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Mesangial cell necrosis in Thy 1 glomerulonephritis – an ultrastructural study

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Abstract Cell death is central to many physiological and pathological processes. As tissue reactions to the two forms of cell death, necrosis and apoptosis, differ, it is critical to distinguish between them. Although ultrastructure is still the definitive means of assessing this, there are very few in vivo studies. Administration of anti-Thy 1 antibody in rats is a model of acute glomerular mesangial cell death due to their expression of the Thy 1.1 epitope. The nature of this process is unclear; apoptosis was suggested from early morphological studies and recent in vitro effects of anti-Thy 1.1 antibody. We have re-examined the changes by electron microscopy, and identified a process of cell necrosis starting within 30 min of anti-Thy1.1 antibody administration. Although there was chromatin condensation, the necrotic features distinctive from apoptosis were: loss of nuclear membranes, cell swelling and degeneration of cytoplasmic organelles, with liberation of chromatin and organelles into the interstitium causing acute inflammation without phagocytic uptake of apoptotic bodies. These findings accord with the known complement dependence of this model. Ultrastructure is a valuable means of differentiating between in vivo necrosis and apoptosis and this is important for understanding the pathogenesis of injury and subsequent tissue responses.

Key words Apoptosis · Cell necrosis · Thy 1 glomerulonephritis

Introduction

Anti-Thy 1.1 glomerulonephritis is a unique rat model of acute immune glomerular injury and repair initiated by fixation of specific antibody to Thy 1.1 epitopes on the rat mesangial cell plasma membrane. This model has proved valuable for understanding the mechanisms of mesangial cell proliferation, glomerular capillary regeneration, and mesangial matrix production [5].

The rapid injury to mesangial cells is complement dependent [3, 14] and results in a loss of up to two-thirds of the mesangial cell population (mesangiolysis) within 24 h. The exact nature of this process is unclear. The first, and only comprehensive, ultrastructural study of this model described early nuclear chromatin condensation in mesangial cells, resembling apoptosis [1], and there have been recent in vitro studies showing that anti-Thy 1 antibodies trigger apoptosis in mesangial cells [9] in a similar fashion to their effects on immature Thy 1 expressing thymocytes [6]. Apoptosis in the glomerulonephritis model has been definitively identified in the later mesangial proliferative and post-inflammatory phases of the model, as a mechanism for clearance of surplus mesangial and inflammatory cells [2, 12].

Although there has been increasing research on the biochemical nature of apoptosis, ultrastructural confirmation is still necessary to distinguish this process from cell necrosis [13] and, in addition, both processes at certain stages have light microscopical appearances of nuclear pyknosis and karyorrhexis. The critical differences between these processes are that in necrosis, which is a pathological process, there is a generalised disintegration of the cell with lysis and dispersal of cell contents into the surrounding extracellular spaces, whereas in apoptosis, which is a feature of physiological regulation of cell number, the preservation of membrane around the nuclear and cytoplasmic components results in programmed removal by phagocytosis. The morphology of the in vivo formation of apoptotic bodies has rarely been described [7]. We have therefore examined the ultrastructure of the acute phase of mesangial cell loss (up to 24 h after antibody administration) to determine whether mesangiolysis is an apoptotic or a necrotic process.

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Materials and methods

Animal treatment

Thy 1.1 gn was induced in 2-month-old male Lewis rats weighing 200–250 g by a single i.v. injection of monoclonal antibody ER4, as previously described [4]. Thirty minutes, and 1, 2, 4, 5, 7 and 24 h after ER4, kidneys were removed from 1 rat at each time point for ultrastructural studies. The experiments were performed according to Home Office regulations.

Fixation and processing of material for electron microscopy

Renal cortex was cut into 1-mm^3 blocks and fixed in 4% gluteraldehyde in Millonig's phosphate buffer at pH 7.3. It was then processed at 5°C on a Lynx tissue processor as follows: rinsed in Millonig's phosphate buffer, post-fixed in 1% osmium tetroxide in Millonig's phosphate buffer, and rinsed in distilled water. The tissue was then stained en bloc in 2% aqueous uranyl acetate for 30 min at 20°C, dehydrated through a series of alcohols, rinsed in a transition solvent, Inhibisol (1,1,1 trichloroethane), at 20°C, and infiltrated with Inhibisol/epoxy resin (Taab 812) 1:1 at 37°C, then blocked out in fresh resin in polythene embedding capsules and polymerized overnight at 70°C.

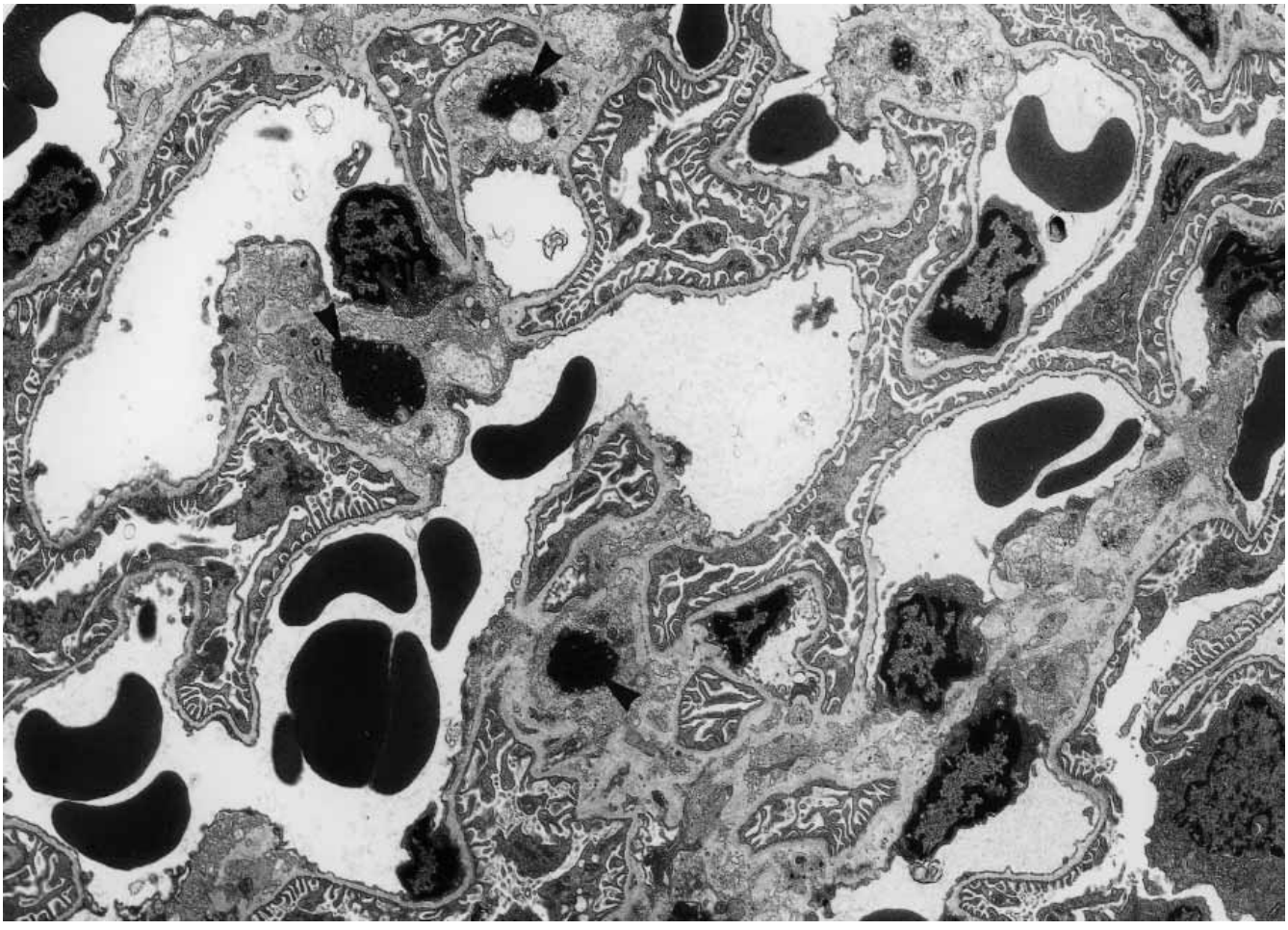
Semi-thin (0.5 µm) sections were cut from the blocks on a Reichert Ultracut ultramicrotome and stained with aqueous 1% Azure II in 1% borax. For each time point at least 50 glomeruli were examined under oil immersion. From these, glomeruli were selected for ultrastructural study. For each time point a minimum of 3 glomeruli (mean 4.4, range 3–7) were cut for ultra-thin sections, picked up on copper grids, stained in Reynold's lead citrate for 22 min and viewed using a Philips 400 transmission electron microscope, and a mean of 26 high-power micrographs (range 12–30) were taken and further examined.

Results

All animals displayed a significant mesangiolysis by light microscopy, as has previously been found in all of our studies using this highly reproducible model. In selecting glomeruli for ultrastructural study from the semi-thin sections using oil immersion light microscopy, we were careful to choose areas where nuclear changes were apparent. At this magnification the appearances between necrosis and apoptosis were indistinguishable.

The ultrastructural abnormalities were confined to the mesangium and to small areas of endothelium. The basement membranes and visceral epithelium were normal. At 30 min there was already clear evidence of mesangial cell loss, oedema, and neutrophil infiltration in some mesangial areas. At low powers rounded dense nuclei resembling apoptotic cells were seen in mesangial areas (Fig. 1). At higher powers these changes in mesangial

Fig. 1 High-power magnification of part of glomerulus 30 min after anti-Thy 1 antibody. The mesangial areas are oedematous, and three have mesangial cells with nuclear chromatin condensation (*arrowheads*). ×4000



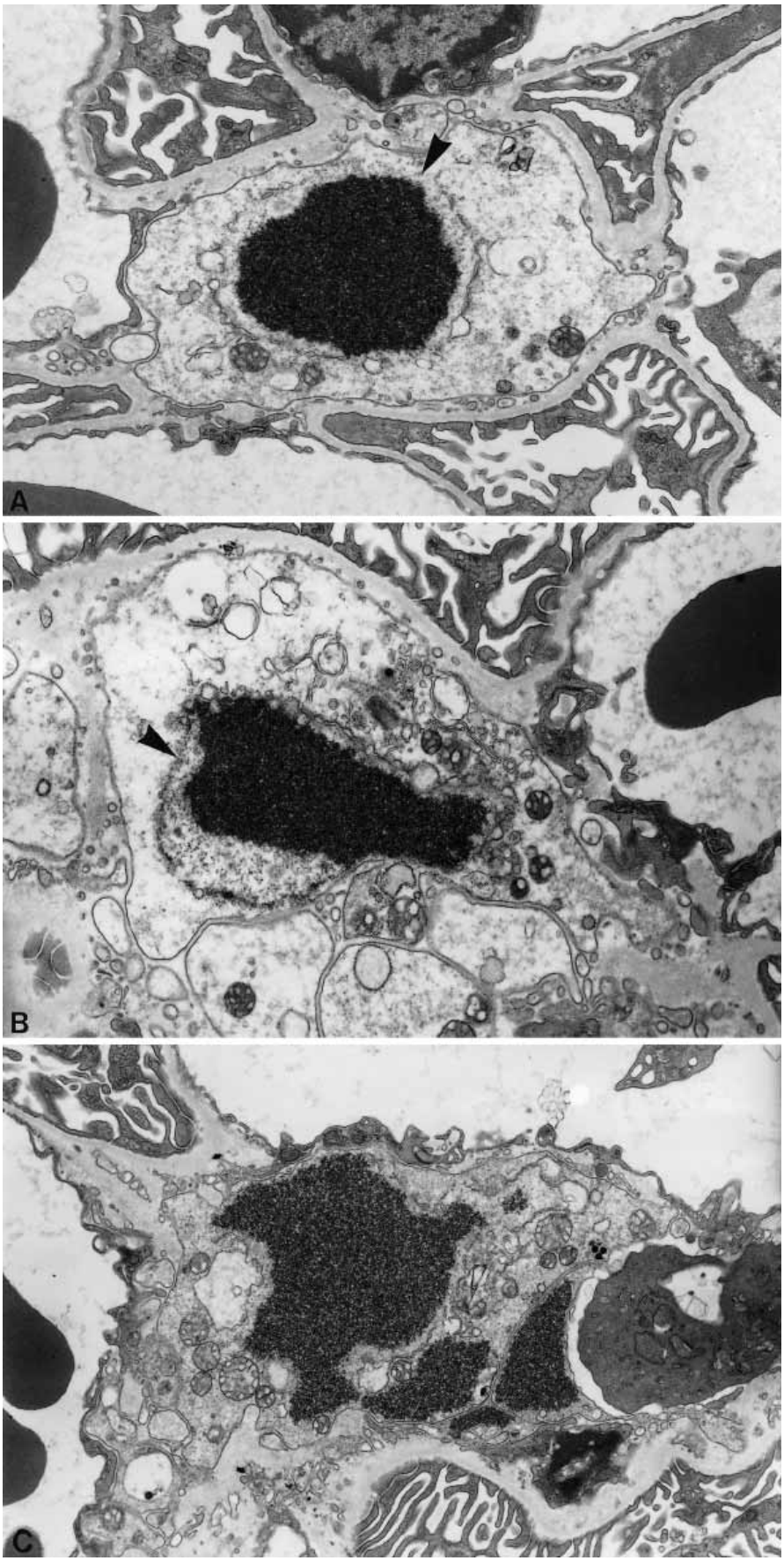


Fig. 2a–c Changes in mesangial cell in Thy 1 gn at 1 h. There is nuclear chromatin condensation, increased cytoplasmic electron lucency, and swelling and loss of organelles. **a**, **b** The nuclear membrane is degenerating but still present (*arrowheads*) and the chromatin has shrunken centrally. **c** The nuclear membrane is no longer present and the chromatin is fragmenting. **a**, **c** ×8000, **b** ×13000

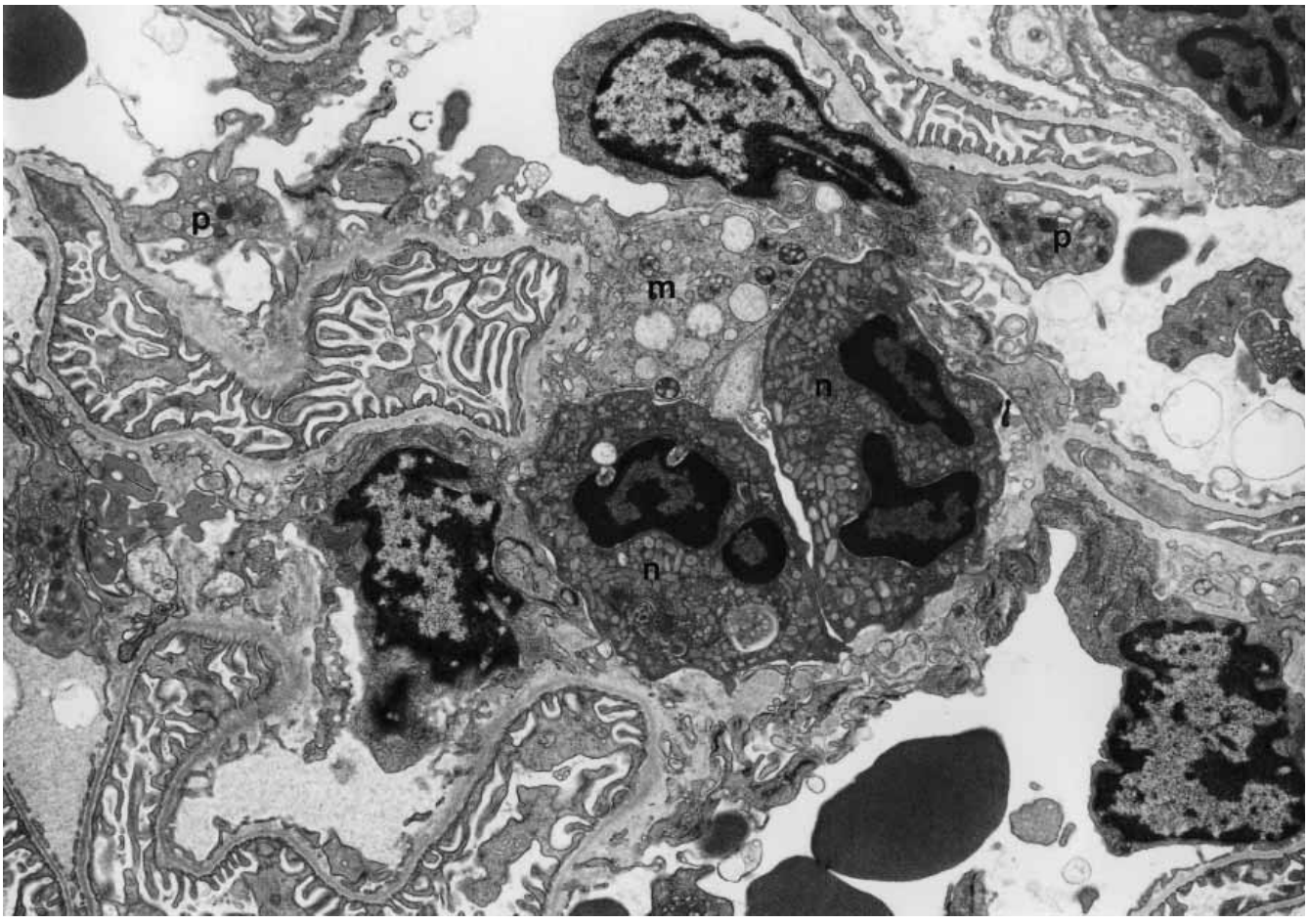


Fig. 3 In this mesangial area (4 h after anti-Thy 1 antibody) there are two intact neutrophils (*n*) adjacent to degenerating mesangial cell cytoplasm (*m*). Platelets (*p*) are adhering to the endothelium. ×7000

cells were (1) an even condensation of nuclear chromatin with loss of nuclear membranes, and (2) cytoplasmic swelling with swelling and loss of organelles, i.e. features of necrosis. Neutrophils with intact granules were closely applied to the borders of degenerating mesangial cells. The endothelium and capillary basement membranes were normal. The changes in mesangial cells at 1 h were similar but more extensive (Fig. 2a–c), and free degenerating organelles were now present in the mesangial matrix. Small numbers of platelets were present in capillary lumens. No apoptotic bodies were identified either within the extracellular spaces or within neutrophils.

By 2 h, there was global mesangiolysis, although the loop structure remained intact. Degenerating mesangial cells were still present. A few residual mesangial cells with normal nuclei remained. Some mesangial areas were acellular with electron-lucent oedema containing clusters of organelles including degenerate mitochondria. In other areas there were neutrophils which appeared normal. There was no evidence of neutrophil uptake of apoptotic bodies. At small defects in the endothelium there were platelet aggregates.

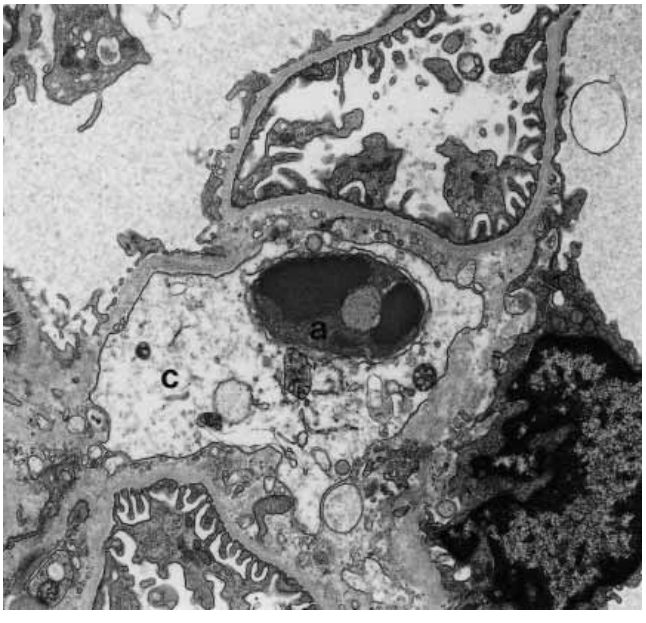
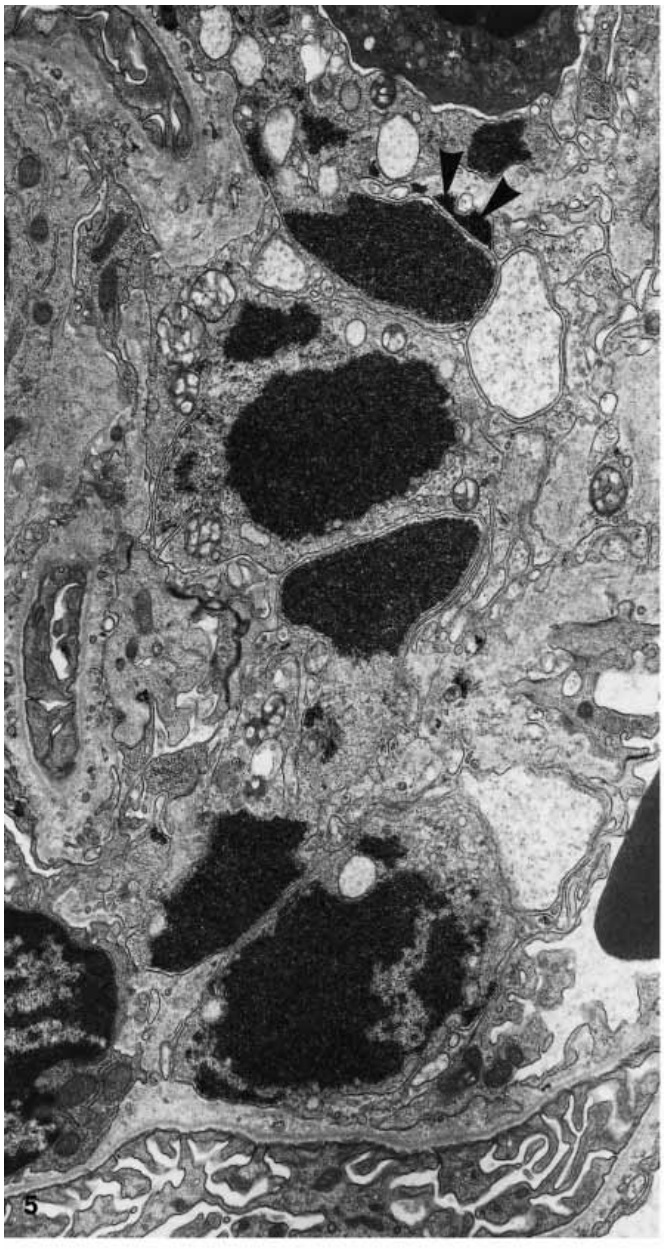
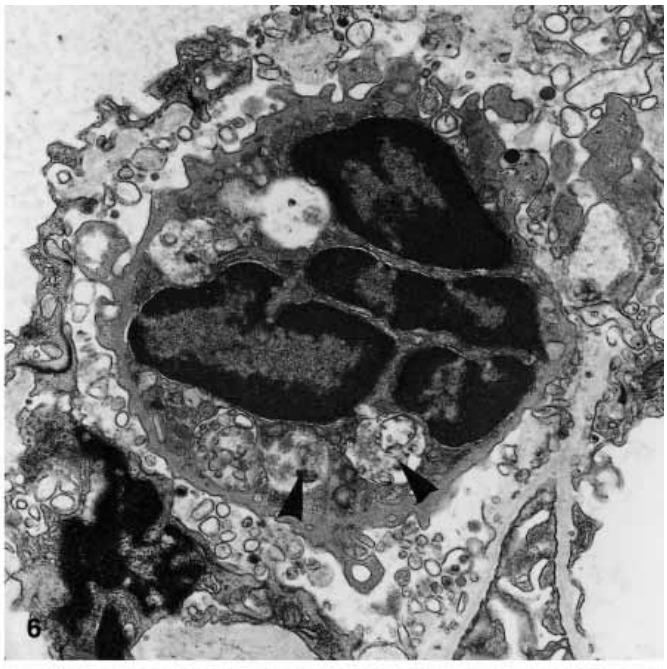


Fig. 4 A neutrophil apoptotic body (*a*). Note the cytoplasmic condensation and the overall shrunken appearance. The body appears to be within the cytoplasm (*c*) of an indeterminate cell. ×7000





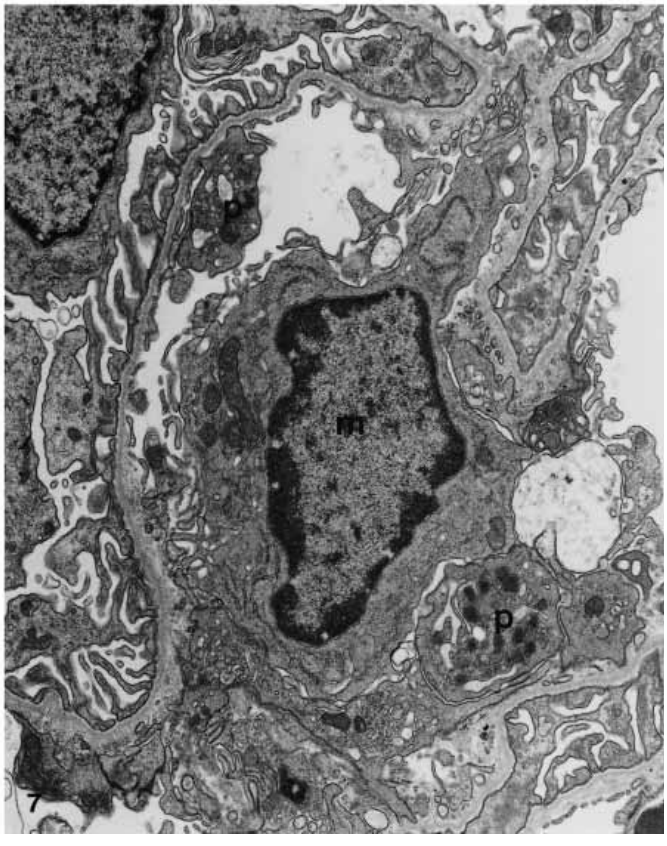


Fig. 5 Mesangial area 5 h after anti-Thy 1 antibody. Clusters of condensed chromatin are present, probably representing several dying mesangial cells. Some of this material is extracellular (*arrowheads*). ×7000

Fig. 6 Seven hours after anti-Thy 1 antibody. An intact neutrophil in the mesangium has several phagosomes containing organelle debris (*arrowheads*), and vesicular debris is scattered in the oedematous mesangial matrix. ×10000

Fig. 7 Twenty-four hours after anti-Thy 1 antibody. A macrophage (*m*) has infiltrated the mesangium. Platelets (*p*) are present both within the mesangium, and adhering to endothelium. The mesangial oedema and debris is now reduced. ×7000

At 4 and 5 h there was increasing mesangial infiltration by neutrophils (Fig. 3) and macrophages, with only occasional infiltrating cells in the lumens. Some neutrophils showed nuclear chromatin condensation consistent with degenerative change, and one instance of an apoptotic neutrophil was seen (Fig. 4). Mesangial cells with large condensed chromatin masses in their cytoplasm were still seen, and some lumps of chromatin appeared to be in the extracellular matrix (Fig. 5). Rare mesangial cell mitoses were present. There were platelets in capillary lumens and adherent to endothelial defects.

At 7 h the disrupted mesangial areas were mainly occupied by neutrophils and some macrophages, surround-

ed by oedema containing cell debris (Fig. 6). Very few mesangial cells, either normal or degenerating, were left. Some neutrophils had phagosomes containing membrane-bound vesicles (Fig. 6). No apoptotic bodies were seen.

At 24 h (Fig. 7), in the mesangium macrophages were now more prominent than neutrophils and there were also small numbers of platelets and red cells, but the degree of oedema and cellular debris was much reduced. Within capillary loops, the number of platelets adhering to the subendothelium had increased. No apoptotic bodies were seen.

Discussion

The early phases of apoptosis and cell necrosis can be identical morphologically, in that both initially show nuclear chromatin condensation, although the nuclear chromatin condensation in apoptosis is characteristically described as adjacent to the nuclear membrane, and as having a crescentic appearance in later stages [13]. Thereafter the sequence of changes in nucleus and cytoplasm is distinct, and the fate of the dead or dying material is also different. The essential differences are two-fold: firstly, early in cell necrosis there are signs of membrane injury to nuclear and plasma membranes and cytoplasmic organelles, whereas in apoptosis the cytoplasm is condensed but organelle and membrane integrity are preserved; secondly, necrosis with liberation of cell debris into the intercellular spaces provokes acute inflammation, whilst apoptosis leads to the break-up of the cell into membrane-bound apoptotic bodies, which are rapidly phagocytosed by adjacent macrophages and/or neighbouring parenchymal cells, and very little of this material remains free in the intercellular spaces. As necrosis leads to inflammation, and apoptosis is a physiological form of programmed cell death which does not incite inflammation, differentiation of these processes is clearly important for understanding of the subsequent tissue reactions.

The changes we have observed in mesangial cells in the acute phase of anti-Thy 1.1 glomerulonephritis have more the appearances described for cell necrosis than for apoptosis, and this is further substantiated by a lack of apoptotic bodies as cell loss progresses. The chromatin condensation in the nucleus does not form dense crescent-like masses, and there is an early loss of the nuclear membrane. Furthermore, there is cytoplasmic swelling rather than condensation with swelling of mitochondria and other cytoplasmic organelles, and liberation of these into the surrounding mesangial matrix, which is a feature of necrosis. The neutrophil infiltration may partly be a response to this. No apoptotic bodies were seen in the adjacent leucocytes, or in residual mesangial cells, which have previously been shown to be capable of uptake of apoptotic bodies [2, 11] apart from one apoptotic neutrophil at 4 h in one section. It is unlikely that apoptotic bodies would not yet have formed within the time scale we have examined, for although there are few studies on apoptosis in tissues in vivo, it is estimated that the maximum time from apoptosis to final digestion of phagocytosed apoptotic bodies would not exceed 12–18 h. There is only one published study examining apoptosis in the acute phase (less than 24 h) of a model of Thy 1.1 nephritis; in this study apoptosis was identified by the typical DNA laddering effect of electrophoresis, and in situ nick end labelling (Tunel technique) and ultrastructure were not performed [8]. Neither of these techniques is now regarded as an absolute criterion of apoptosis, and in addition this model differed from the standard model in that although the total doses of monoclonal antibody were similar, they were administered as three doses at 4-h intervals, and the analysis at 12 h after the first injection was therefore examining a staggered series of insults to the mesangial cells.

The in vivo necrosis is therefore distinct from the apoptotic events that can be induced by the anti-Thy 1 antibody on mesangial cells in vitro, and from the physiological engagement of Thy 1 epitopes in the thymus, which leads to elimination of autoreactive thymocytes and accords with the complement-dependent nature of the in vivo mesangiolysis [3, 14]. Our study also identifies this acute injury phase as distinct from the well-documented later phases of the model (around day 5 onwards) where the mesangial proliferative reaction to injury is controlled by apoptosis [10], with ultrastructural studies clearly showing the uptake of apoptotic bodies by neighbouring cells.

The identification of necrosis as the dominant pathological process causing the acute loss of mesangial cells in Thy 1 glomerulonephritis will increase understanding of this model, and should lead to further examination of the underlying biochemical events. Examination by ultrastructure has clarified the nature of the cell death in this disease model and is a valuable means of differentiating between necrosis and apoptosis.

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