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Regulation of glomerular cell number by apoptosis

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Regulation of glomerular cell number by apoptosis. While commonly viewed as leading to glomerular scarring and end-stage renal failure, glomerular cell proliferation may be a beneficial response that promotes the injured glomerulus to return to its proper function. This brief review looks at the potentially counterbalancing influences that cause glomerular cells to survive, undergo mitosis, differentiate or die.

". . . in this world nothing can be said to be certain, except death and taxes."

BENJAMIN FRANKLIN, 1789

It is now widely recognized that physiological cell death by apoptosis is a normal event in tissue homeostasis [1, 2]. Indeed, apoptosis frequently appears to be a "tax" on the growth of cell populations by mitosis [3, 4]. Therefore, the U.S. statesman and author, Benjamin Franklin, would not have been surprised to find an article on the counterbalancing roles of cell death in a symposium dedicated to increase in cell number. This brief contribution, which concentrates on the mesangial cell, offers a personal view on the role of apoptosis in the tissue kinetics of the diseased glomerulus. It is not intended to provide a review of the molecular mechanisms that mediate apoptosis because these have been described in detail elsewhere [5, 6], but some speculations on the regulation of glomerular cell apoptosis are offered.

RESIDENT CELL NUMBER IN GLOMERULAR DISEASE

Nephrologists are more than familiar with the stereotyped morphology of early glomerular disease, in which infiltration by leukocytes and changes in the nature and amount of extracellular matrix (ECM) are accompanied by an increase in the resident glomerular cell number with evidence of true proliferation of glomerular cells [7, 8]. Quite correctly, there has been intense interest in the molecular mechanisms responsible for excessive glomerular cell proliferation, as this is commonly viewed as an undesirable event that disturbs glomerular function, threatens progression to glomerular scarring and end-stage renal failure, and therefore may warrant blockade as a new approach to treatment [7].

However, a heretical view is that far from being undesirable, glomerular cell proliferation is a beneficial response that promotes healing and restitution of the injured glomerulus. Perhaps the most dramatic evidence in support of this concept comes from the well-described Thy 1.1 model of mesangial proliferative nephritis in the rat [9, 10]. The administration of cross-linking antibody to Thy 1.1 borne by mesangial cells leads to rapid, complement-mediated death of virtually all of the mesangial cells present in glomerular tufts. Remarkably, however, over the ensuing weeks, normal glomerular structure and function are restored by processes that at times exhibit remarkable similarity to events that are commonly regarded as "disease" in human glomeruli. Thus, within a few days, there is a profound "rebound" proliferation of mesangial cells, so that by 10 to 14 days, the mesangial cell complement is double the normal number accompanied by an assumption of a myofibroblast phenotype [11] and deposition of apparently excessive ECM [12]. However, far from inevitably leading to glomerular scarring, provided further insult is not added to initial Thy 1.1-mediated mesangial injury, beyond 14 days there is progressive restitution of normal glomerular structure via remodeling processes that include the return of the mesangial cell number to normal. Indeed, recent data emphasize that much like the myofibroblasts that migrate into tissue deficits in wounded skin [13–15], myofibroblast-like glomerular mesangial cells have the capacity to move into and repair "gaps" left in the tissue by unscheduled cell death. A broadly similar course of events can be observed in human glomerular disease, with resolution occurring spontaneously in postinfectious glomerulonephritis and some cases of IgA nephropathy or after treatment in disorders such as lupus nephritis.

Indeed, the heresy of beneficial glomerular cell prolif-

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eration finds some support in the stereotyped morphology of late, end-stage glomerular disease. In addition to shrinkage and apparent replacement of the glomerular tuft by ECM, there is also a striking loss of glomerular cells. Nevertheless, in what might be interpreted as the last stand of a defense force, there is evidence of increased glomerular cell proliferation despite profound hypocellularity [16].

APOPTOSIS: FRIEND AND FOE

Of course, it is in the nature of debates between the heretic and the orthodox that a balanced and correct view is rarely achieved. Indeed, recent data emphasize that the outcome of glomerular disease is most unlikely to be determined solely by the regulation of resident cell division [8]. Instead, it appears that a crucial factor is the *balance* between cell birth by mitosis, the main subject of this symposium, and cell death by apoptosis.

Apoptosis is a physiological and "programmed" form of cell death, initially identified by highly characteristic morphological changes, including condensation of nuclear chromatin and the cytoplasm, and now known to be the result of an ordered series of biochemical events within the dying cell [1, 2, 17]. Prominent among these is activation of a family of cysteine proteases called caspases, which act on multiple intracellular targets to lead to characteristic changes such as endonuclease-mediated internucleosomal chromatin cleavage ("DNA" laddering) and plasma membrane alterations, including exposure of the anionic phospholipid phosphatidylserine (PS) [18–23]. In turn, PS exposure and other membrane changes rapidly lead to the recognition by phagocytes and swift, histologically inconspicuous removal and degradation of the intact dying cell, preventing injury of surrounding tissues by the uncontrolled release of noxious cell contents [reviewed in 24, 25]. It is now widely recognized that apoptosis is the normal means by which unwanted or surplus cells are deleted during tissue homeostasis.

Consequently, when apoptosis was first described in 1988 in glomerular disease by Harrison [26], it was rational to focus attention on the possible role of apoptosis in deleting unwanted cells from the glomerulus [27], particularly in view of data implicating apoptosis in clearance of infiltrating leukocytes [28, 29]. Indeed, following demonstration *in vitro* that the program of apoptosis was available to the mesangial cell [3], two independent studies implicated mesangial cell apoptosis as the major mechanism mediating beneficial deletion of excess mesangial cells during spontaneous resolution of mesangial hypercellularity in the Thy 1.1 model in the rat [3, 30]. In the second study, the characteristic wave of mesangial cell mitosis in this model was followed by a wave of apoptosis [30], but presumably because of minor experimental differences, we were intrigued to find that glomerular cell apoptosis and mitosis peaked at the same early time point [3]. However, unlike mitosis, which returned to baseline by 14 days, apoptosis remained tenfold above baseline so long as the glomerular cell number was greater than normal. By making some well-substantiated estimates of the duration that an individual apoptotic or mitotic event was histologically detectable [31], we were able to use an "area under the curve" analysis to confirm that the observed incidence of glomerular cell apoptosis at various time points was sufficient to account for the observed rate of change in mesangial cell number. An interesting point was that apoptosis served as a "tax" on mitosis, limiting early increases in mesangial cell number caused by cell proliferation.

However, this evidence for a beneficial role of apoptosis was soon supplemented by data pointing toward undesirable consequences of glomerular cell apoptosis. Sugiyama et al provided human and animal model data supporting a role for unscheduled apoptosis in deleterious cell loss during the progression of glomerular injury to scarring [32]. First, they observed marked increases in glomerular cell apoptosis in renal biopsy specimens from patients with IgA nephropathy. The greatest number, up to one apoptotic cell per cross section, was found in lesions described as "predominantly sclerosing." This represents a very significant rate of cell loss, up to 24 per cross section per day, as the usual time taken for cells to undergo apoptosis and subsequent phagocytic degradation beyond histological recognition is around only one hour [33–35]. Furthermore, in both IgA nephropathy and a smaller series of biopsies from patients with lupus nephritis, there was a statistically significant correlation between the number of apoptotic cells per glomerular cross section and a semiquantitative "sclerosis" score; although mitotic figures were not counted, there was no clear correlation between apoptosis and a semiquantitative score of proliferation. The possibility that there might be a causal relationship between increased apoptosis and the net cell loss from glomeruli undergoing sclerosis was strengthened by additional observations on the rat remnant kidney model [32], in which five-sixths nephrectomy leads rapidly to glomerular and interstitial fibrosis accompanied by progressively impaired renal function. There was clear evidence of significantly increased apoptosis in both glomeruli and tubules compared with sham-operated animals. Furthermore, a study by Shimizu et al of an animal model of severe crescentic glomerulonephritis also emphasized the persistence of increased apoptosis beyond the stage of hypercellularity in glomeruli destined to become hypocellular scars [36].

Consequently, we have proposed that, much as may be the case for glomerular cell proliferation, apoptosis in glomerular cells can be regarded as a "double-edged

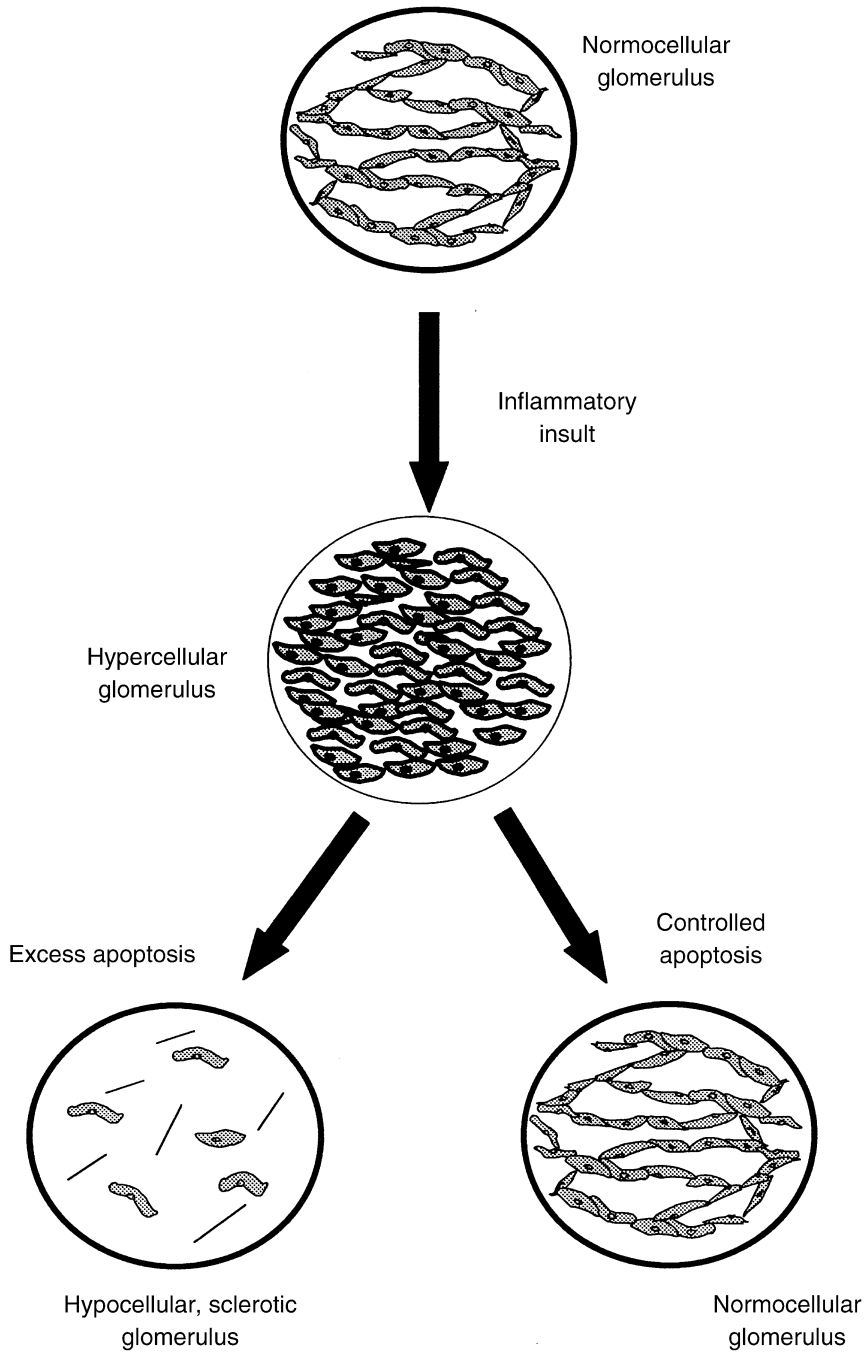

Fig. 1. The "double-edged sword" of apoptosis. Depending upon the response of the cell after glomerular injury, there may be a beneficial deletion of excess resident glomerular cells or a more dramatic increase in cell death that contributes to the progression of renal disease and eventually to end-stage hypocellular kidney.

sword," sometimes promoting resolution of glomerular injury by beneficial deletion of excess resident glomerular cells but, at other times, contributing to the progression to the end-stage hypocellular kidney (Fig. 1) [4]. Therefore, although therapeutic regulation of glomerular cell apoptosis might appear dauntingly complex, understanding the controls on apoptosis in such cells is likely to contribute greatly to the elucidation of the pathogenesis of glomerular disease.

REGULATION OF GLOMERULAR CELL APOPTOSIS

Because techniques for renal cell culture are well established and assays for apoptosis are relatively straightforward *in vitro*, there has been a growing interest in defining factors that might trigger apoptosis in glomerular cells. A number of stimuli have been identified (Table 1), some emphasizing that apoptosis can be an "altruistic" response in cells receiving injury insufficient to cause

Table 1. Stimuli reported to trigger mesangial cell apoptosis in culture

Stimulus	Selected references
Non-specific	
Serum starvation	[3, 37]
Detachment	[38]
Shear stress	[39]
Hydrostatic pressure	[39]
DNA damage	[39]
Reactive oxygen species	[40–42]
Ionizing radiation	[43]
Cytotoxic drugs	[3, 43]
Specific	
Anti-Thy 1.1 antibodies	[44, 45]
Anti-Fas antibodies	[46]
TNFα	[47, 48]
IL-1α	[47]
IL-1β	[43]
C1q	[45]
Anti-dsDNA antibodies	[49]
LDL	[50]
Lovastatin	[51]
Nitric oxide	[52–54]
Superoxide	[53]
Cyclic AMP	[55]

Abbreviations are: TNF, tumor necrosis factor; IL-1, interleukin-1; LDL, low density lipoprotein.

primary necrosis [2], the stricken cell adopting a potentially noninflammatory mode of death. However, as in studies of glomerular cell survival (discussed later in this article), the *in vivo* relevance of many of the reported stimuli remains speculative.

Nevertheless, there are two well-established examples of proapoptotic stimuli that not only induce glomerular mesangial cell death *in vitro* but also trigger apoptosis in this cell type *in vivo*. The relevance to disease of Thy 1.1 ligation as a mechanism for rat mesangial cell apoptosis *in vitro* [44, 56] and *in vivo* [9, 56] is questionable, but Ortiz et al have studied the role of the Fas death receptor [46], which is very likely to be involved in human disease. Fas is a member of the tumor necrosis factor (TNF) receptor family that appears specialized for engaging apoptosis by being "hot-wired" to the caspase cascade through adaptor molecules, including FADD and caspase-8 (FLICE), once the receptor has been trimerized by its TNF-like ligand (FasL) [57–59]. Although the administration of Fas cross-linking antibody to mice ultimately incites fatal liver injury consequent on apoptosis in Fas-expressing hepatocytes, clear evidence was obtained that Fas ligation also induced glomerular cell apoptosis [46, 60]. This important work established a candidate mechanism for (unscheduled) glomerular cell apoptosis because FasL can be expressed by leukocyte types known to infiltrate the glomerulus, such as monocyte/macrophages [61] and CD8-positive cytotoxic T cells [62]. However, *in vitro* studies emphasize that mesangial cell susceptibility to ligation of Fas can be independently regulated by proinflammatory cytokines such as γ-interferon [63, 64].

The potential importance of Fas ligation in unscheduled glomerular cell apoptosis has been further emphasized in studies of mesangial cell "survival factors," agents that promote cell survival by inhibiting apoptosis. Raff has proposed the important hypothesis that all cells in the adult body will inevitably undergo apoptosis unless they receive a sufficient supply of survival signals [65]. By contrast with the highly conserved death pathway in the metazoan cell, there is growing evidence that survival factors are integrated to provide exquisitely lineage-specific signals, which are spatially localized to ensure that cells that wander from their correct home do not prosper but undergo apoptosis instead [66]. We showed that insulin-like growth factor-1 (IGF-1) and IGF-II were able to inhibit apoptosis induced in cultured mesangial cells by serum starvation, etoposide-mediated DNA damage, or cycloheximide-mediated inhibition of protein synthesis [38]. However, the specificity of cytokine survival signals was emphasized by the failure of either platelet-derived growth factor, a potent mesangial cell mitogen, or epithelial growth factor, a potential survival signal for renal tubular cells, to provide survival signals to mesangial cells. Furthermore, there was evidence of IGF-1–mediated "paracrine" survival signaling, which might sustain expanded populations of mesangial cells, as medium conditioned by healthy mesangial cells was also able to inhibit apoptosis in "stressed" cells, a property specifically diminished by neutralizing antibody to IGF-1. Nevertheless, suitably sensitized mesangial cells were not rescued by IGF-1 from Fas-mediated apoptosis, demonstrating that survival signals can be overridden by Fas ligation and reinforcing the potential role of this cell death signaling system in "undesirable" apoptosis in glomerular disease [38, 67].

In nonrenal cell types, it appears that survival signals can also be supplied by ECM via heterodimeric cell surface receptors of the integrin superfamily [66, 68, 69]. Comparable data are now available for glomerular mesangial cells (abstract; Mooney et al, *J Am Soc Nephrol* 7:1760, 1996) [39, 70, 71]. Our own data raise the interesting possibility that changes in glomerular ECM typical of progressive disease might increase the susceptibility of glomerular cells to "undesirable" apoptosis (abstract; ibid) [70]. Thus, although normal constituents of mesangial ECM, such as type IV collagen and laminin supplied β1 integrin-mediated survival signals to serum-starved and etoposide-treated mesangial cells, it was notable that neither type I collagen nor plasma fibronectin signaled survival. These abnormal mesangial ECM constituents, which accumulate in progressive disease, may therefore disrupt survival signaling from matrix.

THE MACROPHAGE: MASTER OF THE DOUBLE-EDGED SWORD?

Accumulating data indicate that there are likely to be many potentially counterbalancing influences on whether glomerular cells survive, differentiate, divide, or die. These factors will be partially controlled by the constituent cells of the glomerulus itself—an example being mesangial cell production of IGF-I, which we have demonstrated to be a mesangial cell survival factor. Indeed, it is possible to speculate that changes in glomerular cell synthesis of ECM constituents, metalloproteinases, and their inhibitors might bring about an irreversible increase in susceptibility of glomerular cells to apoptosis.

Nevertheless, it is tempting to seek a cell type that might play the role of master swordsman, promoting restitution and resolution where these are possible, but deleting irreversibly damaged glomeruli by eliminating glomerular cells and allowing an accumulation of ECM. A few strands of evidence hint that the inflammatory macrophage could serve as a master cell in glomerular injury. First, the seminal work of Leibovich and Ross established a requirement for macrophages in wound healing [72]. We do not have access to a "clean" experiment probing the effects of macrophage depletion in Thy 1.1 nephritis (E. de Heer, personal communication), but this will be of great interest given the similarities between the early stages of this model and wound healing. Second, Lang and Bishop have provided elegant data showing that macrophages can also determine and trigger apoptosis in tissues undergoing remodeling, such as the leash of vessels that is eliminated from behind the lens in the developing rodent eye [73, 74]. This is intriguing given preliminary reports that cells of the macrophage lineage can trigger "cytolysis" of cultured mesangial cells by mechanisms potentially involving nitric oxide (abstract; Pugliese et al, *J Am Soc Nephrol* 8:A3008, 1997) [75, 76], data that we have recently extended to show that macrophages activated *in vitro* and *in vivo* can trigger apoptosis in mesangial cells (abstract; Duffield et al, *Exp Nephrol* 6:152–153, 1998). Given the capacity of the macrophage to both direct glomerular cell death (through release of FasL, TNF-α, IL-1β, nitric oxide, or reactive oxygen species and by degradation of normal mesangial ECM) or promote glomerular cell survival (by release of cytokine survival signals or inhibitors of matrix metalloproteinases), we speculate that "programming" of inflammatory macrophages may be critical in regulating apoptosis in glomerular cells [77].

CONCLUSIONS AND FUTURE WORK

Until the capacity exists to direct or inhibit apoptosis in glomerular cells at particular time points in the evolution and resolution of glomerular disease, no firm conclusions as to the significance of apoptosis in control of glomerular cell number and outcome of glomerular injury can be drawn. There are no data from experiments analogous to those that elegantly demonstrate a key role for mediators such as platelet-derived growth factor (PDGF) in glomerular cell proliferation, either by specific neutralization on the one hand [78] or by PDGF gene transfer on the other [79]. Nevertheless, the capacity of excessive apoptosis to disturb glomerular structure and function *in vivo* has been directly demonstrated by the administration of anti-Fas antibody, and we can look forward to much work in this area. Certainly there is much more to learn about the regulation of apoptosis in all three glomerular cell types [80, 81], and a long-term goal must be to analyze the significance of changes in glomerular cell number "set point" at various stages of glomerular injury, repair, or progression to scarring. This will require an integration of knowledge on control of glomerular cell division, survival, or death. It seems certain, just like being subject to death or taxes, that our current concepts will seem naive and simplistic in retrospect.

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