In Vivo Detection of Deposition of Radiolabeled Lupus Antikidney Antibody and Its Inhibition by Soluble Antigen

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This investigation in an animal model was designed to test the feasibility of using radiolabeled lupus antikidney antibody to show renal deposition in vivo and the ability to block this deposition with a binding peptide. **Methods:** BALB/c mice received injections of radiolabeled murine anti-DNA antibody, antibody with no DNA binding capability, and DNA antibody simultaneously with blocking peptide. **Results:** Significantly higher renal deposition of anti-DNA antibody than of antibody without DNA binding capability occurred in the animals at 48 h after injection (5.21% of the injected dose per gram of tissue versus 2.5%, $P < 0.0004$) and at 7–8 d after injection (1.44% versus 0.20%, $P < 0.00004$). The simultaneous injection of blocking peptide with anti-DNA binding antibody significantly reduced the renal deposition of the anti-DNA antibody at 48 h (1.53%, $P < 0.00001$) and at 7–8 d (0.64%, $P < 0.0017$). **Conclusion:** This study showed the feasibility of using a radiolabeled antibody to evaluate deposition of anti-DNA antibody in the kidney and the successful use of a peptide to block antibody deposition—a strategy that may be useful for renal preservation in lupus. These data support the possibility of using antikidney-labeled antibodies to evaluate immunologic renal disease in vivo in humans.

Key Words: systemic lupus erythematosus; radiolabeled anti-DNA antibody; anti-DNA antibody–blocking peptide; immunologic renal disease

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Kidney disease will develop in approximately 70% of individuals with systemic lupus erythematosus (*1*). Kidney damage in lupus begins with the deposition of antibodies in glomeruli: the antibody specificity most associated with renal disease is directed at double-stranded DNA (*2*). Elevated serum titers of anti-DNA antibody are the best predictor of renal disease. Anti-DNA antibodies can be eluted from the kidneys of patients with lupus (*3*), and anti-DNA antibodies have been shown to deposit in vivo in the kidneys of rodents or ex vivo in renal perfusion studies (*4–8*).

Although the ultimate goal of lupus therapy is the elimination of autoreactivity, protecting the kidneys from immunoglobulin deposition may possibly decrease morbidity without altering the basic immune defect. One of us has previously shown that a peptide can mimic DNA and bind in the DNA binding site of a nephritogenic anti-DNA antibody (*9,10*). This peptide, prepared as a D isoform to minimize the in vivo cleavage of peptide bonds, was shown to inhibit the glomerular sequestration of a monoclonal anti-DNA antibody, suggesting that peptide inhibition of antigen binding activity might protect the kidneys of patients with SLE (*10*). In this study, we show the feasibility of using a radiolabeled antibody to determine if a particular peptide (or combination of peptides) will block antibody deposition in glomeruli.

MATERIALS AND METHODS

Kidney deposition of nephritogenic iodinated R4A antibody was examined after injection of an iodinated antibody into 7- to 9-wk-old female BALB/c mice (Albert Einstein College of Medicine or The Jackson Laboratory, Bar Harbor, ME). R4A is an immunoglobulin G2b (IgG2b) murine anti-DNA antibody and was obtained from a cell culture supernatant or from ascites (*11*). The antibody was purified on a G protein column (Gamma Bind; Pharmacia, Peapack, NJ). Isotype-matched IgG2b with no DNA binding (Sigma, St. Louis, MO) was used as a control antibody. Peptide DWEYS (blocking peptide) was purchased as a D isoform (Research Genetics, Huntsville, AL).

Forty-five mice (body weight range, 17–27 g) were studied. They were given food ad libitum, and iodinated drinking water was added 24 h before the study to block thyroid ^{125}I accumulation. R4A and control antibody were iodinated using the standard iodogenic method (yield, 35%–60%). Fifty to 100 μg DNA-binding R4A or control antibody in 0.1 mL phosphate-buffered saline were mixed with 60–100 μCi sodium ^{125}I and incubated in test tubes coated with 20 μg Iodo-Gen (Pierce Chemical Co., Rockford, IL) for 30–50 min at room temperature.

Iodinated R4A antibody and control antibody were fractionated by chromatography using a G-25M column (Sephadex; Pharmacia Fine Chemicals, Piscataway, NJ) after equilibration with phosphate-buffered saline. Eight fractions were collected. ^{125}I activity

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and antibody concentration in each fraction were determined using, respectively, a gamma counter (model CRC-12; Capintec, Inc., Ramsey, NJ) and a spectrophotometer at 280 μm. The three fractions with both highest ^{125}I activity and highest antibody concentration were pooled and used for this study. Labeling yields less than 40% were not used. The antibody was shown to maintain antigenic specificity after iodination.

Group 1 mice received injections of 3–7 μg ^{125}I R4A (4–6 μCi) through the tail vein. The R4A antibody is an IgG2b anti-DNA antibody that has previously been shown to bind to renal glomeruli in vivo (*6,8,9*). Group 2 mice simultaneously received tail-vein injections of 10–19 μg ^{125}I R4A (2–5 μCi) and 100–240 μg peptide with the sequence DWEYS (blocking peptide). Group 3 mice received injections of 5–10 μg ^{125}I R4A (2–8 μg) iodinated control antibody. R4A deposition in the kidney was measured 48 h and 8 d after administration. The animals were anesthetized with 0.1 mg in 0.1 mL Nembutal sodium solution (Abbott Laboratories, North Chicago, IL) by intramuscular injection and were then exsanguinated before removal of organs to minimize the effect on organ counting rates. The percentage of the dose accumulated in kidney, spleen, liver, and lung was determined in a well scintillation counter (CompuGamma CS; LKB Walloc, Turkee, Finland). All analyses for ^{125}I tissue concentration were expressed as mean ± SE of percentage injected dose per gram of tissue. Statistical analysis was performed using *t* test comparisons of sample means and SE analysis. The study was approved by the Animal Institute Care and Use Committee of the Albert Einstein College of Medicine.

RESULTS

As seen in Table 1, mice given R4A retained 5.21% of the injected dose per gram of tissue antibody in the kidneys after 48 h, whereas mice given control antibody retained only 2.50% ($P < 0.0004$) of the injected antibody in the kidneys. When mice were given R4A antibody and DWEYS peptide that was bound by the antibody, they retained 1.53% ($P < 0.00001$) of the injected antibody in the kidneys at 48 h. The amount of antibody in the total blood volume after 48 h was 22.31% in mice given R4A, 10.69% ($P < 0.030$) in mice given control antibody, and 3.42% ($P < 0.0005$) in mice given both R4A antibody and peptide.

TABLE 1
Percentage Injected Dose per Gram of Tissue
48 Hours After Injection

Organ	Index	R4A (n = 7, 14 kidneys)	R4A + blocking peptide (n = 11, 22 kidneys)	Control antibody (n = 6, 12 kidneys)
Spleen	Mean	0.716	0.123	0.278
	SD	0.333	0.036	0.066
	SE	0.126	0.012	0.027
Liver	Mean	5.805	1.792	5.068
	SD	2.272	0.664	1.148
	SE	0.859	0.200	0.469
Lung	Mean	7.521	7.904	0.468
	SD	2.020	5.024	0.056
	SE	0.764	1.515	0.023
Kidney	Mean	5.207	1.528	2.500
	SD	1.171	0.516	0.308
	SE	0.443	0.156	0.126

R4A = nephritogenic antibody.

TABLE 2
Percentage Injected Dose per Gram of Tissue
7–8 Days After Injection

Organ	Index	R4A (n = 9, 18 kidneys)	R4A + blocking peptide (n = 6, 12 kidneys)	Control antibody (n = 6, 12 kidneys)
Spleen	Mean	0.267	0.031	0.024
	SD	0.211	0.008	0.009
	SE	0.070	0.003	0.000
Liver	Mean	1.788	1.207	0.350
	SD	0.463	0.410	0.154
	SE	0.154	0.167	0.063
Lung	Mean	1.736	0.155	0.336
	SD	1.491	0.095	0.126
	SE	0.497	0.039	0.052
Kidney	Mean	1.440	0.637	0.201
	SD	0.459	0.085	0.075
	SE	0.153	0.035	0.030

R4A = nephritogenic antibody.

Seven to 8 d after injection (Table 2), R4A antibody was still sequestered in the kidneys (1.44% of the injected dose) compared with control antibody (0.20% of the injected dose, $P < 0.00004$) and compared with R4A antibody plus peptide (0.64% of the injected dose, $P < 0.0017$). Likewise, R4A in whole blood was greater (6.19%) than control antibody (1.14%, $P < 0.0011$) and R4A plus peptide (0.40%, $P < 0.003$).

DISCUSSION

These studies show that the sequestration of an anti-DNA antibody in renal tissue can be detected using iodinated antibody. Antigenic peptide will inhibit renal sequestration of antibody. These data suggest the feasibility of using antigen blockade therapeutically to protect kidneys from antibody-mediated damage and to evaluate that effect using nuclear medicine techniques. It seems highly probable that imaging technology can detect potentially pathogenic anti-DNA antibodies and can determine if inhibitors successfully prevent renal sequestration. Furthermore, it is reasonable to expect that nuclear imaging can monitor this intervention. Noninvasive monitoring will be critical if peptide inhibition becomes a therapeutic modality, because the amount of peptide needed to provide blockade will differ among patients.

CONCLUSION

Although most lupus patients with renal disease display high serum titers of anti-DNA antibodies, some patients in whom nephritis develops have no detectable anti-DNA activity. We are now able to ask whether imaging renal deposition of serum immunoglobulin from such individuals can help identify their risk for renal disease and permit early therapy. Although little attention has been directed at the kidney in immunoimaging, these data show the potential of this approach in renal nuclear imaging.

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