Clin. exp. Immunol. (1981) **46**, 35–43.

Specificity of antibodies to heterologous glomerular and tubular basement membranes in various strains of mice with different H-2 types

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(Accepted for publication 24 April 1981)

SUMMARY

C3H, CBA (H-2^k) and NZB (H-2^d) mice were immunized with dog insoluble glomerular (GBM) or tubular basement membrane (TBM). The titre of circulating antibodies was sequentially determined and their specificity was analysed using various soluble antigenic fractions. Glomerular and tubular deposits were studied on serial biopsies by direct immunofluorescence. After elution from whole kidneys, IgG fixation on normal mouse kidney sections was analysed by indirect immunofluorescence. After immunization with insoluble GBM, animals from all three strains develop antibodies mainly directed against collagenous antigenic determinants shared by GBM and TBM. After immunization with insoluble TBM, the antibodies are directed in NZB mice against non-collagenous TBM-specific determinants, in C3H mice against collagenous determinants and in CBA mice against both types of antigenic determinants. Thus the ability to respond to the various antigens of GBM and TBM is genetically determined and does not depend only on the major histocompatibility complex.

INTRODUCTION

It has been shown (Erard *et al.*, 1979) that BALB/c mice (H-2^d type) injected with insoluble dog glomerular basement membranes (GBM) develop circulating and kidney-fixed autoantibodies mainly directed against collagenous antigenic determinants shared by GBM and tubular basement membranes (TBM). The same mice injected with insoluble dog TBM also develop circulating and kidney-fixed autoantibodies mainly directed against non-collagenous, TBM-specific, antigenic determinants (Erard *et al.*, 1979). The production of anti-GBM and/or anti-TBM antibodies, their specificity and the magnitude of the antibody response in mice injected with GBM or TBM may be under genetic control. We have therefore studied the anti-GBM or anti-TBM antibody response in three other strains of mice (C3H, CBA, NZB) with two different H-2 types (C3H, H-2^k; CBA, H-2^k; NZB, H-2^d). The above-mentioned characteristics of the immune response were determined by radioimmunoassay and by immunofluorescent microscopy. It has been found that the three strains of mice immunized with insoluble GBM are able to raise specific antibodies against antigenic determinants shared by GBM and TBM. These antibodies are mainly directed against the collagenous material of basement membranes. Furthermore, the CBA (H-2^k type) and NZB (H-2^d type) mice immunized with insoluble TBM also raise antibodies directed against non-collagenous,

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0009-9104/81/1000-0035$02.00

TBM-specific antigenic determinants while C3H do not. It appears therefore that the ability to respond to the various antigens of GBM and TBM does not depend only on the major histocompatibility complex.

MATERIALS AND METHODS

Animals. Three different inbred strains of mice were used: C3H (H-2^k), CBA (H-2^k) and NZB (H-2^d). The mice (CNRS, Orléans, France) were 4 weeks old at the time of the first injection.

Antigens. Glomerular basement membranes (GBM) and tubular basement membranes (TBM) were purified from dog kidneys as previously described (Erard *et al.*, 1979). The final GBM preparation was contaminated by less than 5% of TBM and vice versa.

Immunization. The lyophilized antigens were suspended in phosphate-buffered saline (PBS) and emulsified volume-to-volume in Freund's complete adjuvant (FCA). C3H and NZB mice were divided into three groups of 16 mice and CBA into three groups of 12 mice. Groups I and II of each strain respectively received eight weekly subcutaneous injections of GBM or TBM (0·2 mg) in FCA (final volume 0·1 ml), whereas groups III received PBS in FCA.

Experimental design. After the second injection each mouse was bled by retro-orbital puncture every 2 weeks. All sera were stored at −20°C for detection of circulating antibodies. Following the same schedule, two or three mice per group were subjected to kidney biopsy. Kidney fragments were immediately frozen in liquid nitrogen and stored at −70°C for immunofluorescent studies. One week after the last injection the animals were killed by bleeding and kidney fragments were taken. In each experiment, two to four mice per group were kept alive, bled 1 month later and killed 3 months later; sera and kidney fragments were taken as above.

Before killing, urine was tested for proteinuria with Ames Labstix. Creatinine was evaluated in sera using a Eurobio kit.

Detection and specificity of circulating anti-DNA, anti-GBM and anti-TBM antibodies. The presence of anti-DNA antibodies has been searched for according to the method of Farr (1968). The presence and the specificity of anti-GBM and anti-TBM antibodies has been searched for by a previously described radioimmunological method (Mahieu, Lambert & Miescher, 1974; Graindorge & Mahieu, 1978). To study the specificity of anti-basement membrane antibodies, type IV procollagen and a non-collagenous glycoprotein fraction purified from basement membrane material secreted by a murine tumour (Timpl *et al.*, 1978) were used. These antigens were kindly provided by Dr J. M. Foidart (NIDR, NIH, Bethesda). The heteropolysaccharide-containing glycopeptides were purified from GBM or TBM, as previously described (Mahieu & Winand, 1970).

Immunofluorescent studies. The kidney biopsies and the kidneys obtained at autopsy were studied for the presence of mouse IgG deposits using fluorescein-conjugated sheep anti-mouse IgG. IgM and C3 deposits were searched for using respectively fluorescein-conjugated goat anti-mouse IgM (Nordic Immunology) and fluorescein-conjugated rabbit anti-mouse β1C (Nordic Immunology). The direct immunofluorescent technique of Lerner, Glassock & Dixon (1967) was used. Kidney sections treated as previously described (Erard *et al.*, 1979) were examined under a Zeiss u.v. microscope equipped with an HbO 50 mercury lamp.

Studies of eluates. Kidneys from each experimental group were pooled and homogenized (Ultra-Turrax, Ika Werck). Antibodies were eluted using 0·02 M citrate buffer, pH 3·2, according to the technique described by Lerner *et al.* (1967). Eluates from each group were concentrated by negative pressure dialysis to a final volume of about 1 ml. The protein content of the eluates was determined by the technique of Lowry *et al.* (1951), using purified human IgG as standard. The presence of mouse IgG was tested for by immunoelectrophoresis using a goat anti-mouse IgG (Hyland Laboratory, Travenol) and a rabbit anti-mouse serum (Dr Pillot, Institut Pasteur, Paris). In each experiment the eluates from the different groups were tested *in vitro* by indirect immunofluorescence performed on kidney sections of normal syngeneic mice. When specified, the eluates were injected intravenously into normal syngeneic mice. Kidney biopsies were performed 1 hr and 6 days after injection for immunofluorescent studies.

RESULTS

In the three strains tested, mice of all experimental groups appeared uniformly healthy at the end of the experiments. The growth of the animals assessed by body-weight measurement was comparable, serum creatinine was identical in control and immunized groups and below 5 mg/l; no protein excretion could be detected.

Results obtained in C3H mice (H-2^k)

Circulating antibodies. As shown in Fig. 1, the percentage of ^{125}I-TBM antigens precipitated remained below 10 in TBM- and GBM-treated mice. In contrast, antibodies against GBM were

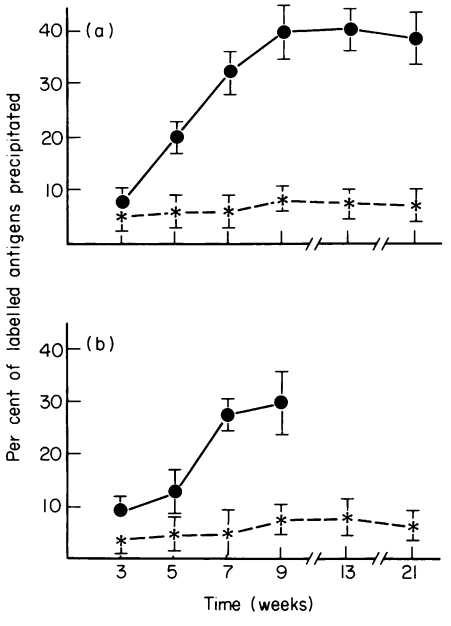

Fig. 1. Circulating anti-GBM and anti-TBM antibodies in C3H mice. Ten nanograms of the labelled GBM or TBM antigens contained in 0·1 ml 0·1 M borate buffer were incubated with 0·4 ml of 1/20 mouse serum in 0·5 ml 40% polyethylene glycol. Percentage of ^{125}I-GBM antigens (●——●) and ^{125}I-TBM antigens (*——*) bound by (a) sera from GBM-treated mice and (b) sera from TBM-treated mice.

Table 1. Results of direct immunofluorescent studies on C3H kidney biopsies

Linear IgG deposits on:	Week No.	Group I	Group II	Group III
GBM	3	++ (1/2)	0 (2/2)	0 (2/2)
	5	++ (2/2)	0 (1/1)	—
	7	++ (2/2)	0 (3/3)	0 (3/3)
	9	+++ (8/8)	+++ (8/11)	0 (8/8)
	21	+++ (2/2)	+++ (2/2)	0 (1/1)
TBM	3	0 (2/2)	0 (2/2)	0 (2/2)
	5	+ (1/2)	0 (1/1)	—
	7	++ (2/2)	+ (1/3)	0 (3/3)
	9	+ (5/8)	+ (2/11)	0 (8/8)
	21	++ (2/2)	++ (2/2)	0 (1/1)

detected in both groups. The percentage of ^{125}I-GBM antigen precipitation reached 40 ± 5 at week 9 in group I and remained stable until week 21. The maximum precipitation of ^{125}I-GBM antigen in group II was 30 ± 4 at week 9.

Specificity of anti-GBM antibodies. To test the specificity of the anti-GBM antibodies elicited in C3H mice of group I, inhibition experiments were performed. One microgram of type IV procollagen, of laminin, and of the disaccharide- or heteropolysaccharide-containing glycopeptides purified from GBM or TBM was added to a solution of anti-GBM antibodies binding specifically 20% of ^{125}I-GBM antigens respectively. The precipitation of labelled GBM antigens was inhibited by 96, 92 and 86% by 1 μg of type IV procollagen and of the disaccharide-containing glycopeptides isolated from GBM and TBM. In contrast, 1 μg of laminin and of the heteropolysaccharide-containing glycopeptides of GBM or TBM did not reduce the specific precipitation of the labelled membrane antigens.

Immunofluorescent studies. The results are summarized in Table 1. Linear IgG deposits were observed on the GBM in group I as soon as week 3. At week 9 a strong linear staining was observed in all glomeruli of all mice (eight out of eight). Light IgG deposits on the cortical tubules appeared at week 5 and five of eight mice exhibited a weak tubular staining at week 9. In group II, no glomerular IgG deposits were obvious before week 9 when all glomeruli of eight of 11 mice exhibited a strong linear staining. At the same time light linear deposits were observed on the cortical tubules in two of 11 mice. No linear deposits on GBM or TBM were observed in control mice of group III. Immunofluorescent studies performed with FITC anti-mouse IgM exhibited a similar pattern of mesangial clusters in immunized and control mice. FITC anti-mouse β1C revealed granular mesangial deposits in glomeruli and segmental linear staining along TBM and Bowman's capsule in all groups. Mice withdrawn at week 9 exhibited a similar pattern of fluorescent deposits when examined at weeks 13 and 21.

Studies of kidney eluates. The protein concentration was about 1 mg/ml in all groups. They were tested for the presence of mouse serum proteins which proved to be IgG only in groups I and II, as demonstrated by immunoelectrophoresis. When tested *in vitro* by indirect immunofluorescence, the eluates from groups I and II fixed linearly on the GBM and the TBM. Eluates from group III

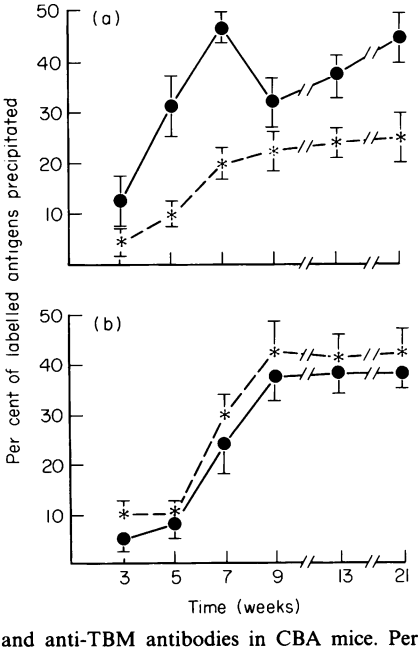

Fig. 2. Circulating anti-GBM and anti-TBM antibodies in CBA mice. Percentage of ^{125}I-GBM antigens (●——●) and ^{125}I-TBM antigens (*——*) bound by (a) sera from GBM-treated mice and (b) sera from TBM-treated mice.

Table 2. Results of direct immunofluorescent studies on CBA kidney biopsies

Linear IgG deposits on:	Week No.	Group I	Group II	Group III
GBM	3	0 (2/2)	0 (2/2)	0 (2/2)
	5	++ (2/3)	0 (3/3)	0 (3/3)
	7	++ (3/3)	+++ (3/3)	0 (3/3)
	9	+++ (10/10)	+++ (10/10)	0 (10/10)
	21	+++ (3/3)	+++ (3/3)	0 (3/3)
TBM	3	0 (2/2)	0 (2/2)	0 (2/2)
	5	++ (3/3)	++ (3/3)	0 (3/3)
	7	++ (3/3)	+++ (3/3)	0 (3/3)
	9	++ (10/10)	+++ (10/10)	0 (10/10)
	21	++ (3/3)	+++ (3/3)	0 (3/3)

exhibited no anti-GBM or TBM reactivity. Three normal C3H mice were injected intravenously with 0·5 ml of the eluates from groups I, II and III respectively. Direct immunofluorescence on kidney biopsies performed 1 hr and 6 days after injection of eluates from groups I and II revealed strong linear IgG deposits along GBM only. After injection of the eluates from group III no specific immunofluorescence could be detected.

Results obtained in CBA mice (H-2^k)

Circulating antibodies. In group I the percentage of ^{125}I-GBM antigens precipitated was $31{\cdot}5 \pm 5$ at week 5, reached a maximum of 47 ± 3 at week 7 and dropped to 32 ± 5 at week 9. In mice withdrawn at the time of killing, the percentage precipitation rose slowly to 45 ± 5 at week 21. The percentage of ^{125}I-TBM antigens precipitated remained between 20 ± 3 at week 7 and 25 ± 5 at week 21 (Fig. 2a). In group II, the labelled TBM precipitated rose rapidly from $10 \pm 2{\cdot}5\%$ at week 5 to $30 \pm 4\%$ at week 7 and to $42{\cdot}5 \pm 6\%$ at week 9, remaining at the same level until week 21 in the mice that had been withdrawn. The precipitation of labelled GBM followed the same course with a plateau of about 38% between weeks 9 and 21 (Fig. 2b). In group III the percentage precipitation of both antigens was always below 6. The specificities of the circulating antibodies have not been determined in this experiment.

Immunofluorescent studies. As summarized in Table 2, linear IgG fluorescent deposits were obvious on GBM and TBM as soon as week 5 in group I. At week 9, all glomeruli and tubules were strongly stained in all mice (10 out of 10). In group II, linear IgG deposits could be observed first on tubules (week 5) and then on the glomeruli (week 7). At week 9, 10 out of 10 mice had intense linear IgG deposits on the GBM and TBM. No specific staining was obvious in control mice.

Studies of kidney eluates. The protein concentration was respectively 1, 0·5 and 0·3 mg/ml in the eluates obtained from groups I, II and III. Only IgG was detected by immunoelectrophoresis in groups I and II. When incubated *in vitro* on normal CBA kidney sections the eluates from groups I and II fixed on the GBM and TBM; however, when injected *in vivo* they fixed on the GBM only.

Results obtained in NZB mice (H-2^d)

Circulating antibodies. In group I, the percentage of ^{125}I-GBM precipitated rose progressively to 30 ± 3 at week 9 and decreased slowly to 23 ± 3 at week 21; no anti-TBM antibodies could be detected (Fig. 3a). In group II (Fig. 3b), the precipitation of labelled TBM antigens reached $42{\cdot}5 \pm 4\%$ at week 9 and dropped to $31{\cdot}5 \pm 5\%$ at week 21. The percentage of labelled GBM antigens precipitated was lower ($26 \pm 3\%$ at week 9). No anti-GBM and anti-TBM antibodies could be detected in FCA-treated mice.

Radioimmunoassay was used to look for circulating anti-DNA antibodies. The percentages of labelled DNA precipitated at week 9 were respectively $17{\cdot}5 \pm 2{\cdot}5$, 28 ± 3 and 35 ± 4 in groups III, I and II and raised to $38 \pm 3\%$ in group I and $45 \pm 5\%$ in group II at week 21. The specificity of

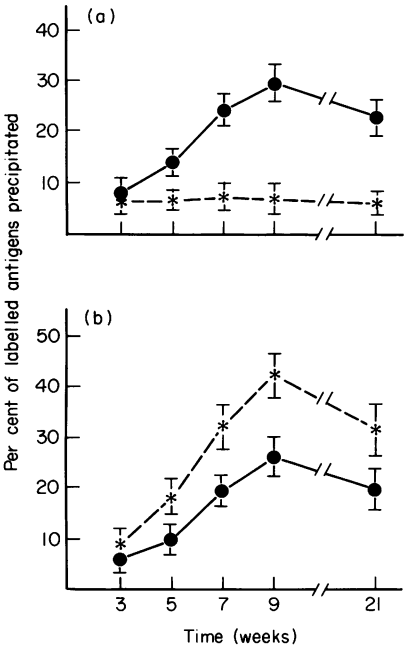

Fig. 3. Circulating anti-GBM and anti-TBM antibodies in NZB mice. Percentage of ^{125}I-GBM antigens (●——●) and ^{125}I-TBM antigens (*——*) bound by (a) sera from GBM-treated mice and (b) sera from TBM-treated mice.

anti-GBM and anti-TBM antibodies was studied by inhibition experiments performed with the above-mentioned antigens. When testing sera from group I the precipitation of labelled GBM antigens was found to be inhibited by 85% by the disaccharide-containing glycopeptide obtained from GBM and TBM and by type IV procollagen. In contrast, with sera from group II, the precipitation of labelled TBM antigens was inhibited by 90% by the heteropolysaccharide-containing glycopeptides obtained from TBM. The inhibition obtained with type IV procollagen was only 16%. The precipitation of labelled DNA was inhibited by 90% by cold DNA but not by GBM or TBM in sera from groups I and II.

Immunofluorescent studies. Results of immunofluorescent studies are summarized in Table 3. In group I, linear IgG deposits were obvious on the tubules as early as week 3 and on the glomeruli at

Table 3. Results of direct immunofluorescent studies in NZB mice

Linear IgG deposits on:	Week No.	Group I	Group II	Group III
GBM	3	0 (2/2)	0 (2/2)	0 (2/2)
	5	++ (3/3)	0 (3/3)	0 (3/3)
	7	+++ (3/3)	0 (3/3)	0 (3/3)
	9	+++ (10/10)	++ (5/11)	0 (10/10)
	21	+++ (3/3)	+++ (3/3)	0 (4/4)
TBM	3	+ (2/2)	+ (2/2)	0 (2/2)
	5	++ (3/3)	+ (3/3)	0 (3/3)
	7	+++ (3/3)	+++ (3/3)	0 (3/3)
	9	+++ (10/10)	+++ (11/11)	0 (10/10)
	21	+++ (3/3)	+++ (3/3)	0 (4/4)

week 5. After eight injections of GBM, all glomeruli and tubules of all mice (10 out of 10) were highly positive. In group II, the tubules were already stained at week 3, all mice (11 out of 11) were positive at week 9, while linear IgG deposits on the GBM were obvious only at week 9 and in four of 11 mice.

All mice of both groups which were withdrawn at week 9 exhibited strong linear IgG deposits on glomeruli and tubules on kidney biopsies performed at week 21.

Studies of eluates. The protein concentrations were respectively 2·5, 3 and 2 mg/ml in the eluates obtained from groups I, II and III. Only IgG was detected in eluates from groups I and II by immunoelectrophoresis. For *in vitro* studies, the eluates were incubated on normal NZB kidney sections at concentrations of 1, 0·5 and 0·25 mg/ml of protein. At these three concentrations, eluates from group I fixed strongly on GBM and TBM while eluates from group II fixed with a stronger intensity on TBM than on GBM at the concentration of 0·25 mg/ml. When the eluates were injected intravenously into normal NZB mice, strong linear IgG deposits were observed on the GBM only while TBM appeared lightly stained on kidney biopsies performed 1 hr and 6 days after injection.

DISCUSSION

Regardless of the dog renal basement membrane preparations used for immunization, all the strains of mice tested produced antibodies (of IgG but not IgM class) to both autologous TBM and GBM, as demonstrated by indirect immunofluorescence performed *in vitro* with kidney eluates. Indeed, in these experiments, linear IgG deposits were observed along the GBM and TBM. All the strains therefore possess the basement membrane antigen(s) that cross-react(s) with the immunizing dog basement membranes. Furthermore, we have shown that there is no correlation between the antibody response to immunization with TBM or GBM and two H-2 types, namely H-2^d and H-2^k. Comparable data have been obtained by Hyman, Colvin & Steinberg (1976) in guinea-pigs and by Rudofsky, Dilwith & Tung (1980) in mice immunized by TBM only. In their studies, no correlation exists between the anti-TBM antibody response and the histocompatibility type of the different strains. On the other hand, we observed that the level of circulating antibodies was not related to the amount of antigen used, as suggested by experiments performed in C3H mice with 2 mg of antigens per injection (Erard *et al.*, unpublished data).

In our work, despite the presence of antibodies in the target organ, all animals exhibited a normal renal function and did not display histological lesions. No significant C3 deposits were observed along the GBM and/or the TBM of mice injected with GBM or TBM antigens. The absence of C3 deposits could explain the lack of detectable kidney lesions. However, this is not a completely satisfactory explanation as glomerular and tubulointerstitial lesions may occur without evidence of complement deposition in certain models of anti-GBM or anti-TBM antibody-mediated nephropathies (Cochrane, 1969; Couser, Stilmant & Lewis, 1973; Bolton, Benton & Sturgill, 1978). Since it has been previously demonstrated in the guinea-pig (Lehman *et al.*, 1974) that pertussis adjuvant is more effective than FCA in inducing interstitial nephritis, we attempted to induce histological lesions by substituting the pertussis adjuvant for FCA in the immunization protocol. In our hands, the use of pertussis adjuvant in mice does not modify the immune response and no renal histological lesions are observed (Erard *et al.*, unpublished data). It should also be pointed out that despite their particular ability to produce autoantibodies, NZB mice injected with TBM or GBM antigens do not develop glomerular or tubulointerstitial lesions. Our results suggest, as do those published by Hyman *et al.* (1976) in guinea-pigs and by Rudofsky *et al.* (1980) in mice, that the magnitude of the antibody response is not the only factor triggering the development of renal lesions or functional impairment. Rudofsky *et al.* (1980) testing mice with seven different haplotypes demonstrated that the ability to develop tubulointerstitial lesions after one injection of TBM was controlled in part by genes of the major histocompatibility complex. Our contrasting results in BALB/c and NZB mice might be explained by the different antigens used and/or by different specificities of the antibodies developed not defined in Rudofsky's work.

Our previous studies on the specificity of antibodies in BALB/c mice have shown that (a) after immunization with insoluble GBM, the antibodies are mainly directed against collagenous

antigenic determinants shared by GBM and TBM; and (b) after immunization with insoluble TBM, the antibodies are mainly directed against non-collagenous TBM-specific antigenic determinants (Erard *et al.*, 1979). In the three other strains tested in the present work, the harvested antibodies are directed against the collagenous material of the membrane, when the immunogen is insoluble GBM. This contention is supported by the *in vitro* studies of the eluates and by radioimmunoassay inhibition experiments. Indeed, IgG eluted from kidneys of GBM-treated mice still bind to GBM and TBM. Antibodies raised in GBM-treated mice are mostly directed against the type IV procollagen of the basement membranes. This fraction has been shown (Graindorge & Mahieu, 1978; Lehman *et al.*, 1974) to be shared by the GBM and TBM. These antibodies can therefore be observed by direct immunofluorescence on the GBM and TBM of GBM-injected mice. The specificity of antibodies developed in TBM-treated mice differs according to the strain of mice. In NZB mice (H-2^d type), as in BALB/c mice (H-2^d type), the antibodies are mainly directed against non-collagenous polypeptides present in TBM. This material is not laminin, a non-collagenous glycoprotein fraction purified from basement membrane secreted by a murine tumour (Timpl *et al.*, 1978). The TBM-specific antigenic determinants, however, are well located in the non-collagenous domain of the membrane, since only the heteropolysaccharide-containing glycopeptides purified from TBM are able to inhibit the binding of labelled TBM antigens to anti-TBM antibodies. These antibodies therefore deposit specifically on tubules as shown by direct immunofluorescence. The CBA mice (H-2^k type) respond to both the collagenous and non-collagenous material of TBM. Accordingly, linear IgG deposits have been observed along the GBM and the TBM of all the mice of this group. The C3H mice do not respond to the non-collagenous material of TBM. With both doses of TBM antigens, linear deposits are only observed along the GBM. These antibodies are directed against the collagenous material shared by the TBM and the GBM. The absence of linear IgG deposits along the tubules may be the consequence of the relatively weak response of these mice to type IV procollagen. The majority of the anti-type IV procollagen antibodies are most probably trapped by the GBM during the ultrafiltration process.

Our results therefore demonstrate that various mouse strains are able to develop an antibody response to the collagenous and/or the non-collagenous polypeptides present in the GBM and TBM. This response does not depend only on the major histocompatibility complex, since there is no correlation between the specificity of the harvested antibodies and the H-2^k or H-2^d types. The existence of antibodies with both collagenous and non-collagenous specificities further rules out the possibility that the chemical nature of basement membrane antigens triggering the immune response plays a major role in the development of renal injury in mice. Other 'facilitating factors' may play a pathogenic role as in human anti-GBM nephritis, such as a persisting impaired humoral and cellular immunity or some intercurrent bacterial or viral infections (Rees, Lockwood & Peters, 1977; Wilson & Smith, 1972; Perez *et al.*, 1974). Experiments are now being developed in order to test the responsibility of other environmental factors in the development of renal lesions in mice.

This study was supported by INSERM CRL 79.5.272.5. We are grateful to Dr P. Galanaud for his valuable advice and to Mrs O. Boudin for typing the manuscript.

REFERENCES

BOLTON, W.K., BENTON, F.R. & STURGILL, B.C. (1978) Autoimmune glomerulotubular nephropathy in mice. *Clin. exp. Immunol.* **33,** 463.

COCHRANE, C.G. (1969) Mediation of immunologic glomerular injury. *Transplant. Proc.* **1,** 949.

COUSER, W.G., STILMANT, M. & LEWIS, J. (1973) Experimental glomerulonephritis in the guinea pig. I. Glomerular lesions associated with anti-glomerular basement membrane antibody deposits. *Lab. Invest.* **29,** 236.

ERARD, D., MOULONGUET-DOLERIS, D., AUFFREDOU, M.T., GRAINDORGE, P., MAHIEU, P., GALANAUD, P. & DORMONT, J. (1979) Specificity of antibodies to heterologous glomerular and tubular basement membranes in Balb/c mice. *Clin. exp. Immunol.* **38,** 259.

FARR, R.S. (1968) A quantitative immunochemical measure of the primary interaction between BSA and antibody. *J. infect. Dis.* **103,** 1412.

GRAINDORGE, P.P. & MAHIEU, P.R. (1978) Radioimmunologic method for detection of antitubular basement membrane antibodies. *Kidney Int.* **14,** 594.

HYMAN, L.R., COLVIN, R.B. & STEINBERG, A.D. (1976) Immunopathogenesis of autoimmune tubulointerstitial nephritis. I. Demonstration of difference susceptibility in strain II and strain XIII guinea-pigs. *J. Immunol.* **116,** 327.

LEHMAN, D.H., MARQUARDT, H., WILSON, C.B. & DIXON, F.J. (1974) Specificity of autoantibodies to tubular and glomerular basement membranes induced in guinea-pig. *J. Immunol.* **112,** 241.

LERNER, R.A., GLASSOCK, R.J. & DIXON, F.J. (1967) The role of antiglomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. *J. exp. Med.* **126,** 989.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265.

MAHIEU, P.R., LAMBERT, P.H. & MIESCHER, P.A. (1974) Detection of antiglomerular basement membrane antibodies by a radioimmunological technique. Clinical applications in human nephropathies. *J. clin. Invest.* **54,** 128.

MAHIEU, P. & WINAND, R.J. (1970) Chemical structure of tubular and glomerular basement membrane of human kidney: isolation and characterization of the carbohydrate units. *Eur. J. Biochem.* **15,** 520.

PEREZ, G.O., BJORNSSON, S., ROSS, A.H., AMATO, J.O. & ROTHFIELD, N. (1974) A mini-epidemic of Goodpasture's syndrome. *Nephron,* **13,** 161.

REES, A.J., LOCKWOOD, C.M. & PETERS, D.K. (1977) Enhanced allergic tissue injury in Goodpasture's syndrome by intercurrent bacterial infection. *Br. Med. J.* **ii,** 723.

RUDOFSKY, V.H., DILWITH, R.L. & TUNG, K.S. (1980) Susceptibility differences of inbred mice to induction of autoimmune renal tubulointerstitial lesions. *Lab. Invest.* **43,** 463.

TIMPL, R., ORKYN, R.W., ROBEY, P.G., MARTIN, G.R. & WICK, G. (1978) Chemical and immunological studies on basement membrane collagen from a murine tumor. In *Biology and Chemistry of Basement Membranes* (ed. by N. A. Kefalides), Vol. 1, p. 143. Academic Press, New York.

WILSON, C.B. & SMITH, R.C. (1972) Goodpasture's syndrome associated with influenza A_2 virus infection. *Ann. intern. Med.* **76,** 91.