Apoptosis in renal disease: a brief review of the literature and report of preliminary findings in childhood lupus nephritis

Keriman Tınaztepe[1], Seza Özen[1], Şafak Güçer[1], Şükrü Özdamar[2]

Departments of [1]Pediatrics, and [2]Pathology, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Tınaztepe K, Özen S, Güçer Ş, Özdamar Ş. Apoptosis in renal disease: a brief review of the literature and report of preliminary findings in childhood lupus nephritis. Turk J Pediatr 2001; 43: 133-138.

Apoptosis, a programmed form of cell death, is an important mechanism that maintains cellular homeostasis. The cellular content of tissues is regulated by a balance between cell proliferation and cell loss. Apoptosis is important not only in physiological conditions but in pathological processes as well.

Apoptosis has been implicated in the pathogenesis of certain renal diseases. In human models, systemic lupus erythematosus (SLE) and IgA nephropathy have been the main interests. These studies have mainly shown that apoptosis is important in the control of mesangial cell population.

We have attempted to define the role of apoptosis in a cohort of childhood lupus nephritis. We have analyzed apoptosis by the terminal deoxynucleotidyl transferase (Tdt)-mediated dUTP-biotin nick end-labeling (TUNEL) method in eight SLE pediatric patients, two of whom had hereditary deficiencies of complement components. Although the sample size was small because of the rarity of hereditary complement deficiencies, we have shown that apoptotic activity was the greatest among these pediatric patients. It has been previously suggested that in lupus, autoimmunity develops as a result of inadequate clearance of apoptotic blebs containing nuclear elements; complement deficiencies are the most important hereditary factors predisposing to the inadequate clearance of apoptotic particles. This is the first time this hypothesis has been evaluated in the tissue samples of hereditary complement deficiency-related proliferative lupus nephritis. On the other hand, apoptosis was not different from the other mesangial proliferative glomerulopathies in the lupus nephritis samples. Further studies are needed to confirm our preliminary findings.

Apoptosis has been implicated in other renal diseases as well, such as autosomal polycystic kidney disease, and in experimental models. A short review of the relevant literature is presented highlighting the role of apoptosis in the pathogenesis and prognosis of certain renal diseases.

Key words: apoptosis, renal disease, systemic lupus erythematosus, hereditary complement deficiency, childhood.

Extensive studies during the past 30 years in histopathology, genetics and molecular biology have shown that virtually all animal cells are armed with the genetic machinery to commit suicide. Under normal conditions damaged or senescent cells sacrifice themselves through a non-inflammatory, energy-dependent form of cell death, termed apoptosis, for body cell homeostasis[1-3]. This type of cell death was first named by Kerr et al[3]. as apoptosis, taken from a Latin word describing the process of leaves falling from the tree or petals from a flower. Apoptosis is a programmed form of cell death. It is required for elimination of unwanted cells in processes as diverse as embryological remodeling, development of the immune repertoire, and resolution of inflammation. In turn, the mechanisms of apoptosis and removal of some cells are required to protect the organism from inflammation and autoreactivity[1].

A Worm's Tale: A Guide for Understanding the Molecular Aspects of Apoptosis

A number of detailed research as in the early 1930's in the nematode worm, Caenorhabditis elegans have shed light on the genetic and

molecular mechanisms of apoptosis[3]. In this worm, specific genes are activated to kill precisely 131 cells, leaving 959 in the adult worm[4,5]. Studies of this worm revealed that apoptosis has four consecutive episodes: 1- Extracellular or intracellular signaling to trigger committed cell death, 2- Cell killing by activation of intracellular proteases, 3- Removal of the apoptotic cell by other cells, and 4- Degradation of the cell corpse within the lysosomes[5]. These stages are controlled by CED (for cell death abnormal) genes in C. elegans which are highly conserved throughout animal evolution from worm to human. The products of CED-3 and CED-4 (homologous to human caspase) are required for execution of apoptosis while CED-9 (homologous to human Bcl-2) hinders apoptosis by inhibiting CED-3 and 4. CED-3 is a cysteine aspartyl protease (caspase) which activates a variety of cellular proteins such as DNA repair enzymes, components of nuclear membranes and endonucleases responsible for cleaving the DNA in the apoptotic cell. CED-3 is homologous to caspases in mammalian cells, while CED-4 is to apoptotic protease activating factor-1 (Apaf-1) and CED-9 is to the Bcl-2 family which protects apoptosis[4].

A number of switches are operative in the control of apoptosis. The main death signals are Fas, Fas-ligand, TNF, c-myc, and the main death promoters are Bad, Bax (Table I, Fig. 1). On the other hand, Bcl-2 is an intracellular, apoptosis inhibitory protein that inhibits Fas/APO-1 -induced apoptosis.

Table I. Genes Controlling Apoptosis in Mammals

Signals leading to apoptotic death	c-myc (myelocytoma oncogene) Fas (APO-1, CD-95) Tumor necrosis factor (TNF, Fas-ligand, etc.) Hid p53 E1A/E1B
Death promoters	Bad Bax Bak (Bcl-2 homologous) Bcl-x_s
Cysteine proteases (caspases)	Ced-3, ced-4 (Caenorhabditis elegans genes)
Survival promoters (anti-apoptotic)	Bcl-2 (B-cell follicular lymphoma, ced-9) Bcl- x_L

The apoptotic process begins with the shrinkage of the cell characterized by condensation of the cell cytoplasm and pyknosis of the nucleus. In the next stage, the nucleus becomes fragmented[4-6]. At the final stage the cell is broken up into several membrane-bound

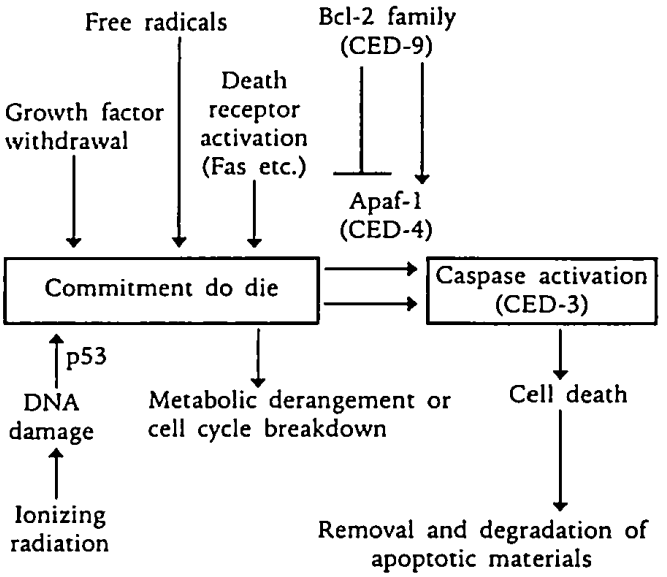

Fig. 1. A simplified scheme of the apoptotic program in mammalian cells[5].

vesicles, which include several intact organelles and nuclear fragments. These are called apoptotic bodies which are then internalized by adjacent cells or macrophages without inciting inflammatory events which might be caused by the uncontrolled release of noxious contents of the cell. Nuclear disintegration is exclusively achieved by a specific endonuclease (CAD-caspase–activated deoxyribonuclease). These enzymes act on multiple intracellular targets causing the formation of DNA laddering (endonuclease-mediated internucleosomal chromatin cleavage) and alterations of plasma membrane, including the exposure of phospholipid phosphotidylserine. Once initiated, the process quickly proceeds. Since the apoptotic cells are rapidly removed by neighboring cells and macrophages within 1-6 hours, only a few examples are visible in tissues. These cells are seen as shrunken cells with a dark pyknotic nucleus usually surrounded by a clear halo visible on light microscopy (Fig. 2). This is an energy–requiring process, differentiating apoptosis from necrosis, which requires no energy. The intracellular levels of adenosine triphosphate (ATP) also affect the form of the cell death[5]. For instance, in experimental studies, it has been demonstrated that apoptosis is the preferable mode of cell death in ATP-supplying conditions, whereas necrosis occurs in ATP-depleted situations. Based on the experimental data, it was concluded that ATP supply by glycolysis and mitochondrial respiration is needed for the final stage of apoptosis leading to nuclear condensation and DNA degradation.

ATP is also an important factor in the activation of executioner caspases that are triggered in the late stages of apoptosis[3-6].

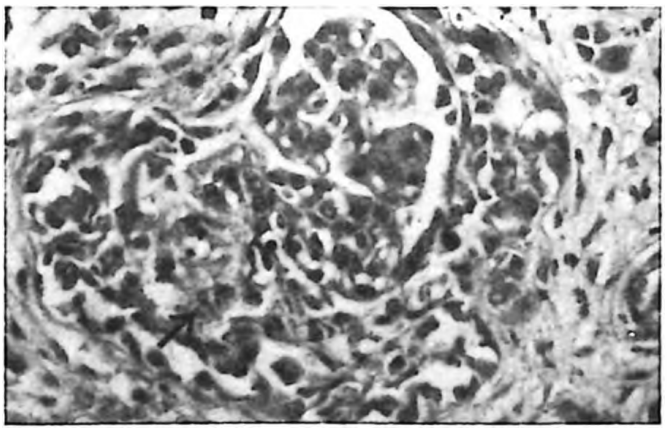

Fig. 2. The glomerulus with endocapillary proliferation with marked variation of its intensity among the segments of glomerular luft, focal karyorrhexis and complete epithelial crescent with partially obliterated Bowman's capsular space where pseudotubular formation is present in a case with C_{1q} deficiency (HE x 400).

Necrosis is different from apoptosis in many aspects (Table II). For example, cellular membranes remain intact during the apoptotic process, whereas cellular swelling, caused by loss of permeability and integrity of cell membrane, is one of the most prominent alterations during necrosis[4]. As a consequence, the disturbance of cellular membranes results in secondary damage of intracellular organelles, such as mitochondria, endoplasmic reticulum and polysomes. In the later stages of necrosis, rupture of plasma membrane and mitochondrial swelling are followed by a spilling out of the cytoplasmic organelles and release of lysosomes. The ruptured lysosomes release hydrolases leading to accelerated cellular disintegration. During the later stages of necrosis, nuclear DNA is degraded in nonspecific fragments.

Apoptotic cell fraction can be easily identified and quantified by Terminal deoxynucleotidyl transferase (Tdt)-mediated dUTP-biotin nick end-labeling (TUNEL) method, as described by Gavrieli et al[7]. The reaction is so specific that only apoptotic nuclei are stained. The method is based on the specific binding of Tdt to 3'-OH ends of DNA following synthesis of a polydeoxynucleotide polymer. Nuclear DNA on renal sections is first exposed to proteolytic treatment, and then Tdt is used to incorporate biotinylated deoxyuridine at sites of DNA breaks. The signals were amplified by avidin-peroxidase system enabling conventional histochemical identification by light microscopy[7,8] (Figs. 3 and 4).

Table II. Comparison of Cardinal Features of Apoptosis and Necrosis

Cardinal features	Apoptosis	Necrosis
Triggers leading to	Physiologic or pathologic conditions without ATP depletion	Severe hypoxia, toxins, conditions of ATP depletion
Biochemistry		
ATP requirement	Yes	No
DNA fragmentation	Laddering of DNA internucleosomal cleavage as 185 base pairs	Randomly
Protein	Caspase activation	Nonspecific degradation
Substrates	Specific substrates	Nonspecific hydrolysis
Histology		
Cell	Chromatin condensation, single deaths, apoptotic bodies	Cellular swelling, death of group of cells, disintegrated nucleus
Organelles	Intact	Damaged
Mitochondria	Swelling, cytochrome-C release	ATP-depleted, swollen, ruptured
Plasma membrane	Intact, blebbed	Disrupted, enhanced permeability
Removal of death cells	Adjacent cells	Immigrant phagocytes
Tissue reaction	Little or none	Inflammation

Fig. 3: Numerous apoptotic bodies with preserved cellular membrane in the glomerular tuft (stained in black) detected by the TUNEL method in the case with C1q deficiency. Note the silhouette appearance of the crescentic glomerulus in the background (x 640).

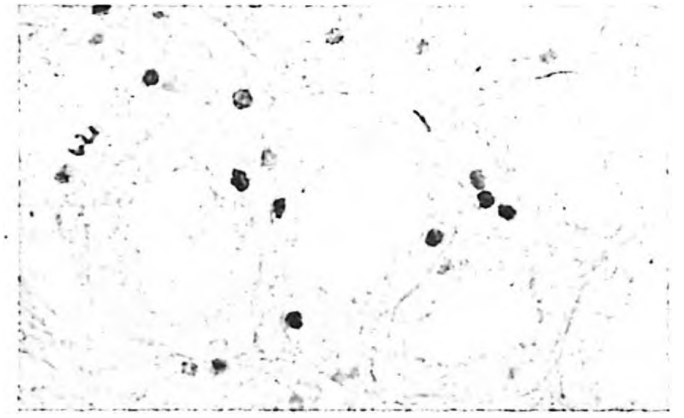

Fig. 4: Same case. Extensive apoptosis in tubuli detected by the TUNEL method (x 400).

Recently, the role of apoptosis in the pathogenesis of renal diseases has attracted much attention[1,2]. Apoptosis has contradictory effects in kidney diseases[2] . In certain experimental models of proliferative glomerulonephritis, apoptosis has been suggested to be an essential process in the regulation of endothelial or mesangial cells, in the repair stage[9-11]. Apoptosis has been assigned a beneficial role in proliferative glomerulopathy induced by anti-Thy-1.1 antibody administration. In this model apoptosis has been suggested to be necessary for regulating the number of intrinsic endothelial cells and for removing the unwanted cells of inflammation[10,11]. On the other hand, apoptosis has been implicated in the sclerotic process within the kidney: apoptotic bodies have been demonstrated in the sclerotic areas of experimental and human renal diseases[10,11]. Makino et al.[12] observed TUNEL-positive cells mainly in the sclerotic lesions. Again, the role of apoptosis in sclerosis was evident in the remnant kidney model in rats[12]. Thus although apoptosis may initially be a regulatory mechanism, 'uncontrolled' apoptosis seems to be involved in the sclerotic process. In the present review, summarize the findings of the various studies on apoptosis in certain renal diseases, and present our data in lupus nephritis in children.

Apoptosis and Glomerulonephritis

The experimental model of Thy-1 glomerulonephritis is a reversible model of glomerulonephritis. In this model it has been demonstrated that apoptosis is the major mechanism mediating the resolution of glomerular hypercellularity. In a study of human IgA nephropathy, there was no correlation between glomerular cell proliferation and Fas antigen expression[11]. In the same study there was remarkable Fas Ag expression in glomeruli of proliferative lupus nephritis.

Apoptosis and Glomerulosclerosis

In the experimental model of anti-GBM glomerulonephritis, induced in WKY rats, apoptosis was associated with sclerosis. In human IgA nephropathy, TUNEL (+) cells were mainly observed in the sclerotic lesions. Makino et al.[12] concluded that in these models, apoptosis in glomerulosclerosis is "uncontrolled apoptosis".

Apoptosis and Systemic Lupus Erythematosus (SLE)

Exciting findings in the study of apoptosis have shed light on the pathogenesis of SLE[13,14]. Increased apoptosis has been detected in the peripheral blood of SLE patients. We and others have shown that lymphocyte apoptosis was significantly increased in SLE patients as compared to controls. In our study, lymphocyte apoptosis was significantly increased, and annexin stainings for lymphocytes in SLE patients (n:6) and healthy controls (n: 12) were 11.3 ± 3.3 and 1.2 ± 0.9, respectively. Lymphocyte apoptosis was significantly increased as compared to controls ($p = 0.008$, CI: 1.9 - 18.2).

Along with this increased apoptosis in SLE, there is impaired removal of apoptotic cells. This is because phagocytosis of apoptotic cells is defective in SLE. Complement system is also required in the processing and disposal of these apoptotic particles. Thus it has become clear that the main reason for the association of complement deficiencies with SLE is the inefficient removal of the apoptotic products.

When endonucleases cleave the chromatin, the resultant product is a unit called nucleosome, with a stretch of DNA covered by histones. Exposure to abnormal normally sequestered amounts of nuclear Ag and nucleosomes elicits the autoimmune response[1]. In fact, it has now become clear that the autoantibodies in SLE nephritis are mainly directed against nucleosomes and not DNA. The positively charged histones may also explain the trapping of these immune complexes at the negatively charged glomerular basement membrane[1].

Subsequently these apoptotically derived self-antigens, particularly nucleosomes, drive a T-cell dependent autoimmune response to formation of autoantibodies. There is persistence of inflammation with these unremoved self-antigens[14].

In SLE, peripheral tolerance to self-antigens is also impaired, resulting in more autoantibody production; recent evidence suggests breakdown of peripheral deletional mechanisms and a lack in control of B cells. This results in aberrant polyclonal activation of B cells favoring autoantibody formation. At this stage, environmental factors such as infections and genetic factors such as MHC repertoire may also be important[14].

The immune complexes comprised of nucleosomes and autoantibodies are the mainstay of the pathogenesis in lupus nephritis. A number of studies in proliferative lupus nephritis point to a decreased apoptosis. In tissue samples of Class IV lupus nephritis, an increased transcription of the Bcl-2 gene, an inhibitor of apoptosis, has been demonstrated[1]. Thus, glomerular trapment of autoimmune complexes is followed by depressed autoinflammatory mechanisms with impaired clearance of infiltrating cells. The persistence of these inflammatory cells provokes secondary molecules of inflammation. When proliferating cell nuclear antigen (PCNA) staining in used for the proliferative index and TUNEL staining for the detection of apoptosis, the imbalance between PCNA and TUNEL+ cells may define the severity of the disease[1].

The importance of regulating of apoptosis has also been confirmed in an experimental model. Bcl-2 is an apoptosis inhibitor. At about one year of age, Bcl-2 transgenic mice develop high titers of antinuclear antibodies and usually die of immune complex glomerulonephritis. Thus, the increased apoptosis with inefficient clearing mechanisms seems to be the main drive for lupus nephritis[5,13,14].

We have attempted to study apoptosis in proliferating lupus nephritis in childhood SLE cases. Of these, two cases had C1q and C3 deficiencies[15,16]. TUNEL staining of proliferative lupus nephritis patients (n: 7) were compared to staining in cases with mesangial proliferative glomerulonephritis (n: 4). There was no significant difference between these two groups. However, the glomerular TUNEL staining in two SLE patients with complement deficiencies was very high. The glomerular TUNEL staining was detected in 42.9 percent of all glomeruli of these two patients, whereas it was 20 percent in the control group. Although the numbers were not sufficient for statistical analysis, we aim to pursue these findings. This is the first tissue representation of increased apoptosis in complement deficient states.

Apoptosis and Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Recent studies have shown that increased apoptosis is a contributing factor for the development of cysts in this disease. Increased Bcl-2 mRNA staining accompanies the increased apoptotic index in the cystic lesions of ADPKD[1]. In conclusion, larger studies in human subjects will enlighten the role of apoptosis in the pathogenesis and/or development of the aforementioned kidney diseases.

REFERENCES

1. Mene P, Amore A. Apoptosis: potential role in renal diseases. Nephrol Dial Transplant 1998; 13: 1936-1943.
2. Savill J. Apoptosis and kidney. J Am Soc Nephrol 1994; 5: 12-21.
3. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with widespread implications in tissue kinetics. Br J Cancer 1972; 26: 239-257.
4. Amore A, Coppo R. Role of apoptosis in pathogenesis and progression of renal disease. Nephron 2000; 86: 99-104.
5. Hetts SW. To die or not to die. An overview of apoptosis and its role in disease. JAMA 1998; 279: 300-307.
6. Savill J, Rees AJ. Mechanism of glomerular injury. In: Davison AM, Cameron JS, Grünfeld JP, Kerr DN, Ritz E, Winearls CG (eds). Oxford Textbook of Nephrology (2nd ed). Vol. I. Oxford: Oxford University Press; 1998: 403-439.
7. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992; 119: 493-501.
8. Negoescu A, Lorimier P, Labat-Moleur F, et al. In situ apoptotic labeling by the TUNEL method: Improvement and evaluation on cell preparations. J Histochem Cytochem 1999; 38: 1177-1183.

9. Saville J. Regulation of glomerular cell number by apoptosis. Kidney Int 1999; 56: 1216-1222.
10. Shimizu A, Kitamura H, Masuda Y, Ishizaki M. Sugisaki Y, Yamanaka N. Glomerular capillary regeneration and endothelial cell apoptosis in both reversible and progressive models of glomerulonephritis. Contrib Nephrol 1996; 118: 29-40.
11. Yashimura A, Sugenoya Y, Ida S, et al. Expression of apoptosis-preventing Bcl-2 protein and inducing Fas antigen in glomeruli of IgA nephropathy. Contrib Nephrol 1996; 118: 48-53.
12. Makino H, Kashihara N, Sugiyama H, Sekikawa T, Ota Z. Role of apoptosis in the progression of glomerulosclerosis. Contrib Nephrol 1996; 118: 41-47.
13. Ozen S. Lupus nephritis (Abstract). Pediatr Nephrol 2000; 14: C29.
14. Salmon M, Gordon C. The role of apoptosis in systemic lupus erythematosus. Rheumatology 1999; 38: 1177-1183.
15. Berkel AI, Petry F, Sanal Ö, et al. Development of systemic lupus erythematosus in a patient with selective complete C1q deficiency. Eur J Pediatr 1997; 156: 113-115.
16. Tınaztepe K, Sanal Ö. Deficiency of the third component of the complement associated with recurrent pyogenic infections and lupus erythematosus-like features. Eur J Ped 1989; 148: 709.