft dysfunction.* Transplantation, 2006. **82**(7): p. 938-44.

303.   Schnuelle, P., et al., *Effects of donor pretreatment with dopamine on graft function after kidney transplantation: a randomized controlled trial.* JAMA, 2009. **302**(10): p. 1067-75.

304.   Otterbein, L.E., et al., *Carbon monoxide suppresses arteriosclerotic lesions associated with chronic graft rejection and with balloon injury.* Nat Med, 2003. **9**(2): p. 183-90.

305.   Kalia, N., et al., *Ketotifen abrogates local and systemic consequences of rat intestinal ischemia-reperfusion injury.* J Gastroenterol Hepatol, 2005. **20**(7): p. 1032-8.

306.   Tamion, F., et al., *Gut ischemia and mesenteric synthesis of inflammatory cytokines after hemorrhagic or endotoxic shock.* Am J Physiol, 1997. **273**(2 Pt 1): p. G314-21.

307.   Magnotti, L.J., et al., *Gut-derived mesenteric lymph but not portal blood increases endothelial cell permeability and promotes lung injury after hemorrhagic shock.* Ann Surg, 1998. **228**(4): p. 518-27.

308.   Haglind, E., et al., *Graded intestinal vascular obstruction. III. An analysis of pathogenic mechanisms.* Circ Shock, 1981. **8**(3): p. 335-41.

309.   Cavriani, G., et al., *Lymphatic system as a path underlying the spread of lung and gut injury after intestinal ischemia/reperfusion in rats.* Shock, 2005. **23**(4): p. 330-6.

310.   Coelho, F.R., et al., *Lymphatic-borne IL-1beta and the inducible isoform of nitric oxide synthase trigger the bronchial hyporesponsiveness after intestinal ischema/reperfusion in rats.* Shock, 2007. **28**(6): p. 694-9.

311.   Poggetti, R.S., et al., *Liver injury is a reversible neutrophil-mediated event following gut ischemia.* Arch Surg, 1992. **127**(2): p. 175-9.

312.   Morse, D. and A.M. Choi, *Heme oxygenase-1: the "emerging molecule" has arrived.* Am J Respir Cell Mol Biol, 2002. **27**(1): p. 8-16.

313.   Ryter, S.W., J. Alam, and A.M. Choi, *Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications.* Physiol Rev, 2006. **86**(2): p. 583-650.

314.   Kimpara, T., et al., *Microsatellite polymorphism in the human heme oxygenase-1 gene promoter and its application in association studies with Alzheimer and Parkinson disease.* Hum Genet, 1997. **100**(1): p. 145-7.

315.   Bethke, K., et al., *Different efficiency of heat shock proteins (HSP) to activate human monocytes and dendritic cells: superiority of HSP60.* J Immunol, 2002. **169**(11): p. 6141-8.

316.   Wallin, R.P., et al., *Heat-shock proteins as activators of the innate immune system.* Trends Immunol, 2002. **23**(3): p. 130-5.

317.   Duquesnoy, R.J., et al., *Evidence for heat shock protein immunity in a rat cardiac allograft model of chronic rejection.* Transplantation, 1999. **67**(1): p. 156-64.

318.   Caldas, C., et al., *Cellular autoreactivity against heat shock protein 60 in renal transplant patients: peripheral and graft-infiltrating responses.* Clin Exp Immunol, 2006. **146**(1): p. 66-75.

319.   Fukata, M., et al., *Innate immune signaling by Toll-like receptor-4 (TLR4) shapes the inflammatory microenvironment in colitis-associated tumors.* Inflamm Bowel Dis, 2009. **15**(7): p. 997-1006.

320. Fukata, M., et al., *Cox-2 is regulated by Toll-like receptor-4 (TLR4) signaling: Role in proliferation and apoptosis in the intestine.* Gastroenterology, 2006. **131**(3): p. 862-77.
321. Zhao, J., et al., *R-spondin1, a novel intestinotrophic mitogen, ameliorates experimental colitis in mice.* Gastroenterology, 2007. **132**(4): p. 1331-43.
322. Francavilla, A., et al., *Augmentation of rat liver regeneration by FK 506 compared with cyclosporin.* Lancet, 1989. **2**(8674): p. 1248-9.
323. Dubernard, J.M., et al., *Functional results of the first human double-hand transplantation.* Ann Surg, 2003. **238**(1): p. 128-36.
324. Gold, B.G., et al., *Neuroregenerative and neuroprotective actions of neuroimmunophilin compounds in traumatic and inflammatory neuropathies.* Neurol Res, 2004. **26**(4): p. 371-80.
325. Pratschke, J., et al., *Improvements in early behavior of rat kidney allografts after treatment of the brain-dead donor.* Ann Surg, 2001. **234**(6): p. 732-40.
326. Pratschke, J., et al., *Brain death and its influence on donor organ quality and outcome after transplantation.* Transplantation, 1999. **67**(3): p. 343-8.
327. Kusaka, M., et al., *Activation of inflammatory mediators in rat renal isografts by donor brain death.* Transplantation, 2000. **69**(3): p. 405-10.
328. Kotsch, K., et al., *Methylprednisolone therapy in deceased donors reduces inflammation in the donor liver and improves outcome after liver transplantation: a prospective randomized controlled trial.* Ann Surg, 2008. **248**(6): p. 1042-50.
329. Rosendale, J.D., et al., *Hormonal resuscitation yields more transplanted hearts, with improved early function.* Transplantation, 2003. **75**(8): p. 1336-41.
330. Follette, D.M., S.M. Rudich, and W.D. Babcock, *Improved oxygenation and increased lung donor recovery with high-dose steroid administration after brain death.* J Heart Lung Transplant, 1998. **17**(4): p. 423-9.
331. Axelsson, J., et al., *Elevated resistin levels in chronic kidney disease are associated with decreased glomerular filtration rate and inflammation, but not with insulin resistance.* Kidney Int, 2006. **69**(3): p. 596-604.
332. Nakajima, T.E., et al., *Adipocytokine levels in gastric cancer patients: resistin and visfatin as biomarkers of gastric cancer.* J Gastroenterol, 2009. **44**(7): p. 685-90.
333. Larochelle, J., et al., *Plasma resistin levels in asthmatics as a marker of disease state.* J Asthma, 2007. **44**(7): p. 509-13.
334. Kunnari, A., et al., *High plasma resistin level is associated with enhanced highly sensitive C-reactive protein and leukocytes.* J Clin Endocrinol Metab, 2006. **91**(7): p. 2755-60.

ABBREVIATIONS

ALT = Alanine aminotransferase

AP-1 = Activator Protein-1

AR = Acute Rejection

AST = Aspartate aminotransferase

ATP = Adenosine Triphosphate

CRP = C - Reactive Protein

CTL = Cytotoxic T Lymphocytes - CD8+ cells

DAMP = Danger-Associated Molecular
  Pattern

DNA = Deoxyribonucleic Acid

DTH = Delayed-type Hypersensitivity

EMSA = Electrophoretic Mobility Shift Assay

GALT = Gut Associated Lymph Tissue

GVHD = Graft Versus Host Disease

HES = Hydroxy Ethyl Starch

HGDF = Hepatoma-Derived Growth Factor

HSS = high sodium solution

HMGB-1 = High Mobility Group Box-1

HO-1 = Heme Oxygenase-1

HSP = Heat Shock Protein

HTK = Histidine-Triptophane-Ketoglutarate

IBD = Inflammatory Bowel Disease

ICAM-1 = Intercellular Adhesion Molecule-1

IFN- γ = Interferon-Gamma

IIF = Irreversible Intestinal Failure

IKK = IκB Kinases

IL = Interleukin

IP = Ischemic Preconditioning

IR = Ischemia and Reperfusion

IκB = Inhibitory-unit κB

LPS = Lipopolysaccharide

LSS = low sodium solution

MHC = Major Histocompatibility Complex

MMP-2 = Matrix metalloproteinase-2

MMP-9 = Matrix metalloproteinase-9

NF-AT = Nuclear Factor of Activated T cells

NF-κB = Nuclear Factor- κB

NK cells = Natural Killer cells

NO = Nitric Oxide

OD = Optical Density units

OFR = Oxygen Free Radicals

PAF = Platelet activating factor

PAMP = Pathogen-Associated Molecular Pattern

PFD = Perfluorodecaline

PMN = Polymorphonuclear cells

PP = Pharmacologic Preconditioning

PTLD = Post-Transplant Lymphoproliferative
  Disease

SBS = Short Bowel Syndrome

SDS-PAGE = Sodium Dodecylsulphate
  Polyacrylamide Gel Electrophoresis

TAC = Tacrolimus

Th1 =T-helper-1 CD4+ cells. Pro-
  inflammatory cells

Th2 =T-helper-2 CD4+ cells. Anti-
  inflammatory cells

TJ = Tight Junction

TLM = Two Layer Method

TLR = Toll-Like Receptor

TNF-α = Tumor Necrosis Factor-Alpha

TPN = Total Parenteral Nutrition

Tregs = T-Regulatory cells or
  CD4+CD25+Foxp3+

UW = University of Wisconsin solution

VAC = Vacuum-Assisted Closure

VCAM-1 = Vascular Cell Adhesion Molecule-1

WBC = White Blood cell Count

ZO-1 = Zonula Occludens-1