Virchows Arch. A Path. Anat. and Histol. 366, 313—330 (1975)

Antibodies Reacting with the Glomerular Mesangium

Isolation and Immunopathology

Hans Peter Seelig, Renate Seelig, Elisabeth Roth, and Ernst Roth
Institute of Immunology and Serology, University of Heidelberg

Received December 16, 1974

Summary. Antigens of porcine lung were prepared by homogenization and ultrasonic treatment of lung tissue. The antigens were digested by collagenase and trypsin and chromatographed on Sephadex G 200. After immunization of rabbits with certain fractions of the chromatographed material, antibodies could be obtained in rabbits which fixed in vitro and in vivo in the glomerular mesangium of rats, hogs and the human, but not in the basement membrane of glomeruli of these species. Following intravenous injection into rats, the antibodies could be observed in the mesangial area without an apparent loss of antigenicity for 55 days. While 5 days after the application of 20 mg of the anti-mesangial IgG-preparation, there were no glomerular changes histologically demonstrable, after 55 days small amounts of rat-IgG could be demonstrated histochemically within the mesangium. Deposits of rat-complement (C3) could not be demonstrated with certainty. Severe morphological lesions were absent. Rats injected with the same dosis of antibody and simultaneously immunized with rabbit-IgG showed a substantially greater deposit of autologous rat-IgG and rat-complement within the mesangial area after 55 days. By histological examination focal and segmental scleroses of the mesangium were determined. A significant pathological proteinuria did not occur. The present model constitutes a new possibility for studying the function of the glomerular mesangium.

Zusammenfassung. Durch Homogenisieren und Ultraschallbehandlung von Schweinelungen wurden wasserlösliche Lungen-Antigene gewonnen. Die Antigene wurden mit Kollagenase oder Trypsin verdaut und anschließend auf Sephadex G 200 chromatographiert. Nach Immunisierung von Kaninchen mit bestimmten Fraktionen der chromatographisch getrennten Antigene wurden Antikörper erhalten, die sich in vitro und in vivo innerhalb des glomerulären Mesangium von Ratte, Schwein und Mensch, nicht aber an der glomerulären Basalmembran anlagerten. Eine unspezifische mesangiale Inkorporation aggregierter Immunglobuline oder Immunkomplexe wurde ausgeschlossen. Nach i.v. Injektion ließen sich die Antikörper über 55 Tage ohne wesentliche Konzentrationsabnahme innerhalb des Mesangium der Rattenniere nachweisen. Lichtoptisch konnten 5 Tage nach der Applikation von 20 mg der antikörperhaltigen IgG-Präparation an der Rattenniere keine Veränderungen nachgewiesen werden. Nach 55 Tagen fand sich immunhistologisch außer den heterologen Antikörpern auch Ratten-IgG intramesangial abgelagert. Ratten-Komplementablagerungen waren nicht eindeutig beurteilbar. Wurde Ratten die gleiche Dosis der Antikörper injiziert und gleichzeitig eine Immunisierung mit reinem Kaninchen-IgG durchgeführt, konnte nach 55 Tagen eine wesentlich stärkere Ablagerung von autologem Ratten-IgG und Ratten-Komplement beobachtet werden. Histologisch fanden sich jetzt fokale und segmentale Sklerosen des Mesangium. Eine bemerkenswerte pathologische Proteinurie trat bei den Versuchstieren nicht ein. Das beschriebene Modell bietet eine neue Möglichkeit zum Studium der Funktion des glomerulären Mesangium.

The immunopathogenesis of glomerulonephritis is based on at least two different immunopathological reactions. The *anti basement membrane glomerulonephritis* is caused by depositions of circulating antibodies along the glomerular basement membrane which react with capillary basement membranes. In the

immune complex glomerulonephritis circulating antigen-antibody-complexes which were formed within the circulation are deposited within the glomerulus and cause an inflammatory reaction by activating the complement system and thereby liberating biologically active mediators (Unanue and Dixon, 1967; Rother, 1967, 1971, 1973). The localization of immune complexes within the glomerulus is determined by factors such as complex-size, liberation of vasoactive amines, rheodynamics and anatomical structure of the glomerular loop. On account of the open communication of the subendothelial and mesangial space as well as the clearing functions of the mesangial cells, in complex glomerulonephritis, we find deposits of immune complexes confined to the basement membrane and the mesangium resp., as well as deposits occurring simultaneously at both sites (Seelig and Seelig, 1974). Whereas the pathological relevance of immune complexes deposited along the basement membrane seems to be established because of an obvious relationship to permeability disturbances of the glomerular capillary wall, the significance of intramesangial deposits remains to be evaluated. In the experimental complex nephritis of the rabbit intramesangial immune complex deposits can be seen in the early phases of immune elimination without the evidence of histological or functional glomerular damage (Wilson and Dixon, 1970). Immune complexes confined to the mesangium were found in patients with lupus erythematodes who—neither by morphology nor by clinical observations—showed the picture of glomerulonephritis (Agnello, Koffler and Kunkel, 1973). Possibly in those early cases there exists a protective phagocytosis of immune complexes by the still unaffected mesangium. The role of persistent deposits of immune complexes in the genesis of mesangial proliferation and sclerosis, which characterizes the picture of numerous glomerulonephritides, has to be investigated by induction of immunopathological reactions confined to the mesangium.

When immunizing rabbits with fractionated antigens of porcine lung we found beside antibodies reacting with the glomerular basement membrane such antibodies which reacted with mesangial antigens only. By means of these mesangial antibodies chronic immunopathological reactions within the mesangium may be induced.

Material and Methods

1. Preparation of Porcine Lung Antigen (PLA)

Porcine lung was dissected from large and medium sized bronchi and vessels and minced. 1500 g lung tissue was homogenized with twice the volume of 0.9% NaCl for 10 min at 4°C (Ultraturrax). The sediment, obtained after centrifugation at 3000 RPM for 30 min, was washed four times with 500 ml 0.9% NaCl resp., resuspended in three volumes phosphate buffered 0.9% .NaCl (PBS), homogenized for 10 min, and washed three times with 250 ml PBS. The sediment was suspended in 200 ml PBS, subjected to ultrasonic treatment for 60 min (Branson Sonifier; 2000 ml Beaker; makro needle, 10, 100 W) and centrifuged at 26000 g for 30 min. The supernatant was dialyzed against aqua dest. for two days and lyophilized. The amount of lyophilized porcine lung antigen (PLA) was 4.9 g.

Digestion with Trypsin. 100 mg PLA were dissolved in 6 ml 0.9% NaCl and pH was brought to pH 8.2 with 0.1 M natrium tetraborate. 5 mg crystalline trypsin (Serva) and 1.5 ml of 3.5% solution of calcium acetate were added. The digestion was performed by constant shaking at 37°C over a period of three hours; the pH was readjusted every 30 min with 0.1 M natrium tetraborate. After inactivation at 60°C for 30 min and centrifugation (3000 RPM) the soluble supernatant was subjected to chromatography on Sephadex G 200.

Digestion with Collagenase. 100 mg PLA were incubated in 6.0 ml 0.1 M Trisacetate buffer pH 7.4 with 1 mg collagenase (2100 PZ units) and 0.004 M calcium acetate as well as NaN_3 (end molarity 0.02 M) for 72 hours at 37° C under constant shaking. 24 hours after the beginning of digestion 0.5 mg, 48 hours later 0.2 mg of collagenase were added to the incubation mixture. Following inactivation (30 min; 60° C) and centrifugation (3000 RPM) the soluble supernatant was subjected to chromatography on Sephadex G 200.

Chromatographic Separation. 100 mg of nontreated, collagenase-digested and trypsin-digested PLA resp. were subjected to chromatography on Sephadex G 200 (2.5×100 cm) in 0.1 M Tris-glycine-HCl buffer pH 7.6. The flow rate was 10.5 ml/h, fractions of 5.25 ml were sampled and protein was recorded at 260 nm continuously. The fractions of the chromatogram were pooled according to the specification in Fig. 1, dialyzed for two days against aqua dest. and lyophilized. Protein was determined by using Folin reagent.

2. Immunization; Preparation of Antisera

Four rabbits (2.5–3.5 kg body weight) were immunized with nontreated PLA. One rabbit each was immunized with each of the fractions of the chromatographed nontreated, trypsin- or collagenase-digested PLA as specified in Fig. 1. Seven rabbits were each immunized with new prepared charges of fraction A of trypsin-digested material. In all instances the immunization followed the pattern of Table 1. The first antigen application was performed in a popliteal lymph node under ether anesthesia. Eight days following the second antigen application the sera were checked for antibodies against kidney tissue as follows:

Table 1. Scheme of immunization

Antigen	Days			
	1	14	28	42
PLA	20 μg + CFA popliteal lymph node	30 mg + CFA s.c.	30 mg in 0.9% NaCl i.m.	30 mg in 0.9% NaCl i.m.
Nat A	20 μg + CFA popliteal lymph node	100 μg + CFA s.c.	20 mg in 0.9% NaCl i.m.	20 mg in 0.9% NaCl i.m.
Try A	20 μg + CFA popliteal lymph node	100 μg + CFA s.c.	20 mg in 0.9% NaCl i.m.	20 mg in 0.9% NaCl i.m.
Nat B Try B Koll A–E	20 μg + CFA popliteal lymph node	100 μg + CFA s.c.		

CFA = Complete Freund Adjuvant.

Two rats[1] each were injected with 1.0 and 2.0 ml of the rabbit serum resp. The kidneys were removed 60 min following the injection, snap frozen in isopentan precooled with liquid nitrogen and subjected to immunohistology for detection of tissue bound deposits of rabbit IgG. In the case of positive test results the rabbits were exsanguinated if there was no antigen material for further immunization. The remaining rabbits were boostered at day 28 and 42. Eight days following the last injection the animals were exsanguinated. All antisera were incubated at 56° C for 20 min, the globulins were precipitated with ammoniumsulfate (40% saturation) and lyophilized after extensive dialysis against tap water and aqua dest. resp.

The lyophilized globulin fraction of the antisera of the rabbits which had been immunized with fraction B of untreated PLA, fraction A of trypsin-digested PLA, and fraction D of

1 Inbred rats of 150–200 g body weight were used for all experiments.

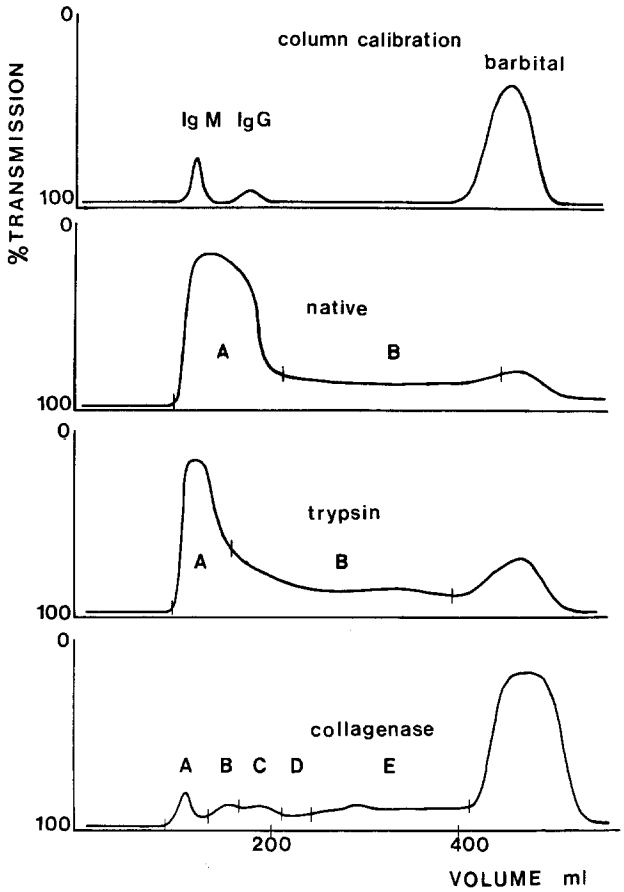

Fig. 1. Elution diagrams (260 nm) of chromatographically separated native, collagenase- and trypsin-digested porcine lung antigen as well as of a control chromatogram with IgM, IgG and barbital on Sephadex G 200 in Tris-glycine-HCl-buffer (0.1 M pH 7.6). A–E designate the pooled fractions

collagenase-digested PLA were subjected to chromatography on DEAE-cellulose in 0.0175 M phosphate buffer pH 6.4 for isolation of IgG. After dialysis against aqua dest. the IgG-fraction was lyophilized.

3. Antibodies against Rat Glomerular Basement Membrane

The isolation of glomerular basement membranes from rat kidneys was performed according to the method described by Spiro (1966) for isolation of bovine glomeruli. Rabbits were immunized with 200 μg lyophilized basement membrane material in complete Freund Adjuvant (Difco) intrapopliteally. Every 14 days 5 mg basement membrane antigens in 0.9% NaCl were given four times subcutaneously at several sites. The animals were exsanguinated 8 days following the last injection. The IgG-fraction was obtained by salt fractionation with ammonium sulfate followed by chromatography on DEAE-cellulose, dialysis and lyophilization. Before injection into rats the IgG-fraction was absorbed with rat serum, cross-linked with glutaraldehyde, following the method of Avrameas and Ternynck (1970).

4. Absorption of Antibodies with Rat Serum

20 mg Anti Try_A-IgG or 20 mg antiglomerular basement membrane IgG (dissolved in 2.0 ml 0.9% NaCl) were absorbed for 4 hours at 37° C with 4 g of rat serum proteins cross-linked by glutardialdehyde (method see Avrameas and Ternynck, 1970). The supernatant was obtained by centrifugation, the cross-linked serum was washed twice with 2 ml 0.9% NaCl resp. All supernatants were pooled and concentrated to the starting volume of 2 ml by pressure dialysis.

5. Absorption of the Mesangium Antibodies with Rat Kidney Antigen and PLA

Rat Kidney Antigen. The cortex of 16 rat kidneys was homogenized in 15 ml 0.9% NaCl and subjected to ultrasonic treatment for 60 min. The suspension was brought to pH 8.2 by addition of 0.1 ml natrium tetraborate. 4.5 ml of 3% calciumacetate solution and 15 mg of crystalline trypsin were added. The digestion was performed for 3 hours at 37° C. The pH was readjusted every 30 min with 0.1 M natrium tetraborate. Following dialysis against aqua dest. the antigen was lyophilized.

For absorption of mesangium antibodies 10 mg anti Try_A-IgG (in 2 ml 0.9% NaCl) were incubated with 5–30 mg untreated PLA or 20–150 mg rat kidney antigen for 48 hours at 4° C. The supernatant obtained by centrifugation was intravenously injected into rats.

6. In vitro Tests for Localisation of Tissue Antigens

For localisation of tissue antigens 4 μ thick acetone-fixed cryostat sections of porcine lung, porcine kidney, rat lung, rat kidney, and human kidney were incubated with Anti Try_A-IgG (10 mg IgG/ml PBS; dilution 1:1–1:32) for 30 min in moist chambers at room temperature. The tissue bound IgG was demonstrated with anti-rabbit IgG labelled with FITC or peroxydase (see 9.). Cryostat sections of the same tissues were incubated with Anti Try_A-IgG (10 mg/ml; dilution 1:1–1:32) labelled directly with peroxydase.

4 μ thick cryostat sections of normal rat kidney were incubated with the enzyme solutions which were used for antigen-digestion by trypsin or collagenase. Incubation periods reached from 10 min to 3 hours at 37° C. After washing in PBS the sections were incubated with Anti Try_A-IgG (10 mg/ml) or anti basement membrane IgG (10 mg/ml) for 30 min at room temperature. Tissue-bound antibodies were demonstrated with anti rabbit IgG labelled with FITC.

7. Agargel Diffusion According to Ouchterlony

The possible cross reaction of Anti Try_A-IgG (10 and 20 mg/ml resp. in 0.9% NaCl) was tested in agargel with the following antigens: porcine serum, human serum, rat serum (dilutions 1:1–1:32), native and lyophilized human and rat urine (10, 20, 30 mg lyophilized urine/ml 0.9% NaCl), native PLA (5.8 mg protein/ml) collagenase- and trypsin-digested PLA (0.74 mg protein/ml) in dilutions of 1:1–1:32.

8. Immunopathological Investigations

Five rats were injected intravenously with 20 mg Anti Try_A-IgG resp., five animals with 20 mg Anti Try_A-IgG concomitantly with a subcutaneous injection of 9 mg of pure rabbit IgG in complete Freund Adjuvant at different sites. Five control animals were given 1 ml of 0.9% NaCl i.v. and five control animals were given 9 mg of pure rabbit IgG in complete Freund Adjuvant subcutaneously at several sites.

Five days following the injection, all animals were subjected to unilateral nephrectomy, 55 days following the injection the remaining kidneys were removed. The urinary protein/24 hours was measured two times before starting the test as well as in weekly intervals following the antibody injection. Protein was determined by the biuret reaction.

9. Histological and Immunohistological Investigations

Tissue was fixed in neutral buffered 10% formaldehyde solution for histological examinations. 2 μ thick paraffin sections were stained with HE, PAS and Methenamin-PAS.

For immunohistological examinations the tissue blocks were snap frozen in isopentan cooled in liquid nitrogen. 4 μ thick cryostat sections were fixed in fresh acetone at 4° C, washed in PBS at room temperature for 10 min and incubated with the corresponding antisera for 30 min. After 3 × 10 min washing the sections were embedded in glycerol: PBS (pH 7.2, 1:10 Vol/Vol). For indirect fluorescence the sections were incubated with the second antiserum for 30 min and washed 3 × 10 min in PBS.

Peroxydase-labelled antibodies were stained by 30 min incubation in a solution of 25 mg 3,3′–4,4′-diphenyl tetramine tetrahydrochloride + 0.001% H_2O_2 in 100 ml 0.05 M Tris buffer pH 7.6. The sections were dehydrated in alcohol and embedded in Caedax.

Antisera

1) Anti-rabbit IgG-serum, FITC labelled from the goat (Behringwerke) dissolved in PBS 1:10 and 1:20.

2) Anti-rabbit IgG, labelled with peroxydase. From anti-rabbit IgG-serum (Behringwerke) the IgG-fraction was isolated by immune adsorption (for method see Jungfer and Tremper, 1972) and labelled with peroxydase (purity degree I, Boehringer, Mannheim) following the method of Avrameas and Ternynck (1969).

3) Anti Try_A-IgG peroxydase labelled. Labelling was performed as described under 2).

4) Anti-rat IgG, FITC-labelled from the rabbit (Hyland), dissolved in PBS 1:10.

5) Anti-rat C3 from the rabbit. Rat C3 (β_{1C}/β_{1A} globulin) was isolated from rat serum following the method of Nilsson and Müller-Eberhard (1965). Rabbits were immunized by injection of 50 μg of C3-globulin in 50 μl 0.9% NaCl and 50 μl complete Freund Adjuvant into both popliteal lymph nodes. The animals were boostered every 14 days for four times with 5 mg C3-globulin in 0.5 ml 0.9% NaCl subcutaneously at several sites. The small amounts of anti-rat IgG contaminating the antisera were eliminated by immune adsorption of the antiserum on Sepharose labelled with rat IgG. The IgG fraction of the antisera was isolated by chromatography on DEAE cellulose (0.0175 M phosphate buffer pH 6.4). The labelling with peroxydase was performed as described under 2).

Results

1. Antigen Fractionation and Antibody Formation

The results of the chromatographic fractionation of untreated as well as trypsin- and collagenase-digested PLA is shown in Fig. 1. Trypsin-digestion was followed by a minor degradation of the antigens whereas collagenase-digestion caused an extensive degradation of the antigens in components of varying molecular weight.

Four rabbits were immunized with untreated PLA. The antibodies produced by these animals were localized along the glomerular basement membrane in a homogeneous linear pattern following intravenous injection into the rat (Fig. 2a). With each of the fractions of chromatographically separated non-treated, collagenase- or trypsin-digested porcine lung antigen which had been sampled according to Fig. 1, one rabbit each was immunized. From these animals, five rabbits produced antibodies, which were localized in a homogeneous linear

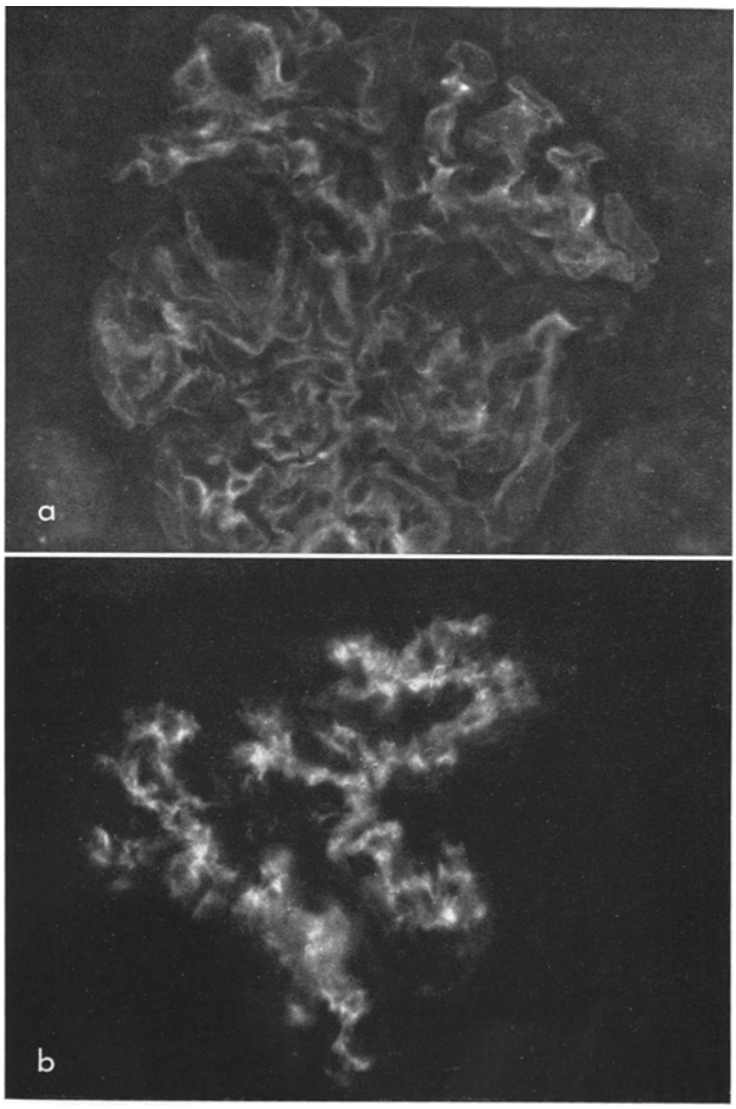

Fig. 2. (a) Rat kidney 60 min following intravenous injection of 1.0 ml Anti-$Coll_A$-serum of the rabbit. Homogeneous-linear deposits of rabbit IgG along the glomerular basement membrane (anti-rabbit IgG, FITC-labelled). (b) Rat kidney 60 min following intravenous injection of 1.0 ml Anti-Try_A-serum of the rabbit. Exclusively intramesangial deposition of rabbit IgG (anti-rabbit IgG, FITC-labelled). (a, b) Enlargment 300 times

pattern along the glomerular basement membrane (see Table 2). Three rabbits (Anti-Nat_B; Anti-Try_A; Anti-$Koll_D$), however, produced antibodies which did not react with the glomerular basement membrane after intravenous injection of the antiserum but were found in a mesangial pattern within the glomerulus (Fig. 2b). The intramesangial localisation was found in all the glomeruli. There

Table 2. Intraglomerular IgG-deposition in rat kidney following injection of 1 ml antiserum after the second immunization

Fraction	Intraglomerular deposition	Intensity of fluorescence
PLA	basement membrane	++++
Nat A	basement membrane	++++
Nat B	mesangium	+
Try A	mesangium	++++
Try B	basement membrane	++
Coll A	basement membrane	++++
Coll B	basement membrane	++
Coll C	basement membrane	++++
Coll D	mesangium	++
Coll E	—	—

was a very fine granular pattern with numerous filamentous processes. The strongest mesangial immunoglobulin deposits were obtained with the antiserum directed against the fraction Try_A.

In order to test the reproduceability of these results, 7 more rabbits were immunized with freshly prepared charges of trypsin-digested PLA (Try_A). Five of the 7 animals produced antibodies which localized within the mesangium following intravenous injection into the rat.

The following investigations were done with the IgG-fraction isolated by chromatography from the Anti-Try_A serum (Anti-Try_A-IgG) first obtained:

a) To exclude the interaction of aggregated immunoglobulins within the Anti-Try_A-IgG preparation 20 mg Anti-Try_A-IgG were subjected to chromatography on Sephadex G 200 (2.5×100 cm) in 0.9% NaCl. The column had been calibrated with IgM, IgG and barbital. A single protein fraction with a molecular weight corresponding to the IgG was obtained. Following intravenous injection of this protein fraction (2.5 ml/rat) an exclusively intramesangial deposition of IgG could be observed within the glomeruli.

b) For the exclusion of an intravasal formation of immune complexes between rat serum proteins and injected antibodies, the possibility of an antigen-antibody-reaction was checked for by agargel double diffusion. Though Anti-Try_A-IgG showed no precipitation with rat serum, Anti-Try_A-IgG was absorbed with cross-linked rat serum. The intramesangial deposition of the Anti-Try_A-IgG was not affected by this treatment.

The localisation of injected Anti-Try_A-IgG confined to the mesangium could be demonstrated by postinjection of a small amount of antibasement membrane IgG (0.5 mg/rat) (Fig. 3).

c) For absorption of the antibodies reacting with the mesangium Anti-Try_A-IgG was incubated with untreated PLA and rat kidney antigen for 48 hours at 4°C. Absorption of the antibodies which were contained in 10 mg of the IgG-fraction was completed by 30 mg untreated PLA or 100 mg rat kidney antigen resp. An intramesangial deposition of the antibodies could not be demonstrated immunohistologically after the intravenous injection of the supernatant obtained from the incubation mixture.

d) Following the incubation of acetone-fixed cryostat sections of kidney of the rat, pig and human with non-labelled as well as with peroxydase-labelled Anti-Try_A-IgG mesangial immunoglobulin deposits were evident in each of these species. No immunoglobulin deposits were found along the glomerular basement membrane (Fig. 4). In contrast to the solitary mesangial deposition after intravenous injection the in vitro-test showed a reaction of the antibodies with the Bowman capsule, tubular basement membrane and perivascular connecctive tissue. Species differences concerning the pattern of immunoglobulin distribution did not exist.

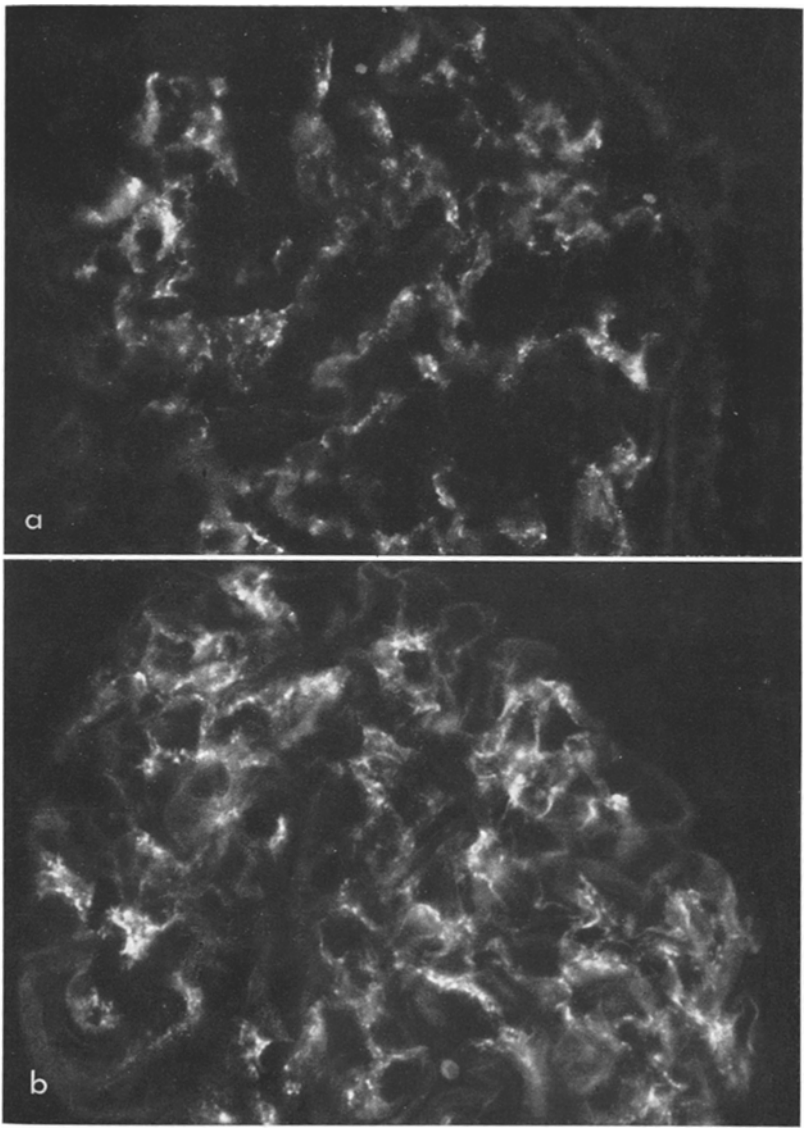

Fig. 3. (a) Rat kidney 2 hours following intravenous injection of 20 mg Anti-Try_A-IgG, unilateral nephrectomy. Intramesangial depositions of Anti-Try_A-IgG, no immunoglobulin deposition along the basement membrane. (b) Kidney of the same animal 2 hours following consecutive anti-basement membrane IgG injection (0.5 mg IgG). Besides intramesangial IgG deposition homogeneous-linear IgG depositions along the glomerular basement membrane can be seen. The intramesangial localisation of Anti-Try_A-IgG can be recognized now very clearly. (a, b) Enlargement 300 times

The mesangial fixation of Anti-Try_A-IgG was abolished after trypsin-digestion of cryostat sections of normal rat kidney, whereas the binding of anti basement IgG was not hampered significantly (Fig. 5). Digestion of cryostat sections with collagenase did not alter the reaction of Anti-Try_A-IgG with the glomerular mesangium.

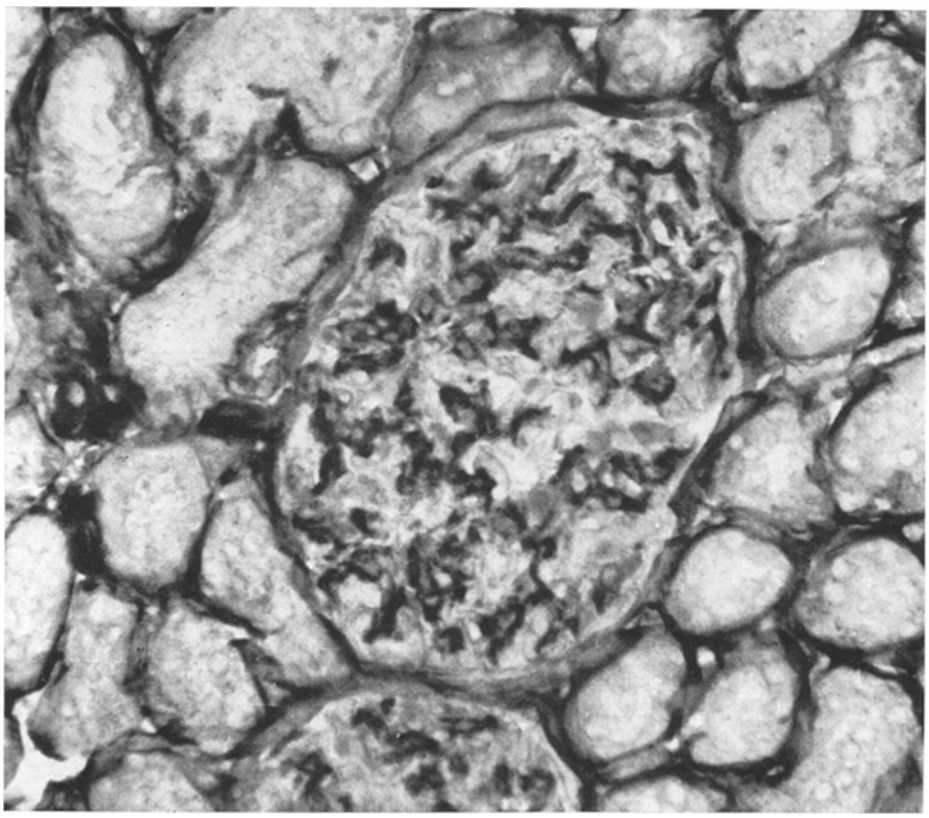

Fig. 4. Rat kidney, acetone-fixed cryostat section, 30 min incubated with peroxydase-labelled Anti-Try_A-IgG. Benzidine-reaction. IgG deposition within the mesangium, along the Bowman capsule and the tubulus capillaries. Enlargement 300 times

Anti-Try_A-IgG reacted in cryostat sections of lung tissue from pig and rat with cartilage cells and peribronchial as well as with perivascular connective tissue. There was no reaction with the basement membrane of the alveolar capillaries.

2. Agargel Diffusion According to Ouchterlony

In the agargel double diffusion according to Ouchterlony Anti-Try_A-IgG showed two precipitation bands with trypsin- and collagenase-digested PLA resp. and three not distinctly separated precipitation bands with untreated PLA. One precipitation band was obtained with porcine serum. This band crossed with the different lung antigens. Precipitation occurred neither with rat nor with human serum, nor with rat and human urine.

3. Immunopathological Properties of the Antibody Reacting with the Mesangium

Five rats were injected intravenously 20 mg Anti-Try_A-IgG, five rats were injected with 20 mg Anti-Try_A-IgG and simultaneously received 9 mg of pure rabbit IgG in complete Freund Adjuvant at several sites subcutaneously. Five control animals were given 1 ml 0.9% NaCl i.v., five control animals received

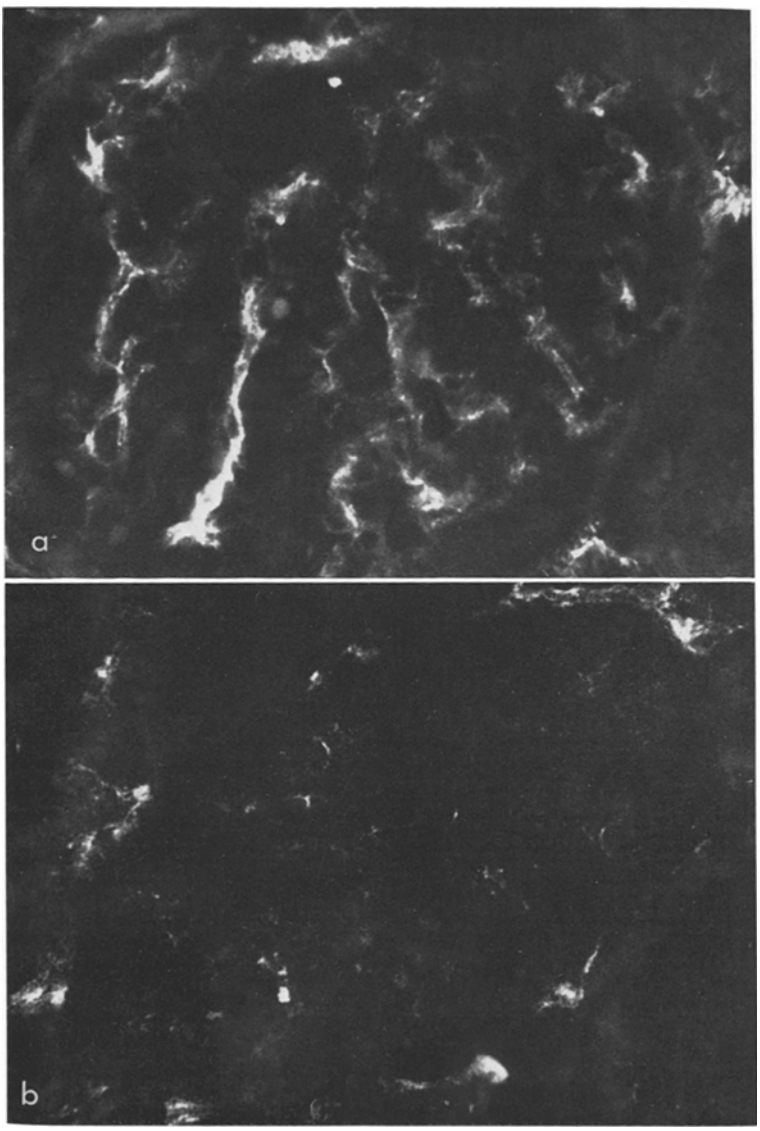

Fig. 5a and b. Rat kidney, cryostat section. (a) Incubation with Anti-Try_A-IgG for 30 min. Mesangial deposition of immunoglobulins (anti-rabbit-IgG FITC-labelled). (b) Before incubation with Anti-Try_A-IgG the section was digested for 3 hours with trypsin. There is loss of antigenic mesangial structures (anti-rabbit-IgG, FITC-labelled). Enlargement 300 times

9 mg rabbit IgG in complete Freund Adjuvant subcutaneously. After five days all animals were subjected to unilateral nephrectomy and were further observed for 50 days.

a) The kidneys of the rats which had been injected with mesangial antibody in all cases showed generalized and diffuse deposits of rabbit IgG within the

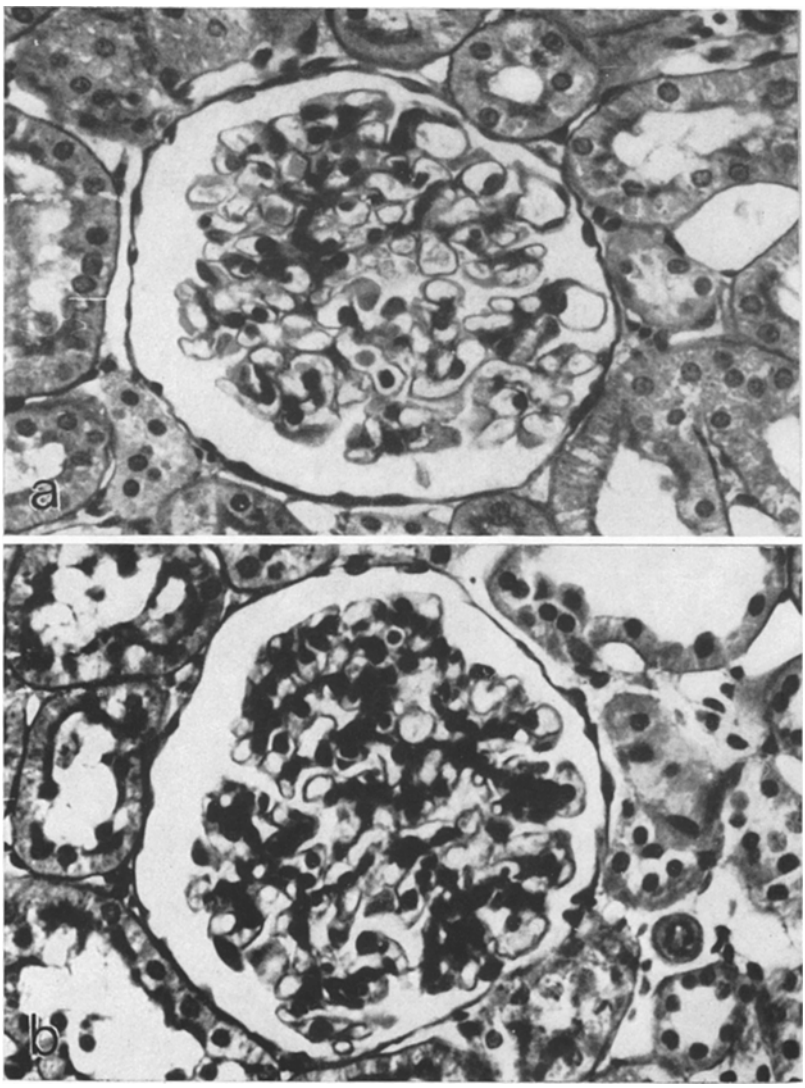

Fig. 6a—d. Rat kidney. (a) Control animal; 50 days following unilateral nephrectomy. (b—d) 55 days after injection of 20 mg mesangial antibodies and simultaneous immunization with rabbit-IgG. Enlargement of the mesangial matrix and deposition of PAS-positive substances. Segmental necroses and partial capillary obstruction. Formalin, Paraffin, PAS. (a, b and d) Enlargement 300 times. (c) Enlargement 500 times

glomerular mesangium five days after injection. Complement deposits within the mesangium could not be detected. Intraglomerular deposits of rat IgG were neither observed in the antibody-treated nor in the control animals. Beside an insignificant enlargement of the mesangium matrix no major histological changes could be seen. Proteinuria did not occur within these five days.

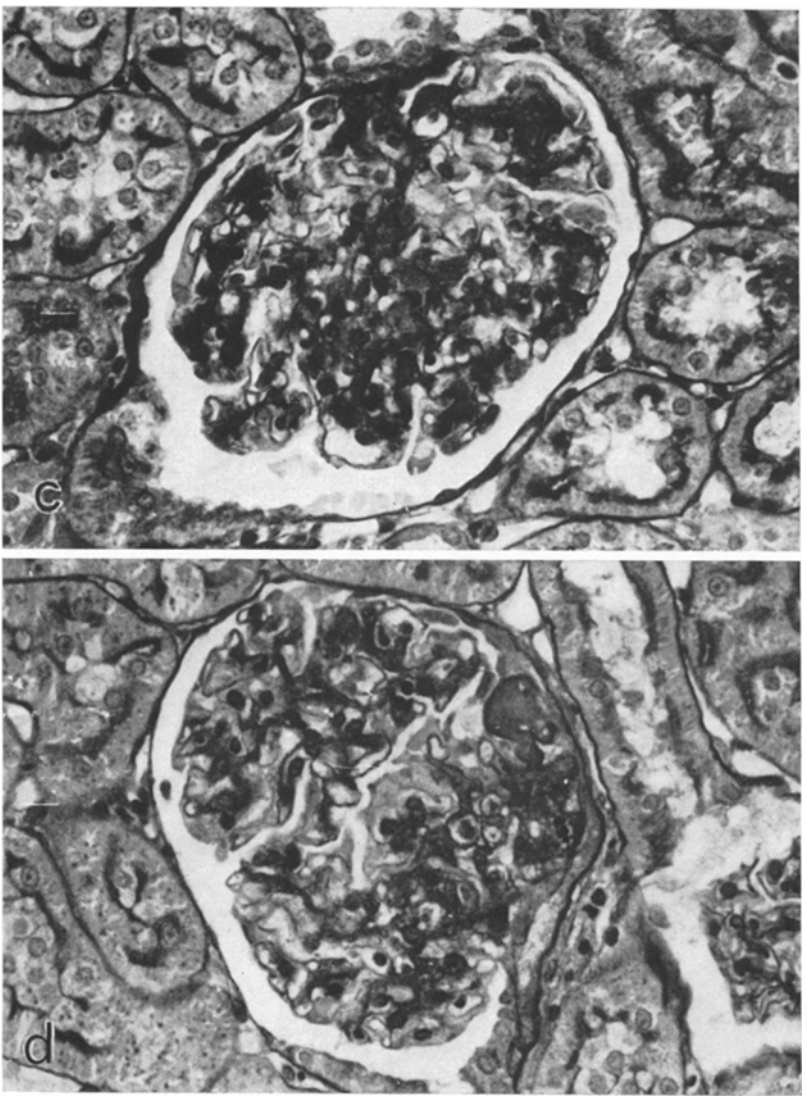

Fig. 6c and d

b) After a period of 55 days the animals injected with mesangium antibody still showed diffuse and generalized intramesangial deposits of rabbit IgG (Anti-Try_A-IgG). At this time minimal diffuse and generalized deposits of rat IgG within the mesangium could be seen. The minimal reaction with anti rat C3 labelled with peroxydase could not be determined unequivocally. There were no deposits of IgG or C3 along the basement membrane. A minor enlargement of the mesangial matrix was seen by means of light microscopy. Infiltration with polymorphonuclear leucocytes or proliferation of mesangial cells were absent.

c) After a period of 55 days the animals which had been immunized simultaneously with rabbit IgG showed diffuse and generalized intramesangial deposits

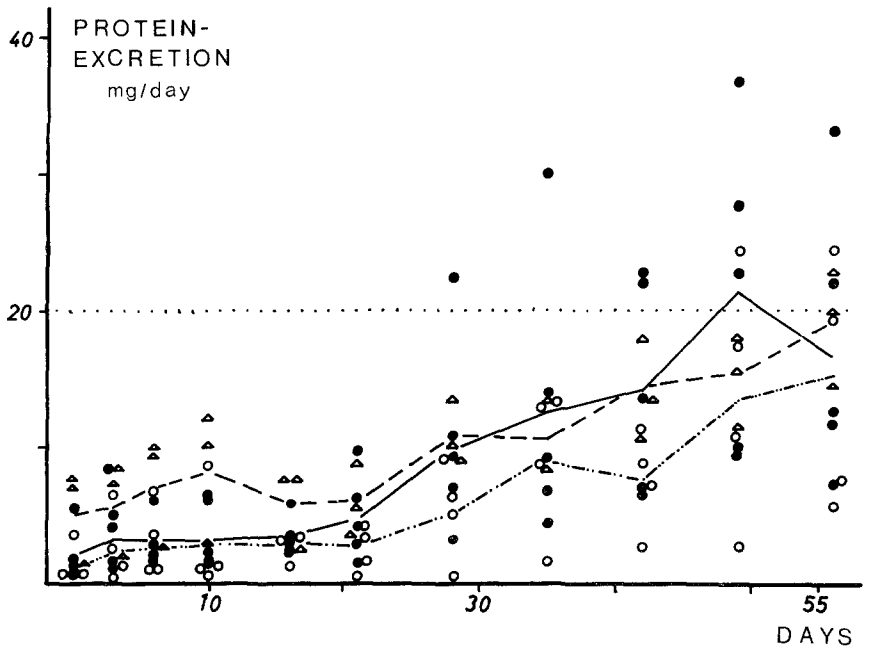

Fig. 7. Excretion of urine protein/day following intravenous injection of 20 mg mesangial antibodies (open triangles; mean values ---), of 20 mg mesangial antibodies and simultaneous immunization with rabbit IgG (closed circles; mean values —) and of control animals (open circles; mean values -.....-). The first two values designate control values before injection of the antibody

of rabbit IgG (Anti-Try_A-IgG) as well as major deposits of rat IgG when compared to the non-immunized animals. Intramesangial complement deposits could be determined now. By histological examination focal and segmental mesangial necroses of different degrees and beginning obliteration of capillaries and deposition of PAS-positive material within the mesangium could be seen. The same section, however, showed glomeruli without evident disturbance (Fig. 6).

d) The control animals neither showed immunoglobulin deposits by immunohistological examination nor histological lesions of the glomeruli.

The daily protein excretion in urine steadily rose in all animals inclusively the control animals after unilateral nephrectomy. The upper norm limit of 20 mg/day was exceeded only by some of the animals which had received rabbit IgG and Anti-Try_A simultaneously (Fig. 7).

Discussion

Experimentally produced intramesangial antigen-antibody-reactions with participation of complement induce lesions of the glomerular mesangium (Maurer, Sutherland, Howard, Fish, Najarian and Michael, 1973). To induce such intramesangial immune reactions Maurer *et al.* (1973) injected aggregated human IgG which deposited within the mesangium within a few hours. They transplanted these kidneys and applicated antibodies against the aggregated immuno-

globulins to the recipient animal. A heavy intramesangial antigen-antibody-reaction took place, activating the complement system and leading to an acute exsudative inflammation with leucocyte infiltration of the mesangium, followed by focal and segmental mesangium cell proliferations and mesangial sclerosis and hyalinosis. Unfortunately, this laborious model did not permit observation for a longer period, because of the transplantate rejection beginning after 8 days.

The production of antibodies reacting with mesangial antigens enables us to induce and to evaluate acute as well as chronic immunopathological reactions within the mesangium. Following immunization of rabbits with trypsin-digested lung antigens, antibodies could be obtained which reacted with mesangial antigens of glomeruli of the rat, the pig, and the human. They showed no reaction with the glomerular basement membrane. Chromatographic analyses of the antibody preparations excluded the existence of immunoglobulins of high molecular weight or of immune complexes. An intravasal formation of immune complexes was unlikely, as the double diffusion test according to Ouchterlony showed no precipitation with rat serum. The antibodies, absorbed with rat serum insolubilized by cross-linking with glutaraldehyde, showed unchanged anti-mesangial properties. The deposition of non-labelled as well as peroxydase-labelled antibodies within the glomerular mesangium of acetone-fixed cryostat sections of the kidneys of several species (rat, hog, human) prove the existence of antibodies reacting with mesangial antigens. In these in vitro tests the antibodies also reacted with the Bowman capsule, tubular capillaries, and the perivascular connective tissue of the kidney. Obviously, the antibodies elicited after an immunization with PLA constitute a population of antibodies with different antigen specificity. After injection, however, the antigenic sites of the mesangium only appear to be accessible to the antibodies.

According to the immunohistological picture the antibodies react with the mesangium matrix which seems to be composed of tropocollagen surrounded by mucoid (Misra and Berman, 1966; Forster and Riad, 1963). The great affinity of the antibodies against the mucopolysaccharid-rich cartilage of bronchi as well as the low protein content of the Try_A antigens used for immunization ($< 20\%$), speaks in favor of an antibody reaction against the mucopolysaccharides of the matrix. The loss of the antigenic sites following trypsin digestion of kidney sections possibly reflects the splitting of sugarcontaining oligopeptides. The nature of these mesangial antigens is to be investigated in further studies. Primarily results have shown that the antigen can be eluted from glomeruli and induces mesangium antibodies in rabbits (Seelig, 1974).

The antibodies reacting with the mesangial antigens possibly belonging to the mucopolysaccharid layer enables the development of an experimental model for intramesangial immunopathological antigen-antibody-reactions. The concentration of antibodies used in the former experiments was not sufficient to produce morphological glomerular lesions within the first five days. In contrast to the limited persistence of deposits of aggregated γ-globulins or immune complexes within the mesangium (Michael, Fish and Good, 1967; Maurer, Fish, Blau and Michael, 1972; Dreesman, Senterfit and Germuth, 1970; Germuth, Senterfit and Dreesman, 1972) the heterologous antibodies persisted for weeks within the mesangium. Antibodies against the foreign mesangial antibodies

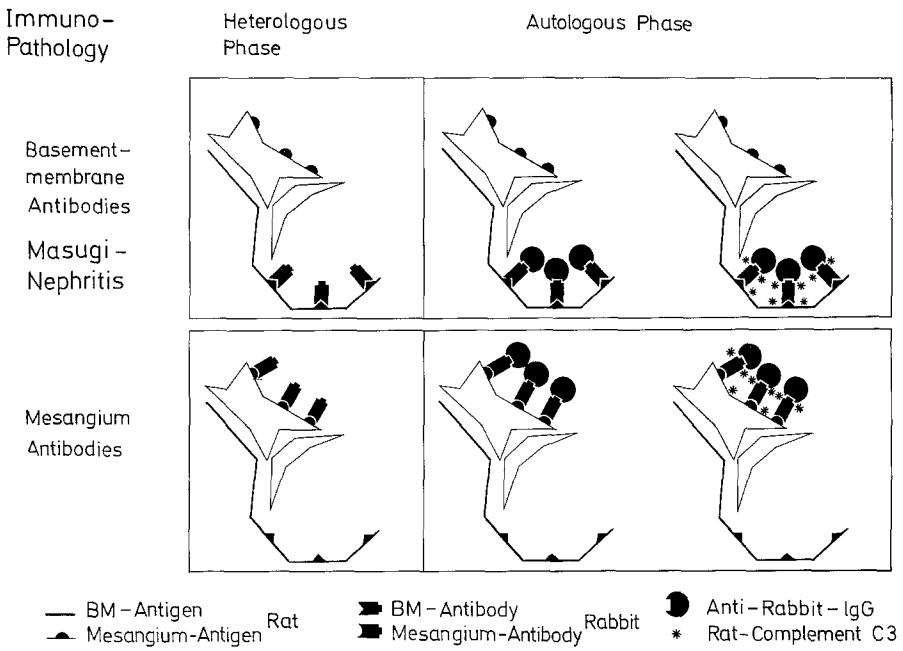

Fig. 8. Diagram of immunopathological reactions in Masugi nephritis and in mesangial lesions induced by mesangial antibodies

produced during this time enable a continuous deposition of autologous immunoglobulins within the mesangium (autologous phase). The activation of the complement system by this antigen-antibody-reaction can generate a chronic inflammation by liberation of biologically active mediators.

The immunopathological reactions confined to the mesangium may be regarded as an analogue to the immunopathological reactions at the basement membrane in Masugi nephritis (Masugi, 1939). In Masugi nephritis a heterologous basement membrane antibody (heterologous phase) reacts with the glomerular basement membrane and causes the generation of antibodies against the foreign IgG. These antibodies (autologous phase) react with the heterologous antibodies fixed to the basement membrane and produce an inflammatory reaction by activating the complement system and consecutive liberation of biologically active mediators (Fig. 8).

An enhancement of the autologous intramesangial immune reaction could be achieved by simultaneous immunization of rats with pure rabbit IgG. In contrast to non-immunized animals enhanced intramesangial deposition of autologous rat IgG and complement (C3-globulin) as well as morphologically manifest glomerular lesions characterized by focal and segmental necroses, matrix deposition and sometimes proliferations of mesangium cells could now be seen.

Many forms of human glomerulonephritis immunohistologically show intramesangial immunoglobulin or immune complex and complement depositions. Even the membranous glomerulonephritis which is characterized by subepithelial granular immune complex deposits in certain cases reveals intramesangial immune

complex and complement deposits, the quantity of which seems to correlated to the degree of mesangium cell proliferation (Portch and Williams, 1973). The pathological relevance of intramesangial immune complex deposits cannot be estimated with certainty in human glomerulonephritis. On the one hand, there are immune complex and complement deposits in patients with lupus erythematodes without any morphologically or clinically manifest glomerular lesion (Baldwin, Lowenstein, Gallo and McCluskey, 1970; Dujovne, Pollack, Pirani and Dillard, 1972; Agnello, Koffler and Kunkel, 1973). In other cases, however, as in the IgA-IgG nephropathy (Berger, 1969) which is characterized by exclusively intramesangial immune complex deposits there are focal and segmental proliferations of the mesagiunm cells, segmental to generalized scleroses or hyalinoses of the glomeruli.

The results of the experimentally induced intramesangial immunopathological reactions by aggregated immunoglobulins (Maurer *et al.*, 1973) or by antibodies against mesangial antigens show, however, that an intramesangial immunopathological reaction may lead to mesangial proliferation, necrosis and sclerosis, the quantity and duration of the immunological reaction thereby coining the morphological picture.

References

Agnello, V., Koffler, D., Kunkel, H. G.: Immune complex systems in the nephritis of systemic lupus erythematosus. Kidney International **3**, 90 (1973)

Avrameas, S., Ternynck, T.: Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. Immunochemistry **6**, 53 (1969)

Baldwin, D. W., Lowenstein, J., Rothfield, N. F., Gallo, G., McCluskey, R. T.: The clinical course of the proliferative and membranous form of lupus nephritis. Ann. intern. Med. **73**, 929 (1970)

Berger, J.: IgA glomerular deposits in renal disease. Transplant. Proc. **1**, 939 (1969)

Dreesman, G. R., Senterfit, L. B., Germuth, F. G.: Experimental immune complex disease. Fed. Proc. 3285 (1970)

Dujovne, I., Pollack, V. E., Pirani, C. L., Dillard, M. G.: The distribution and character of glomerular deposits in systemic lupus erythematosus. Kidney International **2**, 33 (1972)

Forster, C. L., Riad, Z. M.: Some staining properties of the glomerular constituents in normal rabbits. J. Anat. (Lond.) **97**, 403 (1963)

Germuth, F. G., Senterfit, L. B., Dreesman, G. R.: Immune complex disease. I. Experimental acute and chronic glomerulonephritis. Johns Hopk. med. J. **130**, 344 (1972)

Jungfer, H., Tremper, R.: Isolierung der gruppenspezifischen Komponente (Gc) des Humanserums durch Immunadsorption. Z. Immun.-Forsch. **143**, 6 (1972)

Masugi, M.: Über die experimentelle Glomerulonephritis durch das spezifische Antinierenserum. Beitr. Path. Anat. **92**, 429 (1934)

Maurer, S. M., Fish, A. J., Blau, E. B., Michael, A. F.: The glomerular mesangium. I. Kinetic studies of macromolecular uptake in normal and nephrotic rats. J. clin. Invest. **51**, 1092 (1972)

Maurer, S. M., Sutherland, D. E. R., Howard, R. J., Fish, A. J., Najaran, J. S., Michael, A. F.: The glomerular mesangium. III. Acute immune mesangial injury: a new model of glomerulonephritis. J. exp. Med. **137**, 553 (1973)

Michael, A. F., Fish, A. J., Good, R. A.: Glomerular localization and transport of aggregated proteins in mice. Lab. Invest. **17**, 14 (1967)

Misra, R. P., Berman, L. B.: Studies on glomerular basement membrane. I. Isolation and chemical analysis of normal glomerular basement membrane. Proc. Soc. exp. Biol. (N. Y.) **122**, 705 (1966)

Nilsson, U., Müller-Eberhard, H. J.: Isolation of β_{1F}-globulin from human serum and its characterization as the fifth component of complement. J. exp. Med. **122**, 277 (1965)

Portch, P. A., Williams, G.: Mesangial cells in membranous glomerulonephritis. J. clin. Path. **26**, 660 (1973)

Rother, K.: Kidney. In: Miescher, P. and Müller-Eberhard, H. J., eds., Testbook of immunopathology, p. 584. New York, London: Grune & Stratton 1967

Rother, K.: Gefäß- und Nierenläsionen durch Antigen-Antikörper-Komplexe. Pathomechanismus vom Typ der Serumkrankheit. Med. Welt **22** (N.F.), 988 (1971)

Rother, K.: Neue Aufgaben der immunologischen Forschung. Med. Welt **24** (N.F.), 30 (1973)

Seelig, H. P.: Antibodies against the glomerular mesangium. A new approach to the study of the mesangial cell. American society of nephrology, 7th annual meeting, Washington 1974, Abstr. p. 80

Seelig, H. P., Seelig, R.: Das glomeruläre Mesangium: einige immunpathologische Aspekte. Dtsch. med. Wschr. **99**, 1262 (1974)

Spiro, R. G.: Studies on the renal glomerular basement membrane. Preparation and chemical composition. J. biol. Chem. **242**, 1915 (1967)

Unanue, E. R., Dixon, F. J.: Experimental glomerulonephritis: Immunological events and pathogenetic mechanisms. In: Advanc. Immunol. **7**, 1 (1967)

Wilson, C. B., Dixon, F. J.: Antigenic quantitation in experimental immune complex glomerulonephritis. I. Acute serum sickness. J. Immunol. **105**, 279 (1970)

PD Dr. H. P. Seelig
Institut für Immunologie
und Serologie der Universität
D-6900 Heidelberg 1
Im Neuenheimer Feld 305
Federal Republic of Germany