

# The Immunochemical Characterization of Mesangial IgA Deposits

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Mesangial deposits of IgA are found in IgA nephropathy, Schönlein-Henoch purpura (SHP) and in some patients with alcoholic cirrhosis and systemic lupus erythematosus (SLE). In this study the authors characterized the mesangial IgA deposits in patients with the above diseases using antiserums or monoclonal antibodies to A<sub>1</sub>, A<sub>2</sub>, J-chain and secretory component (SC), and examined SC binding *in vitro*. SC was not

present, J-chain was ubiquitous, and A<sub>2</sub> was found (with the use of monoclonal antibodies) rarely but with equal frequency in all groups. The SC binding capacity of the deposits differed between the groups and was found in 13 of 16 patients with alcoholic liver disease, 3 of 4 with SLE, 1 of 10 with primary IgA nephropathy, and none of 6 with SHP. (Am J Pathol 1983, 113:359-364)

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PRIMARY IgA nephropathy, characterized by mesangial deposits of IgA and C<sub>3</sub> occurs most often in young men with synpharyngitic hematuria.<sup>1</sup> Similar immunopathologic findings are found in association with alcoholic cirrhosis,<sup>2</sup> portal-systemic shunts,<sup>3</sup> dermatitis herpetiformis,<sup>4</sup> and celiac disease<sup>5</sup>—all of which may be termed secondary IgA nephropathy—and in Schönlein-Henoch purpura (SHP). Mesangial IgA is also found in lupus nephritis (SLE), but other criteria readily differentiate this from IgA nephropathy.

IgA is present in the circulation as monomeric units, mainly of the A<sub>1</sub> subclass, but at mucosal sites it is secreted actively as A<sub>1</sub> or A<sub>2</sub> dimers<sup>6</sup> polymerized by J-chain and transported by binding to secretory component (SC). The bowel is the main site of IgA production in the body.<sup>6</sup> In secondary forms of IgA nephropathy, the mesangial deposits and the documented increases in both circulating IgA and IgA class immune complexes (CIC) might be accounted for either by a defective mucosal barrier—with increased absorption of floral or dietary antigens and systemic hyperimmunization—or by defective hepatic clearance of antigens, IgA polymers, or complexes (reviewed by Woodroffe et al<sup>7</sup>). The presence of J-chain, subclass A<sub>2</sub> or SC in the mesangium would support a mucosal origin of the IgA. SC has rarely been identified in any form of mesangial IgA nephritis,<sup>8,9</sup> and the reported presence of J-chain<sup>10</sup> must be

qualified because this is also a normal component of IgM, which often accompanies the IgA deposits. The IgA subclass studies so far reported have been contradictory.<sup>10-12</sup>

It has been suggested that primary IgA nephropathy is mediated by the deposition of IgA polymers from the circulation.<sup>13,14</sup> The capacity to bind free SC is an important method of distinguishing polymeric immunoglobulins containing J-chain from monomers.<sup>15</sup> Several groups have shown mesangial IgA deposits to bind free SC<sup>14,16,17</sup> or be of polymeric size.<sup>18</sup> Egido et al<sup>14</sup> showed SC binding capacity in 16 of 20 biopsies with primary IgA nephropathy, compared with 6 of 7 with SHP and none of 3 with SLE. Subsequently Bene et al<sup>16</sup> have shown SC binding capacity in all of a series of 15 patients with IgA nephropathy. Patients with alcoholic liver disease represent a group of patients where a mucosal origin for the mesangial IgA deposits may be predicated.<sup>7</sup> In support of this, Sancho et al<sup>17</sup> found SC binding capacity in all of 6 acid eluates from autopsy kidneys

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and in all of 3 frozen sections from patients with mesangial IgA deposition and alcoholic liver disease.

The present study reexamines the immunochemical characteristics of the IgA mesangial deposits in patients with primary IgA nephropathy, mesangial IgA nephritis secondary to alcoholic liver disease, SLE, and SHP. A<sub>1</sub> and A<sub>2</sub> subclasses were sought by conventional indirect immunofluorescence (IIF) with the use of sheep antisera and by an avidin-biotin system with the use of monoclonal antibody reagents. J-chain and SC were sought by IIF, and the ability of the IgA deposits to bind purified SC was also examined.

### Materials and Methods

The renal tissue selected for study consisted of postmortem specimens from 16 patients with alcoholic cirrhosis shown to have mesangial deposits of IgA and C3 and renal biopsy specimens from patients with primary IgA nephropathy (11), SHP (7) and SLE (5). Immunofluorescence-negative renal tissue from autopsies (2) and biopsies (7) were examined as controls.

Tissue for IF obtained at autopsy within 24 hours of death or immediately after removal of a needle biopsy was snap-frozen in isopentane and liquid nitrogen. Unfixed frozen sections cut at 2  $\mu$  were stained and examined with a Leitz Ortholux microscope fitted with Ploem epi-illumination, an HB 200 mercury lamp as a light source, dichroic mirrors on position 3, and BG 38 and K 510 filters. The reactions were graded as 0 to 4+ by two of the authors (GSH and JDLS).

Direct IF was performed with fluorescein isothiocyanate (FITC)-conjugated monospecific antisera to IgA (Wellcome, Beckenham, UK) and IgM (Hyland-Travenol Laboratories, Costa Mesa, Calif).

IIF was performed with the use of 1:4 rabbit anti-human J-chain (Nordic Immunologic Laboratories, Tilberg, Netherlands) or 1:3 rabbit anti-human SC (Hoechst, Marburg, West Germany) that had been absorbed with 1:9 normal human serum (NHS). The sections were then stained with FITC-conjugated goat anti-rabbit IgG (Dakopatts, Copenhagen, Denmark) that previously had been absorbed with 1:9 NHS. The rabbit antisera were tested with sections from human small bowel biopsies prepared according to the method of Savilahti.<sup>19</sup> Incubation of sections with 1:3 normal rabbit serum was used as a control. Antiserum to human J-chain stained the cytoplasm of lamina propria plasma cells, but not enterocytes. Antiserum to human SC, however, stained both lamina propria plasma cell cytoplasm and enterocytes, the

latter in a "picket fence" distribution along lateral walls and basal surfaces. After absorption with NHS, the SC antiserum stained only enterocytes.

IgA subclasses were examined using 1:3 dilutions of antisera raised in sheep to A<sub>1</sub> and A<sub>2</sub> (Nordic) and stained with FITC-conjugated rabbit anti-sheep IgG (Wellcome) that had been absorbed with 1:9 NHS. Staining was controlled by incubation with 1:3 normal sheep serum. Sheep antisera to A<sub>1</sub> and A<sub>2</sub> subclasses were tested on normal human jejunum and showed similar bright 3+ staining of lamina propria plasma cell cytoplasm. Neither antiserum stained enterocytes. However, staining of an IgA<sub>1</sub> myeloma bone marrow smear gave a 3+ reaction for A<sub>1</sub> and a 1+ reaction for A<sub>2</sub>.

Monoclonal antibodies were obtained from a mouse myeloma cell line, P3-X63-Ag8 653 hybridized with spleen cells from immunized BALB/C mice (IgA<sub>1</sub>) or AJ mice (IgA<sub>2</sub>) (Becton Dickinson, Sunnyvale, Calif). The monoclonal antibodies were tested initially by IIF with the use of FITC-conjugated rabbit anti-mouse (Cappel, Cochranville, Pa) and later in an avidin-biotin amplification system. Subsequently, only the avidin-biotin system was used because this clearly was more sensitive. Sections were incubated with the monoclonal antibody (20  $\mu$ g/ml), biotinylated goat anti-mouse IgG (Tago, Burlingame, Calif) (500  $\mu$ g/ml) and then stained with FITC-conjugated avidin (Becton Dickinson) (50  $\mu$ g/ml protein).<sup>20</sup> Staining was controlled by incubation with 1:9 normal mouse serum. These reagents were tested on human small bowel biopsies and on human IgA<sub>1</sub> myeloma bone marrow smears.

Murine monoclonal antibodies tested on normal small intestine showed a 2+ reaction for large numbers of lamina propria plasma cells stained for subclass A<sub>1</sub>, and a 4+ reaction for lesser numbers of plasma cells stained for A<sub>2</sub>. There was no reactivity with enterocytes. The IgA<sub>1</sub> myeloma bone marrow smear stained with monoclonal antibody to A<sub>1</sub> showed a bright cytoplasmic 4+ reaction, while for A<sub>2</sub> there was faint nuclear but no cytoplasmic staining.

Purified human SC was a gift from Dr. La Brooy, Department of Medicine, Royal Adelaide Hospital. The binding capacity of the SC preparation was tested by incubation at 200  $\mu$ g/ml with a jejunal biopsy in a moist chamber followed by IIF staining for human SC. A control slide preincubated with phosphate-buffered saline (PBS) was used to demonstrate the distribution of native SC in the section. The jejunal section incubated with PBS and stained by IIF with FITC anti-human SC showed localization of SC to the enterocyte basal and lateral walls. Sections

incubated with SC and similarly stained showed enterocyte SC but, in addition, bright staining of mucosal plasma cell cytoplasm. The SC binding capacity of the mesangial IgA deposits in renal sections was tested by incubation of adjacent sections with either SC or PBS followed by IIF for human SC as described above.

The significance of differences in J-chain content or SC binding between the disease groups and in relation to IgM was calculated with the use of a chi-square test with a Yates' correction.

A Mann-Whitney test for nonparametric samples was applied to the incidence of SC binding capacity in primary IgA nephropathy deposits, compared with mesangial IgA deposits secondary to alcoholic liver disease.

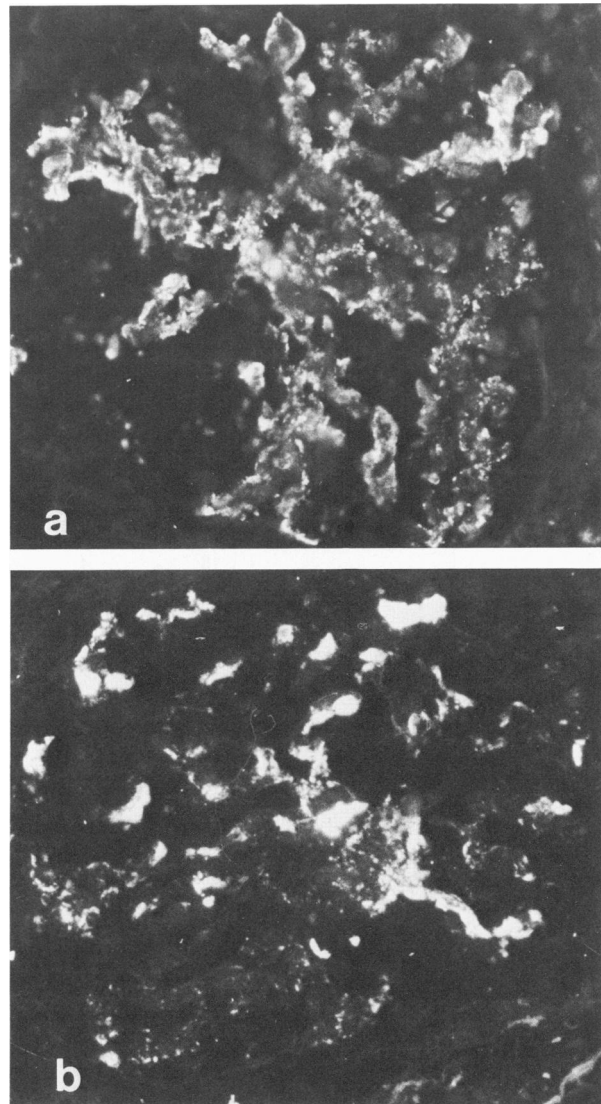
### Results

The results of the renal IF studies are shown in Table 1. With the use of sheep antisera, 10 of the 11 patients with primary IgA nephropathy showed mesangial A<sub>1</sub>, and 9 showed A<sub>2</sub>. Monoclonal antibodies showed A<sub>1</sub> in the same 10 patients but A<sub>2</sub> in only 2. Of the 16 patients with alcoholic cirrhosis, the sheep antisera showed 9 to have both A<sub>1</sub> and A<sub>2</sub>. With monoclonal antibodies, 15 had A<sub>1</sub> and 2 A<sub>2</sub>. Of the 7 patients with SHP, 4 showed staining for A<sub>1</sub> and 2 for A<sub>2</sub> when tested with the sheep antisera. With monoclonal antibody, 5 had A<sub>1</sub>, but only 1 (Patient 29) had A<sub>2</sub> (Figure 1). Of the 5 patients with SLE, sheep antisera showed A<sub>1</sub> in 4 and A<sub>2</sub> in 4. With monoclonal antibodies, A<sub>1</sub> was shown in 5 and A<sub>2</sub> in 3. Overall, monoclonal antibody to A<sub>1</sub> was positive in 35 of 39 patients, compared with 29 of 39 with the sheep antiserum. Monoclonal antibody for A<sub>2</sub>, however, was positive only in 8 compared, with 25, of 39 patients with the sheep antiserum.

J-chain reactions were present in 32 patients. There was no correlation between staining for IgM and J-chain ( $P > 0.5$ ). Twenty-six of 39 kidneys had mesangial IgM staining: 7 of 11 patients with primary IgA nephropathy, 13 of 16 patients with alcoholic cirrhosis, all of those with SLE, and 1 of the 7 with SHP. Of these 26, 4 had no staining for J-chain. Of the 13 patients without IgM, 10 showed staining for J-chain. J-chain staining was not found more frequently in patients with A<sub>2</sub> subclass ( $P > 0.5$ ).

Staining of the mesangium for SC was found in 29 of 39 patients, but this was lost after preincubation of the antiserum with NHS in all but 1 case (Patient 12) where a trace remained.

The SC binding capacity of the mesangial IgA deposits is shown in Table 1. Thirteen of the 16 kid-



**Figure 1**—Kidney from Patient 29, with Schönlein-Henoch purpura, stained with murine monoclonal antibody to A<sub>1</sub> (a) and A<sub>2</sub> (b) and demonstrated by IIF with biotinylated goat anti-mouse IgA and FITC-conjugated avidin. ( $\times 600$ )

neys from patients with alcoholic cirrhosis and mesangial IgA deposits showed SC binding (Figure 2). All of the 9 IF-negative control kidneys and 6 SHP kidneys failed to bind SC, but biopsies from 3 of 4 patients with SLE and 1 of 10 patients with primary IgA nephropathy bound free SC. There was an overall correlation between SC binding and IgM deposits ( $P < 0.05$ ) but no difference in the frequency of IgM in the patients with alcoholic cirrhosis, compared with primary IgA nephropathy ( $P > 0.5$ ), despite a significant difference in SC binding between these two groups ( $P < 0.01$ ). With the use of a Mann-Whitney test for nonparametric samples, the difference in SC binding capacity in patients with primary IgA ne-

Table 1—Mesangial Immunofluorescence Graded from 0 to 4+

Patient category	Patient number	IgA	Sheep antiserum		Murine monoclonal		IgM	J	SC binding capacity
			A <sub>1</sub>	A <sub>2</sub>	A <sub>1</sub>	A <sub>2</sub>			
Primary IgA nephropathy	1	3	3	2	3	0	2	3	0
	2	3	3	3	3	0	0	2	0
	3	3	3	2	3	0	2	2	2
	4	4	3	1	3	0	2	3	0
	5	3	2	1	3	3	0	1	0
	6	3	0	0	0	0	2	1	0
	7	3	1	0	1	0	0	1	0
	8	4	2	1	3	1	0	3	0
	9	4	3	4	4	0	1	3	0
	10	3	1	1	4	0	1	0	NT
	11	4	3	1	3	0	3	3	0
Mesangial IgA nephritis associated with alcoholic liver disease	12	4	4	4	4	0	3	3	3
	13	4	0	0	1	0	0	3	0
	14	2	3	3	4	0	0	3	1
	15	2	1	1	3	0	4	1	4
	16	3	1	1	2	0	3	1	4
	17	3	3	3	3	0	1	3	3
	18	4	2	2	3	0	3	2	0
	19	3	3	0	3	0	3	1	4
	20	2	1	0	1	0	3	0	1
	21	1	0	0	0	0	3	0	0
	22	3	1	1	3	1	1	3	3
	23	3	0	1	2	1	3	2	3
	24	3	0	0	2	0	4	1	4
	25	3	0	0	2	0	3	0	1
	26	3	1	1	2	0	4	2	4
	27	3	1	1	3	0	0	2	3
Schönlein-Henoch purpura	28	2	0	0	2	0	0	1	0
	29	2	4	4	4	4	0	3	NT
	30	3	2	1	3	0	0	2	0
	31	3	0	0	3	0	1	3	0
	32	3	1	0	0	0	0	0	0
	33	2	0	0	0	0	0	0	0
	34	2	1	0	1	0	0	0	0
Systemic lupus erythematosus	35	4	4	3	3	2	2	2	2
	36	4	4	3	3	2	4	2	4
	37	3	2	1	2	0	3	2	NT
	38	3	1	0	2	1	3	3	1
	39	2	0	1	1	0	4	3	0

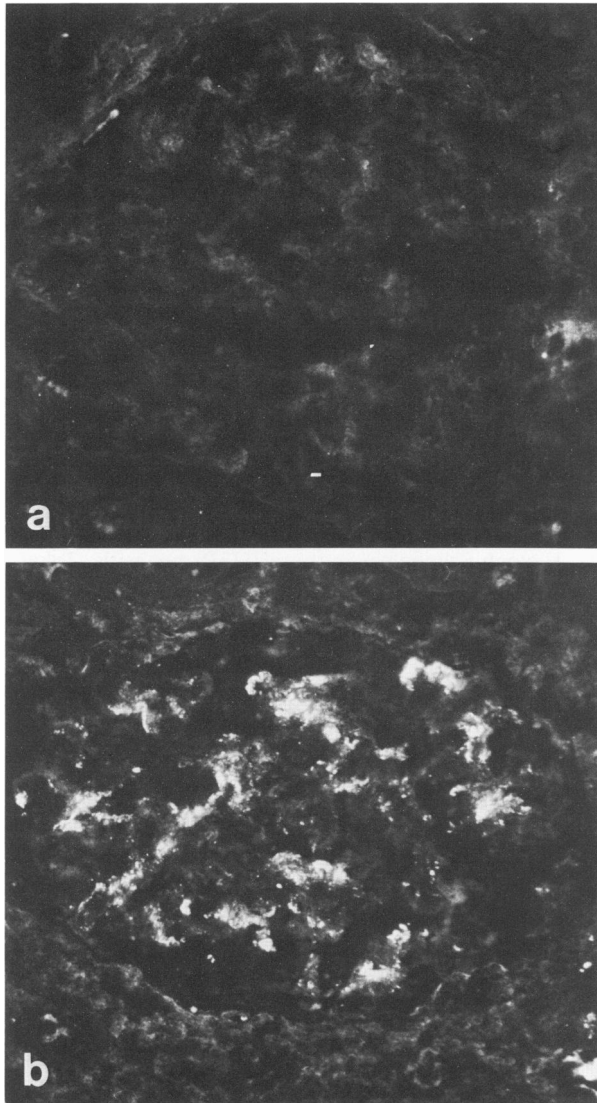
NT, not tested. Nine control biopsies were negative in all tests used.

phropathy, compared with those with IgA nephritis secondary to alcoholic liver disease, was highly significant ( $P = 0.001$ ).

### Discussion

In this study we have examined by IF renal tissue from 39 patients with mesangial IgA deposits to elucidate the immunochemical nature of the IgA. With monoclonal antibodies, we showed that A<sub>1</sub> was present in all but 4 of the 39 patients, with no significant differences between the 4 disease categories examined. IgA<sub>2</sub> was less common, occurring in only 10–20% of patients with primary IgA nephropathy, mesangial IgA nephritis secondary to alcoholic cirrhosis and SHP, and in 3 of the 5 patients with SLE.

A<sub>1</sub> and A<sub>2</sub> subclasses have also been sought by Conley et al,<sup>10</sup> Andre et al,<sup>11</sup> and Tomino et al,<sup>12</sup> with conflicting results. Conley et al, using monoclonal reagents, examined 10 patients with primary IgA nephropathy, 11 with SHP, and 9 with SLE. All were positive for A<sub>1</sub>, but none showed A<sub>2</sub> staining; and, while present, J-chain staining correlated only with the intensity of IgM staining and not with that of IgA. Andre et al found that rabbit antiserum (Nordic) to A<sub>1</sub> stained the mesangium of 5 of 10 patients with primary IgA nephropathy, 8 of 9 with alcoholic cirrhosis, and all of those with SHP and SLE. In contrast to Conley et al, Andre et al, using rabbit antiserum to A<sub>2</sub> (Nordic), demonstrated staining in all 10 patients with primary IgA nephropathy, all 9 patients with alcoholic cirrhosis, both of those with SHP, and



**Figure 2**—SC binding test performed on renal tissue from Patient 24, with mesangial IgA nephritis secondary to alcoholic cirrhosis, incubated with PBS (a) and human SC (b) at 200  $\mu\text{g}/\text{ml}$  and demonstrated by IIF with rabbit anti-human SC and FITC-conjugated goat anti-rabbit IgG. ( $\times 800$ )

all but 1 with SLE. Tomino et al<sup>12</sup> examined 7 patients with primary IgA nephropathy using both Nordic antisera and monoclonal reagents and found A<sub>1</sub> throughout, using both systems but A<sub>2</sub> in only 2 instances using monoclonal antibody. Tomino et al<sup>21</sup> further examined 5 patients with SHP, finding A<sub>2</sub> in none.

We believe, like Tomino et al,<sup>12</sup> that some of these differences relate to the specificity of the reagents. The sheep antiserum to A<sub>2</sub> (Nordic) evaluated in this study cross-reacts with IgA<sub>1</sub> myeloma cells. The relatively high incidence of mesangial A<sub>2</sub> staining with this antiserum was probably caused by this cross-reactivity. We have more confidence in the monoclonal

reagents, particularly when used with the avidin-biotin method.

It has been suggested that staining for J-chain in patients with IgA nephropathy merely represents activity against J-chain in the associated IgM deposits.<sup>10</sup> The finding of mesangial J-chain staining in 10 of the 13 IgM-negative cases and its absence in 4 of the 26 IgM-positive cases does not support this suggestion. This would imply the presence of J-chain containing IgA polymers in the deposits. Like other workers,<sup>8,9</sup> we failed to demonstrate SC in the mesangial deposits.

The study of jejunal SC binding showed that SC bound specifically to plasma cells, most of which in this location might be expected to be producing dimeric IgA. With the use of this technique, SC binding was observed in 13 of 16 kidneys from patients with alcoholic cirrhosis, 1 of 10 patients with primary IgA nephropathy, none of 6 patients with SHP, and 3 of 4 with SLE. The ability of the IgA deposits in alcoholic cirrhosis to bind SC suggests that these are polymeric.<sup>15</sup> However, mesangial IgA is often accompanied by IgM; and it is necessary to interpret SC binding with some caution. Five primary IgA nephropathy patients and 2 with alcoholic cirrhosis had substantial amounts of IgM, yet failed to bind SC. In contrast, 2 patients with alcoholic liver disease but no IgM bound SC. There was a marked difference in the SC binding capacity of mesangial IgA deposits in primary IgA nephropathy and alcoholic cirrhosis, although there was no significant difference in mesangial IgM content.

Our data show that SC binding is almost exclusively restricted to kidneys from patients with alcoholic cirrhosis and SLE. These findings differ from those of other studies.<sup>14,16,17</sup> The reason for this is not known, nor can we explain the discrepancy between SC binding and the presence of J-chain. Irrespective of reagent specificity, we believe that our data demonstrate a functional difference between the IgA deposits in primary IgA nephropathy and mesangial IgA deposits secondary to alcoholic liver disease.

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