Original Article

Significance of glomerular cell apoptosis in the resolution of acute post-streptococcal glomerulonephritis

Takashi Oda¹, Nobuyuki Yoshizawa², Kazuo Yamakami², Aki Ishida³, Osamu Hotta³, Shigenobu Suzuki¹ and Soichiro Miura¹

¹Department of Medicine and ²Department of Public Health, National Defense Medical College, Saitama and ³Department of Nephrology, Sendai Shakaihoken Hospital, Sendai, Japan

Abstract
Background. Glomerular hypercellularity due to resident glomerular cell proliferation and leucocyte infiltration has been described in acute post-streptococcal glomerulonephritis (APSGN). APSGN usually resolves without progression. However, the mechanism of resolution remains to be determined.

Methods. Renal biopsy tissues from 15 patients with APSGN (obtained 1–31 days after disease onset) and five control patients with minor glomerular abnormality were evaluated with respect to glomerular resolution. Apoptotic cells were assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) as well as by immunostaining of single-stranded DNA (ssDNA).

Results. The number of glomerular cells was high in the early-phase of APSGN and decreased over time. No TUNEL+ glomerular cells were found in control subjects, whereas prominent glomerular TUNEL+ cells were observed in APSGN patients, particularly in the early phase of the disease. The number of glomerular TUNEL+ cells decreased exponentially but was still prominent in renal tissue biopsied at 31 days after disease onset. Double staining for ssDNA and glomerular cell markers showed that glomerular apoptotic cells were predominantly mesangium and endothelial cells, with some neutrophils and macrophages.

Conclusions. These results suggest that apoptosis exists in the glomerulus in patients with APSGN from the early to the late stages of the disease and contributes to the resolution of glomerular hypercellularity.

Keywords: acute post-streptococcal glomerulonephritis (APSGN); apoptosis; nephritis-associated plasmin receptor (NAPlr); single-stranded DNA (ssDNA); terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL)

Introduction

Glomerular cell proliferation followed by the development of glomerulosclerosis is a hallmark of various types of progressive human and experimental glomerulonephritis (GN). There have been many studies of accelerated glomerular cell proliferation as a cause of glomerulosclerosis [1,2]. However, recent reports emphasize the importance of inappropriate glomerular cell apoptosis, as disturbed resolution mechanism or as inappropriate cell deletion, in the development of glomerulosclerosis [3–6]. Generally, the development of glomerular injury has been studied extensively, whereas scarce data are available regarding the process of resolution of glomerular damage, particularly in humans. This may be due to difficulties in determining the time of disease onset and the time course of GN in humans.

Acute post-streptococcal glomerulonephritis (APSGN) is one of the sequelae of streptococcus infection and is the prototype acute nephritic syndrome. Although an atypical asymptomatic form has been recognized [7], typical APSGN starts in the form of acute nephritic syndrome and usually resolves without progression. Thus, overt APSGN is one of the few types of human GN with a clearly defined course of events and is the appropriate type of human GN by which to analyse the process of glomerular injury and resolution. The importance of investigation of resolution mechanism in APSGN has been stated [8].

We previously analysed renal biopsy tissues from patients with overt APSGN with respect to glomerular damage [9]. We found accelerated resident glomerular cell proliferation together with immune cell infiltration, particularly in the early phase of the disease. The majority of proliferating cells in the early phase were
endothelial cells. However, as the disease continued, mesangial cells increased to nearly half of the total proliferating cells within the glomeruli.

In the present study, we reanalysed the renal biopsy tissues of the same patients with respect to resolution, with attention to the degree of glomerular cell apoptosis and the cell types involved. We also investigated the relation between glomerular cell apoptosis and localization of nephritogenic antigen of APSGN (nephritis-associated plasmin receptor: NAPIr), which we recently isolated and characterized [10–12]. The results provide valuable information regarding the process of resolution that may lead to elucidation of therapeutic means of human glomerular disease.

Materials and methods

Patients

Renal biopsy tissues obtained from 15 patients with APSGN (Table 1; the same patients previously reported [9]) were used in this study. All patients showed symptoms of overt APSGN, such as facial oedema, hypertension and haematuria. APSGN was diagnosed according to serological and bacteriological evidence of acute streptococcal infection prior to the onset of nephritis as well as to characteristic histological features of renal biopsy tissue determined by light microscopy, immunofluorescence (IF) and electron microscopy. Five patients with an IF-negative minor glomerular abnormality served as age- and sex-matched controls (age: 30.0 ± 15.0 years; male/female = 2/3). Informed consent was obtained from each patient in accordance with the principles of the Declaration of Helsinki.

### Table 1. Patient profiles at the time of biopsy

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age/sex</th>
<th>Onset (days)</th>
<th>S-Cr (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>U-P (g/day)</th>
<th>U-RBC (cells/hpf)</th>
<th>CH50</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.M.</td>
<td>10/F</td>
<td>0.9 (0.9)</td>
<td>1.9</td>
<td>Many</td>
<td></td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>I.M.</td>
<td>16/M</td>
<td>1.0 (1.2)</td>
<td>1.3</td>
<td>Many</td>
<td></td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>H.S.</td>
<td>32/F</td>
<td>1.4 (1.4)</td>
<td>0.3</td>
<td>5 ~ 10</td>
<td>13.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K.H.</td>
<td>33/M</td>
<td>1.0 (1.4)</td>
<td>0.6</td>
<td>Many</td>
<td></td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>N.Y.</td>
<td>42/M</td>
<td>1.4 (1.4)</td>
<td>20</td>
<td>5 ~ 10</td>
<td>21.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K.C.</td>
<td>50/F</td>
<td>1.0 (1.0)</td>
<td>—</td>
<td>Many</td>
<td></td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td>K.T.</td>
<td>28/M</td>
<td>1.4 (1.4)</td>
<td>0.3</td>
<td>10 ~ 20</td>
<td>22.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y.A.</td>
<td>42/F</td>
<td>0.9 (1.1)</td>
<td>23</td>
<td>Many</td>
<td></td>
<td>28.0</td>
<td></td>
</tr>
<tr>
<td>K.K.</td>
<td>33/F</td>
<td>0.7 (0.8)</td>
<td>12</td>
<td>6 ~ 5</td>
<td>27.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.D.</td>
<td>18/M</td>
<td>1.3 (1.3)</td>
<td>0.8</td>
<td>20 ~ 30</td>
<td>28.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S.Y.</td>
<td>33/F</td>
<td>0.8 (0.8)</td>
<td>11</td>
<td>20 ~ 30</td>
<td>25.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O.K.</td>
<td>20/M</td>
<td>1.3 (1.3)</td>
<td>0.8</td>
<td>20 ~ 10</td>
<td>23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H.M.</td>
<td>19/M</td>
<td>1.2 (1.2)</td>
<td>1</td>
<td>1 ~ 3</td>
<td>16.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y.K.</td>
<td>30/F</td>
<td>0.6 (0.8)</td>
<td>12</td>
<td>10 ~ 20</td>
<td>18.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W.K.</td>
<td>45/F</td>
<td>0.9 (0.9)</td>
<td>13</td>
<td>1 ~ 3</td>
<td>16.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The peak serum creatinine value during our treatment is given in parentheses after the values of serum creatinine at the time of biopsy.* Reproduced with permission from ref [9].

### Evaluation of the number of intraglomerular cells

The number of intraglomerular cells with distinct nuclei was counted on PAS-stained sections under light microscopy and was expressed as the total number of glomerular cells per glomerular cross-section (GCS). The number of glomeruli analysed in each patient ranged from 3 to 17 (7.07 ± 4.25) in APSGN patients and from 3 to 8 (5.80 ± 1.92) in control subjects.

### Detection of apoptosis

Apoptotic cells were assessed by three methods. The first method was terminal deoxynucleotidyl transferase–mediated dUTP nick end-labelling (TUNEL) with an in situ apoptosis detection kit (Takara Bio Inc., Tokyo, Japan), principally following the manufacturer’s instructions. In brief, deparaffinized sections were microwaved in 0.01 mol/l citrate buffer (pH 6.0) for 5 min. Then free 3’ hydroxyl ends of fragmented DNA were labelled with FITC-conjugated dUTP by incubating the sections with terminal deoxynucleotidyl transferase for 70 min at 37°C. FITC-labelled cells were detected by incubation with peroxidase-conjugated anti-FITC antibody (Ab) and development with 3,3’-diaminobenzidine (DAB). Counterstaining with either methyl green or PAS was performed. All sections were stained simultaneously. The second method was indirect IF staining of single-stranded DNA (ssDNA), a marker of apoptotic cells [13]. Indirect IF staining was performed on cryostat sections of fresh-frozen renal tissues from 12 APSGN patients. No frozen renal tissue remained for patients 2, 3 and 12. The primary Ab was a polyclonal rabbit anti-ssDNA Ab (Dako Cytomation, Glostrup, Denmark) and the secondary Ab was an Alexa Fluor 488-conjugated goat anti-rabbit IgG Ab (Molecular Probes, Eugene, OR). The third method was light microscopic observation of routine PAS-stained sections. Glomerular cells with typical apoptotic morphologic features, such as condensed and fragmented nuclei and reduced cell volume, were evaluated.

The total numbers of apoptotic cells assessed by these methods were counted in each glomerulus and were expressed as number of apoptotic cells/GCS.

### Characterization of intraglomerular apoptotic cells by double IF staining

To characterize intraglomerular apoptotic cells, we used five murine monoclonal Abs (Dako Cytomation) as markers of intraglomerular cell components: mesangium [anti-human α-smooth muscle actin (α-SMA) Ab; 1A4], endothelial cells (anti-human von Willebrand factor Ab; F8/86), neutrophils [anti-human neutrophil elastase (NE) Ab; NP57], macrophages (anti-human CD68 Ab; EB-M11) and podocytes [anti-human Wilms’ Tumour 1 (WT-1) Ab; 6F-H2]. Indirect double IF staining for ssDNA and these glomerular cells markers was performed on renal tissues from 12 APSGN patients. Cryostat sections of fresh-frozen tissues were fixed in 4% paraformaldehyde for 5 min, treated with 10% normal goat serum in phosphate-buffered saline (PBS) for 5 min, and incubated with the appropriate primary Abs (rabbit anti-ssDNA Ab and each of the five monoclonal Abs listed earlier) for 1 h. Unless otherwise stated, all incubation was performed at 27°C. Sections were then incubated with
the appropriate secondary Ab (Alexa Fluor 594-conjugated goat anti-rabbit IgG Ab for ssDNA and Alexa Fluor 488-conjugated goat anti-mouse IgG Ab for the cell markers; Molecular Probes) for 30 min, washed with PBS, and observed under fluorescence microscopy with appropriate filters for each fluorescent dye. The total numbers of intraglomerular ssDNA+ cells and double-positive cells were counted, and the percentage of ssDNA+ cells that were double positive was calculated.

**Double staining for ssDNA and NAPlr or in situ zymography for plasmin activity**

The relation between glomerular apoptosis and localization of the nephritogenic antigen for APSGN (NAPlr) was investigated by double staining on frozen sections from 9 APSGN patients (patients 1,4–11) that had previously been shown to be positive for NAPlr in the glomeruli [11]. Because NAPlr has the capacity to bind to plasmin and is localized in glomeruli identically to plasmin activity [12], double staining for ssDNA and NAPlr and for ssDNA and plasmin activity was performed. For ssDNA and NAPlr double staining, after incubation with FITC-conjugated monoclonal mouse anti-NAPlr Ab (1F10) for 30 min, sections were fixed in 4% paraformaldehyde for 5 min and then stained for ssDNA as described earlier with the use of Alexa Fluor 594-conjugated goat anti-rabbit secondary Ab. Monoclonal mouse anti-NAPlr Ab (1F10) was generated in our laboratory with recombinant NAPlr and common methods of monoclonal antibody production. The specificity of the Ab was confirmed by western blotting (data not shown). Labelling of 1F10 with fluorescent was performed with the EZ-Label Fluorescein Protein Labelling Kit (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer’s instructions. For ssDNA and plasmin activity double staining, in situ zymography for plasmin activity and indirect IF staining for ssDNA were performed as described previously [12] with the use of P-toluenesulfonyl-L-lysine z-naphthyl ester (Torii Pharmaceutical Co., Ltd., Tokyo, Japan) as the plasmin-sensitive substrate and anti-ssDNA Ab and Alexa Fluor 488-conjugated goat anti-rabbit IgG Ab as the primary and secondary Abs for IF staining, respectively. Sections were observed by light microscopy for plasmin activity and by fluorescence microscopy for ssDNA staining.

**Immunoperoxidase staining for macrophages and scavenger receptors**

Indirect immunoperoxidase staining for CD68 (macrophage marker, receptor for oxidized LDL) and the class A macrophage scavenger receptor (SR-A) was performed on formalin-fixed tissue sections from 15 APSGN patients, with the use of a monoclonal anti-CD68 Ab (PG-M1; Dako Cytomation) and monoclonal mouse anti-human SR-A Ab (SRA-E5; Transgenic Co., Kumamoto, Japan) as primary Abs and secondary Ab supplied with the Envision-HRP system (Dako Cytomation), developed with DAB and counterstained with haematoxylin.

**Statistical analysis**

Data are expressed as the mean±SD. Student’s t-test was used to evaluate differences between means. Differences were considered significant at two-tailed P<0.05.

**Results**

**Time course of glomerular hypercellularity**

The total number of glomerular cells/GCS in patients with APSGN was significantly greater than that in control subjects (224.17±53.81 vs 107.18±23.60 cells/GCS, P<0.001). In addition, there was a negative correlation between the total number of glomerular cells/GCS and duration after onset of APSGN (y = 271.49 – 3.35x, r = −0.52, P<0.05) (Figure 1). Thus, glomerular cellularity decreased (resolved) over time in patients with APSGN.

![Figure 1](image-url)

**Fig. 1.** (A) Total glomerular cell number in PAS-stained biopsy sections from control subjects (minor glomerular abnormality, n = 5) and from APSGN patients (n = 15). Results are expressed as the total number of glomerular cells/GCS. The number of glomerular cells in APSGN patients was significantly higher than that in control subjects. (B) The total number of glomerular cells/GCS (y-axis) in APSGN patients (n = 15) plotted in relation to biopsy date after disease onset (x-axis). There was a significant negative correlation between the number of glomerular cells and disease duration.
Time course and significance of glomerular cell apoptosis

No TUNEL+ cells were found in the glomeruli of control subjects, although tubular epithelial cells were occasionally positive (Figure 2A). Prominent glomerular TUNEL+ cells were observed in patients with early phase APSGN (B, patient 1) as well as in patients with relatively late phase APSGN (C, patient 15). Arrow indicates a TUNEL+ nucleus in the mesangium.

**Fig. 2.** Representative photomicrographs of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) with methyl green (A,B) (original magnification, 200×) or PAS (C) counterstaining (original magnification, 400×) (colour images are available online). No TUNEL+ cells were found in glomeruli of control subjects, although TUNEL+ tubular epithelial cells were occasionally found (A). Prominent glomerular TUNEL+ cells were found in patients with early phase APSGN (B, patient 1) as well as in patients with relatively late phase APSGN (C, patient 15). Arrow indicates a TUNEL+ nucleus in the mesangium.

![Graph 1](image1.png)

**Fig. 3.** (A) The plot of the log value of the number of glomerular TUNEL+ cells/GCS (y-axis) in relation to disease duration (x-axis), accompanied by the plot of the log value of the number of glomerular proliferating cell nuclear antigen (PCNA)+ cells reported previously (21) (n=15) (Modified and reprinted with permission [9]). The decrease in the log value of the number of TUNEL+ cells was slower than that of the log value of the number of PCNA+ cells. Therefore, the ratio of glomerular TUNEL+ cells/PCNA+ cells increased exponentially over time (B, log (y) = 0.51x − 0.74, r = 0.85, P < 0.0001).

**Characteristics of apoptotic cells**

Representative microphotographs of double staining for ssDNA and various glomerular cell markers are shown in Figures 5A–O, and quantified results are shown in Figure 6A. In most APSGN patients, the number of positive cells was generally the greatest by ssDNA staining and the lowest by PAS staining.
majority of ssDNA+ cells were mesangium (Figure 5C) (40.35 ± 13.09%) and endothelial cells (Figure 5F) (33.43 ± 7.78%). Some neutrophils (Figure 5I) (16.30 ± 11.69%) and macrophages (Figure 5L) (8.08 ± 8.74%) were also occasionally positive for ssDNA staining. A few ssDNA+ parietal epithelial cells were observed (Figure 5C), but no ssDNA+ podocytes were identified (Figure 5O). With regard to the time course of cell types in glomerular apoptosis, there was a significant increase in apoptotic mesangial cells \(y = 19.87 + 1.39x, \ r = 0.89, P < 0.0001\) and a significant decrease in apoptotic neutrophils over time \(y = 5.28 - 1.29x, \ r = 0.93, P < 0.0001\) (Figure 6B).

Relation between glomerular apoptotic cells and NAPlR localization or plasmin activity

Most glomerular ssDNA+ cells were localized within areas positive for NAPlR staining (Figures 7A–C) or plasmin activity (Figures 7D–F) in all patients analysed.

Immunostaining for macrophages and scavenger receptors

Phagocytosis of apoptotic cells by macrophages [14] via scavenger receptors is a major pathway for clearance of apoptotic cells [15]. Immunoperoxidase staining for CD68 and the SR-A (Figures 8A and B) revealed prominent infiltration of CD68+ macrophages in the glomeruli of APSGN patients (9.45 ± 5.12 cells/GCS), and most glomerular CD68+ macrophages showed staining for SR-A (10.23 ± 7.22 cells/GCS), regardless of the duration after disease onset.

Discussion

We analysed renal biopsy tissues from APSGN patients with respect to resolution of glomerular hypercellularity. Prominent apoptotic cells were present in APSGN patients, as assessed by TUNEL, ssDNA immunostaining and PAS staining. The trend over time of apoptotic cells detected by these three methods was similar; however, the exact numbers were generally the greatest in ssDNA-stained sections and the lowest in PAS-stained sections. TUNEL is the most popular method for in situ detection of apoptosis, and we previously tried to double-stain paraffin sections by TUNEL to characterize apoptotic cells (Abstract; Oda et al., J Am Soc Nephrol, 1997). However, in our preliminary study, TUNEL staining results varied extremely depending on extent or type of pre-treatment, such as microwave irradiation or proteinase digestion. Therefore, in the present study, we used IF staining of ssDNA on frozen sections for double staining procedures; this method was revealed to be stable and reliable. Actually, immunostaining for ssDNA has been reported to be more sensitive than TUNEL, particularly for early-stage apoptosis, as well as more specific in differentiating between apoptosis and necrosis [13]. Many articles are in agreement on the significant positive correlation between ssDNA staining result and TUNEL result [16–18]. However, the proportion of ssDNA+ cell number to TUNEL+ cell number varies between each report, probably due to the unstableness of the TUNEL against pre-treatment. Watanabe et al. [16] reported approximately three times greater TUNEL+ cells than ssDNA+ cells in human colorectal cancer tissue, while Kumagai et al. [17] and Arai et al. [18] reported obviously greater ssDNA+ cells than TUNEL+ cells in lymphoid tissues of acute canine distemper and in diffuse large B-cell lymphomas, respectively. To confirm the existence of glomerular cell apoptosis, we investigated the expression of activated caspase 3 from selected APSGN patients and control tissues. Indirect immunoperoxidase staining on paraffin sections with rabbit anti-cleaved caspase-3 antibody (ASP-175: Cell Signalling technology, Inc., MA, USA) showed prominent cytoplasmic staining on the glomerular cells of APSGN tissues but no staining on glomeruli of control tissues.
tissues (Supplementary Figure 1, available online), thus confirming the existence of the glomerular cell apoptosis in APSGN.

Regarding the time course, glomerular cell apoptosis was most significant in the earliest phase of APSGN, when cell proliferation was also most prominent. Both proliferating cells and apoptotic cells decreased exponentially over time, but the decrease was more pronounced in proliferating cells than in apoptotic cells. Thus, apoptosis was predominant in the later phase of the disease and may contribute to the decrease in the number of glomerular cells in patients with APSGN. Cell proliferation and cell death are opposing phenomena. Therefore, the simultaneous existence of proliferation and apoptosis in the early phase of APSGN was unexpected. However, recent studies indicate a strong association between cell proliferation and apoptosis, and some investigators regard apoptosis as a part of the cell cycle. As a matter of fact, deregulated expression of the transcription factor...
E2F-1 has been shown to induce both S-phase entry and apoptosis [19]. The time course of glomerular cell proliferation and apoptosis observed in the present study is in surprisingly good accordance with that in experimental GN with successful resolution [20]. The significance of glomerular cell apoptosis in experimental GN has been described as a double-edged sword [5]. A beneficial deletion of excess resident glomerular cells may promote resolution of glomerular injury, while uncontrolled deletion of glomerular cells...
may contribute to the progression of renal disease to the end-stage hypocellular kidney [3,5,6]. The kinetics of glomerular cell proliferation and apoptosis in APSGN in the present study provides evidence that the beneficial effect (resolution mechanism) really occurs in human GN. Moreover, our results are in accordance with the report of Soto et al. [4], which showed correlated up-regulation of apoptosis and proliferation in glomeruli in APSGN.

Prominent up-regulation of cell proliferation and apoptosis from the earliest phase of APSGN suggests that the disease-inducing antigen (nephritogenic antigen) itself may induce proliferation and apoptosis in this disease. The localization of glomerular apoptotic cells within areas of NAPr deposition may supports this concept. Actually, the other leading candidate nephritogenic antigen, streptococcal pyrogenic exotoxin B (SPEB), has been shown to induce apoptosis and proliferation in human mononuclear leukocytes in vitro [21]. However, in the present study, the major cell type involved in glomerular apoptosis was not leukocytes but resident glomerular cells. Alternatively, plasmin may induce glomerular cell apoptosis and proliferation. We proposed a pathogenic role of NAPr-bound plasmin activity in the development of APSGN on the basis of identical distribution of NAPr and plasmin activity in the glomeruli of APSGN patients [12]. Plasmin has been shown to induce proliferation of several cell types [22] and to induce a type of apoptosis termed anoikis in certain cell types via degradation of extracellular matrix proteins [23].

We previously reported that the majority of glomerular-infiltrating cells in patients with APSGN were macrophages and neutrophils, with a small number of T cells [9]. Numbers of these infiltrating cells tended to decrease uniformly, whereas the ratio of macrophages to neutrophils increased as the duration after disease onset increased. In the present study, we found that most glomerular macrophages showed strong staining for SR-A. These features of infiltrating cells appear to correspond with the process of resolution of glomerular hypercellularity, as neutrophil ingestion by macrophages is reported to be a main resolution pathway for experimental GN [14]. However, the percentage of apoptotic neutrophils among total glomerular-infiltrating neutrophils in the early phase of APSGN was not high (Figure 6I). As suggested by Hughes et al. [24], a large proportion of neutrophils should return to the circulation.

In summary, we determined the time course for the resolution of glomerular hypercellularity in human APSGN with attention to the degree and cell types of glomerular cell apoptosis. The process of glomerular resolution shown in the present study provides valuable information with respect to the understanding of progression and resolution of glomerular disease in humans.

Acknowledgements. The authors are grateful to their colleagues Ms Satoko Kiyono and Ms Mami Morisugi for their expert secretarial assistance, to Ms. Tatsuyo Harasawa, Central Research Laboratory, National Defense Medical College, for excellent technical assistance and to Dr Hitoshi Tsuda, Department of Pathology, National Defense Medical College, for valuable advice and discussion.

Conflict of interest statement. None declared.

References

Apoptosis in APSGN


Received for publication: 13.7.06
Accepted in revised form: 2.11.06