The kidney possesses a remarkable capacity for restoring its structure and functional ability following an ischemic or toxic insult. It is unique as a solid organ in its ability to suffer an injury of such magnitude that the organ can fail for weeks and yet recover full function. Studying the natural regenerative process after an acute renal insult has provided new insights into the pathogenesis of acute renal failure (ARF) and possible new therapies. These therapies may limit the extent of injury or even accelerate the regenerative process and improve outcomes for patients suffering with ARF. In this chapter we illustrate some of the molecular responses of the kidney to an acute insult and demonstrate the effects of therapy with growth factors in the setting of experimental models of ARF. We conclude by demonstrating strategies that will provide future insights into the molecular response of the kidney to injury.

The regions of the nephron most susceptible to ischemic injury are the distal segment (S₃) of the proximal tubule and the medullary thick ascending limb of the loop of Henle. Following injury, there is loss of the epithelial lining as epithelial cells lose their integrin-mediated attachment to basement membranes and are sloughed into the lumen. An intense regenerative process follows. Normally quiescent renal tubule cells increase their nucleic acid synthesis and undergo mitosis. It is theorized that surviving cells situated close to or within the denuded area dedifferentiate and enter mitotic cycles. These cells then redifferentiate until nephron segment integrity is restored. The molecular basis that regulates this process is poorly-understood. After an injury, there is a spectrum of cell damage that is dependent on the type and severity of the insult. If the intensity of the insult is limited, cells become dysfunctional but survive. More severe injury results in detachment of cells from the tubule basement membranes, resulting in necrosis. Still other cells have no apparent damage and may proliferate to reepithelialize the damaged nephron segments. Thus, several
17.2 Acute Renal Failure

different processes are required to achieve structural and functional integrity of the kidney after a toxic or ischemic insult: 1) uninjured cells must proliferate and reepithelialize damaged nephron segments; 2) nonlethally damaged cells must recover; and 3) some damaged cells may actually die—not as a result of the initial insult but through a process of programmed cell death known as apoptosis. Figure 17-1 provides a schematic representation of the renal response to an ischemic or toxic injury.

**FIGURE 17-1** Schematic representation of some of the events pursuant to a renal insult and epithelial cell repair. **Subcellular:** Initial events include a decrease in cellular ATP and an increase in intracellular free calcium. There is blebbing of the endoplasmic reticulum with mitochondrial swelling and dysfunction. The brush border of the proximal tubules is sloughed into the tubule lumen, and there is redistribution of membrane proteins with the loss of cellular polarity. **Cellular:** At a cellular level this results in three populations of tubule cells, depending on the severity of the insult. Some cells are intact and are poised to participate in the proliferative process (Pathway 1). Growth factors participate by stimulating cells to undergo mitosis. Nonlethally injured cells have the potential to follow one of two pathways. In the appropriate setting, perhaps stimulated by growth factors, these cells may recover with restoration of cellular integrity and function (Pathway 2); however, if the injury is significant the cell may still die, but through a process of programmed cell death or apoptosis. The third population of cells are those with severe injury that undergo necrotic cell death. **Nephron/Kidney:** With the reepithelialization of damaged nephron segments and cellular recovery of structural and functional integrity, renal function is restored. (Modified from Toback [1]; with permission.)

**FIGURE 17-2** Growth regulation after an acute insult in regenerating renal tubule epithelial cells. Under the influence of growth-stimulating factors the damaged renal tubule epithelium is capable of regenerating with restoration of tubule integrity and function. The growth factors may be 1) produced by the tubule epithelium itself and act locally in an autocrine, juxtacrine or paracrine manner; 2) produced by surrounding cells to work in a paracrine manner; or 3) presented to the regenerating area via the circulation mediated by an endocrine mechanism. Cells at the edge of an injured nephron segment are illustrated on the left. These cells proliferate in response to the growth-stimulating factors. The middle cell is in the process of dividing and the cell on the right is migrating into the area of injury. (Adapted from Toback [1]; with permission.)
Growth Factors in Acute Renal Failure

GROWTH FACTORS IN ACUTE RENAL FAILURE

<table>
<thead>
<tr>
<th>Growth Factor</th>
<th>Ischemic and Toxic</th>
<th>Established ARF</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>Ischemic and Toxic</td>
<td></td>
</tr>
<tr>
<td>IGF-I</td>
<td>Ischemic and Toxic</td>
<td>Established ARF</td>
</tr>
<tr>
<td>HGF</td>
<td>Ischemic and Toxic</td>
<td></td>
</tr>
</tbody>
</table>

ARF—acute renal failure; EGF—epidermal growth factor; HGF—hepatocyte growth factor; IGF-I—insulin-like growth factor.

**FIGURE 17-3**
At least three growth factors have now been demonstrated to be useful as therapeutic agents in animal models of acute renal failure (ARF). These include epidermal growth factor (EGF), insulin-like growth factor I (IGF-I) and hepatocyte growth factor (HGF). All have efficacy in ischemia models and in a variety of toxic models of ARF. In addition, both IGF-I and HGF are beneficial when therapy is delayed and ARF is “established” after an ischemic insult. IGF-I has the additional advantage in that it also ameliorates the course of renal failure when given prophylactically before an acute ischemic insult.

**FIGURE 17-4**
Expression of messenger RNA (mRNA) for prepro–epidermal growth factor (EGF) in kidney. This schematic depicts the localization of mRNA for prepro-EGF under basal states in kidney. Prepro-EGF mRNA is localized to the medullary thick ascending limbs (MTAL) and distal convoluted tubules (DCT). Immunohistochemical studies demonstrate that under basal conditions the peptide is located on the luminal membrane with the active peptide actually residing within the tubule lumen. It is speculated that, during pathologic states, preformed EGF is either transported or routed to the basolateral membrane or can enter the interstitium via backleak. After a toxic or ischemic insult, expression of EGF is rapidly suppressed and can remain low for a long time. Likewise, total renal content and renal excretion of EGF decreases. CTAL—cortical thick ascending limb; IMCD—inner medullary collecting duct; OMCD—outer medullary collecting duct; and PCT—proximal convoluted tubule.
GROWTH FACTOR PRODUCTION

<table>
<thead>
<tr>
<th>EGF</th>
<th>IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submandibular salivary glands</td>
<td>Liver</td>
</tr>
<tr>
<td>Kidney</td>
<td>Lung</td>
</tr>
<tr>
<td>Others</td>
<td>Kidney</td>
</tr>
<tr>
<td>HGF</td>
<td>Heart</td>
</tr>
<tr>
<td>Liver</td>
<td>Muscle</td>
</tr>
<tr>
<td>Spleen</td>
<td>Other organs</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>Other organs</td>
<td></td>
</tr>
</tbody>
</table>

Production of epidermal growth factor (EGF), insulin-like growth factor (IGF-I), and hepatocyte growth factor (HGF) by various tissues. EGF, IGF-I, and HGF have all been demonstrated to improve outcomes in various animal models of acute renal failure (ARF). All three growth-promoting factors are produced in the kidneys and in a variety of other organs. The local production is probably most important for recovery from an acute renal insult. The influence of production in other organs in the setting of ARF has yet to be determined. This chapter deals primarily with local production and actions of EGF, IGF-I, and HGF.

Receptor binding for epidermal growth factor (EGF). EGF binding in kidney under basal conditions is extensive. The most significant specific binding occurs in the proximal convoluted (PCT) and proximal straight tubules. There is also significant EGF binding in the glomeruli (GLOM), distal convoluted tubules (DCT), and the entire collecting duct (OMCD, IMCD). After an ischemic renal insult, EGF receptor numbers increase. This change in the renal EGF system may be responsible for the beneficial effect of exogenously administered EGF in the setting of acute renal failure. CTAL—cortical thick ascending loop.
FIGURE 17-7

Epidermal growth factor (EGF)-mediated signal transduction pathways. The EGF receptor triggers the phospholipase C-gamma (PLC-gamma), phosphatidylinositol-3 kinase (PI3K), and mitogen-activated protein kinase (MAPK) signal transduction pathways described in the text that follows.

Growth factors exert their downstream effects through their plasma membrane–bound protein tyrosine kinase (PTK) receptors. All known PTK receptors are found to have four major domains: 1) a glycosylated extracellular ligand-binding domain; 2) a transmembrane domain that anchors the receptor to the plasma membrane; 3) an intracellular tyrosine kinase domain; and 4) regulatory domains for the PTK activity. Upon ligand binding, the receptors dimerize and autophosphorylate, which leads to a cascade of intracellular events resulting in cellular proliferation, differentiation, and survival.

The tyrosine phosphorylated residues in the cytoplasmic domain of PTK are of utmost importance for its interactions with cytoplasmic proteins involved in EGF–mediated signal transduction pathways. The interactions of cytoplasmic proteins are governed by specific domains termed Src homology type 2 (SH2) and type 3 (SH3) domains. The SH2 domain is a conserved 100–amino acid sequence initially characterized in the PTK-Src and binds to tyrosine phosphorylated motifs in proteins; the SH3 domain binds to their targets through proline-rich sequences. SH2 domains have been found in a multitude of signal transducers and docking proteins such as growth factor receptor–bound protein 2 (Grb2), phosphatidylinositol-3 kinase (p85-PI3K), phospholipase C-gamma (PLC-gamma), guanosine triphosphatase (GTPase)-activating protein of ras (ras-GAP), and signal transducer and activator of transcription 3 (STAT-3).

Upon ligand binding and phosphorylation of PTKs, SH2-domain containing proteins interact with the receptor kinase domain. PLC-gamma on interaction with the PTK, becomes phosphorylated and catalyzes the turnover of phosphatidylinositol (PI3) to two other second messengers, inositol triphosphate (IP3) and diacylglycerol (DAG). DAG activates protein kinase C; IP3 raises the intracellular calcium (Ca2+) levels by inducing its release from intracellular stores. Ca2+ is involved in the activation of the calmodulin-dependent CAM-kinase, which is a serine/threonine kinase.

A more important signal transduction pathway activated by PTKs concerns the ras pathway. The ras cycle is connected to activated receptors via the adapter protein Grb2 and the guanosine diphosphate–guanosine triphosphate exchange factor Sos (son of sevenless). GDP-ras, upon phosphorylation, is converted to its activated form, GTP-ras. The activated ras activates another Ser/Thr kinase called raf-1, which in turn activates another kinase, the mitogen activated protein kinase (MAPK).

MAPK activates the serine/threonine kinases, and extracellular signal-regulated kinases Erk1 and 2. Activation of Erk1/2 leads to translocation into the nucleus, where it phosphorylates key transcription factors such as Elk-1, and c-myc. Phosphorylated Elk-1 associates with serum response factor (SRF) and activates transcription of c-fos. The protein products of c-fos and c-jun function cooperatively as components of the mammalian transcription factor AP-1. AP-1 binds to specific DNA sequences in putative promoter sequences of target genes and regulates gene transcription. Similarly, c-myc forms a heterodimer with another immediate early gene max and regulates transcription.

The expression of c-fos, c-jun, and Egr-1 is found to be upregulated after ischemic renal injury. Immunohistochemical analysis showed the spatial expression of c-fos and Egr-1 to be in thick ascending limbs, where cells are undergoing minimal proliferation as compared with the S3 segments of the proximal tubules. This may suggest that the expression of immediate early genes after ischemic injury is not associated with cell proliferation.

Several mechanisms control the specificity of RTK signaling: 1) the specific ligand-receptor interaction; 2) the repertoire of substrates and signaling molecules associated with the activated RTK; 3) the existence of tissue-specific signaling molecules; and 4) the apparent strength and persistence of the biochemical signal. Interplay of these factors can determine whether a given ligand-receptor interaction leads to events such as growth, differentiation, scatter or survival.
FIGURE 17-8
Expression of mRNA for insulin-like growth factor I (IGF-I). Under basal conditions, a variety of nephron segments can produce IGF-I. Glomeruli (GLOM), medullary and cortical thick ascending limbs (MTAL/CTAL), and collecting ducts (OMCD, IMCD) are all reported to produce IGF-I. Within hours of an acute ischemic renal insult, the expression of IGF-I decreases; however, 2 to 3 days after the insult, when there is intense regeneration, there is an increase in the expression of IGF-I in the regenerative cells. In addition, extratubule cells, predominantly macrophages, express IGF-I in the regenerative period. This suggests that IGF-I works by both autocrine and paracrine mechanisms during the regenerative process. DCT/PCT—distal/proximal convoluted tubule.

FIGURE 17-9
Receptor binding for insulin-like growth factor I (IGF-I). IGF-I binding sites are conspicuous throughout the normal kidney. Binding is higher in the structures of the inner medulla than in the cortex. After an acute ischemic insult, there is a marked increase in IGF-I binding throughout the kidney. The increase appears to be greatest in the regenerative zones, which include structures of the cortex and outer medulla. These findings suggest an important trophic effect of IGF-I in the setting of acute renal injury. CTAL/MTAL—cortical/medullary thick ascending loop; DCT/PCT—distal/proximal convoluted tubule; GLOM—glomerulus; OMCD/IMCD—outer/inner medullary collecting duct.
Figure 17-10

Diagram of intracellular signaling pathways mediated by the insulin-like growth factor I (IGF-I/R) receptor. IGF-I/R when bound to IGF-I undergoes autophosphorylation on its tyrosine residues. This enhances its intrinsic tyrosine kinase activity and phosphorylates multiple substrates, including insulin receptor substrate 1 (IRS-1), IRS-2, and Src homology/collagen (SHC). IRS-1 upon phosphorylation associates with the p85 subunit of the PI3-kinase (PI3K) and phosphorylates PI3-kinase. PI3K upon phosphorylation converts phosphoinositide-3 phosphate (PI-3P) into PI-3,4-P2, which in turn activates a serine-threonine kinase Akt (protein kinase B). Activated Akt kinase phosphorylates the proapoptotic factor Bad on a serine residue, resulting in its dissociation from B-cell lymphoma-X (Bcl-XL). The released Bcl-XL is then capable of suppressing cell death pathways that involve the activity of apoptosis protease activating factor (Apaf-1), cytochrome C, and caspases. A number of growth factors, including platelet-derived growth factor (PDGF) and IGF I promotes cell survival. Activation of the PI3K cascade is one of the mechanisms by which growth factors mediate cell survival. Phosphorylated IRS-1 also associates with growth factor receptor bound protein 2 (Grb2), which bind son of sevenless (Sos) and activates the ras-raf-mitogen activated protein (ras/raf-M AP) kinase cascade. SHC also binds Grb2/Sos and activates the ras/raf-M AP kinase cascade. Other substrates for IGF-I are phosphotyrosine phosphatases and SH2 domain containing tyrosine phosphatase (Syp). Figure 17-7 has details on the other signaling pathways in this figure. MBP—myelin basic protein; nck—an adaptor protein composed of SH2 and SH3 domains; TF—transcription factor.
Acute Renal Failure

FIGURE 17-11
Expression of hepatocyte growth factor (HGF) mRNA and HGF receptor mRNA in kidney. While the liver is the major source of circulating HGF, the kidney also produces this growth-promoting peptide. Experiments utilizing in situ hybridization, immunohistochemistry, and reverse transcription-polymerase chain reaction (RT-PCR) have demonstrated HGF production by interstitial cells but not by any nephron segment. Presumably, these interstitial cells are macrophages and endothelial cells. Importantly, HGF expression in kidney actually increases within hours of an ischemic or toxic insult. This expression peaks within 6 to 12 hours and is followed a short time later by an increase in HGF bioactivity. HGF thus seems to act as a renotrophic factor, participating in regeneration via a paracrine mechanism; however, its expression is also rapidly induced in spleen and lung in animal models of acute renal injury. Reported levels of circulating HGF in patients with acute renal failure suggest that an endocrine mechanism may also be operational.

The receptor for HGF is the c-met proto-oncogene product. Receptor binding has been demonstrated in kidney in a variety of sites, including the proximal convoluted (PCT) and straight tubules, medullary and cortical thick ascending limbs (MTAL, CTAL), and in the outer and inner medullary collecting ducts (OMCD, IMCD). As with HGF peptide production, expression of c-met mRNA is induced by acute renal injury.
Molecular Responses and Growth Factors

**FIGURE 17-12**

Model of hepatocyte growth factor (HGF)/c-met signal transduction. In the extracellular space, single-chain precursors of HGF bound to the proteoglycans at the cell surface are converted to the active form by urokinase plasminogen activator (uPA), while the matrix soluble precursor is processed by a serum derived pro-HGF convertase. HGF, upon binding to its receptor c-met, induces its dimerization as well as autophosphorylation of tyrosine residues. The phosphorylated residue binds to various adaptors and signal transducers such as growth factor receptor bound protein-2 (Grb2), p85-P13 kinase, phospholipase C-gamma (PLC-gamma), signal transducer and activator of transcription-3 (STAT-3) and Src homology/collagen (SH C) via Src homology 2 (SH 2) domains and triggers various signal transduction pathways. A common theme among tyrosine kinase receptors is that phosphorylation of different specific tyrosine residues determines which intracellular transducer will bind the receptor and be activated. In the case of HGF receptor, phosphorylation of a single multifunctional site triggers a pleiotropic response involving multiple signal transducers. The synchronous activation of several signaling pathways is essential to conferring the distinct invasive growth ability of the HGF receptor. HGF functions as a scattering (dissociation/motility) factor for epithelial cells, and this ability seems to be mediated through the activation of STAT-3.

Phosphorylation of adhesion complex regulatory proteins such as ZO-1, beta-catenin, and focal adhesion kinase (FAK) may occur via activation of c-src. Another Bcl2 interacting protein termed BAG-1 mediates the antiapoptotic signal of HGF receptor by a mechanism of receptor association independent from tyrosine residues.

**FIGURE 17-13**

Mechanisms by which growth factors may possibly alter outcomes of acute renal failure (ARF). Epidermal growth factor, insulin-like growth factor, and hepatocyte growth factor (HGF) have all been demonstrated to improve outcomes when administered in the setting of experimental ARF. While the results are the same, the respective mechanisms of actions of each of these growth factors are probably quite different. Many investigators have examined individual growth factors for a variety of properties that may be beneficial in the setting of ARF. This table lists several of the properties examined to date. Suffice it to say that the mechanisms by which the individual growth factors alter the course of experimental ARF is still unknown.

**DETERMINANT MECHANISMS FOR OUTCOMES OF ACUTE RENAL FAILURE**

<table>
<thead>
<tr>
<th>Mitogenic</th>
<th>Anabolic</th>
</tr>
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<tbody>
<tr>
<td>Morphogenic</td>
<td>Alter leukocyte function</td>
</tr>
<tr>
<td>Cell migration</td>
<td>Alter inflammatory process</td>
</tr>
<tr>
<td>Hemodynamic</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Cytoprotective</td>
<td>Others</td>
</tr>
</tbody>
</table>
Acute Renal Failure

**ACTIONS OF GROWTH FACTORS IN ACUTE RENAL FAILURE**

<table>
<thead>
<tr>
<th>Actions</th>
<th>IGF-I</th>
<th>EGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>↓/↑</td>
<td>↑/↓</td>
</tr>
<tr>
<td>mRNA</td>
<td>↓/↑</td>
<td>↓</td>
</tr>
<tr>
<td>Receptors</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Vascular</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Anabolic</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Mitogenic</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>↓</td>
<td>↑↑↑</td>
</tr>
</tbody>
</table>

**FIGURE 17-14**
Selected actions of growth factors in the setting of acute renal failure (ARF). After an acute renal injury, a spectrum of molecular responses occur involving the local expression of growth factors and their receptors. In addition, there is considerable variation in the mechanisms by which the growth factors are beneficial for ARF. After an acute renal insult there is an initial decrease in both insulin-like growth factor (IGF-I) peptide and mRNA, which recovers over several days but only after the regenerative process is under way. The pattern with epidermal growth factor (EGF) is different in that a transient increase in available mature peptide from cleavage of pre-formed EGF is followed by a pronounced and prolonged decrease in both peptide and message. Both peptide and message for hepatocyte growth factor (HGF) are transiently increased in kidney after a toxic or an ischemic insult. The receptors for all three growth factors are increased after injury, which may be crucial to the response to exogenous administration.

The mechanism by which the different growth factors act in the setting of acute renal injury is quite variable. IGF-I is known to increase renal blood flow and glomerular filtration rate in both normal animals and those with acute renal injury. To the other extreme, EGF is a vasoconstrictor and HGF is vasoneutral. IGF-I has an additional advantage in that it has anabolic properties, and ARF is an extremely catabolic state. Neither EGF nor HGF seems to affect nutritional parameters. Finally, both EGF and HGF are potent mitogens for renal proximal tubule cells, the nephron segment is most often damaged by ischemic acute renal injury, whereas IGF-I is only a modest mitogen. Likewise, both EGF and HGF appear to be more effective than IGF-I at inhibiting apoptosis in the setting of acute renal injury, but it is not clear whether this is an advantage or a disadvantage.

**Clinical Use of Growth Factors in Acute Renal Failure**

**FIGURE 17-15**
Rationale for the use of insulin-like growth factor IGF-I in the setting of acute renal failure (ARF). Of the growth factors that have been demonstrated to improve outcomes after acute renal injury, the most progress has been made with IGF-I. From this table, it is evident that IGF-I has a broad spectrum of activities, which makes it a logical choice for treatment of ARF. An agent that increased renal plasma flow and glomerular filtration rate and was mitogenic for proximal tubule cells and anabolic would address several features of ARF.

**FIGURE 17-16**
Serial serum creatinine values in rats with ischemic acute renal failure (ARF) treated with insulin-like growth factor (IGF-I) or vehicle. This is the original animal experiment that demonstrated a benefit from IGF-I in the setting of ARF. In this study, IGF-I was administered beginning 30 minutes after the ischemic insult (arrow). Data are expressed as mean ± standard error. Significant differences between groups are indicated by asterisks.

This experiment has been reproduced, with variations, by several groups, with similar findings. IGF-I has now been demonstrated to be beneficial when administered prophylactically before an ischemic injury and when started as late as 24 hours after reperfusion when injury is established. It has also been reported to improve outcomes for a variety of toxic injuries and is beneficial in a model of renal transplantation with delayed graft function and in cyclosporine-induced acute renal insufficiency. (From Miller et al. [2]; with permission.)
Body weights of rats with ischemic acute renal failure (ARF) treated with insulin-like growth factor (IGF-I) or vehicle. Unlike epidermal growth factor or hepatocyte growth factor (HGF), IGF-I is anabolic even in the setting of acute renal injury. These data are from the experiment described in Figure 17-16. As the data in this figure demonstrate, ARF is a highly catabolic state: vehicle-treated animals experience 15% weight reduction. Animals that received IGF-I experienced only a 5% reduction in body weight and were back to baseline by 7 days. Data are expressed as mean ± standard error. Significant differences between groups are indicated by asterisks. (From Miller et al. [2]; with permission.)

Photomicrograph of kidneys from rats with acute renal failure (ARF) treated with insulin-like growth factor (IGF-I) or vehicle. These photomicrographs are of histologic sections stained with hematoxylin and eosin originating from kidneys of rats that received vehicle or IGF 1 after ischemic renal injury. Kidneys were obtained 7 days after the insult. There is evidence of considerable residual injury in the kidney from the vehicle-treated rat (A): dilation and simplification of tubules, interstitial calcifications, and papillary proliferations the tubule lumen of proximal tubules. The kidney obtained from the IGF-I–treated rat (B) appears almost normal, showing evidence of regeneration and restoration of normal renal architecture. In this experiment the histologic appearance of kidneys from the IGF-I–treated animals was statistically better than that of the vehicle-treated controls, as determined by a pathologist blinded to therapy. (From Miller et al. [2]; with permission.)
RATIONALE FOR INSULIN-LIKE GROWTH FACTOR I (IGF-I) IN ACUTE RENAL FAILURE

Reported therapeutic trials of insulin-like growth factor (IGF-I) in humans. Based on the compelling animal data and the fact that there are clearly identified disease states involving both over- and underexpression of IGF-I, this is the first growth factor that has been used in clinical trials for kidney disease. Listed above are a variety of studies of the effects of IGF-I in humans. This peptide has now been examined in several published studies of both acute and chronic renal failure. Additional studies are currently in progress.

In the area of acute renal failure there are now two reported trials of IGF-I. In the initial study IGF-I or placebo was administered to patients undergoing surgery involving the suprarenal aorta or the renal arteries. This group was selected as it best simulated the work that had been reported in animal trials of ischemic acute renal injury. Fifty-four patients were randomized in a double-blind, placebo-controlled trial of IGF-I to prevent the acute decline in renal function frequently associated with this type of surgery. The primary end-point in this study was the incidence of renal dysfunction, defined as a reduction of the glomerular filtration rate as compared with a preoperative baseline, at each of three measurements obtained during the 3 postoperative days. Modern surgical techniques have decreased the incidence of acute renal failure to such a low level, even in this high-risk group, so as to make it impractical to perform a single center trial with enough power to obtain differences in clinically important end-points. Thus, this trial was intended only to offer “proof of concept” that IGF-I is useful for patients with acute renal injuries.

FIGURE 17-19
Incidence of postoperative renal dysfunction treated with insulin (IGF-I) or placebo. IGF-I significantly reduced the incidence of postoperative renal dysfunction in these high-risk patients. Renal dysfunction occurred in 33% of those who received placebo but in only 22% of patients treated with IGF-I. The groups were well-matched with respect to age, sex, type of operation, ischemia time, and baseline renal function as defined by serum creatinine or glomerular filtration rate. The IGF-I was tolerated well; no side effects were attributed to the drug. Secondary end-points such as discharge, serum creatinine, length of hospitalization, length of stay in the intensive care unit, or duration of intubation were not significantly different between the two groups. (Adapted from Franklin, et al. [3]; with permission.)

FIGURE 17-20
Receptors are present on proximal tubules
Regulates proximal tubule metabolism and transport

Increases renal plasma flow and glomerular filtration rates
Mitogenic for proximal tubule cells
Enhanced expression after acute renal injury
Anabolic

THERAPEUTIC TRIALS OF INSULIN-LIKE GROWTH FACTOR I IN HUMANS

Summary of an abstract describing the trial of insulin-like growth factor (IGF-I) in the treatment of patients with established acute renal failure (ARF). Based on the accumulated animal and human data, a multicenter, double-blind, randomized, placebo-controlled trial was performed to examine the effects of IGF-I in patients with established ARF. Enrolled patients had ARF of a wide variety of causes, including surgery, trauma, hypertension, sepsis, and nephrotoxic injury. Approximately 75 patients were enrolled, treatment being initiated within 6 days of the renal insult. Renal function was evaluated by iodothalilate clearance. Unfortunately, at an interim analysis (the study was originally designed to enroll 150 patients) there was no difference in renal function or survival between the groups. The investigators recognized several potential problems with the trial, including the severity of many patients’ illnesses, the variety of causes of the renal injury, and delay in initiating therapy [4].
**Future Directions**

**FIGURE 17-22**
Advantages of insulin-like growth factor (IGF-I) in the treatment of acute renal failure. The limited data obtained to date on the use of IGF-I for acute renal failure demonstrate that the peptide is well-tolerated and may be useful in selected patient populations. Additional human trials are ongoing including use in the settings of renal transplantation and chronic renal failure.

**FIGURE 17-23**
Limitations in the use of growth factors to treat acute renal failure (ARF). The disappointing results of several recent clinical trials of ARF therapy reflect the fact that our understanding of its pathophysiology is still limited. Screening compounds using animal models may be irrelevant. Most laboratories use relatively young animals, even though ARF frequently affects older humans, whose organ regenerative capacity may be limited. In addition, our laboratory models are usually based on a single insult, whereas many of our patients suffer repeated or multiple insults. Until we gain a better understanding of the basic pathogenic mechanisms of ARF, studies in human patients are likely to be frustrating.

**HUMAN IGF-I IN PATIENTS WITH ARF**

<table>
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<th>*No difference between the groups were observed in final values or changes in values for glomerular filtration</th>
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<tr>
<td>ARF secondary to surgery, trauma, hypertensive nephropathy, sepsis, or drugs</td>
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<tr>
<td>Evaluated renal function and mortality</td>
<td>1 Hour: ↑, ↔</td>
</tr>
<tr>
<td>Treated within the first 6 days for 14 days</td>
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<tr>
<td>Evaluated renal function and mortality</td>
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<tr>
<td>Treated within the first 6 days for 14 days</td>
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</tr>
<tr>
<td></td>
<td>1 Hour: ↑, ↔</td>
</tr>
</tbody>
</table>

References:
- Bardella et al. [5]
- Ouellette et al. [6]
- Bonventre et al. [7]
- Witzgall et al. [8]
- Safirstein et al. [9]
- Goes et al. [10]
- Singh et al. [11]
- Soifer et al. [12]
- Firth and Ratcliffe [13]
Well-tolerated

<table>
<thead>
<tr>
<th>In Acute Renal Failure</th>
<th>Growth Factor Limitations</th>
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<tbody>
<tr>
<td>Safe in short-term studies</td>
<td>Lack of basic knowledge of the pathophysiology of ARF</td>
</tr>
<tr>
<td>Experience with diseases of overexpression and underexpression</td>
<td>No screening system for compounds to treat ARF</td>
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<tr>
<td>Did not worsen outcomes</td>
<td>Animal models may not be relevant</td>
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</tr>
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<td>Difficulty of identifying appropriate target populations</td>
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**FIGURE 17-24 (Continued)**

Several genes have already been identified to be induced or down-regulated after ischemia and reperfusion. This table lists genes whose expression is altered as a result of ischemic injury. It is not clear at present if the varied expression of these genes plays a role in cell injury, survival, or proliferation.
Molecular Responses and Growth Factors

MOLECULAR RESPONSE TO RENAL ISCHEMIC/REPERFUSION INJURY

Genes
- Transcription factors:
  - c-Jun
  - c-fos
  - Egr-1
  - Kid 1

Cytokines
- JE
- KC
- IL-2
- IL-10
- IFN-γ
- GM-CSF
- MIP-2
- IL-6
- IL-11
- LIF
- PTHrP
- Endothelin 1
- Endothelin 3

FIGURE 17-25
Schematic representation of differential display. In a complex organ like the kidney, ischemic renal injury triggers altered expression of various cell factors and vascular components. Depending on the severity of the insult, expression of these genes can vary in individual cells, leading to their death, survival, or proliferation. A better understanding of the various factors and the signal transduction pathways transduced by them that contribute to cell death can lead to development of therapeutic strategies to interfere with the process of cell death. Similarly, identification of factors that are involved in initiating cell migration, dedifferentiation, and proliferation may lead to therapy aimed at accelerating the regeneration program. To identify the various factors involved in cell injury and regeneration, powerful methods for identification and cloning of differentially expressed genes are critical. One such method that has been used extensively by several laboratories is the differential display polymerase chain reaction (DD-PCR).

In this schematic, mRNA is derived from kidneys of sham-operated (controls) and ischemia-injured rats, some pretreated with insulin-like growth factor (IGF-I). The mRNAs are reverse transcribed using an anchored deoxy thymidine-oligonucleotide (oligo-dT) primer (Example: dT[12]-MX, where M represent G, A, or C, and X represents one of the four nucleotides). An anchored primer limits the reverse transcription to a subset of mRNAs. The first strand cDNA is then PCR amplified using an arbitrary 10 nucleotide-oligomer primer and the anchored primer. The PCR reaction is performed in the presence of radioactive or fluorescence-labeled nucleotides, so that the amplified fragments can be displayed on a sequencing gel. Bands of interest can be excised from the gel and used for further characterization. ARF—acute renal failure.

FIGURE 17-26
Schematic representation of a differential display gel in which mRNA from kidneys is reverse-transcribed and polymerase chain reaction (PCR) amplified (see Figure 17-25). The PCR amplification is conducted in the presence of radioactive nucleotides. The cDNA fragments corresponding to the 3’ end of the mRNA species are displayed by running them on a sequencing gel, followed by autoradiography. The arrows show bands corresponding to mRNA transcripts that are expressed differentially 1) in response to insulin-like growth factor (IGF-I) treatment and induction of ischemic injury; 2) in an IGF-I–dependent manner; 3) in response to induction of ischemic injury; and 4) to genes that are down-regulated after induction of ischemic injury. ARF—acute renal failure.
References