Isolation of the Target Antigen of Human Anti-Tubular Basement Membrane Antibody–associated Interstitial Nephritis

Michael D. Clayman, Lauren Michaud, Jan Brentjens, Guiseppe A. Andres, Nicholas A. Kefalides, and Eric G. Neilson
Renal Electrolyte and Connective Tissue Research Sections, Department of Medicine, University of Pennsylvania School of Medicine; University City Science Center, Philadelphia, Pennsylvania 19104; and Departments of Microbiology, Pathology and Medicine, State University of New York at Buffalo, Buffalo, New York 14214

Abstract

Using a monoclonal anti-tubular basement membrane antibody (αTBM-Ab) affinity column, we isolated from collagenase-solubilized human renal tissue (HSRTA) a predominantly 48,000-mol-wt moiety (H3M-1) which is selectively recognized by antisera from two patients with αTBM-Ab–associated interstitial nephritis (αTBM disease). Whereas both antisera had αTBM-Ab titers of 1:64–1:128 by immunofluorescence on tissue sections, their reactivity with H3M-1 in a solid-phase radioimmunoassay was demonstrable at dilutions up to 1:10,000. While these sera displayed some reactivity with pre-column HSRTA, this was markedly less than with H3M-1. HSRTA depleted of H3M-1 by passage over the αTBM-Ab affinity column was almost completely depleted of reactivity. Neither pooled normal human sera nor sera from patients with a variety of renal lesions not associated with αTBM-Ab (including interstitial nephritis and anti-glomerular basement membrane disease) were reactive with H3M-1. Both patient antisera containing αTBM-Ab were also highly reactive with R3M-1, the 48,000-mol-wt rabbit glycoprotein antigen of experimental αTBM disease. Furthermore, a competitive inhibition radioimmunoassay revealed that αTBM-Ab from rodents with experimental αTBM disease could inhibit 45–98% of the R3M-1 binding reactivity of patient antisera and 85% of the H3M-1 binding reactivity of patient antisera, thus suggesting paratypic cross-reactivity. We conclude, therefore, that tubular basement membrane target epitopes and their paratypic recognition are highly conserved among mammals.

Introduction

Interstitial nephritis represents an important group of kidney diseases which accounts for up to 25% of patients reaching end-stage renal failure (1). Because a mononuclear cell infiltrate is the typical histologic feature of this lesion and because anti-tubular basement membrane antibodies (αTBM-Ab)[1] are present in some cases, an autoimmune response is thought to play an important role in disease expression (2, 3). This view has been reinforced by studies in experimental interstitial nephritis associated with αTBM-Ab. This is a well-characterized model in which pathogenic autoimmune responses have been clearly documented in rodents (4–7). We have also recently isolated and characterized from rabbits the nephritogenic antigen used in this model (8). This current study provides new immunologic information on human interstitial nephritis associated with αTBM-Ab. Such an entity has been described in association with drugs (9), after renal transplantion (10), and in idiopathic form (11). The target antigen of the human autoimmune response has not been formally identified and the relationship of this human response to the model of experimental αTBM disease is not entirely clear.

We now report the isolation of this tubular antigen from human kidney using a relevant monoclonal αTBM-Ab affinity column. αTBM-Ab from two patients with αTBM disease specifically reacted with the isolated moiety from both human and rabbit tubular basement membrane. These human antibodies also share paratypic specificity with αTBM-Ab from rats and mice with experimental αTBM disease. Our findings establish two important links between human αTBM disease and its experimental model.

Methods

Antisera. We obtained antisera from two patients with αTBM disease. The first patient was a 36-yr-old white male who presented with end-stage renal failure. Extensive evaluation did not reveal an etiology and he was placed on chronic maintenance hemodialysis. 3 mo later he received a cadaveric kidney transplant which functioned well initially. 2 mo later he presented with signs of rejection, and a biopsy of the transplanted kidney was performed. The second patient was a 27-yr-old white female who presented with acute renal failure of unknown cause. Extensive evaluation was unrevealing. Hemodialysis was instituted and a kidney biopsy was performed. In both patients light microscopy revealed interstitial nephritis with mononuclear cell and polymorphonuclear leukocyte infiltration. The glomeruli were largely normal. Immunofluorescence demonstrated linear deposition of Ig and C3 along the tubular basement membrane, and there was no glomerular staining. In addition to these two patient antisera, serum was also obtained from normal volunteers and from seven patients with acute renal disease in the absence of demonstrable αTBM-Ab. These included two patients with primary interstitial nephritis, two patients with anti-glomerular basement membrane (αGBM) disease without αTBM-Ab, two patients with lupus nephritis, and one patient with membranous glomerulonephritis. αTBM-Ab from SJL mice and BN rats were eluted from kidneys of animals immunized to produce αTBM disease (6, 7).

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Address reprint requests to Dr. Clayman, Renal Electrolyte Section, 860 Gates Pavilion, Hospital of the University of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104.

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1. *Abbreviations used in this paper:* αTBM-Ab, anti-tubular basement membrane antibody or antibodies; GBM, glomerular basement membrane; H3M-1, moiety derived from HSRTA; HSRTA, human collagenase-solubilized renal tubular antigen; R3M-1, moiety derived from RSRTA; RSRTA, rabbit collagenase-solubilized renal tubular antigen; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBM(s), tubular basement membrane(s).

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Immunofluorescence analysis. Indirect immunofluorescence was performed with both antisera from patients with αTBM disease. The titer of circulating αTBM-Ab was assessed using cryostat sections of normal human kidney which were incubated with serial dilutions of serum. Normal human serum served as a negative control. Deposited Ig was visualized with a fluoresceinated anti-human IgG (Cappel Laboratories Inc., Cochransville, PA). Patient sera (at a 1:10 dilution) were tested on the following tissues: 6 normal human kidneys (obtained from accident victims); 48 human kidney biopsies, covering a wide spectrum of renal diseases and including two cases of nail-patella syndrome and one case of familial nephropathy (Alport's syndrome); 4 human skin biopsies; 6 human lung biopsies; normal monkey esophagus; and normal kidney tissue from C57BL mice, BN and LEW rats, guinea pigs, and NZW rabbits.

Preparation of renal tubular antigen. Rabbit and human renal tubular basement membranes (TBMs) were isolated by a differential sieving technique (5). Human kidneys, which were intended for transplantation but were deemed unusable for reasons of defective vascular anatomy, were obtained from cadaveric donors. Highly enriched basement membrane fragments were isolated, sonicated, lyophilized, and stored at −70°C. Solubilized tubular antigen (human-HSRTA; rabbit-RSRTA) was made from these lyophilized membranes using bacterial collagenase (CLS IV; Worthington, Division of Cooper Biomedical, Inc., Malvern, PA) in the presence of protease inhibitors (12).

Monoclonal antibody affinity chromatography. α3M-1 (αTBM-Ab) and α29M-1 (αTBM/αGBM-Ab) monoclonal antibodies have been described previously (8). These were originally obtained from mice immunized with rabbit renal tubular antigen. α3M-1 does not recognize glomerular basement membrane nor other renal structures. It stains the TBM of BN rats (a strain susceptible to the induction of experimental αTBM disease) but not that of LEW rats (a strain generally acknowledged to be lacking the target antigen of disease). α3M-1 also binds the TBM of normal human tissue. α29M-1 recognizes both TBM and glomerular basement membrane (GBM), and stains LEW rat kidneys. The preparation and use of an α3M-1 Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, NJ) immunoaffinity column has also been previously described in detail (8). A moiety was derived from RSRTA (R3M-1) and from HSRTA (H3M-1).

Gel electrophoresis and antigen elution. 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels were performed by the slab technique of Laemmli (13). The gel bands were developed with silver stain (14). In some experiments, after silver staining of end lanes, selected bands in central lanes were excised and incubated with 0.1% SDS for 72 h at 4°C with constant mixing. The eluate was collected, dialyzed extensively against distilled water, and lyophilized for future use.

Solid-phase radioimmunoassay. This methodology has been previously described in detail (8). In essence, the wells of a polyvinyl chloride microtiter plate were incubated with an antigen of interest, blocked, incubated with test antisera, and developed with the appropriate species-specific ^{125}I-anti-IgG. Competitive inhibition radioimmunoassays were performed by admixing serial dilutions of eluted kidney-bound antibodies (15) from SJL mice or BN rats with αTBM disease, or IgG from normal controls, with a dilution of human antisera which gave 50% of maximal binding reactivity. The reaction was then developed with ^{125}I-anti-human IgG.

Results

Immunofluorescent analysis of αTBM-Ab. Incubation of cryostat sections of normal human kidney with serum from either patient with αTBM disease resulted in the binding of IgG exclusively to the basement membrane of proximal tubules and of Bowman's capsule. These sera reacted with the TBMs of all normal and pathologic human kidney specimens. Binding to the GBM was never observed. Binding was also restricted to the kidney, as no reactivity was observed with basement membranes in human lung and skin or monkey esophagus. The αTBM-Ab of both patients also localized along the basement membrane of normal proximal tubules and Bowman's capsule in C57BL mice, BN rats, guinea pigs, and NZW rabbits. No binding, however, was observed when normal LEW rat kidney was used as the reaction substrate. All normal human sera as well as sera from renal patients without αTBM disease failed to bind the TBM.

Isolation of H3M-1 and its recognition by human αTBM antisera. In an initial screening, solid-phase radioimmunoassay antisera from both patients with αTBM disease displayed strong and specific reactivity with R3M-1, the rabbit antigen of experimental αTBM disease (Fig. 1 *A*). HSRTA was also passed over the α3M-1 immunoaffinity column. The eluate, H3M-1, was compared with R3M-1 for reactivity with the reference monoclonal α3M-1 in a solid-phase radioimmunoassay (Fig. 1 *B*). H3M-1 displayed ~30% of the reactivity with α3M-1 compared with R3M-1. SDS-PAGE (Fig. 2) revealed H3M-1 (lane *C*) to be comprised predominantly of a 48,000-mol-wt band, similar to R3M-1 (lane *B*). Collagenase (lane *A*) and pre-column HSRTA (lane *D*) are shown for comparison. The heterogeneous nature of HSRTA is evident.

Since the 3M-1 moiety denatures with Western blotting (data not shown), we demonstrated antibody recognition of electrophoresed material using elution techniques. The dominant band of H3M-1 was eluted in 0.1% SDS and assessed for reactivity with patient antisera containing αTBM antibodies in a solid-phase radioimmunoassay. Fig. 2 *B* demonstrates strong recognition of eluted material by patient antisera and minimal reactivity with normal human serum. Background activity of both sera against an irrelevant protein of approximately similar size (bovine serum albumin) was negligible. No measurable amounts of protein could be obtained from other areas of the H3M-1 gel

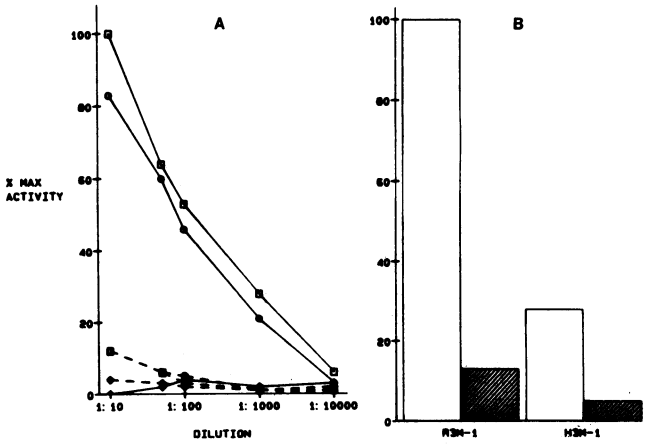

Figure 1. (*A*) Solid-phase radioimmunoassay of human αTBM antisera vs. R3M-1 and RSRTA. Wells were lined with R3M-1 (——) or RSRTA (- - -) and were reacted with serial dilutions of patient 1 (○), patient 2 (□), or normal human serum (◇). Raw data are figured as a percentage of maximum activity by dividing by the maximum counts per minute obtained in that assay. In this and other assays, reactivity of serum with bovine serum albumin (as a specificity control) is negligible and has been subtracted. Curves of patient 1 and patient 2 antisera against RSRTA are identical. (*B*) Recognition of H3M-1 by α3M-1 monoclonal antibody. Wells were lined with R3M-1 or H3M-1 and were reacted with a 1:100 dilution of α3M-1 (□) or α29M-1 (■) monoclonal antibody ascites (1.9 μg/well). Maximum counts per minute in *A* and *B* were 3,727 and 5,257, respectively.

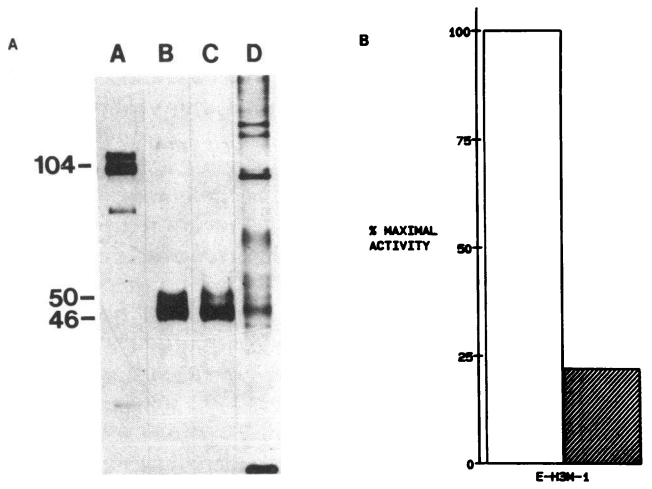

Figure 2. (*A*) SDS-PAGE gel of H3M-1: (lane *A*) bacterial collagenase; (lane *B*) R3M-1; (lane *C*) H3M-1; and (lane *D*) HSRTA. All are reduced. (*B*) Solid phase radioimmunoassay of the dominant band of H3M-1 eluted from an 8% SDS-PAGE gel. Wells were lined with the eluted material (E–H3M-1) and were reacted with an optimal dilution (1:500; see Fig. 3) of patient antisera containing αTBM antibodies (□), or normal human serum (▨). Maximum counts per minute in this assay were 11,203.

so that additional comparisons could not be made. Nevertheless, the dominant 48,000-mol-wt band of H3M-1 specifically reacted with patient antisera.

Sera from both patients were reactive with H3M-1 up to dilutions of 1:10,000 (Fig. 3 *A*). This compares with immunofluorescent titers of 1:64–1:128. Neither pooled normal human sera, nor individual sera from seven patients with acute renal disease without αTBM antibodies, displayed reactivity with H3M-1. The possibility that the human αTBM antisera might recognize determinants in HSRTA other than H3M-1 was then investigated. Fig. 3, *B* and *C* demonstrate that whereas reactivity is quite strong with H3M-1, binding to HSRTA is much less, and column filtrate is almost completely depleted of reactivity (compared with normal human sera). This suggests that H3M-1 is the relevant moiety in HSRTA recognized by both patient antisera.

Shared paratypic specificity between human and experimental animal αTBM antisera. In a solid-phase radioimmunoassay the ability of αTBM-Ab (IgG fraction) from experimental animals (immunized with rabbit tubular antigen) to inhibit binding of human αTBM antisera to R3M-1 and H3M-1 was examined (Fig. 4, *A* and *B*). αTBM-Ab eluted from the kidneys of both SJL mice and BN rats substantially inhibited the binding of human αTBM-Ab to H3M-1, as well as to R3M-1. Interestingly, with SJL αTBM-Ab, inhibition of binding to H3M-1 was greater than that to R3M-1. Maximal inhibition ranged from 45 to 98%, and normal mouse and rat IgG were not inhibitory.

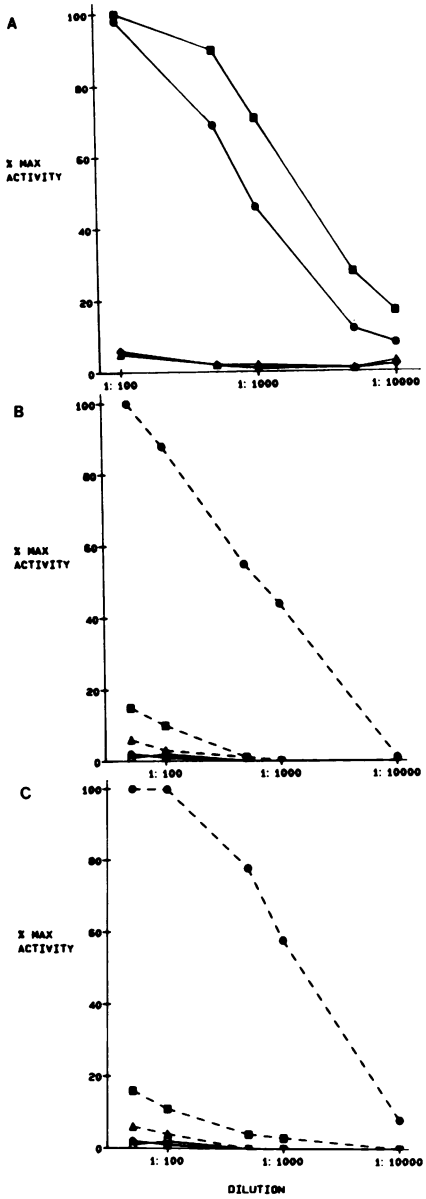

Figure 3. (*A*) Solid-phase radioimmunoassay of H3M-1. Wells were lined with H3M-1 and were reacted with serial dilutions of serum from patient 1 (●), serum from patient 2 (■), pooled normal human serum (▲), and serum from patients with acute renal disease without αTBM antibodies (♦). These included two patients with interstitial nephritis, two patients with αGBM disease, two patients with lupus nephritis, and one patient with membranous glomerulonephritis. Each of these sera were tested individually. Results are presented as pooled data since reactivity did not vary by more than 5% from patient to patient. (*B*) Solid-phase radioimmunoassay of patient 1 serum reactivity. Wells were lined with H3M-1 (●), HSRTA (■), or column filtrate (▲), and were reacted with serial dilutions of patient 1 serum (- - -) or normal human serum (——). (*C*) Solid-phase radioimmunoassay of patient 2 serum reactivity. Details of assay are as described in 2 *B*. Maximum counts per minute in A, B, and C were 20,310; 33,847; and 38,523, respectively.

Discussion

Implicit in the fine analysis of the immunopathogenesis of an autoimmune disease is the definition of the target antigen. We have recently isolated from a complex rabbit renal tubular antigen preparation the glycoprotein responsible for inducing αTBM disease in experimental animals (8). While a comparable disease entity is well described in patients, few data are currently available regarding the basic immunologic aspects of this renal lesion in humans, including the identification of the target antigen.

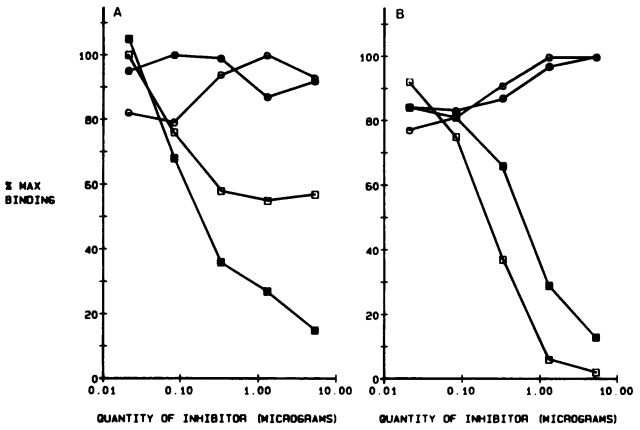

Figure 4. Competitive inhibition of human αTBM-Ab recognition of R3M-1 and H3M-1 by αTBM-Ab from experimental animals. Wells were lined with R3M-1 (open symbols) or H3M-1 (closed symbols) and were reacted with a dilution of patient 1 antisera which represented 50% of maximal binding activity (1:4000 vs. R3M-1; 1:2000 vs. H3M-1). (*A*) Increasing quantities of IgG from normal SJL mouse serum (●), pooled mouse IgG (○), or SJL αTBM-Ab (□, ■) were comixed with an equal volume of appropriately diluted patient 1 antisera and then added to individual R3M-1- or H3M-1-lined wells. Uninhibited counts per minute vs. R3M-1 and vs. H3M-1 were 2,841 and 6,713, respectively. (*B*) Increasing quantities of IgG from normal BN rat IgG (○, ●) or BN αTBM-Ab (□, ■) were used as competitive inhibitors. Uninhibited counts per minute vs. R3M-1 and vs. H3M-1 were 4,263 and 5,434, respectively.

The most detailed report which attempts to address this issue described a 70,000-mol-wt glycoprotein isolated from human TBM, and demonstrated that antisera from two patients with glomerulonephritis complicated by interstitial nephritis and linear αTBM immunofluorescence precipitated this moiety (16). Whereas normal human sera did not react to any significant degree, the majority of patients with αGBM disease who also had αTBM linear immunofluorescence also recognized the TBM moiety. The possibility that the isolated TBM antigen was partially cross-reactive with the GBM could not be entirely excluded. Further, while this antigen elicited an immune response in BN rats, its relationship, if any, to heterologous antigens classically used to induce experimental αTBM disease was not analyzed. In addition, the specificity of the αTBM antisera, determined as differential recognition of BN, but not LEW, rat TBM was not evaluated. Finally, this study did not formally address the possibility that moieties within the TBM preparation other than the 70,000 mol-wt antigen were recognizable by the αTBM antisera.

We have previously reported on the 48,000-mol-wt nephritogenic antigen (R3M-1) of experimental αTBM disease (8). This was isolated from a complex (>15 bands on SDS-PAGE) rabbit renal tubular antigen preparation usually used to induce disease. R3M-1 is a noncollagenous glycoprotein localized to the most lateral aspects of the TBM. The present study also used immunoaffinity chromatography to isolate from normal human renal tissue a similarly sized moiety (H3M-1) which is the target antigen of αTBM-Ab from patients with well documented αTBM disease. Although one patient developed disease after renal transplantation and the other did so spontaneously, both patients had almost identical reactivity with H3M-1 by solid-phase radioimmunoassay. Subtraction experiments demonstrated that the likely single recognition antigen in HSRTA is H3M-1. Neither pooled normal human sera, nor sera from seven patients with acute renal disease without αTBM antibodies recognized H3M-1. This latter group included two patients with primary interstitial nephritis and two patients with αGBM disease, indicating that an αH3M-1 response is not engendered nonspecifically as a part of interstitial nephritis and that H3M-1 is not cross-reactive with GBM determinants recognized by αGBM antisera. Several lines of evidence point to similarities between H3M-1 and R3M-1. First, not surprisingly, they are both recognized by the α3M-1 monoclonal antibody, suggesting R3M-1 shares at least one similar epitope with H3M-1. Second, patient antisera are highly reactive with both R3M-1 and H3M-1. Third, while the patient antisera stains TBM of BN rat kidney in linear fashion, they are negative on LEW rat kidney, a strain generally acknowledged to be lacking the nephritogenic antigen of experimental αTBM disease. Lastly, antisera from experimental animals immunized with rabbit renal tubular antigen to produce αTBM disease competitively inhibit the binding of human αTBM antibody to R3M-1 and H3M-1. It is intriguing that αTBM-Ab from SJL mice are more effective at inhibiting the binding of human αTBM-Ab to H3M-1 than to R3M-1. This suggests the possibility that human αTBM-Ab recognize some R3M-1 epitopes not seen by SJL antibodies. Alternatively, the human antibodies may recognize the same epitopes on R3M-1 that the mouse antibodies do, but the former antibodies do so with greater affinity.

It is, of course, impossible to state that H3M-1 is the target antigen of human αTBM disease in all patients. This must await the evaluation of additional αTBM-Ab. Our findings, nevertheless, document an important and rare relationship between a human autoimmune renal lesion and its experimental counterpart.

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References

1. Burton, B. T., and G. H. Hirschman. 1979. Demographic analysis: end stage renal disease and its treatment in the United States. *Clin. Nephrol.* 11:47–51.

2. McCluskey, R. T. 1983. Immunologically mediated tubulointerstitial nephritis. *Contemp. Issues Nephrol.* 10:121–149.

3. Brentjens, J. R., B. Noble, and G. A. Andres. 1982. Immunologically mediated lesions of kidney tubules and interstitium in laboratory animals and in man. *Springer Semin. Immunopathol.* 5:357–378.

4. Steblay, R. W., and U. Rudofsky. 1971. Renal tubular disease and autoantibodies against tubular basement membrane induced in guinea pigs. *J. Immunol.* 107:589–594.

5. Neilson, E. G., and S. M. Phillips. 1979. Cell-mediated immunity in interstitial nephritis. I. T lymphocyte systems in nephritic guinea pigs: the natural history and diversity of the immune response. *J. Immunol.* 123:2373–2380.

6. Neilson, E. G., and S. M. Phillips. 1982. Murine interstitial ne-

phritis. I. Analysis of disease susceptibility and its relationship to pleiomorphic gene products defining both immune-response genes and a restrictive requirement for cytotoxic T cells at H-2K. *J. Exp. Med.* 155: 1075–1085.

7. Neilson, E. G., D. L. Gasser, E. McCafferty, B. Zakheim, and S. M. Phillips. 1983. Polymorphism of genes involved in anti-tubular basement membrane disease in rats. *Immunogenetics.* 17:55–65.

8. Clayman, M. D., A. Martinez-Hernandez, L. Michaud, R. Alper, R. Mann, N. A. Kefalides, and E. G. Neilson. 1985. Isolation and characterization of the nephritogenic antigen producing anti-tubular basement membrane disease. *J. Exp. Med.* 161:290–305.

9. Border, W. A., D. H. Lehman, J. D. Egar, H. J. Sass, J. E. Glode, and C. B. Wilson. 1974. Anti-tubular basement membrane antibodies in methicillin associated interstitial nephritis. *N. Engl. J. Med.* 291:381–384.

10. Klassen, J., K. Kano, F. Milgrom, A. B. Menno, S. Anthone, R. Anthone, M. Sepulveda, C. M. Elwood, and G. A. Andres. 1973. Tubular lesions produced by autoantibodies to tubular basement membrane in human renal allografts. *Int. Arch. Allergy Appl. Immunol.* 45:675–689.

11. Bergstein, J., and N. Litman. 1975. Interstitial nephritis with anti-tubular-basement-membrane antibody. *N. Engl. J. Med.* 292:875–878.

12. Kefalides, N. A. 1972. The chemistry of antigenic components isolated from glomerular basement membrane. *Connect. Tissue Res.* 1: 3–13.

13. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

14. Morrisey, J. H. 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced sensitivity. *Anal. Biochem.* 117:307–310.

15. Neilson, E. G., E. McCafferty, S. M. Phillips, M. D. Clayman, and C. J. Kelly. 1984. Anti-idiotypic immunity in interstitial nephritis. II. Rats developing anti-tubular basement membrane disease fail to make an antiidiotypic regulatory response: the modulatory role of an $RT7.1^+$, $OX8^-$ suppressor T cell mechanism. *J. Exp. Med.* 159:1009–1026.

16. Graindorge, P. P., and P. R. Mahieu. 1978. Radioimmunologic method for detection of antitubular basement membrane antibodies. *Kidney Int.* 14:594–606.