Nuclear Localization of Immunoglobulins in Renal Biopsies of Patients with Lupus Nephritis

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Immunofluorescent evaluation of renal biopsies from 19 patients with lupus nephritis revealed nuclear localization of immunoglobulins (IgG and IgM) in 6 patients. Homogeneous, nuclear rim and speckled patterns of nuclear localization were observed. The extent of localization varied, with only occasional nuclei fluorescing in 1 case, whereas approximately 50% of the nuclei exhibited fluorescence in the most extreme case. The phenomenon of nuclear localization of immunoglobulins was not observed in immunofluorescent studies of 225 renal biopsies from patients with conditions other than lupus nephritis. The possibility that nuclear localization of immunoglobulins occurred artifactually in the 6 patients was considered and was discounted by determining antinuclear antibody titers on serum obtained concurrently with the renal biopsy. Nuclear localization was not confined to areas of histologically evident parenchymal destruction, indicating that antinuclear antibodies do not react only with nuclear antigens after tissue breakdown, but may gain access to intracellular antigens prior to cell dissolution. (Am J Pathol 68:469-478, 1972).

INDIRECT IMMUNOFLUORESCENT TESTS employing tissue substrates as a source of nuclear antigen, patient’s serum and antihuman immunoglobulins have become a well-recognized method for detecting antinuclear antibodies in patients with suspected systemic lupus erythematosus. Using this technic, three distinct patterns of nuclear localization (homogeneous, nuclear rim and speckled) have been characterized, and these patterns have been correlated with antibody activity to specific nuclear materials.1-2 In spite of this characterization, the exact significance of these antibodies in the pathogenesis of systemic lupus erythematosus remains unclear.3 Most studies in which direct immunofluorescent technics were used to evaluate immune complex lesions in renal tissue from patients with lupus nephritis have not described nuclear localization of immunoglobulins in the kidney.4-8 Indeed, it is held that no such nuclear localization occurs in the kidney9 or in other organs.10,11 This negative find-
ing has supported the concept that antinuclear antibodies do not bind in vivo with nuclear material of intact cells but only react with products of tissue breakdown to produce nuclear antigen-antibody complexes.12

The present report describes nuclear localization of immunoglobulins in renal biopsies from 6 of 19 patients with lupus nephritis studied by immunofluorescent technics at the Duke University Medical Center from August 1970 through January 1972. Serum from each of the patients, obtained concurrently with the biopsy, was evaluated for the presence and titer of antinuclear antibodies. The results were then correlated with nuclear localization of immunoglobulins in renal biopsies.

Materials and Methods

Immunofluorescent Studies of Renal Biopsies

Tissue from each of the 19 patients was obtained by percutaneous needle biopsy. The tissue specimens were divided into three portions: one for light microscopy, one for electron microscopy and one for immunofluorescent microscopy. Tissue for immunofluorescent microscopy was immediately frozen, unfixed, at −70°C in a gelatin matrix. Sections for immunofluorescent evaluation were cut at 4 μm on an International Harris cryostat. One set of slides was then air-dried, fixed for 5 minutes in acetone and washed twice in phosphate-buffered saline. These sections were then incubated for 30 minutes with fluorescein-labeled antibody preparations, washed again in phosphate-buffered saline and mounted under glass coverslips with 10% glycerin. A second set of sections was washed for 5 minutes in phosphate-buffered saline immediately after they had been cut and before they were fixed in acetone. This set was then incubated with antibody preparations and subsequently treated in the same manner as the first set. Sections were examined with a Zeiss Photoscope I equipped with an HBO-200W mercury vapor light source, UG-1 exciter filter and a K-490 barrier filter. Specimens were photographed with high speed Ektachrome film (ASA 160). The coordinates of photographed fields were recorded, and the coverslips floated from sections in phosphate-buffered saline. Sections were dehydrated in ether-alcohol, fixed in Zenker's solution and stained with hematoxylin and eosin. Nuclei were identified in the hematoxylin and eosin sections and compared with sites of labeled immunoglobulin localization in the same field.

Antibody preparations used in the immunofluorescent evaluations included goat or rabbit antihuman IgG, IgM, IgA, β1C-globulin, fibrinogen and albumin, which were tested for monospecificity by immunoelectrophoresis. Sodium sulfate-precipitated immunoglobulin fractions were labeled with fluorescein isothiocyanate by the method of Marshall et al.13 Control preparations for the immunofluorescent studies consisted of: a) conjugated antibody preparations absorbed with specific antigen prior to incubation with tissue sections and b) tissue sections incubated with unlabeled antibodies before incubation with respective labeled antibody preparations. The former control procedure usually completely abolished, while the latter only moderately diminished specific fluorescence.
Serum Antinuclear Antibody Titers
The indirect immunofluorescent technic was used to determine antinuclear antibody titers on each patient’s serum. Nuclear antigen substrate consisted of fresh rat kidney snap-frozen at −70°C and sectioned at 4 μm on an International Harris cryostat. Sections were fixed in acetone for 5 minutes, according to Tan’s method¹ and then incubated for 30 minutes with serial dilutions of each patient’s serum. Sections were washed in phosphate-buffered saline, incubated with goat antihuman IgG for 30 minutes, washed again in phosphate-buffered saline and mounted under glass coverslips with 10% glycerin. Specimens were examined and photographed with the equipment described above. The pattern of antinuclear antibody localization was recorded; the titer was reported as the highest dilution in which nuclear localization was observed.

Serum Anti-DNA Antibody Levels
Serum levels of antibody to DNA were determined by the nitrocellulose membrane filter method.¹⁴

Results
Features of the renal histopathology, pattern and class of nuclear immunoglobulin localization and results of serum antibody studies are given in Table 1, for each of the 6 patients in whom renal biopsy revealed nuclear localization of immunoglobulins.

Glomerular histopathology was varied. Membranous and proliferative glomerulonephritis, the latter in both a focal and diffused pattern, was observed in the 6 patients who exhibited nuclear localization of immunoglobulins. All biopsies contained some degree of parenchymal destruction, with either focal or diffuse interstitial inflammatory cell infiltrates, interstitial fibrosis and tubular destruction. Sites of nuclear localization of immunoglobulins did not always coincide with these areas of parenchymal destruction, however. In each of the 6 patients, the pattern of nuclear immunoglobulin localization in the biopsy was the same as the pattern observed by indirect immunofluorescent tests using patient’s serum and rat kidney tissue as substrate. Speckled and homogenous patterns of nuclear antihuman immunoglobulin localization occurred most commonly in renal biopsies. In one case, however, a membranous or nuclear rim pattern was observed. Localization of antihuman immunoglobulins was observed in nuclei of glomerular visceral and parietal epithelium, tubular epithelium and interstitial cells (Figures 1–4). Antihuman IgG and IgM localized in the nuclei in 4 patients (CO, EE, AT, and BM), while 2 (LM and DH) demonstrated antihuman IgG localization only. There was no detectable localization of antihuman IgA, β1C-globulin, fibrinogen or albumin in nuclei of any of the 6 patients. The extent of nuclear localization in each case
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Race</th>
<th>Type of glomerular lesion</th>
<th>Degree of parenchymal destruction*</th>
<th>Pattern of nuclear localization and class of immunoglobulin in renal biopsy</th>
<th>Pattern of indirect serum nuclear localization</th>
<th>Serum antinuclear antibody levels¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>27 N F</td>
<td></td>
<td>Membranous glomerulonephritis</td>
<td>+ Focal</td>
<td>Speckled IgG, IgM</td>
<td>Speckled</td>
<td>2 µg/ml anti-DNA, 1:4 antinuclear antibody</td>
</tr>
<tr>
<td>EE</td>
<td>34 N F</td>
<td></td>
<td>Diffuse proliferative glomerulonephritis</td>
<td>+++ Focal</td>
<td>Homogenous and speckled IgG, IgM</td>
<td>Homogenous and speckled</td>
<td>0 µg/ml anti-DNA, 1:256 antinuclear antibody</td>
</tr>
<tr>
<td>AT</td>
<td>22 N F</td>
<td></td>
<td>Focal proliferative glomerulonephritis</td>
<td>++ Focal</td>
<td>Membranous IgG, IgM</td>
<td>Membranous</td>
<td>600 µg/ml anti-DNA, 1:1024 antinuclear antibody</td>
</tr>
<tr>
<td>BM</td>
<td>29 N F</td>
<td></td>
<td>Membranous glomerulonephritis</td>
<td>++ Diffuse</td>
<td>Speckled IgG, IgM</td>
<td>Speckled</td>
<td>10 µg/ml anti-DNA, 1:256 antinuclear antibody</td>
</tr>
<tr>
<td>LM</td>
<td>34 N F</td>
<td></td>
<td>Focal proliferative glomerulonephritis</td>
<td>+ Focal</td>
<td>Speckled and homogenous IgG</td>
<td>Speckled and homogenous</td>
<td>7.5 µg/ml anti-DNA, 1:128 antinuclear antibody</td>
</tr>
<tr>
<td>DH</td>
<td>39 W F</td>
<td></td>
<td>Membranous glomerulonephritis</td>
<td>+ Focal</td>
<td>Speckled IgG</td>
<td>Negative¹</td>
<td>0 µg/ml anti-DNA, 0 antinuclear antibody¹</td>
</tr>
</tbody>
</table>

* Graded 1-4+  
¹ Negative at biopsy, antinuclear antibody titer was 1:8; speckled pattern on followup 3 weeks postbiopsy  
² Normal 0 6 µg/ml, see text
was variable. In 2 patients (CO and DH), only occasional nuclei in focal areas showed antihuman immunoglobulin localization (Figures 1, 4), while, at the other extreme, approximately half of all nuclei in the biopsy from patient EE revealed localization of antihuman IgG and IgM (Figures 2, 3).

Antinuclear antibody titers in patient's serum at the time of renal biopsy varied from 0 to 1:1024. Anti-DNA levels varied from 0 to 1400 μg/ml (normal level: 0 to 6 μg/ml). Negative tests for serum antibody were obtained in the sixth patient, DH, who presented with Raynaud's syndrome, proteinuria and hematuria. Lupus erythematosus cell preparations, antinuclear antibody determinations and anti-NDA determination were negative in initial tests. A renal biopsy revealed membranous glomerulonephritis with immunofluorescent findings of antihuman IgG and β1C-globulin localization in a granular pattern along glomerular capillary walls and antihuman IgG localization in a speckled pattern in nuclei. Subsequent examination of the renal biopsy by electron microscopy confirmed the membranous glomerular changes and revealed virus-like structures in the endoplasmic reticulum of glomerular endothelial cells identical to those described in lupus nephritis. The antinuclear antibody titer, 3 weeks after the biopsy, was 1:8.

During the period in which these cases were observed (August 1970 through January 1972), immunofluorescent studies were performed on 225 renal biopsies from patients in whom there was no clinical diagnosis or suggestive evidence of systemic lupus erythematosus. Histopathologic diagnoses in that group included proliferative glomerulonephritis, membranous glomerulonephritis, membranoproliferative glomerulonephritis, focal glomerulonephritis, chronic glomerulonephritis, arteriolar nephrosclerosis, amyloidosis, diabetic glomerulosclerosis and chronic pyelonephritis. Nuclear localization of immunoglobulins was not observed in any of those cases which were processed with initial acetone fixation prior to washing with phosphate-buffered saline.

Discussion

Although localization of immunoglobulins has been observed in nuclei of nonkeratotic cells in skin biopsies from patients with systemic lupus erythematosus, reports of nuclear immunoglobulin localization in kidney are almost nonexistent. In immunofluorescent studies utilizing autopsy kidney specimens, Paronetto and Koffler reported nuclear localization in 3 of 16 patients with systemic lupus erythematosus.
Rapp, on the other hand, working with HeLa cells in tissue culture, concluded that antinuclear antibodies did not penetrate the viable cell. The question thus arises, in the patients described by Paronetto and Koffler, whether nuclear localization may have occurred during the postmortem period, with antinuclear antibodies diffusing from serum and localizing in nuclei as autolysis occurred.

In the present study, biopsies from 4 patients exhibited nuclear localization of antihuman IgG and IgM, while nuclear localization of only antihuman IgG occurred in biopsies from 2 patients. Nuclear localization of other serum components, specifically IgA, β1C-globulin, fibrinogen or albumin was not observed in any of the biopsies. In each instance, the pattern of nuclear localization in the kidney was the same as that observed by indirect immunofluorescent antinuclear antibody tests using the patient's serum.

The possibility must be considered that nuclear localization observed in renal biopsies in this report was artificial. It is conceivable that in patients possessing high titers of antinuclear antibodies, these might diffuse from the vascular compartment of the biopsy, during sectioning and washing procedures, and localize in nuclei artificially at the time of processing. To evaluate this possibility, two methods of processing were used in each case. In the first method, sections were fixed in acetone for 5 minutes immediately after they were cut and before they were incubated with specific antibody preparations or washed. The initial acetone treatment in this technic was designed to fix serum proteins in the section and prevent leaching of nuclear antigens during subsequent rinsing procedures after incubation with antibody preparations. In the second method the cut sections were washed in phosphate-buffered saline for 5 minutes immediately after the tissue was sectioned before it was fixed in acetone or incubated with the specific antibody preparations. In this latter technic, the sections were rinsed in order to wash unbound γ-globulin from the specimen. Rinsing was confined to 5 minutes, as recommended by Tan, to minimize leaching of saline-soluble components, such as DNA and phosphate-extractable Sm antigen from nuclei. There was no significant difference in localization with either method.

The strongest evidence that this nuclear staining represents true in vivo and not artificial immunoglobulin localization which occurred during sectioning is derived from serum antinuclear antibody titers obtained at the time of renal biopsy. The antinuclear antibody titers varied from a low of 1:4 to a high of 1:1024 in 5 patients. However, in the sixth patient, DH, serum antinuclear antibody and
anti-DNA antibody determinations were both negative at the time of renal biopsy. The latter finding would seem to suggest that nuclear localization did not occur during the processing procedure, since there was no measurable serum antibody to diffuse from the vascular compartment of the biopsy.

Since nuclear localization was not confined to areas of histologically evident parenchymal destruction, the above findings suggest that antinuclear antibodies not only react with nuclear antigens released during tissue breakdown, but may gain access to nuclei in the kidney before the cell disintegrates. The mechanism by which this phenomenon occurs remains to be elucidated. Since there is little evidence to suggest that immunoglobulins normally penetrate the intact cell, an alternate hypothesis is proposed. Nuclear localization of antinuclear immunoglobulins may occur in cells injured sublethally during the progressive parenchymal destruction which accompanies immune complex nephritis. This nuclear localization in injured cells could result either from antinuclear immunoglobulins which have been actively reabsorbed from the glomerular filtrate by epithelial cells or from immunoglobulins diffusing from the vascular and interstitial compartments through cell membranes that have lost their functional integrity.

Whatever the mechanism, the phenomenon of immunoglobulin nuclear localization in renal biopsies should be appreciated and anticipated by those performing immunofluorescent studies, since it might prove helpful in establishing the diagnosis of systemic lupus erythematosus in previously unconfirmed cases.

References

6. Burkholder PM: Complement fixation in diseased tissues. II. Fixation of

Acknowledgments
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Fig 1—Biopsy from patient DH revealing antihuman IgG localization in a diffuse granular pattern along glomerular capillary walls, in a speckled pattern in two nuclei of proximal tubular epithelium (arrows, upper left) and in nuclei of visceral epithelial cells of the glomerulus (arrows, right) (x 475). Fig 2—Biopsy from patient EE revealing localization of antihuman IgG in a speckled and homogenous pattern in a large portion of tubular epithelial nuclei (x 315). Fig 3—Biopsy from patient EE showing localization of antihuman IgM in a speckled and homogenous pattern in tubular epithelial nuclei (x 315). Fig 4—Biopsy from patient CO revealing antihuman IgG localization in a diffuse, confluent granular pattern along the glomerular capillary walls and localization in a speckled pattern in occasional nuclei of tubular epithelial cells (x 315).
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