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Antibodies to Saline Extractable Tissue Antigen in Sera of Patients with Renal Diseases[1] (42470)

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Abstract. Multiple serum samples originating from 110 renal allograft recipients were examined against saline extract of normal human kidney by means of double diffusion gel precipitation. Eleven recipients were found to be positive; 99 of 106 sera from these patients were positive. Pretransplantation sera were available from 7 of these recipients and 6 patients were found "positive." The precipitation reaction was composed of one line. Identity reactions were formed between the lines produced by sera from all patients except 1. Sera of patients from end-stage renal disease produced similar reaction; however, only 3 of 234 sera from patients with nonrenal diseases precipitated the kidney extract. None of 154 normal sera were positive. Several positive sera also were positive in complement fixation tests with human kidney extract. Evidence was presented that the antibodies under study combined with a nonorgan-specific but species-restricted tissue antigen. The hypothesis was advanced that these antibodies are autoantibodies formed in response to a sequestered antigen released as a result of tissue damage. Apparently, the antigen is released frequently in immunogenic form from injury to kidney but infrequently from injury to other organs.

The destruction of tissues may lead to the production of autoantibodies in response to the release of sequestered tissue antigens; such autoantibodies are frequently organ specific. Antibodies to thyroglobulin may be found in the sera of patients with hyperthyroidism, adenocarcinoma of the thyroid, and in patients who have undergone surgery on the thyroid (1). Antibodies to sperm or testicular antigens are detected in the sera of male patients after vasectomy, and after traumatic injury or inflammatory disease of the genital tract (2). Antibodies to cardiac antigens are found in the sera of a majority of patients who have undergone cardiac surgery and in a lesser percentage of patients who have experienced pathological or vascular injury to cardiac or pericardial tissues (3, 4). In addition to autosensitization due to disease or trauma, organ-specific autoantibodies have been elicited experimentally in animals through immunization with heterologous, homologous, or autologous lens, brain, thyroid, testicle, adrenal, or kidney (5).

The production of autoantibodies to non-organ-specific antigens in response to tissue destruction or immunization is also a well-established phenomenon, even if it has been studied less extensively than the formation of organ-specific autoantibodies. It has been proposed that the Wassermann antibodies associated with syphilis are produced as a result of stimulation by autologous cardiolipin released during the course of infection (5, 6). The autoantibodies to non-organ-specific connective tissue matrix antigens elicited during the course of mercuric chloride-induced glomerulonephritis in experimental animals are believed to result from stimulation by altered endogenous antigens released through the toxic effects of the heavy metal (7, 8).

Allografting has been shown to exert an adjuvant effect for the production of antibodies to weak antigens (9, 10). Furthermore, destruction of the graft during the process of rejection may result in the release of antigens shared by the donor and recipient eliciting the production of autoantibodies (11).

In this study, sera of recipients of renal allografts and patients with end-stage renal disease were examined for antibodies to saline-extractable antigens of normal human kidney to determine whether the destruction of kidney

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parenchyma mediated by processes of chronic renal disease and/or renal graft rejection resulted in the production of antibodies to tissue antigens.

Materials and Methods. *Saline extracts.* Human organs were obtained from autopsies at the Erie County Medical Center (Buffalo, NY). Bovine organs were obtained from a local slaughter house. Guinea pig and chicken organs were purchased from Pel-Freeze Biological, Inc. (Rogers, AR). The organs were rinsed in phosphate-buffered saline (PBS), pH 7.2. After adipose tissue and large vessels had been removed, the organs were minced using scissors. PBS was added to make a 20 or 40% (w/v) suspension of tissue. The suspension, submerged in an ice bath, was homogenized in a Sorvall Omnimixer for 3–4 min and sonicated using a sonifer cell disrupter (Heat Systems Ultrasonics, Inc., Plainview, NY) at 20,000 cps for four 30-sec intervals. The sonicated tissue preparation was then centrifuged at 30,000 rpm for 30 min in a Spinco No. 40 head in a Beckman Model L-2 ultracentrifuge. The recovered supernatant, referred to as 20 or 40% saline extract, was kept at −20°C until used.

Sera. Pre- and post-transplantation sera from renal allograft recipients were obtained from patients at The Buffalo General Hospital (Buffalo, NY). Sera from patients with end-stage renal disease were obtained from the Tissue Typing Laboratory of the Erie County Medical Center through the kind cooperation of Dr. D. Muirhead. Other pathological sera were from the collection of this laboratory. Normal human sera were supplied by Dr. R. M. Lambert of the American Red Cross Blood Service (Buffalo, NY). Additional normal human sera were obtained from healthy staff members and students of this department.

Serological procedures. Double diffusion gel precipitation (DDGP) tests employed in these studies were conducted in 0.6% Seakem ME agarose (FMC Corp., Rockland, MD) in 0.15 *M* NaCl pipetted into 9 × 50-mm plastic Petri dishes (Becton–Dickinson and Co., Oxnard, CA) to form an approximately 4-mm-thick layer. Wells with a diameter of 5 mm were cut in the solidified agarose with brass tubing. The distance between the wells was 3 mm. Wells were filled with sera or tissue extracts, which were allowed to diffuse for 2 hr at room temperature and then for 48–72 hr at 4°C.

Immunoelectrophoresis was performed according to the procedure outlined by Grabar and Burtin (12) employing a barbital acetate buffer system (pH 8.6) and 1.0% (w/v) Ionagar No. 2 (Difco Laboratories, Detroit, MI). Sera were electrophoresed at 50 mA for 2–4 hr and subsequently diffused against kidney extract or goat antiserum to whole human serum (Cappel Laboratories, Division of Cooper Biomedical, Inc., Cochrane, PA) for 24 hr at room temperature and an additional 24 hr at 4°C.

For the enhancement of precipitation lines to facilitate photography, slides were washed in several changes of 0.15 *M* NaCl and submerged in 6.0% (w/v) saline solution of polyethylene glycol (Carbowax PEG 6,000, Union Carbide Corp., South Charleston, WV) for 16 hr at 4°C. Additional enhancement of precipitation lines was accomplished by layering a goat antiserum to human γ globulins onto individual slides after the slides had been washed in several changes of saline. The goat antiserum was kindly supplied by Mr. P. Bronson of this department.

Complement fixation tests were performed according to the procedure routinely used in this laboratory (13), employing a triethanolamine buffer system (0.015 *M*, pH 7.2) and a 2% suspension of sheep red blood cells (RBC) sensitized with a subagglutinating dilution of rabbit anti-sheep hemolysin (Hyland, Division of Travenol Laboratories, Costa Mesa, CA). Guinea pig serum employed as a source of complement (Difco Laboratories, Detroit, MI) was used at a 1:20 dilution.

Immunofluorescence tests were performed in the laboratory of Dr. B. Albini of this department, using frozen sections of rabbit, human, and monkey kidney, and an FITC-conjugated goat antiserum to human IgG (Cappel Laboratories). The procedure outlined in (14) was followed.

Results. *Precipitins against kidney extract in sera of patients with renal diseases.* Multiple serum samples from each of 110 renal allograft recipients were examined against saline extracts of normal human kidney by means of DDGP tests. As shown in Table I, 99 of 106 sera originating from 11 recipients contained precipitins for kidney extract. In most "positive" cases, a single precipitation line was noted between serum and kidney extract. Over 500 sera originating from 99 recipients gave negative results. Pretransplantation sera from

TABLE I. DOUBLE DIFFUSION GEL PRECIPITATION TESTS: REACTIONS WITH KIDNEY EXTRACT OF SERA FROM "POSITIVE" RENAL GRAFT RECIPIENTS

Renal allograft patient	Number of positive sera/number of sera tested	
	Post-transplantation	Pre-transplantation
E.C.	10/14	1/3
K.F.	12/12	1/4
A.G.	1/1	NA[a]
D.G.	9/12	3/6
J.G.	11/11	0/3
J.H.	18/18	3/4
R.H.	7/7	NA
D.L.	12/12	NA
J.P.	5/5	NA
R.S.	6/6	3/3
L.Y.	8/8	3/3
Total	99/106	14/26

[a] Not available.

7 of the aforementioned 11 positive patients were available for testing in DDGP tests. As seen in Table I, 14 of 26 pretransplantation sera originating from 6 of the 7 patients tested were found to contain precipitins to kidney extract. In only 1 patient (J.G.) did the post-transplantation sera contain the precipitin, while the pretransplantation sera did not. Pre-transplantation sera from the 99 renal allograft recipients whose post-transplantation sera did not contain precipitins were not tested.

As seen in Fig. 1, precipitation lines produced by sera originating from different patients merged into reactions of identity when examined against kidney extract in DDGP tests. The notable exception was patient J.G. whose serum samples formed precipitation lines with kidney extract, but reactions of nonidentity[2] were noted with lines formed by sera from all other patients. When sequential serum samples, which were taken from the same patient over a period of several months, were tested against the kidney extract, identity reactions always were observed. Furthermore, when pre- and post-transplantation sera from any "positive" patients were simultaneously tested against kidney extract, reactions of identity were noted. It should also be pointed out that in observations made thus far, the position of precipitation lines did not indicate any major changes in antibody titers during the observation period.

Upon finding the precipitins in a pretransplantation sera of six renal allograft recipients, 119 serum samples obtained from 60 patients with end-stage renal disease were examined in DDGP tests against kidney extracts. In addition, sera from patients with various nonrenal diseases and sera from normal human subjects were examined. As summarized in Table II, none of the 154 normal sera and only 3 of 234 sera from patients with nonrenal diseases precipitated kidney extract. However, 22 of 119 sera originating from 11 of 60 patients with end-stage renal disease contained the precipitins. When sera from these patients were tested in DDGP along with precipitin-containing sera from renal allograft recipients, the precipitation lines formed with kidney extract merged into reactions of complete identity (Fig. 2A). Of the three "positive" sera obtained from patients with nonrenal diseases, only a serum of one of the patients (A.B.) from the group with head and neck tumors gave a strong enough reaction with kidney extract to permit further study. Interestingly, the precipitation line formed by this serum and by the serum of renal allograft recipient, J.H., formed a reaction of complete nonidentity (Fig. 2B).

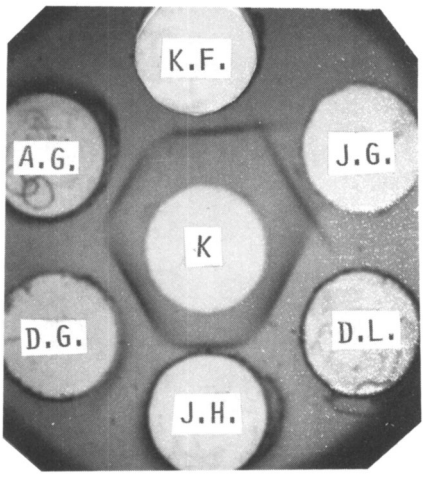

FIG. 1. Double diffusion gel precipitation. Sera from patients K.F., J.G., D.L., J.H., D.G., and A.G. were diffused against a 20% extract of human kidney (K).

[2] In reviewing the galley proof it was noted that the line formed by J.G. appears as partial identity reactions in Fig. 1. In the original photograph, weak but definite nonidentity reactions are discernible.

TABLE II. DOUBLE DIFFUSION GEL PRECIPITATION TESTS: REACTIONS OF PATHOLOGICAL AND NORMAL SERA WITH KIDNEY EXTRACT

Sera of patients with	No. of sera tested	No. of patients tested	No. positive sera/ No. positive patients
End-stage renal disease	119	60	22/11
Syphilis	35	35	0/0
Leprosy	43	43	0/0
Multiple sclerosis	30	30	0/0
Rheumatoid arthritis	36	36	1/1
G.I. carcinoma (CEA +)	16	16	0/0
Liver cirrhosis	9	9	0/0
Hodgkin's disease	15	15	0/0
Leukemia	19	19	1/1
Lymphoma	8	8	0/0
Head and neck tumor	23	23	1/1
Normal subjects	154	154	0/0

Selected sera from renal allograft recipients were electrophoresed in ionagar and diffused against a preparation of kidney extract. Representative results are shown in Fig. 3. Reaction lines formed between patient sera and kidney extract were noted in the γ globulin region corresponding to IgG.

Selected antibody-containing sera from renal allograft recipients and from patients with end-stage renal disease were examined in complement fixation tests against kidney extracts. As seen in Table III, definite positive reactions were noted with sera K.F., D.L., J.P., and N.M., and a weakly positive reaction with serum R.S. Sera J.H., R.W., and T.G. and the normal human control serum gave doubtful or negative results.

To determine whether the antibodies under investigation were directed toward alloantigens, patients' sera were reacted with extracts of individual human kidneys in DDGP. Lines of precipitation merged into reactions of identity as exemplified in Fig. 4. In this way sera of patients K.F., J.H., D.L., and L.Y. each were tested against eight individual kidney preparations and in all instances the lines merged into reactions of complete identity. This suggested that the antibodies under study were "panreactive" rather than "alloreactive."

Patients' sera which precipitated kidney extract were examined against saline extracts of various normal human organs in DDGP tests. As seen in Fig. 5, serum of patient J.H. precipitated not only kidney extract, but also extracts of human liver, heart, lung, and spleen. Precipitation lines formed with extracts of all these organs and with extract of kidney merged into reactions of complete identity. Similar

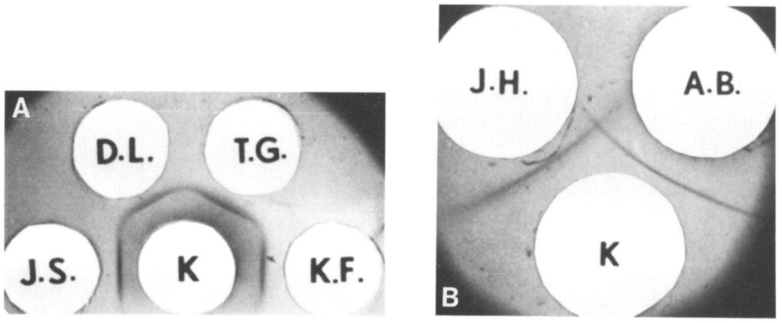

FIG. 2. Double diffusion gel precipitation. (A) Sera from renal allograft recipients D.L. and K.F. and sera from patients J.S. and T.G. with end-stage renal disease were tested against a 20% extract of human kidney (K). (B) Serum from renal allograft recipient J.H. and serum from patient A.B. with head and neck tumor were tested against a 20% extract of human kidney (K).

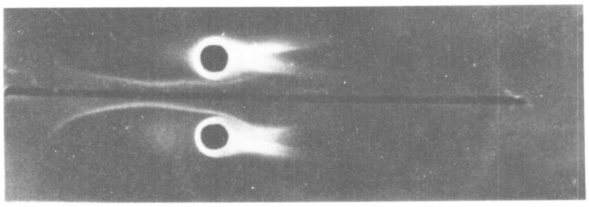

FIG. 3. Immunoelectrophoresis. Sera of renal allograft recipients K.F. and D.L. were electrophoresed from circular wells. The trough was filled with human kidney extract (K).

results were obtained with sera from other renal allograft recipients and patients with end-stage renal disease.

Selected antibody-containing sera were examined against extracts of kidney of chicken, guinea pig, and bovine origin as well as human kidney extracts in DDGP tests. Only some serum samples from patient J.G., but not from other renal allograft patients, reacted with chicken kidney extract. All patient sera tested reacted with guinea pig and bovine kidney extracts. In most instances, when patient sera were examined against human and nonhuman kidney extracts simultaneously, precipitation lines formed reactions of nonidentity or partial identity, as seen in Figs. 6A and B.

TABLE III. COMPLEMENT FIXATION TESTS: REACTIONS OF SERA FROM RENAL ALLOGRAFT RECIPIENTS AND PATIENTS WITH END-STAGE RENAL DISEASE WITH HUMAN KIDNEY EXTRACT

Patient	Serum dilution	Kidney extract at a dilution of 1:1	1:3	Diluent
K.F.[a]	1:5	++++[c]	++++	±
	1:15	+++	+++	−
D.L.[a]	1:5	++++	++++	−
	1:15	++++	++++	−
J.P.[a]	1:5	++++	++++	±
	1:15	++	+	−
R.S.[a]	1:5	++++	+	−
	1:15	+++	+	−
J.H.[a]	1.5	+	±	±
	1:15	+	±	−
R.W.[b]	1:5	±	±	−
	1:15	±	−	−
N.M.[b]	1:5	++++	++++	±
	1:15	++++	++++	−
T.G.	1:5	±	±	±
	1:15	±	−	−
Normal	1:5	+	±	−
	1:15	−	−	−
Diluent		±	−	−

[a] Allograft recipient.
[b] Patients with end-stage renal disease.
[c] Reactions were graded from − (complete hemolysis) to ++++ (no hemolysis).

Selected sera which contained the antibodies to kidney extract were examined for antibodies to glomerular basement membrane (GBM) and tubular basement membrane (TBM) antigens. No GBM or TBM staining was noted in indirect immunofluorescence tests using kidney sections of human, rabbit, or monkey origin.

Discussion. A precipitin which reacted with saline extract of normal human kidney was detected in the post-transplantation sera of 11 of 110 renal allograft recipients. This precipitin, however, was detected also in the pretransplantation sera from 6 of 7 patients tested and it was also found in the sera of 11 of 60 patients with end-stage renal disease. The precipitin was shown to be of the IgG class. Five of eight precipitin-containing sera studied reacted in complement fixation tests with kidney extracts, suggesting that in most cases the antibodies were capable of binding complement *in vitro*. The antibodies did not demonstrate any allospecificity in their reactions with individual human kidney extracts. They were not directed toward an organ-specific antigen in that they reacted also with extracts of human liver, spleen, heart, and lung. However, the antibodies under investigation did demonstrate a degree of species restriction, since they did not react with extract of chicken kidney and gave reactions of nonidentity or partial identity when examined simultaneously against human kidney extract and bovine or guinea pig kidney extract.

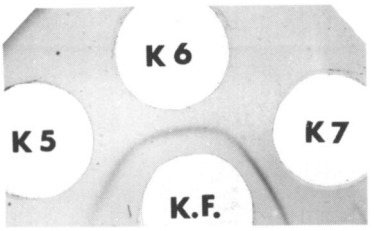

FIG. 4. Double diffusion gel precipitation. Serum of renal allograft recipient K.F. tested against 20% extracts of human kidneys from three donors, K5, K6, and K7.

Although actual autoreactivity of the antibodies to kidney extract was not demonstrated in this study because the patient's own kidney or other tissues were not available, there is a strong probability that the antibodies were autoantibodies, considering their panreactivity with all human kidney extracts tested. Wassermann antibody, directed towards a ubiquitous lipid antigen, is one of the few autoantibodies which have been known that are devoid of organ specificity (5). The antibodies examined in the present study resembled Wassermann antibody in that they were not organ specific or allospecific in their reactions. Unlike the Wassermann antibody, antibodies to kidney extract were species restricted in their reactions. In this respect, antibodies to kidney extract were more akin to the species-restricted autoantibodies described by Milgrom *et al.* (15) and by Centeno *et al.* (16). According to these authors, immunization of rabbits or guinea pigs with homologous or autologous kidney homogenates often elicited the production of non-organ-specific, species-restricted autoantibodies which were detected by DDGP and complement fixation tests employing saline extracts of various homologous and autologous tissues.

The production of antibodies to kidney extract was associated with chronic or end-stage renal disease. Considering the reactivity of these antibodies with extracts of various human organs, one might have anticipated that these antibodies would be found in the sera of patients with numerous disease states involving chronic destruction of tissues such as lepromatous leprosy, chronic liver disease, syphilis, and malignancies. This, however, was not the case in as much as the antibodies under investigation were noted virtually only in renal diseases. It may well be that even though the antigen in question is not restricted to kidneys, it is frequently released in immunogenic form when the kidney is involved in a pathological process, but hardly ever when an organ other than the kidney is involved. Some analogy with Wassermann antibodies cannot be overlooked, which also are directed against a non-organ-specific antigen, cardiolipin, and still virtually are restricted to syphilis, in which cardiolipin is apparently released in an immunogenic form from lesions.

There is no evidence for the role of antibodies to kidney extract in the pathogenesis of chronic renal disease or graft rejection. These antibodies initially were detected in the sera of patients with functioning renal allografts, suggesting that they were not binding actively to the kidneys *in vivo.* This observation, coupled with the fact that the antigen(s) is present in saline extracts of human kidneys, suggests that the antigen(s) is a cytoplasmic or intracellular antigen and that a rejection crisis or other processes of tissue damage resulting in local release of sequestered renal antigens is required before the antibodies would be capable of reacting *in vivo.* Additional studies are needed to determine the possible pathogenic or physiological role of antibodies to kidney extract. The purification and characterization of antigen(s) in kidney extracts with

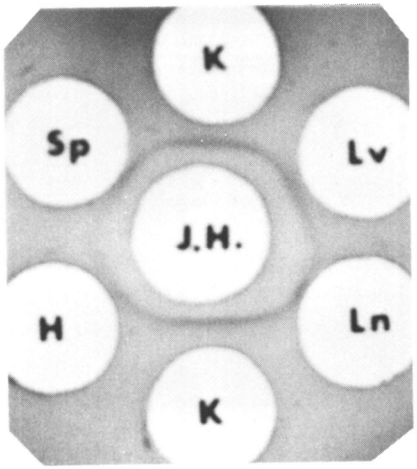

FIG. 5. Double diffusion gel precipitation. Serum of renal allograft recipient J.H. tested against 20% extracts of human kidney (K), spleen (Sp), heart (H), liver (Lv), and lung (Ln).

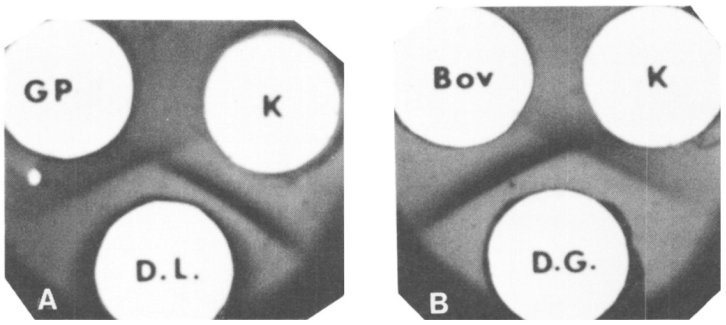

FIG. 6. Double diffusion gel precipitation. (A) Serum of renal allograft recipient D.L. was tested against extracts of human (K) and guinea pig (GP) kidneys. (B) Serum of renal allograft recipient D.G. was tested against extracts of human (K) and bovine (Bov) kidneys.

which the antibodies react will be necessary in order to understand the ramifications for the production of these antibodies.

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