

# Apoptosis: A mechanism for regulation of the cell complement of inflamed glomeruli

JOHN SAVILL

Renal Unit, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, England, United Kingdom

“Apoptosis” or “programmed cell death” is a regulated mechanism for deletion of “unwanted” cells. Recent research indicates a crucial role for apoptosis in a number of fields of biological science, including immunological tolerance, embryology and neoplasia. This article emphasizes the potential importance of this process in determining the glomerular response to inflammation. Thus, there is evidence that apoptosis in infiltrating leucocytes is a mechanism for clearance of such cells which is likely to promote resolution rather than persistence of glomerulonephritis. Furthermore, programmed death of intrinsic cells which have undergone unwanted proliferation may enable a pathologically hypercellular glomerulus to return to normal. However, before discussing these potential roles of apoptosis, current concepts of this process are summarized.

## Cell death: Necrosis versus apoptosis

Death of a nucleated eukaryotic cell can be classified into two distinct types [1, 2].

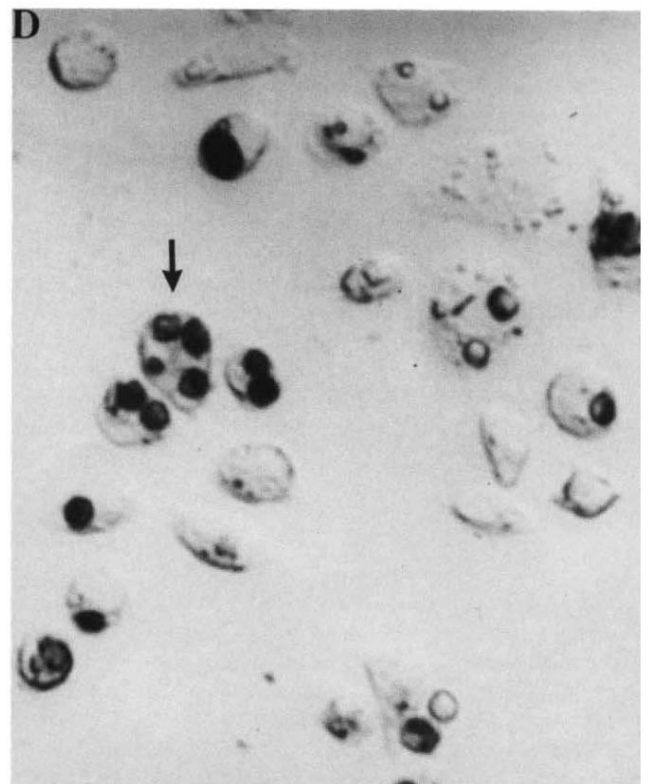
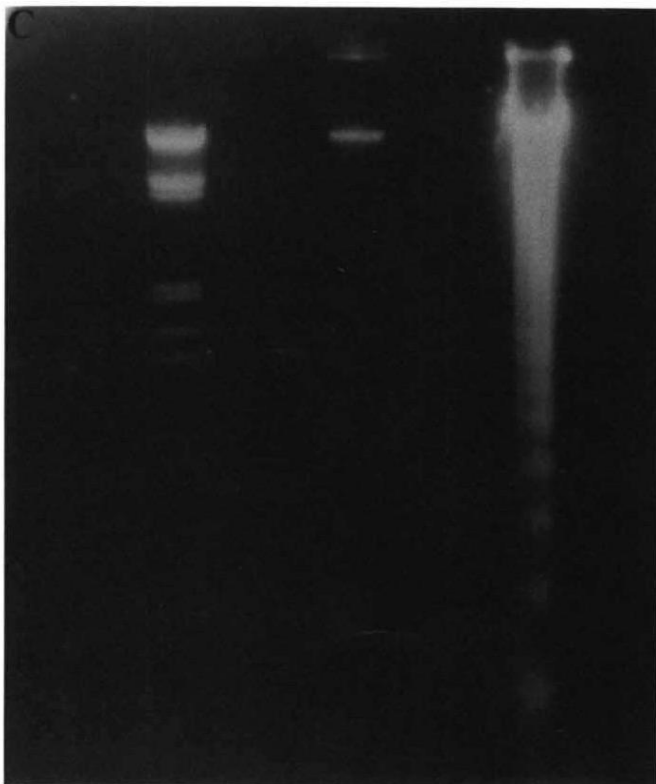
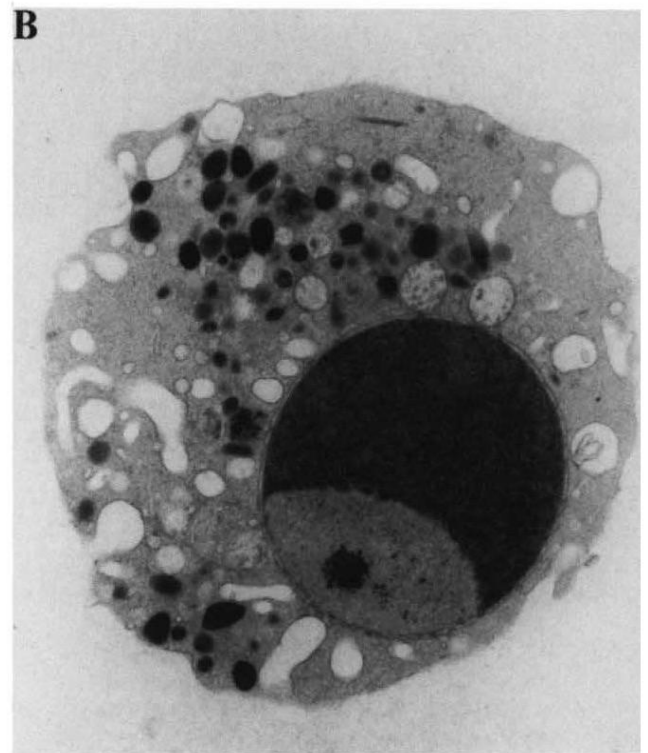
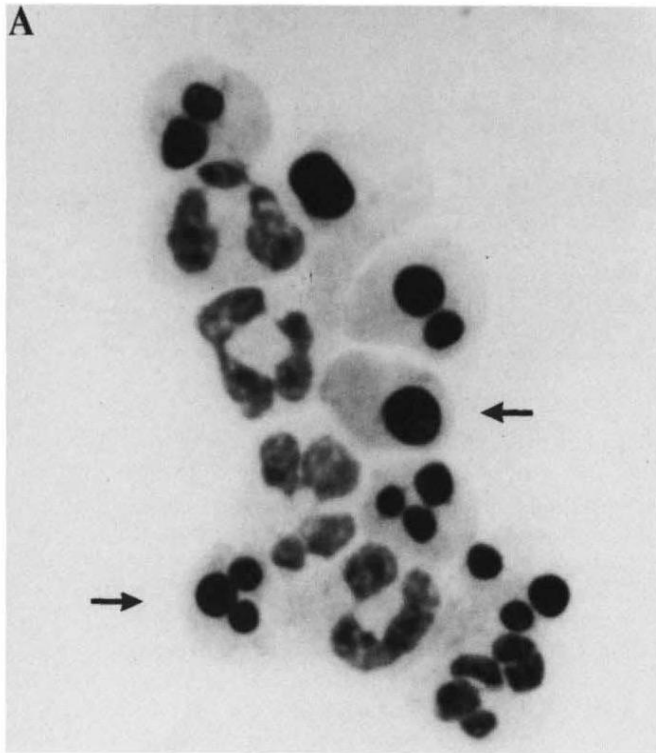
**Necrosis.** Necrosis occurs after gross insults to the cell, such as hypoxia, high temperature, and exposure to high concentrations of toxins, all of which inhibit membrane cation pumps. This type of cell death can also follow “attack” by complement components or lytic viruses, which directly damage the plasma membrane. In either circumstance, the cell becomes abnormally permeable to ions and water, undergoes reversible and then irreversible swelling and blebbing, and finally disintegrates. The structure of organelles such as mitochondria is disrupted, protein synthesis declines and there is flocculation of nuclear chromatin, which nevertheless remains in an approximately normal pattern. In an intact organism, necrosis is precipitated by stimuli which affect contiguous tracts of cells; consequently there is usually sufficient tissue damage to incite an acute inflammatory response.

**Apoptosis.** By contrast, apoptosis is a tightly controlled mode of cell death which is “programmed” in two ways [1, 2]. First, although there may be variations in detail, all cells undergoing apoptosis follow a basic program of biochemical and morphological events. Second, in many circumstances there appears to be an organized program of deletion of cells by apoptosis within an individual. This concept is best illustrated by developmental cell death in the embryo; in an exquisitely regulated manner, apoptosis leads to elimination of interdigital cells during remod-

elling of the solid limb paddle to form fingers or toes. Apoptosis programmed in this sense also occurs in endocrine-dependent tissue atrophy, metamorphosis, tumor regression and in the control of normal tissue turnover in many organs. Unlike necrosis, apoptosis frequently occurs in cells apparently distributed at random deep within a tissue. A further critical difference is that programmed cell death does not incite inflammation—indeed it is often thought of as being physiological rather than pathological. This connotation is implicit in the meaning of “apoptosis”, which in ancient Greek is the “falling off” as of leaves from an autumnal tree—a regulated, physiological event subject to control by external stimuli.

Apoptosis was first defined on the basis of a highly characteristic series of morphological changes [1, 2]. These begin with rounding up and shrinking of the cell associated with cytoplasmic condensation and loss of microvilli from the surface. The endoplasmic reticulum dilates but mitochondria and other organelles remain intact. There is rapid condensation of chromatin into dense crescent-shaped aggregates at the periphery of the nucleus, and complex nuclei such as that of the neutrophil may coalesce into a single body (Fig. 1A, B). Critically, the cell membrane remains intact for several hours (excluding vital dyes such as Trypan blue), and in vivo the apoptotic cell is swiftly recognized and ingested while still intact by local phagocytes, before final destruction by the lysosomal enzymes of the phagocyte. In some cells undergoing programmed death there is nuclear segmentation and budding off from the cell of membrane-bound “apoptotic bodies” containing cytoplasmic organelles and nuclear fragments. Phagocytes also recognize and ingest these bodies. Macrophages are the “professional phagocytes” removing apoptotic cells/bodies, but other cell types (including neighboring cells in epithelia or tumors) can participate. In vitro, apoptotic cells deprived of contact with phagocytes eventually undergo swelling and disintegrate.

In 1980 Wyllie associated the morphological changes of apoptosis with internucleosomal cleavage of chromatin into low molecular weight double-stranded fragments which are integer multiples of 180 to 200 base pairs of DNA [3]. These form a highly characteristic “ladder” on gel electrophoresis of extracted DNA, and this is now regarded as the biochemical hallmark of the process (Fig. 1C). This pattern is not seen in necrotic cell death, where prior to disintegration there may be digestion of chromatin into a continuous spectrum of sizes, indicating destruction of histones by proteases and exposure of the entire length of DNA to endonucleases. The cleavage



pattern of apoptosis can be mimicked by micrococcal nuclease in the presence of protease inhibitors, and reflects access of enzyme to linker DNA between nucleosomes, which may be

particularly vulnerable in transcriptionally active regions [4]. Consequently, it is widely assumed that the key event in apoptosis is activation of an endogenous endonuclease. Present

**Fig. 1. Features of neutrophil apoptosis.** **A.** Morphology. Photomicrograph of cytopspin preparation of human neutrophils isolated from inflammatory joint fluid and "aged" in culture for 8h. Many cells (examples arrowed) show condensation of cytoplasm and nuclear chromatin typical of apoptosis. Note ease of identification of apoptotic cells (approx  $\times 790$ ). **B.** Ultrastructure. Transmission electron micrograph of apoptotic neutrophil. Note intact plasma membrane, retention of granules, cytoplasmic vacuolation, and crescentic condensation of nuclear chromatin (approx  $\times 7900$ ). **C.** The ladder pattern of internucleosomal DNA fragmentation in apoptotic neutrophils (right lane). One percent agarose gel electrophoresis of DNA extracted from low molecular weight chromatin (resisting centrifugation at 25,000 g) of freshly isolated neutrophils (center lane) and apoptotic neutrophils (right lane). Left lane shows size standards [ref. 11 for details]. Note lack of fragmentation in low molecular wt DNA of freshly isolated cells. **D.** Recognition by glomerular inflammatory macrophages. Macrophages were isolated by enzymatic digestion of glomeruli from kidneys of rats with nephrotoxic nephritis, attached to tissue culture wells and interacted with apoptotic neutrophils for 30 minutes. Non-ingested neutrophils were washed away, and the monolayer fixed and stained for myeloperoxidase. The dark stained (orange-brown) globules within most macrophages (example arrowed) represent ingested neutrophils; the techniques used do not stain macrophage nuclei [11]. This appearance was not seen in macrophages incubated with freshly isolated neutrophils (inverted microscope, approx  $\times 316$ ).

evidence suggests that this enzyme is dependent upon  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  and inhibited by  $\text{Zn}^{2+}$ , but its full biochemical characterization has yet to be achieved [5]. A number of laboratories are hoping to clone and sequence this enzyme in the near future.

There has been intense interest in the intracellular events controlling apoptosis [6-9]. Cycloheximide and other inhibitors of protein synthesis do not have consistent effects upon model systems of programmed cell death; some are inhibited, others enhanced, and sometimes there is no effect. These observations have prompted Cohen et al [7] to propose that there may be (at least) three classes of control mechanism which regulate initiation of the final common pathway of endogenous endonuclease activation. Cycloheximide inhibits apoptosis induced in thymocytes by exposure to glucocorticoid, or low dose irradiation, indicating that there may be induction mechanisms which require new protein synthesis before apoptosis can proceed (a marked distinction from necrosis). Such controls are likely to play critical roles in deletion of T cell clones during the development of immunological tolerance. Thus, antigen receptor stimulation of lymphocytes of thymic lineage can induce apoptosis by such cycloheximide-sensitive mechanisms. Whether these pathways or those leading to proliferation are chosen by the cell may depend upon the balance of signalling pathways activated [8]. However, protein synthesis inhibitors have no effect on the more rapid, but nevertheless typical, morphological and biochemical changes of apoptosis which occur in cells exposed to cytotoxic T lymphocytes or agents such as gliotoxin, a fungal product. Thus there must be a class of transduction mechanisms which bypass the "induction" pathways and the associated requirement for gene expression. Finally, cycloheximide can accelerate apoptosis in cells apparently destined to die, such as neutrophils and other cells of myeloid lineage, which undergo apoptosis spontaneously. A number of investigators have postulated that this effect of cycloheximide reflects interference with synthesis of short half-life proteins which might normally keep either induction or transduction mechanisms in check; therefore Cohen has suggested the existence of a third class of release mechanisms involved in governing apoptosis. For example, although the mechanisms are not yet clear, both the bcl-2 oncogene and viral genes can suppress apoptosis [9].

Depending on cell type, these intracellular tiers of control may be subject to regulation by extracellular factors, just as climatic stimuli can influence autumn leaf fall. Thus programmed cell death can be initiated by hormones such as steroids or TNF, both may also follow withdrawal of growth factors, as in mature T cells deprived of IL-2. Furthermore,

IL-1, endotoxin and other agents can inhibit apoptosis in cells undergoing the process spontaneously or following exposure to inducing stimuli. These observations have fuelled interest in the cell surface receptors, signalling pathways and newly expressed genes involved in apoptosis. In therapeutic terms, these studies hold the ultimate prospect of selective induction of apoptosis in "undesirable" cells. For example, binding of a 52 kD surface protein by the monoclonal antibody APO-1 can induce programmed death in leukemic lymphocytes. However, this example also illustrates problems of specificity inherent in attempted therapeutic manipulation of a fundamental biological process; the APO-1 antigen is borne by a wide range of cell types [10].

#### Inflammatory leucocyte clearance by apoptosis

Recent studies indicate that inflammatory leucocytes undergo apoptosis leading to uptake by phagocytes. It appears that this is a mechanism for the safe disposal of leucocytes from inflamed sites [11].

Our work has focused on the archetypal inflammatory leucocyte, the neutrophil polymorphonuclear granulocyte. Summoned from the blood by rapidly generated chemotactic factors, it is the first cell to arrive at perturbed sites. The neutrophil has evolved systems which generate reactive oxygen intermediates, powerful degradative granule enzymes and toxic cationic proteins. However, these weapons of defence against invading micro-organisms can also inflict injury upon host tissues. Indeed, there is growing evidence that neutrophils and their toxic contents play a critical role in a number of inflammatory diseases.

Clearly, the removal of neutrophils is a prerequisite for the resolution of acute inflammation. Many have assumed that this is invariably achieved by neutrophil necrosis and disintegration in situ. However, this would inevitably expose tissues to toxic neutrophil contents with risk of exacerbation of injury, and could amplify leucocyte infiltration by the enzymatic generation of chemotactic fragments from extracellular matrix proteins such as fibronectin. An alternative disposal mechanism for inflammatory neutrophils, which avoids uncontrolled release of toxic contents, involves recognition and ingestion by macrophages of the senescent but intact granulocyte. This mode of neutrophil removal was first described around 100 years ago by Metchnikoff in his seminal studies of the resolution phase of experimentally-induced inflammation in animals, and has since been reported at inflamed sites on many occasions. Indeed, in models such as the experimentally inflamed peritoneum, which allow repeated sampling of the cellular infiltrate, phagocytosis

of intact cells appears to be the dominant route of neutrophil disposal.

In the last few years the mechanisms responsible for macrophage phagocytosis of senescent neutrophils have begun to be elucidated. Newman, Henson and Henson [12] reported that macrophages recognize and ingest intact neutrophils which have been aged overnight in culture; freshly isolated neutrophils are not recognized. Subsequently, using improved methods of neutrophil isolation and culture (such that after 24 hours *in vitro* > 98% of cells were viable by Trypan blue dye exclusion) we in Haslett's group were able to show that aging neutrophils spontaneously undergo typical morphological and biochemical changes of apoptosis, and that such apoptotic neutrophils are those recognized by macrophages [11]. *In vitro*, phagocytosis of apoptotic cells is extremely rapid, occurring in a few minutes, and once ingested, neutrophils are quickly broken down. Consequently, even if there is a large flux of cells undergoing apoptosis and recognition, neither free apoptotic cells nor macrophages containing very recently ingested intact neutrophils (when they can be identified as being apoptotic) will be frequent *in vivo*. This point is well illustrated by studies of tissue involution and resorption, where large areas of tissue undergo programmed death but few phagocytes contain recognizable apoptotic cells at any one time. Nevertheless, there is histological evidence of neutrophil apoptosis leading to uptake by macrophages in the inflamed human knee joint and respiratory tract, confirming the *in vivo* relevance of these processes. We view these events as constituting a neutrophil disposal mechanism which has potential to limit tissue injury. Thus, from the point of view of the neutrophil, undergoing apoptosis *in vitro* does not lead to spontaneous discharge of granule contents, indicating that neutrophils can be cleared without inevitable release of these histotoxic molecules. Furthermore, macrophage ingestion of apoptotic neutrophils does not incite secretion by the phagocyte of proinflammatory mediators such as eicosanoids or granule enzymes [13]. Indeed, these *in vitro* findings are entirely in accord with the general observation that removal of cells by apoptosis does not incite inflammation.

The lack of responsiveness on the part of the macrophage appears to be determined by the mechanism by which the apoptotic cell is recognized rather than the particle itself—opsonic receptor-mediated ingestion of apoptotic neutrophils coated with a rabbit antiserum does induce mediator release. The normal recognition of apoptotic neutrophils by monocyte-derived macrophages is specifically inhibited by cationic amino sugars and amino acids in a charge-dependent manner, which suggests that anionic groups on the neutrophil surface are important. These appear to be unaffected by broad-spectrum proteases. On the macrophage, a critical role for the  $\alpha_v\beta_3$  vitronectin receptor integrin was indicated by the inhibitory effects of peptides and proteins bearing the Arg-Gly-Asp tripeptide adhesion signal and monoclonal antibodies to both subunits of this integrin heterodimer [14]. Data presented in abstract form, elsewhere in this symposium, suggest that macrophage-secreted thrombospondin may be a component of a "molecular bridge" between macrophage  $\alpha_v\beta_3$  and the apoptotic neutrophil, but the cell surface structure determining recognition of apoptotic neutrophils as "senescent-self" remains to be determined. There is evidence from studies of rodent thymocytes induced to undergo apoptosis that either exposure of N-acetyl

glucosamine residues normally hidden in the mature glycan chains of the cell surface [2], or exposed phosphatidylserine (V. Fadok, P.M. Henson, personal communication), may underlie recognition by macrophages. However, lack of effect of the appropriate inhibitors suggests that different structures are involved in our system. Consequently, different cell types may employ different cell surface markers to signal their apoptotic status to phagocytes.

The potential benefits of apoptosis leading to the removal of senescent neutrophils before they disintegrate are self-evident. The apoptotic program is also available to monocyte/macrophages. For example monocytes cultured in the absence of serum or LPS spontaneously undergo programmed death, while inflammatory monocyte-derived macrophages can be induced to undergo apoptosis by agents such as gliotoxin. However, it is not known whether monocyte/macrophages undergo such a fate *in vivo*, or which factors might lead to this. Much work has been done on programmed death in cells of the lymphoid lineage, but is unclear whether antigen or other stimuli may lead to apoptosis rather than proliferation of lymphocytes at an inflamed site. However, some similarity with mechanisms relating to neutrophils is to be expected, since human peripheral blood lymphocytes induced to undergo apoptosis by irradiation are recognized via macrophage  $\alpha_v\beta_3$ .

#### Apoptosis of leucocytes in glomerular inflammation

In common with inflammation at other sites, neutrophils are implicated in the pathogenesis of glomerulonephritis. These cells can injure glomerular components *in vitro*, damage isolated perfused kidneys, and injure glomeruli *in vivo*, as shown by the effects of specific depletion and reconstitution of circulating neutrophils upon the initial self-limited phase of nephritis induced by heterologous nephrotoxic globulin. Neutrophils may also play an important role in the initiation of the later monocyte-dependent autologous phase (caused by development of antibodies to heterologous antibody fixed to basement membrane), where persistent inflammation progresses to scarring. Neutrophil infiltration of the glomeruli is much less striking than in the heterologous phase, but nevertheless, specific neutrophil depletion just prior to the onset of the autologous phase abrogates tissue injury. Moreover, in a model of lung inflammation monocyte influx was shown to depend upon initial neutrophil accumulation. Therefore, despite an apparent lack of neutrophil infiltration in certain types of human glomerulonephritis, this does not preclude a pathogenetic role for the cell at an earlier stage of the disease. Furthermore, neutrophils are prominent in the glomeruli of patients with acute renal injury associated with a wide spectrum of diseases ranging from systemic vasculitis to IgA nephropathy. Consequently, safe disposal of neutrophils is likely to be an important event promoting resolution of glomerulonephritis.

There is now evidence that neutrophil apoptosis leading to clearance by macrophages occurs in inflamed glomeruli. It should be appreciated that identification of apoptotic cells within glomeruli is not easy, given the likely speed of removal of these cells, the difficulties of picking them out within a densely cellular tissue, and the fact that at the light microscopic level it may be hard to differentiate the cell type undergoing apoptosis. Despite these difficulties, Harrison reported apoptotic cells in the glomeruli of patients with various forms of

nephritis, most frequently in those infiltrated by neutrophils [15]. With hindsight many of these cells appear to be apoptotic neutrophils, but their paucity precluded identification by electron microscopy, since the very thin tissue slices employed necessarily sample only a small volume of tissue. Recently, we have observed that inflammatory macrophages isolated from the glomeruli of rats with nephrotoxic nephritis contain apoptotic neutrophils in various stages of degradation, indicating that these had been ingested *in vivo*. Furthermore, glomerular macrophages preferentially recognize apoptotic neutrophils *ex vivo* (Fig. 1D). Taken together with Harrison's findings these data indicate that neutrophil apoptosis leading to uptake by inflammatory macrophages can occur in glomeruli, as at other inflamed sites [16].

However, cells other than the macrophage can ingest apoptotic cells. For over 20 years it has been known that the glomerular mesangial cell can clear particulate debris reaching the glomerulus. Attracted by the concept of the mesangial cell as a "glomerular dustbin", we have recently shown that cultured human mesangial cells can also ingest neutrophils undergoing apoptosis [16]. Furthermore, there is evidence that this occurs *in vivo*. In rats with nephrotoxic nephritis we have been able to detect neutrophil debris within mesangial cells immunopurified on the basis of Thy 1 expression [16]. Finally, albeit in a single case of acute glomerular injury, Arends and Harrison have obtained electron micrographs showing apoptotic cells within mesangial cells [17].

Our current hypothesis is that in glomerulonephritis, both inflammatory macrophages (derived from blood monocytes) and intrinsic mesangial cells mediate clearance of infiltrating leucocytes undergoing apoptosis. The quantitative contribution of each type of phagocyte, and the mechanisms and consequences of uptake of apoptotic neutrophils are under study.

#### Apoptosis of intrinsic glomerular cells

Abnormal proliferation of intrinsic glomerular cells, particularly mesangial cells, is a common consequence of glomerular inflammation. This may be a pathway leading to abnormal deposition of extracellular matrix and sclerosis or scarring of the glomerulus. However, a glomerulus can recover from abnormal proliferation of intrinsic cells. This is well illustrated by clinical examples such as post-streptococcal glomerulonephritis, or animal models such as the self-limited mesangial proliferation in the rat which follows mesangiolysis induced by antiserum to Thy 1 antigen.

Harrison suggested that apoptosis of unwanted intrinsic glomerular cells might allow a hyperplastic glomerulus to return to normal [15]. At present this attractive postulate remains highly speculative. However, this possibility draws support from evidence, detailed above, that programmed cell death can occur in the diseased glomerulus, and that glomerular cells can take up apoptotic cells. Furthermore, it is clear that apoptosis is a remodelling mechanism available to the kidney: a wave of apoptosis in tubular epithelial cells is observed during recovery from lead nitrate-induced renal hyperplasia. Indeed, there is evidence which suggests that proliferating mesangial cells can undergo apoptosis. Firstly, both mesangial cells in injured glomeruli and fibroblasts in injured skin acquire myofibroblast characteristics such as  $\alpha$ -smooth muscle actin. Elegant studies by Gabbiani's group show that during resolution and remodel-

ling of the inflammatory response induced by a skin wound there is a wave of deletion of myofibroblasts by apoptosis. This implies that stimulated myofibroblast-like mesangial cells might also be capable of undergoing apoptosis. Secondly, preliminary data from our laboratory and others indicates that cultured mesangial cells displaying myofibroblast phenotypic features can indeed undergo apoptosis. At present, it is not known if other glomerular cells are "turned over" by apoptosis, but there are indications that they have the program available; for example, TNF can induce apoptosis in vascular endothelial cells.

Finally, there are hints that alterations in the program of apoptosis may promote scarring in the kidney. For example, Muller and colleagues have shown that fibroblasts from human kidneys with interstitial fibrosis not only undergo more rapid growth and division in culture than cells from normal kidneys, but having entered the post-mitotic phase, they also take up to 20 weeks longer before they finally undergo apoptosis. Therefore, delay of apoptosis (by unknown factors) represents a mechanism by which "unwanted" cells could accumulate in the diseased kidney, just as the *bcl-2* oncogene can contribute to oncogenesis by promoting tumor cell longevity.

#### Conclusions and future prospects

The concepts and preliminary data presented here indicate that apoptosis is likely to play an important role in the inflamed glomerulus. In particular, it appears that programmed death may have beneficial effects, by promoting removal of infiltrating leucocytes and, perhaps, "unwanted" intrinsic cells. Since apoptosis can be regulated by external stimuli there is the (distant) prospect that this process might be regulated for therapeutic effect. It would be essential to be able selectively to delete a particular cell type, to know which cells to eliminate at any particular stage of nephritis, to understand how to persuade just the right number of cells to undergo suicide, and above all to do minimum harm to the patient! A more realistic prospect is that work on apoptosis in the glomerulus may provide new experimental approaches to unravelling the pathogenesis of persistent inflammation and scarring. Thus it may be possible to define factors which lead to death of neutrophils by disintegration and release of toxic contents likely to promote persistence of inflammation. This might occur either because of failure of phagocyte uptake of apoptotic cells, or by interruption of the death program such that senescent neutrophils undergo necrosis. Manipulation of such factors might prove to be of therapeutic benefit.

#### Acknowledgments

The author is in receipt of a Wellcome Trust Senior Research Fellowship in Clinical Science. Professor Chris Haslett, Dr. Andrew Wyllie (both of the University of Edinburgh), Dr. Laura Meagher and Professor Andrew Rees have played a crucial role in development of the ideas discussed here, while Frances Abbot, Jenny Smith and Yi Ren have made valuable experimental contributions.

*Reprint requests to John Savill, M.R.C.P., Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0NN, England, United Kingdom.*

## References

1. WYLLIE AH, KERR JFR, CURRIE AR: Cell death: the significance of apoptosis. *Int Rev Cytol* 68:251-306, 1980
2. DUVALL E, WYLLIE AH: Death and the cell. *Immunol Today* 7:115-119, 1986
3. WYLLIE AH: Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284:555-556, 1980
4. ARENDS MJ, MORRIS RG, WYLLIE AH: Apoptosis. The role of the endonuclease. *Am J Pathol* 136:593-608, 1990
5. COHEN JJ, DUKE RC: Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. *J Immunol* 132:38-42, 1984
6. GOLSTEIN P, OJCIUS DM, YOUNG JD-E: Cell death mechanisms and the immune system. *Immunol Rev* (in press)
7. COHEN JJ, OWENS GP, SMITH PA, TAYLOR MK, LANG JA: Programmed cell death and apoptosis in the immune system: Induction, transduction and release mechanisms. (abstract) *FASEB J* 5:A519, 1990
8. MCCONKEY DJ, ORRENIUS S, JONDAL M: Cellular signalling in programmed cell death (apoptosis). *Immunol Today* 10:120-121, 1990
9. WILLIAMS GT: Programmed cell death: Apoptosis and oncogenesis. *Cell* 65:1-2, 1991
10. TRAUTH BC, KLAS C, PETERS AMJ, MATZUKU S, MOLLER P, FALK W, DEBATIN K-M, KRAMMER PH: Monoclonal antibody-mediated tumour regression by induction of apoptosis. *Science* 245:301-305, 1989
11. SAVILL JS, WYLLIE AH, HENSON JE, WALPORT MJ, HENSON PM, HASLETT C: Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. *J Clin Invest* 83:865-875, 1989
12. NEWMAN SL, HENSON JE, HENSON PM: Phagocytosis of senescent neutrophils by human monocyte-derived macrophages and rabbit inflammatory macrophages. *J Exp Med* 156:430-442, 1982
13. MEAGHER LC, SAVILL JS, BAKER A, FULLER R, HASLETT C: Macrophage secretory responses to ingestion of aged neutrophils. *Biochem Soc Trans* 17:608-609, 1989
14. SAVILL J, DRANSFIELD I, HOGG N, HASLETT C: Vitronectin receptor-mediated phagocytosis of cells undergoing apoptosis. *Nature* 343:170-173, 1990
15. HARRISON DJ: Cell death in the diseased glomerulus. *Histopathology* 12:679-683, 1988
16. SAVILL J, SMITH J, REN Y, SARRAF CE, ABBOTT F, REES AJ: Glomerular macrophages and mesangial cells ingest neutrophils undergoing apoptosis. (submitted for publication)
17. ARENDS MJ, HARRISON DJ: Novel histopathologic findings in a surviving case of hemolytic uremic syndrome. *Hum Pathol* 20:89-91, 1989