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Formation and clearance of tubulointerstitial immune complexes in kidneys of rats immunized with heterologous antisera to Tamm-Horsfall protein

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Formation and clearance of tubulointerstitial immune complexes in kidneys of rats immunized with heterologous antisera to Tamm-Horsfall protein. Tubulointerstitial immune complex nephritis was produced by passive immunization of rats with antisera to rat Tamm-Horsfall protein (TH), a surface membrane glycoprotein of the cells of the thick ascending limb of Henle's loop. Circulating anti-TH antibodies were deposited in the kidney after an intravenous injection of rabbit antisera to TH. These anti-TH antibodies combined with TH at the base of tubular cells in the thick ascending limb of Henle's loop and formed granular immune complexes in situ in the space between basal cell surface membranes and tubular basement membranes. Immune complexes were also selectively formed in this site during perfusion of isolated kidneys with antisera to TH. Tubular immune complexes containing immunoglobulin, complement, and TH were maximal during the first week after an intravenous injection while high circulating anti-TH antibody titers were present. As the antibody titers subsequently fell to undetectable levels, tubular immune complexes were rapidly cleared and were virtually absent 4 weeks after the injection. During this clearance phase, rabbit IgG and rat TH were detected in the renal interstitium and in renal hilar lymph nodes. The rapid clearance of subepithelial TH immune complexes contrasts with the prolonged persistance of both glomerular subepithelial immune complexes and basement membrane deposits formed after injection of heterologous antisera to other renal components. The process of rapid clearance of tubulointerstitial immune complexes may allow rapid reversibility of immune injury in tubulointerstitial nephritis.

Formation et disparition d'immuns-complexes tubulo-interstitiels dans les reins de rats immunises avec des anti-serums heterologues contre la proteine de Tamm-Horsfall. Une néphrite tubulo-interstitielle à immuns complexes était produit chez des rats par immunisation passive avec un anti-sérum anti-protéine de Tamm-Horsfall de rat (TH), une glycoprotéine de la surface membranaire des cellules de la partie ascendante large de l'anse de Henle. Des anticorps anti-TH circulants se déposaient dans le rein après injection intraveineuse d'antisérum anti-TH de lapin. Ces anticorps anti-TH se combinaient avec la protéine de TH à la base des cellules tubulaires de la partie large ascendante de l'anse de Henlé et formaient in situ des immuns-complexes granuleux dans l'espace entre les membranes basales cellulaires et la basale tubulaire. De même, des immuns complexes se formaient sélectivement dans cette zone lors de la perfusion de reins isolés avec un antisérum anti-TH. La quantité d'immuns complexes tubulaires contenant des immunoglobulines, du complément et de la protéine de TH était maximale pendant la première semaine après l'injection i.v. au moment ou on trouvait des titres élevés d'anticorps anti-TH. Lorsque les anticorps retombaient à un taux indétectable, les immuns complexes tubulaires disparaissaient rapidement, et on n'en trouvait pratiquement plus 4 semaines après l'injection. Pendant cette période d'élimination on détectait des IgG de lapin et de la protéine de TH de rat dans l'interstitium et les ganglions hilaires rénaux. La disparition rapide des immuns complexes à TH sous épithéliaux contrastait avec la persistance prolongée des immuns complexes sous épithéliaux et des dépôts dans la membrane basale glomérulaire formés après injection d'anti-sérums hétérologues contre d'autres constituants du rein. La disparition rapide des immuns complexes tubulo-interstitiels pourrait permettre une régression rapide des lésions immunes au cours des néphrites tubulo-interstitielles.

Recent clinical and experimental studies have provided evidence that immunologic mechanisms may play important roles in renal tubulointerstitial diseases. Immune complexes or antibodies to tubular basement membranes may be involved in antibody-mediated tubular and interstitial lesions [1]. Granular deposits of immunoglobulin and complement comparable to those observed in glomerulonephritis have been identified along proximal and distal tubular basement membranes in human renal diseases [2; 3] and are most frequently seen in systemic lupus erythematosus [4]. Similar deposits have been observed in rats and rabbits immunized with homologous renal tissue [5–7].

The sera of some patients with renal disease [8–10] and the sera of rats immunized with homologous renal tissue [5] have been shown to contain autoantibodies reacting with distal tubular epithelium. We have recently demonstrated that rats immunized with Tamm-Horsfall protein (TH), a surface membrane glycoprotein of the cells of the thick ascending limb of the loop of Henle (ALH) [11] develop an immunologically mediated tubulointerstitial nephritis [12]. The extent of immune complexes at the base of ALH cells and histologic lesions corresponded to serum titers of antibodies to TH. In order to better define this relationship, we studied the renal lesions and circulating anti-TH antibody titers in rats passively immunized by intravenous injection of heterologous antisera to TH. In this new model, renal lesions and levels of circulating antibodies to TH could be sequentially evaluated after injection of a known quantity of antisera. Our studies provide further evidence relating tubular

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immune deposits to circulating levels of anti-TH antibodies and demonstrate that tubular immune complexes are rapidly cleared after the fall of circulating anti-TH antibodies.

Methods

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, Massachusetts), 5 weeks old and weighing 135 to 185 g were used in studies involving intravenous injections. Male Sprague-Dawley rats weighing 275 to 300 g were used for unilateral renal perfusions and studies involving injections of 10 times the standard dose of antisera to Tamm-Horsfall protein (TH). TH was isolated from normal Sprague-Dawley rat urine as described previously [13]. New Zealand white rabbits were initially immunized with 100 to 200 μg of rat TH emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Michigan) and subsequently reimmunized with rat TH in incomplete Freund's adjuvant at approximately monthly intervals, as described previously [13]. Antisera to rat TH was frozen at −20° C and pooled prior to isolation of the IgG fraction. After ammonium sulfate precipitation, the IgG fraction of this rabbit antisera was separated by DEAE cellulose column chromatography with a .01 M phosphate, .025 M sodium chloride, pH 7.0 buffer solution, and dialyzed against .01 M phosphate, .15 M sodium chloride, pH 7.4 (PBS) at a protein concentration of 10 mg/ml prior to use. Protein concentrations were determined using the method of Lowry et al [14]. The IgG fraction of serum from normal rabbits was obtained by the same procedures. These IgG preparations demonstrated a single line by immunoelectrophoresis against anti-whole rabbit serum. Portions of tissue samples were obtained by open biopsy, unilateral nephrectomy, or at the time of sacrifice. Renal tissue was fixed in 10% buffered formalin, sectioned and stained with periodic acid Schiff (PAS)-hematoxylin for light microscopy. Additional portions of renal tissue were mounted on cellulose sponges, snap-frozen in isopentane prechilled in liquid nitrogen and stored at −70° C. Cryostat sections were cut at 4 μ and stained by direct and indirect immunofluorescence methods as described previously [15, 16]. Sections containing both cortex and medulla were stained with fluorescein conjugated antisera to rat TH, IgG, IgM, albumin, C3, and fibrinogen and to rabbit IgG prepared in our laboratories also described previously [13, 15, 16]. Tissue was processed for double label immunofluorescence using fluorescein and rhodamine-conjugated antibodies [12]. Rhodamine-conjugated goat anti-rabbit IgG was obtained from Cappel Laboratories, Inc., Downington, Pennsylvania. Immunofluorescence microscopy was performed using a Zeiss Universal microscope equipped with filter combinations [12]. Immunofluorescence staining was graded on a scale from negative (0) to the most strongly positive (3+) with trace staining being graded as 1+. In evaluating basal ALH deposits, the percentage of tubules with each grade of staining was determined, and an average score was assigned to the cortical zone and to an outer medullary zone (inner stripe). Portions of renal tissue for electron microscopy were fixed by immersion in 2% paraformaldehyde, 2.5% glutaraldehyde in .15 M sodium phosphate buffer, processed, and examined [17]. Serum titers of rabbit anti-TH antibodies were determined by indirect immunofluorescence in a manner analogous to that already described for rat IgG anti-TH antibodies [12], using fluorescein-tagged goat anti-rabbit IgG after prior incubation of normal rat kidney with the test serum. The titer of the IgG fraction of rabbit anti-TH serum (10 mg/ml) was 1:2560. Staining of this antiserum and of an antisera to rat proximal tubular Fx1A with comparable titer was evaluated by indirect immunofluorescence after absorption with highly purified rat TH (1.5 mg/ml). The titer of the antiserum to TH was decreased more than 50% by absorption with 15 μg of TH/mg IgG, and by 99% with 30 μg/mg IgG; staining was completely blocked after absorption with 75 μg of TH/mg IgG. There was no decrease in the titer of proximal tubular brush border staining after absorption of the antisera to Fx1A with 300 μg of TH/mg IgG. The level of rabbit IgG in rat serum was determined by single radial immunodiffusion by the method of Mancini, Carbonara, and Heremans [18] using goat anti-rabbit IgG with normal rabbit IgG isolated by DEAE cellulose chromatography as a standard.

Experimental design. Thirty-eight rats were given an i.v. injection of 10 mg of an IgG fraction of serum obtained from a rabbit immunized with Tamm-Horsfall protein or from normal rabbits. Serum was obtained at the following times after immunization: 4 hr, 1, 3, 5, 7, 10, 14, 21, and 28 days and frozen at −20° C prior to assay. Levels of rabbit IgG and titers of anti-TH antibodies in the sera of 4 to 11 experimental rats were determined at each of the above times except days 3 and 28 when sera of three rats were studied. Rabbit IgG levels in the sera of 4 to 7 control rats given normal rabbit IgG were determined at the above times except that two sera of control rats were studied at 1, 3, 21, and 28 days.

Renal tissue samples were obtained for evaluation by immunofluorescence and light microscopy at 1, 3, 5, 7, 10, 14, 21, 28, and 49 days after an intravenous injection. A total of 38 samples from 29 experimental rats given anti-TH were evaluated by immunofluorescence. Sequential samples of renal tissue obtained from two rats at 7 and 28 days, from three rats at 7 and 49 days, and from two additional rats at 7, 28, and 49 days were studied. The kidneys of nine control rats obtained 7 days after injection of normal rabbit IgG were evaluated by immunofluorescence. Kidney tissues from three of these control rats at 3 days, from three rats at 28 days, and from two rats at 49 days were also studied bringing the total to 17 specimens from control rats. Left renal hilar lymph nodes from 24 rats and lymph nodes from near the aortic bifurcation of seven rats were examined by immunofluorescence.

The left kidneys of three rats were isolated and selectively perfused as previously described [15] with 1.0 ml of antisera to rat TH containing 10 mg/ml of IgG, followed 5 min later by 5.0 ml of isotonic saline; renal circulation was reestablished after a total of 10 min. One hr after perfusion, portions of the left and right kidneys were obtained for evaluation by immunofluorescence microscopy.

Because our previous studies of rats actively immunized with TH had shown that the size and number of electron dense deposits at the base of tubular cells correlated closely with serum anti-TH titers [17], ultrastructural studies were performed in rats given 10 times the standard dose of anti-TH to facilitate detection of deposits. Two rats were each given a total of 100 mg of the IgG fraction of antiserum to rat TH by intravenous injections during a 3-day period. Renal tissue for immunofluorescence and electron microscopy was obtained 7 days after the first injection.

Results

Immunofluorescent microscopy. We found granular deposits of rabbit IgG at the base of tubular cells of the ALH in all kidneys examined between 1 and 21 days after we injected rats

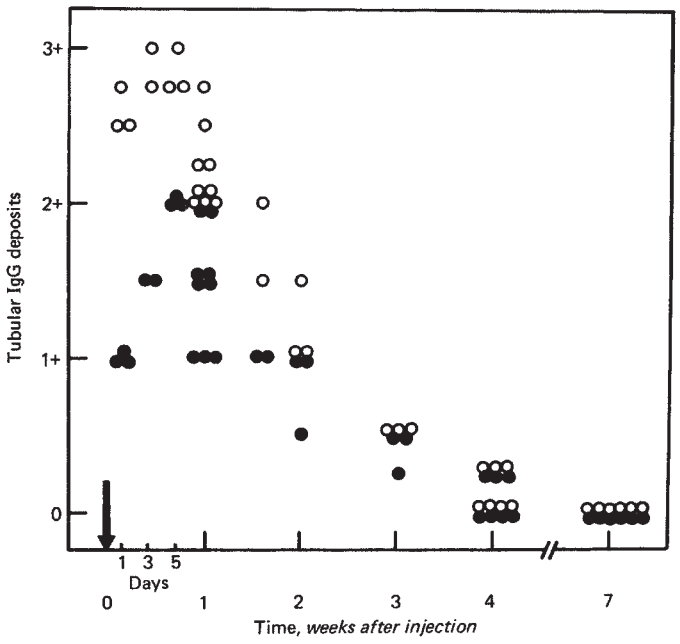

Fig. 1. *Time course of tubular deposits of rabbit IgG in rat kidneys after an injection of antisera to TH.* Deposits at the base of ALH cells in the cortex are shown by *open circles* and in the medulla are shown by *closed circles* (See text for grading method).

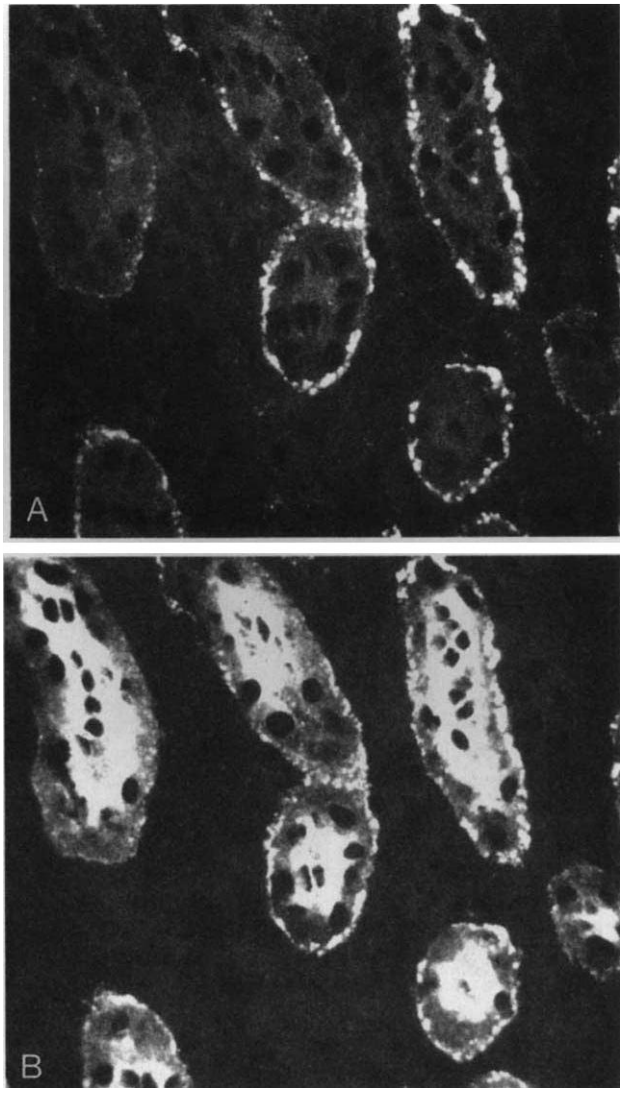

Fig. 2. *Double-label immunofluorescent micrographs of the same section of kidney from a rat 5 days after an injection with antisera to TH.* **A** *Granular deposits of rabbit IgG shown at the base of ALH cells (Rhodamine-labelled goat anti-rabbit IgG, × 400).* **B** *Diffuse TH staining of ALH cells in addition to granular basal TH deposits in a distribution identical to rabbit IgG deposits (Fluorescein-labeled rabbit anti-rat TH, magnification, ×400).*

intravenously with 10 mg of IgG fraction of rabbit antisera to rat TH (Fig. 1), whereas none of the rats injected with an equal quantity of IgG from normal rabbit serum showed tubular deposits. Tubular deposits of rabbit IgG were maximal during the first week after an injection of antisera and then rapidly decreased during the next 2 weeks. By 4 weeks, tubular deposits of rabbit IgG were no longer present in kidneys of the majority of the rats given antisera and were absent from all kidneys examined at 7 weeks. Sequential evaluation of the kidney tissue of individual rats also demonstrated that the extensive tubular deposits of rabbit IgG at 1 week were markedly reduced or absent by 4 weeks and absent at 7 weeks.

Within individual kidneys, the amount of tubular immunoglobulin deposition was always closely related to distance along the ALH; deposits were always less prominent in the first portion of the ALH, the inner stripe of the outer medulla, than in the juxtamedullary region (Fig. 1) where deposits were maximal. Basal rabbit IgG deposits along the course of the entire ALH to the parent glomerulus were present in kidneys with the most extensive lesions. ALH cells directly opposite and beyond the macula densa had basal deposits, although immune deposits were not observed at the base of macula densa cells. During the first week, the distribution of deposits associated with individual tubules was relatively even and became more focal at subsequent times. Basal ALH deposits of rat TH and C3 were found in the same distribution as rabbit IgG deposits. The identity of localization was confirmed in double-label studies on the same section (Fig. 2). The time course of the formation and clearance of TH and C3 deposits at the base of ALH cells did not differ from that of rabbit IgG deposits. Small quantities of granular rabbit IgG and rat TH staining in an identical distribution were frequently observed in the interstitial areas surrounding clusters of ALHs and were most prominent during the second week after injection. Kidneys of rats injected with 10 times the standard dose of rabbit antisera showed much larger deposits of rabbit IgG and rat TH at the base of ALH cells and greater quantities of these proteins in the surrounding interstitial areas (Fig. 3). Finely granular basal tubular deposits of trace quantities of rat IgG and IgM were first seen 5 to 7 days after an injection of antisera, reached a maximum at 7 to 14 days, and were usually cleared by 21 days. Fibrinogen and albumin deposits were not observed in either experimental or control rats. Renal tissue obtained 1 hr after unilateral renal perfusion with rabbit antisera to rat TH showed finely granular basal ALH deposits of rabbit IgG in the perfused kidney (Fig. 4). Deposits of rabbit IgG were not present in the contralateral kidney.

Granular deposits of rat TH were observed in a reticular pattern within the germinal centers and within superficial cortical sinuses of renal lymph nodes of rats injected with rabbit

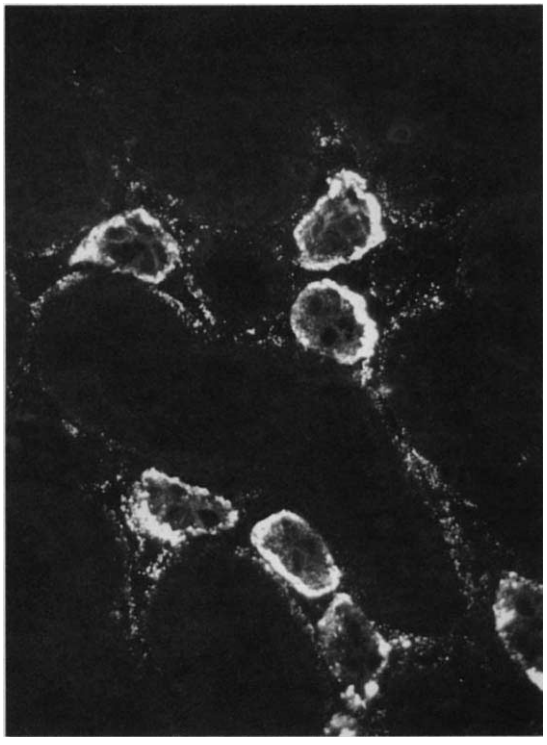

Fig. 3. *Immunofluorescent micrograph of kidney tissue from rat given 10 times the standard dose of antisera to TH, obtained 7 days after the first injection.* Large granular and nodular deposits of rabbit IgG were present at the base of ALH cells and frequently appeared confluent. Smaller, finely granular deposits were present in the interstitial areas near the tubules with large basal deposits. Rat TH was present in an identical distribution at the base of ALH cells and in the interstitium. (Fluorescein-labelled goat anti-rabbit IgG, magnification, ×250).

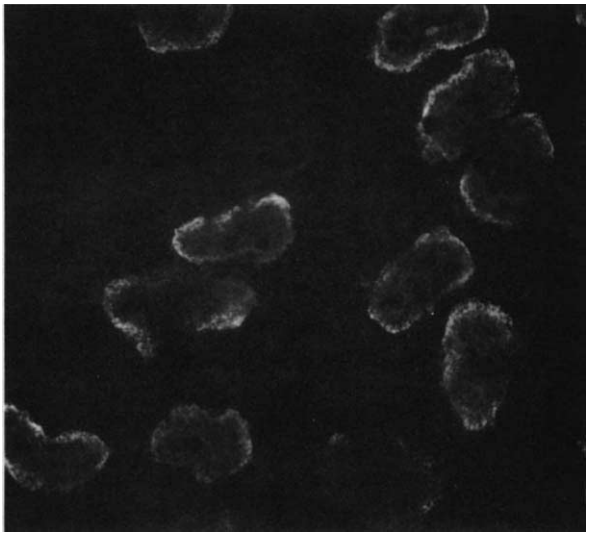

Fig. 4. *Immunofluorescent micrograph of rat kidney tissue obtained 1 hr after isolated unilateral renal perfusion with rabbit antisera to rat TH.* Finely granular deposits of rabbit IgG were present at the base of ALH cells in the outer medulla. (Fluorescein-labelled goat anti-rabbit IgG, magnification, ×250).

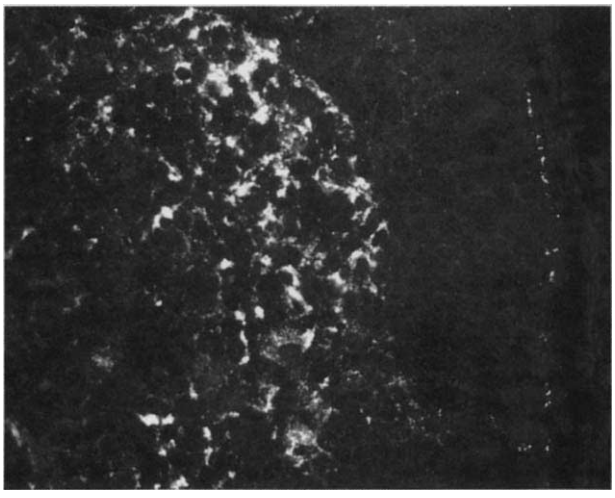

Fig. 5. *Immunofluorescent micrograph of renal hilar lymph node of a rat obtained 28 days after an intravenous injection of antisera to rat TH.* Granular staining for rat TH was present in a reticular pattern within the germinal center and in the superficial sinus. Rabbit IgG was present in an identical pattern. (Fluorescein-labelled rabbit anti-rat TH, magnification, ×275).

antisera to TH studied during the clearance phase. These TH deposits and rabbit IgG deposits in an identical distribution were present in renal lymph nodes from four of five rats studied at 21 days and in three of four rats studied 28 days after an injection of antisera and in the renal lymph nodes of the two rats studied 4 weeks after an injection of 10 times the standard dose of anti-TH (Fig. 5). This pattern of rat TH and rabbit IgG staining in germinal centers and superficial sinuses in renal lymph nodes was blocked by prior absorption of the fluoresceinated antisera with the respective antigen. In contrast, rat TH and rabbit IgG were not detected in renal lymph nodes from eight normal rats, two rats studied 28 days after an injection of 10 mg of normal rabbit IgG, and three rats studied 49 days after an injection of rabbit anti-TH; these proteins were not detected in more distal aortic lymph nodes from seven rats injected with anti-TH.

Electron microscopy. Ultrastructural examinations were performed on sections from the outer stripe of the outer medulla because the largest deposits by immunofluorescence were demonstrated in this zone. Numerous electron dense deposits were detected at the base of ALH cells in each of the kidneys obtained from rats 7 days after an injection of antisera to TH. The largest electron dense deposits were present in the extracellular space between the tubular basement membrane (TBM) and the base of ALH cells (Fig. 6). In addition, many electron dense deposits were present in the extracellular space between basolateral ALH cell membranes quite near the TBM (Fig. 7). Electron dense deposits were not detected within the TBM or at the base of cells of other tubular segments and were not present at the base of ALH cells in kidneys from four normal rats studied in a similar fashion.

Light microscopy. Mild focal mononuclear cell infiltration was occasionally seen in the areas surrounding ALHs in the kidneys of experimental rats during the first week after an injection of the standard dose of antisera to TH. There was no PAS positive basal tubular deposits; abnormalities by light

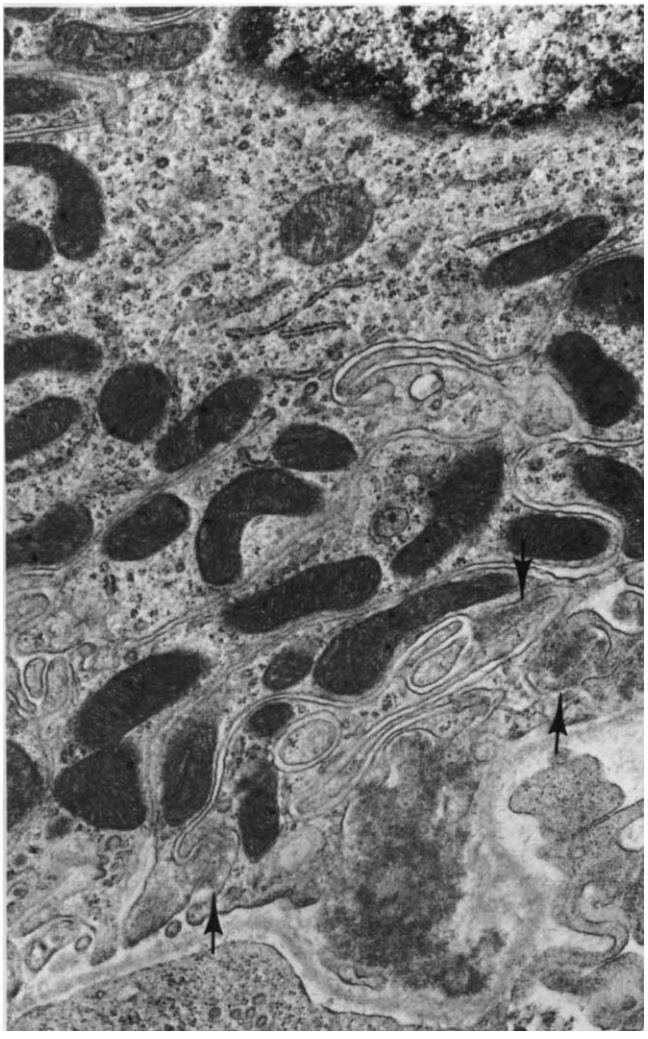

Fig. 6. *Electron micrograph of an outer medullary section of rat 7 days after an injection of rabbit antisera to rat TH.* A large electron dense deposit is present in the extracellular space between the basal surface membranes of the ALH cell and the tubular basement membrane. Smaller electron dense deposits *(arrows)* were present in the extracellular space between basolateral ALH cell membranes. (Uranyl acetate and lead citrate, magnification, ×20,000).

microscopy were not observed in kidneys of experimental or control rats at later times.

Other studies. The mean levels of rabbit IgG in the serum of control and experimental rats were very similar throughout the 28 days of the study (Fig. 8), with a calculated half-life of approximately 8.5 days. The slightly greater mean weight of control rats (161 g versus 144 g in experimental rats) may be responsible for the slightly lower mean levels for controls, although the differences were not statistically significant at any point. The circulating titers of antibodies to TH showed a more rapid removal from the serum of rats than the total rabbit IgG levels (Fig. 9), with an overall half-life after initial equilibration of roughly 3.5 days. None of the experimental rats given antisera to TH had detectable anti-TH antibodies in either undiluted serum or in a 1:5 dilution at 21 and 28 days.

Discussion

The present study demonstrates the formation of tubular immune complexes in the kidneys of rats passively immunized with heterologous antisera to rat TH and the rapid removal of these subepithelial immune complexes after the clearance of circulating anti-TH antibodies from the serum of these rats. Thus, circulating antibodies are required not only for forming but also for maintaining tubular complexes in this passive model. Only trace quantities of rat immunoglobulins were detected transiently at the base of tubular cells, and there was no evidence for autoimmune amplification of the type implicated in the perpetuation of tubulointerstitial nephritis in guinea pigs injected with isologous anti-TBM sera [19]. Immune complexes in the passive TH model were present in the same distribution as immune complexes in the kidneys of rats actively immunized with TH [12] and appear to be the consequence of the combination of circulating IgG anti-TH antibodies with an antigen (TH) present at the base of ALH cells. The mechanism leading to the granular basal tubular immune deposits in rats and rabbits immunized with homologous renal tissue [5–7] also appears to involve in situ immune complex formation similar to that observed in other tissues [20–23]. Immune complexes are formed in situ in the thyroid after passive immunization [20] as well as after active immunization [21], and similar immune deposits have been demonstrated in human thyroid disease [22]. In guinea pigs given heterologous antisera to thyroglobulin, electron dense deposits were observed in the space between basal plasma membranes and the basement membrane of thyroid follicle cells [20]. This localization is directly analogous to that of the electron dense deposits observed at the base of ALH cells in the present study and the active TH model [17]. The formation of immune complexes in this site in the TH models is favored by the limited permselectivity of peritubular capillaries. Because peritubular capillaries are much less restrictive than glomerular capillaries to the movement of macromolecules larger than IgG [24], molecules of TH associated with ALH basal surface membranes and in the extracellular spaces at the base of these cells [11] are accessible to circulating IgG antibodies. The formation of similar immune complexes during the isolated perfusion of kidneys with antisera to TH as well as the close relationship between circulating levels of anti-TH antibodies and the extent of immune deposits in both active and passive models also favor an in situ mechanism of formation [17, 25]. Immune deposits in glomeruli suggesting a role for circulating immune complexes are not present in the TH models.

The clearance of tubular immune complexes in the passive TH model described in this report was much more rapid than the clearance of the immune deposits formed after rats were given an intravenous injection of heterologous antisera to other renal components such as basement membranes [26] or proximal tubular antigens [27, 28]. The glomerular subepithelial immune deposits formed after an injection of antisera to proximal tubular antigen persisted for months [27] even after renal transplantation to isogeneic recipients [28].

Possible explanations for the differences in clearance rates of immune complexes warrant consideration. Relatively less is known concerning clearance mechanisms than the various factors responsible for the renal localization of immune complexes [25, 29]. The solubilization of large latticed complexes by

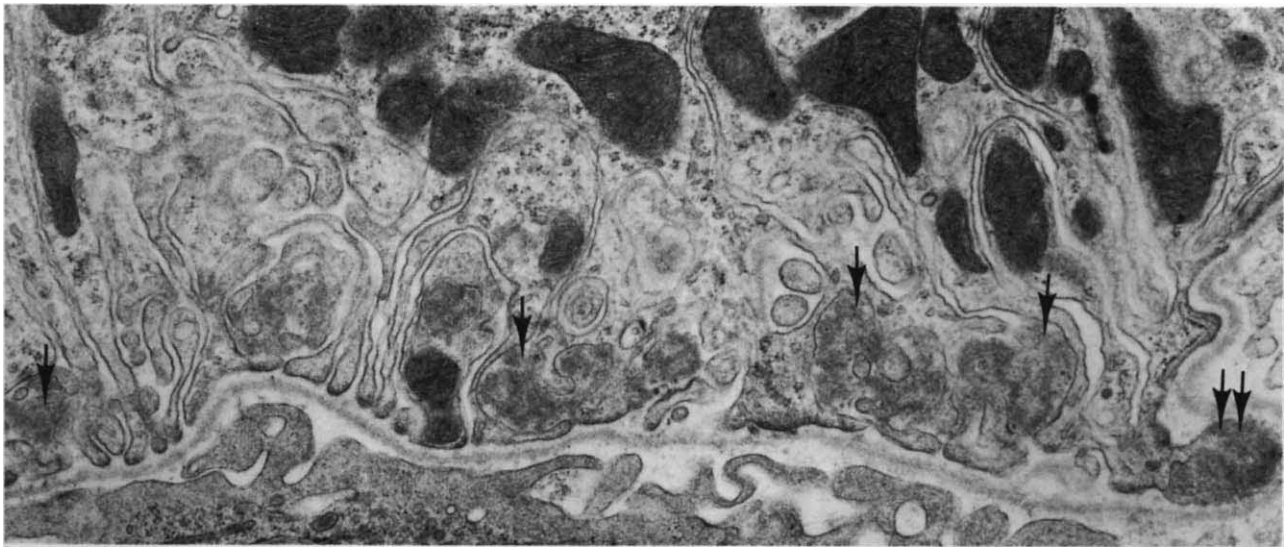

Fig. 7. *Electron micrograph of renal tissue from same kidney as Figure 6.* A few of the numerous electron dense deposits present in the extracellular space between basolateral ALH cell membranes very near the tubular basement membrane are indicated by *single arrows*. A single large electron dense deposit is present in the space between the basal cell membrane and the tubular basement membrane *(double arrows)* (Uranyl acetate and lead citrate, magnification, ×21,000).

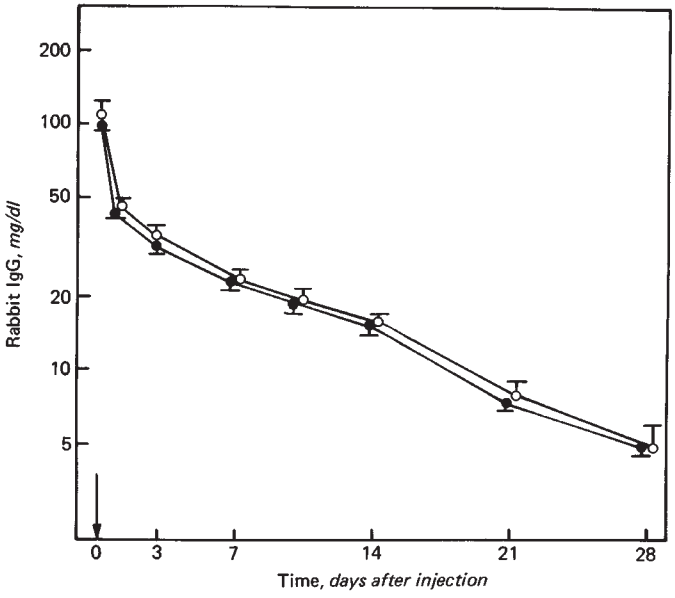

Fig. 8. *Sequential comparison of the levels of rabbit IgG in the sera of rats after an injection of rabbit anti-TH* (open circles) *or normal rabbit IgG* (closed circles).

excess antigen is an important potential component of clearance mechanisms in immune complex nephritis. Studies of serum sickness in rabbits have shown both a decrease in renal disease and glomerular immune deposits [30] and shortening of the half-life of the antigen in kidneys [31] after giving large doses of excess antigen. More recent studies in mice given preformed immune complexes have also shown a rapid removal of extracellular glomerular immune complexes following the administration of excess antigen [32]. Immune complexes in the TH models are extracellular and thus potentially accessible to the solubilizing effects of excess antigen. Substantial quantities of anti-TH antibodies are present in the serum only during the first week in the passive TH model and thereafter, conditions of antigen excess are likely to be present. Basal tubular immune complexes appear to contain relatively large quantities of antigen because the antigens in these tubular complexes are readily demonstrated by immunofluorescence [12, 33]. In contrast, prior elution of antibody is frequently required to demonstrate the antigenic component of glomerular subepithelial immune complexes [34]. The rapid turnover and solubility properties of TH [35] have been recently reviewed and would appear to favor achieving antigen excess at the base of ALH cells. In contrast, the prolonged persistence of renal immune deposits in other models after the clearance of circulating antibodies may be related to a much slower turnover of relatively insoluble antigens in basement membranes [36] or to more limited quantities of the antigenic component of glomerular subepithelial deposits within normal and diseased glomeruli [25, 34].

It appears that the permeability characteristics of peritubular capillaries that allow a movement of macromolecules such as ferritin [24] could also facilitate the movement of small immune complexes from the extracellular space between tubular cells and basement membranes. The presence of small finely granular deposits of both rabbit IgG and TH in the interstitial areas surrounding tubules with large basal immune deposits during the clearance phase in this model is consistent with such a mechanism. Our further demonstration that TH and rabbit IgG deposits are present together in the renal lymph nodes of passively immunized rats during the clearance phase indicates that one pathway for the subsequent removal of TH anti-TH immune complexes involves the renal lymphatics. However, other pathways such as re-entry into the circulation through peritubular capillary walls or excretion into the urine are also

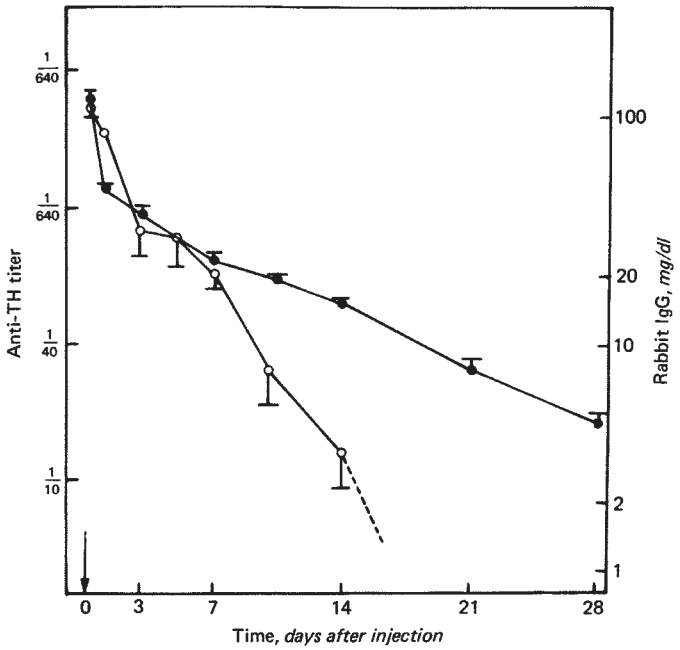

Fig. 9. *Sequential comparison of the levels of rabbit IgG* (closed circles) *and titer of rabbit antibodies to TH* (open circles) *in the serum of rats injected with rabbit anti-TH.* Antibodies to TH were not detectable after day 14.

possible. Because movement of basal immune deposits toward the tubular lumen was not detected during the clearance phase in the present study, urinary excretion seems less likely.

Rapid immune complex clearance similar to that described here should be considered as a potential factor favoring the reversibility of tubular injury mediated by immune complexes in human tubulointerstitial nephritis. Sequential serologic evaluation of antibodies to renal antigens such as TH [37] may be of value in evaluating patients with tubulointerstitial diseases. Many aspects of the immunologic responsiveness to renal antigens such as Tamm-Horsfall protein and its role in disease, remain to be clarified by further clinical and experimental studies.

Acknowledgments

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References

1. Andres GA, McCluskey RT: Tubular and interstitial renal diseases due to immunologic mechanisms. *Kidney Int* 7:271–289, 1975
2. Klassen J, Andres GA, Brennan JC, McCluskey RT: An immunologic renal tubular lesion in man. *Clin Immunol Immunopathol* 1:69–83, 1972
3. Lehman DH, Wilson CB, Dixon FJ: Extraglomerular immune deposits in human nephritis. *Am J Med* 58:765–786, 1975
4. Brentjens JR, Sepulveda M, Baliah T, Bentzel C, Erlanger BF, Elwood C, Montes M, Hsu KC, Andres GA: Interstitial immune complex nephritis in patients with systemic lupus erythematosus. *Kidney Int* 7:342–350, 1975
5. Klassen J, Sugisaki T, Milgrom F, McCluskey RT: Studies on multiple renal lesions in Heymann nephritis. *Lab Invest* 25:577–585, 1971
6. Unanue ER, Dixon FJ, Feldman JD: Experimental allergic glomerulonephritis induced in the rabbit with homologous renal antigens. *J Exp Med* 125:163–175, 1967
7. Klassen J, McCluskey RT, Milgrom F: Nonglomerular renal disease produced in rabbits by immunization with homologous kidney. *Am J Pathol* 63:333–350, 1971
8. Pasternack A, Linder E: Renal tubular acidosis: An immunopathological study on four patients. *Clin Exp Immunol* 7:115–123, 1970
9. Ford PM: A naturally occurring human antibody to loops of Henle. *Clin Exp Immunol* 14:569–572, 1973
10. Chanarin I, Loewi G, Tavill AS, Swain CP, Tidmarsh E: Defect of renal tubular acidification with antibody to loops of Henle. *Lancet* 2:317–318, 1974
11. Hoyer JR, Sisson S, Vernier RL: Tamm-Horsfall glycoprotein: Ultrastructural immunoperoxidase localization in rat kidney. *Lab Invest* 41:168–173, 1979
12. Hoyer JR: Tubulointerstitial immune complex nephritis in rats immunized with Tamm-Horsfall protein. *Kidney Int* 17:284–292, 1980
13. Hoyer JR, Resnick JS, Michael AF, Vernier RL: Ontogeny of Tamm-Horsfall urinary glycoprotein. *Lab Invest* 30:757–761, 1974
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275, 1951
15. Hoyer JR, Mauer SM, Michael AF: Unilateral renal disease in the rat: I. Clinical, morphologic and glomerular mesangial functional features of the experimental model produced by renal perfusion with the aminonucleoside of puromycin. *J Lab Clin Med* 85:756–768, 1975
16. Hoyer LW, DeLos Santos RP, Hoyer JR: Antihemophilic factor antigen: Localization in endothelial cells by immunofluorescent microscopy. *J Clin Invest* 52:2737–2744, 1973
17. Seiler MW, Hoyer JR: Ultrastructural studies of tubulointerstitial immune complex nephritis in rats immunized with Tamm-Horsfall protein. *Lab Invest,* 45:321–327, 1981
18. Mancini G, Carbonara AO, Heremans JR: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235–254, 1965
19. Hall CL, Colvin RT, Carey K, McCluskey RT: Passive transfer of autoimmune disease with isologous IgG_1 and IgG_2 and antibodies to the tubular basement membrane in strain XIII guinea pigs. Loss of self-tolerance induced by auto antibodies. *J Exp Med* 146:1246–1260, 1977
20. Kåresen R: Induction of thyroiditis in guinea pigs by intravenous injection of rabbit anti-guinea-pig thyroglobulin serum III. Electron microscopic study. *Immunology* 19:301–318, 1970
21. Clagett JA, Wilson CB, Weigle WO: Interstitial immune complex thyroiditis in mice. The role of autoantibody to thyroglobulin. *J Exp Med* 140:1439–1456, 1974
22. Kalderon AE, Bogaars HA: Immune complex deposits in Grave's disease and Hashimoto's thyroiditis. *Am J Med* 163:729–734, 1977
23. Bigazzi PE, Kosuka LL, Hsu KC, Andres GA: Immune complex orchitis in vasectomized rabbits. *J Exp Med* 143:382–404, 1976
24. Venkatachalam MA, Karnovsky MJ: Extravascular protein in the kidney: An ultrastructural study of its relation to renal peritubular capillary permeability using protein tracers. *Lab Invest* 27:435–444, 1972
25. Couser WG, Salant DJ: In situ immune complex formation and glomerular injury. *Kidney Int* 17:1–13, 1980
26. Unanue E, Dixon FJ: Experimental glomerulonephritis IV. Participation of complement in nephrotoxic nephritis. *J Exp Med* 119:965–982, 1964

27. FEENSTRA K, LEE RVD, GREBEN HA, ARENDS A, HOEDEMAEKER PJ: Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens: I. The natural history: A histologic and immunohistologic study at the light microscopic and ultrastructural level. *Lab Invest* 32:235–242, 1975
28. VAN ES LA, BLOK APR, SCHOENFELD L, GLASSOCK RJ: Chronic nephritis induced by antibodies reacting with glomerular bound immune complexes. *Kidney Int* 11:106–115, 1977
29. COCHRANE CG, KOFFLER D: Immune complex disease in experimental animals and man. *Adv Immunol* 16:185–264, 1973
30. VALDES AJ, SENTERFIT LB, POLLAK AD, GERMUTH FG, JR: The effect of antigen excess on chronic immune complex glomerulonephritis. *John Hopkins Med J* 124:9–17, 1969
31. WILSON CB, DIXON FJ: Quantitation of acute and chronic serum sickness in the rabbit. *J Exp Med* 134:s7–18, 1971
32. MANNIK M, STRIKER GE: Removal of glomerular deposits of immune complexes in mice by administration of excess antigen. *Lab Invest* 42:483–489, 1980
33. KLASSEN J, MILGROM FM, MCCLUSKEY RT: Studies of the antigens involved in an immunologic renal tubular lesion in rabbits. *Am J Pathol* 88:135–144, 1977
34. EDGINGTON TS, GLASSOCK RJ, DIXON FJ: Autologous immune complex pathogenesis of experimental allergic glomerulonephritis. *Science* 155:1432–1434, 1967
35. HOYER JR, SEILER MW: Pathophysiology of Tamm-Horsfall protein. *Kidney Int* 16:279–289, 1979
36. PRICE RG, SPIRO RG: Studies on the metabolism of the renal glomerular basement membrane. Turnover measurements in the rat with the use of radio-labeled amino acids. *J Biol Chem* 252:8597–8602, 1977
37. HANSON LA, FASTH A, JODAL U: Autoantibodies to Tamm-Horsfall protein, a tool for diagnosing the level of urinary-tract infection. *Lancet* 1:226–228, 1976