Virchows Arch [Pathol Anat] (1985) 406:285–300

Virchows Archiv A

Anti-GBM nephritis in the mouse: severe proteinuria in the heterologous phase

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Summary. Highly reproducible anti glomerular basement membrane (GBM) nephritis has been induced in the mouse after a single injection of rabbit or goat antibody against purified homologous GBM. The severity of albuminuria was closely related to the amount of antibody given. With doses of 4 mg or more, low serum albumin concentrations, sometimes accompanied by ascites and oedema, were observed after 1 week. Glomerular injury was characterized by an initial accumulation of polymorphonuclear granulocytes followed by thrombosis and necrosis, the extent of which defined the outcome of the glomerulonephritis. With high doses of antibody the exudative lesions entered a chronic phase, while at doses lower than 2 mg remission of the lesions occurred. Immunofluorescence studies showed prompt linear fixation of the injected antibodies to the glomerular capillary wall, accompanied by immediate binding of C3 in a fine granular pattern. Fibrin deposits appeared at 2 h in some glomeruli, increased thereafter, and were present after one day in more than 90% of the glomeruli in mice that had received 4 mg of antibody. This new reproducible model in the mouse is suited for the study of the relationship between activation of mediator systems, histological lesions, and proteinuria.

Key words: Anti-GBM nephritis – Complement – Polymorphonuclear granulocytes – Mouse

Introduction

One of the first experimental models in which immunological pathways of glomerular injury were studied was the anti-glomerular basement membrane (GBM) nephritis or nephrotoxic nephritis, originally described by Masugi et al. (1932), and induced by the injection of antibodies against antigens from the GBM. The disease has been most extensively studied in the rabbit and the rat, because the lesions induced in these species proved

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to be highly reproducible. Two distinct phases can be identified. The first socalled heterologous phase is the result of a rapid fixation of the injected antibodies to the glomerular wall. Concomitantly with morphological changes an immediate proteinuria can occur, the severity of which depends on the amount of antibody given. The second or autologous phase is characterized by the immune response of the host to the heterologous antibodies. Binding of these antibodies to the heterologous proteins already present in the glomerulus leads to an exacerbation of the disease. The glomerular changes in both phases are variable and depend on the animal species, source and amount of administered antibodies, and the intensity of the immune response of the host. The anti-GBM nephritis of the rabbit, for instance, is usually characterized by transient proliferative alterations, but seldom enters a chronic phase. However, a chronic glomerulonephritis frequently develops in the rat, despite mild, predominantly exudative lesions during the heterologous phase (Kondo and Shigematsu 1980; Unanue and Dixon 1967).

The mouse would be an attractive model to study this disease, because so much is known of its immunogenetic background. Unfortunately, only a few detailed studies have been carried out in this species, mainly because it is difficult to induce lesions in the heterologous phase in the absence of reproducible changes in the autologous phase (Douglas et al. 1969; Nagai et al. 1982; Nishihara et al. 1981; Okada et al. 1982; Russell et al. 1969; Unanue et al. 1967). Proteinuria and morphological lesions usually developed only several days after injection of the nephrotoxic antibodies, in most cases probably as part of the autologous phase. Unanue et al. in one of the most extensive reports of this model in mice, did not observe a correlation between the amount of antibodies injected and the severity and time of appearance of the proteinuria (Unanue et al. 1967). A chronic glomerulonephritis characterized by thrombotic lesions slowly developed in the autologous phase and was sometimes accompanied by proteinuria, ascites, and renal insufficiency. The severity of the nephritis was strain-dependent which was most probably caused by difference in the immune response of the host.

Only a few studies have been published in which brief mention is made of the induction of significant morphological lesions and immediate proteinuria in mice in the heterologous phase after injection of anti-GBM antibodies (Arana et al. 1964; Blair et al. 1965; Okada et al. 1982; Russell et al. 1969). In this study we describe the heterologous phase of a highly reproducible anti-GBM nephritis in a mouse strain, evoked by intravenous injection of antibodies against homologous GBM. The severity of the immediate proteinuria and of the morphological changes depended on the amount of antibody injected.

Materials and methods

Animals. Swiss mice, randomly bred, were bought from the Central Institute for the breeding of laboratory animals, TNO, Zeist, The Netherlands. The inbred strain of C57Bl/10 mice

was originally obtained from the Jackson Laboratory, Bar Harbor, Maine, USA, and was kept by continuous brother-sister matings. New Zealand white rabbits and goats were bought from a local breeder.

Antigens. Mouse basement membranes were prepared from Swiss mouse kidneys by a differential sieve technique, followed by sonication and detergent treatment as previously described (Assmann et al. 1983). The resulting preparation of basement membranes was designated GBM/TBM, because the glomerular basement membranes were contaminated with tubular segments. GBM/TBM was washed extensively in distilled water, lyophilized, and stored at −30° C.

Antisera. Two adult male rabbits were initially immunized with 1 mg GBM/TBM, emulsified in complete Freund's adjuvant (CFA, Difco Laboratory, Detroit, MI, USA) at multiple subcutaneous sites. Four and six weeks later subcutaneous booster injections of 0.2 and 1 mg respectively were given, and the animals were bled 10 days later. A goat was immunized by the same procedure. The pooled antisera were heated at 56° C for 45 min and IgG fractions were prepared from a 50% ammonium sulphate precipitate. After dialysis against PBS, (pH 7.2) the rabbit antibody was further purified by affinity chromatography on a Sepharose-4B coupled protein-A column (Pharmacia, Uppsala, Sweden). The purified IgG antibodies were concentrated to 20 mg/ml by ultrafiltration with a XM-50 Diaflow membrane (Amicon Corporation, Scientific System Division; Lexington, Massachusetts), sterilized by passage through a sterile 0.2-μm filter and stored at −30° C. Rabbit and goat IgG from non-immunized animals were obtained by the same procedure and used for the control studies. Analysis of purity and specificity was carried out by micro-Ouchterlony and immunoelectrophoresis in 1.3% agarose (Ouchterlony and Nilsson 1978). Protein concentrations were measured by the method of Lowry or by the radial immunodiffusion technique (Lowry et al. 1951; Mancini et al. 1965). The antibody specificity was tested by indirect immunofluorescence on normal mouse kidneys and by absorptions of the antiserum with mouse GBM/TBM (5 mg GBM/TBM per ml of antiserum).

An antiserum against mouse albumin raised in a goat was prepared according to our previously described method (Assmann et al. 1983). A 50% ammonium sulphate precipitate of this antiserum was used in the radial immunodiffusion technique for the determination of urinary albumin concentration.

Experimental protocol

Experiment A. Two groups of 7 female C57Bl/10 mice received 8 mg of rabbit or goat antibody. Albuminuria was measured at days 0, 1, 2, 3, 4, and 8. Thereafter a dose response study was done in other groups of 7 mice that received increasing amounts of goat antibody. Albuminuria was determined at days 0, 1, and 8. Kidneys were processed for light microscopy and immunofluorescence at the time of killing.

Experiment B. Two groups of 21 female C57Bl/10 mice were injected with 1 and 4 mg of goat anti-mouse GBM antibodies and 3 mice were sacrificed at selected intervals: 15 min, 1, 2, and 6 hours, days 1, 4, and 8. Parts of their kidneys were processed for light microscopy, immunofluorescence, and electronmicroscopy, and serum was stored for determination of albumin and urea concentrations. At days 0, 1, 4, and 8, urine was collected during 18 h for the determination of albuminuria, while during the first day albumin concentration was measured in samples obtained by bladder puncture. Two other groups of 21 female C57Bl/10 mice, that received 1 or 4 mg of non-immune goat Ig, served as controls. At each interval 3 mice were sacrificed and examined according to the same procedures.

Tissue processing

Light- and electronmicroscopy. The fixation and staining techniques have been previously described (Assmann et al. 1983). The number of polymorphonuclear granulocytes (PMNs) were

counted in 40 glomeruli of each mouse. The average counts per glomerulus of 3 mice are reported.

Immunofluorescence. For the direct immunofluorescence techniques, tissue fragments were snap frozen in liquid nitrogen and in 2 μm sections cut in a cryostat. We used monospecific, fluorescein-labelled goat anti-mouse IgG (heavy and light chains) and C3 (Cappell Laboratories, Downingtown, Pennsylvania, USA) rabbit anti-human fibrinogen that cross-reacted strongly with mouse fibrinogen, swine anti-rabbit Ig (Dakopatt, Copenhagen, Denmark) and rabbit anti-goat Ig (Nordic, Tilburg, The Netherlands). The antisera against rabbit and goat Ig were also used as a second layer in the indirect immunofluorescence technique, and absorbed with 500 mg/ml lyophilized non-immune mouse serum. The staining intensity and quantity of the immune reactants were recorded semiquantitatively (0 = negative, 1+ = moderate, 2+ = relatively strong, 3+ = strong, 4+ = maximum intensity) and the patterns classified as mesangial, GBM/TBM, and granular or linear. Sections were examined in a Leitz fluorescence microscope with a Ploemopac epi-illumination.

Other procedures

Albuminuria, as an index of glomerular protein leakage, was measured by radial immunodiffusion on 18-h urine samples obtained in metabolic cages (Hoffsten et al. 1975). During the urine collections only tap water was provided ad libitum. Of 46 normal female C57Bl/10 mice, the mean albuminuria determined over this period was 22 μg + SD 33. Pathological albuminuria was defined as a value greater than the normal mean plus two standard deviations. Albumin concentration in the serum was assayed by the bromecresol-green method (Doumas et al. 1971). Blood urea was measured by use of the CoBasBio Autoanalyzer (Roche, Basel, Switzerland).

Results

Clinical and laboratory findings

The mice showed no untoward systemic reactions immediately after the intravenous injection of 8 mg of rabbit or goat antibody. However, albuminuria developed after injection with peak values at day 1 (Fig. 1). All animals became seriously ill during the subsequent days and some mice died of renal failure. The surviving mice were sacrificed at day 8. At that time they had low concentrations of serum albumin (18 + 4 g/L, $n = 5$, normal value 33.3 + 1.6 g/L), sometimes accompanied by ascites and foot pad swelling. None of the mice that received lower doses of antibody died of renal insufficiency. The results of the dose response study with goat-anti GBM antibody are depicted in Fig. 2. They show a correlation between the quantity of goat Ig injected and the degree of albuminuria both at day 1 and day 8. A transient albuminuria was found after administration of 0.4 mg, while a plateau was reached with 4 mg or more. Albuminuria did not decrease to normal values after 8 days if more than 0.5 mg of antibody had been administered. Measurements in two urine samples, obtained by bladder puncture at the time of killing, 6 h after antibody injection, yielded albumin concentrations as high as 5.8 + 3.9 mg/ml (1 mg group) and 25 + 20.2 mg/ml (4 mg group), while in the first 2 h the albumin concentrations remained normal, not exceeding the amount of 45 μg/ml. Mice injected with 4 mg of normal goat Ig demonstrated amounts of albumin excretion just above

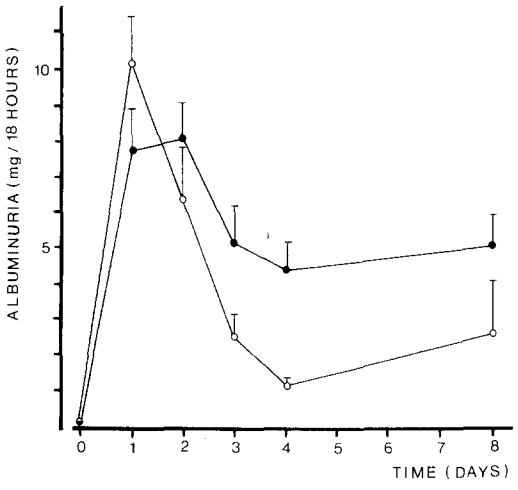

Fig. 1. Albuminuria (mg albumin excreted during 18 hours) after administration of 8 mg of rabbit-(o——o), or goat anti-mouse GBM antibodies (●——●). Each point represents the mean +SEM of 7 animals

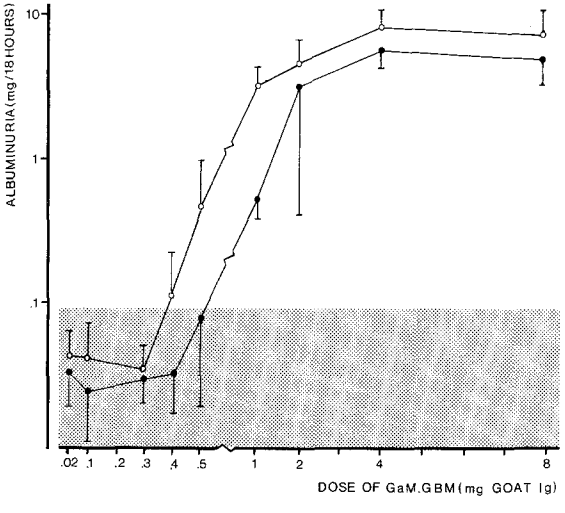

Fig. 2. Dose dependency of albuminuria (mg albumin excreted during 18 hours). Albuminuria is measured on day 1 (o——o), and day 8 (●——●). Shaded area represents the physiological range of albuminuria in this mouse strain. Each point represents the mean +SD of 7 animals

the upper limit of physiological albuminuria, while no pathological albuminuria was seen after administration of 1 mg of goat Ig.

The albumin concentration in serum slightly decreased in all mice immediately after injection of antibody as well as control Ig, but it rose to normal values at the end of the experiment in all groups, except in the one injected with 4 mg of antibody. This group showed a gradual reduction of the albumin concentration to one third of the normal range (Table 1). These mice also had raised concentrations of urea from the first day on.

Morphological changes

Light microscopy. The extent of the morphological changes were also related to the amount of the antibody administered. No distinct changes were pres-

Table 1. Albumin and urea concentrations in serum after injection of 1 and 4 mg of goat anti-mouse GBM antibodies (experiment) and normal goat Ig (control)[a]

Dose (mg)	Time	Albumin (g/l)		Urea (mmol/l)	
		Experiment	Control	Experiment	Control
1	15	28.0+2.0	26.3+0.6	6.4+0.1	8.7+2.0
	1 h	28.7+1.5	27.0+1.0	7.9+2.2	6.5+1.4
	2 h	28.3+2.1	24.7+1.5	5.7+1.2	5.4+1.2
	6 h	28.7+1.2	27.3+1.5	10.8+0.5	11.1+0.4
	1 d	30.3+2.1	34.3+1.5	14.4+2.2	12.5+1.0
	4 d	32.0+0.0	28.7+0.6	13.9+2.3	15.0+4.5
	8 d	34.0+0.0	32.0+ − [b]	12.5+0.6	10.7+ − [b]
4	15	23.3+0.6	24.0+1.0	8.2+1.0	10.4+1.0
	1 h	24.5+0.7	23.5+0.7	7.9+1.8	11.0+2.0
	2 h	25.0+1.0	24.5+2.1	7.9+0.6	6.0+1.1
	6 h	24.3+0.6	27.0+1.0	12.0+1.2	9.3+1.0
	1 d	19.7+3.5	28.7+0.6	32.8+10.8	12.8+2.1
	4 d	17.7+2.5	30.3+3.0	43.6+25.8	11.8+1.8
	8 d	13.3+2.5	31.7+0.6	43.2+3.9	9.7+0.2

[a] Means +S.D. of 3 observations each. Normal albumin concentration 33.3+1.6 g/liter, normal uria concentration 11.0+1.4 mmol/l

[b] Single observation

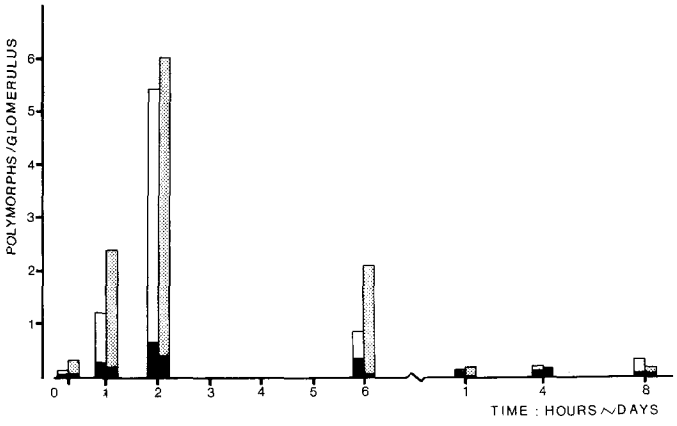

Fig. 3. Number of polymorphonuclear granulocytes (PMNs) per glomerulus after administration of 1 mg (*open bars*) and 4 mg (*shaded bars*) of goat anti-mouse GBM antibodies. Black areas in the bars represent control numbers after injection of 1 and 4 mg of normal goat Ig. Number of PMNs were counted in 40 glomeruli of each mouse. The average counts per glomerulus of 3 mice are reported

ent within 15 min following the injection of 1 or 4 mg of antibody. PMNs appeared in the glomerular lumina in small numbers at 1 h, were most prominent at 2 h, dropped considerably at 6 h, and remained at normal levels during the subsequent days (Fig. 3). An increase in monocytes was not observed. After administration of 4 mg of antiserum, focal and segmen-

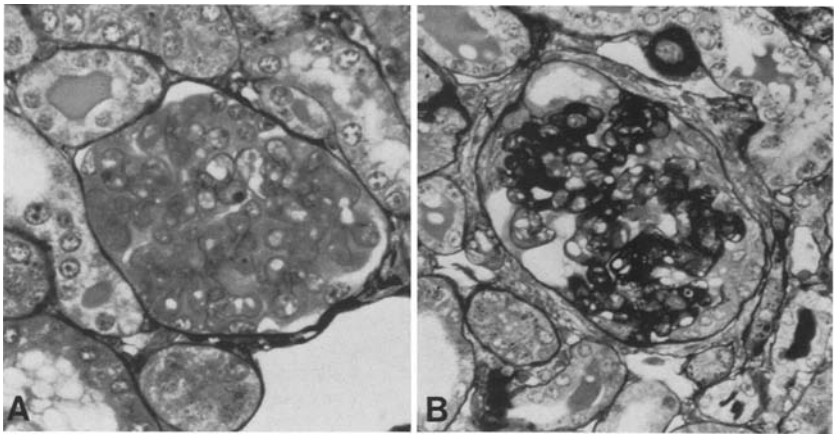

Fig. 4A, B. Histological changes after injection of 4 mg goat anti-GBM. **A** Extensive intravascular coagulation in the glomerulus at day 1. **B** Progressive glomerulonephritis at day 15. Increase of mesangial cells and matrix in the glomerulus with swelling of the endothelia cells. Adhesions to the Bowman's capsule are present with an increase of epithelial cells. Tubular epithelia are swollen of flattened and some casts fill the lumina. ×380

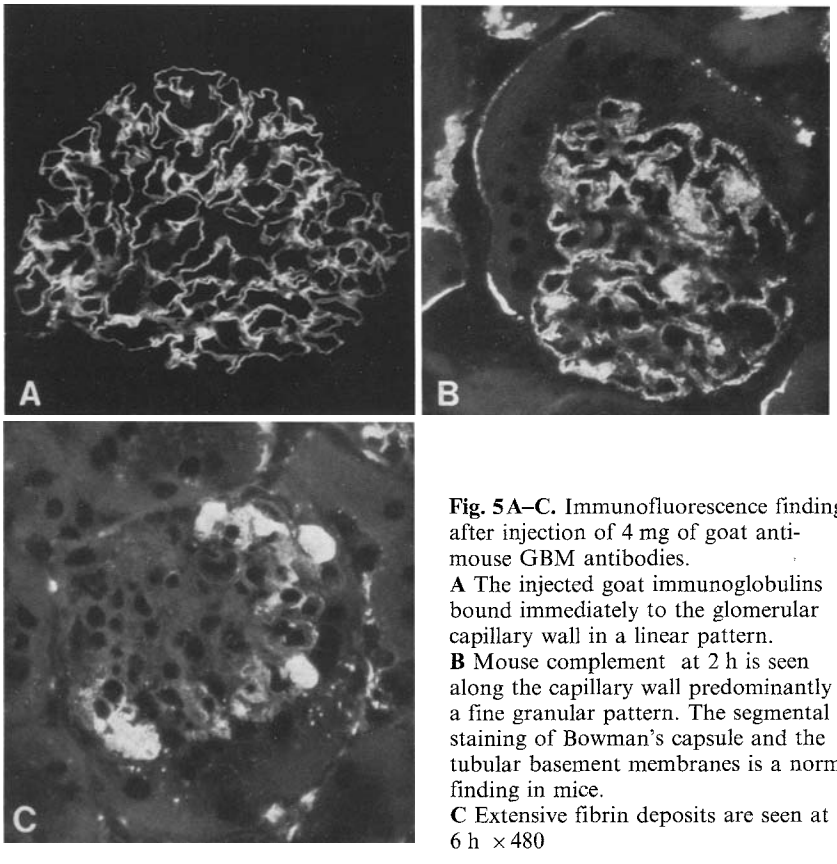

Fig. 5A–C. Immunofluorescence findings after injection of 4 mg of goat anti-mouse GBM antibodies.
A The injected goat immunoglobulins bound immediately to the glomerular capillary wall in a linear pattern.
B Mouse complement at 2 h is seen along the capillary wall predominantly in a fine granular pattern. The segmental staining of Bowman's capsule and the tubular basement membranes is a normal finding in mice.
C Extensive fibrin deposits are seen at 6 h ×480

Table 2. Immunofluorescence findings. Deposition of immune reactants in the glomerular capillary wall after injection of 4 mg of goat anti-mouse GBM antibodies

Time[a]	Goat Ig[b]	Mouse Ig[c]	Mouse C3[d]	Fibrin
15	++++[L]	−	++[G]	−
1 h	++++	−	+++	−
2 h	++++	−	++	+
6 h	++++	−	++	++
d 1	++++	−	+	+++
d 4	++++	−	+	+++
d 8	++++	+[L]	+[L]	++

[a] Three mice were killed at each time
[b] (L) linear and (G) granular staining of the glomerular capillary wall
[c] Small amounts of mouse Ig and C3 in the mesangium being a normal finding in this mouse strain, are not listed
[d] Predominantly fine granular deposits in the capillary wall

tal glomerular thrombosis and swelling of endothelial cells could be seen coinciding with the peak value of PMNs at 2 h. Thereafter, many glomeruli showed segmental or global thrombosis, leading to overt necrosis (Fig. 4A). From day 1 on, the tubules contained many homogeneous or granular casts, while the tubular epithelial cells were swollen, flattened or focally necrotic. Eight days after administration of the antiserum a marked swelling of epithelial and endothelial cells, and a slight increase of mesangial cells were still present along with thrombosis and necrotic lesions. The GBM looked fluffy, irregular or showed occasional splitting with a few adhesions to Bowman's capsule. Mice receiving 1 mg of antiserum initially showed comparable, but less extensive lesions of the glomeruli. At day 8 the exudative lesions were notably reduced. Mice given 1 or 2 mg of antiserum and sacrificed at day 15, demonstrated an almost normal morphology, while higher doses had caused a progressive glomerulonephritis at that time (Fig. 4B).

Immunofluorescence (Table 2). A rapid linear fixation of goat Ig was observed along the glomerular capillary wall after injection of 4 mg of antibody (Fig. 5A). Immediate binding of C3 to the capillary wall could also be seen. However the pattern was more fine granular although it was sometimes difficult to distinguish it from a faint linear pattern (Fig. 5B). The binding of C3 became weak or hardly visible at day 4 and had increased again to a weak linear pattern on day 8, concomitant with the appearance of mouse Ig. Focal and segmental deposits of fibrin were seen after 2 h along the capillary wall, as small trombi in the lumina, and in the mesangium. The amount of fibrin increased considerably in the subsequent hours (Fig. 5C), and it was present in more than 90% of the glomeruli at day 1. Although most thrombi gradually disappeared, fibrin deposits generally did not tend to regress. The proximal tubular epithelia contained granules

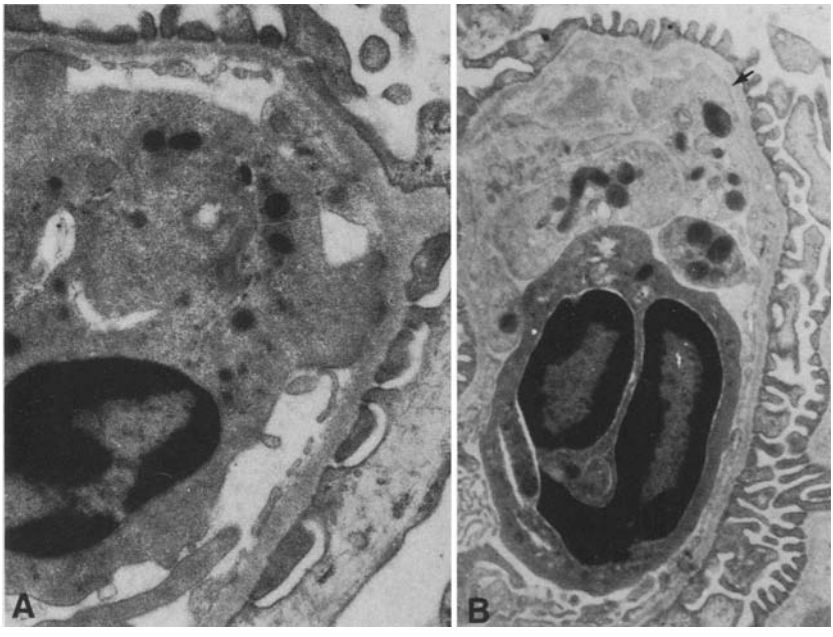

Fig. 6A, B. Ultrastructural findings 2 h after the injection of 4 mg of goat anti-mouse GBM antibody. **A** PMN inserts its cytoplasmic processes into the subendothelial space. **B** Platelets with granular material in a glomerular capillary loop close to the denuded basement membrane (*arrow*). **A** ×16,420. **B** ×9,700

of goat and mouse Ig from 6 h on, while C3 and fibrin predominantly stained the tubular casts after 1 day. Mice injected with 1 mg of goat Ig showed comparable immunofluorescence findings with only quantitative differences. Fibrin deposits affecting about 50% of the glomeruli at day 1, demonstrated an enhanced clearing on day 8. No fibrin was seen with doses of 0.5 mg or less, despite a linear fixation of goat Ig. Mice of the control groups showed only small or trace amounts of mouse Ig and C3 in the mesangium, which is a normal finding in this mouse strain.

Electronmicroscopy. The earliest change following the injection of 4 mg of antibody and preceding the highest influx of PMNs, was a slight and patchy swelling of the endothelium, especially of the attenuated layer at 1 h. Many PMNs present at 2 h in the glomerular capillary lumina, inserted their cytoplasmic processes into the subendothelial space or replaced the endothelium (Fig. 6A). At the same time platelets in combination with granular material or strands of fibrin were observed in some glomerular segments mostly related to a strong swelling of the endothelium or a denuded basement membrane (Fig. 6B). The epithelial foot processes were partly fused in these areas. The lesions at 6 h were characterized by a decline of the number of PMNs and an increase of the platelet-fibrin thrombi along with a marked swelling and exfoliation or necrosis of the endothelium, and deposition of

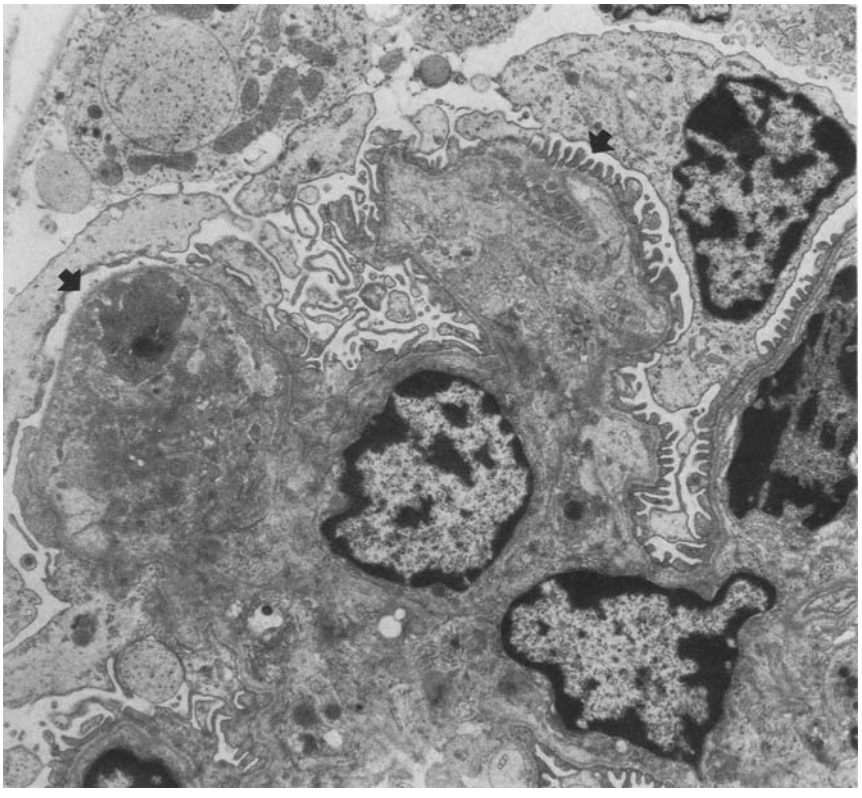

Fig. 7. Ultrastructural changes six hours after the injection of 4 mg of goat anti-mouse GBM antibodies. Two capillary loops occluded by plateled-fibrin thrombi along with swollen or necrotic endothelia (*arrows*). The epithelia foot processes are partly fused. × 5,800

a fluffy or dense granular material in the subendothelial space, mesangium, and between swollen endothelia (Fig. 7). After 1 day these changes had largely increased with occlusion and collapse of many glomeruli, a slight increase of mesangial cells, and hypertrophy of the epithelium. At day 4 many glomeruli showed global necrosis. On the other hand repair of the glomerular alterations was seen in the 1 mg group, marked on day 4 by disappearance of the platelets, cellular debris and by more patent capillary lumina, although some swelling of the endothelia and a widened subendothelial space filled with granular material in it were still present at day 8. Mice of the control groups did not show changes.

Discussion

In this report we describe the induction of immediate morphological and functional alterations in a mouse strain after the administration of both a rabbit antiserum and a goat antiserum directed against purified mouse GBM/TBM material. Determination of albumin concentrations on urine samples from the bladder during the first hours after injection of the antise-

rum, showed that already at 6 h severe albuminuria occured. A close correlation was observed between the amount of albumin excreted in the urine during 18 h on day 1 and 8 after injection and the administered dose of goat antibodies. Although we did not use the paired isotope labeling technique to quantify more precisely the amount of glomerular fixed antibodies to which an enhanced glomerular permeability can be related, our results indicate that a certain amount of antibody bound to the glomerular basement membrane is essential to produce albuminuria and that with increasing doses of antibody a maximum of albuminuria is reached. We measured albuminuria instead of total proteinuria as the hallmark of enhanced glomerular permeability in mice, because many mouse strains have physiological proteinuria, the extent of which depends on the sex and age of the mice (Finlayson and Bauman 1958; Hoffsten et al. 1975). Furthermore, the amount of excreted albumin reflects the enhanced permeability caused by failure of the charge selective barrier of the glomerular capillary wall more precisely. This occurs, most probably, in the initial phase of the nephrotoxic nephritis (Cochrane et al. 1965; Gang et al. 1970; Kreisberg et al. 1979).

The induction of the first phase of the anti-GBM nephritis in mice was highly reproducible and appeared not to depend on the species in which the antibodies were raised. With both rabbit and goat anti-mouse GBM antibodies an identical glomerular injury could be evoked. Most attempts to induce clinical and morphological changes in the heterologous phase in mice mention inconsistent results as distinct from the highly reproducible anti-GBM nephritis in rats and rabbits. A few attempts to induce of immediate glomerular injury in the mouse have been reported in abstract form (Arana et al. 1964; Blair et al. 1965). Most studies, although sometimes containing a description of short-lasting enhanced glomerular permeability in the first days, only focus on the clinical and morphological lesions of the autologous phase (Douglas et al. 1969; Lindberg and Rosenberg 1968; Nagai et al. 1982; Okada et al. 1982; Russell et al. 1969). Unanue et al. did not observe glomerular changes in the heterologous phase after administration of an anti-mouse GBM antiserum (Unanue et al. 1967). In a more recently published study morphological alterations in the glomeruli and ascites were seen after injection of a rabbit anti-rat GBM antiserum, but not after injection of anti-mouse GBM antibodies (Nishihara et al. 1981). In another study glomerular injury after injection of a rabbit anti-mouse GBM antiserum was only observed in mice in which an accelerated autologous phase was induced by pre-immunization with normal rabbit Ig (Nagai et al. 1982).

The causes of these inconsistent results in mice are unknown. One of these could be the potency of the antiserum. In most reports antibodies were raised by injecting rabbits with kidney homogenates or sediments, while sometimes GBM material obtained by sonication was used. We injected rabbits and goats with mouse GBM rendered free from cellular remnants by detergent treatment. Houser et al. have shown that antisera against glomerular membranes, isolated by detergent extraction, detect many different antigens located in site-specific arrays in and along the lamina densa

(Houser et al. 1982). It is possible that our way of preparing the antigenic fraction of the GBM leads to a more potent antiserum by eliciting antibodies against more antigenic determinants.

Lack of activation of one of the mediator systems in the mouse, such as complement, might be another cause for the variable findings during the heterologous phase. This is suggested by the observations of Unanue who could not evoke glomerular injury in mice, but who could induce a classical anti-GBM nephritis with acute proteinuria and fixation of complement to the glomerular capillary wall in rats with the same antiserum (Unanue et al. 1967). In our studies we saw fixation of mouse C3 in a fine granular pattern concomitant with a prompt linear fixation of goat immunoglobulins along the capillary wall. This fixation of C3 was transient, reaching its maximum in the first 2–6 h. These findings are in contrast to the results obtained in rats in which C3 did not disappear, but fixation even became more intense after a few days (Unanue et al. 1964). From studies in a model of acute antibody mediated rejection of skin grafts in the mouse, it is known that mouse skin allografts can be successfully destroyed by alloantibody only when at the same time heterologous complement, i.e., rabbit complement is administered (Koene et al. 1973). Mouse endogenous complement is inefficient in this model and this correlates well with the low efficiency of this complement species in in vitro cytolytic systems (Berden et al. 1978a). Complement activation depended upon the degree of histo-incompatibility between donor and recipient, because mouse complement could be activated in a xenogeneic system, in which antigenic targets are more abundantly available for binding with the antibody (Baldamus et al. 1973; Berden et al. 1978b; Bogman et al. 1982). Thus, mouse complement seems to be less easily activated and this might explain why induction of a heterologous phase of anti-GBM nephritis has proved to be difficult in the mouse in contrast to rats or rabbits.

Like the albuminuria, the morphological changes were also proportional to the amount of antibody given. Besides the dose related accumulation of PMNs the most prominent feature after two hours was the deposition of fibrin along with the aggregation of platelets that ended in varying degrees of necrosis depending upon the amount of antiserum given. After a low dose of antiserum there was a tendency to recovery in the lesions, however a large amount of antibody caused irreversible damage to the glomeruli, with progression to a chronic glomerulonephritis. At the highest amounts of antiserum given most of the mice became seriously ill and developed renal insufficiency, sometimes accompanied by an overt nephrotic syndrome. The histological pictures seen in these mice and the outcome of the lesions resembled the Shwartzman reaction as it can occur in the hyperacute rejection of human kidney grafts (Starzl et al. 1968). The heterologous phase of the anti-GBM nephritis in rats is also characterized by exsudative lesions, but generally a recovery takes place within a couple of days. Only when high doses of antibodies are given is the outcome of the glomerular changes determined by the extent of the thrombotic process affecting the glomeruli.

The mouse with its many inbred strains has several advantages over the rat or rabbit in a systematic study of the role of secondary mediator systems in tissue destruction in both the heterologous and autologous phase. A complement-neutrophil dependent injury is thought to play a key role in the heterologous phase as shown especially from studies in the rat (Cochrane et al. 1965; Unanue and Dixon 1967). It is assumed that lysosomal enzymes released from PMNs, chemotactically attracted by complement factors, cause the glomerular damage with resulting proteinuria. Groggel et al. however demonstrated that in the heterologous phase of rabbits a complement-dependent, neutrophil-independent component of injury, in which the terminal complement components, including the membrane attack complex, was essential for the full development of proteinuria (Groggel et al. 1984). Activation of the complete lytic pathway seemed also to be necessary for evoking the proteinuric effect in the passive Heymann nephritis (Adler et al. 1983; Salant et al. 1980). The anti-GBM nephritis in the mouse provides a good model to study further these interrelationships between complement and PMNs. The role of the terminal complement components can be delineated in normal and C5 deficient mice. Furthermore, treatment of C5 deficient mice with Cobra Venom Factor, can give additional information on the role of C3. The role of PMNs can be studied in mice depleted of PMNs by total body irradiation (600 rad). This method is preferable to treatment with anti-PMN sera which always induces concomitant depletion of complement levels (Bogman et al. 1984). The conspicuous Shwartzman-like morphological lesions occurring in the first phase of the anti-GBM nephritis in mice, renders this model suitable for the examination of the effects of anti-platelet and fibrinolytic therapy on the outcome of the glomerulonephritis. The reproducibility of this new model of anti-GBM nephritis in the mouse offers an opportunity to obtain more insight in the relationship between activation of mediator systems, histological lesions and proteinuria.

Acknowledgements. The authors acknowledge the technical assistance of Mrs. M. Blokpoel-de Ruyter, Mrs. O. Wolf-Eupen, Mr. L. Burgers, and Mr. J. Koedam. We thank Mr. A. Reynen, head of the Department of Medical Photography, and Mr. W. Jansen for their skillful help. Measurements of serum albumin, and urea were done in the Clinical Laboratory of the Department of Pediatrics (head Dr. L. van Munster). Miss A. de Vries typed the manuscript.

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Accepted March 8, 1985