ANTIGLOMERULAR BASEMENT MEMBRANE NEPHRITIS IN BEIGE MICE

Deficiency of Leukocytic Neutral Proteinases Prevents the Induction of Albuminuria in the Heterologous Phase

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The glomerular damage during the early phase of the passive antiglomerular basement membrane (GBM)[1] nephritis, a well-known model of experimental renal disease, is dependent on complement and polymorphonuclear granulocytes (PMN) in most animal species studied (1–3). Recently we have described a murine model of anti-GBM nephritis, in which the massive albuminuria occurring after the injection of heterologous antibody is not mediated by complement, but is completely dependent on the presence of PMN in the glomeruli (4). Lysosomal proteinases and reactive oxygen metabolites (ROM) from activated PMN, acting either alone or synergistically, have been implicated as agents contributing to the glomerular injury and enhanced permeability for proteins (5–12). Activated PMN secrete lysosomal enzymes, especially when they are firmly attached to extracellular matrices (8–10, 13, 14). It has been established that neutral proteinases are major factors in tissue destruction at sites of inflammation. These enzymes can cleave many if not all of the constituents of the extracellular matrix, including the GBM (13–17). Not only lysosomal proteinases but also ROM, generated during a respiratory burst from activated PMN, can function as mediators of tissue injury (16, 18, 19). Several in vitro and in vivo studies have reported the participation of ROM in neutrophil-dependent glomerular disease (6, 7, 11, 12, 17). ROM have been shown to degrade GBM in vitro in concert with lysosomal proteinases, although others could not confirm this synergistic effect (20–22). In most experiments the evidence of their mediator function of glomerular injury is largely derived from the inhibitory effects of ROM scavengers on the damage produced by PMN (5–7, 17, 23).

To examine more precisely the role of leukocytic neutral proteinases, we induced an anti-GBM nephritis in C57BL/6J,bg/bg (beige) mice with a genetic defect analogous to the Chédiak-Higashi syndrome in man, in which PMN are reported to be deficient for elastase and cathepsin G (24, 25). The severe albuminuria after injec-

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[1] *Abbreviations used in this paper:* DFO, desferrioxamine; GBM, glomerular basement membrane; PMN, polymorphonuclear leukocyte; RaM-GBM, rabbit anti-mouse GBM antibody; ROM, reactive oxygen metabolites.

tions of anti-GBM antibodies in normal control mice, did not occur in this particular beige mouse strain, despite the presence of a comparable influx of PMN in the glomeruli. Since the in vitro superoxide production of PMN from beige mice was normal, it is concluded that leukocytic neutral proteinases are responsible for the GBM damage that leads to albuminuria in this model. Furthermore, results of inhibitory studies with ROM scavengers in anti-GBM nephritis induced in B10.D2 new mice suggest that ROM do not participate in the induction of the albuminuria in this model.

Materials and Methods

Reagents. The following materials were obtained from the indicated sources: catalase (Sigma Chemical Co., St. Louis, MO); PMSF, cetrimide, and PMA (Sigma Chemical Co.); desferrioxamine mesylate (DFO) (Ciba-Geigy, Basel, Switzerland); methoxysuccinyl-alanyl-alanyl-propyl-valyl-aminomethylcoumarin (Bachem, Bubendorf, Switzerland); FITC (BDH, Poole, UK); FITC-labeled swine anti-rabbit Ig, and FITC-labeled rabbit anti-human fibrinogen (Dako Corp., Copenhagen, Denmark); FITC-labeled goat anti-mouse IgG (heavy and light chains), and FITC-labeled goat anti-mouse C3 (Cappel Laboratories, West Chester, PA); glutaraldehyde (LADD, Burlington, VT); sodium cacodylate and lead citrate (BDH Chemicals Ltd.); osmium tetroxide (OsO_4) (Johnson Matthey Chemicals Ltd., Roystone, UK); IFA (Difco Laboratories, Detroit, MI); hematoxylin, eosin, Epon 812, and uranyl acetate (Merck, Darmstadt, FRG); paraplast (Amstelstad, Amsterdam, The Netherlands); Sepharose 4B-coupled protein-A (Pharmacia, Uppsala, Sweden); XM-50 diaflow membrane (Amicon Corp., Scientific System Division, Lexington, MA); Dulbecco's PBS (Flow Laboratories, Irvine, Scotland).

Animals. C57BL/6J,bg/bg (beige), C57BL/6J,+/+ (control) and B10.D2 new mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME). Only male mice were used at ages of 3–4 mo. Randomly bred Swiss mice were purchased from the Central Institute for the Breeding of Laboratory Animals, TNO, Zeist, The Netherlands. Rabbits, used for the preparation of the antiserum, were bought from a local breeder.

Production of Anti-mouse GBM Antibodies. GBM was prepared from Swiss mouse kidneys by a differential sieve technique, followed by sonication and detergent treatment as previously described (26, 27). Antisera against this basement membrane suspension were raised in rabbits. The pooled antisera were heated at 56°C for 45 min and the IgG fractions were purified by affinity chromatography on a Sepharose 4B-coupled protein-A column. The IgG antibodies were concentrated by ultrafiltration with an XM-50 Diaflow membrane, sterilized by passage through a sterile 0.2-μm filter and stored at −30°C. The IgG concentration was measured by the radial immunodiffusion technique (28).

Induction of Anti-GBM Nephritis. Anti-GBM nephritis in the mouse was induced by intravenous injection of rabbit anti-mouse GBM (RaM-GBM) antibodies, as described previously (4, 26). A dose-response study was performed in C57BL/6J,bg/bg (beige) and C57BL/6J,+/+ (control) mice that received increasing amounts (1.4–22 mg) of RaM-GBM antibodies. Albuminuria, as a sign of glomerular protein leakage, was measured in 18-h urine samples collected 1 d before, and between 6 and 24 h after the injection of the RaM-GBM antiserum. During their confinement in individual metabolic cages the mice received only tap water ad lib. Pathological albuminuria was defined as a value greater than the normal mean plus two standard deviations. Urinary albumin concentrations were measured by radial immunodiffusion in 18-h urine samples, using a goat antiserum against mouse albumin (27). The kidneys of four mice, killed at 2 and 24 h after injection of 2.8 and 11 mg of the anti-GBM serum, were processed for histological examinations.

Isolation of Peritoneal PMN. Mouse peritoneal cells were elicited by injection of 0.5 ml i.p. of IFA in beige and control mice. After 24 h the cells were harvested by lavaging the peritoneal cavity with ice-cold saline. The cell suspension, containing >90% of PMN, was washed twice in saline and resuspended in Dulbecco's PBS when used for the determination of superoxide production. For the measurement of the elastase activity, the cells were disrupted

by sonication in 0.5 M NaCl, 0.4% cetrimide, 0.1 M Tris, pH 8.0, as described before (PMN extract) (29).

Superoxide Production and Elastase Activity from Isolated PMN. The superoxide production by peritoneal PMNs (10^6/ml) was determined upon stimulation with 100 ng/ml PMA in Dulbecco's PBS at 37°C for 15 min. Superoxide dismutase inhibitable cytochrome *c* reduction was measured as described before (30), assuming an extinction coefficient of 21,000/M/cm (difference between reduced and oxidized form). The rate of superoxide production is expressed as nmol cytochrome *c* reduced per minute per 10^6 cells. To confirm the reported deficiency of leukocytic neutral proteinases, we measured the elastase activity from the isolated peritoneal PMN fluorimetrically using methoxysuccinyl-alanyl-alanyl-prolyl-valyl-aminomethylcoumarine as a substrate (31). The enzyme content of the PMN is expressed as pkatal per 10^6 cells, 1 pkatal being the amount of enzyme that converts 1 pmol of substrate per second under the given assay conditions.

Degradation of GBM by PMN Extracts. GBM from mouse kidneys (2 mg/ml) was labeled with 0.5 mg/ml FITC for 6 h at 37°C, yielding a preparation of ~1.6 μg FITC per milligram GBM. Breakdown of GBM-FITC was measured as follows: 50 μl of GBM-FITC (2 mg/ml) suspended in phosphate buffer (pH 7.4) was added to 50 μl of a PMN extract (10^7 cells/ml, pool of five mice). The mixture was incubated at 37°C for 60 min, 1 ml phosphate buffer was added, and after centrifugation (5 min, 10,000 *g*) the amount of solubilized GBM in the supernatant was measured fluorimetrically at 488 nm (excitation) and 520 nm (emission). Maximum fluorescence was determined by exhaustive trypsinisation of the GBM-FITC preparation. Degradation of GBM by PMN extracts was expressed as a percentage of the total fluorescence. PMSF, a serine proteinase inhibitor, was added at 1 mM to the PMN extracts for 60 min, before the incubation with GBM-FITC.

Effect of Catalase and Desferrioxamine on Albuminuria in B10.D2 New Mice. Thymol-free bovine catalase, which converts H_2O_2 to O_2 and H_2O, was obtained in a crystalline form containing 11,000 U/mg. 10 mg of catalase was injected intravenously together with 5.3 mg of antibodies in B10.D2 new mice. The iron chelator desferrioxamine mesylate was dissolved in distilled water at a final concentration of 75 mg/ml. B10.D2 new mice received 7.5 mg s.c. and 2.5 mg i.v. of DFO before the administration of 5.3 mg of antibodies. Albuminuria was measured as described before. Morpholical studies were done on kidneys of mice killed 2 h after the administration of antibody.

Light Microscopy, Immunofluorescence, and Electron Microscopy. Kidney fragments were fixed in Bouin's solution, dehydrated, and embedded in paraplast (Amstelstad, Amsterdam, The Netherlands) and 4-μm sections were stained with hematoxylin and eosin, Periodic Schiff and Silver methenamine, as mentioned earlier (27). The glomerular influx of PMNs was determined by counting of PMNs in at least 40 glomeruli per mouse in four mice per observation point, and expressed as the average number of PMN per glomerulus. For immunofluorescence, kidney fragments were snap frozen in liquid nitrogen, and 2-μm cryostat sections were stained with FITC-labeled swine anti-rabbit IgG, goat anti-mouse IgG (heavy and light chains), and C3, and rabbit anti-human fibrinogen, which crossreacts with mouse fibrinogen. The sections were examined in a Leitz fluorescence microscope equipped with a Ploemopak epiillumination and the staining intensity was recorded semiquantitatively (on a scale from 0 to 4+) as described before (27). For electron microscopy small pieces of cortex were fixed in 2.5% glutaraldehyde dissolved in 0.1 M sodium cacodylate buffer, pH 7.2, for 4 h at 4°C, and washed in the same buffer. The tissue fragments were postfixed in phosphate-buffered 2% OsO_4 for 2 h, dehydrated, and embedded in Epon 812. Ultrathin sections were cut in an ultratome (LKP Produkter, Bromma, Sweden) and stained with 5% uranyl acetate for 45 min and with lead citrate for 2 min at room temperature. The sections were examined in an electron microscope (ELMISKOP 101; Siemens, Berlin, FRG).

Statistical Analysis. For statistical analysis, Wilcoxon's rank sum test was used. p values <0.05 were regarded as significant. All values are expressed as means ± SD.

Results

Albuminuria. Previous studies have shown that the early phase of anti-GBM nephritis in C57BL/10, B10.D2 new, and B10.D2 old mice is characterized by a dose-

TABLE I

Albuminuria in C57BL/6J,bg/bg and C57BL/6J, +/+ Mice at Day 1 after the Intravenous Injection of RaM-GBM

Dose of RaM-GBM	Albuminuria	
	C57BL/6J,bg/bg	C57BL/6J, +/+
mg	*μg/18 h*	
1.4	61 ± 26 (5)*	63 ± 23 (5)
2.8	78 ± 41 (5)‡	344 ± 87 (6)
5.5	64 ± 15 (5)‡	1,634 ± 713 (6)
11	144 ± 95 (6)‡	4,747 ± 1,548 (6)
22	434 ± 414 (6)‡§	7,913 ± 3,989 (6)
None	112 ± 90 (24)‡	51 ± 23 (30)

* Number of mice in parentheses.

‡ Significantly different from C57BL/6J, +/+ ($p < 0.01$).

§ Significantly different from untreated C57BL/6J,bg/bg mice, ($p < 0.02$).

dependent albuminuria, that is at its maximum 24 h after administration of antibody (4, 26). This albuminuria develops after a transient glomerular influx of PMN during the first hours. Table I shows that doses of RaM-GBM antibody ranging from 2.8 to 22 mg induced an albuminuria in control C57BL/6J,+/+ mice that was related to the amount of antibody given. By contrast, the albuminuria of C57BL/6J,bg/bg mice was not significantly different from the physiological albuminuria at nearly all doses of antibody used. Only at the highest antibody dose of 22 mg was the albuminuria in beige mice significantly higher than that in untreated animals of the same strain ($p < 0.02$). Table I also shows that the physiolog-

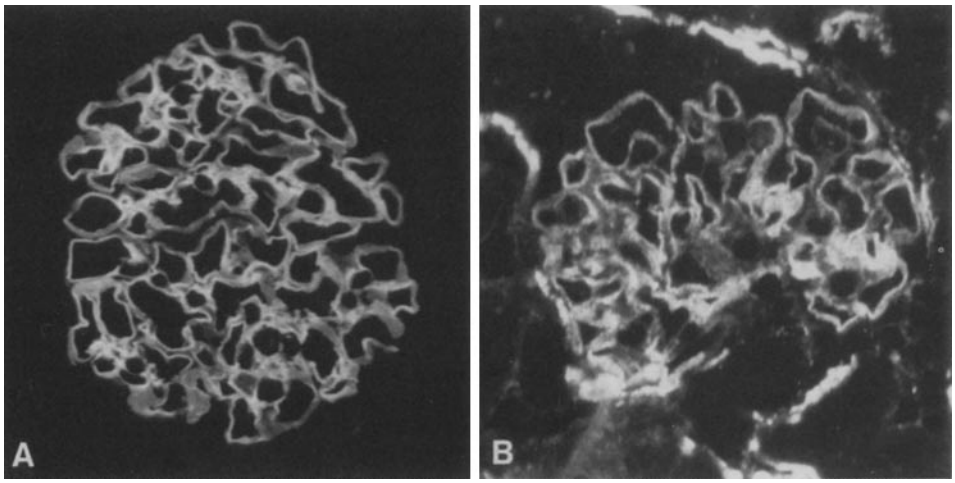

FIGURE 1. Detection of rabbit Ig (*A*) and mouse C3 (*B*) by direct immunofluorescence at 2 h after injection of 5.3 mg of RaM-GBM antibody into a C57BL/6J,bg/bg mouse. Rabbit Ig is localized in a strong linear pattern along the GBM (*A*), whereas mouse C3 is distributed in a more fine granular pattern (*B*). (*A*) ×160, (*B*) ×200.

TABLE II
Immunofluorescence Findings in C57BL/6J,bg/bg Mice 2 h after Injection of RaM-GBM

Dose	Rabbit IgG*	Mouse C3‡	Fibrin
mg			
2.8	+ + +	+	–
11	+ + + +	+ + / + + +	– / +

Identical results were obtained in C57BL/6J, + / + control mice.
* Linear staining.
‡ Fine granular staining along the capillary walls.

ical albumin excretion in untreated beige mice was slightly, but significantly, higher than that of normal control mice.

Histology. We studied the glomerular inflammatory response in beige and control mice by injecting a low dose (2.8 mg) and a high dose (11 mg) of RaM-GBM antibody. By immunofluorescence the two mouse strains showed comparable linear binding of rabbit Ig (Fig. 1 *A*), and a fine granular binding of the third component of the complement system to the glomerular capillary wall (Fig. 1 *B* and Table II). As in our previous studies, the amount of mouse C3 was greatly diminished after 24 h, while at that time small fibrin deposits had appeared in some glomerular loops. 2 h after injection of the antibodies a massive glomerular influx of PMNs was seen, the number of which was related to the dose of antibody (Fig. 2). Beige mice showed a more prominent influx of PMN in their glomeruli than control mice, especially at the low dose of antibody (Table III). 24 h after the injection of antibody the number of intraglomerular PMN had returned to normal values in both groups. The glomerular changes at 2 h were studied in more detail by electron microscopy. In both

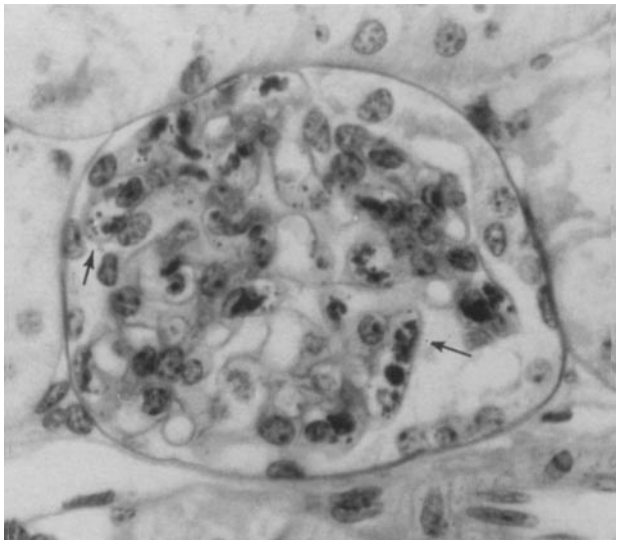

FIGURE 2. A prominent influx of polymorphonuclear granulocytes (*arrows*) is seen in a glomerulus of a C57BL/6J,bg/bg mouse by light microscopy at 2 h after injection of 5.3 mg of RaM-GBM antibody (Periodic Schiff staining, × 210).

TABLE III
Influx of PMN in the Glomeruli 2 h after Intravenous Injection of RaM-GBM

RaM-GBM	PMN per glomerulus*	
	C57BL/6J,bg/bg	C57BL/6J, + / +
mg		
2.8	7.2 ± 4.8	3.2 ± 1.5
11	9.1 ± 1.0	7.9 ± 2.8
None	0.2 ± 0.1	0.1 ± 0.1

* PMN in 40 glomeruli per mouse were counted. The number is expressed as the mean ± SD of PMN per glomerulus of four mice per experimental group.

strains of mice PMN were observed, either lying free in the glomerular capillary lumina, or making contacts of varying extent with the inner side of the GBM, pushing aside or damaging the endothelial cells (Fig. 3). In some glomerular loops, PMN were seen making contact with the GBM through long pseudopods penetrating the endothelial fenestrae (Fig. 4); at other sites PMN were lying in close apposition to a denuded GBM. Occasionally, a few platelets were also seen attached to the GBM. Endothelial cells more remote from the sites of GBM-fixed PMN had a normal appearance or were swollen and detached from the underlying basement membrane. Electron dense deposits or fibrin fibrils were not observed at this stage of the disease. Thus, despite the absence of albuminuria in beige mice, comparable damage of endothelial cells was noted with PMN lying in close contact to the GMB.

Superoxide Production, Elastase Content, and GBM-degrading Capacity of Peritoneal PMN. Since differences in ROM released from activated PMNs could theoretically also explain the observed difference in albuminuria between normal and beige mice, we measured the superoxide production of peritoneal PMNs in both strains. In addition, we measured the elastase content of peritoneal PMNs in order to verify the reported deficiency of neutral proteases in the beige mice (24). Table IV shows that PMA-induced superoxide production of beige PMNs is not significantly different from that in normal mice. We could confirm the deficiency of neutral proteinases in the beige mice. The elastase content was shown to be sixfold lower as compared with normal C57BL/6 mice.

The capacity of PMN-extracts of both strains to degrade purified murine GBM fragments is shown in Table V. FITC-labeled, insoluble GBM was readily broken down by a PMN-extract from control mice, whereas the extracts from beige PMN were significantly less effective. Pretreatment of the PMN extracts of beige and control mice with 1 mM of the serine proteinase inhibitor PMSF completely prevented the breakdown of GBM, indicating that the enzymes involved belong to the class of serine proteinases.

Effects of ROM Scavengers on Albuminuria in B10.D2 New Mice. As shown in the previous section, superoxide production by beige mice was not significantly different from control mice, suggesting that ROM are not responsible for the difference in glomerular injury between the two strains. It is, however, still possible that ROM are involved in inducing the observed damage, by acting synergistically with neutral

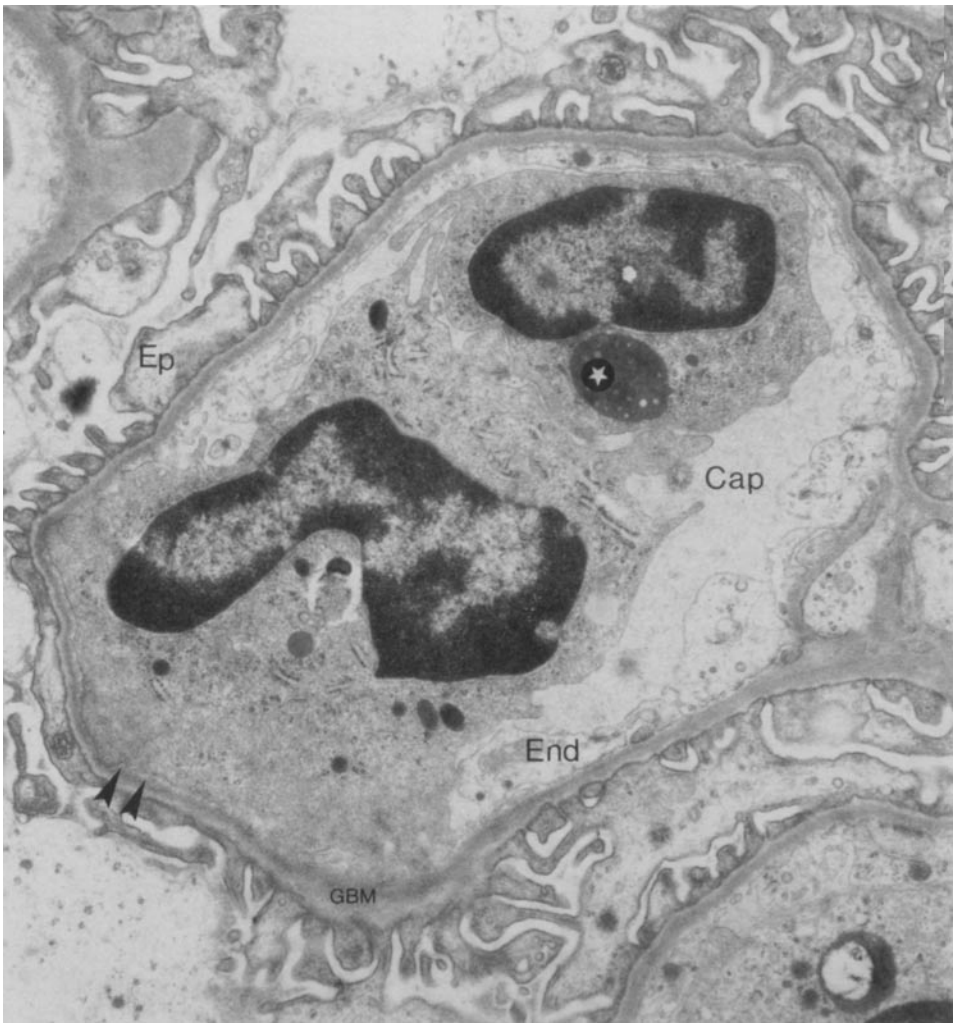

FIGURE 3. Electron photomicrograph of a glomerular capillary loop of a C57BL/6J,bg/bg mouse at 2 h after administration of 2.8 mg of RaM-GBM antibody showing two polymorphonuclear granulocytes (PMN) in the capillary lumen. One of the PMN is directly attached to the inner side of the GBM (*arrowheads*) replacing the endothelium. The other PMN containing a giant lysosome (*asterisk*) in its cytoplasm lies against an intact endothelial layer. Identical results were seen in C57BL/6J,+/+ (control) mice injected with the same amount of RaM-GBM antibody (×16,640). Abbreviations: *US*, urinary space; *Ep*, foot processes of the visceral epithelial cell; *Cap*, capillary lumen.

proteases. To investigate the possible role of ROM, we tested the effect of scavengers of hydrogen peroxide and hydroxyl radicals in our original anti-GBM nephritis model in B10.D2 new mice. Neither catalase, which eliminates hydrogen peroxide, nor DFO, which chelates iron and thereby prevents hydroxyl radical formation, were able to prevent albuminuria in anti-GBM nephritis, as shown in Table VI.

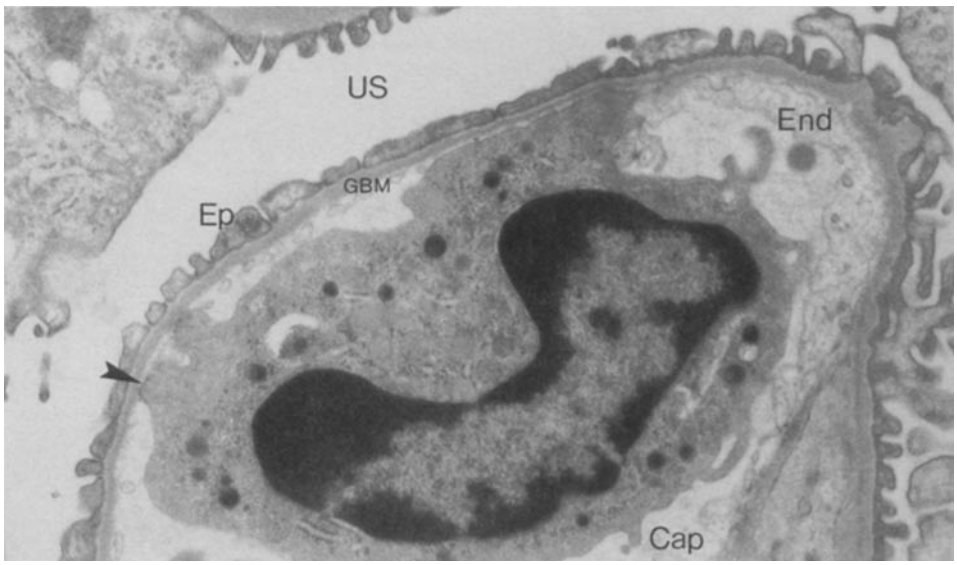

FIGURE 4. Electron photomicrograph of a glomerular capillary loop of a C57BL/6J,bg/bg mouse 2 h after administration of 2.8 mg of RaM-GBM antibody. The endothelial cell (*End*) has been partly pushed aside by a PMN, that adheres directly to the denuded surface of the GBM. Pseudopods of the same PMN also make contact with the GBM through endothelial fenestrae (*arrowhead*). The endothelial cell layer between these two points of contact is intact (×15,300). Abbreviations: *US*, urinary space; *Ep*, foot processes of the visceral epithelial cell; *Cap*, capillary lumen.

TABLE IV

Superoxide Production and Proteinase Content of PMN from C57BL/6J,bg/bg and C57BL/6J, +/+ Mice

	C57BL/6J,bg/bg	C57BL/6J, +/+
Superoxide production*		
(nmol/10^6/min)	3.4 ± 0.5	2.9 ± 0.7
Elastase (pKatal/10^6)‡	6 ± 1	37 ± 5

* Figures represent means ± SD of six mice.
‡ Figures represent the results of separate measurements on three pools of five peritoneal exudates.

TABLE V

Degradation of GBM by PMN Extracts from C57BL/6J,bg/bg and C57BL/6J, +/+ Mice

PMN extract obtained from:	Treatment	Degradation of GBM
		%
Buffer	-	3 ± 2 (4)*
Beige mice	-	10 ± 5 (4)‡
Control mice	-	34 ± 13 (6)
Beige mice	PMSF	4 ± 2 (4)
Control mice	PMSF	4 ± 2 (6)§

* Number of experiments in parentheses.
‡ Significantly different from degradation by normal PMN, ($p < 0.01$).
§ Significantly different from degradation by normal PMN without PMSF ($p < 0.01$).

TABLE VI
Effect of Inhibitors of Reactive Oxygen Metabolites on Albuminuria in Anti-GBM Nephritis in B10.D2 New Mice

Inhibitor*	RaM-GBM	Albuminuria‡
	mg	*μg/18 h*
Catalase	5.3	2,650 ± 578 (8)
Catalase	–	27 ± 21 (6)
DFO	5.3	2,808 ± 1,626 (6)
DFO	–	106 ± 79 (5)
None	5.3	2,769 ± 1,062 (8)

* 10 mg of catalase were injected intravenously along with the anti-GBM antibodies. DFO was given subcutaneously (7.5 mg) and intravenously (2.5 mg) before the administration of the antibodies.

‡ Albumin excretion measured on urine samples obtained from 6–24 h after injection of the antibodies. Number of mice examined in parentheses.

Discussion

The data presented in this paper demonstrate the involvement of leukocytic neutral proteinases in the induction of albuminuria during the acute phase of anti-GBM nephritis in the mouse. It is shown that mice deficient in leukocyte elastase do not exhibit significant albuminuria in the early phase of anti-GBM nephritis compared with normal mice, notwithstanding an identical glomerular infiltration of PMN in both groups. Surprisingly, electron microscopy showed many PMNs attached to the denuded inner side of the GBM concomitant with damage to endothelial cells in both strains of mice. These results suggest that unlike the findings in other models with endothelial injury (32, 33), glomerular endothelial damage per se does not lead to an enhanced glomerular permeability for proteins. Other mediators than neutral proteinases seem to be responsible for the destruction of the endothelia. This is supported by recent observations in rat kidneys, where perfusion with elastase and cathepsin G led to proteinuria without any visible injury of endothelial cells (34). No definite role of ROM, the generation of which is not inhibited in PMN of beige mice, was found in the induction of albuminuria in this model, although they may have been involved in the observed cell injury. In vitro studies have demonstrated that ROM are indeed directly cytotoxic to a wide variety of normal cells including endothelial cells (35).

Several ROM have been implicated in the induction of PMN-dependent glomerular disease in rats, such as hydrogen peroxide, hydroxyl radicals, and hypohalous acid, the toxic derivatives of the reaction of the leukocytic myeloperoxidase with hydrogen peroxide (5, 6, 11, 12). The evidence of their action is largely based on the effects of ROM scavengers on the glomerular injury by circulating PMN. In our murine model the albuminuria was not inhibited by catalase, which catalyzes the conversion of hydrogen peroxide to water and oxygen, nor by the iron chelator DFO, which prevents the generation of hydroxyl radicals from hydrogen peroxide. Equivalent dosages of their agents in rats have been shown to be highly effective (6). Whether this discrepancy is caused by differences in effector mechanisms between rats and mice, or by insufficient delivery of the scavengers to the site of action

in mice, remains to be investigated. In vitro superoxide production by activated PMN of beige mice was not significantly different from control values. Recently, it was shown by others that also hydrogen peroxide production in both strains was similar (36). They observed, however, a reduced production of hydroxyl radicals in vitro by PMN of beige mice, a phenomenon that is not easy to explain, since hydroxyl radicals are derived from hydrogen peroxide, which is produced in equal amounts (36).

Several studies both in man and in experimental animals suggest a role for neutral proteinases in glomerular damage. It has been shown that lysosomal enzymes from PMNs, especially elastase and cathepsin G, can digest GBM in vitro (8, 9, 15, 21, 22, 37). In addition, PMN-derived neutral proteinases along with fragments of the GBM have been detected in the urine of rabbits with a passive anti-GBM nephritis (8). Both these components have also been found in the urine of patients with a proliferative glomerulonephritis in which PMN were involved (38). Recently, a linear correlation between urinary excretion of neutral proteinases and proteinuria was found in a model of accelerated autologous phase anti-GBM nephritis and in aminonucleoside nephrosis (39). In these latter models, but also in the above-mentioned clinical study, the neutral proteinases recovered from urine were able to degrade GBM in vitro. It has also been shown that administration of amidine-type proteinase inhibitors in vivo protected against glomerular injury during immune complex–mediated glomerulonephritis (40). It was suggested that these inhibitors act on proteinases, such as Hageman factor, kallikrein, plasminogen activator and plasmin, and thereby dampen the inflammatory response. More direct evidence that neutral serine proteinases can mediate glomerular damage stems from the recent finding that perfusion of rat kidneys with elastase and cathepsin G caused massive proteinuria (34).

The possible role of PMN neutral proteinases has been investigated in several other models of inflammation. Using the beige mouse as a model, no significant differences were found in experimental alveolitis, compared with normal mice (41). In a reversed passive Arthus reaction, used as a model of immune complex vasculitis, no difference was found in the degree of vascular damage (42). In PMN from humans with emphysema an enhanced chemotaxis in response to a chemotactic peptide was found compared with control cells. PMN from patients with bronchiectasis contained significantly more of the serine proteinase elastase. In both these forms of chronic obstructive lung disease this "overload" of PMN may have contributed to their greater extracellular proteolysis (43). These findings illustrate that the role of neutral proteinases might be dependent on the model, i.e., the type of inflammation (tissue localization), and the animal that is used.

There are several factors that enhance the role of PMN neutral proteinases as important mediators in glomerular damage. The close apposition of the PMN to the GBM facilitates not only the binding of released enzymes to it, but may also exclude plasma proteinase inhibitors such as α-1-proteinase inhibitor and α-1-antichymotrypsin from the site of enzyme action. Binding of elastase to its substrate is furthermore promoted by virtue of their cationic nature (44). Since this enzyme has an isoelectric point higher than 8.8, it will exhibit affinity for the GBM, which is negatively charged due to the glycosaminoglycan chains of the heparan sulphate proteoglycans. Affinity of cationic proteins and exclusion of anionic protease inhibitors from GBM might be analogous to the interaction of this protein with negatively charged cartilage matrix as we have shown before (45, 46).

We would propose that this particular anti-GBM nephritis model is a useful screening system for antiproteinase drugs, that ultimately might prove to be effective in a number of human diseases including pulmonary emphysema, arthritis and glomerulonephritis.

Summary

Antiglomerular basement membrane (GBM) nephritis with massive albuminuria can be induced in mice by injection of heterologous antibodies against mouse GBM. The albuminuria and the glomerular lesions in this model are not mediated by complement, but are dependent on the presence of polymorphonuclear granulocytes (PMN) in the glomeruli. Neutral serine proteinases and reactive oxygen metabolites produced by activated PMN have been implicated as agents contributing to tissue damage. We examined the role of leukocytic neutral proteinases by comparing the glomerular damage and albuminuria after injection of rabbit anti-mouse GBM antibodies in normal control mice (C57BL/6J,+/+) and in beige mice (C57BL/6J,bg/bg) in which PMN are deficient of the neutral proteinases elastase and cathepsin G. The dose-dependent albuminuria that occurred in control mice after injection of 1.4–22 mg of anti-GBM antibodies was not observed in beige mice, despite a comparable influx of PMNs in the glomeruli. By electron microscopy both strains showed a similar attachment of PMN to the denuded GBM together with swelling and necrosis of endothelial cells. Elastase activity of extracts from PMN of beige mice was only 10–15% of the activity of control mice. In vitro, GBM degradation by PMN extracts of beige mice was 70% lower than that seen in control experiments. PMNs of beige and control mice showed no differences in superoxide production. In addition, administration of scavengers of reactive oxygen metabolites, such as catalase and desferrioxamine, did not prevent the albuminuria in this model. These findings support the important contribution of leukocytic neutral proteinases to the induction of albuminuria in the acute phase of anti-GBM nephritis in the mouse.

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