Selective Deposition of Immunoglobulin A₁ in Immunoglobulin A Nephropathy, Anaphylactoid Purpura Nephritis, and Systemic Lupus Erythematosus

M. E. Conley, M. D. Cooper, and A. F. Michael, Cellular Immunobiology Unit, Tumor Institute, Departments of Pediatrics and Microbiology, and The Comprehensive Cancer Center, University of Alabama in Birmingham, Birmingham, Alabama 35294; Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455.

Abstract To further characterize the IgA deposits found in glomeruli of patients with IgA nephropathy, anaphylactoid purpura nephritis, and systemic lupus erythematosus, renal biopsies from patients with these disorders were stained by immunofluorescence with monoclonal anti-IgA subclass reagents, anti-light chain reagents, and anti-J chain. The mesangium and pericapillary capillaries were brightly stained for IgA₁ and were negative for IgA₂. IgA₁ and, to a lesser extent, IgA₂ were contained in tubular casts. Both kappa and lambda light chains were found in all deposits. The intensity of J chain staining correlated with the intensity of IgM and not IgA staining. Biopsies brightly stained for IgA but negative for IgM were negative for J chain. These results indicate that glomerular IgA deposits in these disorders consist predominantly of monomers of IgA₁.

Introduction

IgA nephropathy is characterized by IgA deposits in the renal mesangium and recurring episodes of hematuria (1–3). It occurs most frequently in adolescent boys and young men (2) and is frequently preceded by an episode of upper respiratory or gastrointestinal infection of presumed viral etiology (1, 3).

Deposition of IgA within the glomerulus has also been observed in patients with systemic lupus erythematosus (SLE) (4), liver disease (5), and anaphylactoid or Henoch-Schönlein purpura (HSP) nephritis (6). The latter disorder is also more commonly observed in males and is frequently preceded by a viral infection (7).

The mechanism(s) responsible for the deposition of IgA in these disorders is unclear. IgA immune complexes have been found in the serum of patients with HSP (8) and elevated levels of IgA have been noted in the serum of patients with HSP (9) and IgA nephropathy (3). The antigenic specificity of this IgA is unknown. When the IgA was eluted from kidneys of patients with IgA nephropathy, one eluate showed weak binding of intraglomerular components of a normal kidney, the other three failed to bind (2).

The presence of glomerular deposits of properdin (10) and C3 (1–3) in IgA nephropathy often unassociated with Cl and C4 suggest participation of the alternative complement pathway although the precise role of complement activation in this syndrome is unknown. In a murine model of IgA nephropathy, complement depletion by cobra venom factor did not influence the IgA deposition (11).

In humans there are several important differences between serum IgA and secretory IgA. 90% of the serum IgA is monomeric, whereas most of the secretory IgA is made up of dimers composed of two monomers of IgA, a J chain and secretory component. In addition, IgA₂ constitutes 10–20% of the serum IgA but makes up 40–60% of the secretory IgA (12). Because IgA nephropathy and HSP often follow infections of the mucosal surfaces, it appeared possible that renal deposition of IgA might be the result of increased levels of IgA₂ or dimeric IgA of either subclass in the serum. To explore these possibilities we have used indirect immunofluorescence to determine the subclass of IgA in glomerular deposits and to search for the presence of J chains.

Address reprint requests to Dr. Conley, Children's Hospital of Philadelphia, Pa.

Received for publication 28 August 1980 and in revised form 6 October 1980.

*Abbreviations used in this paper: HSP, Henoch-Schönlein purpura; SLE, systemic lupus erythematosus.
METHODS

Patients. All patients included in this study were treated at the affiliated hospitals of the University of Minnesota between 1974 and 1980. The diagnosis of IgA nephropathy or HSP was made on the basis of clinical and histopathological findings. The group with IgA nephropathy included two females and eight males with ages ranging from 6 to 33 yr. Five of the patients with HSP were females and six were males. Their ages ranged from 5 to 47 yr. The nine patients with SLE were included in this study because immunofluorescent staining of their renal biopsies demonstrated IgA deposits. In contrast to the IgA nephropathy, and HSP patients’ biopsies where IgM and IgG staining was normally faint or absent, the SLE biopsies consistently showed substantial staining for IgM and IgG. The SLE patients were selected from ~50 patients with SLE biopsied between 1974 and 1980. Intestinal tissue was obtained by biopsy during diagnostic evaluation, the tonsilar tissue was obtained after therapeutic tonsilectomy.

Antibodies. Monoclonal hybridoma anti-IgA subclass antibodies were prepared by described methods (13). Briefly, lymphocytes from the lymph nodes of mice immunized with purified IgA myelomas were fused to tumor cells from the HAT sensitive, nonproducing myeloma line P815Ag8653 (14). IgA subclass specific hybridomas were detected by an enzyme-linked immunosorbent assay. The anti-IgA\textsubscript{1} designated clone 1-155-1, is a mouse y3\textalpha of BALB/c allotype. The anti-IgA\textsubscript{2}, designated as 14-3-26, is a mouse y2B\textkappa of A\textalpha allotype. These monoclonal antibodies have been shown to be IgA subclass specific by several techniques. In an enzyme-linked immunosorbent assay on 37 IgA myelomas, 30 IgA\textsubscript{1}, and 7 IgA\textsubscript{2} including both Am2\textsuperscript{*} and Am2\textsuperscript{-} allotypes, the monoclonal hybridoma antibodies recognized only the appropriate subclass. In a direct binding radioimmunoassay the antibodies can detect as little as 1 ng/ml of the IgA subclass. This binding is not inhibited by as much as 100 \mu g/ml of IgG or 10 \mu g/ml of IgM. By indirect immunofluorescent staining, the anti-IgA\textsubscript{1}, 1-155-1, stained plasma cells from a patient with IgA\textsubscript{1} myeloma but not those from a patient with IgA\textsubscript{2} myeloma. The reverse was true with the anti-IgA\textsubscript{2}, 14-3-26.

Rhodamine-tagged goat anti-mouse y2B and y3 were a gift from Dr. J. F. Kearney, Birmingham, Ala. These goat antimouse reagents do not stain normal or abnormal renal tissue in the absence of mouse anti-human antibodies. The rhodamine-tagged rabbit anti-J chain was a gift from Dr. J. Mestecky, Birmingham, Alabama. Fluorescein isothiocyanate conjugated anti-human IgA, IgM, kappa chain and lambda chain were purchased from Meloy Laboratories, Inc., (Springfield, Va.). The anti-IgA and IgM were absorbed with IgG before use to insure lack of reactivity with light chains. These antisera were shown to be heavy chain specific by reactivity with the appropriate antigen in gel diffusion and by immunohistochemical studies on kidney tissue.

Immunofluorescence studies. Tissue was obtained by percutaneous or open kidney biopsy, snap frozen in isopentane precooled in liquid nitrogen, and stored at ~70°C. Sections (4 \mu m) were fixed to slides with acetone and washed three times in phosphate-buffered isotonic saline before immunofluorescent staining. The intestinal tissue, the tonsil, and the 19 renal biopsies that had been obtained more than 6 mo earlier were resectioned. The intensity of the staining and the background staining on these stored tissues were indistinguishable from that obtained with fresh tissue.

Indirect immunofluorescent staining was used to detect the IgA subclasses. The hybridomas antibody was layered over the tissue and allowed to incubate for 20 min at room temperature and the slides were then washed twice in phosphate-buffered isotonic saline. The slides incubated with 1-155-1, the mouse y3\textalpha anti-human IgA\textsubscript{1}, were then stained with rhodamine-tagged goat anti-mouse y3. Those incubated with 14-3-26, the mouse y2B\textkappa anti-human IgA\textsubscript{2}, were stained with rhodamine-tagged goat anti-mouse y2B. Tissues were directly

TABLE 1

<table>
<thead>
<tr>
<th>Disease</th>
<th>Intensity of staining</th>
<th>Mesangium</th>
<th>Peripheral capillary*</th>
<th>Tubular casts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgA</td>
<td>IgA\textsubscript{1}</td>
<td>IgA\textsubscript{2}</td>
</tr>
<tr>
<td>IgA Nephropathy</td>
<td>3+</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>trace</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HSP</td>
<td>3+</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>9</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>trace</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>SLE</td>
<td>3+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>trace</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

* Subendothelial or glomerular basement membrane.
stained with fluorochrome-tagged anti-IgA, IgM, kappa, lambda, or J chain. To enhance J chain staining some slides stained with the rhodamine-tagged rabbit anti-human J chain were counterstained with fluorescein-tagged goat anti-rabbit IgG. Slides were mounted under a coverslip and examined with a Zeiss fluorescent microscope (Carl Zeiss, Inc., New York) with epi-illumination and appropriate exciter and absorbing filters. All slides were read by two investigators.

RESULTS

The glomerular mesangium in renal biopsies from all patients with IgA nephropathy or HSP nephritis stained brightly for IgA (Table I). Mesangial staining was also seen in five of the nine patients with SLE. In all of these cases the fluorescence was equally intense on sections stained with the anti-IgA\textsubscript{1} antibodies. In contrast there was no mesangial staining for IgA\textsubscript{2} in any of the biopsies (Fig. 1).

The peripheral capillary was positively stained for IgA in 8 of the 10 biopsies from patients with IgA nephropathy, 9 of the 11 HSP biopsies, and in all 9 of the specimens from patients with SLE. This staining was less intense than the mesangial staining and was also limited to IgA\textsubscript{1} without evidence of IgA\textsubscript{2} deposition.

Tubular casts were positively stained for IgA in 5 of 10 patients with IgA nephropathy, 6 of the 11 patients with HSP nephritis and 6 of the 9 patients with SLE (Table I). These casts contained IgA\textsubscript{1} and to a lesser extent IgA\textsubscript{2}.

To verify that the antigenic sites recognized by the anti-IgA subclass antibodies were not modified by the freezing and fixation techniques used in these experiments, intestinal tissue from four different patients and tonsilar tissue from a child with IgA nephropathy were processed and stained by the same methods. Submucosal plasma cells demonstrating intense and equivalent fluorescence could be found in all tissues using either anti-subclass reagent.

Because the IgA in glomerular deposits was exclusively of the IgA\textsubscript{1} subclass, biopsies were stained with anti-kappa and anti-lambda antibodies to determine whether the IgA was monoclonal in origin. Glomerular deposits in biopsies from four patients with IgA nephropathy and four patients with HSP could be stained with both anti-kappa and anti-lambda. In four cases the lambda staining was brighter; in the remaining four the intensity of staining was equivalent using either reagent.

![Figure 1](http://www.jci.org) Photomicrographs of a renal biopsy from a patient with IgA nephropathy stained with heavy chain-specific heterologous anti-IgA (A), monoclonal anti-IgA\textsubscript{1} (B), monoclonal and anti-IgA\textsubscript{2} (C); and anti-J chain (D). Note the absence of IgA\textsubscript{2} and J chain ×400.
To detect the presence of IgA dimers in glomeruli of patients with IgA deposits, renal biopsies were stained with rabbit anti-human J chain. Because J chain is also a component of IgM and IgM is frequently found in association with IgA in these glomerular deposits, the intensity of J chain staining was correlated with the intensity of IgM staining and IgA staining. J chain could be detected in glomeruli of patients with IgA nephropathy but the intensity of staining correlated closely with that of IgM and not IgA. J chain was not detected in two biopsies with intense (3+) mesangial staining for IgA and either no or trace staining for IgM (Fig. 1).

**DISCUSSION**

By immunofluorescent staining we have shown that the glomerular IgA deposits in patients with IgA nephropathy, HSP, and SLE consist predominantly of monomers of IgA2. Immunofluorescent staining provides only a semiquantitative estimate of the amount of antigen present, and therefore we cannot rule out the possibility that small amounts of IgA2 or dimeric IgA are also present. However, in the normal individual IgA2 constitutes 10–20% of the total serum IgA. If the serum IgA were deposited in the glomeruli irrespective of subclass, IgA2 should have been detected. This assumption is strengthened by our ability to detect IgA2 in tubular casts from these patients since the protein in tubular casts is thought to reflect a nonspecific filtration defect of damaged glomeruli. The predominance of IgA1, is even more surprising when one considers that IgA nephropathy and HSP usually follow infections of the mucosal surfaces and the IgA produced at the mucosal surfaces is predominantly dimers of IgA2. These results indicate that the mesangial deposition of IgA is not simply the result of an “overflow” of secretory IgA into the peripheral circulation.

A small proportion of patients with lupus nephropathy have renal deposits of IgA (4). The glomerular staining of the SLE biopsies included in this study was less intense than that seen in IgA nephropathy and HSP but was also limited to IgA1. The tubular casts were most brightly stained in this group and five of six biopsies that showed IgA and IgA2 in the casts could also be stained for IgA2. This positive staining for IgA2 in the renal tubular casts provides a good internal control demonstrating that IgA2 can be preserved and stained in renal tissue.

Several factors known to affect immune complex deposition might be responsible for the remarkable predominance of IgA1 in glomerular deposits. IgA1 may have an increased tendency to aggregate. Although neither IgA subclass activates complement by the classical pathway, both may activate by the alternative pathway and IgA1 may do this more efficiently than IgA2 (15). It is possible that the antigens that elicit the IgA response in IgA nephropathy and HSP selectively trigger an IgA1 response. This type of subclass specificity has been demonstrated by the predominant IgG2 response to carbohydrate antigens in humans (16).

Recent studies have also shown that charge may influence the location of renal immune complex deposition. In the rat, cationic complexes were found in the renal mesangium, whereas anionic complexes localized to the capillary walls (17). However, there are only small charge differences between IgA1 and IgA2 making this an unlikely differentiating mechanism.

Past studies characterizing the IgA deposits in IgA nephropathy and HSP have shown that secretory component cannot be found in the glomerular lesions (18). This report provides further evidence that the IgA in these deposits does not reflect the IgA produced at the mucosal surfaces for transport into secretions under normal circumstances. The pathologic mechanisms that result in the selective deposition of monomers of IgA1 in the renal mesangium remain unknown.

**ACKNOWLEDGMENTS**

This investigation was supported by National Institutes of Health grants CA 16673, AI 10704, AM 25518, and T32GM07090 and grant 1-608 from the March of Dimes, Birth Defects Foundation.

**REFERENCES**


*IgA Subclass-specific Deposits in Glomerulonephropathies*


