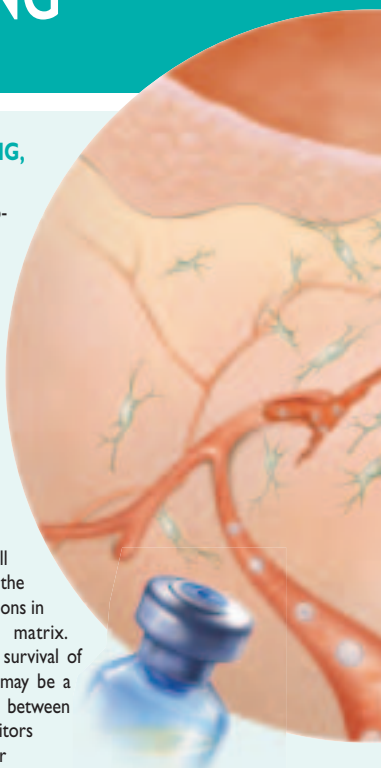
TISSUE REPAIR AND ULCER/WOUND HEALING



▷ INTRAVITAL MULTI-PHOTON MICROSCOPY TO VISUALIZE RENAL ENDOTHELIAL CELL INJURY AND DYSFUNCTION

Three and four dimensional (time) intravital multi-photon microscopy has been utilized to study the effects of ischemia on endothelial cell injury including actin cytoskeletal alterations. In addition, fluorescent dextrans were used to evaluate microvascular flow patterns and permeability defects as a measure of endothelial cell dysfunction. Endothelial cell swelling, microthrombi formation, vasoconstriction and WBC endothelial attachment resulted in reduced cortical microvascular blood flow. The permeability defect was maximal at 24 hours post injury and its extent correlated with persistent reductions in microvascular flow.

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▷ ROLE OF INFLAMMATION/INFECTION IN CHRONIC IDIOPATHIC INFLAMMATORY BOWEL DISEASES (IBD)

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory conditions of the gastrointestinal tract of unknown aetiology. IBD seems to be the result from complex interactions among susceptibility genes, the environment and the immune system. The relationship between infections and IBD encompasses many aspects including differential diagnosis, complications and especially the pathogenesis of IBD. Several studies indicate a major role for the intestinal bacterial flora in the development of IBD as an endogenous stimulus generating inflammation through a variety of immune abnormalities. T-cells and monocytes and related cytokines play an important role in the disturbed regulation of the immune response. CD is essentially a transmural inflammation with repercussions for the enteric nervous system and smooth muscle while UC is essentially limited to the mucosa. The natural history of both conditions is characterized by alternating episodes of remission and relapse of symptoms. This evolution is responsible for major structural alterations of the bowel wall and loss of structural integrity.

Karel Geboes
University Hospital, Leuven, Belgium

▷ KIDNEY ISCHEMIC PRECONDITIONING

Once exposed to a noxious stimulus the kidney is protected against similar or dissimilar toxic influences. Although there is frequent clinical concern that a prior insult might predispose the kidney to a subsequent insult we have found, in the mouse, that prolonged protection against subsequent ischemic injury can be produced by prior ischemia or prior ureteral obstruction. We have explored cellular mechanisms responsible for this "preconditioning" in epithelial cells *in vitro*. Stress proteins have been identified which act via interactions with the mitogen activated protein kinases (MAPKs) to induce protection against a subsequent reactive oxygen species-mediated insult *in vitro*. A genomic approach has been taken to identify candidate genes that may be involved in this protective response. There is compelling evidence in other organs that preconditioning occurs in humans. It therefore behooves us to understand the endogenous processes which the kidney has developed to protect itself against an ischemic insult. Armed with this understanding we can then attempt to mimic these processes and thereby prevent and treat ischemic acute renal failure.

Joseph V. Bonventre
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▷ CELLULAR AND MOLECULAR MECHANISMS OF ULCER HEALING 2005: LESSONS FROM GASTROINTESTINAL ULCERS

Ulcer healing, genetically programmed repair process, includes inflammation, proliferation, reepithelialization, formation of granulation tissue, angiogenesis, interactions between various cell, matrix and tissue remodeling, all resulting in scar formation. All these events are controlled by cytokines, growth factors (EGF, PDGF, KGF, HGF, TGFß, bFGF, VEGF, angiopoietins) and transcription factors activated by tissue injury in spatially and temporally coordinated manner. These growth factors trigger mitogenic, motogenic and survival pathways utilizing Ras, MAPK, PI-3K/Akt, PLC-γ and Rho/Rac/actin signaling. Hypoxia activates angiogenic genes (e.g. VEGF, angiopoietins) via HIF, while serum response factor (SRF) is critical for VEGF-induced angiogenesis and muscle restoration. Circulating progenitor cells are also important for ulcer healing. We demonstrated that local gene therapy with VEGF + Ang1 and/or SRF cDNAs dramatically accelerates esophageal and gastric ulcer healing and improves healing quality. Future therapeutic directions include use of stem cells and tissue engineering.

Andrzej S. Tarnawski
VAMCLB & University of California, Irvine, USA

▷ MECHANISMS OF RENAL CELL REPAIR AND REGENERATION

Although much research has focused on the cellular events leading to acute renal failure (ARF), less emphasis has been placed on the mechanisms of renal cell repair and regeneration. Studies using *in vivo* and *in vitro* models have demonstrated the importance of proliferation, migration, and repair of physiological functions of injured renal proximal tubular cells (RPTC) in the reversal of ARF. In particular, recent studies provide strong evidence that the epidermal growth factor receptor plays a key role in the signaling of RPTC proliferation and migration following injury. Further, interactions between integrins and extracellular matrix protein collagen IV were shown to promote the repair of physiological functions in injured RPTC. Elucidation of the signaling pathways of renal cell repair and regeneration may identify new targets for pharmacological intervention.

Rick G. Schnellmann
Medical University of South Carolina, Charleston, USA

▷ TISSUE REMODELING DURING HEALING, ROLE OF APOPTOSIS

Normal wound healing includes a number of overlapping phases. After injury, there is an early inflammatory step characterized by hemorrhage and clotting. In the next phase allowing the development of the granulation tissue, fibroblasts invade the wound and commence replacing the provisional matrix with a more mature wound matrix. As the granulation tissue phase proceeds, fibroblasts start showing a new phenotype with prominent microfilament bundles. These typical myofibroblasts have been shown to express α-smooth muscle actin, and are responsible for wound contraction. Lastly, in the resolution phase of healing, there is considerable loss of various cell types including myofibroblasts, by apoptosis. The signal for this cell death is unknown but may be related to reductions in the concentrations of local trophic factors or to modifications in myofibroblast adhesion to the extracellular matrix. Inappropriate delay of apoptosis, and thus increased survival of myofibroblasts activated during the healing process, may be a factor which leads to excessive scarring. The balance between matrix metalloproteinases and their endogenous inhibitors plays an important role early in wound repair and later as remodeling occurs; deregulation in this balance could favor excessive matrix deposition.

Alexis Desmoulière
INSERM E362, Université Victor Segalen, Bordeaux, France

▷ STEM CELLS AND MYOCARDIAL REGENERATION

Cardiac stem and early committed cells (CSCs-ECCs) are present throughout the myocardium, express c-Met and IGF-1 receptors and through a feed-back loop respond by secreting the corresponding ligands, HGF and IGF-1. HGF mobilizes CSCs-ECCs and IGF-1 promotes their survival and proliferation. Therefore, HGF and IGF-1 were injected in the heart of infarcted mice to favor, respectively, translocation of CSCs-ECCs from the surrounding myocardium to the dead tissue, and enhanced viability, growth and differentiation within the damaged area. The newly formed myocardium contained arterioles, capillaries and functionally competent myocytes, which with time increased in size, ameliorating ventricular performance at healing and long thereafter. Myocardial regeneration induced by growth factors improved survival and rescued animals with infarcts up to 86% of the ventricle, which are commonly incompatible with life. Thus, the heart has an endogenous reserve of CSCs-ECCs that can be activated to reconstitute dead myocardium and obviate the need to introduce exogenous stem cells.

Piero Anversa
Cardiovascular Research Institute, New York Medical College, Valhalla, USA

▷ NOVEL THERAPIES FOR CELL PROTECTION AND REGENERATION IN THE NERVOUS SYSTEM

The adult central nervous system exhibits little capacity to spontaneously recover from injury or disease. Over the past 20 years, growth factors have emerged as a potential means of significantly enhancing cell survival and promoting neural repair. The delivery of these proteins to the brain or spinal cord has been a chief obstacle to testing their potential in human trials, however. Recently, gene therapy has emerged as a potential means of effectively delivering and accurately targeting growth factors to treat various models of human neurological disease. Research has progressed from animal models to human clinical trials currently in progress.

Mark H. Tuszynski
University of California, San Diego, La Jolla, USA

▷ LIVER REPOPULATION STRATEGIES

Hepatocytes have the unique capacity to self-renew and repair the liver *ad integrum* when stimulated to proliferate by a liver injury. However, transplantation of isolated hepatocytes is usually not efficient enough to achieve a therapeutic purpose. We conferred a survival advantage to transplanted hepatocytes and showed that they were able to repopulate an almost entire mouse liver submitted to a repeated liver injury. Using bone marrow stem cells instead of hepatocytes, we then demonstrated that participation of adult bone marrow cells to liver regeneration was far below any therapeutic level. Current data about liver stem cells will be discussed.

Hélène Gilgenkrantz
INSERM U 567, Institut Cochin, Paris, France

▷ TREATMENT OF GASTRODUODENAL ULCERS. STATE-OF-THE-ART 2005

Recent years brought new effective strategy for the management of peptic ulcer disease: acid inhibition, *H. pylori* eradication, selective NSAIDs less damaging gastrointestinal mucosa and new endoscopic therapies for bleeding. Also, new drugs enhancing mucosal defense and reducing mucosal inflammation were developed and new cellular and molecular mechanisms of ulcer

development, healing and recurrence were uncovered. I will provide new insight into the ulcer recurrence, including the roles of cytokines (IL-1ß, TNFα and MCP-1) in this process, the current state-of-the-art update on diagnosis and therapy of esophageal, gastric and duodenal ulcers and novel methods for assessing the quality of ulcer scar using quantitative histology, intravital staining and highly magnifying endoscopy. In addition, I will introduce potential new molecular targets for ulcer treatment such as gastrin antagonists, ligands to histamine H3 receptors, CCK-2 receptor antagonists and GRP targeting drugs.

Tetsuo Arakawa
Osaka City University Graduate Medical School, Japan

▷ NEW THERAPEUTIC ADVANCES IN INFLAMMATORY BOWEL DISEASES

The advance in understanding the dysregulated immune response and the pathogenesis of IBD provide the basis of new therapeutic modalities. The role of established anti-cytokine strategies such as anti-TNFα antibodies and new strategies using anti-inflammatory cytokines will be discussed. The trials with anti sense oligonucleotides; the place of probiotic bacteria and the mechanisms responsible for their beneficial effects will be reviewed. Finally, the recent established immunostimulatory effects of oligonucleotides containing the CpG motif, their modulation of experimental colitis and possible usage as a new therapeutic modality for IBD will be highlighted.

Daniel Rachmilewitz
Shaare Zedek Medical Center, Jerusalem, Israel

▷ HEALING AND REPAIR OF THE INJURED LUNG

Acute and chronic lung disorders such as the Acute Respiratory Distress Syndrome (ARDS), asthma, chronic obstructive pulmonary diseases and pulmonary fibrosis a major cause of morbidity and mortality and an enormous burden on world health systems. A feature of these diseases is the destruction and remodelling of the lung's support structures with deleterious effects on lung function. This presentation will explore our current concepts of the molecular mechanisms regulating this response and current approaches to prevent these changes using pharmaceutical intervention.

Geoffrey J. Laurent
Centre for Respiratory Research, London, United Kingdom

▷ BONE AND CARTILAGE REPAIR

Destruction of bone and cartilage tissue due to disease and inefficient bone healing after traumatic injury may be addressed by tissue engineering techniques. The stimulation of bone production is often required to treat loss of bone tissue brought about by trauma, osteonecrosis and tumours. Currently, bone grafting procedures are employed to promote healing of fracture non-union (where the ends of the bone to do not heal together), in cranio-facial reconstruction procedures and in fusion techniques for spinal surgery. The clinical gold standard for bone repair is an autologous graft which although effective, is limited by the availability of sufficient donor tissue and problems with donor site morbidity. These limitations have led to the development of both biological agents to induce bone growth and substitute tissue grafts using different cell types, attached to a variety of matrices or scaffolds. In diseases such as osteoarthritis, repair or replacement of damaged cartilage may directly benefit the patient by increased quality of life. Growth factor and cytokine protein and gene therapies are being developed, which in conjunction with suitable carriers will regenerate missing bone or help in defective healing of cartilage. This presentation will focus on latest advances in stem cell technology and gene therapy, tissue engineering and tissue grafting and bioactive bone substitutes.

Martin Braddock
AstraZeneca R&D Charnwood, Loughborough, United Kingdom

▷ DERMAL ULCER HEALING: ADVANCES IN UNDERSTANDING THEIR PATHOGENESIS

Uncomplicated dermal wound healing is normally a rapid process characterized by the stages of inflammation, cell proliferation and matrix deposition, and finally remodeling and resolution. However there are large numbers of patients with chronic wounds, which occur in the elderly in the setting of cellular hypoxia and recurrent ischemia reperfusion injury. Insights into the altered tissue repair response in these settings will provide new therapeutic targets for chronic wounds.

Thomas Mustoe
Northwestern University, Chicago, USA

▷ SURGICAL MANAGEMENT OF WOUNDS

Recent advances in surgical wound management drastically changed the algorythm of care when dealing with resurfacing exposed or cavity wounds. Debridement has demonstrated its efficiency in chronic as well as in acute wounds, and several techniques of sharp, surgical debridement can be proposed. Non surgical debridement has also been developed, using different techniques autolytic, biosurgical or enzymatic. Promotion of the granulation tissue formation has been extensively developed, especially since the development of the VAC technique (Vacuum Assisted Closure) which are able now to enhance the granulation tissue in cavity wounds. This technique is now proposed in acute traulatic wounds and in chronic situations. Recently, articles resuming the different clinical indications of this negative pressure therapy have been published. Skin replacement was developed in several complementary directions; dermal substitutes offer now an improvement in skin elasticity, reconstituting the dermal component; not cellularized dermal substitutes (Integra, Matriderm) are proposed in Europe, acting like a frame for proliferation of fibroblasts. They are secondarily grafted using split-thickness skin grafts. Other dermal and complete skin substitutes are available in US, with allogenic cells included (Dermagraft, Apligraf). Keratinocyte cultures are available, based on the Green technique. Essentially orientated towards large burnt areas coverage, these cell cultures obtain a high 75% rate of take. These promising solutions, transforming the surgical attitude nowadays based on an extensive use of flaps into a staging recontruction, are under evaluation.

Luc Téot
Hôpital Lapeyronie, Montpellier, France

▷ TISSUE PERFUSION AND OXYGENATION IN WOUND HEALING AND INFECTION

Energy produced by oxygenation is required for healing of tissue lesions. A correlation between oxygen supply and production of collagen and the development of infection has been described. The healing process becomes a function of the capacity of the lungs, the cardiovascular system and oxygen diffusion in the tissue. The effect of hyperbaric oxygen is primarily based on an increased diffusion distance of oxygen in the tissue. External factors as smoking and pain decrease the peripheral oxygen supply. Hyperbaric oxygen and use of stem cells may improve angiogenesis and increase perfusion and oxygenation of ischemic tissue. A correct assessment of perfusion and oxygenation is essential for optimal wound treatment.

Finn Gottrup
University Center of Wound Healing, Odense, Denmark

▷ HGF IN TISSUE REGENERATION AND ANTIFIBROSIS: MECHANISMS AND CONCEPT

Liver regeneration is the most dramatic phenomenon in mammalian tissue regeneration. Hepatocyte growth factor (HGF), originally identified as a hepatotrophic growth factor, plays definitive role in regeneration of various tissues, including the liver. Supplement of HGF exerts potent therapeutic effects in acute and chronic disease models. Particularly, HGF induces/accelerates recovery from tissue fibrosis, including liver cirrhosis, renal sclerosis, and lung fibrosis, through its multiple biological activities involved in dynamic rearrangement of tissue architecture. HGF gene therapy in clinical trial has been successfully done. Mechanisms and concept in tissue regeneration and therapeutics involved in HGF are focused.

Toshikazu Nakamura
Osaka University Graduate School of Medicine, Japan

▷ MULTI-DIMENSIONAL OPTICAL IMAGING IN THICK TISSUES: CONTRAST ACROSS SPATIAL SCALES

Quantitative characterization of tissue structure and function across spatial scales is one of the most challenging problems in Biomedical Imaging. Field of view, depth of interrogation, and resolution are critical features that dramatically impact image quality and information content. Optical methods can potentially provide a single platform for imaging biological tissues with resolution and depth sensitivity ranging from microns to centimeters. Multi-dimensional near-infrared (NIR) techniques will be described that allow scalable resolution and depth based on fundamental light-tissue interaction mechanisms. Emphasis will be placed on intrinsic signal imaging of structure and biochemical composition, and multi-modality image co-registration. Examples will be discussed that highlight the sensitivity of optical methods to various components of wound repair and disease processes, including cellular metabolism, extracellular matrix composition, and vasculature.

Bruce J. Tromberg
Beckman Laser Institute, Irvine, USA

▷ DELIVERY OF INDUCTIVE MOLECULES, GENES AND STEM CELLS

Tissue regeneration is dependent on the localized action of multiple inductive molecules, and cells capable of responding to these factors. We have recently demonstrated that the appropriate temporal presentation of growth factors, either by delivery of the protein or genes encoding the factors, allows one to drive multiple steps in the tissue formation process. Further, controlling the local population of responding cells and the mechanism of cell adhesion to polymeric delivery materials can allow one to regulate their ability to form new tissues. This work may hasten the development of clinically useful engineered tissues, and is providing novel systems to study tissue development.

David Mooney
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