Int. J. Exp. Path. (1993) **74**, 9–19

Anti-glomerular basement membrane glomerulonephritis (anti-GBM GN) in the mouse: BrdU-labelling indices and histological damage

JANICE WHEELER*, HELEN ROBERTSON*, A.R. MORLEY† AND D.R. APPLETON‡

*Departments of *Microbiology, †Histopathology and ‡Medical Statistics, University of Newcastle upon Tyne, UK*

Received for publication 19 March 1992
Accepted for publication 14 August 1992

Summary. In-vivo BrdU incorporation and visualization by immunohistochemistry, previously reported in normal mouse kidney, were applied to a mouse model of anti-GBM GN, induced by immunization with rabbit anti-mouse GBM antiserum, to assess the contribution of capsular cell proliferation in the development of crescents.

A significant increase ($P=0.003$) in the BrdU-labelling index (LI) for capsular cells was observed, as compared to normal mice (5.76 ± 1.1 *vs* $0.70\% \pm 0.12\%$). Elevated LI were also observed for tuft and tubular cells but these increases were not statistically significant. It was concluded that, in this model, capsular cell proliferation is a major contributory factor to the formation of cellular crescents.

In addition, other pathological features, indicative of glomerular damage, were assessed semi-quantitatively alongside numbers of labelled capsular cells per glomerulus. It was found that podocyte vacuolation is strongly associated with, and may precede, proliferation, suggesting some common causative factor. Fibrin, when present, was confined within the tuft capillary loops and was only weakly associated with either podocyte vacuolation or capsular cell proliferation. It was concluded that this protein does not play a major role in the initiation of pathological damage.

Finally, glomerular lesions were found to be randomly distributed. Thus, the idea of intraglomerular signalling, resulting in 'clustering' of damaged glomeruli, is not supported.

Keywords: anti-GBM, bromodeoxyuridine, glomerulonephritis, labelling index, mouse

The use of animal models of crescentic glomerulonephritis (CGN) has made a major contribution to understanding the role of crescents in loss of renal function due to glomerular occlusion. The aetiology underlying CGN is poorly understood in man but the extent of glomerular occlusion and proportion of glomeruli involved are of considerable importance (Zollinger & Mihatsch 1978).

Many studies of CGN, in man and experimental

Correspondence: Dr Janice Wheeler, Department of Microbiology, University of Newcastle upon Tyne, The Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK.

animals, have indicated that both proliferation and accumulation of cells in Bowman's capsule play some part in crescent development. However, despite their apparent importance, there have been few studies of cell proliferation in CGN, in either man or animals, which exploit powerful cell kinetic techniques such as bromodeoxyuridine (BrdU) incorporation and visualization.

Incorporation of BrdU into DNA of S-phase cells has now been established as a method for the unequivocal identification of such cells (Cawood & Savage 1983; Dean *et al.* 1984). A number of studies in man have been undertaken using in-vitro BrdU incorporation into rapidly proliferating tissues, both normal and neoplastic (Thornton *et al.* 1988; Veronese *et al.* 1989). However, studies involving in-vivo BrdU incorporation are less frequent (Danova *et al.* 1988) and are largely limited to experimental animals because of the mutagenic properties of BrdU.

Previous studies in this laboratory have established a method for measuring BrdU-labelling indices in the slowly proliferating normal mouse kidney by in-vivo incorporation of BrdU. The label is detected with a monoclonal antibody to BrdU and visualized by an immunoperoxidase technique (Robertson *et al.* 1990). With this method, we have now measured the labelling indices in a mouse model of anti-glomerular basement membrane glomerulonephritis (anti-GBM GN) to evaluate the contribution of cell proliferation to crescent development. In addition, we have assessed the histological damage within glomeruli and examined the relationship between damage and labelling. The model, in male T70 (Beige) mice, has been developed in this laboratory (Wheeler *et al.* 1990) and involves immunization with rabbit anti-mouse GBM antiserum.

Materials and methods

Experimental animals

Inbred T70 (Beige) mice [(C3H × 101)F_1 hybrids] were originally obtained from the MRC Radiobiology Unit, Harwell, Didcot, Oxon and were then maintained by continuous brother–sister mating.

One New Zealand White male rabbit was purchased from Ranch Rabbits (Crawley Down, Sussex).

All animals were housed in the Comparative Biology Centre of the University of Newcastle upon Tyne and had unrestricted access to food and water.

Preparation of mouse GBM

Lyophilized GBM was obtained from normal mouse kidneys, as detailed previously (Morley & Wheeler 1985) and stored at −30°C.

Production of rabbit anti-mouse GBM antiserum

Rabbit anti-mouse GBM antiserum was obtained as detailed previously (Wheeler *et al.* 1990) and stored at −20°C. A pool of the first four bleeds, obtained 44, 67, 162 and 288 days after the initial injection of GBM, was used in this study.

Each bleed had been shown to have a similar high level of anti-mouse GBM antibodies, as determined by a modification of the ELISA of Wheeler *et al.* (1988). Furthermore, a dose of 0.2 ml of each bleed had been shown to induce a high daily weight gain, associated with the nephrotic syndrome and capsular cell proliferation, in the anti-GBM GN mouse model of Wheeler *et al.* (1990).

Experimental design for the induction of anti-GBM GN

The following protocol was based upon earlier studies with the anti-GBM GN mouse model (Wheeler *et al.* 1990; J. Wheeler, H. Robertson & A.R. Morley, unpublished observations).

T70 (Beige) male mice, of known age and weight, were immunized with the pooled rabbit anti-mouse GBM antiserum. Two 0.1 ml injections (alternate intravenous/intraperitoneal) were given on days −1 and 0 to seven mice (Group A) and four animals had one 0.1 ml intravenous injection on day 0 (Group B). All animals were weighed daily and carefully monitored for evidence of ascites, tissue oedema and haematuria. One hour prior to sacrifice, mice were injected with BrdU, as detailed below. Mice were killed by cervical dislocation and kidneys removed and fixed in formal sublimate for BrdU immunohistochemistry and histological assessments, as detailed below.

BrdU incorporation

One hour prior to sacrifice, mice were injected intraperitoneally with 50 mg/kg BrdU (Sigma Chemical Company Ltd, Poole, Dorset), at a concentration of 6 mg/ml in 0.05 M phosphate-buffered saline, pH 7.4 (PBS).

BrdU immunohistochemistry and general histology

Methodology for the detection of BrdU-labelled cells was as detailed previously (Robertson *et al.* 1990). In brief, the kidneys were removed from BrdU-injected mice immediately after killing and fixed in formal sublimate for 3 hours at room temperature. Fixation was followed by processing and embedding in paraffin wax (m.p. 56°C).

Sections at 2–4 μm, on lysine-coated slides, were dewaxed and treated with 0.3% hydrogen peroxide in methanol to inactivate endogenous peroxidase. They were then covered with a rat monoclonal antibody to BrdU (Sera-Lab Ltd, Crawley Down, Sussex: code MAS 250b), at a 1 in 20 dilution, and incubated overnight at 4°C. The monoclonal antibody was detected with rabbit anti-rat immunoglobulins conjugated to horse-radish peroxidase (Dako Ltd, High Wycombe, Bucks: code P162), at a 1 in 200 dilution, and incubation for 1 hour at room temperature. The peroxidase reaction was developed with 3,3-diaminobenzidine (1 mg/ml), plus imidazole (0.68 mg/ml), containing 0.02% hydrogen peroxide, for 2–3 minutes at room temperature. The sections were counterstained with periodic acid–Schiff and Mayer's haematoxylin. With this method, BrdU-labelled nuclei appear brown, unlabelled nuclei purple and basement membranes deep pink.

Sections treated as above were used to measure BrdU-labelling indices, as detailed below. The same sections were assessed qualitatively for general histological damage and semi-quantitatively for glomerular damage, also detailed below.

Measurement of labelling indices (LI)

Labelled and unlabelled nuclei were counted in the tufts and capsules of the first 25 glomeruli encountered on scanning a section, from left to right, in each of two distinct areas. These areas were located at one of the poles and in the mid-region of the kidney. In the tubules, labelled and unlabelled nuclei were counted in approximately 5000 cells (15–17 fields), spanning a section in the mid-region of the kidney, cut longitudinally. In this way, tubular cells of both cortex and medulla were included. All counts were performed at × 400 magnification. The labelling indices (LI) of the three populations of cells were then calculated as percentages.

Semi-quantitative assessment of glomerular damage

This was performed independently of the measurement of the labelling indices and was undertaken at × 200 magnification. Each glomerulus in two adjacent 'strips' of kidney, located at a pole and in the mid-region (Figure 1), was visualized in turn and was assessed for histological damage previously observed in this model (between 12 and 33 glomeruli in each pair of strips). Podocyte vacuolation and fibrin deposition in the glomerular tuft were scored on a 4-point scale of 0 (absent) to 3 (severe). BrdU-labelled nuclei were counted in the capsules and expressed as per glomerulus.

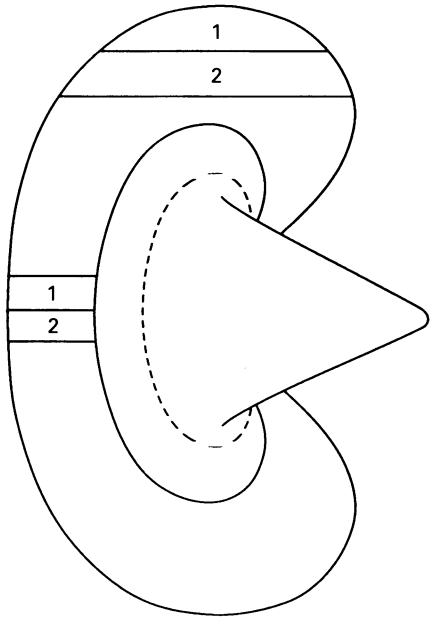

Figure 1. Diagrammatic representation of kidney cross-section, illustrating the two adjacent strips at a pole and in the mid-region, from which glomerular damage was assessed.

Statistical methods

Paired and two-sample *t*-tests, analysis of variance and a permutation test were used as detailed.

Results

Induction of GN

The seven mice (A1–A7) immunized with 0.2 ml of rabbit anti-mouse GBM antiserum all showed a rapid gain in weight immediately following the last injection, with average daily weight gains between days 0 and 3 (DWG) ranging from 1.22 to 2.44 g/day (Figure 2). As previously demonstrated (Wheeler *et al.* 1990), this parameter is a useful in-vivo guide to the severity of the renal lesion, the gain in weight being associated with ascites, oedema and haematuria. The weights of all seven animals subsequently either plateaued or peaked, at which point the mice were sacrificed. All mice in this group were sacrificed between days 5 and 7.

Of the four mice given the reduced 0.1 ml dose (Fig. 2), mouse B4 showed the same response to that observed in the Group A animals, with DWG of 1.41 g/day. This animal was sacrificed on day 7. Mice B1 and B3 showed similar responses but these were slower initially, as reflected by

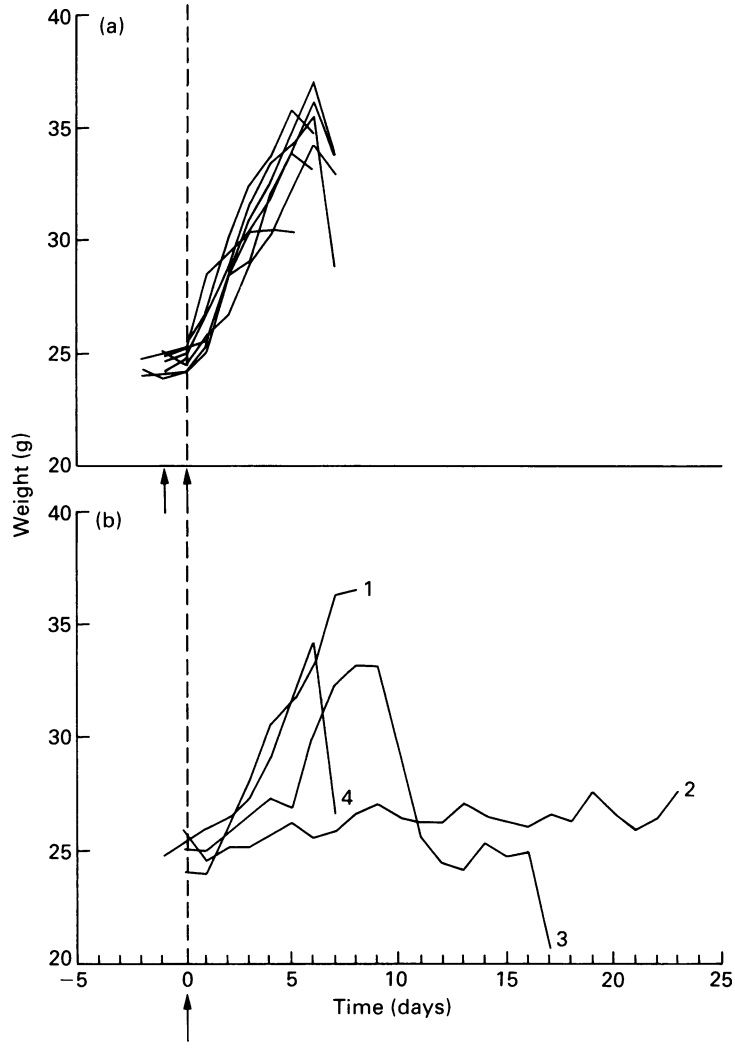

Figure 2. Daily weight gain of mice following immunization (↑) with rabbit anti-mouse GBM antiserum. a, Group A (0.2 ml dose); b, Group B (0.1 ml dose).

their respective DWGs, which were 0.61 and 0.53 g/day. Mouse B1 was sacrificed on day 8. Although showing a marked weight loss between days 9 and 13, mouse B3 remained active during this period and, thus, was not sacrificed at this time. The weight of this animal appeared to stabilize between days 13 and 16 but subsequently fell again and the animal was sacrificed on day 17. Mouse B2 appeared not to respond to the antiserum. Its DWG was −0.15 g/day and, at sacrifice on day 23, this animal showed no evidence of ascites, oedema or haematuria.

BrdU-labelling indices (LI)

The LI in the glomerular tuft and Bowman's capsule for all 11 test mice, as derived from counts made at a pole and in the mid-region of the kidney, are shown in Figure 3. These are compared with similar counts previously determined from five normal mice (Robertson *et al.* 1990). As can be seen, the assessments at two areas gave very similar LI for both the tufts and capsules, within both the normal and test groups. Furthermore, paired *t*-tests indicated no significant differences between the pole and mid-region LI for the normal tuft ($P=0.79$), normal capsule ($P=0.52$), test tuft ($P=0.41$) and test capsule ($P=0.19$).

As statistical evaluation showed no significant differences between the LI determined from counts made at a pole or in the mid-region of a kidney, a combined LI was determined. These combined LI for the tuft, capsule and tubular cell populations, from the normal, Group A and Group B animals are shown in Table 1 and Figure 4.

As can be seen, the LI of the three populations of cells in the normal mice, one hour after flash labelling with

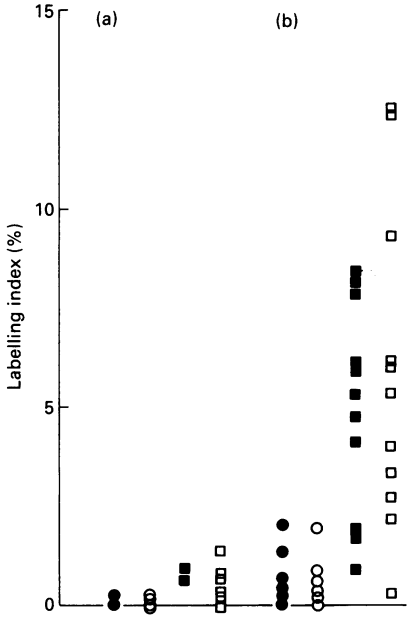

Figure 3. BrdU-labelling indices for tuft and capsular cells from a, normal and b, test mice (Groups A and B) as determined by counts made at a pole and in the mid-region. ●, Tuft cells pole; ○, tuft cells mid-region; ■, capsular cells pole; □, capsular cells mid-region.

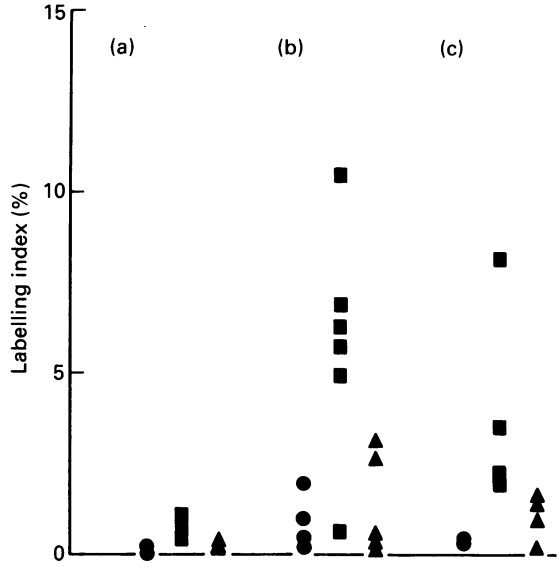

Figure 4. Combined (pole and mid-region) BrdU-labelling indices for ●, tuft; ■, capsular and ▲, tubular cells from a, normal; b, Group A; c, Group B mice.

Table 1. Combined (pole and mid-region) BrdU-labelling indices in normal and test mice

Mouse	Day of kill	Labelling index (%)		
		Tuft	Capsule	Tubule
Normal				
1		0.09	0.45	0.38
2		0.10	0.49	0.28
3		0.22	0.67	0.23
4		0.05	1.12	0.20
5		0.14	0.77	0.14
Group A				
1	7	0.48	5.74	0.23
2	7	1.01	5.73	2.62
3	5	0.18	0.63	0.07
4	6	1.96	10.19	3.09
5	7	0.45	4.91	0.15
6	7	0.30	6.28	0.35
7	6	0.36	6.85	0.51
Group B				
1	8	0.35	8.18	1.32
2	23	0.38	1.96	0.08
3	17	0.43	3.53	0.89
4	7	0.37	2.21	1.61

BrdU, were all very low (1.12% or less). Randomized-block analysis of variance indicated a significant difference ($P=0.003$) in mean values between tuft (0.12%), capsule (0.70%) and tubule (0.25%).

A wide range of LI was found for all three populations of cells in Group A mice. However, in comparison with the normal mice, the mean LI for each population of cells was elevated. The most marked increase in LI was noted in the capsular cells, with a mean LI of 5.76% as compared to 0.70% for the normals. A two-sample *t*-test (using logarithms) showed this difference to be statistically significant ($P=0.0012$). The LI for the tuft cells was also increased significantly compared to the normals (0.68 *vs* 0.120%, $P=0.0038$). A similar, but non-significant observation was made for the tubular cells (1.00 *vs* 0.246%, $P=0.35$).

With the Group B mice, increased LI were again noted, especially in the capsular cell population, as compared to the normal animals. Even mouse B2, which appeared not to respond to the antiserum by virtue of a gain in weight, showed increased LI in the tuft and capsular cells but not in the tubular cell population. However, as this group was both smaller and more heterogeneous with respect to the time of sacrifice, statistical evaluations were not made.

Histological damage

The histological features were similar in all Group A

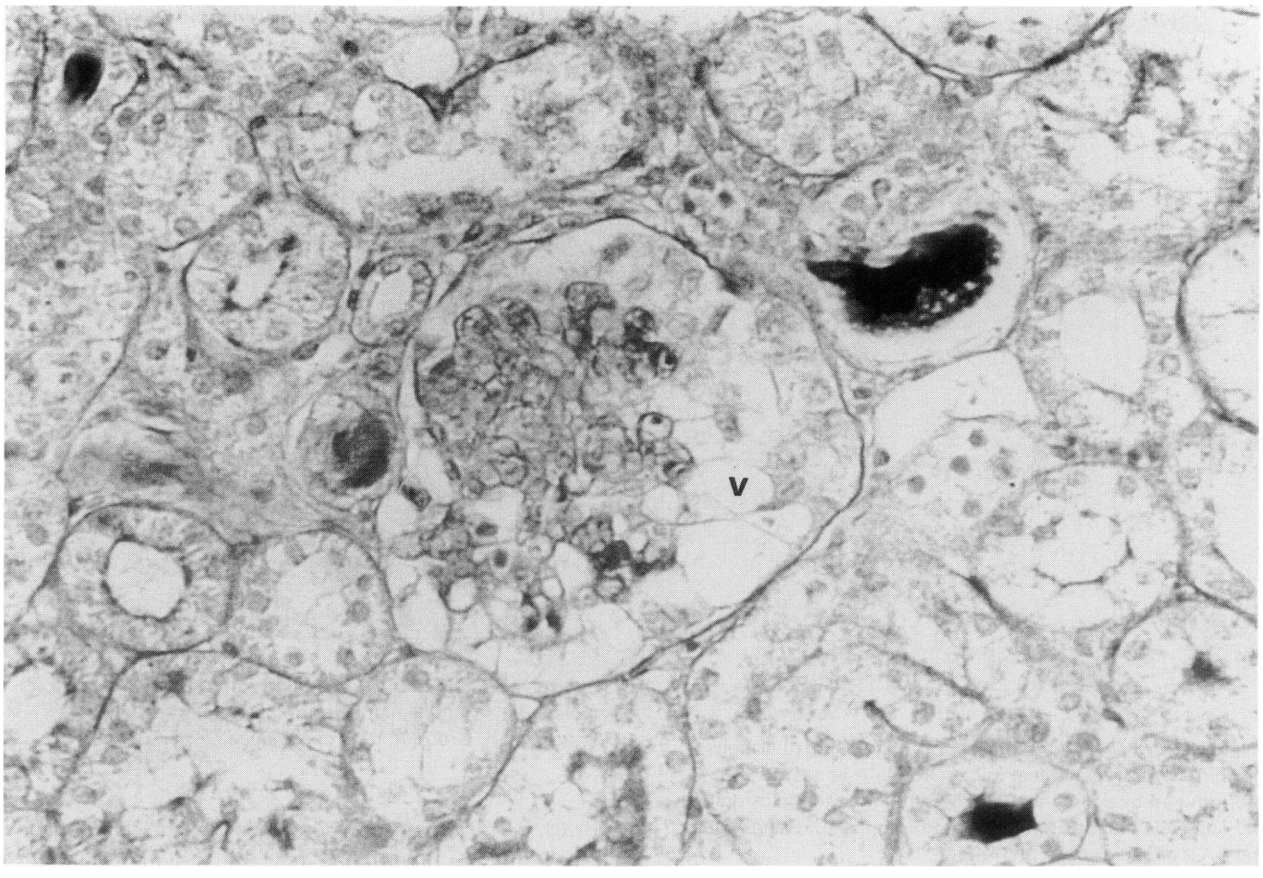

Figure 5. Glomerulus of Group A mouse showing podocyte vacuolation (V). × 200.

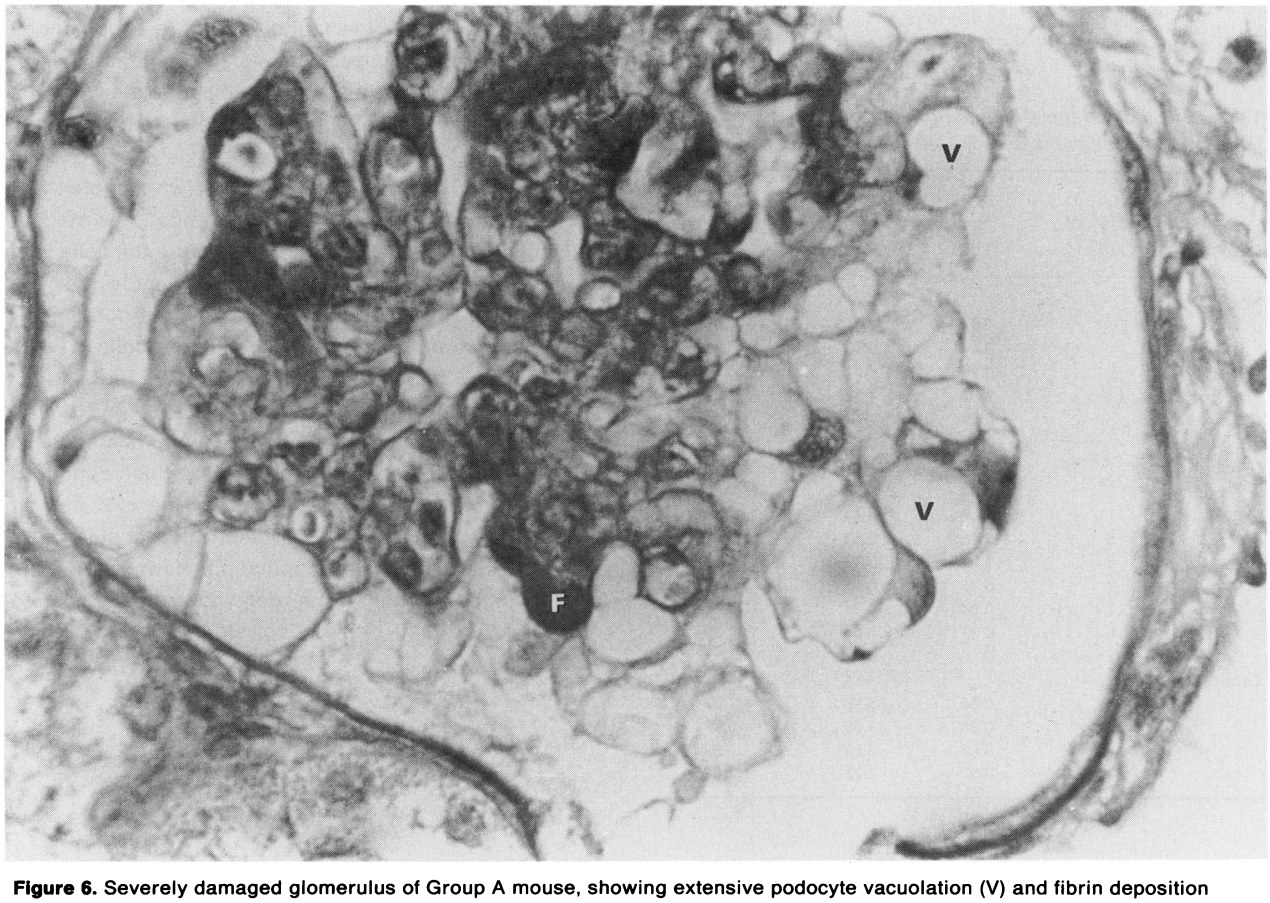

Figure 6. Severely damaged glomerulus of Group A mouse, showing extensive podocyte vacuolation (V) and fibrin deposition within capillary loops (F). × 400.

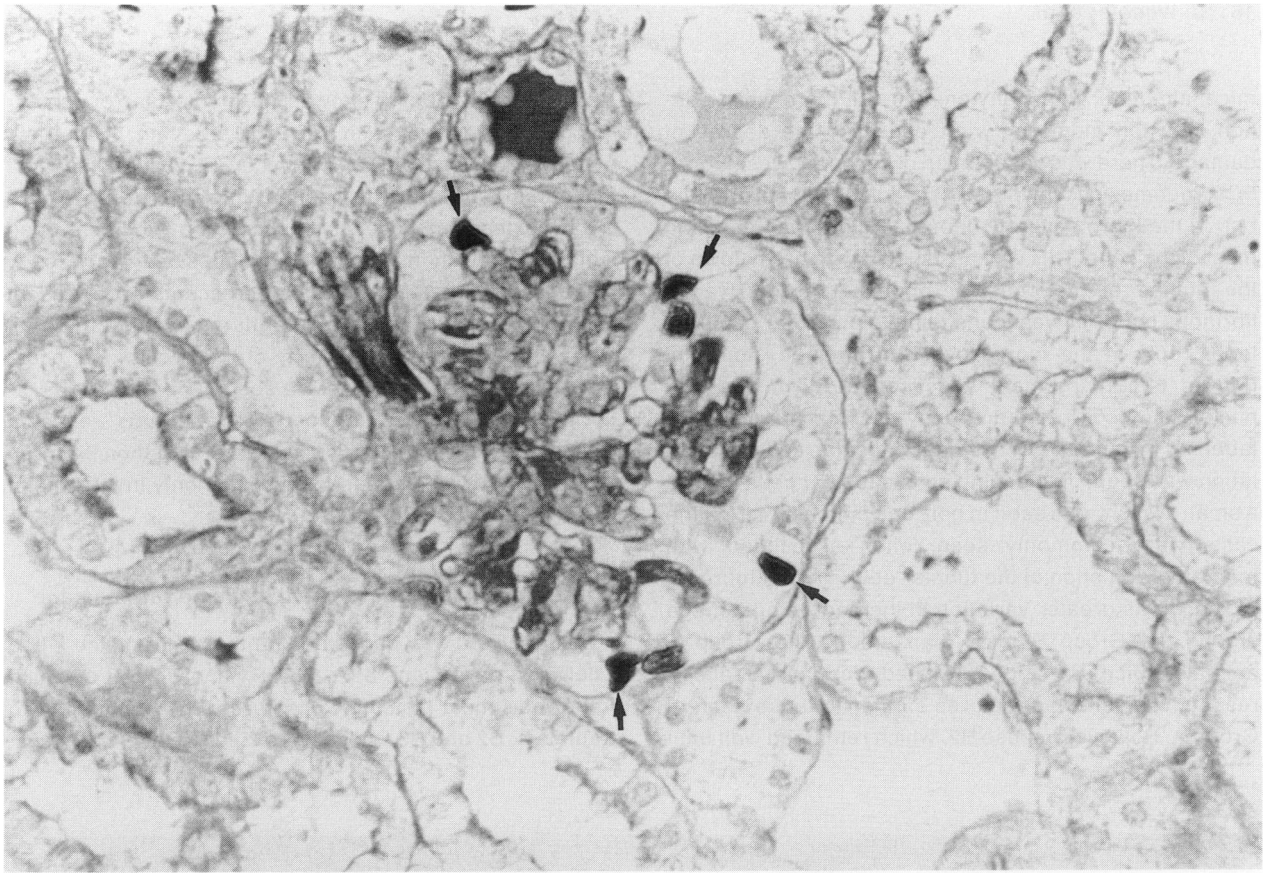

Figure 7. Glomerulus of Group A mouse showing enlargement and irregularity of BrdU-labelled capsular nuclei (↑). × 200.

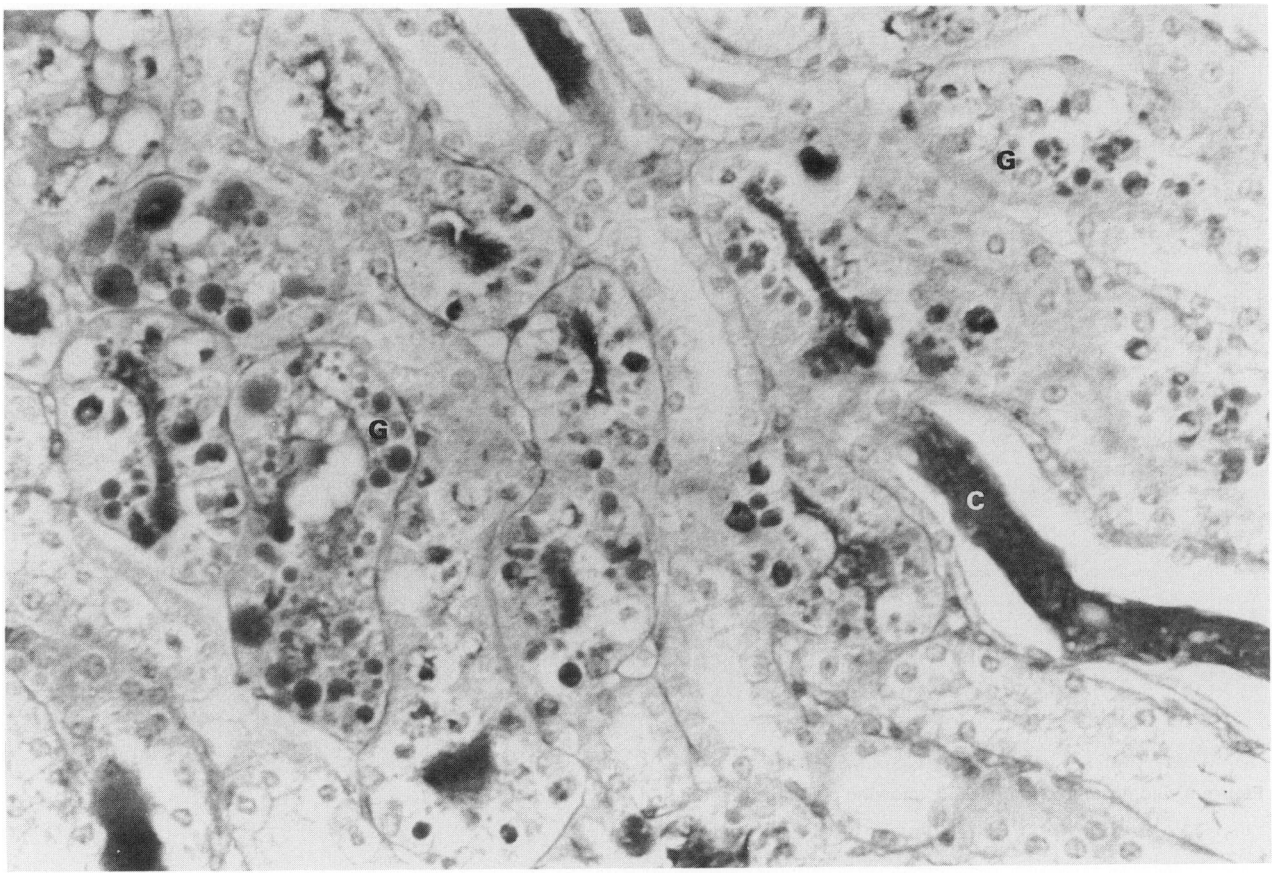

Figure 8. Tubular damage in Group A mouse, illustrated by hyaline casts (C) and granulation (G) of tubular epithelium. × 200.

mice, although, within any one animal, there was variation between glomeruli with respect to the degree of damage. There was vacuolation of the podocytes (Figure 5), sometimes occupying only part of the capsular space but, in the more severely damaged glomeruli, affecting most of the cells. This was associated with breakdown of the outline of the capsular epithelial cells. Hyaline fibrinous material was found filling glomerular capillary loops, only in the more severely damaged glomeruli (Figure 6). Enlargement and irregularity of capsular nuclei also occurred, apparently associated with BrdU labelling (Figure 7). Occasional capsular cell accumulation was seen, but heaping up of capsular cells to form a small cresent was seen in only one animal, A4. Hyaline casts were commonly seen, often associated with marked granulation of the tubular epithelium (Figure 8).

There was greater variation in the degree of damage among the Group B mice than among the Group A animals. Mice B1 and B4, killed on days 8 and 7 respectively, showed features similar to those seen in Group A. However, mouse B2, which remained well until sacrifice at 23 days, showed no glomerular or tubular abnormalities and much less BrdU labelling. In contrast, mouse B3, which was killed on day 17, showed the most marked changes of any animal in either group. There was extensive podocyte vacuolation and enlarged glomeruli, with many capillary loops showing heavy deposits of fibrin. Large cellular crescents were present in approximately 5% of glomeruli examined (Figure 9). However, despite the severity of these changes, occasional normal glomeruli were seen.

In summary, comparison of the groups revealed similar lesions, but with much greater variation in Group B, and with well developed crescents only in the animal which survived 17 days.

Semi-quantitative assessment of glomerular damage

This analysis was performed on those nine mice killed between days 5 and 8 (A1–A7, B1 and B4), all of which showed similar histological features. The remaining two animals, B2 and B3, which were killed at 23 and 17 days,

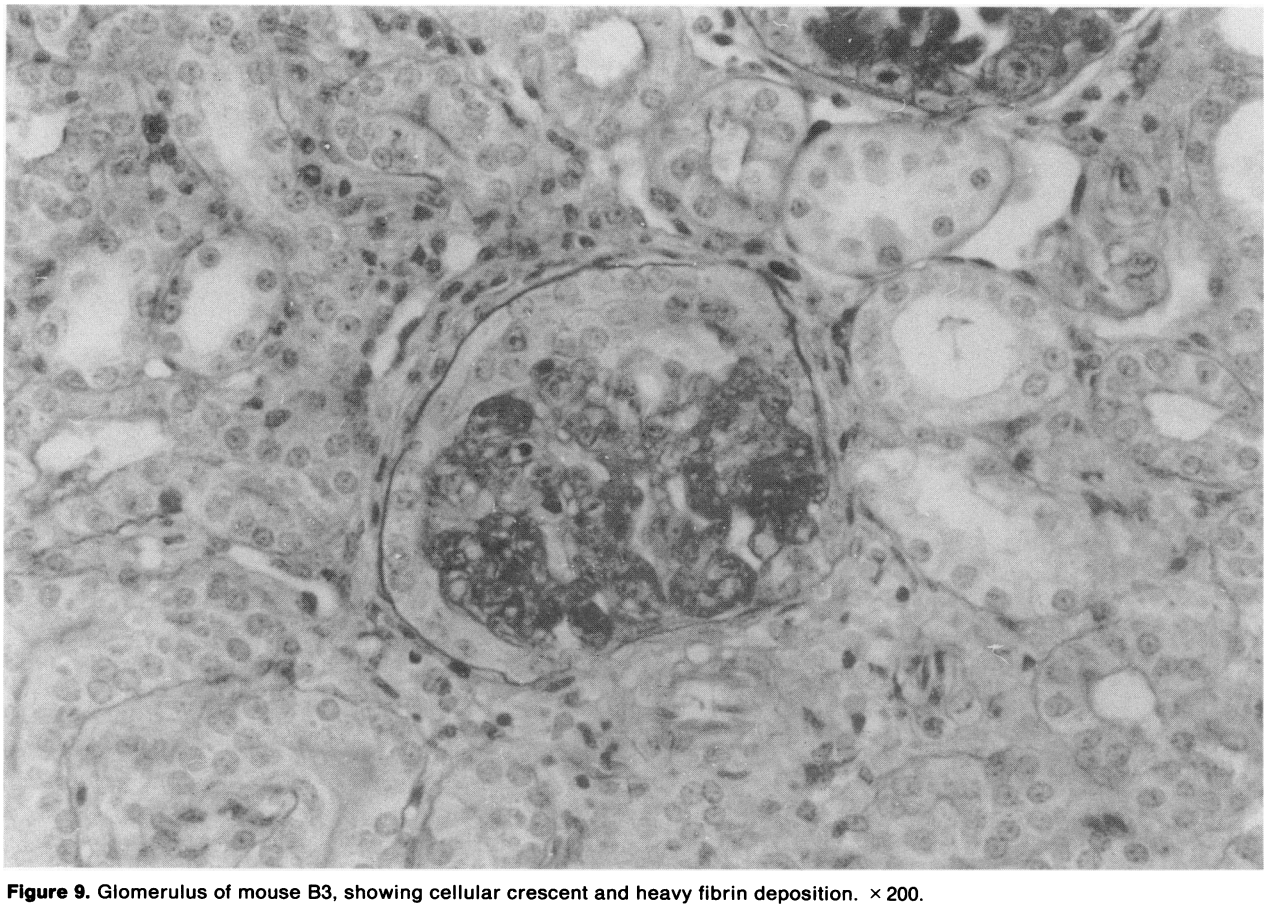

Figure 9. Glomerulus of mouse B3, showing cellular crescent and heavy fibrin deposition. × 200.

Table 2. Semi-quantitative assessment of glomerular damage

Mouse	A3	A4	A7	A1	A2	A5	A6	B4	B1
Day of kill	5	6	6	7	7	7	7	7	8
Number of glomeruli counted									
Pole	25	31	21	33	19	19	24	33	25
Mid-region	29	20	28	31	15	18	12	20	24
Total	54	51	49	64	34	37	36	53	49
Podocyte vacuolation: mean score per mouse. Scale: 0 (absent) to 3 (severe)									
Pole	0.60	0.84	0.95	0.91	0.58	0.74	1.00	0.94	0.68
Mid-region	0.72	1.15	1.25	1.13	1.00	1.11	1.25	0.75	0.88
Fibrin deposition: mean score per mouse. Scale: 0 (absent) to 3 (severe)									
Pole	0.08	0.16	0.05	0.21	0.37	0.26	0.17	0.06	0.20
Mid-region	0.03	0.65	0.50	0.32	0.47	0.11	0.33	0.05	0.25
BrdU-labelled capsular cells per glomerulus: mean count per mouse									
Pole	0.08	0.45	0.43	0.54	1.20	0.47	0.46	0.61	0.64
Mid-region	0.03	1.65	0.86	0.84	1.60	0.28	0.75	0.60	0.62

respectively, and appeared to have different characteristics, were excluded from statistical analysis. The results are shown in Table 2.

A paired *t*-test showed that there was significantly less podocyte vacuolation at the poles of the kidneys (mean score 0.80) than in the mid-regions (mean score 1.02, $P=0.006$). However, no significant differences were observed between polar and mid-region glomeruli with respect to the amount of fibrin (mean scores 0.17 and 0.30 respectively, $P=0.11$) or the numbers of BrdU-labelled cells per glomerulus (mean counts 0.54 and 0.80, $P=0.096$).

To examine whether there was a relationship *within animals* between podocyte vacuolation, fibrin deposition and BrdU-labelled capsular cells/glomerulus, analyses of variance were carried out on the combined results from the pole and mid-region of the kidney for each animal. The results showed that the presence of fibrin had a significant but weak association with both podocyte vacuolation and capsular labelling ($P=0.05$). There was, however, a very strong relationship between the latter two variables ($P=<0.001$). This varied somewhat between animals but can be expressed briefly as follows:

(1) as podocyte vacuolation increased by 1, the number of BrdU-labelled cells in the capsule increased by 0.71 ± 0.05;
(2) as the number of labelled cells increased by 1, the podocyte vacuolation score increased by 0.43 ± 0.03.

These alternative modes of expression are equally valid since we have no reason to regard either of the factors as the dependent variable.

An alternative method of evaluating these data is to construct the contingency tables for each of the nine animals, relating the two abnormalities (Table 3). These results show that it is unusual to find capsular labelling in the absence of podocyte vacuolation. However, when capsular labelling is present podocyte vacuolation is also a common feature.

Glomerular clustering

The glomeruli scored in the above investigation had been recorded sequentially in two strips at the pole and mid-region of the kidney. In order to test the possibility of clustering of abnormal glomeruli, a permutation test was devised (cf, one-sample runs test of randomness (Siegel & Castellan 1988)). This test was applied to all strips in which there were at least two 'abnormal' and at least two 'normal' glomeruli; other strips were deemed incapable of yielding useful information. Three criteria of 'abnormal' were defined: (1) glomeruli having any labelled

Table 3. Contingency table for presence or absence of podocyte vacuolation and BrdU-labelling of capsular cells

		Capsular cell BrdU-labelling					
		−	+	−	+	−	+
Glomerular podocyte vacuolation	−	23	2	25	2	16	1
	+	10	14	17	20	16	16
	−	23	0	27	0	17	0
	+	14	16	25	2	11	9
	−	13	0	20	3	12	8
	+	12	11	15	13	3	11

Table 4. *P*-values of permutation test for glomerular clustering

Variables	Pole	Mid-region	Combined
Capsule labelling	0.91	0.97	0.99
Podocyte vacuolation	0.52	0.58	0.46
Fibrin	0.40	0.10	0.14

cells in the capsule; (2) a podocyte vacuolation score greater than zero; (3) a fibrin score greater than zero.

From nine animals, 28 strips were available for analysis of capsular labelling (15 polar, 13 mid-region); for podocyte vacuolation, 33 strips were analysed (17 polar, 16 mid-region); and for fibrin, only 20 strips possessed a suitable mix of normal and abnormal glomeruli (nine polar, 11 mid-region).

In summary, the test counted the number of changes in each strip from normal to abnormal, and abnormal to normal. Thus, low counts were indicative of clustering. A one-tailed probability (P_i) was found for each strip, and these were combined by assuming that k strips were independent and treating $-2\,\Sigma(\log P_i)$ as a χ^2 value with $2k$ degrees of freedom. The P values obtained are given in Table 4 and show that there was no evidence of clustering.

Discussion

The mouse model of anti-GBM GN used in this study has been developed and extensively investigated in our laboratory (Morley & Wheeler 1985; Wheeler *et al.* 1990). As with most animal models, some inter-animal variability has been encountered. Further, as yet unpublished, observations have indicated that the major source of this variability is the efficacy of the particular antiserum/bleed employed. Thus, in this study, to minimize variability, an antiserum pool of proven nephrotoxicity was used.

The results indicate that, with this antiserum pool, a dose of 0.2 ml achieved a reproducible response in the seven Group A mice in terms of DWG. With a dose of less than 0.2 ml (Group B), the response was much more variable between the four mice, ranging from no apparent response (B2) to the rapid response characteristic of Group A mice (B4). As all animals in both groups were of similar starting weights and ages, this variability must be attributed to the individual's ability to deal with this immunological insult.

In-vivo BrdU-labelling of S-phase cells is now widely regarded as an excellent parameter for cell proliferation. Previous studies with this technique in normal male mice have allowed the measurement of LI for the various populations of cells within the kidney (Robertson *et al.* 1990). The unambiguous localization of labelled nuclei, inherent in this method, makes it particularly suitable for application to a model of anti-GBM GN, in which crescents may develop.

Initially, LI for the tuft and Bowman's capsule were determined from counts made at a pole and in the mid-region of each kidney. Counts made at two sites would highlight any differences in LI, which might reflect variations in renal blood flow and, consequently, exposure to immunological damage. No significant difference was noted in the LI for the tuft or Bowman's capsule determined at these two sites. In addition, evaluation of labelled cells per glomerulus and fibrin deposition, which was performed independently, also showed no significant differences between the pole and mid-region. However, podocyte vacuolation was significantly higher in the mid-region compared to the pole.

Reproducibility of the model was achieved in the seven mice in Group A of this study, in terms of DWG, but a wide range of LI was found in all populations of cells and most markedly within the capsule. Although the mean LI for the tubular cells was elevated in the Group A mice as compared with normal animals, this difference was not significant. However, a comparison of the mean LI for the group with those measured in normal mice showed a significant sixfold increase in tuft LI and an eightfold increase in capsular LI. The capsular epithelium in the male mouse is of proximal tubular epithelial type. Because of the considerably greater labelling in the capsular epithelium as compared to the tubular epithelium, one may speculate that this site is especially sensitive to stimuli such as release of coagulation factors or cytokines and reacts by cell proliferation.

Whilst statistical evaluations were not made of the LI of tuft, capsular and tubular cells in Group B mice, because of the heterogeneity of the group, the greatest increase above 'normal' was also in the capsular cells. It is notable that one animal in Group B (B2), which appeared to show no histological or functional response and was sacrificed after 23 days, showed a modest increase in capsular LI compared to normals.

A concurrent study to identify macrophages within Bowman's space has been performed with the rat monoclonal antibody to the mouse macrophage-specific antigen F4/80. No evidence has been obtained to support the infiltration of these cells in this model (data not shown).

All the Group A mice showed some degree of podocyte vacuolation, fibrin deposition and capsular proliferation. The degree of podocyte vacuolation was striking and similar to that described by Furness *et al.* (1989). Despite

the level of capsular proliferation, well defined crescents were not apparent in these animals killed between days 5 and 7.

The variability of histological damage in the Group B animals suggests that, at the lower dose, individual susceptibility is important. The observation that one animal in this group (B3) showed both high LI and severe morphological damage with large cellular crescents probably reflects the longer period of survival of this animal (17 days) compared with the Group A animals.

Examination of the main morphological and proliferative features within glomeruli indicates that podocyte vacuolation is strongly associated with capsular labelling. Furthermore, the results of the contingency table (Table 3) support the suggestion that podocyte vacuolation precedes proliferation of capsular cells, and then persists as proliferation continues. The observation that these features are likely to coexist in the same glomerulus suggests some common causative factor. The nature of any such factor remains speculative. Previous observations of proteinuria and nephrotic syndrome in this model (Wheeler *et al.* 1990) suggest that damage to the GBM plays an important part. Other studies have suggested a role for fibrin (Vassalli & McCluskey 1971; Min *et al.* 1974). However, in this model we have observed that fibrin, when present, is largely confined within the tuft capillary loops rather than in Bowman's space. In this study, we have, in addition, noted a weak association between fibrin deposition and either podocyte vacuolation or capsular proliferation. All these observations are against a major role for this protein.

It is tacitly assumed that glomerular lesions are randomly distributed, but an observer will frequently find adjacent glomeruli showing damage. Few attempts have been made to test this objectively. The method used here is deficient in ignoring the three-dimensional structure of the kidney and fails to detect the obvious non-random arrangement of glomeruli in relation to the intralobular arteries. Our failure to demonstrate evidence of clustering in capsular proliferation, podocyte vacuolation, or fibrin deposition may, therefore, be due to inadequate methodology. Alternatively, our results might indicate random variation in blood supply or individual glomerular susceptibility. The result does not support the idea of the existence of any kind of interglomerular signalling.

Acknowledgements

We are indebted to the National Kidney Research Fund for financial support.

References

Cawood A.H. & Savage J.R.K. (1983) A comparison of the use of bromodeoxyuridine and [^{3}H] thymidine in studies of the cell cycle. *Cell Tissue Kinet.* **16,** 51–57.

Danova M., Riccardi A., Brugnatelli S., Fiocca R., Girino M., Villani L., Giordano P., Dionigi P., Giordano M., Buttini R., Ucci G. & Mazzini G. (1988) *In vivo* bromodeoxyuridine incorporation in human gastric cancer: a study in formalin fixed paraffin embedded sections. *Histochem. J.* **20,** 125–130.

Dean P.N., Dolbeare F., Gratzner H., Rice G.C. & Gray J.W. (1984) Cell cycle analysis using a monoclonal antibody to BrdU. *Cell Tissue Kinet.* **17,** 427–436.

Furness P.N., Turner S.N., Appleby P. & Turner D.R. (1989) A morphological study of experimental proteinuria using a novel form of surface fixation. *J. Pathol.* **157,** 37–46.

Min K.W., Gyorkey F., Gyorky P., Yium J.J. & Eknoyan G. (1974) The morphogenesis of glomerular crescents in rapidly progressive glomerulonephritis. *Kidney Int.* **5,** 47–56.

Morley A.R. & Wheeler J. (1985) Cell proliferation within Bowman's capsule in mice. *J. Pathol.* **145,** 315–327.

Robertson H., Wheeler J. & Morley A.R. (1990) *In vivo* bromodeoxyuridine incorporation in normal mouse kidney: immunohistochemical detection and measurement of labelling indices. *Histochem. J.* **22,** 209–214.

Siegel S. & Castellan N.J. (1988) *Nonparametric Statistics for the Behavioral Sciences.* Second edition. New York: McGraw-Hill. p. 58.

Thornton J.G., Wells M. & Hume W.J. (1988) Flash labelling of S-phase cells in short term organ culture of normal and pathological human endometrium using bromodeoxyuridine and tritiated thymidine. *J. Pathol.* **154,** 321–328.

Vassalli P. & McCluskey R.T. (1971) The pathogenetic role of the coagulation process in glomerular disease of immunological origin. *Adv. Nephrol.* **1,** 47–63.

Veronese S., Gambacorta M. & Falini B. (1989) *In situ* demonstration of tissue proliferative activity using anti-bromodeoxyuridine monoclonal antibody. *J. Clin. Pathol.* **42,** 820–826.

Wheeler J., Simpson J. & Morley A.R. (1988) Routine and rapid enzyme linked immunosorbent assays for circulating anti-glomerular basement membrane antibodies. *J. Clin. Pathol.* **41,** 163–170.

Wheeler J., Morley A.R. & Appleton D.R. (1990) Anti-glomerular basement membrane (GBM) glomerulonephritis in the mouse: development of disease and cell proliferation. *J. Exp. Path.* **71,** 411–422.

Zollinger H.U. & Mihatsch M.J. (1978) General aspects of glomerulonephritis. In *Renal Pathology in Biopsy.* First edition. Eds H.U. Zollinger & M.J. Mihatsch. Berlin, New York: Springer-Verlag. pp. 182–183.