Apoptosis and the Kidney

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(J. Am. Soc. Nephrol. 1994; 5:12–21)

ABSTRACT

Apoptosis is a programmed form of cell death mediating the precisely controlled deletion of "unwanted" cells. This review discusses the key features of this cell death program, emphasizing that apoptosis is regulated by factors extrinsic and intrinsic to the dying cell. Furthermore, because apoptosis leads to the swift phagocytic clearance of intact cells, tissues are protected against the noxious effect of cell contents. Apoptosis occurs in the developing and adult kidney, and nephrologists now need to consider whether abnormalities of this program may contribute to renal disease. Evidence suggests that such defects could contribute to developmental abnormalities including polycystic disease, induce autoimmunity to renal tissue, and exacerbate renal inflammation and scarring. Finally, apoptosis may offer new avenues for therapy.

Key Words: Apoptosis, kidney, cell death, cell survival

A poptosis came of age in 1993, 21 yr after this "programmed" form of cell death was first described by Kerr et al. (1). The term apoptosis, which the originators pronounce appo-toe-stis, was suggested by a Scottish professor of ancient Greek and means "the dropping off as of leaves from a tree." This emphasizes that apoptosis is a physiologic form of cell death, occurs in the individual cell (or leaf) in an endogenously programmed pattern, can be triggered in the whole organism according to a program regulated by external stimuli (autumn), but at any one time may appear to be occurring at random. In keeping with the recent 21st birthday, research into apoptosis has grown into an exciting and rapidly expanding field that will have an important effect on the understanding of renal disease. This article will briefly outline the features and regulation of this mode of cell death, review the occurrence of apoptosis in the developing and adult kidney, and consider how this cell clearance mechanism might be perturbed in renal disease processes such as autoimmunity and inflammation. The objective is to stimulate readers' interest in the scope and significance of apoptosis, rather than to dwell on details that have been admirably reviewed elsewhere (2–4).

FEATURES OF APOPTOSIS

Apoptosis can be distinguished from the other major type of cell death, necrosis, according to various criteria that have evolved somewhat since the original description. These will be described very briefly.

First, apoptosis is a physiologic, programmed death mediated by the controlled deletion of "unwanted" cells. A powerful example of how cell elimination by apoptosis is normal and indeed desirable is provided by morphogenetic death during embryonic and postembryonic development (5). Necrosis is accidental, unplanned death occurring in response to sudden and severe cell injury due to hypoxia, extremes of temperature, toxins, and attack by complement and lytic viruses.

Second, apoptosis is defined by highly characteristic and remarkably stereotyped morphologic changes best confirmed by electron microscopy. As illustrated in Figure 1, which shows an apoptotic granulocyte within a glomerular capillary, there is condensation of nuclear heterochromatin, cytoplasmic condensation, and cell shrinkage with retention of organelles. Although videomicroscopy shows that these changes can occur in minutes (6), apoptotic cells maintain the integrity of the plasma membrane for hours, possibly because this may be strengthened by the cross-linking of proteins mediated by the activation of transglutaminases (7). In many cell types, the membrane may become ruffled and the cell may fragment into membrane-bound apoptotic bodies. By contrast, in necrosis, the plasma membrane is damaged and becomes abnormally permeable with the result that the cell swells and disintegrates. Organelles such as mitochondria are disrupted, and nuclear chromatin flocculates.

Third, apoptosis is an "active" form of cell death, in which the cell can be thought of as committing suicide rather than being the passive victim of a murderous attack, as in necrosis. A powerful illustration is that, whereas necrotic cells lose metabolic activity, apoptosis in certain situations requires...
Savill

Journal of the American Society of Nephrology

Figure 1. Apoptotic granulocyte in glomerular capillary (transmission electron micrograph; original magnification, x5,000). A case of vasculitis in which the glomerular capillary lumen is occluded by leukocytes. The apoptotic granulocyte (marked by white arrow overlying an erythrocyte) displays typical condensation of nuclear heterochromatin and cytoplasm and the retention of granules and other organelles.

Spicuous, as explained in later sections. Necrosis is a messy and conspicuous death.

Where the load of apoptotic cells is high, the clearance job is done by macrophages or "professional" phagocytes. However, in many tissues, cells undergoing apoptosis are ingested by neighboring cells capable of acting as "semiprofessional" phagocytes, such as glomerular mesangial cells (12) or renal tubular cells (Figure 2). Much remains to be defined of the cell surface molecules that allow phagocytes to recognize the "eat me" status of apoptotic cells (13). However, at least three phagocytic mechanisms have been partially characterized. The heterodimeric phagocyte αvβ3 "vitronectin" receptor mediates the ingestion of a number of different types of apoptotic cell by phagocytes such as macrophages and mesangial cells (14). This member of the integrin superfamily of adhesion receptors binds the glycoprotein thrombospondin (15), which "bridges" unknown moieties on the apoptotic cell to the phagocyte surface, where binding to receptors such as CD36 may cooperate with the ligation of αvβ3 (16). Some types of macrophages may use receptors recognizing phosphatidylserine, which may be exposed on apoptotic cells because of a loss of membrane asymmetry that normally limits this anionic phospholipid to the inner leaflet of the bilayer (17). Finally, macrophages and other cell types may be able to recognize changes in cell surface carbohydrate (18,19).

Last, apoptosis leads to the safe removal of cells by phagocytes, whereas necrosis leads to the inevitable leakage of cell contents, which provokes tissue injury and inflammation. Intact cells undergoing apoptosis and membrane-bound apoptotic bodies are swiftly recognized and degraded by phagocytic cells in vitro, protecting tissues against the release of the contents of dying cells. Apoptotic cells die a clean death, eaten and digested by phagocytes with such speed and efficiency that their passing is histologically inconspicuous, as explained in later sections. Necrosis is a messy and conspicuous death.

Figure 2. Apoptotic tubular cell within neighbor (light micrograph; original magnification, x1,000) from normal human kidney. The tubular lumen is marked by an asterisk. Note the typical shrunken cell with condensed chromatin within the phagolysosome of the normal tubular cell (arrow).
REGULATION OF APOPTOSIS BY EXTERNAL FACTORS

Stimuli external to the cell regulate apoptosis. Many cells require "survival" factors to prevent them from undergoing apoptosis by default (20). Indeed, in developmental neurobiology, the idea that cells may compete for limiting amounts of survival or " trophic" factors is well established (21). For example, both the in vivo and the in vitro administration of cytokines demonstrated that platelet-derived growth factor (PDGF) and insulin-like growth factor-1 are important survival factors for oligodendrocytes (22). Similarly, lymphocyte lines may undergo apoptosis if deprived of interleukin-2 (IL-2), whereas hematopoietic cell lines may require erythropoietin, IL-3, and colony-stimulating factors (23–25). In terminally differentiated cells incapable of mitosis, it is easy to separate the "survival" effect of a cytokine from the ability to stimulate cell division (i.e., a "growth factor" effect). In cells capable of division, a single cytokine may serve both purposes, perhaps signaling either survival or mitosis via different parts of the same transmembrane receptor (26). The dissection of intracellular second messengers keeping apoptosis in check is awaited with interest.

External stimuli can also initiate apoptosis. In many cases, these may be low levels of stimuli such as ionizing radiation or toxins that at higher levels would induce necrosis (3). The "suicide" of a stricken cell is probably preferable to eventual necrosis. The immune system exploits the external regulation of apoptosis in order to kill foreign cells. For example, cytotoxic T cells contain various agents capable of inducing apoptosis in targets, whereas tumor necrosis factor (TNF) can activate this suicide mechanism in susceptible cells (4). Cytotoxic T cells may also express a transmembrane protein of the TNF family known as the Fas ligand (27) because it binds to Fas (also known as APO-1; 28), a 45-kd member of a superfamily of transmembrane proteins including the TNF and nerve growth factor receptors (29). The ligation of Fas, which may be mimicked by the binding of antibodies, induces apoptosis in target cells bearing this receptor (29,30). Susceptible cells include thymocytes and activated mature peripheral T cells, and as will be discussed below, it is likely that Fas may have an important part to play in the elimination of self-reactive T cell clones. However, Fas is also expressed in the liver, lung, ovary, and heart. Because the Fas ligand is not only expressed by activated lymphoid cells but is also expressed in gut, lung, and kidney, it is clear that much remains to be understood about how apoptosis in a number of organs may be regulated by the coming together of cells bearing the "death signal" ligand and susceptible cells bearing Fas. Furthermore, the intracellular signaling pathways stimulated by the ligation of Fas will also be of great interest. Finally, in hepatocytes and other cells, the familiar cytokine transforming growth factor-β, may also act as a "death signal" (31).

INTERNAL REGULATION OF APOPTOSIS

The demonstration that cells contain genes capable of regulating their death by apoptosis has been one of the strongest stimuli for the recent explosion of interest in the field (32). Such genes have been discovered by a number of routes, including the dissection of the molecular pathology of cancer and viral infection. However, a fascinating path into the genetic regulation of programmed cell death has also been beaten by those studying genes identified by abnormalities of developmental cell death in the nematode Caenorhabditis elegans, in which the fate of every cell has been mapped (33).

One of the most surprising and instructive examples of a gene controlling apoptosis is provided by c-myc. This is a proto-oncogene thought to be crucial in signaling cell division after stimulation with growth factors and known to be deregulated and constitutively active in many tumors. Elegant work from two laboratories, one using cells expressing a DNA construct of c-myc regulated by β-estradiol (6) and the other using antisense oligonucleotide (34), has shown that c-myc expression can also drive cells into apoptosis. However, this only occurs when cells are deprived of survival factors (6). This mechanism probably serves to protect the organism from cancer due to mutations activating c-myc; such cells die unless they have some other mutation that enables them to live without survival factors.

A second example of interaction between internal and external factors is provided by the tumor suppressor protein p53. A mass of data, including elegant p53 gene knockout experiments, indicates that, in addition to many other functions, p53 protein may serve as a "guardian of the genome," promoting apoptosis in cells when DNA damage is inflicted by ionizing radiation or drugs (35). Intriguingly, p53 does not seem to affect apoptosis induced in thymocytes by other stimuli.

However, "anti-apoptosis" genes exist. The best established example is provided by the bcl-2 proto-oncogene, which was identified in patients with follicular cell lymphoma. A t(14:18) translocation results in the deregulated expression of bcl-2 because this gene is juxtaposed with the immunoglobulin heavy chain locus. A large body of data from gene transfection experiments has shown that bcl-2 can protect cells from a variety of stimuli inducing apoptosis, such as growth factor deprivation, activation of apoptosis-inducing genes such as c-myc, and DNA-damaging drugs (32,36–39). This ability may relate to antioxidant or other cytoprotective properties of
the Bcl-2 protein (40), which resides in the nuclear and mitochondrial membranes (37, 41). Intriguingly, the death-repressing activity of Bcl-2 may be counteracted by dimerization with a second protein, Bax (42), suggesting that relative levels of these two proteins may act as some form of "rheostat" governing cell survival or death. Indeed, such a rheostat may comprise products of bcl-x, a gene related to bcl-2: bcl-x RNA transcripts are alternately spliced to yield bcl-xL, which inhibits apoptosis like bcl-2, and bcl-xS, a smaller mRNA that remarkably promotes rather than inhibits apoptosis (43). The importance of the bcl-2 family of genes is emphasized by the demonstration that the ced-9 gene in C. elegans appears similar in both function and structure to bcl-2 (33), as does Epstein-Barr virus BHFRF-1, which may be important in "immortalizing" infected cells (44).

In outlining the features and regulation of apoptosis, I hope to have emphasized the scope and significance of this type of cell death. The following sections will now consider evidence that apoptosis occurs in the kidney and that disruptions of this program may promote renal disease.

**RENAL DEVELOPMENT**

Cell death in the development of the kidney has attracted little attention, even in comprehensive studies of renal organogenesis. However, prompted by the importance of developmental cell death in other organs, Coles et al. sought morphologic evidence of apoptosis in the embryonic and postnatal rat kidney (45). Their findings and those of others spell out important points relevant to apoptosis in the adult kidney.

First, to detect apoptosis, one needs to look in the right place at the right time. Waves of cell death occurred in two particular regions of the kidney but at different stages of development. In the medullary papilla, death peaked at postnatal Day 7, whereas in the nephrogenic zone, most cell death was observed several days before birth. However, at both sites, apoptosis had diminished from peak values, where ~3% of nuclei had typical changes, to about 0.1% within a few days. Apoptosis may be overlooked unless histologic studies are timed correctly.

Second, a small amount of detectable apoptosis indicates a large amount of cell death because dying cells are rapidly ingested and degraded by phagocytes. Electron microscopy of developing rat kidneys revealed that almost all of the apoptotic cells had already been ingested by other cells, usually neighbors. Furthermore, when epidermal growth factor (EGF) was administered in an attempt to prevent apoptosis (see below), the number of detectable apoptotic cells fell by 50% in less than 2 h, implying that the average "clearance time" of dying kidney cells is at most 2 h (45). An identical estimate was made in the developing rat eye, where the proportion of rat retinal ganglion cells undergoing apoptosis was only 0.5%, and yet about 50% (i.e., 100,000) are known to die over several days (46).

Third, apoptosis in susceptible cells is regulated. The administration of a candidate exogenous "survival" factor such as EGF reduced apoptosis in the nephrogenic zone, particularly among "surplus" mesenchymal cells that had not been induced to form nephron epithelium (45). Indeed, in a transfiber organ culture system, Koseki et al. found that EGF "rescued" explanted rat metanephric mesenchyme from apoptosis, as did embryonic spinal cord, a ureteric bud substitute (47).

Last, there is strong circumstantial evidence that disrupted apoptosis may cause abnormality or disease. The bcl-2 proto-oncogene plays a key role in protecting cells against apoptosis (see above). The Bcl-2 protein is expressed in the developing rodent kidney, where Coles et al. (45) suggested that apoptosis may play an important function in controlling epithelial differentiation by "balancing" numbers of metanephric mesenchyme cells and ureteric bud cells. The disruption of this balance may underlie the exciting discovery by Korsmeyer's group that bcl-2-/- "knockout" mice develop polycystic kidneys soon after birth (48). The interested reader is referred to their elegant article for a detailed discussion of the possible relationships between disordered apoptosis and animal models of polycystic disease (48). However, a causal relationship between dysregulated apoptosis and human polycystic disease is as yet unproven, although it is intriguing that increased tubular cell apoptosis has been reported in human polycystic kidney disease (49). Whether abnormalities of bcl-2 gene family expression occur in human polycystic disease requires urgent examination, as does the possibility that bcl-2 family genes subserve functions in the kidney in addition to known roles in controlling apoptosis.

**GLOMERULAR APOPTOSIS IN THE ADULT**

Harrison was the first to report light microscopical evidence of apoptosis in the diseased human glomerulus (50). The paucity of apoptotic cells precluded identification of their lineage by electron microscopy. However, it was suggested that not only resident glomerular cells but also infiltrating leukocytes might be eliminated by apoptosis, because apoptotic bodies were especially prominent in glomeruli containing numerous neutrophils. Harrison proposed that apoptosis serves as a homeostatic mechanism allowing hypercellular glomeruli to return to normal. A few years later, it appears that he was right.

First, in rat nephrotoxic nephritis, we found clear morphologic evidence that infiltrating neutrophils undergo apoptosis, leading to rapid ingestion and
degradation by phagocytes (12). Furthermore, this occurs in humans with nephritis of similar intensity (Figure 1). The potential significance of this leukocyte clearance mechanism is discussed below in the section on inflammation.

Second, we and Yamanaka’s group now have preliminary evidence that, during the resolution of mesangial proliferative nephritis, excess glomerular cells are indeed removed by apoptosis (Baker et al., unpublished observation; 51). Both groups studied nephritis induced in rats by Thy1.1 antiserum (52), in which brisk mesangiolysis is followed by mesangial cell proliferation, peaking about 5 days later. This results in an up to twofold increase in the number of mesangial cells, but both cell number and glomerular morphology eventually return to normal, indicating that “unwanted” cells are eliminated. At various times during the evolution and resolution of Thy1.1 nephritis, we counted apoptosis by light microscopy, by fluorescence microscopy of sections stained with propidium iodide, and by in situ end-labeling of fragmented DNA with terminal deoxytransferase. In normal rat glomeruli, about 0.01% of glomerular cells appeared apoptotic, but this rose to a peak of about 0.25% soon after maximum proliferation. Given a clearance time of about 2 h, this suggests that, at this stage, about 3% of glomerular cells are eliminated each day. Because the peak number of all types of glomerular cell reached in the model was about 25% above baseline, it is easy to appreciate that the apparently low incidence of apoptosis will nevertheless allow rapid clearance of excess cells.

We also investigated potential mechanisms triggering glomerular cell apoptosis. Because antibody blockade suggests that the expansion of mesangial cell numbers in Thy1.1 nephritis is driven in large part by the acute release of PDGF (54), it is possible that subsequent apoptosis reflects decreased availability of “survival” factors, which could include PDGF in view of its capacity to block oligodendrocyte apoptosis (22). We modeled lack of survival factors by depriving cycling cultured mesangial cells of serum and growth factors. Within 8 h, about 10% of cells had undergone apoptosis, as confirmed by morphology, DNA fragmentation into a typical “ladder,” and recognition of the apoptotic cells by healthy mesangial cell cultures (54). Furthermore, Ortiz et al. have preliminary in vitro data indicating that cultured mesangial cells express proteins regulating apoptosis such as Bcl-2 and Fas (55,56).

Obviously, much remains to be discovered about the incidence, regulation, and significance of glomerular apoptosis. In particular it will be necessary to confirm that excess epithelial and endothelial cells may be deleted by apoptosis. Nevertheless, available data reinforce lessons learned from the developing kidney. One needs to look at the right time and to appreciate the kinetic consequences of the rapid clearance of dying cells. Furthermore, glomerular cell apoptosis is likely to be regulated by external signals such as survival factors or Fas ligand and by endogenous proto-oncogenes.

**TUBULOINTERSTITIAL COMPARTMENT**

Studies of tubular cell apoptosis suggest that these same principles apply but also emphasize the potential importance in the kidney of another general characteristic of apoptosis—triggering by low-grade injury insufficient to cause necrosis. Thus, a seminal study by Gobe and Axelsen documented that tubulointerstitial atrophy after unilateral ureteric occlusion results from cell deletion by apoptosis, leading to the phagocytosis of apoptotic bodies by neighboring tubular cells (Figure 2) and the direct shedding of apoptotic cells into the tubular lumen (57). Subsequent work indicated that ureteric obstruction results in diminished EGF mRNA and protein in distal tubules and that the administration of EGF reduced tubular cell apoptosis (58). This suggests that low-grade insults such as obstruction may trigger tubular cell apoptosis by interfering with the local production of EGF or other survival factors.

Indeed, there is an impressive body of morphologic and molecular evidence implicating apoptosis in tubular injury by toxins and ischemia (56,59); when injury is severe, however, necrosis is probably the dominant mode of cell loss (60). A further note of caution is that, although injured tubules can increase the expression of clusterin (also known as SGP-2 and TRPM-2) in common with other cell lineages engaging the death program, this does not signify that tubular cells are destined to undergo apoptosis. Indeed, it may be a marker of survival because clusterin has complex cytoprotective properties, including the inactivation of complement fragments (61). Nevertheless, a preliminary study of folic acid nephropathy reveals a 50% reduction in renal bcl-2 mRNA expression and a large increase in Fas mRNA, suggesting that susceptibility to apoptosis may be increased by toxins (55,56). It will be particularly interesting to define which cells in the injured kidney express Fas because (at least in mice) Fas ligand mRNA is also expressed in renal tissue (30), although the cell type(s) responsible are also unknown. Nevertheless, these observations raise the intriguing possibility that populations of renal cells bearing the Fas ligand may “police” the size of other kidney cell populations in which pathologic stimuli induce Fas expression.

In keeping with studies of glomerular apoptosis, it also appears that apoptosis is a means whereby tubular hyperplasia may resolve (62). Whether this is also due to decreases in local survival factor supply is unclear, although apoptosis is induced in tubular
cells *in vitro* by serum deprivation in a manner similar to mesangial cells (56). At present, there is no direct evidence that programmed death may mediate the removal of interstitial renal cells such as fibroblasts, but this is to be expected in view of the extensive *in vitro* data indicating that fibroblasts are susceptible to apoptosis (6).

Having discussed apoptosis in the developing and adult kidney, I will now focus on the possible roles of perturbed programmed cell death in the major disease processes affecting the kidney—autoimmunity, inflammation, and scarring.

**AUTOIMMUNITY**

Autoimmunity to kidney tissue is increasingly regarded as an important early event in the pathogenesis of renal diseases such as glomerulonephritis. Apoptosis probably plays a key role in the regulation of the immune response (63–65), and there is now evidence that defects in the regulation of this program of cell deletion may be important in the development, maintenance, and severity of autoimmune responses. Furthermore, apoptosis may be triggered in cells under immune attack.

First, defects in apoptosis may result in a failure to delete self-reactive T cells. Normally, one important step in this process is likely to be the programmed death of thymocytes triggered by the ligation of the T cell receptor by self-antigen (64,65). A role for Fas also seemed likely because thymocytes express this receptor. Furthermore, murine Fas was shown to be encoded by the gene at the locus of the mouse lymphoproliferative locus *lpr*, which is an autosomal recessive mutation. MLR mice homozygous for this mutation develop lymphadenopathy and a lupus-like autoimmune disease (30). Such mice were shown to have defective expression of Fas protein as a result of a gene rearrangement, and their lymphoid cells resisted attempts to induce death by the ligation of Fas (30). However, although these data suggested that the ligation of Fas might be important in the thymic deletion of self-reactive T cells, thymic selection proved to be normal in *lpr/lpr* mice, the defect in the elimination of self-reactive clones being postthymic (67). This was in keeping with the subsequent failure to detect Fas ligand mRNA in the thymus (27), despite earlier suggestion that the thymic stroma might express this molecule and regulate thymocyte death (30). Furthermore, the propensity of MLR/*lpr* mice to develop autoantibodies and widespread tissue injury including nephritis may require interaction with additional genetic factors (67).

Nevertheless, resistance to apoptosis resulting in the failure to eliminate self-reactive T cell clones may also underlie the lymphadenopathy and autoimmune disease of transgenic mice overexpressing *bcl-2* (36).

Second, the defective clearance of intact dying cells by apoptosis resulting in the leakage of immunogenic contents may not only trigger, but also perpetuate an autoimmune response. This could occur if cells dying by apoptosis failed to develop surface changes recognized by phagocytes, or if there were a specific defect in macrophage capacity to clear dying cells in individuals susceptible to autoimmune disease. Although highly speculative, these hypotheses find some support in reports of circulating nucleosomes in lupus (68). This is not only evidence of the leakage of cell contents, but also emphasizes that this event may amplify autoimmune responses, because in *in vitro* evidence suggests that nucleosomes from noninjected disintegrating apoptotic cells have a potent capacity to induce lymphocyte proliferation (69). Furthermore, were the leakage of contents from uncleared cells undergoing apoptosis a hitherto unrecognized risk factor for the development of autoimmunity, it might help to explain the well-described link between infection and the initiation/exacerbation of autoimmunity because both viral and bacterial infections can trigger apoptosis in host tissues (6).

Third, the defective clearance of apoptotic cells and the undesirable inhibition of leukocyte apoptosis might both exacerbate tissue injury mediated by humoral and cellular autoimmunity. Because circulating nucleosomes may be bound in glomerular capillaries and the mesangium (67,70), the failure of phagocytosis of apoptotic cells with leakage of contents may target phlogistic autoantibodies to the kidney in diseases such as lupus. Furthermore, evidence from resolving viral infections indicates that activated cytotoxic CD8-positive T cells are normally eliminated by undergoing apoptosis (71). This is likely to be beneficial, reducing the number of cytotoxic cells and therefore reducing the risk of tissue injury. The "tissue load" of cytotoxic cells might therefore be increased with deleterious effects should apoptosis be inhibited by the undesirable availability of survival factors such as IL-2, the apposition of T cells to "activated" fibroblasts, or other stimuli leading to increased T cell expression of *bcl-2* (71).

Last, apoptosis can be a manifestation of (auto)immune attack by both antibody (54) and T cells (4). Thus, ironically, impaired elimination by apoptosis of potentially autoreactive immune cells might ultimately damage other tissues by the undesirable engagement of apoptosis.

**INFLAMMATION**

Inflammation evolved as a beneficial, self-limited response to tissue injury or infection. The elimination by apoptosis of intact infiltrating leukocytes is likely to be an important mechanism governing the reso-
olution of inflammation (72,73). Indeed, although both in vivo and in vitro evidence strongly supports apoptosis being an "injury-limiting" mechanism for the safe removal of senescent neutrophils and their injurious contents from inflamed sites (7,12), there is also growing evidence to suggest that this cell deletion mechanism operates in or near inflamed sites for eosinophils, monocytes, and lymphocytes (71,74,75). However, in renal disease, inflammation often appears to be uncontrolled, in that tissue injury is severe, or the response becomes persistent and leads to scarring.

"Uncontrolled" inflammation could arise from defects in the phagocyte uptake of leukocytes undergoing apoptosis. Supportive evidence in the form of circulating nucleosomal components in lupus, a persistent inflammatory disease, has already been cited (68). An extreme example of the failure of leukocyte clearance by apoptosis is provided by an abscess, in which neutrophils disintegrate. This presumably releases toxic contents such as potent degradative enzymes with potential not only to destroy tissue but also to amplify leukocyte infiltration by the cleavage of extracellular matrix proteins to yield chemotactic fragments, adding to the intrinsic chemoattractant activity of neutrophil contents such as the monocyte chemotaxin CAP 37 and the neutrophil attractant IL-8 (73). An abscess also emphasizes the importance of the inflammatory microenvironment in regulating cell clearance by apoptosis, because the pH of abscess fluid is known to be sufficiently acidic to block the phagocytosis of apoptotic neutrophils in vitro (76). Furthermore, other factors known to be present at inflamed sites such as fibronectin and vitronectin fragments might also block neutrophil clearance by interfering with the function of the macrophage \( \alpha_\beta_3 \) integrin (14).

In vitro data also point to a second, more subtle way in which leukocyte clearance by apoptosis might be perturbed to exacerbate rather than control inflammation. The "injury-limiting" potential of this leukocyte clearance is emphasized by the failure of cultured macrophages taking up apoptotic neutrophils to release proinflammatory mediators such as eicosanoids, granule enzymes, or cytokines. However, the macrophage uptake of cell debris consequent on the undesirable disintegration of apoptotic cells can trigger proinflammatory responses (77). Therefore, cell debris leaking from noningested dying cells may be doubly "dangerous to tissue health," causing injury both directly and indirectly by amplifying the inflammatory response.

SCARRING

The end result of poorly controlled autoimmunity and inflammation is scarring and loss of organ function. Possible links between disrupted apoptosis and scarring are the most speculative described in this article but may also be the most important. This is because, despite a growing understanding of the mediators driving renal scarring, such as transforming growth factor-\( \beta \) (78), we still have little insight into the initiation of irreversible scarring and no effective treatments.

However, it is important to appreciate that a key event in scarring, the accumulation of (myo)fibroblasts, is reversible. For example, myofibroblasts involved in the repair of skin wounds are eliminated by apoptosis (79). Whether this occurs in glomerular or interstitial renal disease is not known but is important to determine. Certainly, in glomerulonephritis, myofibroblasts can be recruited directly to inflamed glomeruli, and intrinsic mesangial cells may assume the characteristics of myofibroblasts in glomerular disease, including Thy1.1 nephritis in rats (80). Given the evidence cited above that myofibroblast-like mesangial cells can be eliminated by apoptosis, it is likely that this mechanism could be important in the elimination of myofibroblasts from the kidney. Whether this is the case and whether deficient myofibroblast elimination contributes to scarring remain to be determined.

CONCLUSIONS AND FUTURE PROSPECTS

Apoptosis is a precisely regulated and physiologic form of cell death that leads to the elimination of intact cells by phagocytes without inciting tissue injury. Apoptosis in any particular cell lineage is governed by exogenous influences, such as survival factors, and by the genes of the susceptible cell. Recently, it has been recognized that typical programmed cell death occurs the kidney and may be important in regulating the cell complement in both health and disease. Indeed, new data suggest that defects in the program of apoptosis might be hitherto unrecognized factors in the pathogenesis of a number of important renal disease processes. However, it should not be forgotten that apoptosis is a "double-edged sword"—the death program may be engaged in renal cells subjected to injury.

Future work on renal apoptosis will undoubtedly increase the understanding of the developmental abnormality and diseases involving autoimmunity, inflammation, and scarring. Indeed, dissecting how cell death is regulated in the kidney may prove to be just as important as defining how renal cell division is controlled. Ultimately, hastening the removal of "unwanted" cells might be a new mode of treatment for renal disease.

ACKNOWLEDGMENTS

The author is grateful for present and previous support from the Wellcome Trust and the Medical Research Council of Great Britain.
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