The morphology of acute renal failure and the tubular cell exfoliation phenomenon

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Acute renal failure may result from injury to any of the structural elements of the kidney-glomeruli, vessels, interstitium or tubules. With severe injury, morphologic changes may be widespread, and involve more than one structural component. The renal tubule, in particular, may be injured as a direct result of a pathologic process which primarily affects tubular epithelium, such as ischemic or toxic injury. This results in «acute tubular necrosis», the most common cause of clinical acute renal failure. However, tubular injury may also occur secondary to severe interstitial, glomerular or vascular diseases, presumably as a result of compromised blood flow to the tubules in these conditions or direct injury by inflammatory cells or mediators. In this review, we will focus on characteristics of tubular injury in acute renal failure.

In clinical acute renal failure due to direct tubular injury, a range of morphologic changes may be seen in renal tubular epithelial cells. These include manifestations of sublethal cell injury such as loss of PAS-positive proximal tubular brush border, blebbing of the apical cytoplasm, vacuolization, and cell swelling[1-3]. Ultrastructural studies confirm loss of brush border. Internalization of intact brush border fragments into the cell cytoplasm has also been documented, particularly in experimental animals[4]. There is also simplification of the complex infoldings of the basolateral surfaces of tubular cells that serve to amplify cell surface area and enhance tubular transport/reabsorption processes[5,6].

Despite use of the term «acute tubular necrosis» to denote the anoxic/toxic type of human tubular cell injury, actual necrosis of tubular cells is usually not widespread in this clinical condition in the native kidney[1-3,7]; necrotic cells are seen with greater frequency in «ATN» in the transplanted kidney[6]. Instead, there are gaps along the tubular basement membrane which represent sites of cell loss and non-replacement (see figure 1). These gaps are among the few morphologic alterations which correlate with decreased renal function, being significantly more numerous during ARF, and disappearing with recovery from ARF[8]. At one time, these gaps were felt to represent sites where cells had undergone necrosis and subsequently been sloughed into the tubular lumen. However, there is now evidence that at least some of these gaps may represent sites where viable tubular cells have lost their normal epithelial attachments and exfoliated from the tubular basement membrane.

In recent studies in both clinical «acute tubular necrosis»[9,10], and in experimental models of ischemic or toxic injury[9], voiding of numerous viable tubular cells has been documented. In studies by Racusen et al.[9] cells were shown to be viable by Trypan blue exclusion and by ability to grow and form epithelial monolayers when placed in appropriate culture conditions. Shed cells appeared to originate from both proximal and distal tubules using morphologic and histochemical criteria, consistent with observations made in clinical and experimental studies of tubular cell injury with ischemia. In some voids from patients with ARF, 100 % of the identifiable tubular cells were viable by the criteria indicated above.

Graver et al.[10] have reported that urinary sediments of the majority of patients with acute renal failure due to intrinsic renal injury contained vacuolated cells which excluded Trypan blue. Electron microscopy of these cells revealed morphologically intact cells with characteristics of renal tubular cells, including some with surface microvilli. The highest incidence of these cells were seen in the sediment from patients with «acute tubular necrosis», but were also detected in nephrotic syndrome, chronic renal insufficiency, hepatorenal syndrome, and acute interstitial nephritis.

In other studies, while actual viability of exfoliated tubular cells has not been ascertained, there are numerous reports of the voiding of morphologically intact tubular cells in many types of renal injury[11-13]. Segasothy et al.[11] using immunoperoxidase techniques, were able to identify tubular cells from proximal and distal nephron as well as the loop of Henle in urine from patients with «acute tubular necrosis», consistent with morphologic findings in biopsies from these patients. These investigators also reported substantial numbers of tubular cells from all segments of the nephron with glomerulonephritis, especially of the crescentic type, and in cases of acute interstitial

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nephritis, consistent with the tubular injury documented in these setting[14, 15].

Reflecting injury to and loss of tubular epithelial cells there are also regenerative/reparative changes in the surviving tubular epithelium (see figure 1), including flattening of cells, and presence of hyperchromatic nuclei, basophilic cell cytoplasm, and cell mitoses. These regenerative tubular cells often coexist with evidence of tubular cell injury, suggesting that the injury process is an ongoing one, at least in clinical «acute tubular necrosis». The flattening and lateral spreading of surviving epithelial cells tend to cover the gaps along the tubular basement membrane, leading to some restitution of epithelial integrity, and to an underestimation of sites of cell loss on examination of biopsies by light microscopy[7]. The mechanism of and stimulus signals for these in situ regeneration/repair processes are not fully defined. However, a number of factors mitogenic for tubular cells in vitro have been identified, including known growth factors such as epidermal growth factor and platelet-derived growt factor, altered extracellular cation concentrations, adenosine mono and diphosphate, and cytokines. Growth factors may act via endocrine, autocrine or paracine mechanisms to enhance cell regeneration at sites of injury. The role of activation of early-response genes and genes encoding growth factors is also being investigated[16, 17].

Dilatation of the tubular lumen in the proximal nephron is also seen in «acute tubular necrosis», a probable reflection of nephron obstruction and/or elevations in intratubular pressure. Hyaline, granular or pigmented casts are often seen in the distal nephron, which may serve to obstruct the tubule. It is likely that cells and cell debris contribute to cast formation and blockage of the tubule.

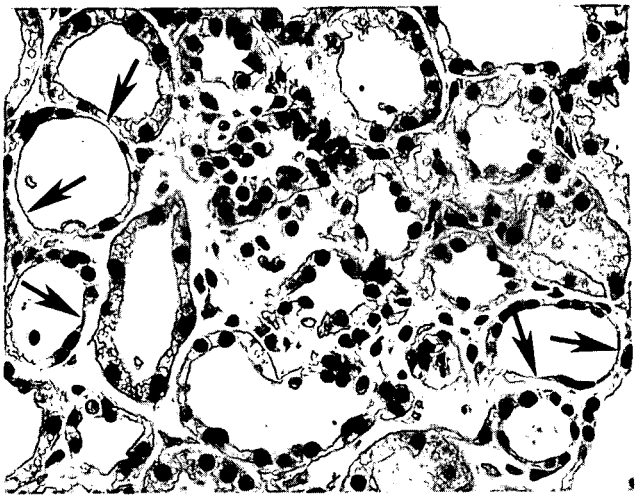

Fig. 1.—Micrograph of cortical tubules in a biopsy from a young woman with acute tubular necrosis developing in the setting of toxic shock syndrome. Arrows indicate areas along the tubular basement membrane where tubular cells have been lost and the basement membrane is denuded or covered by only a thin layer of alternated tubular epithelial cell cytoplasm. Note also tubular dilatation loss of apical brush border and tubular cell vacuolation and reactive/regenerative changes including hyperchromatic nuclei, basophilic cytoplasm, and flattening of surviving cells.

As alluded to above, tubular injury may also be seen with primary interstitial nephritis or primary glomerular disease. Interstitial nephritis is characterized by moderate or severe interstitial inflammation, often including polymorphonuclear leukocytes and eosinophils as well as mononuclear cells, and is often associated with infection or drug hypersensitivity reactions. Concomitant tubular injury is frequent and presumed to be due to a number of factors including a direct tubulotoxic effect of the inciting drug, inflammatory/immunologic injury, and/or ischemia. Interestingly, features of tubular injury, and especially loss of individual tubular cells and thining of the PAS-positive brush borders are the morphologic features which correlate most closely with level of renal dysfunction in interstitial nephritis just as they do in «acute tubular necrosis»[15]. With primary tubular injury (ATN), interstitial edema and an interstitial inflammatory infiltrate may be seen — there is some evidence thar inflammatory cells may contribute to tubular injury and renal dysfunction in this setting as well[18, 19].

With primary glomerular diseases, tubular injury is most commonly seen in the more florid inflammatory processes such as diffuse proliferative and especially, crescentic glomerulonephritis[11]. Since all peritubular capillary blood flow comes from the post glomerular efferent arteriole, nutrient blood supply to the tubules may be profoundly affected by primary glomerular disease. Tubular alterations are presumably secondary to ischemia and drastically reduced tubular fluid flow and/or to the interstitial inflammation accompanying the glomerular process. Acute tubular injury may also be seen, however, with minimal change nephropathy, when the nephrotic syndrome leads to systemic fluid derangements and circulatory insufficiency. As noted above, voiding of tubular cells from all segments of the nephron has been documented in ARF due to acute glomerular disease[11].

Injury to and loss of tubular cells would contribute to renal dysfunction in acute renal failure via a number mechanisms[7]. Gaps along the basement membrane where cells have exfoliated or gaps between cells which have lost normal attachments to adjacent cells, or areas of frank cell necrosis would serve as sites for backleak of tubular fluid into the peritubular interstitial space and thence into the capillaries. Cells and cell debris from sites of injury may become impacted in the tubular lumen and produce obstruction. Loss of ephitelial cells and ephitelial integrity, and/or more subtle alterations in cell-cell attachments would disrupt the normal vectorial transport of electrolytes and fluid from the tubular lumen, leading to filtration failure via tubuloglomerular feedback and afferent arteriolar vasoconstriction.

There is now accumulating preliminary data on the cellullar mechanisms of alterations in tubular cell attachment that occur with sublethal injury. In vitro cell culture systems

have proved particularly useful in studying altered cell adhesion in response to anoxia/ATP depletion or oxidant injury. Changes in the normal cell-cell and cell-substrate adhesion molecules of tubular epithelium have been documented in cultured mammalian tubule cells. Bacallao and Mandel[20] have documented internalization of the cell-cell attachment molecule E-cadherin (L-CAM) as well as Na-K-ATPase with energy depletion in cultured renal cells. Using cultured BSC-1 cells, Goligorsky and Gailit[21] have reported decreased basal expression of a subunit of integrin molecules, which mediate cell-substrate adhesion, with oxidant stress, a change associated with altered cell adhesion in their system.

Other recent studies have implicated alterations in microfilaments in altered attachments of renal tubular epithelial cells with injury. Kellerman and coworkers have documented early alterations in tubular actin staining with renal ischemia[22]. In human proximal tubule cell cultures, Racusen has reported alterations in cellular microfilaments associated with alterations in cell shape reflecting altered cell attachments with anoxia; cell shape changes were largely prevented when microfilaments were stabilized with phalloidin[23]. Hanson et al. have also used a cultured human tubule cell model to study oxidant injury, and found altered cell adhesion associated with disruption of cellular actin and vimentin filaments[24]. ATP depletion in mouse proximal tubule cell cultures also produces alterations in cell actin filaments, with marked reduction in cell-substrate adhesion[25].

Much remains to be done in defining the cellular and molecular pathophysiology of altered tubule cell adhesion following injury. Investigations in this area, as well as in the area of progression to irreversible injury and of tubule cell regeneration and repair phenomena, should contribute importantly to our understanding of the pathology and pathophysiology of acute renal failure, and will likely lead to new strategies to reduce functional and morphologic damage and enhance recovery following renal tubular cell injury.

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