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Immunoglobulin-A distribution in glomerular disease: Analysis of immunofluorescence localization and pathogenetic significance

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Immunoglobulin-A distribution in glomerular disease. Analysis of immunofluorescence localization and pathogenetic significance. Renal biopsies from 470 patients with various glomerulonephropathies were studied for patterns and frequency of glomerular bound IgA. Correlations of IgA with IgG, IgM, C3, and C4 were made. Glomerular deposits of IgA were observed in five of six cases of Henoch-Schoenlein anaphylactoid nephritis (83%), stalk proliferative glomerulonephritis (73%), lupus nephritis (60%), and focal proliferative glomerulonephritis (57%). In addition, IgA was less frequently observed in diffuse (acute) proliferative (33%), membranoproliferative (42%), membranous (32%), focal sclerosing (25%) crescentic (26%), and chronic glomerulonephritides (23%) as well as malignant arterionephrosclerosis, amyloidosis, and a group of patients with minimal glomerular alteration and no determinable diagnosis (40%). IgA was not specifically associated with IgG or IgM in any one diagnostic category but was often present with both. Deposits containing C3 and C4 most closely paralleled those of IgG and/or IgM. Presence of IgA appeared to correlate with variable degrees of increased glomerular mesangial cellularity in "minimal," stalk proliferative, and focal-segmental glomerular lesions. The cause and immunopathogenetic significance of mesangial or peripheral glomerular capillary localization of IgA is unknown. Though a number of apparent examples of what has been referred to as IgA-IgG nephropathy were observed in this study, this entity, characterized by mesangial deposits of IgA, IgG, and C3, could not always be specifically identified or differentiated on histopathologic criteria alone from a variety of other glomerulopathies in which variable proportions of IgA, IgG, IgM, C3, and C4 globulins were localized.

Distribution de l'immunoglobuline-A dans les affections glomérulaires: Analyse de la localisation par immunofluorescence et de la signification pathogénique. Les biopsies rénales de 470 malades atteints de néphropathies glomérulaires diverses ont été étudiées du point de vue des aspects et de la fréquence des dépôts glomérulaires d'IgA. Les corrélations de l'IgA avec l'IgG, l'IgM, C3 et C4 ont été recherchées. Les dépôts glomérulaires d'IgA ont été observés dans 5 cas sur 6 de néphropathie anaphylactoïde de Henoch-Schoenlein (83%), dans les proliférations mésangiales (73%), dans 60% des néphropathies lupiques et 57% des glomérulonéphrites focales prolifératives. L'IgA a été moins fréquemment observée dans les glomérulonéphrites prolifératives diffuses (33%), membranoprolifératives (42%), membraneuses (32%), sclérosantes focales (25%), avec croissants (26%) et les néphropathies glomérulaires chroniques (23%) de même qu'au cours de l'artério-néphro-sclérose, de l'amyloïdose et dans un groupe de malades qui avaient des altérations glomérulaires minimes sans diagnostic défini (40%). L'IgA n'était pas spécifiquement associée à l'IgG ou à l'IgM dans l'une de ces catégories de diagnostics mais était souvent présente en même temps que l'IgG et l'IgM. Les dépôts contenant C3 et C4 étaient les plus étroitements corrélés à ceux d'IgG et/ou IgM. La présence d'IgA est apparue être en corrélation avec les divers degrés d'augmentation de la cellularité mésangiale dans les lésions glomérulaires minimes, les proliférations mésangiales et les lésions segmentaires focales. La cause et la signification immunopathogénique des localisations de l'IgA dans le mésangium ou à la périphéric des capillaires glomérulaires est inconnue. Bien que de nombreux exemples apparents de ce qui a été décrit sous le nom de néphropathie à IgA-IgG aient été observés dans cette étude, cette entité — caractérisée par des dépôts mésangiaux d'IgA, d'IgG et de C3 — n'a pas toujours pu être identifiée spécifiquement ou différenciée en fonction des seuls critères histopathologiques de diverses autres néphropathies glomérulaires dans lesquelles des proportions variables d'IgA, d'IgM de C3 et de C4 ont été localisées.

Fluorescent antibody reagent reactive with human immunoglobulin-A (IgA) is often used in conjunction with a battery of fluorescent antibody reagents monospecifically reactive with other serum proteins in evaluation of renal biopsies, but diagnostic and pathologic significance of the presence of IgA in kidney disease has not been clearly established. Recent reports indicate that IgA is frequently detected in selected pathologic entities such as the nephritis of Henoch-Schoenlein anaphylactoid purpura, lupus glomerulonephritis and in some types of focal proliferative glo-

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merulonephritis [1–9]. Furthermore, Berger has recently described a newly recognized glomerulopathy characterized immunohistochemically by predominantly mesangial deposits of IgA, IgG, and C3 in the setting of mild stalk or focal segmental proliferative glomerular lesions [3].

This report constitutes a detailed description of the incidence, patterns, and distribution of glomerular bound IgA and other associated immunoglobulins and C3 in 470 renal biopsies from patients with clinicopathologically defined glomerular diseases.

Methods

Immunofluorescence microscopic evaluation for the presence and distribution of glomerular localization of IgA was performed on 470 of over 1,500 renal biopsies from Duke University Medical Center, the University of Wisconsin Medical Center, and the Veterans Administration Hospital, Madison, Wisconsin. Diagnoses were established on the basis of a combination of clinical data and histopathologic findings on examination of renal biopsies by light, immunofluorescence, and electron microscopy. The general categories of renal diseases evaluated are listed in Table 2, and in instances where some confusion may arise regarding given diagnostic labels, are further defined in the Results section.

Morphologic terminology. There is considerable confusion created by the lack of standard descriptive terms for conveying impressions about the distribution and extent of renal glomerular lesions and immunohistochemically identified deposits. In an effort to clarify this problem, Table 1 provides a glossary of descriptive terminology used in this paper for presentation and discussion of glomerular lesions and distribution, patterns, and localization of glomerular bound plasma proteins.

Light microscopy. Portions of renal biopsy specimens were fixed in buffered 10% formalin, embedded in paraffin, and sectioned at 2 to 3 μ. The sections were stained routinely with hematoxylin and eosin and periodic acid Schiff reaction. Sections from most cases were also stained with periodic acid-methenamine silver-Masson stain and others were stained with either periodic acid-alcian blue, or Masson stains.

Immunofluorescence microscopy. Another portion of the renal biopsy specimens was immediately frozen in a matrix of 7.5% aqueous gelatin [14] or mouse liver and stored at −70° C until sectioned at 4 μ and stained. Prior to staining with fluorescein-labeled antibody reagents, sections were "fixed" in acetone or alcohol for 5 min and washed twice in phosphate buffered saline, pH 7.3. A drop of respective fluorescent reagent was placed on the sections which were then incubated at room temperature for 30 min followed by two 15 min rinses in buffered saline. Sections were mounted with buffered 10% glycerol and examined and photographed with a Zeiss or Leitz fluorescence microscope.

Table 1. Glossary of descriptive morphology for immunohistochemically identified deposits

Terminology	Definition
Localization of deposits in glomeruli:	
Mesangial	Within stalk areas of the glomerular tuft
Peripheral	Along the glomerular capillary walls
Capsular	In Bowman's capsule
Distribution of a lesion or deposits in glomeruli:	
Segmental	Portions of individual glomerular tufts
Diffuse	Entirety of individual glomerular tufts
Focal	Some glomeruli in the total specimen
Generalized	All glomeruli in the total specimen
Pattern of deposits in glomeruli:	
Granular	Beaded or "lump-bumpy" fluorescence varying from fine granular to coarse globular
Linear	Delicate and continuous fluorescence along contours of capillary walls
Interrupted, linear segments	Occasionally fluorescence may involve interrupted, short streaks in segments of capillary walls
Quantitation of intensity and/or extent of distribution of immunofluorescence:	
Negative	Complete absence of fluorescence
1+	Mild amount, often widely interspersed and of weak intensity
2+	Moderate amount of fluorescence
3+	Heavy, bright, maximum fluorescence intensity

Immunofluorescence reagents and control evaluations. Immune sera monospecifically reactive with selected components of human sera were purchased from a variety of commercial sources (Hyland, Behring-Werke, Cappel Laboratories, Kallestadt, and Miles Laboratories) or were prepared in our laboratories. After immunoelectrophoretic analysis and verification of monospecificity of reaction, the immunoglobulin fractions of the various immune sera were conjugated with fluorescein isothiocyanate [15], freed of free fluorescein by passage through a Sephadex G-25 column, and then depleted of nonspecific fluorescent reactants by absorption with beef liver powder. The major immunofluorescent reagents used in this study were immunoglobulins reactive with heavy chain moieties of human IgA, IgG, and IgM, and with human C3 and fibrinogen.

In later phases of this study an immunoglobulin reactive with human C4 was also used.

The specificity of immunohistochemical reactivity of the various fluorescent conjugates was verified in several ways in many but not all cases and phases of this study. In those instances where labeled antibodies were absorded with respectively specific immunoglobulin, complement component, or fibrinogen, subsequent immunofluorescent reaction with tissues was abolished. Alternatively, in those few instances where sections of kidney were first treated with unlabeled antibodies, subsequent immunofluorescence reaction with respective labeled antibody was markedly or moderately inhibited. Evaluation of absence of any apparent cross reactivity or possible antilight chain activity of the antiimmunoglobulin reagents was achieved in several ways. Though interpretations of negative agar immunodiffusion reactions are inconclusive, no precipitation reactions were detectable between selected batches of antiheavy chain antibodies and kappa or lambda myeloma proteins in Ouchterlony immunodiffusion. Furthermore, in microimmunoelectrophoresis, only single bands of immunoprecipitation were observed between antiheavy chain antibodies and respective class of immunoglobulin, implying that such antibodies could not react with light chains on other classes of immunoglobulins. And finally, lack of reaction or strikingly different patterns of reaction of one labeled antiimmunoglobulin with glomeruli containing deposits of one or another of the other two classes of immunoglobulins in many cases, implies lack of cross reactivity or light chain reactivity of each antiglobulin conjugate.

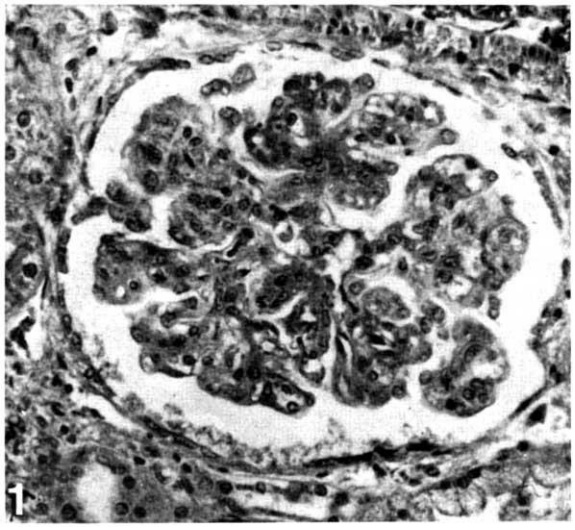
Results

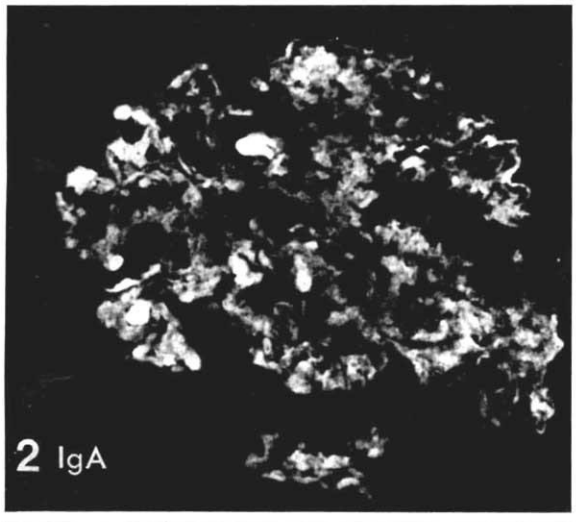
Results of this immunohistochemical investigation of the distribution of glomerular deposits of IgA and incidence of companion immunoglobulins in a variety of renal diseases are summarized in Table 2. Correlative analyses of immunoglobulin and complement component deposition will be presented categorically for each major diagnostic category.

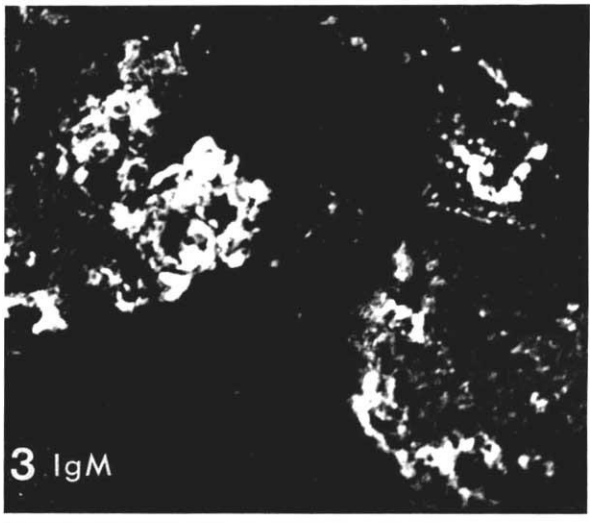
Glomerulonephritis associated with systemic lupus erythematosus (SLE) (Figs. 1–5). In the main this diagnosis

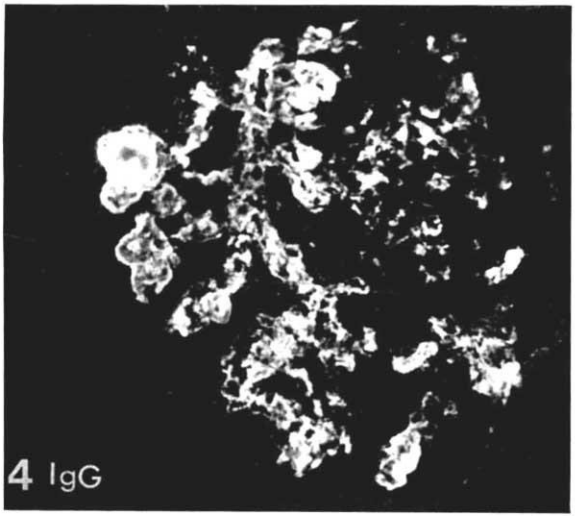
Table 2. Frequency of interassociation of localized immunoglobulins in diseased glomeruli

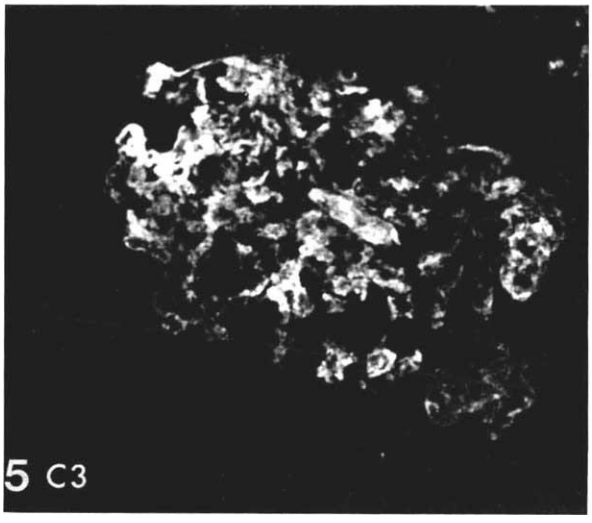
Disease	Number of Cases	IgA				IgG		IgM	
		Alone	With IgG	With IgM	With IgG+IgM	Alone	With IgM	Alone	Negative
Glomerulonephritides associated with systemic lupus erythematosus	57				34	3	18	1	1
Diffuse proliferative (acute) glomerulonephritis	40		1	2	10	6	14	1	6
Membranous glomerulopathy	28		1		8	5	14		
Membranoproliferative glomerulonephritis	43	1		3	14	3	15	6	1
Transplant rejection	41	1			3	4	12	10	11
Hyperacute rejection	1								1
Goodpasture's syndrome	13				3	2	8		
Rapidly progressive proliferative glomerulonephritis	22				6	5	9	1	1
Chronic glomerulonephritis	47			2	8	2	24	6	5
Stalk proliferative glomerulonephritis	11			1	7	1	1		1
Focal proliferative glomerulonephritis	21		1	1	10	1	4	1	3
Henoch-Schoenlein purpura	6		1		4		1		
Focal sclerosing glomerulonephropathy with hyalinosis	24		1		5	1	10	4	3
Minimal change glomerulonephropathy ("Nil disease")	22								22
Minimal glomerular alterations with indeterminable diagnosis	58		9	2	12	6	18	11	
Amyloidosis	6				3	1		1	1
Wegener's granulomatosis	4				1		1		2
Malignant arteriolonephrosclerosis	17				3 3 (art)		10		4
Diabetic glomerulosclerosis	4					2	1	1	
Chronic lobular glomerulonephritis	3					1	1		1
Thrombotic thrombocytopenic purpura	1								1











Figs. 1 to 5. *Lupus glomerulonephritis, with diffuse peripheral mesangial localization of immunoglobulins and C3 by immunofluorescence microscopy.* Fig. 1 (H & E × 200); Fig. 2 (IgA × 180); Fig. 3 (IgM, 2 glomeruli × 180); Fig. 4 (IgG × 100); Fig. 5 (C3 × 150).

was established on the basis of standard substantiating clinical features of SLE complicated by signs and histopathologic evidence of renal involvement. Though findings on renal biopsy occasionally were diagnostically insufficient or otherwise not typical of glomerulonephritis complicating SLE, more often the biopsy was substantially confirmatory and occasionally primarily responsible for the diagnosis of SLE. We recognize that glomerular alterations in renal

disease associated with SLE may consist of minimal glomerular changes, variable degrees of stalk proliferative glomerulitis, focal-segmental proliferative glomerulonephritis, a variety of mixed forms of segmental and diffuse focal proliferative glomerulonephritis, diffuse generalized proliferative glomerulonephritis, crescentic proliferative glomerulonephritis, membranoproliferative (lobular) glomerulonephritis, membranous glomerulonephropathy, and chronic glomerulonephritis. This report does not present the immunofluorescence data with reference to these various histopathologic varieties of glomerular alterations, but rather considers together all patients with SLE that have renal involvement.

Thirty-four of 57 biopsies from patients with SLE nephropathy (60%) had glomerular deposits of IgA. Localization of IgA was peripheral (focal, segmental) in 12 biopsies, mesangial (generalized, diffuse) in four biopsies, and both peripheral and mesangial (generalized, diffuse) in 18 biopsies. In all cases, IgA fluorescence patterns were granular and intensity was 2 to 3+. Focal, granular arteriolar localization of IgA was observed in four of the glomerular IgA positive biopsies. All IgA positive biopsies had associated IgG, IgM, and C3 glomerular deposits (Figs. 2–5 and Table 2). In 23 of these biopsies, patterns of IgG were the same as IgA (Figs, 2, 4) and in the remaining 11, the patterns of IgG were different than IgA. IgM was similar to IgA in 24 biopsies and was more variable in the other ten IgA positive biopsies. Glomerular localization of C3 and C4 (when tested) generally paralleled that of IgG and IgM.

Diffuse proliferative (acute) glomerulonephritis (AGN). In this category are included examples of acute glomerulonephritis of classical poststreptococcal type as well as those of unproven or idiopathic etiology which clinically and histopathologically are indistinguishable from the latter. Among 40 biopsies of patients with AGN, 13 biopsies (33%) had glomerular deposits of IgA; in only three of these was it 3+ and in the other only 1+. Typically, IgA was localized within mesangial regions in segmental, granular patterns. Peripheral localization (segmental, granular) was observed in only two biopsies. Glomerular IgG was present in all but two IgA-positive biopsies and IgM was observed in all but one IgA-positive biopsy. The patterns of IgG and IgM in these biopsies were granular, but their localization was far more diffuse and peripheral than mesangial as IgA. The C3 deposition paralleled that of IgG and IgM.

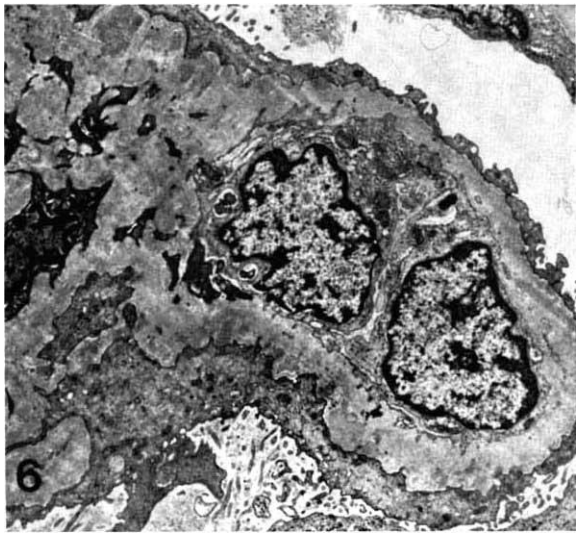
Membranous glomerulonephropathy (MbGN) (Figs. 6 to 10). Nine of 28 biopsies (32%) of MbGN had granular glomerular deposits of IgA never in excess of 2+. IgA localization was peripheral in eight biopsies (Fig. 7), focal segmental in seven, and generalized diffuse in the other. In the ninth biopsy IgA was observed focally and within mesangial regions in a granular, segmental pattern. IgG and IgM (but for one biopsy) as well as C3 were observed in diffuse or sometimes segmental, peripheral, granular patterns in all IgA-positive biopsies (Figs. 8–10). Patterns of C4, when tested, were similar to C3, IgG, and IgM.

Membranoproliferative Glomerulonephritis (MbPGN). Patients included in this recently fully characterized category of glomerulonephritides [10, 11] usually have mixed nephritic-nephrotic features, often with C3 hypocomplementemia. By light microscopy, glomeruli display variable degrees of mesangial hypercellularity and increase in mesangial membrane matrix material, often resulting in lobular accentuation of the tufts as well as variable, often marked thickening of peripheral portions of glomerular capillary walls with regions of lamination, lacy, or tramtrack alterations.

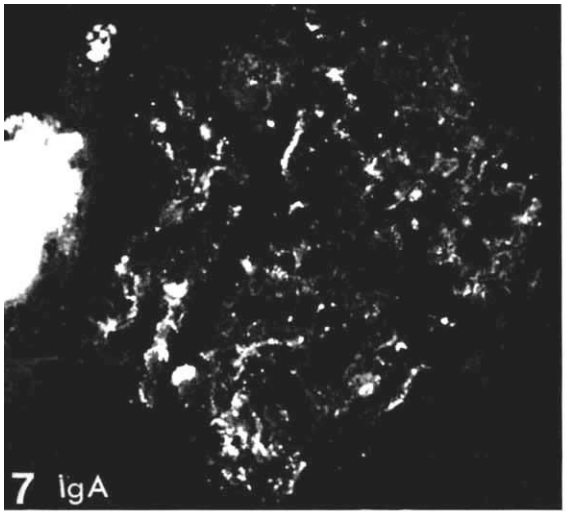
Eighteen of 43 patients (42%) with MbPGN had glomerular deposits of IgA in their kidney biopsies. In eight biopsies, IgA was localized along peripheral capillary walls in a 1 to 2+ diffuse, often widely interspersed, granular pattern. In the remaining ten biopsies IgA was present focally, most frequently within mesangial regions in 1 to 3+ segmental, granular or globular patterns. In those biopsies where IgA was peripherally located, IgM and IgG were noted in peripheral and less impressively in mesangial regions. In those biopsies where IgA was localized mainly to mesangial regions, IgG and/or IgM were similarly located. Patterns of C4 were similar to IgG or IgM. C3, however, was always of impressively greater intensity than C4, more diffusely located within mesangial and peripheral capillary walls, and although frequently correlating with localization of complement-fixing immunoglobulins, was often of much greater magnitude [10–11].

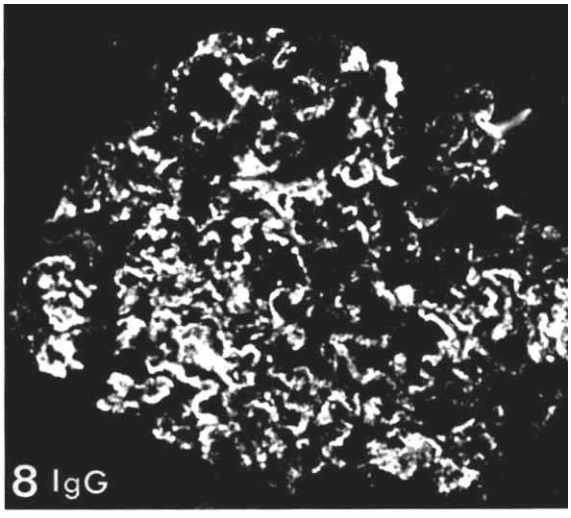
Renal allografts (Tx). Glomerular localization of IgA was present in only four of 41 Tx kidneys in various stages of rejection with or without evidence of recurrence of glomerular disease. In one of the four, there were rare 1+ focal, segmental, granular deposits of IgA within peripheral capillary walls and mesangium. No IgM or IgG was observed in this particular case. A second kidney had 1+ focal, segmental granular IgA deposits only along peripheral capillary walls with similar associated pattern of IgG, IgM, and C3. The remaining two kidneys had predominantly mesangial, 2 to 3+ segmental granular deposits of IgA along with IgG, C3, and to a lesser extent, IgM. One renal graft with hyperacute rejection reaction was negative for all immunoglobulins and complement components [16].

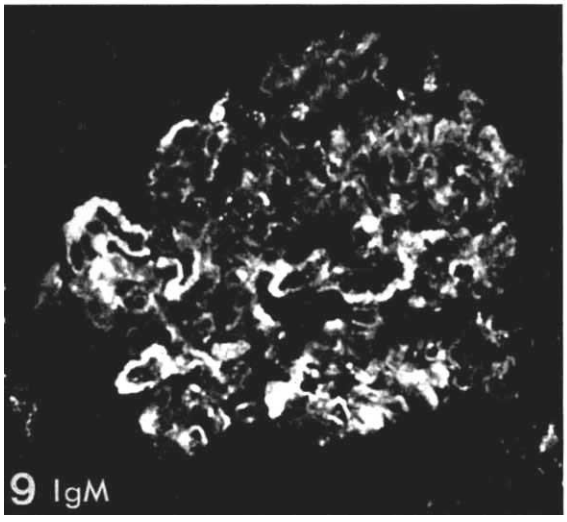
Goodpasture's syndrome. Renal biopsies from three of 13 patients with Goodpasture's syndrome had focal rare 1+ segmental granular deposits of IgA along the peripheral glomerular capillary walls. In these biopsies there was focal, both peripheral and mesangial, segmental granular deposits of IgM. All kidneys had diffuse linear IgG along glomerular capillary walls. C3 and C4 were distributed in a fashion similar to IgM rather than the IgG in the IgA positive biopsies.

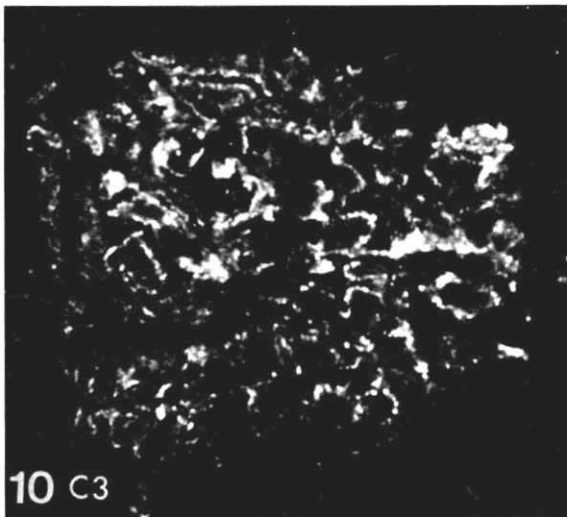


Figs 6 to 10. *Membranous glomerulonephropathy, with diffuse peripheral glomerular localization of immunoglobulins and C3 by immunofluorescence microscopy.* Fig. 6, electron micrograph, showing typical subepithelial-intramembranous electron dense deposits (× 10,300); Fig. 7, IgA (× 180); Fig. 8, IgG (× 220); Fig. 9, IgM (× 180); Fig. 10, C3 (× 190).









Rapidly progressive proliferative glomerulonephritis (RPGN). These patients usually have rapidly progressive glomerular disease clinically with extracapillary crescentic proliferation of glomerular epithelial cells. In some instances frank necrosis of glomeruli may be observed and glomerular membrane reactive immunoglobulins are demonstrable far less frequently than in Goodpasture's syndrome. This category of glomerular diseases used to be called subacute

glomerulonephritis and is variably referred to as malignant glomerulonephritis or extracapillary crescentic proliferative glomerulonephritis.

Kidney biopsies from six of 22 patients with rapidly progressive proliferative glomerulonephritis (RPGN) had glomerular deposits of IgA. Two of the six had 1 to 2+ diffuse fine granular, peripheral capillary deposits and the others had segmental or diffuse granular deposits of IgA within mesangial regions and along the peripheral capillary walls. When associated with IgA, IgG was present either in a diffuse linear pattern (two biopsies) or a diffuse fine granular pattern (four biopsies). IgM was present focally in a segmental granular pattern within the mesangium in all six IgA-positive biopsies.

Chronic glomerulonephritis (CGN). Kidney biopsies from ten of 47 patients (23%) with CGN had focally distributed IgA deposits. In all ten biopsies, deposition was segmental in both peripheral and mesangial regions as globs or clusters of granules. IgG, IgM, and C3 were present in all ten biopsies in variable localization and distribution with distinctive patterns of interassociation.

Stalk proliferative glomerulonephritis (SPGN). This category includes all those biopsies with variable but significant degrees of increased glomerular mesangial cellularity and matrix material without lobular accentuation or thickening of glomerular capillary walls in patients with nonspecific clinical features of proteinuria and mild hematuria. This morphologic category implies no specific disease entity and in fact undoubtedly comprises a heterogeneous mixture of patients with insufficient clinical and pathologic findings to render a satisfactory diagnosis. Early phases of a variety of diseases that might be recognizable later in more progressive stages, transient insignificant forms of mild glomerulitis, resolving stages of previous clinically unrecognized proliferative glomerulonephritis, or insufficient quantity of biopsied tissue missing a focal variety of glomerular disease may be conditions constituting this category of glomerular alteration.

Kidney biopsies from eight of 11 patients (73%) with SPGN had 1 to 3+ granular IgA deposits within the mesangial regions of their glomerular tufts. Five of these were focal and segmental, the other three were generalized and diffuse 2 to 3+ in mesangium. IgG, IgM, C3, and C4 (where done) were similar to IgA in seven biopsies. In one biopsy where IgG was absent, IgM and C3 were similar to IgA.

Focal proliferative glomerulonephritis (FPGN) (Figs. 11 to 20). This group comprises patients with focal-segmental proliferative or sclerotic glomerular lesions in whom systemic disease or specific renal disease have been ruled out (i.e., SLE, Henoch-Schönlein purpura, Goodpasture's syndrome, focal embolic glomerulonephritis of bacterial endocarditis, polyarteritis, etc.). Most of these cases, characterized by proteinuria and mild or recurrent hematuria, are idiopathic in origin and some presumably represent stages of resolution of previous, diffuse proliferative glomerulonephritis.

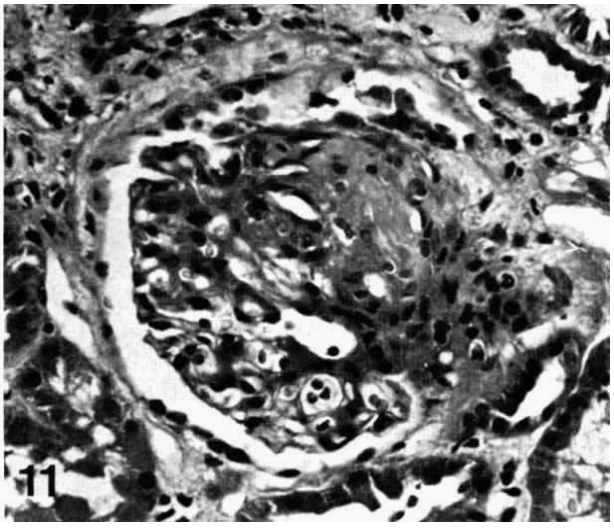
Twelve of 21 renal biopsies (57%) with FPGN were positive for IgA. Three of the 12 had generalized, diffuse mesangial, 3+ granular deposits of IgA (Fig. 12); IgG was segmentally localized in mesangial regions in two of these biopsies, and was similar to IgA in the other. Six of the 12 had generalized segmental mesangial deposits of IgA and IgG. IgM was extremely variable and did not correlate with either IgA or IgG in those nine biopsies. In the three remaining biopsies, IgA along with IgG and IgM was located in globs within one to three lobular segments of tufts, corresponding to the segmental proliferative glomerular lesions seen on light microscopy (Figs. 16–17). Distribution of C3 and C4 was similiar to that of IgG and IgM.

Henoch-Schönlein purpura (HSP). Five of six patients with clinically diagnosed HSP and associated FPGN on renal biopsy had diffuse and generalized mesangial localization of IgA. Four of these also had both IgG and IgM with C3, and one had IgG with C3 in similar distribution. The one patient who was negative for IgA but who had both IgG and IgM was a 42-year-old male with an atypical clinical presentation and a more chronic FPGN with extensive glomerular sclerosis.

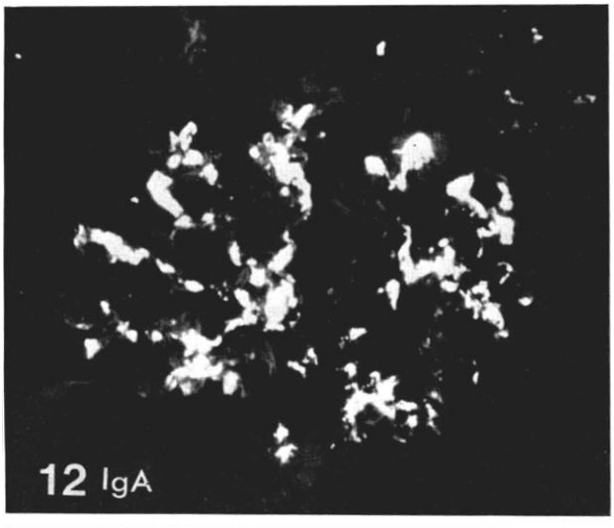
Focal sclerosing glomerulonephropathy with segmental hyalinosis (FSGN). This is a recently described, now clearly recognizable renal disease [12, 13], clinically characterized by nephrotic syndrome, usually with onset in young persons, that is progressively complicated by hypertension and renal failure. The glomerular lesion consists of focal-segmental, nonproliferative capillary sclerosis and collapse with locally prominent endocapillary foam cells and hyaline insudate containing clear vacuoles.

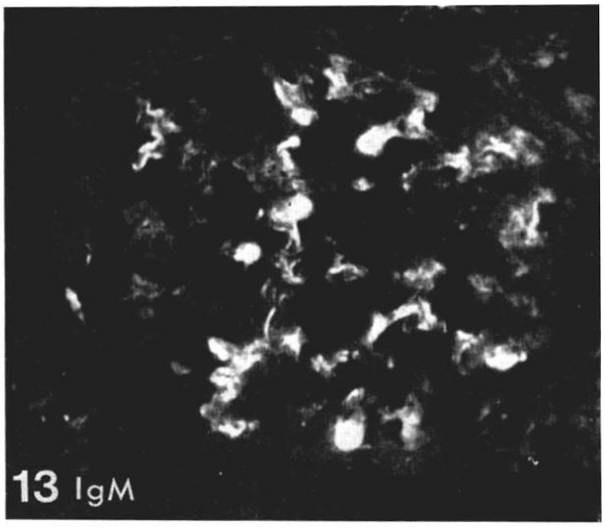
Renal biopsies from six of 24 patients (25%) with FSGN were positive for IgA. In two biopsies IgA was diffuse 3+ along with IgG and C3 within mesangial regions of all glomeruli examined. IgM was negative in one of these two biopsies and only minimally present in the other. Two other biopsies had segmental 2+ globs of IgA, IgM, IgG, and C3 in capillary walls and mesangium of most glomeruli. The remaining two biopsies had IgA, IgM, IgG, and C3 in some glomeruli only in a segmental portion of peripheral capillary walls, but not within mesangial regions.

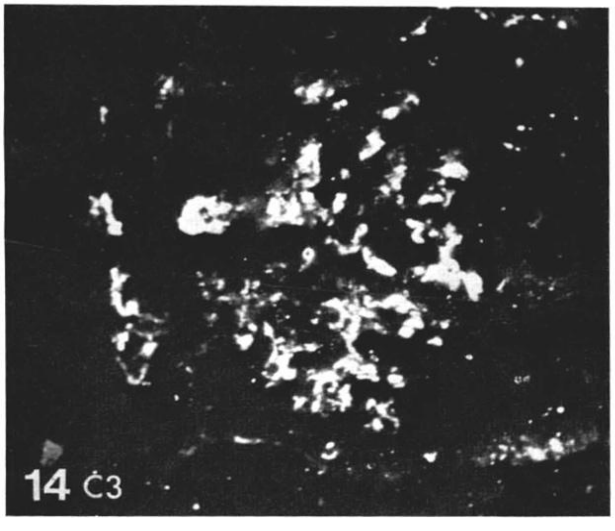
Minimal-change glomerulonephropathy (MCGN). This disease, characterized by nephrotic syndrome of idiopathic origin, commencing most often in young individuals and usually responding satisfactorily to steroid therapy, has very little in the way of glomerular alterations recognizable by light microscopy, hence the designation minimal-change glomerulonephropathy, light negative or "nil-disease." The glomeruli are usually immunofluorescence negative, and ultrastructurally, there is intermittent, often extensive, loss of epithelial foot processes, hence the alternative designation "foot-process disease."

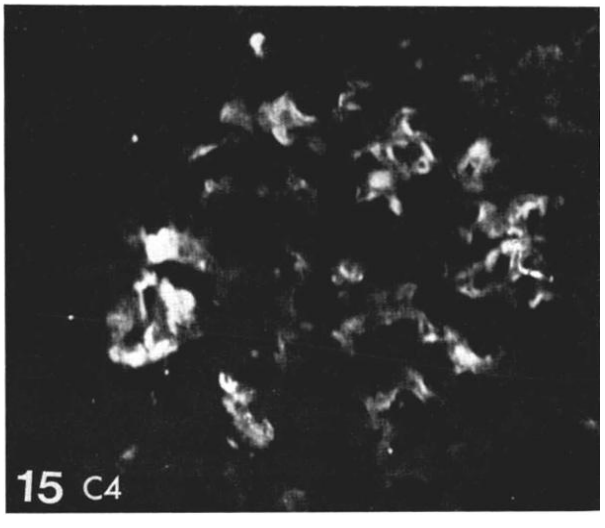


Figs. 11 to 15. *Focal proliferative glomerulonephritis, with diffuse mesangial glomerular localization of immunoglobulins, C3, and C4 by immunofluorescence microscopy.* Fig. 11, H & E (× 245); Fig. 12, IgA (× 240); Fig. 13, IgM (× 230); Fig. 14, C3 (× 170); Fig. 15, C4 (× 240).



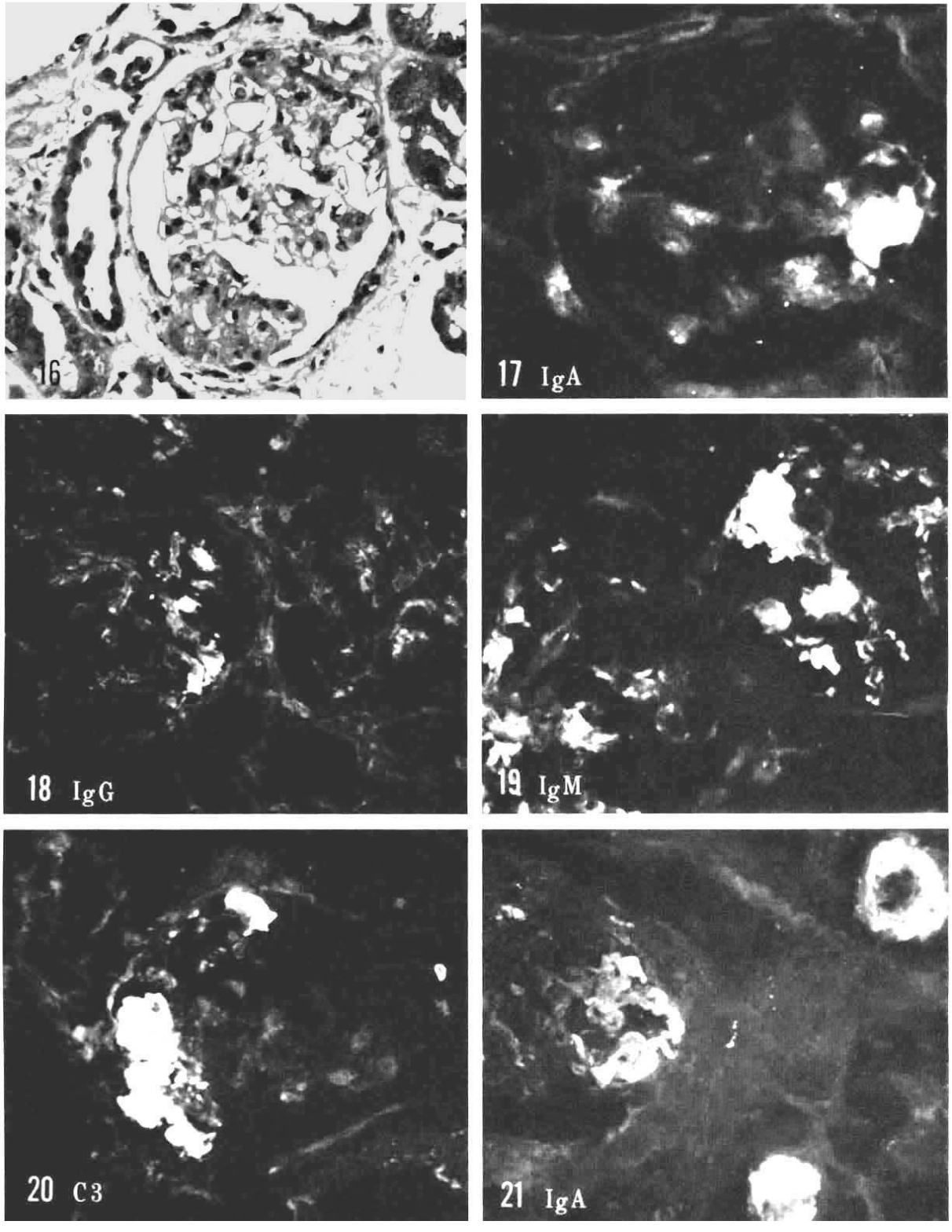






Twenty-two patients were considered by clinicopathologic criteria to have MCGN with nephrotic syndrome and these were totally negative for deposits of any immunoglobulins or C3.

Minimal glomerular changes with indeterminable diagnosis. Patients included in this group comprise those that apparently do not have idiopathic nephrotic syndrome with minimal-change glomerulonephropathy described above



Figs. 16 to 20. *Focal proliferative glomerulonephritis with segmental sclerosis, with focal, segmental, mesangial and peripheral glomerular localization of immunoglobulins and C3 by immunofluorescence microscopy.* Fig. 16, H & E (× 260); Fig. 17, IgA (× 180); Fig. 18, IgG, 2 glomeruli (× 160); Fig. 19, IgM, 2 glomeruli (× 170); Fig. 20, C3 (× 230).

Fig. 21. *Malignant arteriolonephrosclerosis showing IgA within the wall of an arteriole, mesangium and capillary walls of a glomerulus, and tubular cast.* Immunofluorescence microscopy (× 180).

and do not have sufficient glomerular alterations to be labeled as stalk or focal proliferative glomerulonephritis. These patients consitute a diagnostic problem and are included in this category because they cannot be placed in the other better defined clinical and pathologic categories included in this study. As in stalk glomerulonephritis, some biopsies in this category may represent examples either of insufficient biopsy sample, which missed a focal glomerular lesion, or of early disease yet to progress to a clinical and pathologic stage with accumulation of sufficiently diagnostic features.

Fifty-eight patients in this category had recurrent microscopic hematuria, nephrotic syndrome, or minimal persistent proteinuria with minimal glomerular changes, often indistinguishable by light microscopy from "light-negative disease." Some minimal glomerular changes were observed in this group and consisted, at the maximum, of only mildly increased numbers of mesangial cells with minimal segmental increases of PAS positive mesangial membrane matrix. Six patients had rare focal global glomerulosclerosis without any segmental glomerular lesions. Twenty-three (40%) had granular, predominantly mesangial, glomerular deposits of IgA and IgG. IgM was also present in 14 of these 23 patients, although in inconsistent, heterogenous patterns. Deposits of C3 and C4 were distributed and localized within the IgG/IgM positive regions.

Amyloidosis. Three of six patients with amyloidosis had glomerular deposits of IgA along with IgG, IgM, and C3 in segmental granular patterns in mesangium and capillary walls.

Wegener's granulomatosis. One of four patients with Wegener's granulomatosis had glomerular localization of IgA, IgG, IgM, and C3 in a limited segmental, granular distribution along peripheral capillary walls of some glomeruli. Occasional arterioles were also positive for IgA, IgM, IgG, and C3.

Malignant arteriolonephrosclerosis (Mal ANS). Three of 17 patients with Mal ANS had IgA localization along with IgG, IgM, and C3 within the walls of small arterioles (Fig. 21). Two of these three patients also had granular IgA, segmentally along peripheral portions of capillary walls in some glomeruli (Fig. 21).

Miscellaneous. Kidney biopsies from four patients with diabetic glomerulosclerosis, three with chronic lobular glomerulonephriis, and one with thrombotic thrombocytopenic purpura were all negative for glomerular deposits of IgA.

Discussion

In a group of 470 kidney biopsies from patients with various glomerulonephropathies, glomerular deposits of IgA were observed in 159 (34%) biopsies. Among these 159 biopsies, IgA was associated with IgG, IgM, and C3 in 83%, with IgM and C3 in 7%, and with IgG and C3 in 9% and occurred alone in only 1% of the biopsies. C4, where studied, generally followed the patterns of IgG and/or IgM. Glomerular deposition of IgA in five of six patients with HSP is in accordance with similar observations reported by others [1–5]. The 60% incidence of IgA in SLE is somewhat higher than that reported by others [1, 6–8]. IgA localization, though striking in SLE, was not specifically diagnostic due to marked variability in patterns of localization. In AGN, IgA was frequently observed within mesangial regions, whereas complement-fixing immunoglobulins as well as C3 were observed more characteristically along peripheral capillary walls. The incidence of IgA in MbPGN is in agreement with the findings of others [17].

Within the past five years there have been several reports [1–4] of a recently described entity: IgG-IgA glomerulopathy. This diagnosis was made primarily on the findings of IgG, IgA, and C3 deposits within glomerular mesangial regions. IgM was reported to be notably absent in most cases. Histologic changes described on light microscopy were variable, but the predominant lesion consisted of focal-segmental, proliferative and sclerotic glomerular changes with a background of minimal stalk changes or later of advanced chronic sclerotic glomerular changes. In our present series, 18 biopsies have immunofluorescence findings superficially compatible with so-called IgA-IgG nephropathy. By light microscopy one of these 18 biopsies was interpreted as AGN, one as MbGN, and one as FSGN. In addition, four of these biopsies were consistent with SLE with predominantly mesangial IgA, IgG, and less IgM and C3. One positive biopsy was obtained from a patient with HSP. If these cases with specific clinicopathologic diagnoses are excluded from consideration, this leaves ten patients as possibly having IgG-IgA glomerulopathy. Nine were interpreted as having minimal glomerular changes with indeterminable diagnosis and one as FPGN. The maximum incidence of so-called IgA-IgG nephropathy in our total series of 470 patients, therefore, would be 2.1%, significantly less than the 18% reported by Berger [1]. Whether IgA-IgG nephropathy can or should be singled out as a distinct entity is questionable on the grounds that immunofluorescence and light microscopic glomerular alterations, as well as presumably etiologic and pathogenetic mechanisms, are mimicked more frequently by a variety of other well characterized glomerular diseases.

In light of this present study, deposition of IgA apparently is not diagnostically specific as indicated by the heterogeneous variety of glomerulopathies, patterns, and incidence of companion globulins with which it is observed. Moreover, demonstration of glomerular deposition of IgA, though sometimes helpful, is basically of little practical value in the differential diagnosis of similar appearing glomerular lesions. For example, the presence and distribution of IgA may be similar in focal, segmental, or proliferative glomerular lesions of SLE, HSP, and FPGN, and in some examples of minimal glomerular alterations or SPGN. However, comparative identification and characterization of IgA deposition with other immunoglobulins

may be of some diagnostic value. Therefore, it appears more logical to continue using a system of diagnostic nomenclature based on combined clinical and histopathologic findings, and to use IgA positivity along with the presence or absense of other immunoglobulins in an attempt to further elucidate possible immunologic mechanisms in the pathogenesis of glomerulonephropathies.

The immunopathologic role of IgA in renal glomerular disease is unclear. Seldom does IgA appear as the only localized immunoglobulin in diseased glomeruli, and therefore, if localized complement-fixing immunoglobulins (IgG and IgM) do play a pathogenetic role in certain glomerulonephropathies (and considerable evidence favors this), then it is difficult to implicate IgA itself as a single or major cause of glomerular lesions. A recent report indicating that aggregated IgA may initiate complement activity at the C3 reaction step [18] suggests a possible synergistic pathogenetic contribution by deposits of IgA. However, it was quite apparent in this study that patterns of localized host C3 (and parenthetically *in vitro* fixation of guinea pig C3 and C4) parallel more closely those patterns of IgG and/or IgM rather than IgA. Furthermore, since IgA is most often found with other immunoglobulins in glomeruli in a wide variety of glomerular lesions and disease processes, it is difficult to ascribe any primary pathogenetic mechanism or specific significance to IgA localization.

Very little is known about mediator mechanisms associated with IgA complexes or about immunologic activity of IgA in regions other than mucosal surfaces, and so it is difficult to assess the consequences of IgA localization in glomeruli. In selected cases of HSP, high levels of circulatory IgA have been identified and have led to speculation of a possible IgA-mediated hypersensitivity or immune-complex phenomenon related to predominant IgA immune response in respiratory or gastrointestinal microbial infections or allergies [19]. In a few cases of lupus erythematosus, antinuclear antibodies of the IgA class have been identified, and it therefore is possible that selected individuals might localize IgA antinuclear complexes in glomeruli during development of SLE nephropathy [20]. Also the rare occurrence of rheumatoid factors of the IgA class might on occasion account for localization of IgA antiglobulin at glomerular sites of previously bound IgG or IgM immunoglobulins [21]. The common mesangial location of IgA may, on the one hand, reflect preferential phagocytosis of IgA complexes by mesangial cells, or on the other hand, possible migration from capillary walls of noncomplement fixing IgA complexes which, in contrast to complement-fixing immune aggregates, might be less likely to remain bound in peripheral portions of glomerular capillary walls.

An unlikely, but tantalizing explanation for the predominant mesangial localization of IgA is possible local synthesis and secretion of IgA by mesangial cells in selected glomerular injuries. Whether the glomerular bound IgA is of the circulating or secretory class of IgA is not yet clear, but results of preliminary studies would suggest that secretory IgA may be present in deposits since secretory piece has been identified with specific antisecretory piece antibody in the same location as IgA in a few cases [Hyman, L.R., Burkholder, P.M., Zimmerman, S., and Hong, R., unpublished observations].

If IgA is deposited in glomeruli in immune aggregates, then the nature of the possible antigens involved is unknown. IgA antibodies reactive with diphtheria [22], coliform, or other common bacteria, viruses (commonly enteric or respiratory) [23] or nuclear antigens [20] have been identified and could be responsible for immune-complex deposition in glomerular capillaries in selected circumstances. Of possible correlative significance is exacerbation of symptoms of renal disease in MbPGN, SLE nephritis, minimal glomerular changes, FPGN, or HSP in apparent association with upper respiratory infections of viral or other microbial etiology. Even though presence of IgA in glomerular lesions is not clearly diagnostically specific, and the nature and significance of localized IgA are not understood, it is hoped that continued assessment for the presence and possible participation of IgA in glomerulonephropathies will provide new clues.

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