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Chapter I—Cytokines

Role of Leukemia Inhibitory Factor (LIF) in Experimental Sepsis	1
Susan I. Brundage, MD, Mark I. Block, MD, Kevin G. Billingsley, MD, Douglas L. Fraker, MD, and H. Richard Alexander, MD	
Ciliary Neurotrophic Factor Is a Novel Inducer of Cachexia	3
N. Joseph Espot, MD, Troy Auffenberg, BS, Michael A. Rogy, MD, David Martin, PhD, Edward M. Copeland III, MD, FACS, and Lyle L. Moldawer, PhD	
Tumor Necrosis Factor (TNF) Mediates Bacterial Translocation from the Gut	5
Lior Heller, MD, Gideon Goldman, MD, Itamar Shalit, MD, Arie Merhav, MD, FACS, and Joseph M. Klausner, MD	
TNF and IL-1, But Not Endotoxin Stimulate Glutamate Transport in Lung Epithelial Cells	7
Masafumi Wasa, MD, Ming Pan, MD, PhD, and Wiley W. Souba, MD, ScD, FACS	
GM-CSF Is Protective Against Sepsis in Protein-Calorie Malnutrition	11
Arnold D. K. Hill, FRCSI, Alex Cech, MD, Hassan Naama, FRCSI, Jian Shou, MD, and John M. Daly, MD	

TGF β Pretreatment, But Not Conditioned Medium from Endotoxin-pretreated Macrophages, Inhibits Subsequent Endotoxin-stimulated Macrophage TNF Release 14

Susan C. Seatter, MD, Janet Bellingham, BS, Terriel Bennett, MS, Melvin P. Bubrick, MD, FACS, Michael A. West, MD, PhD, FACS

Anti-murine IL-6 Antibody Treatment Improves Survival in Gut-derived Sepsis in a Time-dependent Manner, by Enhancing Host Defense 16

Roberto Gennari, MD, and J. Wesley Alexander, MD, ScD, FACS

Endogenous Interleukin-10 Protects Against Death in Septic Peritonitis in Mice 18

Tom van der Poll, MD, PhD, Arnaud Marchant, MD, Lisa Berman, Douglas D. Lazarus, PhD, Christopher V. Keogh, Lan Nguyen, Michel Goldman, PhD, Lyle L. Moldawer, PhD, and Stephen F. Lowry, MD, FACS

Human TNF Receptor and Interleukin-10 Gene Transfer in the Mouse Reduces Mortality to Lethal Endotoxemia 21

Michael A. Rogy, MD, FACS (Aust), Benjamin Lutge, N. Joseph Espat, MD, Edward M. Copeland III, MD, FACS, and Lyle L. Moldawer, PhD

IL-1 Receptor Antagonist (IL-1RA) Attenuates Activation of the Complement, Coagulation, and Fibrinolytic Systems in Patients With Sepsis 23

Marja A. Boermeester, MD, Paul A. M. van Leeuwen, MD, Susette M. Coyle, RN, Alexander P. J. Houdijk, MD, Anke J. M. Eerenberg, BS, Gert Jan Wolbink, MD, David M. Stiles, MS, John P. Pribble, PharmD, Robert I. C. Wesdorp, MD, C. Erik Hack, MD, and Stephen F. Lowry, MD, FACS

Increased Plasma Levels of Anti-inflammatory Mediators After Injury and Their Clinical Significance	26
Wolfgang Ertel, MD, Mario Bonaccio, MD, Florence A. Scholl, PhD, John S. Kenney, MA, Harald Gallati, PhD, Hans-Peter Friedl, MD, and Otmar Trentz, MD	

Chapter II—Nutrition/Immunomodulation

Peritoneal Macrophage Priming During TPN Feeding Is Not Related to Bacterial Translocation, Operation, or an Intravenous Line	29
W. Scott Helton, MD, Richard M. Garcia, BS, Iris A. Garcia, BS, and Ronald V. Maier, MD, FACS	
Effect of Hypertriglyceridemia on Endotoxin-induced Cytokine Production in Humans	31
Carla C. Braxton, MD, Tom van der Poll, MD, PhD, Susette M. Coyle, RN, Walton J. Montegut, MD, John Wang, MD, Ashwini Kumar, Steve E. Calvano, PhD, and Stephen F. Lowry, MD, FACS	
Enteral Glutamine Augments Host Immune Function	33
Jian Shou, MD, and John M. Daly, MD, FACS	
Vitamin E Inhibits Atherosclerosis in the Diet-induced Hypercholesterolemic Rabbit Model	36
A. J. Matthews, MD, John Belcher, PhD, Jozef Balla, MD, Jozef Murar, MD, Howard Bourdages, MD, Hector Menchaca, MD, Van Michalek, BA, Gregory Vercellotti, MD, and Henry Buchwald, MD, PhD	

- Chronic Central Nervous System Exposure to Interleukin-1, But Not Interleukin-6, Mediates Catabolism in the Rat 38
 Andrew G. Hill, MBChB, Jorge Gonzalez, MD, Jan D. Rounds, BS,
 and Douglas W. Wilmore, MD, FACS
- Differential Expression of Ubiquitin mRNA in Red and White Skeletal Muscle Reflects Differences in Sepsis-induced Muscle Proteolysis 41
 Cheng-Hui Fang, MD, Gregory M. Tiao, MD,
 Josef E. Fischer, MD, FACS, and Per-olof Hasselgren, MD, FACS
- Extrahepatic Expression of Rat Lipopolysaccharide Binding Protein ... 44
 Stewart C. Wang, MD, PhD, Grace L. Su, MD,
 Paul D. Freeswick, MD, David A Geller, MD, Qi Wang, BS,
 Richard A. Shapiro, BS, Timothy R. Billiar, MD, and
 Richard L. Simmons, MD, FACS
- Endotoxin Tolerance: Suppression of Tumor Necrosis Factor, Interleukin-6 and Thromboxane B₂ and Improved Survival Following Hemorrhagic Shock 47
 Patrick J. O'Neill, MD, Patrick G. Mullen, MD, FRCSI,
 Brian Spangelo, PhD, Hong Chen, MS, James A. Cook, PhD,
 and T. Karl Byrne, MD, FRCSI
- Protective Effect of Monophosphoryl Lipid A (MPLA) During Systemic Candidiasis in Neutropenic and Normal Mice 50
 Sung K. Kim, MD, Richard J. Battafarano, MD,
 Peter S. Dahlberg, MD, and David L. Dunn, MD, PhD, FACS
- A Novel Nonanticoagulant Heparin (GM1892) Restores Cardiovascular and Hepatocellular Function Following Trauma-Hemorrhage and Decreases Susceptibility to Sepsis 52
 Ping Wang, MD, Zheng F. Ba, BS, Stephen S. Reich, BS,
 Mian Zhou, MD, Kevin R. Holme, PhD, and
 Irshad H. Chaudry, PhD

Inappropriate Th2 Response to <i>Candida</i> After Hemorrhage Correlates With Reduced Survival	55
Alex C. Cech, MD, James F. Markmann, MD, PhD, Hassan Naama, FRCSI, and John M. Daly, MD	
Specific, Not Global, Defect of Lymphocyte Function in Injured Adults With Elevated <i>Candida</i> Antigen Titers	57
John F. Sweeney, MD, Alexander S. Rosemurgy, MD, FACS, Sheng Wei, MD, and Julie Y. Djeu, PhD	

Chapter III—Sepsis/Shock

The Perfused Mouse Liver Produces TNF, IL-1, and IL-6 After Endotoxin	59
Norman H. Kumins, MD, Julia L. Hunt, BS, Richard L. Gamelli, MD, FACS, and James P. Filkins, PhD	
The Role of Nitric Oxide in Subcutaneous and Transmural Gut Tissue Oxygenation	62
David D. Zabel, MD, Harriet W. Hopf, MD, and Thomas K. Hunt, MD, FACS	
Hemodynamic and Molecular Evidence that the Vascular Decomensation in Hemorrhagic Shock Is Not Mediated by Nitric Oxide	65
Edward Kelly, MD, Nishit Shah, MD, Nathan N. Morgan, BS, Simon C. Watkins, PhD, Andrew B. Peitzman, MD, FACS, and Timothy R. Billiar, MD	

- Nitric Oxide Scavenging: An Alternative Therapeutic Approach to Nitric Oxide Synthesis Inhibition in Nitric Oxide-Mediated Hypotension of Sepsis** 67
 Hae Won Kim, PhD, Jennifer K. Hughes, BS, Paul Breiding, BS, and A. Gerson Greenburg, MD, PhD, FACS
- Hemofiltration in Human Sepsis: Evidence for Elimination of Immunomodulatory Substances** 69
 Johannes N. Hoffmann, MD, Reinhold Deppisch, PhD, Eugen Faist, MD, Wolfgang H. Hartl, MD, Marianne Jochum, PhD, and Dietrich Inthorn, MD
- Translocating Gut Bacteria Reach the Pulmonary Artery by Means of the Thoracic Duct During Hemorrhagic Shock** 72
 Alex Shnaper, MD, Franklin Greif, MD, FACS, Moshe Micowitz, MD, David Feodorov, MD, Patrick Sorkin, MD, and Shlomo Lelcuk, MD
- Cardiopulmonary Effects of Raised Intra-abdominal Pressure** 74
 Philip C. Ridings, MD, FRCS, Charles R. Blocher, BS, and Harvey J. Sugerman, MD, FACS
- Biochemical Markers of Cardiac Injury After Burn Shock** 76
 Nilda M. Garcia, MD, Jureta W. Horton, PhD, and Joseph Keffer, MD
- Right Ventricular End-diastolic Volume Index Is Superior to Cardiac Filling Pressures in Determining Preload Status** 79
 Michael L. Cheatham, MD, Karen Safcsak, RN, and Loren D. Nelson, MD, FACS
- Pulmonary Hypertension in Endotoxin Shock: Evidence for a Starling Resistor-mediated Component** 81
 H. Kenith Fang, MD, Rick L. Kraemer, MS, Joseph Vitello, MD, Eric B. Rypins, MD, FACS, and William R. Law, PhD

Artificial Surfactant Improves Experimental Adult Respiratory Distress Syndrome (ARDS) in Subhuman Primates 84
 Francis G. Duhaylongsod, MD, Philip J. Fracica, MD,
 Yuh-Chin T. Huang, MD, Claude A. Piantadosi, MD, and
 Walter G. Wolfe, MD, FACS

Predictors of Survival and Death for Injured Children Receiving Pre-hospital Cardiopulmonary Resuscitation 87
 Matthew Moront, MD, Catherine S. Gotschall, ScD,
 Martin R. Eichelberger, MD, FACS, Gilberto Maksoud, MD,
 and J. René Morrissey, BA

Chapter IV—Inflammation

Low-dose Endotoxin Augments Alveolar Macrophages to Platelet-activating Factor Stimulation 90
 Chong-Jeh Lo, MD, Iris Garcia, BS, and
 Ronald V. Maier, MD, FACS

Differential Modulation of Macrophage pH Regulation by Acute Oxidant Stress 93
 Guy F. Brisseau, MD, Sergio Grinstein, PhD, Aye Aye Khine, MSc,
 and Ori D. Rotstein, MD, FRCSC, FACS

Activated Macrophages Impair Sodium Channel Activity in Alveolar Epithelial Cells: A Mechanism Contributing to ARDS 96
 Jin Wen Ding, MD, Hugh O’Brodivich, MD, FRCPC,
 Paul Y.-C. Cheung, MSc, and Ori D. Rotstein, MD, FRCSC, FACS

Beneficial Effects of a Novel C-terminal Fragment of Rabbit CAP18 (Cationic Antimicrobial Protein of 18 kDa) in a Porcine Model of Endotoxocosis	98
Thomas J. VanderMeer, MD, Jing Zhuang, MD, Michael J. Menconi, PhD, Hailong Wang, MD, Carmen Bouza, MD, Robert J. Murtaugh, DVM, Paul Stevens, PhD, and Mitchell P. Fink, MD, FACS	
Early Discrepancy Between Plasma Interleukin-8 (IL-8) Levels and Neutrophil (PMN) Priming in Postinjury Multiple Organ Failure (MOF) Suggests PMN Dysfunction	100
Abraham J. Botha, MD, FRCS(Eng), Frederick A. Moore, MD, FACS, Ernest E. Moore, MD, FACS, Belchor Fontes, MD, Reginald Franciose, MD, and Anirban Banerjee, PhD	
Activated Neutrophils Inhibit Hepatocyte Acute-phase Protein Synthesis	103
Paul E. Bankey, MD, PhD, Kevin Kadesky, MD, and Richard Turnage, MD	
Gut Ischemia/Reperfusion Promotes Sequestration of Activated Neutrophils in the Lung but Not in the Liver	104
Belchor Fontes, MD, Ernest E. Moore, MD, FACS, Frederick A. Moore, MD, FACS, Abrie Botha, MD, Virginia Carl, MS, and Anirban Banerjee, PhD	
Attenuation of Lung Injury in a Rabbit Acid Aspiration Model Using GM-1925, A Novel Selectin Inhibitor	107
Kelley M. Cornell, MD, and Mark W. Bowyer, MD	
Cellular Mechanisms of Endothelial Cell Death During the Systemic Inflammatory Response Syndrome	110
Jiang Huai Wang, PhD, H. Paul Redmond, FRCSI, R. William G. Watson, BSc, and David Bouchier-Hayes, FRCSI	

- Accelerated Thymic Apoptosis During Polymicrobial Sepsis Is Driven by Corticosteroids but Not by Tumor Necrosis Factor (TNF)112**
 Alfred Ayala, PhD, Donna L. Lehman, BS, Crystal D. Herdon, BS, and Irshad H. Chaudry, PhD
- Cytokine-induced Nitric Oxide Synthesis in Hepatocytes Involves the Transcription Factor NF- κ B115**
 David A. Geller, MD, Paul D. Freeswick, MD, Hartmut Luss, MD, Andreas Nussler, PhD, and Timothy R. Billiar, MD
- Nitric Oxide Biosynthesis in the Absence of L-Arginine: The Role of Recycling L-Citrulline in Cytokine-stimulated Smooth Muscle Cells117**
 Amy C. Heidenreich, MD, Stephen E. Morrow, MD, Sidney M. Morris, PhD, Paul Davies, PhD, Bruce R. Pitt, PhD, Timothy R. Billiar, MD, and Don K. Nakayama, MD, FACS

Chapter V—Alimentary Tract

- Epidermal Growth Factor Is a Potent Stimulus to Esophageal Mucosal Growth120**
 Brian J. Dunkin, MD, Hamdy Aly, MD, PhD, Faiz Bhora, MD, Lloyd D. McKie, MB, FRCS, John W. Harmon, MD, FACS, and Barbara L. Bass, MD, FACS
- CGRP Mediates Visceral Afferent Nerve-induced Esophageal Hyperemia123**
 Lloyd D. McKie, MB, FRCS, Brian J. Dunkin, MD, John W. Harmon, MD, FACS, and Barbara L. Bass, MD, FACS

- Superoxide Dismutase Attenuates PAF-induced Gastric Microvascular Injury by Reducing Neutrophil Uptake126**
 John R. Schwappach, MD, John G. Wood, PhD, and
 Laurence Y. Cheung, MD, FACS
- Expression of mRNA Encoding the Basal Na-K-Cl Cotransporter in Amphibian Gastric Mucosa and Its Regulation by Feeding128**
 David I. Soybel, MD, and Eric Delpire, PhD
- Cholecystokinin (CCK) and Secretin Stimulate Implantation and Growth of a Human Gastric Adenocarcinoma130**
 Guillermo Gomez, MD, Luis A. Padilla, MD, Richard J. Bold, MD,
 Robert R. Chen, George H. Greeley, Jr, PhD,
 Courtney M. Townsend, Jr, MD, FACS, and
 James C. Thompson, MD, FACS
- Cholecystokinin-induced Protection Against Gastric Mucosal Injury by Acidified Ethanol Is Negated by Type "A" CCK Receptor Blockade133**
 David W. Mercer, MD, James M. Cross, MD, Jose C. Barreto, PhD,
 Nathaniel H. P. Strobel, BS, and Thomas A. Miller, MD, FACS
- Role of Nitric Oxide and Prostacyclin in Angiotensin II-induced Gastric Vasoconstriction135**
 Michael P. Darnell, MD, Jay Beyer, MD, John G. Wood, PhD, and
 Laurence Y. Cheung, MD, FACS
- Microsomal 7- α -Hydroxylase Activity Is Decreased in Miniswine on Total Parenteral Nutrition137**
 Joseph P. Muldoon, MD, Mary A. Greiner, MS,
 David L. Nahrwold, MD, FACS, and
 Lillian G. Dawes, MD, FACS

Bile Acid Gastric Mucosal Injury Is a Neutrophil-dependent Process	139
Michael W. Grabowski, MD, Daniel T. Dempsey, MD, FACS, Jim Lee, MD, George Tsai, MD, and Wallace P. Ritchie, Jr, MD, PhD, FACS	
Progesterone Alters Biliary Flow Dynamics	141
Sean Tierney, FRCSI, Attila Nakeeb, MD, Melvin Reinhardt, CNMT, Oliver Wong, MD, Pamela A. Lipsett, MD, FACS, Samuel Sostre, MD, Henry A. Pitt, MD, FACS, and Keith D. Lillemo, MD, FACS	
Platelet-activating Factor (PAF) Stimulates Release of PGI ₂ from Inflamed Rabbit Gallbladder Cell Cultures	144
Lori Bartula, BS, Angela Riva, BS, R. Turnage, MD, K. Kadesky, MD, and S. Myers, MD, FACS	
Crystalline Cholesterol Has Inflammatory Properties in the Guinea Pig Gallbladder in vivo	146
Jay B. Prystowsky, MD, FACS, and Robert V. Rege, MD, FACS	
Rapid Elevation of Systemic Cytokines During Acute Pancreatitis and Their Origination Within the Pancreas	148
James Norman, MD, Michael Franz, MD, Adam Riker, MD, Peter J. Fabri, MD, FACS, and William R. Gower, PhD	
The Influence of Diabetes on the Growth of Pancreatic Cancer	151
William E. Fisher, MD, Laszlo G. Boros, MD, Thomas M. O'Dorisio, MD, M. Sue O'Dorisio, MD, PhD, and William J. Schirmer, MD	
Human Pancreatic Thread Protein Is Mitogenic to Pancreatic-derived Cells in Culture	153
Michael E. Zenilman, MD, Thomas H. Magnuson, MD, Kevin Swinson, BA, Josephine Egan, MD, Riccardo Perfetti, MD, and Alan R. Shuldiner, MD	

- Adenovirus-mediated Pancreatic Gene Transfer in the Rat156
 Ronald P. DeMatteo, MD, James M. Wilson, MD, PhD, and
 Steven E. Raper, MD, FACS
- Functional Responses of the Endocrine Cell Line (BON) Transfected
 With the Human Bombesin Receptor158
 Richard J. Bold, MD, B. Mark Evers, MD, FACS,
 Jin Ishizuka, MD, PhD, Courtney M. Townsend, Jr, MD, FACS,
 and James C. Thompson, MD, FACS
- Pituitary Adenylate Cyclase-activating Peptide Stimulates Dual Signal
 Transduction Mechanisms160
 Ambrosio Hernandez, BS, Beth Kimball, MD, and
 Michael W. Mulholland, MD, PhD
- Alpha_{1b}-adrenergic Receptor Expression Is Increased Following Hepatic
 Ischemia–Reperfusion162
 Gene D. Branum, MD, Sean R. Sue, BA, Ravi S. Chari, MD,
 William C. Meyers, MD, Sue J. Lee, MD, Richard Whalen, PhD, and
 Thomas D. Boyer, MD
- Transcriptional Activation of the iNOS Gene in Hepatocytes Is Isolated
 to a 1.7-kb Promoter Region164
 Paul D. Freeswick, MD, David A. Geller, MD,
 Charles J. Lowenstein, MD, Richard A. Shapiro, BS, and
 Timothy R. Billiar, MD
- Liver Plays an Inhibitory Role on Hypothalamic Dopamine Release
 During Eating and TPN166
 Zhong-jin Yang, MD, Albert Oler, MD,
 Michael M. Meguid, MD, PhD, FACS, and
 John R. Gleason, PhD
- Bile, IgG Isotype Switch: A Clue to Gallstone Pathogenesis169
 Pamela A. Lipsett, MD, FACS, Lisa M. Nipkow, BS,
 James Hildreth, MD, PhD, Keith D. Lillemoe, MD, FACS,
 and Henry A. Pitt, MD, FACS

Insulin Extraction During Liver Regeneration	171
Kelly A. Kogut, MD, L. Wiley Nifong, MD, Mary Jo Witt, BA, Seymour I. Schwartz, MD, FACS, and David A. Krusch, MD, FACS	
Changes in Expression of TGF β 1, TGF β Type II Receptor, and IGFII Receptor in Human Hepatocellular Carcinoma	174
Sean R. Sue, BA, Ravi S. Chari, MD, Feng-Ming Kong, MD, Jeremy R. Mills, PhD, Robert Fine, MD, Randy L. Jirtle, PhD, and William C. Meyers, MD	
Intraluminal Peptide YY Is Proabsorptive in the Canine Ileum in vivo	176
Carson D. Liu, MD, Edward E. Whang, MD, Oscar J. Hines, MD, Todd R. Newton, BS, Michael J. Zinner, MD, FACS, Stanley W. Ashley, MD, FACS, and David W. McFadden, MD, FACS	
Gastrin Regulates Lactase Gene Expression in the Intact Rat	178
Richard A. Hodin, MD, Shufen Meng, MD, and Amy Shei, BA	
Retainment of the Postprandial Intestinal Hyperemia Response After Small Bowel Transplantation	181
Rong Yang, MD, Qi Liu, MD, Joseph L. Unthank, PhD, Mark D. Pescovitz, MD, FACS, and Jay L. Grosfeld, MD, FACS	
Massive Enterectomy Alters Brush Border Amino Acid and Glucose Transport	184
Timur P. Sarac, MD, Anna S. Seydel, MD, Wiley W. Souba, MD, ScD, FACS, Charlotte K. Ryan, MD, Jennie H. Miller, MS, Palmer Q. Bessey, MD, FACS, and Harry C. Sax, MD, FACS	

- Brush-border Rather Than Basolateral Na⁺/H⁺ Exchange Plays a Predominant Role in Basal and Meal-stimulated Ileal Absorption187**
 Michael M. Maher, MB, FRCSI, Jacqueline D. Gontarek, BA,
 Ramon E. Jimenez, MD, Mark Donowitz, MD, and
 Charles J. Yeo, MD, FACS
- Growth Effect of Bombesin on Normal and Neoplastic GI Tissue190**
 Kyo U. Chu, MD, Jin Ishizuka, MD, PhD,
 Courtney M. Townsend, Jr, MD, FACS, and
 James C. Thompson, MD, FACS
- The Metabolites of Different Dietary Fibers Exert Distinct Effects on Human (Caco-2) Colonocytes192**
 Marc D. Basson, MD, PhD, and Fu Hong, MD
- Potassium Ion Channels and Sodium Absorption in Human Colon195**
 Donal D. Maguire, MB, Gerald C. O'Sullivan, MB, MSc, MCh, FRCS,
 and Brian J. Harvey, PhD, DSc
- Bombesin Mediates Calcium Fluxes in Myenteric Neurons Via a Phospholipase C-dependent Mechanism197**
 Diane M. Simeone, MD, Beth C. Kimball, MD, and
 Michael W. Mulholland, MD, PhD
- Ammonia Selectively Inactivates cAMP-mediated Cl⁻ Secretion in Cultured Human Intestinal Epithelial Monolayers199**
 Madhu Prasad, MD, and Jeffrey B. Matthews, MD
- Skeletal Muscle Force Deficit After Transposition for Anal Sphincter Reconstruction202**
 Edwin G. Wilkins, MD, William M. Kuzon, Jr, MD, PhD, FRCS(C),
 Richard T. Hinkle, MS, Mohammad Khaled H. Youssef, MD,
 Paul Guelinckx, MD, PhD, and John A. Faulkner, PhD

Mechanisms of Peptide YY-induced Colonic Absorption	204
Edward E. Whang, MD, Oscar J. Hines, MD, Carson D. Liu, MD, Joseph R. Reeve, Jr, PhD, Anton J. Bilchik, MD, PhD, James A. Moser, MD, Michael J. Zinner, MD, FACS, David W. McFadden, MD, FACS, and Stanley W. Ashley, MD, FACS	

Chapter VI—Cardiothoracic

Ischemic Cardiac Preconditioning Is Mediated Through Protein Kinase C	207
Elizabeth C. Brew, MD, Max B. Mitchell, MD, Thomas F. Rehring, MD, Fabia Gamboni-Robertson, PhD, Anirban Banerjee, PhD, and Alden H. Harken, MD	
Hypoxic Preconditioning Preserves Antioxidant Reserve and Prevents Calcium Overload in the Ischemic/Reperfused Working Rat Heart	209
Daniel T. Engelman, MD, Masazumi Watanabe, MD, Richard M. Engelman, MD, FACS, John A. Rousou, MD, FACS, Joseph E. Flack III, MD, David W. Deaton, MD, Elena Kisin, MS, Valerian Kagan, PhD, and Dipak K. Das, PhD	
Is the Myoprotective Ischemic Preconditioning Response Age and/or Model Dependent?	212
Paul G. Burns, MD, Irvin B. Krukenkamp, MD, Christopher A. Caldarone, MD, Glenn R. Gaudette, MSc, and Sidney Levitsky, MD, FACS	
The Differential Effects of Ischemic Preconditioning on Clinically Relevant Cardioplegia	214
Richard W. Illes, MD, Shane Haynes, MS, and Karen Inners-McBride, BS	

- Multiple Parallel Signal Transduction Pathways Can Mediate Cardiac
Preconditioning216
Mary M. Wollmering, MD, Anirban Banerjee, PhD,
Christopher B. Winters, MD, David A. Campbell, MD,
and Alden H. Harken, MD
- Adenosine Administration at Reperfusion Improves Left Ventricular
Performance in Neonatal Lambs Following Cardiopulmonary Bypass and
Two Hours of Global Hypothermic Ischemia219
Joseph M. Forbess, MD, Takuya Miura, MD, Takeshi Hiramatsu, MD,
and John E. Mayer, Jr, MD
- Comparison of the Cardioprotective Effects of Acadesine and Adenosine
on in situ Porcine Myocardial Stunning222
Mohinder P. S. Randhawa, Jr, MD, Robert D. Lasley, PhD,
Lisa M. Karpinski, BS, Julia Hegge, BS, and
Robert M. Mentzer, Jr, MD, FACS
- Glycolytic Flux During Myocardial Ischemia: An Adenosine
Mechanism225
Steven F. Bolling, MD, FACS, Keith Childs, BS,
and Xue-Han Ning, MD
- Cardioplegia With Glutamate and Aspartate: Effect on TCA Cycle
Metabolism227
Mark K. Reed, MD, Chen Barak, MS, Craig R. Malloy, MD,
Stephen P. Maniscalco, BS, and
Michael E. Jessen, MD, FACS, FRCSC
- Pretreatment With Lazaroid (U-74500A) Prevents Ischemic and
Reperfusion Injury in Blood-Perfused Rabbit Hearts230
Kazuyuki Miyamoto, MD, Shigeki Morita, MD,
Takahiro Nishida, MD, Munetaka Masuda, MD, Yasuo Kanegae, MD,
Atsuhiro Nakashima, MD, Manabu Hisahara, MD, Kouji Fukae, MD,
Shigehiko Tokunaga, MD, Yoshito Kawachi, MD, and
Hisataka Yasui, MD

- The Mechanism by Which Pyruvate Protects Myocardium from Ischemia
Reperfusion Injury: A Dual Mechanism232
Juan A. Crestanello, MD, Joseph Kamelgard, MD,
and Glenn J. Whitman, MD, FACS
- A New Animal Model of LV Dysfunction Due to Regional
Infarction235
Hiroshi Maruyama, MD, Lynda L. Mickleborough, MD, FRCSC,
Richard Szwarc, MSc, Gregory Wilson, MD, and
Shanas Mohamed, RN
- Durability of Donor-specific and Organ-specific Heart Transplant
Tolerance Induced by Intrathymic Pretreatment With Allogeneic Spleen
Cells237
Zhenya Shen, MD, Muhammad Mohiuddin, MD,
and Verdi J. DiSesa, MD, FACS
- Complete Atrioventricular Orthotopic Cardiac Transplantation Improves
Ventricular Mechanics239
Simon W. Kendall, BSc, MBBS, FRCS(C/Th),
Hartmuth B. Bittner, MD, Damian Craig, MS, Shim Youngtak, PhD,
David S. Peterseim, MD, and Peter VanTrigt III, MD
- Permanent Drug-free Cardiac Allograft Survival Using a Nonlethal
Approach to Mixed Bone Marrow Chimerism242
Yolonda L. Colson, MD, PhD, Kathy J. Zadach,
and Suzanne T. Ildstad, MD, FACS
- Jarvik 2000 Ventricular Assist Device: A Potential Bridge to Pediatric
Transplantation244
Richard J. Kaplon, MD, Mehmet C. Oz, MD,
Pawel A. Kwiatkowski, MD, Matthew Williams, BA,
Howard R. Levin, MD, Robert K. Jarvik, MD, and
Eric A. Rose, MD, FACS

- High-resolution Quantitative Flow Maps of a Pediatric VAD247
 Timothy W. Pettitt, MD, Reinhold A. Gerbsch, PhD, and
 Bill B. Daily, MD, PhD
- Intraoperative Autologous Donation: Volume-dependent Red Cell
 Preservation249
 Robert E. Helm, MD, John D. Klemperer, MD,
 Todd K. Rosengart, MD, Jeffrey P. Gold, MD, Powers Peterson, MD,
 Steven J. Thomas, MD, and Karl H. Kreiger, MD
- The Hemostatic Defect After Cardiopulmonary Bypass Is Not an Intrinsic
 Platelet Function Defect252
 Herbert Reich, MD, Victor A. Ferraris, MD, PhD, FACS,
 Suellen P. Ferraris, PhD, Evelio Rodriguez, Mark Huang,
 Atul Gupta, MD, James A. Bennett, PhD, Thomas T. Andersen, PhD,
 and John W. Fenton II, PhD
- Extracellular Concentrations of Neuroexcitatory Amino Acids During
 Spinal Cord Ischemia255
 David B. Glick, MD, Stephen W. Downing, MD,
 Gregorio A. Sicard, Jr, Douglas C. Lobner, PhD,
 Dennis W. Choi, MD, PhD, Nicholas T. Kouchoukos, MD, FACS,
 and Brent T. Allen, MD, FACS
- Aorto-pulmonary Collaterals Decrease the Rate of Cerebral Cooling and
 Alter Regional Cerebral Perfusion During Cardiopulmonary Bypass258
 Paul M. Kirshbom, MD, Lynne A. Skaryak, MD,
 Louis R. DiBernardo, MD, Frank H. Kern, MD,
 William J. Greeley, MD, J. William Gaynor, MD,
 and Ross M. Ungerleider, MD
- Low Flow Bypass Versus Circulatory Arrest: Similar Vulnerability to
 Postbypass Cerebral Metabolic Injury260
 Lynne A. Skaryak, MD, Paul M. Kirshbom, MD,
 Louis R. DiBernardo, MD, Frank H. Kern, MD,
 William J. Greeley, MD, Ross M. Ungerleider, MD, and
 J. William Gaynor, MD

- Brain Protection During Circulatory Arrest263
 Carlos L. Filgueiras, MD, MSc, BeAtrice Winsborrow, MSc, PhD,
 Jian Ye, MD, MSc, Alexander Aronov, MD, PhD,
 Piotr Kozlowski, PhD, John K. Saunders, PhD,
 Roxanne Deslauriers, PhD, and Tomas A. Salerno, MD, MSc
- The Differential Effect of Brain Death on Right and Left Ventricular
 Mechanics and β -Adrenergic Receptor Function265
 Hartmuth B. Bittner, MD, Simon W. H. Kendall, FRCS(C/Th),
 Carmelo A. Milano, MD, Robert D. Davis, MD, and
 Peter Van Trigt, MD
- Novel Technique of Orthotopic Lung Transplantation in Rats in Which
 Survival and Hemodynamic Assessment Can Be Measured Independent of
 the Native Lung268
 Nepal C. Chowdhury, MD, Yoshifumi Naka, MD, PhD,
 David J. Pinsky, MD, Osvaldo J. Yano, MD,
 Craig R. Smith, MD, FACS, Eric A. Rose, MD, FACS,
 David M. Stern, MD, Robert E. Michler, MD, FACS,
 and Mehmet C. Oz, MD
- Mixed Chimerism to Achieve Donor-specific Transplantation Tolerance
 for Lung Allografts in Rats271
 Wook Youm, MD, Yolanda L. Colson, MD, PhD,
 Kanshi Komatsu, MD, Suzanne T. Ildstad, MD,
 Samuel A. Yousem, MD, Bartley P. Griffith, MD,
 and Si M. Pham, MD
- Ischemic Injury Does Not Increase Rejection in Rat Lung Allografts ..273
 Takeshi Shiraishi, MD, Takatoshi Mizuta, MD,
 Steve R. DeMeester, MD, Jon H. Ritter, MD, Paul E. Swanson, MD,
 Mark R. Wick, MD, Joel D. Cooper, MD, FACS, FRCS(C),
 and G. Alexander Patterson, MD, FACS, FRCS(C)

- Inhaled Nitric Oxide Reduces Lung Allograft Reperfusion Injury276
 Kan Okabayashi, MD, Anastasios N. Triantafyllou, MD,
 Motohiro Yamashita, MD, Motoi Aoe, MD,
 Joel D. Cooper, MD, FACS, FRCS(C), and
 G. Alexander Patterson, MD, FACS, FRCS(C)
- Inhaled Nitric Oxide Improves Transpulmonary Transport Function and
 Right Ventricular Performance in Hypoxic Pulmonary Artery
 Hypertension279
 Neal D. Hillman, MD, Donald R. Black, MD, Damian M. Craig, MS,
 Jon N. Meliones, MD, and Peter K. Smith, MD, FACS
- Single Lung Transplant Improves Right Ventricular Function in
 Pulmonary Hypertensive Rats283
 Osvaldo Juniti Yano, MD, Daniel Burkhoff, MD, PhD,
 Marc Louis Dickstein, MD, Robin Baradaran, BA,
 Henry Michael Spotnitz, MD, FACS, and
 Craig Richey Smith, MD, FACS
- Effects of Endotoxin on Pulmonary Gas Exchange and Aerodynamic and
 Hemodynamic Function in an Isolated Blood-perfused Rat Lung
 Model286
 Shigeyuki Sasaki, MD, PhD, James D. McCully, PhD,
 Joseph LoCicero III, MD, FACS, Sidney Levitsky, MD, FACS,
 R. Armour Forse, MD, PhD, FACS, and John D. Palombo, DSc
- Platelet-Activating Factor (PAF) Antagonism With SDZ HUL-412
 Markedly Reduces Lung Reperfusion Injury via Decreased Leukocyte-
 mediated Oxidative Injury288
 Rick A. Low, MD, Kay Uthoff, MD, Kenton J. Zehr, MD,
 and R. Scott Stuart, MD
- Attenuation of Rat Lung Isograft Reperfusion Injury With Antiadhesion
 Molecule Monoclonal Antibodies291
 Steven R. DeMeester, MD, Maria A. Molinari, Takeshi Shiraishi, MD,
 Kan Okabayashi, MD, Jill Manchester,
 Joel D. Cooper, MD, FACS, FRCS(C), and
 G. Alexander Patterson, MD, FACS, FRCS(C)

Isolated Lung Perfusion With Carboplatin for Metastatic Sarcoma in the F344 Rat	294
Jennifer L. Ellis, MD, Bruce Ng, MS, Jeffery Port, MD, and Michael E. Burt, MD, PhD	
The Role of Tumor Necrosis Factor-alpha in a Rat Lung Model of Ischemia-Reperfusion Injury	297
Michael J. Eppinger, MD, Michael L. Jones, G. Michael Deeb, MD, FACS, Steven F. Bolling, MD, FACS, and Peter A. Ward, MD	
A Simple Model for Isolated Lung Function Studies	300
Futing Zhang, MD, Guanghan Wu, MD, Zuyi Zhang, MD, Robert Salley, MD, and Sufan Chien, MD	
Overexpression of a Constitutively Active α_1 -Adrenergic Receptor in Transgenic Mice Induces Cardiac Hypertrophy	304
Carmelo A. Milano, MD, Lee F. Allen, MD, PhD, Paul C. Dolber, PhD, and Robert J. Lefkowitz, MD	
Maximizing Dissolved Oxygen Optimizes Tissue Oxygenation During Cardiopulmonary Bypass	307
Edward R. Ferguson, MD, Walter V. A. Vicente, MD, PhD, Russell D. Spruell, BSEE, and William L. Holman, MD, FACS	
Inflammatory Injury to the Heart: The Effect of Protein Kinase C Activation	309
Percival Buenaventura, MD, Koh Takeuchi, MD, Hung Cao-Danh, PhD, Paul Glynn, PhD, Francis McGowan, MD, and Pedro del Nido, MD, FACS	
Vascular Cell Adhesion Molecule-1 (VCAM-1) Up-regulation Precedes and Predicts Cellular Rejection of Human Cardiac Allografts	312
Abbas Ardehali, MD, Hillel Laks, MD, FACS, Davis Drinkwater, MD, Jon Kobashigawa, MD, Jaime Moriguchi, MD, and Thomas A. Drake, MD	

Myocardial Ischemia Triggers Rapid Expression of Mitochondrial Genes	315
Ion I. Moraru, MD, PhD, Daniel T. Engelman, MD, Richard M. Engelman, MD, FACS, John A. Rousou, MD, FACS, Joseph E. Flack III, MD, David W. Deaton, MD, and Dipak K. Das, PhD	
Association of DNA Aging and Enhanced Endonuclease Activity in Senescent Ischemic Intolerance	317
Takuro Tsukube, MD, PhD, James D. McCully, PhD, Elizabeth A. Faulk, MD, Irvin B. Krukenkamp, MD, and Sidney Levitsky, MD, FACS	
Carotid Ligation Alters Myocardial Gene Expression	320
Thomas Yeh, Jr, MD, John W. C. Entwistle III, MD, Laura J. Graham, LVT, Andrew S. Wechsler, MD, FACS, and Emma R. Jakoi, PhD	
Congestive Heart Failure Impairs Coronary Endothelial Release of Prostacyclin and Nitric Oxide	323
Matthew S. T. Chow, MD, Pei-Chin Yao, BS, Barbara L. Robinson, MD, James J. Morris, MD, FACS, and Hartzell V. Schaff, MD, FACS	
The Depressive Effects of Protamine on Myocyte Contractile Function Are Enhanced With Chronic Left Ventricular Dysfunction	325
R. Barry Hird, MD, Francis G. Spinale, MD, PhD, and Fred A. Crawford, MD	
3,5,3'Triiodo-L-Thyronine (T ₃) Pretreatment Provides a Direct and Beneficial Effect on Myocyte Function Following Hypothermic Cardioplegic Arrest and Rewarming	328
Jennifer D. Walker, MD, Fred A. Crawford, MD, and Francis G. Spinale, MD, PhD	

A Three-Dimensional Analysis of Papillary Muscle Spatial Relationships in Acute Postinfarction Mitral Insufficiency	330
Robert C. Gorman, MD, James S. McCaughan, MD, Mark B. Ratcliffe, MD, Krishanu B. Gupta, PhD, T. Sloane Guy, MD, and L. Henry Edmunds, Jr, MD, FACS	
LVAD Support Alters Regional Right Ventricular Systolic Mechanics	334
Marc R. Moon, MD, Abe DeAnda, MD, Luis J. Castro, MD, George T. Daughters, MS, Neil B. Ingels, PhD, and D. Craig Miller, MD, FACS	

Chapter VII—Vascular

Vascular Smooth Muscle Cell Growth Factors Activate Mitogen-activated Protein Kinase by Multiple Intracellular Pathways	338
Shinsuke Mii, MD, J. Anthony Ware, MD, Sheila Mallette, BS, and K. Craig Kent, MD, FACS	
Glutamine Transport in Human Endothelium Is Inhibited by Protein Kinase C Activation	340
Ming Pan, MD, PhD, Masafumi Wasa, MD, and Wiley W. Souba, MD, ScD, FACS	
In vivo Endothelial-specific Adenoviral Vector-mediated Gene Transfer into Rat Carotid Arteries	343
Andrew H. Schulick, MD, Gang Dong, MD, PhD, Kurt D. Newman, MD, and David A. Dichek, MD	
Acute and Chronic Vascular Compensation to Abrupt Occlusion of the Rat Femoral Artery Is Inhibited by <i>N</i> ω -Nitro-L-Arginine Methyl Ester	345
Joseph L. Unthank, PhD, J. Craig Nixon, MS, and Michael C. Dalsing, MD, FACS	

Intracellular Mechanism and Cell Cycle Effects of Nitric Oxide Inhibition of Vascular Smooth Muscle Cell Mitogenesis348
 Rajabrata Sarkar, MD, R. Clinton Webb, PhD,
 Louis M. Messina, MD, FACS, and James C. Stanley, MD, FACS

Gene Transfer of Human Inducible Nitric Oxide Synthase: Delivery to Vascular Endothelial Cells351
 Edith Tzeng, MD, David A. Geller, MD, Hideaki Tahara, MD, PhD,
 Paul D. Robbins, PhD, Richard L. Simmons, MD, FACS, and
 Timothy R. Billiar, MD

Blocking Type I Collagen Formation Prevents Smooth Muscle Cell Proliferation by Causing Late G₁ Cell Cycle Arrest353
 Eric T. Choi, MD, Niraj L. Sehgal, BA, Una S. Ryan, PhD, and
 Allan D. Callow, MD, PhD

Smooth Muscle Cell Inhibition With Endothelial Cell Stimulation by Growth Regulators Within a Fibrin Glue Delivery Vehicle355
 Steven S. Kang, MD, Dewei Ren, MD, Claire Gosselin, MD, and
 Howard P. Greisler, MD, FACS

Synergism Between Vascular Endothelial Growth Factor and Basic Fibroblast Growth Factor in the Induction of Angiogenesis in vivo ...358
 Takayuki Asahara, MD, Christophe Bauters, MD, Lu P. Zheng, MD,
 Jeffrey M. Isner, MD, FACC, and
 James F. Symes, MD, FRCS(C), FACS

Neutrophil (PMN) Chemotaxis Is Stimulated by Domain-specific Antibodies to PECAM-1 (Platelet-Endothelial Cell Adhesion Molecule-1)360
 Michael M. Farooq, MD, Richard E. Carballo, MD,
 Dan Yamini, BS, Jonathan B. Towne, MD, Peter J. Newman, PhD,
 and Julie A. Freischlag, MD

Production of Platelet-derived Growth Factor B Chain (PDGF-B) by Endothelial Cells Subjected to Cyclic Strain in vitro362
 Gary L. Gallagher, MD, and Bauer E. Sumpio, MD, PhD, FACS

Transforming Growth Factor- β 1 Inhibits Human Arterial Smooth Muscle Cell Proliferation and Induces 1α (I) Procollagen Gene Expression	364
Brian G. Halloran, MD, Greg D. Prorok, MS, Yi Ping Wang, MD, Thomas G. Lynch, MD, FACS, and B. Timothy Baxter, MD, FACS	
Correlation Between Pathology and Electrical Activity During Acute Intestinal Ischemia	368
C. Louis Garrard, MD, Susan Halter, MD, and William O. Richards, MD, FACS	
The Critical Aortic Wall Structural Defect in Abdominal Aortic Aneurysm Formation	372
Connie J. Campbell, MD, John Oh, MD, and John V. White, MD	
A Soluble Extract from Abdominal Aortic Aneurysm Wall Stimulates Protein Secretion and Gelatinase Expression in Cultured Macrophage-like Cells (U937)	375
Anil Hingorani, MD, Karen Newman, PhD, Anita Gregory, MD, and M. David Tilson, MD, FACS	
Administration of Intraperitoneal Silica Enhances Regression of Atherosclerosis in a Rabbit Model	378
Howard Bourdages, MD, Hector Menchaca, MD, A. J. Matthews, MD, Van Michalek, BA, Rebecca Rose, DVM, PhD, Jeffry D. Shearer, MA, and Henry Buchwald, MD, PhD, FACS	
Comparison of Platelet-derived Growth Factor-alpha Gene Expression in Human Carotid Plaque, Normal Artery, and Venous Tissue	380
J. Gordon Wright, MD, FACS, J. Chadwick Tober, MD, D. Blaine Nease, BS, Donald E. Kuhn, PhD, Papachen E. Kolattukudy, PhD, and William L. Smead, MD, FACS	
Distribution of Tenascin in Human Neointimal Hyperplastic Lesions	382
Changyi Chen, MD, Carolyn Suwyn, BS, Samer Mattar, MD, Kellie A. Coyle, MD, David N. Ku, MD, PhD, and Alan B. Lumsden, MB, ChB	

Effects of Chronic Nitric Oxide Inhibition on the Evolution of Chronic Renal Ischemia in Two-Kidney, One-Clip Hypertension385

Hidetomo Nakamoto, MD, Stanley B. Fuller, MD,
David L. Robaczewski, MD, Eric Winicov, MBA,
Carlos M. Ferrario, MD, and Richard H. Dean, MD, FACS

P-selectin Mediates Local Reperfusion Injury After Lower Torso Ischemia389

Martin R. Weiser, MD, Simon A. L. Gibbs, FRCS,
Lester Kobzik, MD, C. Robert Valeri, MD, David Shepro, PhD,
and Herbert B. Hechtman, MD, FACS

Ischemia Is Associated With Increased Expression of the Angiogenic Protein Acidic Fibroblast Growth Factor: Implications for “Biologic” Revascularization392

Todd K. Rosengart, MD, Martin Duenas, BS, Jeffrey Winkles, PhD,
Karl Krieger, MD, and O. Wayne Isom, MD

Role of Platelet-Activating Factor in Skeletal Muscle Ischemia Reperfusion Injury395

John G. Adams, Jr, MD, Animesh Dhar, PhD,
Michael J. Kikta, MD, Shariff Tarazi, MS, and
Donald Silver, MD, FACS

Aspirin Increases Platelet and Arterial Wall Thromboxane A₂ Receptor Density Following Prosthetic Grafting397

Thomas E. Brothers, MD, Jacob G. Robison, MD, FACS,
Bruce M. Elliott, MD, FACS, Janet M. Boggs, LVT, and
Perry V. Halushka, MD, PhD

Neutrophil Activation on ePTFE Surfaces Triggers Platelet Activation399

Daniel Katz, MD, Constantinos Stratoulas, MD,
Beatrice Haimovich, PhD, and Ralph S. Greco, MD, FACS

Chapter VIII—Transplantation

- CTLA4-Ig Induced Transplantation Tolerance: Analysis of Donor Cell Chimerism402
 Diane Z. Alexander, MD, Thomas C. Pearson, MD, DPhil,
 Rose Hendrix, BA, Peter S. Linsley, PhD, and
 Christian P. Larsen, MD, DPhil
- Bone Marrow Stem Cell Engraftment Across Major Histocompatibility Barriers: Radiation Sensitivity of a Novel Facilitating Population in Donor Bone Marrow404
 Christina L. Kaufman, PhD, Yolonda Colson, MD, PhD, and
 Suzanne T. Ildstad, MD
- Viral IL-10 Gene Transfer Prolongs Cardiac Allograft Survival by Promoting T_H2-mediated Suppression407
 Kenneth D. Chavin, MD, PhD, Lihui Qin, MD, Yaozhong Ding, MD,
 Hideaki Tahara, MD, PhD, Paul D. Robbins, PhD,
 Michael T. Lotze, MD, FACS, and
 Jonathan S. Bromberg, MD, PhD, FACS
- Antibodies to the Adhesion Molecule VLA-4 Enhance Small Bowel Allograft Survival in Rats409
 Sayeed Ikramuddin, MD, David Bruch, MS,
 John D. Halverson, MD, FACS, and Diane G. Tice, PhD
- The Intact Thymus Is Necessary to Induce Cardiac Allograft Prolongation With Intravenous Donor Lymphocytes and Cyclosporine411
 Shafqat M. Ahmed, MD, A. Joseph Tector, MD,
 Shu-Xin Zheng, MD, RD, Clarke Forbes, MD, and
 Jean I. Tchervenkov, MD, FRCS(C)
- Molecular Biology of Cardiac Allograft Rejection and Tolerance: Changes in the T-cell Repertoire Induced by Intrathymic Pretreatment With Allogeneic Cells415
 Muhammad Mohiuddin, MD, Zhenya Shen, MD, and
 Verdi J. DiSesa, MD, FACS

MHC-deficient Allograft Rejection by Indirect Antigen Presentation	417
James F. Markmann, MD, PhD, Avinash Bhandoola, MD, PhD, Craig Seidman, MD, Luis Campos, MD, Mark I. Greene, MD, PhD, and Clyde F. Barker, MD	
Effect of CsA on the MHC Class II Autoreactive T-Cell Repertoire	419
Anne C. Fischer, MD, PhD, Peter P. Ruvolo, PhD, Louis Horwitz, MS, and Allan D. Hess, PhD	
Human TCR V β Usage After in vitro Exposure to Porcine Lymphocytes	422
Peter Mattei, MD, and Paul M. Colombani, MD, FACS, FAAP	
Rat T-Cell Receptor Repertoire in Xenogenic Chimeras (Rat \rightarrow Mouse): Evidence for TCR-V β -Specific Deletion of V β 16	426
Minzhi Chen, MD, and Suzanne T. Ildstad, MD	
Studies of Class I and II Antigens, CD4 ⁺ and CD8 ⁺ T Lymphocytes in Allograft and Xenograft Rejection	428
John P. Henretta, MD, Dorian Araneda, RT, Keith Pittman, BS, Judith Thomas, PhD, and Francis T. Thomas, MD, FACS	
Metabolic Function of Long-Term Canine Islet Autografts: Intraperitoneal Unpurified Islets Versus Intraportal Purified Islets	431
David C. Wahoff, MD, Thao M. P. Tran, BS, Lindsey A. Nelson, BA, Basil E. Papalois, MD, PhD, Paul F. Gores, MD, FACS, and David E. R. Sutherland, MD, PhD, FACS	
Islet Transplantation (ITXP) Normalizes Chronic Diabetic Vascular Endothelial Dysfunction	433
Milan Jordan, MD, MPH, Galen Pieper, PhD, Chris Johnson, MD, FACS, Mark Adams, MD, FACS, and Allan Roza, MD, FACS, FRCSC	

Induction of Tolerance by Simultaneous Blockade of Multiple Coreceptors	436
Prabhakar Baliga, MD, Jennifer Woodward, MS, Lihui Qin, MD, Kenneth Chavin, MD, PhD, Peter Linsley, PhD, and Jonathan Bromberg, MD, PhD, FACS	
Donor-Specific Transplantation Unresponsiveness in Sensitized Rats Following Treatment With a Nondepleting Anti-CD4 Monoclonal Antibody	438
Jochen Binder, MD, Mohamed H. Sayegh, MD, Bruno Watschinger, MD, Wayne W. Hancock, MD, PhD, Manfred Lehmann, MD, Hans D. Volk, MD, PhD, and Jerzy W. Kupiec-Weglinski, MD, PhD	
Inhibition of Macrophage Activation Prevents Functional and Morphological Changes of Chronic Rejection in Rat Kidney Allografts	442
Haruhito Azuma, MD, Michio Ishibashi, MD, Christof Schmid, MD, Uwe Heemann, MD, and Nicholas L. Tilney, MD, FACS, FRCS	
The Inoculation of Cytomegalovirus Accelerates Transplant-associated Coronary Atherosclerosis in Heterotopic Heart Allograft Model	445
Nobuhiro Handa, MD, Masataka Hatanaka, MD, Keisuke Ueda, MD, Ryozo Omoto, MD, William A. Baumgartner, MD, FACS, Bruce A. Reitz, MD, FACS, and Ahvie Herskowitz, MD	
Development of Cytotoxic Antibodies Post-Lung Transplantation Correlates With the Development of Bronchiolitis Obliterans Syndrome	447
Sudhir Sundaesan, MD, FRCS(C), Thalachallour Mohanakumar, PhD, Donna Phelan, BA, CHS, Elbert P. Trulock, MD, Neil Ettinger, MD, Mary Pohl, RN, Joel D. Cooper, MD, FACS, and G. Alexander Patterson, MD, FACS	

- Study of the Pathogenesis of Delayed Xenograft Rejection in the Guinea Pig-to-Rat Model450**
 Joseph R. Leventhal, MD, Bradley Berry, MS, Pamela Simone, BS, Augustin P. Dalmaso, MD, R. Morton Bolman, MD, and Arthur J. Matas, MD
- Marked Prolongation of Cardiac Xenograft Survival by Adaptive Transfer of Xenoactivated Spleen Cells452**
 Paul Quarantillo, MD, Dorian Araneda, RT, Emil Cekeda, MD, Tor Ljung, MD, John Henretta, MD, and Frank Thomas, MD
- Long-Term Normalization of Albumin Levels in Nagase Analbuminemic Rats Following Selective Intrahepatic Transplantation of Normal Hepatocytes455**
 Albert D. Moscioni, PhD, Jacek Rozga, MD, PhD, Henri Scott, BS, Eugenio Morsiani, MD, and Achilles A. Demetriou, PhD, MD, FACS
- Glutamine Protects Function and Improves Preservation of Small Bowel Grafts459**
 Kazuaki Sasaki, MD, Wei Zhang, MD, Allison Bain, BA, Kathleen Reilly, MD, William T. Adamson, MD, and John L. Rombeau, MD, FACS
- Burn Injury Selectively Impairs Host Sensitization to Cultured Keratinocyte Allografts461**
 C. Scott Hultman, MD, Bruce A. Cairns, MD, Suzan deSerres, BA, Lisa A. Brady, BS, and Anthony A. Meyer, MD, PhD, FACS

Chapter IX—Surgical Oncology

- Could Glutamine-supplemented Nutrition Adversely Modulate Tumor Biology in Cancer Patients?464
 Gregory A. Turowski, MD, PhD, Zaihan Rashid, MD,
 Fu Hong, MD, and Marc D. Basson, MD, PhD
- Matrix Therapeutic Implant Increases Intratumoral Cisplatin Levels and Enhances Tumorcidal Activity466
 B. Scott Davidson, MD, George M. Fuhrman, MD,
 Zahid H. Siddik, PhD, and Steven A. Curley, MD
- Exogenous Human Growth Hormone Does Not Influence Growth, Protein Kinetics, or Cell Cycle Kinetics of Human Pancreatic Carcinoma in vivo469
 Lawrence E. Harrison, MD, David Blumberg, MD,
 Russell S. Berman, MD, Bruce Ng, MS, Steven Hochwald, MD,
 Murray F. Brennan, MD, and Michael Burt, MD, PhD
- Monocytes Augment Production of Interleukin-6 (IL-6) and Leukemia Inhibitory Factor (LIF) by Human Tumor Cell Lines471
 Kevin G. Billingsley, MD, Gideon Strassmann, PhD,
 W. Scott Arnold, MD, Douglas L. Fraker, MD, and
 H. Richard Alexander, MD, FACS
- Comparative Evaluation of Disseminated Tumor Cells in Bone Marrow of Breast and Gastric Cancer Patients475
 Ilona Funke, MD, Karl W. Jauch, MD, PhD, Stefanie Fries,
 Markus M. Heiss, MD, Friedrich W. Schildberg, MD, Prof
- p21ras-GTP Activation Is Weak in Colon Cancer Cell Lines Containing Aspartate-13 k-ras Mutations477
 Philip B. Paty, MD, Michael D. Lieberman, MD, Gargi Debnath, PhD,
 Sidney D. Finkelstein, MD, Neal Rosen, MD, PhD, and
 Alfred M. Cohen, MD, FACS

Nitric Oxide Attenuates Interleukin-2-induced Lung Injury479
 David Bouchier-Hayes, MB, Hazem Abdih, MB,
 Cathal J. Kelly, FRCSI, M. Barry, FRCSI, H. Paul Redmond, FRCSI,
 Paul E. Burke, FRCSI, W. Watson, BSc, and
 David J. Bouchier-Hayes, FRCSI, FACS

Screening for Mutations of the RET Protooncogene in Men 2A
 Families480
 Andrea Frilling, MD, PhD, Wolfgang Höppner, PhD,
 Anya Stenger, MD, and Christoph E. Broelsch, MD, FACS

Prognostic Value of New Functional Risk Factors in Gastric Cancer
 Defined by the Urokinase-Type Plasminogen Activator System482
 Mark M. Heiss, MD, Rudolf Babic, MD, Heike Allgayer,
 Uwe Gruetzner, Karl-Walter Jauch, MD, Udo Loehrs, MD, and
 Friedrich W. Schildberg, MD

Permissive Tumor Growth After Laparotomy Versus Laparoscopy Is
 Associated With Altered TNF Levels486
 Marc Bessler, MD, John D. F. Allendorf, BA, Jerome D. Chao, AB,
 Scott D. Oesterling, BS, Mark L. Kayton, MD, Michael R. Treat, MD,
 Roman Nowygrod, MD, and Richard L. Whelan, MD

In vivo Fast Fourier Transform Analysis of the Autofluorescence of
 Normal and Neoplastic Upper Aerodigestive Mucosa488
 Dido Franceschi, MD, Joydeep Haldar, MD, Robert Alfano, PhD,
 Howard E. Savage, PhD, and Stimson Schantz, MD

Interleukin-12 (IL-12) Induces Specific Antitumor Immunity in Animals
 Bearing Established Subcutaneous Murine Sarcoma492
 Chet L. Nastala, MD, Thomas J. McKinney, BA,
 Howard D. Edington, MD, Hideaki Tahara, MD, PhD,
 Walter J. Storkus, PhD, and Michael T. Lotze, MD, FACS

Gene Therapy in Malignant Mesothelioma495
 Daphne J. Y. Mew, MD, PhD, FRCS, Mary Jo Mulligan-Kehoe, PhD,
 and Harvey I. Pass, MD, FACS

- Significant Survival Benefit Following Adenovirus-mediated Gene Therapy for Experimental Human Mesothelioma499**
 W. Roy Smythe, MD, Harry C. Hwang, BS, Kunjlata M. Amin, PhD, James M. Wilson, MD, PhD, Steven M. Albelda, MD, and Larry R. Kaiser, MD, FACS
- Antisense to the Focal Adhesion Kinase Gene Disrupts Human Sarcoma Growth and Adhesion501**
 Lewis V. Owens, MD, Tim Weiner, MD, LiHui Xu, MD, Glenn C. Sturge, BS, Edison T. Liu, MD, and William G. Cance, MD
- MDR-1 Expression in Soft Tissue Sarcoma503**
 Edward A. Levine, MD, Igor B. Roninson, PhD, Dong K. Kim, PhD, Christian Bolliger, BS, Sarah Bacus, PhD, and Tapas K. Das Gupta, MD, PhD
- Chromosomal Deletions in Early Stages of Tumor Progression: Search for a Novel Tumor Suppressor or Angiogenesis Suppressor Gene505**
 Sareh Parangi, MD, Eric H. Radany, MD, PhD, Gerhard Christofori, PhD, William Dietrich, PhD, Eric S. Lander, PhD, Judah Folkman, MD, FACS, and Douglas Hanahan, PhD
- A Functional Cadherin/Catenin Complex Is Associated With a Decrease in Colon Carcinoma Cell Proliferation507**
 Elizabeth Breen, MD, Glenn Steele, Jr, MD, PhD, and Arthur M. Mercurio, PhD
- A Rapid Presymptomatic Genetic Test for the Detection of Mutations in the RET Protooncogene Associated With Men 2A and FMTC510**
 David D. Chi, MD, Shenshen Dou, MS, Katrin Carlson, BS, Koji Toshima, MD, Helen Donis-Keller, PhD, and Samuel A. Wells, Jr, MD, FACS

- Changes in Host T-Cell Concentrations but Not in Donor TIL Concentrations at the Tumor Site Following Adoptive Immunotherapy513
 Ulrike L. Burger, MD, Maximilian P. Chang, BA,
 Peter S. Goedegebuure, PhD, and Timothy J. Eberlein, MD, FACS
- p21Ras and MAP Kinase Respond to Epidermal Growth Factor (EGF) Stimulation in Pancreatic Adenocarcinoma Cell Lines516
 Michael D. Lieberman, MD, Benjamin O. Anderson, MD,
 Gargi Debnath, PhD, Sidney Finkelstein, PhD,
 Neal Rosen, MD, PhD, Murray F. Brennan, MD, FACS, and
 Philip Paty, MD
- Cytokine Gene Expression in Metastatic Melanoma519
 Markus Zuber, MD, Urs Luescher, MD, Giulio G. Spagnoli, MD,
 Luis Filgueira, MD, Antonio Juretic, MD, PhD, Thomas Kocher, MD,
 Elke Schultz, MTA, Verena Caetano, MTA,
 Felix Harder, MD, FACS, and Michael Heberer, MD
- Application of the Polymerase Chain Reaction to Study Phenotype Expression in an in vitro Model of Colonic Tumor Cell Differentiation521
 Julio Faria, MD, CM, Judith L. Trudel, MD, MSc, FRCSC, FACS,
 and Gary E. Wild, MD, CM, PhD, FRCPC
- Induction of Chemosensitivity in Human Cancer Cells in vivo by Adenovirus-mediated Transfer of the Wild-type p53 Gene524
 Toshiyoshi Fujiwara, MD, PhD, Elizabeth A. Grimm, PhD,
 Tapas Mukhopadhyay, PhD, Wei Wei Zhang, MD, PhD,
 Laurie B. Owen-Schaub, PhD, and Jack A. Roth, MD
- Role of Interferon- α (IFN- α) in the Active Specific Immunotherapy of Vaccinia Colon Oncolysate Plus IFN- α 527
 Nobuyuki Tanaka, MD, PhD, M. Sivanandham, PhD, and
 Marc K. Wallack, MD, FACS

- Adeno-associated Virus Plasmid: Cationic Liposomal-mediated Gene Transfer Results in Significant Cytokine Gene Expression in Human Tumor Cells Following Lethal Irradiation530
 Bryan Clary, MD, Johannes Vieweg, MD, Ramila Phillip, PhD, Eamonn Coveney, MD, FRCSI, J. Michael DiMaio, MD, Eli Gilboa, PhD, and H. Kim Lyerly, MD
- IV Recombinant Vaccinia Virus Preferentially Infects Murine Pancreas Cancer, Produces Interleukin-1 β , and Inhibits Tumor Growth534
 Gary R. Peplinski, MD, Kangla Tsung, PhD, Jennifer B. Meko, MD, Eric D. Whitman, MD, and Jeffrey A. Norton, MD, FACS
- Mitogenic Basis of Gene Therapy for Hepatic Metastases537
 Deepak Narayan, MD, Daniel Herz, MD, Sara Hougen, MD, and Thanjavur S. Ravikumar, MD, FACS
- Inhibition of Breast Cancer Metastasis by Cytokine Gene-modified Tumor Vaccination in Tumor-bearing Mice540
 Eamonn Coveney, MD, FRCSI, Bryan Clary, MD, J. Michael DiMaio, MD, Johannes Vieweg, MD, Eli Gilboa, PhD, and H. Kim Lyerly, MD
- A Proposed Mechanism for Estrogen-induced Pulmonary Metastases in Estrogen Receptor-negative Human Breast Cancer543
 Anthony Joseph Palazzo, MD, Kevin Craig Marler, MD, Tonglia Jia, MD, Kambiz Jahadi, MD, Carol Ann Flanagan, BS, and Michael Allen Schwalke, MD
- Growth Inhibition by Transforming Growth Factor β (TGF β): Role of the Retinoblastoma Protein (pRB) and the TGF β Receptor545
 Maria Frexes-Steed, MD, PhD, Agnes Gorska, BS, Karl Munger, PhD, and Harold L. Moses, MD

Effects of Human Fibrosarcoma Tumor Supernatant on Endothelial Cells (EC): Induction of Tissue Factor (TF) Expression and Augmented Expression of Adhesion Molecules in Response to Tumor Necrosis Factor (TNF)548

W. Scott Arnold, MD, Susan H. Tannenbaum, MD,
H. Richard Alexander, MD, FACS, and Douglas L. Fraker, MD

Production of Metalloproteinase-2 (MMP-2) in Metastatic Colorectal Cancer (CRC) Is Stromal (Fibroblastic) in Origin551

Masaru Kuranami, MD, PhD, Alfred M. Cohen, MD, FACS, and
Jose G. Guillem, MD, MPH

Inhibition of Nitric Oxide Synthesis and Alteration in T-helper Cytokine Pattern Induced by B16 Melanoma554

Hassan A. Naama, FRCSI, Alex Cech, MD, Sun Yong Lee, MD,
Arnold Hill, FRCSI, Linda Callans, MD, and
John M. Daly, MD, FACS

Hepatic Pyruvate Carboxylase Activity Is Increased in Tumor Hosts556

Katherine J. M. Liu, MD, FACS, Yuying C. Hwang, PhD,
Jehad Jarad, BS, and Rong-Ping Yang, PhD

Human Carcinogenesis in SCID/Human Chimeras: A Cutaneous Model559

Peter W. Soballe, MD, FACS, John M. Daly, MD, FACS, and
Meenhard Herlyn, DVM, DSci

Chapter X—Gynecology/Obstetrics

A Model of Amniotic Band Syndrome in Fetal Lambs562

Kathryn Dirkes, MD, Timothy M. Crombleholme, MD,
Timothy M. Whitney, MD, Sara H. Garmel, MD,
Benjamin A. Alman, MD, and Raymond J. Connolly, PhD

- Ovarian and Breast Cancer Express Shared Antigenic Peptides
Recognized by Tumor-specific Cytotoxic T Lymphocytes565
George E. Peoples, MD, Roy C. Smith, PhD,
David C. Linehan, MD, Peter S. Goedegebuure, PhD, and
Timothy J. Eberlein, MD, FACS
- Ovarian and Breast Tumor-associated T Lymphocytes Stimulated
With an Antigenic Peptide Derived from HER2/*neu* Show Enhanced
Cytotoxicity Against Autologous Tumor568
David C. Linehan, MD, George E. Peoples, MD, Akhil S. Parikh, MD,
Peter S. Goedegebuure, PhD, and Timothy J. Eberlein, MD, FACS
- Dexamethasone Inhibits Trophoblast Production of Cytokines570
Michael E. Cunningham, MD, Marvin A. McMillen, MD, FACS, and
Harvey J. Kliman, MD, PhD
- Restoration of “Tired” Neutrophil Antimicrobial Function by Aprotinin
Is Dependent on the Presence of Taurine573
H. Paul Redmond, FRCSI, Jiang Huai Wang, PhD,
R. William G. Watson, BSc, and David Bouchier-Hayes, FRCSI
- Uterine Tone: Does Nitric Oxide Play a Role?575
Andrea P. Metkus, MD, and Michael R. Harrison, MD, FACS

Chapter XI—Neurologic Surgery

- 8-Isoprostane Production in Normal and Transformed Glia: Assessment of
Oxidative Activity577
Timothy C. Ryken, MD, Paul D. Sawin, MD, Terrence C. Runge, BS,
and Vincent C. Traynelis, MD

- Neurologic and Histopathologic Outcome in a Highly Reproducible Model of Secondary Spinal Cord Injury in the Rat579
Christopher B. Dechet, Simcha J. Weller, MD, Larry I. Benowitz, PhD, and Eugene Rossitch, Jr, MD
- Cerebral Endothelial Cell Production of TNF- α and IL-1 β After Percussive Trauma581
Christine G. Gourin, MD, Steven R. Shackford, MD, FACS, and Marie A. Shatos, PhD
- Changes in Intracranial Pressure During Carbon Dioxide Pneumoperitoneum in Normovolemic and Hypovolemic Animals583
José L. Mijangos, MD, MSc, Nyein Thwin, DVM, E. John Hinchey, MD, MSc, FRCSC, FACS, and Christopher M. Oung, MD, MSc, FRCSC
- A New Animal Model for Myelomeningocele: Studies in the Fetal Sheep587
Martin Meuli, MD, Claudia Meuli-Simmen, MD, Charles D. Yingling, PhD, Kathleen McBiles Hoffman, DVM, Michael R. Harrison, MD, FACS, and N. Scott Adzick, MD, FACS
- TGF- β 1, - β 2, - β 3, and IGF-1 Localization in Rat Cranial Suture Development and Fusion589
Michael T. Longaker, MD, Douglas A. Roth, MD, Heather F. McMullen, MD, Arnold S. Breitbart, MD, Jeffrey H. Wisoff, MD, Victor K. Han, MD, Leslie I. Gold, PhD, and Joseph G. McCarthy, MD, FACS
- A Simplified in vitro Assay of Cellular Invasiveness591
William C. Welch, MD, Paul L. Kornblith, MD, Ruth Zolock, BS, and Ronald H. Goldfarb, PhD
- Comparative Evaluation of Suture and Non-penetrating Metal Clips for Vascular Reconstruction593
Yong Hua Zhu, MD, Robert Steckel, DVM, and Wolff M. Kirsch, MD, FACS

Temporary Occlusion Time Relative to Outcome in Patients With Giant Intracerebral Aneurysms	598
Richard D. Fessler, MD, Robert R. Johnson II, MD, and Fernando G. Diaz, MD, PhD	
Aminoguanidine Attenuates Focal Cerebral Ischemia	600
Gary A. Zimmerman, MD, Ona Bloom, BA, Malcolm Meistrell, BA, David Ford, MD, Marina Bianchi, MD, and Kevin J. Tracey, MD	
The Effect of Ammonium Sulfate Injection on Peripheral Nerves in Rats of Varying Ages	603
M. Catherine Hertl, MD, Jun Kobayashi, MD, Patricia K. Hagberg, MS, Jacob C. Langer, MD, and Susan E. Mackinnon, MD, FACS	
The Effect of Axoplasmic Fluid on Regeneration of Conventionally Repaired Peripheral Nerves	606
Ferit Demirkan, MD, Clifford C. Snyder, MD, Osman Latifoglu, MD, and Maria Siemionow, MD, PhD, DSc	

Chapter XII—Orthopaedic

Endoscopic Versus Open Carpal Tunnel Release: A Retrospective Review	609
John H. Chrostowski, MD, Scott W. Wolfe, MD, and Richard Bernstein, MD	
Matched Hemiresection Interposition Arthroplasty of the Distal Radioulnar Joint	612
David M. W. Pugh, MD, Gregory I. Bain, MBBS, FRACS, Joy MacDermid, BSc(Pt), MSc, and James H. Roth, MD, FACS, FRCSC	

Arthroscopy in Distal Radial Fractures614
 Robert S. Richards, MD, FRCSC,
 James H. Roth, MD, FACS, FRCSC, and
 John D. Bennett, MD, CM, FRCPC

Verapamil Protects Postischemic Skeletal Muscle Function but Does Not
 Preserve High Energy Phosphates616
 Paul-Martin Sutter, MD, Axel Marx, MD, Lorenz Gürke, MD,
 Jonas Landmann, MD, Felix Harder, MD, FACS, and
 Michael Heberer, MD

Flurbiprofen as an Inhibitor of Reactive Fibrous Membrane Surrounding
 Joint Arthroplasty Components619
 Paul G. Perona, MD, Frederick H. Wezeman, PhD, and
 Terry Light, MD

The Effect of Calcitonin Gene-related Peptide on Reperfusion
 Injury621
 Diane M. Allen, BS, Long-En Chen, MD, Anthony V. Seaber, and
 James R. Urbaniak, MD

Meniscal Fibrochondrocytes: In vitro Characterization in a Polymer
 Scaffold, Response to Cytokines, and the Potential for Meniscal
 Repair/Grafting624
 William Ertl, MD, Daniel Grande, PhD, Robert Schwartz, MD,
 Ryhana Manji, BA, and Carl Paulino, BS

The Extracellular Matrix as a Component of Chondrocyte Regulatory
 Milieu627
 Wen-Ning Qi, MD, Edwin C. Bryson, BA, Alison P. Toth, BS, and
 Sean P. Scully, MD, PhD

Force Deficit After Skeletal Muscle Reinnervation630
 William M. Kuzon, Jr, MD, PhD, FRCS(C), Hirotaka Asato, MD,
 and Mohammad Khaled H. Youssef, MD

- Complications of the Gamma Nail Osteosynthesis in Per- and Subtrochanteric Femur Fractures. Consequences for the Development of a New Implant632
 Wilhelm Friedl, MD, FACS, and Christian Fritz, MD
- Pulse Oximetry in the General Surgical Ward—Is It Worthwhile?637
 Jacob Rosenberg, MD, Jørgen Koch, MD, and
 René Kirchhoff-Jensen, MD
- Nutritional Assessment in Total Joint Patients639
 Brian J. Daley, MD, Michael Stecker, MD,
 James Bordley IV, MD, FACS, and
 Michael Ries, MD, FAAOS

Chapter XIII—Otorhinolaryngology

- Effects of Sleep Apnea Treatment Upon Enuresis in Children643
 Robert C. Wang, MD, FACS, and Jonathan Vordemark, MD, FACS
- Endoscopic Transethmoid Approach for Medial Orbital Masses645
 R. James Koch, MD, MS, and
 George T. Simpson II, MD, MPH, FACS
- The Role of the p53 Tumor Suppressor Gene in Head and Neck Carcinogenesis647
 Eric J. Lentsch, MD, and Fred J. Hendler, MD, PhD
- Structural Stability and Airway Patency Following Extensive Laryngotracheal Reconstruction by Collagen/Hydroxyapatite-injected Nonskeletal Myocutaneous Flaps649
 Frank A. Papay, MD, and Isaac Eliachar, MD

Localization of TGF- β Isoforms in Adult and Fetal Mouse Lip Wounds	651
David J. Whitby, MD, Heather F. McMullen, MD, Joann J. Sung, BS, Leslie I. Gold, PhD, John W. Siebert, MD, and Michael T. Longaker, MD	
Patterns of Island Flap Survival	654
Michael F. Angel, MD, Srdan Babovic, MD, Andrew M. Ress, MD, Michael J. Im, PhD, and Paul N. Manson, MD, FACS	

Chapter XIV—Pediatric Surgery

Cyclic GMP Relaxes the Internal Anal Sphincter in Hirschsprung's Disease	657
Karen J. VanderWall, MD, John F. Bealer, MD, N. Scott Adzick, MD, FACS, and Michael R. Harrison, MD, FACS	
Species-specific in situ Hybridization Demonstrates That the Human Fetal Fibroblast Modulates Scarless Repair	659
Richard Y. Lin, BS, Kerry M. Sullivan, MD, Peter A. Argenta, BS, H. Peter Lorenz, MD, and N. Scott Adzick, MD, FACS	
An Adult-Fetal Skin Interface Heals Without Scar Formation in Sheep	662
Kerry M. Sullivan, MD, Martin Meuli, MD, Thomas E. MacGillivray, MD, and N. Scott Adzick, MD, FACS	
Lung Liquid Production in Fetal Sheep With Diaphragmatic Hernia	664
Elizabeth A. Beierle, MD, Max R. Langham, Jr, MD, FACS, and Sidney S. Cassin, PhD	

- Lung Liquid From Fetal Lambs With Tracheal Occlusion Is Mitogenic to Pulmonary Cells in vitro666
 John W. DiFiore, MD, and Jay M. Wilson, MD, FACS
- Neutrophil Infiltration Is Reduced During Partial Perfluorocarbon Liquid Ventilation in the Setting of Lung Injury668
 Danny M. Colton, MD, Ronald B. Hirschl, MD,
 Kent J. Johnson, MD, FACP, Gerd O. Till, MD, Shay B. Dean, BS,
 and Robert H. Bartlett, MD, FACS
- Nitric Oxide Reverses Elevated Pulmonary Vascular Resistance After Oleic Acid Injury in Neonatal Piglet Lungs Perfused by Extra Corporeal Membrane Oxygenation (ECMO)671
 Samuel Weinstein, MD, Eric L. Lazar, MD, Evan C. Lipsitz, MD,
 Marilyn Butler, MD, Arthur Smerling, MD, and
 Charles J. H. Stolar, MD, FACS
- Minimal Drug Resistance After Prolonged Antiangiogenic Therapy With AGM-1470674
 Harold Brem, MD, Fumimasa Goto, MD, PhD, Andrew Budson, MD,
 Lawrence Saunders, BA, and Judah Folkman, MD, FACS
- Defective Nonessential Amino Acid Synthesis in Premature Infants ..677
 Ronna G. Miller, MD, Farook Jahoor, PhD, and
 Tom Jaksic, MD, PhD, FRCS(C)
- Taurine Up-regulates Antimicrobial Function of Human Inflammatory Cells Through a Calcium-dependent Mechanism679
 R. William G. Watson, BSc, H. Paul Redmond, FRCSI, and
 David Bouchier-Hayes, FRCSI
- Are DA₁ Dopamine Receptors Functional in Newborn Piglet Mesenteric or Renal Vasculature?682
 R. James Pearson, MD, BSc,
 Dennis W. Jirsch, MD, MSc, PhD, FRCSC,
 Keith J. Barrington, MB, ChB, FRCPC, and
 Po-Yin Cheung, MBBS

Antisense to *c-myb* Inhibits Neointimal Hyperplasia in Vein Grafts ...684
 Alexander D. Soutter, MD, and Judah Folkman, MD, FACS

Chapter XV—Plastic Surgery/Wound Healing

Regulation of Fibroblast Protein Synthetic Activity in the Wound Environment688
 Mark C. Regan, MD, FRCSI, Stephen J. Kirk, MD, FRCSI,
 Moira Hurson, MPhil, David R. Holt, MD, Hannah L. Wasserkrug, BA,
 and Adrian Barbul, MD, FACS

Production of Nitric Oxide from Citrulline by Wound-derived Macrophages690
 Romeo B. Mateo, MD, and Jorge E. Albina, MD

Hyaluronan and Inhibition of Granulation Tissue Deposition692
 James H. Blackburn II, MD, Christopher J. Blewett, MD,
 Robert E. Cilley, MD, FACS, Peter W. Dillon, MD, FACS,
 H. Paul Ehrlich, PhD, and Thomas M. Krummel, MD, FACS

Inhibition of Wound Contraction Using Colchicine and Penicillamine Applied Locally694
 Hazel L. Joseph, BS, Gary L. Anderson, PhD,
 John H. Barker, MD, PhD, Leonard J. Weiner, MD,
 Fred J. Roisen, PhD, and Gordon R. Tobin, MD, FACS

Hyperglycemia-induced Abnormal Nitric Oxide (NO) Production by Vascular Endothelial Cells: Implications in Diabetic Wound Healing ..697
 Prasad V. Gade, MD, Tai-Lan Tuan, PhD, Marcel Nimni, PhD,
 Bill Subin, MD, and Anthony Sank, MD

- EGF Ameliorates Doxorubicin-mediated Inhibition of DNA Synthesis in a Human Skin Explant Model699
 Faiz Y. Bhora, MD, Shmuel Batzri, PhD, Brian J. Dunkin, MD,
 B. L. Bass, MD, FACS, and John W. Harmon, MD, FACS
- Topical Diphenylhydantoin Increases Wound Tensile Strength and Collagen Deposition702
 M. L. Da Costa, MB, M. C. Regan, FRCSI, and
 D. Bouchier-Hayes, FRCSI
- Hyaluronic Acid Synthesis in Keloid Fibroblasts Before and After Administration of Triamcinolone704
 Samuel M. Alaish, MD, Dorne R. Yager, PhD,
 Robert F. Diegelmann, PhD, and I. Kelman Cohen, MD, FACS
- Cellular and Molecular Pathogenesis of Defective Healing in Diabetes Mellitus707
 Bill Subin, MD, Tai-Lan Tuan, PhD, David Cheung, PhD,
 Marcel Nimni, PhD, Prasad Gade, MD, and Anthony Sank, MD
- Growth Factors and Cell Protein Concentration in Cultured Diabetic Rat Fibroblasts709
 Masayuki Yamamoto, MD, Richard H. C. Harries, MD,
 Francis Ko, BS, Martin C. Robson, MD, FACS, and
 Linda G. Phillips, MD, FACS
- Increased Expression of Insulin-like Growth Factor-I Receptors Occurs During Wound Healing by Secondary Intention711
 Brian F. Lane, MD, Douglas A. Hale, MD, FACS,
 Mark Molloy, MD, FACS, John Bilello, MD, and Shmuel Batzri, PhD
- Retinoic Acid Restores Transforming Growth Factor- β 1 Concentrations in a Steroid-impaired Wound-healing Model714
 Anders E. Ulland, MD, Madeline H. Gartner, MD,
 John R. Richards, MS, and Michael D. Caldwell, MD, PhD, FACS

- Improved Sciatic Nerve Regeneration in Rat Hindlimb Allografts
 Immunosuppressed With RS-61443717
 Prosper Benhaim, MD, James P. Anthony, MD, Jon C. Lewis, PhD,
 and Stephen J. Mathes, MD, FACS
- The Use of "Sliding Epineurial Sheath Tube" for Repair of Peripheral
 Nerve Defects719
 Kenan Atabay, MD, Chull Hong, MD, Brian V. Heil, BS,
 Michael L. Bentz, MD, and J. William Futrell, MD, FACS
- Microcirculatory Changes Associated With Standardized Electrical Injury
 of Skeletal Muscle in a Rat Gracilis Muscle Model723
 Jurgen Hussmann, MD, William A. Zamboni, MD,
 John O. Kucan, MD, FACS, Robert C. Russell, MD, FACS, FRACS,
 Hans Suchy, BS, and Allan Roth, PhD
- Effect of WEB 2170, a Platelet-activating Factor Antagonist, on Skin
 Flap Survival in the Rat725
 Laura K. Knox, MD, Alastair G. Stewart, PhD,
 Wayne A. Morrison, MD, FACS, FRACS, and
 Peter G. Hayward, MD, FACS
- Basic Fibroblast Growth Factor Improves the Survival of Random Skin
 Flaps728
 Athan Roumanas, MD, MS, and D. Byron McGregor, MD
- T-cell Response in Silicone Gel Breast Implant Capsules730
 Daniel A. Ladin, MD, Ghassan M. Saed, PhD, and
 David P. Fivenson, MD
- Age-dependent Proliferation, Gene Expression, and Growth Factor
 Production in Cultured Calvarial Osteoblasts732
 Richard A. Bartlett, MD, Franz Josef Kramer, BS,
 Ernesto Canalis, PhD, and Louis Gerstenfeld, PhD

- The Analysis of TGF- β 1, - β 2, - β 3 and IGF-1 in Premature Cranial Suture Fusion in Humans734**
 Douglas A. Roth, MD, Michael T. Longaker, MD,
 Victor K. Han, MD, Arnold S. Breitbart, MD, Leslie I. Gold, PhD,
 and Joseph G. McCarthy, MD
- Ex vivo Evaluation of an Implantable and a Percutaneous Mandibular Distractor Complex in the Canine Model737**
 Steven I. Reger, PhD, Frank A. Papay, MD, James E. Zins, MD, and
 Steven G. Sanderson, MS
- The Passive Intracranial Translocation of Microplates and Screws—An Experimental Model Using Infant Pigs739**
 Jack C. Yu, MD, DMD, David S. Goldberg, MD,
 Scott P. Bartlett, MD, FACS, and Linton A. Whitaker, MD, FACS
- The “Fetal” Macrophage and Its Response to Hypoxia743**
 Lisa B. Bootstaylor, MD, Thomas K. Hunt, MD, and
 Michael J. Banda, PhD
- Particle-mediated DNA Transfer With EGF-plasmid Promotes Wound Healing746**
 Christoph Andree, MD, Curtis Page, MS, Jaromir Slama, MS,
 Dimitrios Hatzis, MD, PhD, William F. Swain, PhD, and
 Elof Eriksson, MD, PhD, FACS
- The Effect of PDGF-AB on Interstitial Collagenase and Gelatinase ..749**
 Lisa J. Gould, MD, PhD, Robert F. Diegelmann, PhD,
 Wolfgang Meyer-Ingold, PhD, Andreas Albrod, MD, and
 I. K. Cohen, MD, FACS
- In vivo Analysis of TGF- β Isoforms Injected Individually and in Combinations751**
 Karyn S. Bouhana, BS, Michael T. Longaker, MD,
 Michael J. Banda, PhD, Anita B. Roberts, PhD, H. Peter Lorenz, MD,
 David J. Whitby, MD, James A. Weatherbee, PhD, and
 Harold F. Dvorak, MD

- Hyaluronidase Activity in Fetal and Adult Wounds753**
 David C. West, PhD, David M. Shaw, BS, H. Peter Lorenz, MD,
 N. Scott Adzick, MD, FACS, and Michael T. Longaker, MD
- Fetal Pig Platelets Contain Lower Levels of TGF- β 1, TGF- β 2, and PDGF-AB: A Possible Explanation for Reduced Scarring in the Fetus755**
 Oluyinka O. Olutoye, MD, Dorne R. Yager, PhD,
 Robert F. Diegelmann, PhD, and I. Kelman Cohen, MD, FACS
- Suspension Organ Culture: Comparison of Human Fetal and Adult Wound Repair757**
 Nicole S. Gibran, MD, Lynne T. Smith, PhD,
 Richard P. Rand, MD, FACS, David M. Heimbach, MD, FACS,
 and Karen A. Holbrook, PhD
- Endothelin-1 Levels Increase in Burn Patients Concurrent With Prostaglandin E₂761**
 Marsel Huribal, MD, Michael E. Cunningham, MD,
 Michael L. D'Aiuto, MD, FACS, Walter E. Pleban, MD, FACS,
 and Marvin A. McMillen, MD, FACS
- The Importance of Epithelial–Mesenchymal Interactions: Stimulation of Fibroblasts by Hypoxic, KGF-treated Keratinocytes764**
 Liancun Wu, MD, Jeffrey S. Rubin, MD, PhD, Robert D. Galiano, BA,
 Glenn F. Pierce, MD, PhD, and Thomas A. Mustoe, MD, FACS
- Full-thickness Porcine Wounds Produce Keratinocyte Growth Factor (KGF) and Additional Topical KGF Does Not Accelerate Healing in vivo767**
 Jaromir Slama, MS, Christoph Andree, MD, Norvin Perez, BS,
 Curtis Page, BA, Dimitrios Hatzis, MD, PhD, Julia Tseng, BS, MS,
 Dimitry M. Danilenko, DVM, PhD, and Elof Eriksson, MD, PhD, FACS

- The Effect of Graft Thickness on Return of Functional Sensation in Rat Split-thickness Skin Grafts770
 W. G. Williams, MD, B. P. Rogers, RN, PhD,
 D. N. Herndon, MD, FACS, and L. G. Phillips, MD, FACS
- Delayed Wound Healing Is Associated With Enhanced Collagen Degradation in Ischemic Rat Flap Wounds772
 Daniel A. Schwarz, MD, William J. Lindblad, PhD,
 Belinda Adamson, MEd, and Riley S. Rees, MD
- The Use of Gene Transfer of Viral IL-10 to Facilitate Allogeneic Skin Transplantation776
 Christoph Höhnke, MD, Chet L. Nastala, MD,
 Hideaki Tahara, MD, PhD, Tadamichi Suzuki, MD, PhD,
 Howard D. Edington, MD, and Michael T. Lotze, MD

Chapter XVI—Urology/Reproductive Biology

- Biochemical, Immunocytochemical, and Molecular Characteristics of Human Testis and Sperm Galactosyl Receptor780
 Erik T. Goluboff, MD, James Mertz, PhD, Laura L. Tres, PhD, and
 Abraham L. Kierszenbaum, MD, PhD
- Successful Surgical Treatment of Cryptorchidism in the Long-Evans Cryptorchid Rat Model785
 James Lugg, MD, Farshid Sadeghi, BA, Andrew Freedman, MD,
 Néstor González-Cadavid, PhD, and Jacob Rajfer, MD
- Maturation Response of Normal Human Urothelial Cells in Culture Is Dependent on Extracellular Matrix and Serum Additives786
 Matthew S. Tobin, MD, Michael R. Freeman, PhD, and
 Anthony Atala, MD

- Basic Fibroblast Growth Factor Is a Wilms' Tumor Marker789**
 Peter A. Argenta, BS, Richard Y. Lin, BS, Kerry M. Sullivan, MD,
 and N. Scott Adzick, MD, FACS
- The Use of Rhein to Augment Fluid and Electrolyte Secretion in the
 Continent Jejunum Reservoir791**
 Debora K. Hade, MD, James F. Donovan, Jr, MD,
 Eugene D. Kwon, MD, John B. Stokes, MD, Michael P. Bertholf, BS,
 Michael J. Flanigan, MD, and Harold P. Schedl, MD
- Renal Dysfunction and Tubular Apoptosis in a Rat Pneumoperitoneum
 Model794**
 Andrew J. Kirsch, MD, Terry W. Hensle, MD, FACS, FAAP,
 David T. Chang, MD, Mark L. Kayton, MD,
 Carl A. Olsson, MD, FACS, and Ihor S. Sawczuk, MD, FACS
- Loss of Heterozygosity at Chromosomal Locus 7q22 in Metastatic
 Prostate Cancer797**
 Daniel Lee Watson, MD, Robert Mashal, MD, Krishna Krithivas,
 Christopher Corless, MD, Philip Kantoff, MD, and
 Jeffrey Sklar, MD, PhD
- Specific Sequences of Fibronectin Activate Protein Kinase C Signal
 Transduction Pathway in Invasive Bladder Cancer: Implications for
 Novel Therapeutic Modalities800**
 Eric J. Margolis, MD, Jimmy C. S. Choi, BS, Wei-Ping Shu, MS,
 Michael J. Droller, MD, FACS, and Brian C.-S. Liu, PhD
- The Prostate-specific Antigen Promoter Permits Androgen-mediated
 Expression of Transfected Genes in Prostate Cancer Cell Line
 LNCaP802**
 Samir S. Taneja, MD, Kambiz Dardashti, Arie Belldegrun, MD, FACS,
 and Shen Pang, PhD
- Chromosome 9 LOH Analysis in Transitional Cell Carcinoma805**
 S. Bruce Malkowicz, MD, Lee Pressler, MD, Shira Robbins, BS,
 Alan J. Wein, MD, FACS, and Alban J. Linnenbach, PhD

- Accelerated Growth of Orthotopically Placed Prostate Tumors809
Peter Bridges, MD, Michael Shaw, MS, Marvin Rubenstein, PhD, and
Patrick Guinan, MD, FACS
- Alterations of Invasive Behaviors in Prostatic Adenocarcinoma Cell Lines
by *N*-(4-Hydroxyphenyl)retinamide811
Jae H. Kim, MD, Tetsuyuki Tanabe, MD, Gerald W. Chodak, MD,
and Daniel B. Rukstalis, MD
- Interstitial Photodynamic Therapy for the Treatment of Prostate Cancer:
A Canine Feasibility Study815
Sugandh D. Shetty, MD, FRCS, Qun Chen, PhD, Larry T. Sirls, MD,
Fred Hetzel, PhD, and Joseph C. Cerny, MD, FACS
- Increased Detection of Prostate Cancer Using MRI-guided Transrectal
Ultrasound Biopsies817
Allan J. Pantuck, MD, Michael R. Lobis, MD, Joseph G. Barone, MD,
Reuben S. Mezhich, MD, PhD, and Kenneth B. Cummings, MD, FACS
- Evaluation of the Need for Staging Pelvic Lymphadenectomy for
Clinically Localized Prostate Cancer: A Retrospective Multivariate
Analysis of 303 Consecutive Patients819
Madhu Alagiri, MD, Marc D. Colton, MD, E. James Seidmon, MD,
Richard E. Greenberg, MD, and Philip M. Hanno, MD

HEMOFILTRATION IN HUMAN SEPSIS: EVIDENCE FOR ELIMINATION OF IMMUNOMODULATORY SUBSTANCES

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Eugen Faist, MD, Wolfgang H. Hartl, MD,
Marianne Jochum, PhD, and Dietrich Inthorn, MD

CONTINUOUS HEMOFILTRATION (HF) is widely used for renal replacement therapy in patients who develop kidney failure as part of a multiple organ dysfunction syndrome (MODS). In these patients we demonstrated a correlation between the daily amount of ultrafiltrate and the survival rate.¹ It has been suggested that HF may eliminate toxic mediators of MODS.² The present study examined whether HF can activate or eliminate established mediators in patients with septic MODS. Because the exact nature of the factors removed remains unclear, it appeared feasible to evaluate the biological effect of ultrafiltrate by exposing it to several white blood cell subfractions *in vitro*.

MATERIALS AND METHODS

Continuous isovolemic veno-venous HF was performed using a fiber hemofilter (FH 66, Gambro, Hechingen, FRG) and a flow-controlled roller pump (filtration rate: 2 L/min) in 16 patients with septic MODS (Elebute and Stoner³ scorepoints ≥ 20) and in five healthy volunteers. Pre- and post-filter and ultrafiltrate concentrations of cytokines (interleukin [IL]-1 β , IL-6, IL-8, tumor necrosis factor [TNF]- α) and of complement compounds (C3, C3a, C5a, C5b-9) were measured shortly after the beginning of HF (t_0) and 60 minutes later (t_{60}). Healthy peripheral blood mononuclear cells (PBMC) and lymphocytes were incubated with ultrafiltrate and stimulated with endotoxin and phytohemagglutinin (PHA), respectively. Cell function was determined by measuring concentrations of several released cytokines in the supernatants and by PHA (0.5 $\mu\text{g}/\text{mL}$)-induced ³H-thymidine uptake. Isotonic saline solution (NaCl) served as control. All data are expressed as

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Table 1—TNF, IL-6, and IL-1 release of human peripheral blood mononuclear cells (PBMC) and IL-2 and IL-6 release of lymphocytes in vitro

PBMC	Septic patients (n = 16)			Healthy volunteers (n = 5)		
	Ultrafiltrate at t ₀	Ultrafiltrate at t ₆₀	NaCl controls	Ultrafiltrate at t ₀	Ultrafiltrate at t ₆₀	NaCl controls
TNF (U/mL)	41.0 ± 10.0†	40.2 ± 10.7†	12.8 ± 4.3	26.5 ± 8.6	31.2 ± 12.1	23.8 ± 7.8
IL-6 (U/mL)	2,015 ± 466	1,485 ± 244	2,006 ± 255	1,518 ± 212	1,471 ± 294	1,398 ± 175
IL-1 (pg/mL)	966 ± 256	n.d.	946 ± 183	935 ± 211	n.d.	936 ± 173
Lymphocytes						
IL-2 (U/mL)	0.35 ± 0.07†	n.d.	0.66 ± 0.07	0.48 ± 0.18	n.d.	0.41 ± 0.12
IL-6 (U/mL)	4,295 ± 896*	3,093 ± 741†	6,496 ± 873	5,879 ± 2,670	5,316 ± 1,833	5,433 ± 1,497

Cultures were costimulated with endotoxin (1 µg/mL) and phytohemagglutinin (2.5 µg/mL), respectively. Cells were incubated with septic or healthy ultrafiltrate collected at t₀ and t₆₀. Ultrafiltrate and corresponding NaCl incubations were always performed in the same cell preparation.

**P* < 0.01 vs. NaCl; †*P* < 0.001 vs. NaCl; n.d. = not determined.

mean \pm SEM. The differences between the incubation procedures and between means of different time points were compared with the Student's *t*-test. A significance level of $P = 0.01$ was used throughout the study.

RESULTS

HF showed no signs of mediator activation, since the most sensitive parameter (pre/postfilter C5b-9 concentration difference) remained unchanged during treatment. Prefilter concentrations of cytokines were constant during HF, although IL-1 and IL-8 were detected in the ultrafiltrate. Prefilter C3a concentration significantly declined during HF (patients: $t_1 = 676.9 \pm 99.7$ ng/mL, $t_2 = 545.4 \pm 83.2$, $P < 0.001$; volunteers: $t_1 = 54.82 \pm 13.25$ ng/mL, $t_2 = 33.9 \pm 10.68$, $P < 0.001$) and C3a appeared in ultrafiltrates. Septic ultrafiltrate enhanced endotoxin-induced TNF- α release in PBMC and suppressed PHA-induced lymphocyte IL-2 and IL-6 production (Table 1). PBMC proliferation was suppressed by septic ultrafiltrate compared to NaCl (septic ultrafiltrate at t_0 : $14,754 \pm 1,534$ cpm vs. NaCl: $37,418 \pm 160$ cpm; $P < 0.001$). Ultrafiltrate from volunteers did not cause significant changes.

CONCLUSIONS

Blood-membrane contact during hemofiltration does not appear to activate mediators of MODS. Certain factors such as C3a, but not established cytokines, are effectively eliminated by HF. Ultrafiltrate from patients with MODS contains a mixture of mediators, which induces changes in healthy white blood cell function with similarity to the septic response. HF represents a new modality of mediator removal in situations calling for an attenuation of an exaggerated monocyte TNF production and a stimulation of suppressed lymphocyte function.

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