Selective Deposition of Immunoglobulin A_1 in Immunoglobulin A Nephropathy, Anaphylactoid Purpura Nephritis, and Systemic Lupus Erythematosus

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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 peripheral capillary were brightly stained for IgA_1 and were negative for IgA_2. IgA_1 and, to a lesser extent, IgA_2 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 IgA_1.

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 preceeded 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)[1] (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 preceeded 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 four 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 C1 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, IgA_2 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 seemed possible that renal deposition of IgA might be the result of increased levels of IgA_2 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.

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

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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 P63xAg8653 (14). IgA subclass specific hybridomas were detected by an enzyme-linked immunoadsorbent assay. The anti-IgA_1, designated clone 1-155-1, is a mouse $\gamma 3\lambda$ of BALB/c allotype. The anti-IgA_2, designated as 14-3-26, is a mouse $\gamma 2B\kappa$ of A/J allotype. These monoclonal antibodies have been shown to be IgA subclass specific by several techniques. In an enzyme-linked immunoabsorbent assay on 37 IgA myelomas, 30 IgA_1, and 7 IgA_2 including both $Am2^+$ and $Am2^-$ 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 μg/ml of IgG or 10 μg/ml of IgM. By indirect immunofluorescent staining, the anti-IgA_1, 1-155-1, stained plasma cells from a patient with IgA_1 myeloma but not those from a patient with IgA_2 myeloma. The reverse was true with the anti-IgA_2, 14-3-26.

Rhodamine-tagged goat anti-mouse $\gamma 2B$ and $\gamma 3$ were a gift from Dr. J. F. Kearney, Birmingham, Ala. These goat anti-mouse 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 μM) 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 $\gamma 3\lambda$ anti-human IgA_1, were then stained with rhodamine-tagged goat anti-mouse $\gamma 3$. Those incubated with 14-3-26, the mouse $\gamma 2B\kappa$ anti-human IgA_2, were stained with rhodamine-tagged goat anti-mouse $\gamma 2B$. Tissues were directly

TABLE I

Intensity of Immunofluorescent Staining for IgA and IgA Subclasses in the Mesangium, Peripheral Capillaries or Basement Membranes, and Tubular Casts in Patients with IgA Nephropathy, HSP or SLE

Disease	Intensity of staining	Mesangium			Peripheral capillary*			Tubular casts		
		IgA	IgA_1	IgA_2	IgA	IgA_1	IgA_2	IgA	IgA_1	IgA_2
		No. patients demonstrating staining pattern								
IgA Nephropathy	3+	6	6	0	0	0	0	0	0	0
	2+	4	4	0	1	1	0	1	1	0
	1+	0	0	0	3	4	0	2	1	1
	trace	0	0	0	4	3	0	2	3	0
	0	0	0	10	2	2	10	5	5	9
HSP	3+	1	1	0	0	0	0	0	0	0
	2+	9	10	0	4	4	0	2	2	0
	1+	1	0	0	3	3	0	3	2	2
	trace	0	0	0	2	2	0	1	1	1
	0	0	0	11	2	2	11	5	6	8
SLE	3+	0	0	0	0	0	0	3	3	0
	2+	3	4	0	4	4	0	2	1	1
	1+	1	0	0	5	5	0	1	1	2
	trace	1	1	0	0	0	0	0	1	2
	0	4	4	9	0	0	9	3	3	4

* 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_1 antibodies. In contrast there was no mesangial staining for IgA_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_1 without evidence of IgA_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_1 and to a lesser extent IgA_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_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.

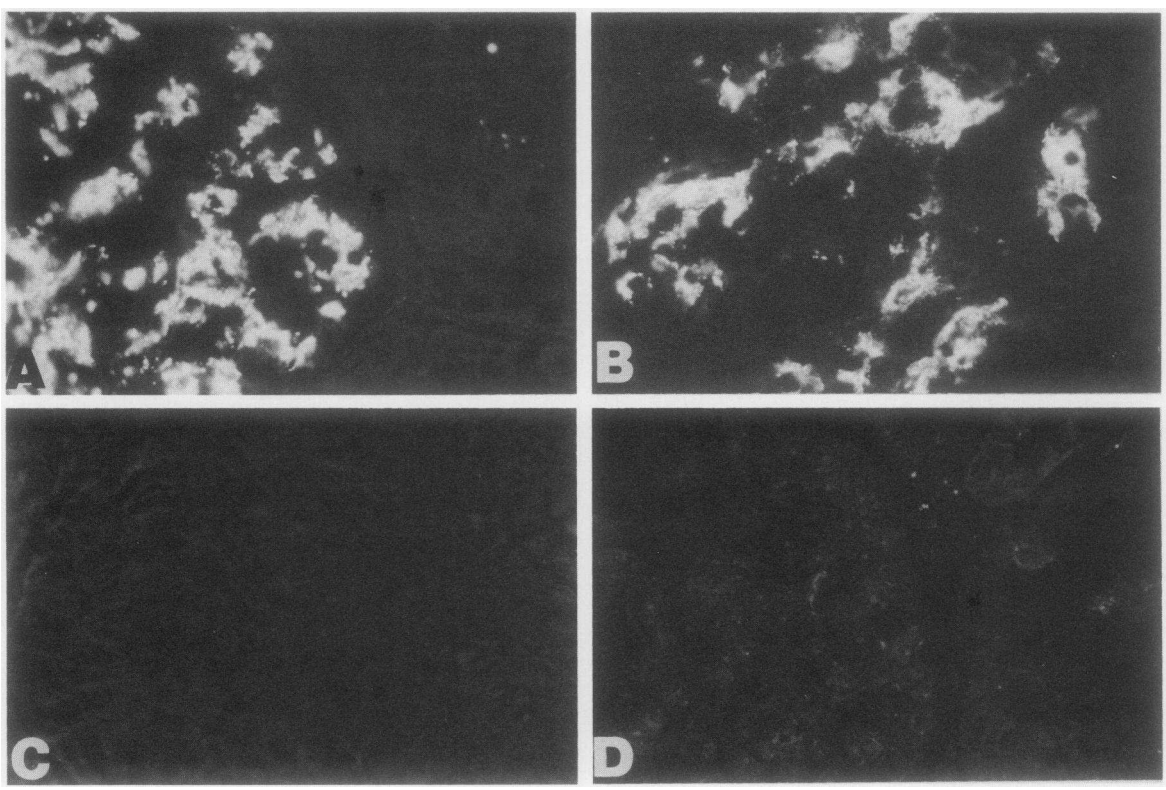

FIGURE 1 Photomicrographs of a renal biopsy from a patient with IgA nephropathy stained with heavy chain-specific heterologous anti-IgA (A), monoclonal anti-IgA_1 (B), monoclonal and anti-IgA_2 (C); and anti-J chain (D). Note the absence of IgA_2 and J chain ×400.

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 IgA_1. 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 IgA_2 or dimeric IgA are also present. However, in the normal individual IgA_2 constitutes 10–20% of the total serum IgA. If the serum IgA were deposited in the glomeruli irrespective of subclass, IgA_2 should have been detected. This assumption is strengthened by our ability to detect IgA_2 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 IgA_1 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 IgA_2. 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 IgA_1. The tubular casts were most brightly stained in this group and five of six biopsies that showed IgA and IgA_1 in the casts could also be stained for IgA_2. This positive staining for IgA_2 in the renal tubular casts provides a good internal control demonstrating that IgA_2 can be preserved and stained in renal tissue.

Several factors known to affect immune complex deposition might be responsible for the remarkable predominance of IgA_1 in glomerular deposits. IgA_1 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 IgA_1 may do this more efficiently than IgA_2 (15). It is possible that the antigens that elicit the IgA response in IgA nephropathy and HSP selectively trigger an IgA_1 response. This type of subclass specificity has been demonstrated by the predominant IgG_2 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 IgA_1 and IgA_2 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 IgA_1 in the renal mesangium remain unknown.

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