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NEPHROLOGY FORUM

Apoptosis in post-streptococcal glomerulonephritis

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CASE PRESENTATION

An 18-year-old white female student presented to Hope Hospital, Salford, England, two years ago with a four-week history of general malaise and weight gain. The illness started with a seven-day episode of anorexia, sore throat, cervical lymphadenopathy, and pyrexia. The illness progressed over the next three weeks with the development of vomiting, diarrhea, and headache. One week prior to admission, she noticed swelling of her legs and abdomen; the swelling spread to her face, particularly the peri-orbital region. A 10 kg weight gain was noted during the week prior to presentation. Her primary care physician noted her blood pressure to be 170/105 mm Hg, and urinalysis disclosed 3+ proteinuria and 2+ hematuria.

None of the symptoms were referable to the urinary tract. Her medical history was unremarkable apart from a right lower lobe pneumonia two years previously. The patient was a non-smoker and was taking no regular medications.

On examination, the patient weighed 69 kg. She had peri-orbital edema and pitting edema of both ankles. Her blood pressure was 160/100 mm Hg. Funduscopy was unremarkable. No murmurs were audible on auscultation of the precordium, and the chest was free of rales and rhonchi.

The abdomen and oropharynx were normal. Dipstick urinalysis revealed 3+ protein and 2+ blood. Microscopy of the urine confirmed the presence of hematuria with >100 red cells and 60 white cells/mm^3. Red cell casts were present. Hemoglobin was 13.2 g/dL, with a white blood cell count of 6.6 $\times$ 10^9/L and platelets of 260 $\times$ 10^9/L. Clotting was normal, with a PT of 11 seconds and a PTT of 27 seconds. Urinary protein excretion was 1.7 g/24 h with a measured creatinine clearance of 64 mL/min. Laboratory values were as follows: serum sodium, 136 mmol/L; potassium, 4.3 mmol/L; urea, 8.4 mmol/L; and creatinine, 1.1 mg/dL (97 μmol/L). Liver function tests were within normal limits: AST, 32 U/L (normal, 5-45); alkaline phosphatase, 230 U/L (normal, 70-330); and ALT, 24 U/L (normal, 5-40). Corrected calcium was normal at 8.5 mg/dL (2.2 mmol/L; normal, 2.2-2.6 mmol/L) as was serum phosphate at 1.2 mmol/L (normal, 0.7-1.4 mmol/L).

Immunologic studies revealed an elevated ASO titer of 1200 IU/mL (normal, 0-200); normal immunoglobulin concentrations; depressed C3, 0.26 g/L (normal, 0.83 to 1.46); and borderline low levels of C4 at 0.19 g/L (normal, 0.20 to 0.52); ANCA, anti-GBM, ANA, and rheumatoid factor were negative. An atypical infection screen was reported as follows: herpesvirus < 16 IU; mycoplasma < 16 IU; Coxiella, < 8 IU; and Legionella < 8 IU. A throat swab sample taken on admission failed to grow any organisms.

Ultrasonography demonstrated two kidneys, 11 cm and 12 cm in length, with normal echotexture. A chest radiograph reported a cardiothoracic ratio of 15/29 with bilateral prominence of the upper lobe vasculature. The lung fields were otherwise clear. An electrocardiogram revealed sinus rhythm and was otherwise unremarkable.

The patient was treated with bumetanide, which resulted in a good diuresis. Her blood pressure declined to 145/88 mm Hg with the onset of the diuresis. Renal biopsy performed on day 5 revealed 11 glomeruli, none of which was sclerosed. All showed a global increase in cells, and the inflammatory infiltrate included polymorphonuclear leukocytes. There was no tuft necrosis, and no crescents were seen. The tubules, interstitium, and blood vessels all appeared normal. Immunoperoxidase stains revealed heavy basement membrane and mesangial staining for C3 with some of the basement membrane staining having a "hump"-like appearance. IgG stained only weakly. IgM, IgA, C1q, and fibrinogen were negative. Electron microscopy showed the presence of subepithelial humps; some subendothelial and mesangial deposits were also present. The pattern was consistent with post-infectious endocapillary proliferative glomerulonephritis.

Fourteen days after presentation, the patient was seen in the outpatient clinic. Her blood pressure was 130/80 mm Hg and body weight was 55 kg. She had no peripheral edema; diuretic therapy was discontinued. Dipstick urinalysis showed 1+ protein and 1+ blood. Serum creatinine was 1.22 mg/dL (108 μmol/L); creatinine clearance was measured at 55 mL/min. Red cells were noted in the urine but no casts were visualized. C3 was 0.4 g/L; C4, 1.1 g/L. Virology was repeated with no change in titer noted.

Three months after presentation, the patient was seen again.

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Her blood pressure was 120/75 mm Hg and her body weight was 56 kg; serum creatinine, 0.9 mg/dL (77 μmol/L); creatinine clearance, 75 mL/min; and 24-hour urinary protein excretion less than 0.05 g/day. Urinalysis was clear of blood and protein. The C3 had returned to normal at 1.1 g/L and the C4 was 0.3 g/L. Six months later the patient remained well with no alteration in weight, serum creatinine, or urinary protein excretion. Both C3 and C4 were normal. Urinalysis was clear and blood pressure was 125/75 mm Hg. Creatinine clearance was measured at 78 mL/min. The patient was discharged from follow-up.

DISCUSSION

Dr. John Savill (*Professor of Medicine and Director of the University of Edinburgh/Medical Research Council Centre for Inflammation Research, University of Edinburgh Medical School, Edinburgh, UK*): Although post-streptococcal glomerulonephritis (PSGN) may not be the problem it once was in the developed world, this condition remains important because it provides fascinating insights into the glomerular response to immune injury and because it remains a significant cause of nephritis in the developing world [1, 2]. The case exemplifies many of the clinical features widely considered to be typical of PSGN in a cooler climate (readers not expert in European weather patterns can be assured that Manchester and its environs enjoy a climate that many people would consider very cool). Thus, initial development of a sore throat of likely streptococcal origin, a delay of around two weeks, subsequent onset of a nephritic illness with salt and water retention, glomerular hematuria, and significant albuminuria with little impairment of excretory renal function all are characteristic of PSGN. Similarly, it comes as no surprise that this patient had marked suppression of circulating C3 levels, deposition of C3 and some immunoglobulin G in the glomeruli, infiltration by neutrophils, and increases in the number of intrinsic glomerular cells; these findings also can be regarded as "typical."

My discussion of this case will focus on what I regard as the most important characteristic of PSGN—the propensity for apparently complete resolution of the clinical abnormalities and, presumptively, the histologic changes. My discussion will focus on cellular mechanisms likely to mediate resolution of leukocyte infiltration and glomerular hypercellularity in PSGN, as the condition is an important exemplar of mechanisms that can promote spontaneous resolution of glomerular inflammation in other forms of post-infectious glomerulonephritis, in IgA nephropathy, and in Henoch-Schönlein purpura. Furthermore, similar mechanisms might mediate glomerular repair after immunosuppressive/anti-inflammatory therapy of systemic vasculitis and lupus nephritis. But I do not presume that PSGN always resolves. In light of evidence that a minority of patients with PSGN progress to end-stage renal failure [1, 3, 4], we will consider potential mechanisms for the persistence of glomerular hypercellularity that predisposes to glomerular scarring. Nevertheless, my approach is selective because it is impossible to cite all relevant primary studies. I need not review comprehensively the immunopathogenesis of PSGN, because Nordstrand and colleagues masterfully covered the topic in a 1999 article [5]. Indeed, since the data on cellular mechanisms mediating resolution of PSGN are so scanty, I will illustrate key principles by reference to animal model data on the resolution of nephritis induced by formation of complement-fixing immune complexes in situ in the glomerulus. Such models are broadly compatible with prevalent hypotheses on the immune mechanisms triggering PSGN.

It is widely assumed that nephritogenic streptococcal antigens such as streptokinase are deposited in glomeruli, leading to complement activation, C3 deposition, recruitment of immune cells, IgG deposition, and subsequent exacerbation of local glomerular inflammation [5]. Streptokinase, as everyone knows, has now entered the therapeutic armamentarium as a thrombolytic agent and, when administered to pre-immune individuals, can elicit the formation of circulating immune complexes and consequent "serum sickness" [6]. As this represents a mechanism by which immunity to streptococci can injure glomeruli, I also will present relevant unpublished data from studies of experimental glomerular injury by deposition of circulating immune complexes, studies previously reported by Professor Guy Neild of University College London Medical School [7].

Cell clearance

The established glomerular lesion of PSGN clearly poses a problem of cell clearance if the histologic abnormalities are to resolve. The first problem is marked infiltration with inflammatory myeloid leukocytes, mainly neutrophils and monocyte/macrophages. In the seminal studies by Hooke and colleagues, acute post-infectious cases of glomerulonephritis exhibited approximately 17 neutrophils and 18 monocytes per glomerular cross-section [8]. These data bolstered an earlier study by Ferrario et al, in which approximately 9 monocytes per glomerular cross-section were observed in a set of patients with documented PSGN [9]. Although some evidence suggests local monocytic cell proliferation [10], the vast majority of these leukocytes are likely to have been recruited through mechanisms including generation of chemoattractant complement fragments and local secretion of chemokines [11]. For resolution to occur, these infiltrating leukocytes must be cleared.

Second, established PSGN not only exhibits dramatic evidence of glomerular cell proliferation [10] but also displays obvious increases in the number of certain types of resident glomerular cells. For example, Ludwigsen and Sørensen reported peak increases in glomerular endo-

thelial cell number to 97% above baseline (that is, a near doubling) and in mesangial cell number to 67% above baseline [12]. The lineage-specific nature of the mitogenic factors locally released (or of the cellular response to such factors) is emphasized by the lack of a significant increase in glomerular visceral epithelial cells [12]. However, more work is required to characterize the proliferative factors operating in PSGN, although some data point to involvement of glomerular cell mitogens defined in vitro [13]. Nevertheless, whatever the mechanisms responsible for proliferation of glomerular mesangial and endothelial cells, resolution of PSGN clearly requires that the glomerulus remodel and delete excess intrinsic cells.

Cell kinetics

Investigation of inflammatory and resident cell kinetics in single renal biopsies from patients with PSGN is possible because the time of onset of the clinical abnormalities usually can be determined with some accuracy; thus, data from a series of patients can be assessed according to a time course of the disease. A study in Japanese patients, which employed immunohistochemical detection of proliferating cell nuclear antigen (PCNA), emphasized that proliferation of glomerular endothelial cells, mesangial cells, and macrophages was a short-lived phenomenon detectable only during the first three weeks or so of the nephritic illness, an excess of neutrophils still being apparent once this proliferative phase was complete [10]. Proliferation was most evident in glomerular endothelial cells, which represented approximately 80% of proliferating glomerular cells in the first week of the illness [10]; this finding is consistent with the peak in endothelial cell number (~90% above baseline) reported during weeks one to three in an earlier study of Danish patients [12]. Similarly, the later increase in mesangial cell proliferation to about 50% of all proliferating cells in the second and third weeks of clinical illness [10] was also consistent with earlier data indicating sustained increases in mesangial cell number (to ~60% above baseline) between the fourth and eighth week of illness [12]. Finally, low-grade macrophage proliferation, which disappeared after the third week of illness, was consistent with a Pan-American series of patients who exhibited a peak in monocyte/macrophage number to approximately 3 per 100 glomerular cells between two and four weeks [14].

Nevertheless, cessation in cell birth by mitosis cannot of itself explain the clear and dramatic fall in expanded populations of glomerular cells as PSGN resolves. Thus, in the series of Danish patients I mentioned, glomerular endothelial cell and mesangial cell numbers had returned to normal by approximately 8 and 24 weeks, respectively [12], whereas in the Pan-American series, macrophage numbers fell threefold when patients with disease of longer than four weeks in duration were compared with those biopsied two to four weeks after onset of symptoms [14]. Obviously, potent mechanisms must account for clearance of leukocytes and excess resident glomerular cells as PSGN resolves.

Apoptosis (programmed cell death), the physiologic mechanism for safe deletion of unwanted or excess cells [15, 16], is therefore an obvious candidate for returning the glomerular cell complement to normal as PSGN resolves. Intensive study of the molecular "program" of apoptosis over the last decade has achieved a remarkably complete characterization of the molecular mechanisms that result in the classic morphologic changes of apoptotic cell death [17]. These include condensation of cytoplasm and nuclear heterochromatin, cellular shrinkage with preservation of organelles and, in some cell lineages, very active blebbing of the dying cell, a process that sometimes leads to reduction in cell volume by the release of membrane-bound apoptotic bodies. A key event is activation of cysteine proteases termed "caspases" (because they cleave a variety of target proteins at aspartate residues), which are thought to disassemble the cell [18-20]. By cleaving an inhibitor [21], caspases also activate an endonuclease that leads to typical internucleosomal fragmentation of chromatin (so-called caspase-activated DNAse). This event can be detected in tissues by the TUNEL technique [22], which detects DNA cleaved by endonucleases, although great care is required to avoid artefactual DNA cleavage during tissue preparation. Oda and colleagues reported greatly increased TUNEL labeling in glomeruli in PSGN [10], thus reinforcing the candidacy of this programmed type of cell death in the resolution process.

We must recognize, however, that apoptosis is much more than programmed cell death—it is a program for swift and safe cell clearance. By contrast with accidental cell death or necrosis, in which pro-inflammatory release of injurious cell contents is inevitable, apoptosis in vivo almost invariably leads to recognition and rapid uptake by phagocytes of intact, membrane-bound apoptotic cells or bodies [16, 23]. In many tissues, this clearance job is carried out by healthy neighbors acting as "semi-professional" phagocytes, but where large numbers of apoptotic cells must be removed, the clearance job is performed by the professional scavengers of the body, macrophages. Phagocytic clearance of apoptotic cells in vivo is highly efficient and remarkably rapid; various strands of evidence indicate that in most tissues it takes an hour or less for dying cells to become recognizably apoptotic, be taken up by phagocytes, and be degraded beyond histologic detection after ingestion [23].

Clearance of glomerular leukocytes

Compelling evidence indicates that neutrophils and their toxic granular contents injure glomeruli, but until recently it was widely assumed that the usual fate of neu-

trophils recruited to inflamed sites was eventual disintegration before clearance of cellular fragments by macrophages [24]. This view ran counter to classic microscopic studies by Metchnikoff undertaken in the late 19th century, in which he identified the fate of inflammatory, extravasated neutrophils as uptake of intact cells by macrophages (the "big eaters" of the inflammatory response) [25]. However, after nearly a century of neglect, Newman and colleagues rejuvenated interest in Metchnikoff's seminal work with their observation that intact neutrophils "aged" overnight in culture were recognized and ingested by macrophages [26]. Haslett discovered that the change in aging neutrophil populations that led to their progressively increasing susceptibility to phagocytosis was constitutive apoptosis (defined as a spontaneous program of death arising from within the cell itself) and that an increasing proportion of neutrophils exhibited typical features of apoptosis with increasing time in culture [27, 28]. We demonstrated that apoptosis in aged neutrophils derived from blood or inflamed sites directed their recognition by macrophages, including human inflammatory macrophages ex vivo [28]. Furthermore, evidence clearly indicated that neutrophil apoptosis leads to clearance by macrophages in aspirates of joint fluid from patients with arthritis [28]. Indeed, subsequent work by Cox and colleagues reinforced the importance of constitutive granulocyte apoptosis leading to phagocytic removal in a model of self-limited lung inflammation [29]. These data were supported by clinical studies on neonatal respiratory distress [30] and asthma [31].

Does neutrophil clearance by apoptosis play an important role in resolution of PSGN? Animal models of immune-mediated glomerular injury suggest that it does. First, in nephrotoxic-globulin-induced nephritis in rats, we found clear evidence of neutrophil apoptosis leading to clearance both by glomerular inflammatory macrophages and mesangial cells acting as semi-professional phagocytes [32]. Second, in the ConA/anti-ConA model of immune complex nephritis, in which brief accumulation of neutrophils is almost exclusively limited to the lumen of glomerular capillaries and resolves in about 24 hours, we found clear histologic evidence of neutrophil apoptosis and phagocytosis of apoptotic neutrophils by intraluminal macrophages, especially where neutrophils were "trapped" in thrombosed capillary loops [33]. Indeed, the ability of intraluminal macrophages to ingest apoptotic cells has been beautifully demonstrated in previously unpublished electron microscopic images from Professor Guy Neild's studies of glomerular vascular injury in rabbits with a model of serum sickness (Fig. 1) [7]. Therefore, there is a prima facie case that neutrophil deletion by apoptosis could occur in PSGN.

Our work on ConA/anti-ConA nephritis in rats further emphasized that histologic studies might markedly underestimate the quantitative significance of neutrophil clearance from glomeruli by apoptosis [33]. First, when we followed the fate of infused radiolabeled syngeneic neutrophils, we found that during resolution of glomerular injury, about two-thirds of recruited neutrophils meeting their fate locally were no longer histologically detectable at 24 hours, presumably because they were rapidly degraded by phagocytes: 50% of detectable autoradiographic foci representing neutrophils retained at 24 hours were inside macrophages. We also concluded that about three-quarters of neutrophils sequestered in the lumen of glomerular capillaries left the kidney to meet their fate elsewhere; the methods employed did not allow us to track these neutrophils, but we had strong indications that the neutrophils had returned to the circulating blood pool, possibly because apoptosis had resulted in loss of adhesion to the glomerular capillary wall [33]. Nevertheless, despite the clear inference that histologically detectable neutrophil apoptosis in glomeruli represents "the tip of an iceberg," an important preliminary study of post-infectious glomerulonephritis by Szabolcs and colleagues (abstract; Szabolcs et al, *J Am Soc Nephrol* 5:844A, 1994) revealed an average of about one apoptotic cell per glomerular cross-section, 300-fold greater than the number in control healthy glomeruli. Thus, it does indeed seem likely that what we can now regard as the "normal" means of deletion of inflammatory neutrophils—efficient clearance by in-situ apoptosis—does operate in PSGN. Clearly, further work will be required to confirm this important hypothesis; the recent development of a mouse model of nephritis consequent upon slow leakage of streptococcal antigens from infected sites, a model based on subcutaneous implantation of a cage containing a streptococcal innoculum, is likely to be useful [34].

Is neutrophil deletion by apoptosis likely to promote resolution of PSGN? Answering this question will not be possible until we have experimental means by which to block selectively phagocytic clearance of apoptotic neutrophils in a good animal model of PSGN. But our data do emphasize the injury-limiting potential of this means of neutrophil deletion. In particular, in vitro studies revealed that neither macrophages nor mesangial cells taking up apoptotic neutrophils released pro-inflammatory mediators, such as eicosanoids [35] or chemokines [36]. Furthermore, elegant work in other laboratories has demonstrated that deliberately activated macrophages receive an important "anti-inflammatory" signal from ingesting apoptotic cells, which results in marked inhibition of the release of pro-inflammatory cytokines such as tumor necrosis factor α; this signal is mediated in part by autocrine/paracrine effects of the anti-inflammatory cytokine transforming growth factor-β1 (TGF-β1) [37, 38]. Therefore, deletion of neutrophils undergoing apoptosis via uptake by activated macrophages could be a key pro-resolution event in self-limited inflammation such as PSGN.

Although freshly isolated blood monocytes are suscep-

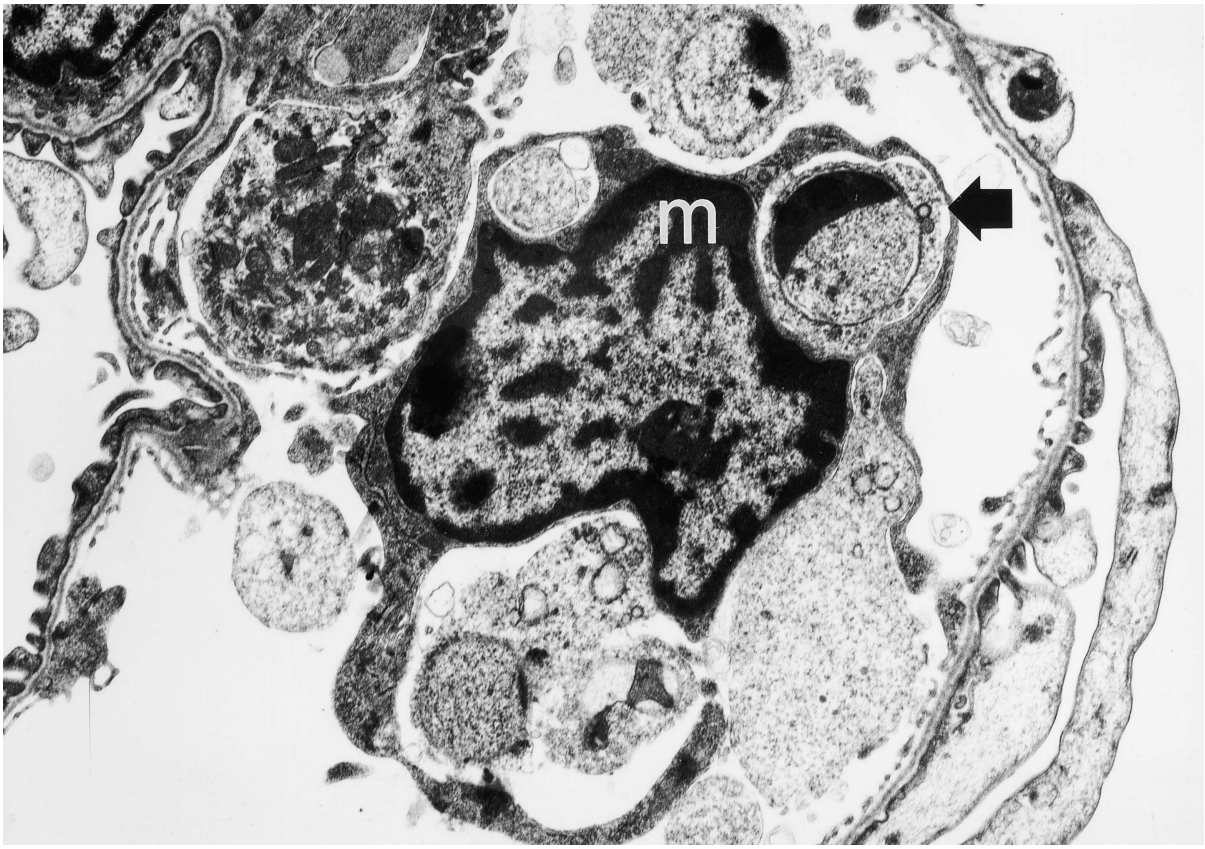

Fig. 1. Intraglomerular clearance of apoptotic cells. Electron micrograph of glomerular capillary from rabbit with glomerular injury induced by serum sickness/cyclosporine A [7]. Note intraluminal macrophage (m) that has ingested several dying cells; one ingested cell (arrow) has classic nuclear condensation of apoptosis (From Professor Guy Neild; magnification ×8000).

tible to apoptosis, maturation into macrophages coincides with development of resistance to many pro-apoptotic stimuli. Nevertheless, important studies by Lan and colleagues in a rat model of crescentic glomerulonephritis (crescents are, of course, observed in some cases of PSGN) not only emphasize the proliferative capacity of extravasated cells of the monocyte lineage [39], but also demonstrate that monocyte/macrophages can undergo apoptosis in situ [40]. This mode of elimination therefore might operate in PSGN.

Careful studies of the fate of labeled macrophages also have emphasized that, unlike neutrophils, emigration via the lymphatic vessels is a major route for removal of macrophages from inflamed sites [41]. Indeed, one of the earliest descriptions of this phenomenon—which urgently requires mechanistic characterization—was in nephrotoxic nephritis [42]. It seems possible that the ultimate fate of emigrating macrophages is to undergo apoptosis in draining lymph nodes, but this speculation requires formal testing.

Cells of the monocyte/macrophage lineage emigrating from inflamed sites might carry with them intracellular "parcels" of inflammatory cells that undergo degradation after recognition as apoptotic and ripe for removal. For example, in our study of ConA/anti-ConA nephritis, occasional macrophages bearing radiolabeled foci (compatible with earlier uptake of dying neutrophils) were observed in hilar lymph nodes [33]. Such observations could have great significance for regulation of immune responses in glomeruli in view of recent evidence that macrophage-like immature myeloid dendritic cells can ingest apoptotic cells and present certain antigens derived from the apoptotic "meal" via both major histocompatibility complex (MHC) class I and class II to CD8+ and CD4+ T lymphocytes, respectively [43–45]. This antigen presentation might promote tolerance rather than incite ongoing immune responses [46].

Clearance of resident glomerular cells

In PSGN, as in many other forms of glomerular injury, mesangial cells adopt a myofibroblast-like phenotype, expressing α-smooth muscle actin [47]. Abundant evidence from experimental proliferative glomerulonephritis indicates that excess myofibroblast-like mesangial cells are deleted from glomeruli by undergoing apoptosis [48, 49], which leads, in the main, to phagocytosis by

healthy neighbors [48]. Therefore it seems likely that some of the TUNEL-positive apoptotic cells identifed in PSGN [10] represent mesangial cells, although double-staining for α-smooth muscle actin would help confirm this possibility.

I recently reviewed the diverse stimuli that regulate mesangial cell apoptosis in vitro [50], so I wish to concentrate here on the possible importance of activated macrophages in directing mesangial cell clearance by apoptosis. Mené and colleagues reported that activated cells of the U937 macrophage line could induce "cytolysis" of mesangial cells upon co-culture [51]. Their work, together with that of Lang on macrophage-directed developmental cell death [52, 53], prompted us to examine whether activated macrophages isolated from experimentally inflamed rat glomeruli could regulate mesangial cell fate in co-culture experiments. Such macrophages and lipopolysaccharide/γ-interferon-activated bone-marrow-derived macrophages (but not quiescent macrophages) induced both suppression of mitosis and a dramatic increase in apoptosis, with the result that the mesangial cell population in co-culture was reduced [54]. Experiments with inhibitors and macrophages from relevant "knock-out" mice revealed that the "downsizing" effects on mesangial cells were mediated by products of macrophage-inducible nitric oxide synthase (iNOS or NOS2) in concert with another factor likely to be tumor necrosis factor-α (TNF-α). Our current hypothesis is that the macrophage "beats as it sweeps as it cleans" (Fig. 2). Thus, although activated macrophages can trigger apoptosis in unwanted or damaged resident cells [52-54] and accelerate apoptosis in bystander leukocytes [55], we speculate that when these cells ingest apoptotic cells, they are "deactivated" [38] or "reprogrammed" [56] to become "reparative" (for example, secreting the repair cytokine TGF-β1 [38]) and/or "emigratory" [33, 57].

Hints suggest that macrophage-directed mesangial cell apoptosis operates in PSGN, but the data are tenuous and require careful examination. Nevertheless, Hooke and colleagues' report of a relative dearth of glomerular macrophages in a small series of PSGN patients exhibiting mesangial hypercellularity with little neutrophil infiltration is intriguing [8].

In his early work on glomeruli injured by serum sickness/cyclosporine A, Prof. Guy Neild encountered microscopic evidence of glomerular endothelial cell apoptosis (Fig. 3) [7]. Furthermore, Shimizu and coworkers obtained clear evidence of (undesirable) endothelial cell apoptosis in progression to scarring of glomerular injury experimentally induced by nephrotoxic globulin [58, 59]. Indeed, Lang reported definitive evidence that macrophages can dock onto and induce apoptosis in unwanted microvascular endothelial cells in the developing rodent eye [52, 53]. It therefore appears likely that studies of glomerular endothelial cell apoptosis will yield new insights into the resolution of PSGN.

Potential mechanisms for failed resolution

Little is known about the mechanisms by which a small proportion of PSGN patients progress to persistent glomerular inflammation and scarring with eventual loss of renal function [1, 3, 4]. However, if apoptosis of infiltrating leukocytes and excess resident cells plays an important role in the resolution of PSGN, then derangements in cell clearance by apoptosis could be critical in promoting the persistence of tissue injury [60]. First, failed clearance of leukocytes undergoing apoptosis results in secondary necrosis of non-ingested apoptotic cells and leads to direct tissue injury by noxious cell contents as well as to indirect exacerbation of inflammatory injury due to stimulation of macrophage secretion of pro-inflammatory mediators [27, 60]. Additionally, failure of macrophage clearance of apoptotic leukocytes bearing ingested (streptococcal) antigens might allow such cells to be taken up by dendritic cells so that antigen is presented to T-cells, setting up a persistent and potentially damaging immune response [46]. Although a number of cell surface recognition mechanisms ensure that macrophages can safely ingest apoptotic cells [reviewed in 23 and 61], recent data suggest that lectin-like functions of C1q, the first component of complement, are particularly important in binding apoptotic cells in glomeruli and "bridging" them to C1q receptors on glomerular phagocytes [62, 63]. Indeed, since C1q "knockout" mice spontaneously develop a lupus-like disorder with glomerulonephritis, as observed in C1q-deficient humans [63], exhibit an excess of apoptotic cells in glomeruli consistent with diminished clearance [63], and possess diminished capacity to clear labeled apoptotic cells administered to the more accessible inflamed peritoneum [64], one can hypothesize that diminished C1q-mediated clearance of apoptotic cells in PSGN leads to persistent inflammatory injury, consequent upon leakage of leukocyte contents and dendritic cell presentation of antigens derived from ingested apoptotic cells, and to increased risk of progression to scarring.

Second, progression to hypocellular scar also could ensue if clearance of excess resident cells by apoptosis were undesirably prolonged, as in the case of glomerular endothelial cell loss in progressive nephrotoxic nephritis in rats [59]. Many possible stimuli could increase the role of unscheduled glomerular cell loss by apoptosis [reviewed in 50]. However, a unifying hypothesis is that failed clearance of apoptotic cells denies activated macrophages a crucially important anti-inflammatory signal (Fig. 2), so that they continue to trigger apoptosis in resident cells, with the result that excess glomerular endothelial and mesangial cells are lost and a hypocellular scar is formed.

CONCLUSIONS

Post-streptococcal glomerulonephritis warrants continuing detailed investigation because defining mechanisms by which inflammation resolves will provide exciting new insights into what goes wrong when glomerular inflam-

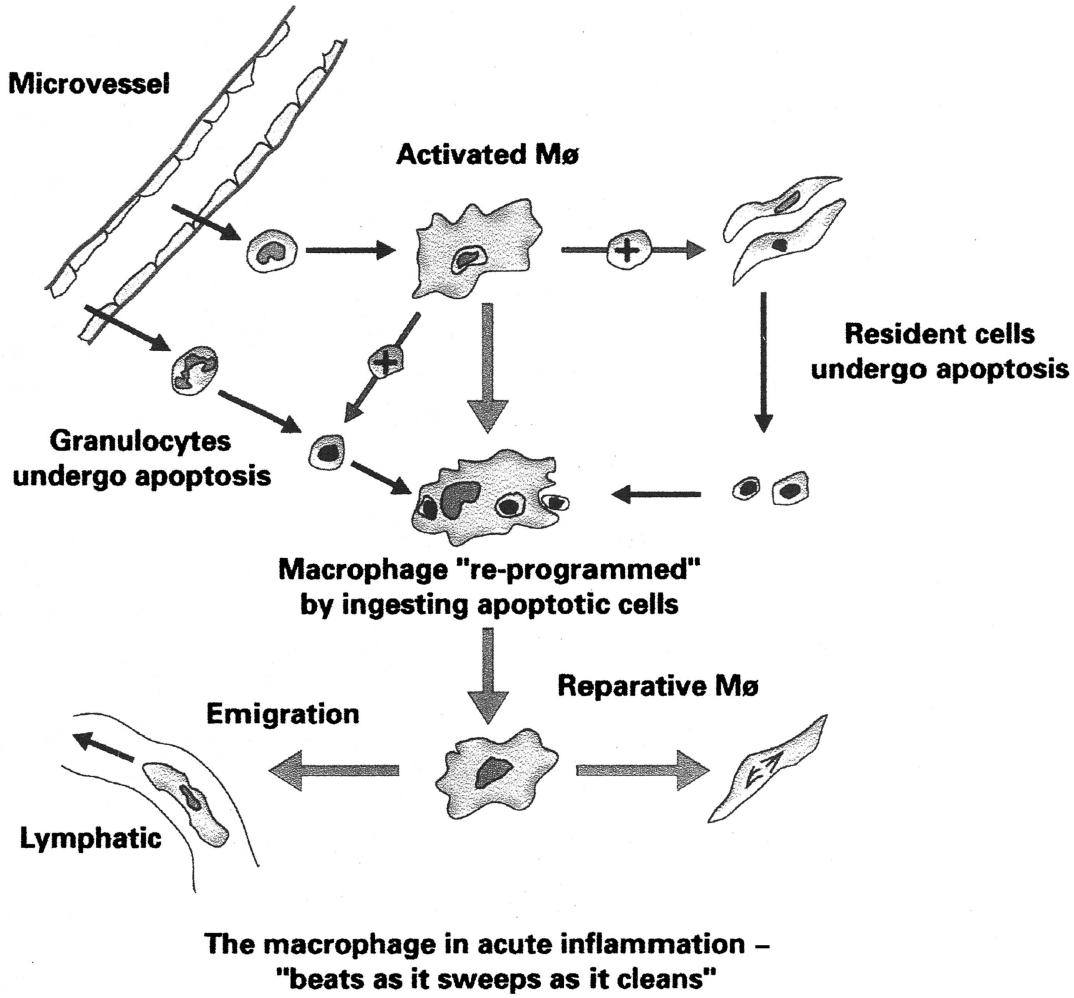

Fig. 2. Hypothesis for regulation of macrophage activation by interaction with apoptotic cells. Activated macrophages (Mø) can accelerate leukocyte apoptosis and trigger resident cell apoptosis. Subsequent phagocytosis of the apoptotic progeny deactivates or "reprograms" the macrophage, which then receives signals to promote, repair, and/or emigrate. Mø, macrophages.

mation persists and leads to scarring. Perhaps the most important insight is that disappearance of the original stimulus, streptococcal infection, might not be enough to promote resolution. Although much more work needs to be done to substantiate the role of apoptosis in resolution of this and other forms of nephritis, the discovery that this cell clearance program is involved generates new hypotheses for mechanisms promoting progression to chronic renal failure.

QUESTIONS AND ANSWERS

DR. NICOLAOS E. MADIAS (*Executive Academic Dean, Tufts University School of Medicine, Boston, Massachusetts*): Has the development of the pathologic process in post-streptococcal glomerulonephritis been studied in experimental models?

PROF. SAVILL: Yes, Norstrand's group has successfully modeled slow leakage of streptococcal antigen from an infected site and the subsequent development of immune complex glomerulonephritis by putting a streptococcal inoculum inside a cage implanted under the skin in rodents [34]. This model might allow experimental studies of programmed cell death in this condition.

DR. MADIAS: Would you speculate on the determinants of the variable severity of disease in post-streptococcal glomerulonephritis from sub-clinical to severe crescentic disease? What factors might mediate this spectrum?

PROF. SAVILL: Variable disease severity might reflect differences in the initiation of the inflammatory response, differences in its regulation once established, or differences in its resolution, three processes that could overlap in some types of inflammatory response. Multiple influences might be expected to impinge on these processes, ranging from cytokine polymorphisms to levels of C1q [64].

DR. MADIAS: Can you tell us a bit more about the exit of macrophages in the resolution phase?

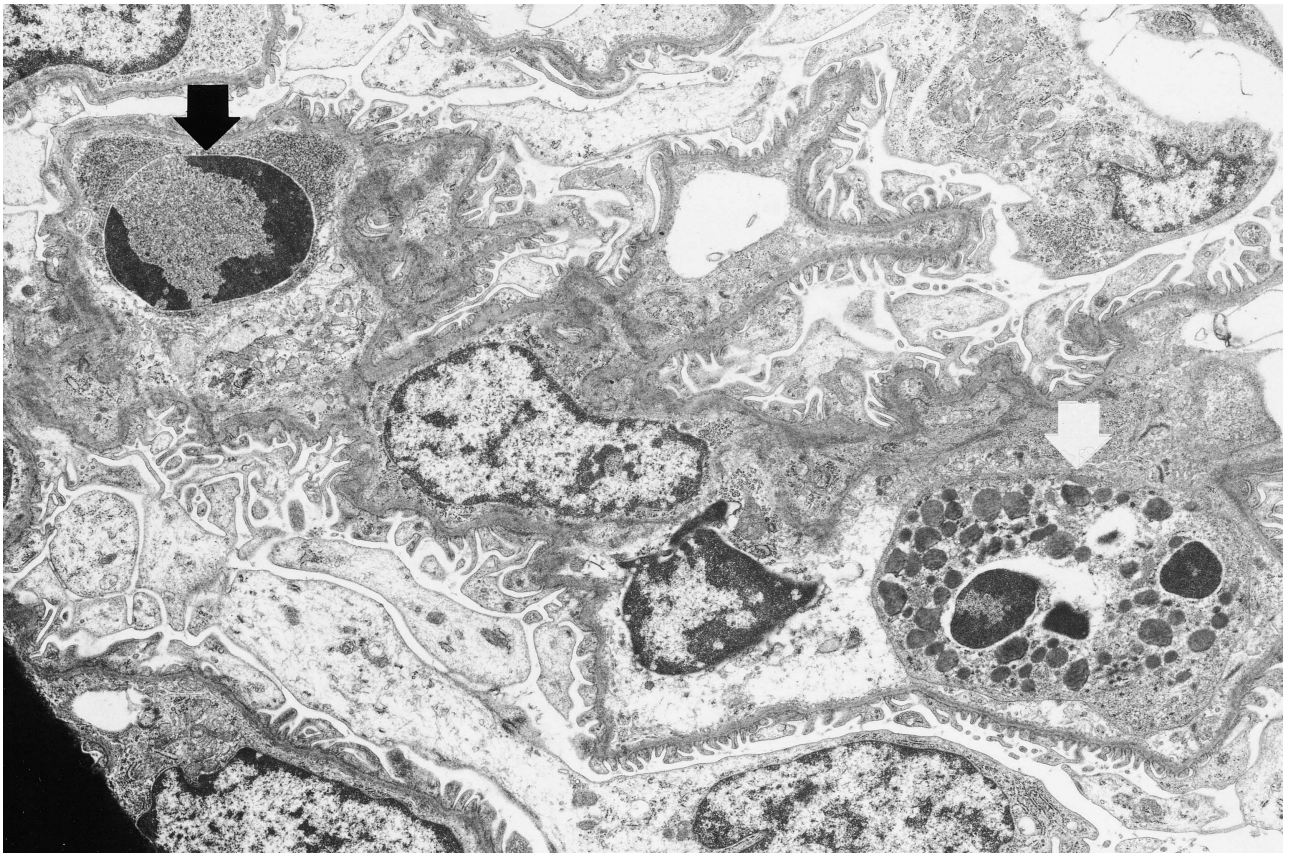

Fig. 3. Glomerular endothelial cell apoptosis. Electron micrograph of glomerular capillary from rabbit with glomerular injury induced by serum sickness/cyclosporine A [7]. On the left (black arrow) an injured glomerular endothelial cell displays typical nuclear morphology of apoptosis. On the right (white arrow) a neutrophil exhibits early apoptotic changes (From Professor Guy Neild; ×2000).

Prof. Savill: As I mentioned, this issue was first examined in experimental nephritis by Atkins' group, who reported migration of macrophage-like cells from inflamed glomeruli to draining lymph nodes [42]. Subsequently Bellingan labeled macrophages in experimental peritonitis and then tracked macrophage emigration, and found that macrophages leave the peritoneum by migration into the lymphatics and passage to the draining nodes, where it is speculated that they undergo apoptosis [41]. The mechanisms controlling macrophage emigration from inflamed sites are poorly understood but will be important to define.

Dr. Rodney Gilbert (*Department of Paedriatrics, St. James University Hospital, Leeds, England*): There are important differences in the outcomes in certain glomerular diseases in children and adults. Post-streptococcal glomerulonephritis is an obvious example, and hepatitis B-associated membranous nephropathy is another. Are there differences in apoptosis and control of apoptosis in children and adults that might explain these discrepancies?

Prof. Savill: This would be an interesting area for future studies.

Dr. Patrick Naish (*North Staffordshire Hospital Centre, Stoke on Trent, England*): John, you touched briefly on a fascinating topic—the mechanism of control of cell numbers within an organ. Is that mechanism related to the glomerulus?

Prof. Savill: No definitive data have determined what governs how many cells should be in a glomerulus. One important influence is the macrophage [54], but we don't know where the computer lies that tells the macrophages to regulate cell number. Another favored hypothesis is that microvascular endothelial cells also have the ability to govern cell number by determining oxygen supply [65].

Prof. John Feehally (*Leicester General Hospital, Leicester, England*): I appreciated your response to Dr. Madias about variations in initiation, regulation, and resolution. Nevertheless, the disease we are discussing is extraordinarily striking compared to any other human glomerular disease for its intensity and its complete recovery. Why is it so different? Is it just because neutrophils are so dominant in this disease and that they are particularly susceptible to clearance? Or are other things going on?

Prof. Savill: Post-streptococcal glomerulonephritis is not merely influx of neutrophils, as there is a very typical

inflammatory response in terms of resident cell proliferation. I think this is the glomerular equivalent of other severe but self-limited inflammatory disorders such as pneumococcal pneumonia, which sometimes resolves completely without antibiotics, and comparably severe inflammatory conditions in the skin or the bowel, which also can resolve completely. I would speculate that this propensity for resolution reflects the fact that post-streptococcal glomerulonephritis and comparable conditions are examples of perfect elimination of a "one-shot" initiating stimulus by a classic, and therefore apparently severe, acute inflammatory response, which can then resolve completely with perfect "healing." I suspect that the longevity of the initiating stimulus is one important determinant of the outcome of glomerulonephritis, diminished clearance of the stimulus by a suboptimal inflammatory response being a risk factor for persistence. However, you will have gathered that I believe that defective resolution mechanisms should also be sought in types of nephritis that persist.

Dr. Michael Venning (*University of South Manchester, South Manchester, England*): I would be interested in your comments about the analogous but different disease, proliferative nephritis associated with bacterial endocarditis. We have sporadic and unpublished evidence suggesting that vigorous intervention in this setting is beneficial: first, valve replacement if it can be achieved, and second, the use of steroids. In settings in which valve replacement hasn't been used and steroids have not been administered, our feeling is that the disease grumbles on and causes scarring. My first question is, what is your view about therapies in the setting of bacterial endocarditis? Second, in patients with post-streptococcal nephritis whose disease doesn't seem to be resolving, should we be using therapeutic maneuvers to try and control the progression to scarring?

Prof. Savill: Those are very good questions, and both are relevant to a related issue that I would like to address first. Not only is there in vitro evidence [66], but we also have preliminary in vivo evidence that high concentrations of glucocorticoids drive noninflammatory phagocyte clearance of apoptotic cells from inflamed sites (Thomas G, Clay M, Savill J, unpublished data). However, in the context of post-streptococcal glomerulonephritis, this potentially pro-resolution effect of glucocorticoids needs to be set against the potentially deleterious effects of prolonging neutrophil lifespan [67].

Let me return to your first question. My bias is that judicious immunosuppression likely helps in infectious disorders such as endocarditis with immunologic complications like nephritis. I would view these situations as being analogous to ANCA-positive vasculitis, in which relapse or exacerbation frequently is triggered by infection, and one needs to both increase the immunosuppressive therapy and treat the infection.

Your second question addressed the place of immunosuppressive or anti-inflammatory therapies in post-streptococcal nephritis. My bias would be to rely on the powerful natural resolution processes unless renal function declines or many crescents are present, in which case I would advise immunosuppression, including glucocorticoids. In Edinburgh we have recently seen surprisingly complete resolution upon follow-up biopsy after immunosuppressive treatment of a patient with post-streptococcal glomerulonephritis presenting with 100% crescents, but we will never know whether the treatment was necessary!

Prof. Neil Turner (*Royal Infirmary of Edinburgh*): You suggested that a difference between cases that resolve versus cases that progress to scarring is closely related to the duration of the initiating stimulus. Yet you also suggested that later scarring could be a problem with the mechanism of apoptosis. Isn't it more likely to be related to continuing injury or some problem with the resident cells?

Prof. Savill: In fact, I favor a hypothesis subtly different from those you mention, namely, that progression to scarring occurs because of dysregulated control of resident cell number by apoptosis, and that this dysregulated control is engendered by the persistence of cytocidal macrophages. Although I cannot be sure on the basis of the published data, I have the impression that progression to scarring can sometimes occur despite clinical evidence of complete resolution of well-validated post-streptococcal glomerulonephritis. Consequently, progression to scarring in this disorder might be one of the best arguments against a prolonged initiating stimulus being central to a bad outcome. It might be that elimination of the initiating stimulus is universal in post-streptococcal glomerulonephritis, but what might not be universal is complete resolution. I don't really have time to go into a large number of experimental studies that suggest that discrete events very early in an inflammatory response can govern subsequent events many days, weeks, and years down the line. Nevertheless, I suspect that in most cases of progressive nephritis, the injurious stimulus is long gone and yet aspects of post-inflammatory remodeling such as macrophage-directed, resident cell apoptosis persist undesirably.

Dr. Paul Brenchly (*Manchester Royal Infirmary, Manchester, England*): John, we know a lot about the cytokine growth factor profile that can activate macrophages to become more interested in removing cells by apoptosis, but what do we know about the cytokine profile that might actually switch them out of that phase? Or is there a normal default pattern that, once macrophages find there aren't any more apoptotic cells, they naturally default to a more quiescent phase?

Prof. Savill: I'm not sure that we know enough about factors that govern acquisition by monocytes of the abil-

ity for large-scale clearance of apoptotic cells as the monocytes mature into macrophages, but I would like to emphasize that the act of eating apoptotic cells is a potent stimulus towards "quiescence," as evidenced by decreased production of pro-inflammatory cytokines upon exposure to endotoxin [37, 38], suppression of antigen presentation by dendritic cells [43, 46], and our preliminary observation of macrophages' diminished capacity to kill mesangial cells. It seems likely that all of these phenomena of quiescence could reflect the autocrine/paracrine effects of TGF-β1, which is released after uptake of apoptotic cells [38]. I speculate that activated macrophages that fail to ingest apoptotic cells are denied an important "turn off" signal, so perhaps the default pattern is to remain injurious rather than become quiescent or reparative.

Dr. Brenchly: May I ask a second question? I was interested to see the very potent endothelial cell proliferation very early, and this case is a good example of glomerular endothelial proliferation. We don't know, but I suspect that this proliferation might be driven by vascular endothelial growth factor (VEGF), and the neutrophil carries quite a load of VEGF. Would you see that as the main source of the endothelial mitogenesis?

Prof. Savill: That is an extremely interesting suggestion, which could be tested by neutrophil depletion in an animal model, and supported by immunohistochemical studies in human cases.

Prof. Jean-Pierre Grünfeld (*Hôpital Necker, Paris, France*): In recent years it has been shown that post-infectious or maybe infectious glomerulonephritis was more prevalent and more severe in alcoholic patients. Might this difference be related to a defect in apoptosis? Do we have any data on the relationship between alcoholism and apoptosis?

Prof. Savill: You will be intrigued to hear that I have the impression that my own neutrophils exhibit accelerated constitutive apoptosis after I have taken alcohol, but definitive studies are required!

Prof. Grünfeld: My second question is very simple and naïve. What is your opinion on the indication of renal biopsy in patients with typical post-streptococcal glomerulonephritis? Is it useful or not?

Prof. Savill: It probably isn't useful in specific patient management other than for excluding severe crescentic change or another, unsuspected cause of severe glomerular injury, either of which might need heavy immunosuppression. In the case presented today, I would have wanted to biopsy to ensure that I was not missing an ANCA-negative vasculitis or some such condition.

Dr. Donal O'Donoghue (*Hope Hospital, Manchester*): I am interested in the epidemiology of post-streptococcal glomerulonephritis, in which quite a variability exists in the number or percentage of patients who go on to develop end-stage renal failure. How valid do you think those observations are? Second, if they are valid, do they provide us with any clues as to the genetic basis of some of these failures of resolution?

Prof. Savill: These are two very important questions. First, although I also have noticed that much of the evidence that this disorder can "go bad" comes from studies undertaken in the developing world, where investigation of patients like this, for obvious reasons, isn't as fastidious as it might be in richer countries, I think that there is well validated evidence that post-streptococcal glomerulonephritis can progress. Moving on to the second part of your question, it would be of great interest to study such patients by modern molecular genetic techniques such as microarray expression profiling, using patients whose disease did resolve properly as controls. As I emphasized at the beginning, I think we can learn a lot about the pathogenesis of nephritis from patients with post-streptococcal nephritis.

Dr. Philip Kalra (*Hope Hospital, Manchester*): In patients with proliferative glomerular diseases that are more commonly associated with scarring and end-stage renal failure, is there any evidence of disturbed control of cell number by apoptosis within the tubulointerstitial compartment rather than in the glomerulus?

Prof. Savill: That is a particularly apposite question because, although I emphasized the classic observation of minimal histologic evidence of tubulointerstitial involvement in this case of post-streptococcal glomerulonephritis, sharp-eyed members of the audience will have seen that the beautiful studies by Oda and colleagues [10] have demonstrated increased PCNA staining in the tubulointerstitial compartment of such patients. This finding emphasizes the possible influence of changes in number of tubulointerstitial cells, even in such a "clean" glomerular injury. There is growing evidence of unscheduled tubulointerstitial cell apoptosis in many forms of renal injury, but an experimental priority will be to show that modulating such apoptosis alters the outcome of renal injury.

Dr. Rupert Smith (*Birch Hill Hospital, Rochdale, England*): As you say, post-streptococcal glomerulonephritis is rare in Scotland and England, but most units see a lot of Henoch-Schönlein purpura, and we have to follow up those patients for long periods if they get hematuria. I realize it is not the same disease, but are there any parallels with post-streptococcal glomerulonephritis in terms of what is going on in the kidney?

Prof. Savill: I would speculate that there are. I don't know of any studies, histologic or otherwise, of the injured kidney in Henoch-Schönlein purpura with regard to whether there is cell clearance by apoptosis, but obviously the relationship to IgA nephropathy would encourage us to believe there are powerful resolution processes in operation in patients with Henoch-Schönlein purpura.

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