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THE SECRETORY IMMUNE SYSTEM AND RENAL DISEASE

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SUMMARY

An immunopathological analysis of renal tissue from 105 patients was undertaken: (1) to clarify the relationship of the secretory immune system to renal diseases in which glomerular deposits of immunoglobulin A, (α chain), occurred; (2) to determine the lower nephron localization of secretory component and α chain in renal disease. This study, which included twenty-four patients with glomerular deposits of alpha chain, failed to reveal glomerular localization of secretory IgA. Secretory component was not found in renal tubular cells in kidneys with normal or minimally abnormal renal histology. In contradistinction to these findings, significant amounts of secretory component were found in tubular epithelial cells and casts in tissue from fifty-one patients with morphological evidence of significant renal damage; this localization had no correlation with glomerular deposits of IgA, IgM or other immunoreactants. α Chain was rarely found in the tubular epithelium or in interstitial round cells; fifteen patients had α chain in casts. We conclude that the glomerular localization of immunoglobulin in glomerulonephritis is not derived from the secretory immune system, and the IgA present in glomeruli is not secretory IgA. The finding of secretory component in tubular cells in diseased kidneys without α chain may support an hypothesis for an independent role for secretory component in renal disease, apart from its function in the transport and stabilization of secretory IgA.

INTRODUCTION

IgA is the major immunoglobulin in secretions that bathe, or are contiguous with, mucous-epithelial lining membranes (Tomasi *et al.*, 1965). Secretory IgA (S-IgA) possesses a unique glycoprotein called secretory component (SC), that enables this immunoglobulin to resist proteolytic enzyme digestion, acid breakdown and reductive inactivation, and facilitates transport onto lumenal surfaces (Tomasi *et al.*, 1969; Tomasi & Grey, 1972; Jerry, Kunkel

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& Adams, 1972). In addition, SC has been found to combine with 19S IgM (Thompson, 1970); this immunoglobulin shares the property of being selectively transmitted into human exocrine secretions (Eskeland & Brandtzaeg, 1974; Radl *et al.*, 1971). SC is produced independently by epithelial cells and has been the identifying protein for the secretory immune system; an immunological barrier which is thought to represent the first line of defense against both replicating and non-replicating antigens.

Recently, some clinical and experimental observations have suggested a possible interrelationship between the secretory immune system and the pathogenesis of some forms of immune mediated renal disease: (1) the onset and/or exacerbations of certain types of glomerulonephritis with mesangial deposits of IgA, IgM and IgG are in close temporal proximity to an upper respiratory illness (Urizar *et al.*, 1968; Berger, 1969; Lowance, Mullins & McPhaul, 1972; Morel-Maroger, Leathem & Richet, 1972; Hyman *et al.*, 1973; McCoy, Abramowsky & Tisher, 1974; Van De Putte, De La Riviere & Van Breda Vriesman, 1974); (2) serum levels of IgA have been found to be elevated in patients with anaphylactoid purpura (Trygstad & Steihm, 1971) and a majority of patients with idiopathic recurrent macroscopic haematuria; (3) serum levels of S-IgA have been detected in 59–96% of normal controls, while elevated levels of S-IgA have been found in certain patients with immune complex-associated disease states, e.g. rheumatoid arthritis (Thompson, Asquith & Cooke, 1969; Thompson & Asquith, 1970; Waldman *et al.*, 1971; Brandtzaeg, 1971); (4) the IgA fraction from the serum of a patient with recurrent gross haematuria and episodes of renal insufficiency has been shown to trigger *in vitro* the alternative pathway of complement activation in the cold (Day *et al.*, 1973).

It seemed particularly important, therefore, not only to clarify whether the IgA that was involved in glomerulonephritis was S-IgA but to establish whether SC could be localized within the glomerulus coincidentally with IgM. We thus undertook an immunohistochemical analysis of renal tissue from patients with a variety of diseases during a period of 1 year in order to investigate the role of the secretory immune system in the pathogenesis of glomerulonephritis, and to determine the comparative lower nephron distribution of S-IgA, SC and α chain.

MATERIALS AND METHODS

Patients

All 105 patients were seen at the affiliated hospitals of the University of Minnesota or outlying area hospitals which send biopsy specimens for immunopathological diagnosis.

These patients were divided into three groups.

Group 1 consisted of seventeen living-related renal allograft donors without known renal disease. These patients were considered to be normal controls. Their ages ranged from 25–40 years. Biopsies of the kidney were taken prior to anastomosis of the graft to the recipient.

Group 2 consisted of twenty-four patients with a variety of diseases known to have α chain deposits within the glomerulus (Table 1). Their age range was 5–50 years.

Group 3 consisted of sixty-four patients with a variety of renal diseases not shown to be associated with glomerular deposits of α chain. Twenty patients had a μ chain glomerular deposits. Also within this group were fourteen patients with renal allografts; all fourteen had a clinical and/or pathological diagnosis of rejection; nine of these patients had documented cytomegaloviruria (see Table 2). Their age range was 2–55 years.

TABLE 1. Comparative relationships of a α chain and secretory component in the human nephron. Immunopathological analysis of group 2: twenty-four patients with glomerular deposits of α chain

Number of patients	Disease	Glomerular locale of α chain*	Other immune reactants in the glomerulus	Number of patients with deposition of secretory component in:	
				Glomeruli	Tubular epithelium
6	Anaphylactoid purpura	Mesangial-peripheral capillary loop	C3, fibrin, IgG, IgM	0	3
7	Recurrent haematuria and focal nephritis	Mesangial	C3, fibrin, IgG, IgM	0	3
5	Systemic lupus erythematosus	Mesangial-glomerular basement membrane	C3, IgG, IgM	0	3
1	Vasculitis	Peripheral capillary loop	C3, IgG	0	1
1	Idiopathic nephrotic syndrome and focal nephritis	Peripheral capillary loop	IgM	0	0
1	Chronic glomerulonephritis	Mesangial	C3, properdin	0	1
2	Familial hepatic fibrosis & glomerulonephropathy	Glomerular basement membrane	C3, IgM	0	2
1	Recurrent hematuria & renal insufficiency associated with IgA-mediated complement activation in the cold	Mesangial	C3, IgG, properdin	0	1

* α Chain was not demonstrated in tubular epithelium.

Hospital charts were retrospectively analysed for the following parameters: protein and lysozyme excretion; serum creatinine and creatinine clearance on the day of, or the day preceding, the biopsy.

Pathology—immunopathology

Tissue was obtained by percutaneous or open renal biopsy in 101 out of 105 patients; there were four autopsy specimens. Four-micron paraffin sections were stained for Haematoxylin–Eosin, periodic acid-Schiff and azocarmine. Tissue was also frozen in isopentane precooled in liquid nitrogen, and sectioned at 4 μm in a Lipshaw cryostat. The methods have been described elsewhere (Michael *et al.*, 1966, 1971). Frozen sections were stained with fluorescein (FITC) conjugated antisera to α chain, SC and other antigens; in some tissues indirect immunofluorescence was employed for detection of α chain and SC. In addition, the distribution of lactoferrin (LF) was analysed using rabbit anti-lactoferrin (RALF) and the indirect immunofluorescent technique (see below).

Antigens

IgA was isolated from both IgA myeloma and normal serum by the method of Litman &

Good (1972). IgM was isolated by the method of Chaplin, Cohen & Press (1965). The methods for isolation of IgG, C3, properdin and fibrin have been previously reported (Michael *et al.*, 1966, 1971; Westberg *et al.*, 1971, McLean & Michael, 1973).

Human colostrum was collected from approximately 100 volunteers within 36 hr, postpartum. All specimens were immediately frozen at −70°C, stored at −20°C, thawed once, and then pooled and divided into two batches. The first batch was used to isolate LF by the method of Querinjean, Masson & Heremans (1971), with the following modification: the CM—Sephadex (Pharmacia, Fine Chemicals, A.B., Uppsala, Sweden) was serially eluted with 20 mM of phosphate-buffered saline (PBS), pH 7·0, containing 0·25 M NaCl and 0·33 M NaCl. In addition, 0·5 M NaCl was added twice; the last peak yielded 162·8 mg after lyophilization. Using the immunoreagents described below, this eluate was shown to contain lactoferrin and trace amounts of human IgG.

S-IgA was prepared by a modification of Tomasi's original method (Tomasi, *et al.*, 1965; Tomasi & Bienenstock, 1968; Mestecky, Kulhary & Kraus, 1972; Jerry *et al.*, 1972). The ammonium sulphate-precipitated colostrum, previously decaseinated and freed from lipid, was dialysed against PBS (pH 7·4) and then sequentially passed through columns of DEAE (Biorad, Richmond, California), Sephadex G-200, (Pharmacia, Fine Chemicals, A.B., Uppsala, Sweden) and Biogel A 1·5 m (Biorad, Richmond, California).

Antisera

Fluorescein-conjugated immunoglobulin to human IgG, C3, fibrin, properdin, albumin, factor B and alpha-2-macroglobulin were prepared by previously described methods (Michael *et al.*, 1966, 1971; Westberg *et al.*, 1971; McLean & Michael, 1973). Goat antisera were prepared against normal human IgA and normal human IgM. Both antisera were passed through a Sepharose 4B column (Pharmacia, Fine Chemicals, A.B., Uppsala, Sweden) conjugated with IgG. In addition, the anti-IgM antisera was passed through a Sepharose column conjugated with IgM-deficient serum in order to remove a contaminating antibody to an 11S beta-globulin. Each antisera reacted by immunodiffusion (Ouchterlony, 1958) and immunoelectrophoresis (IEP) (Scheidegger, 1955), selectively with the respective immunizing antigen. No cross-reactivity could be demonstrated between antisera to IgA, IgM and IgG by immunofluorescent microscopy of tissue from patients with renal disease having varying distributions and intensities of immunoglobulin (Ig) glomerular deposits (Michael & McLean, 1974).

Commercially prepared anti-whole breast milk was obtained from Kallestad Laboratories, Minneapolis, Minnesota.

Rabbit anti-human lactoferrin (RALF) was obtained from Nil-Nordic Diagnostics, Tilburg, Holland. The purity and specificity of this reagent was proven as follows: (1) IEP and Ouchterlony double diffusion analysis revealed no precipitin reaction against normal human serum at serial dilutions; (2) with the double diffusion technique a single line was demonstrated against whole human colostrum, and this line showed identity with isolated lactoferrin; (3) by indirect immunofluorescence the RALF could be shown to react with human leucocytes and human placental tissue, as previously described by Tourville *et al.* (1969) and Masson, Heremans & Schonne (1969), but did not react with human lymph node tissue. Fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit IgG was utilized in the second layer of the indirect technique with proper controls as described elsewhere (Michael *et al.*, 1966, 1971).

Goat antiserum, specific for the hidden 1 and exposed A_2 determinants of SC was given to us by Dr Per Brandtzaeg, Oslo, Norway. Goat antiserum to S-IgA which was isolated originally from human colostrum, was prepared by a modification of the methods of Tomasi *et al.* (1965) and Tomasi & Bienenstock (1968), by Dr Richard Hong, University of Wisconsin School of Medicine, Madison, Wisconsin. This antiserum was passed successively through a Sepharose 4B column (Pharmacia, Fine Chemicals, A.B., Uppsala, Sweden) conjugated to IgA myeloma serum* and lactoferrin. Immunodiffusion analysis revealed no reactivity with normal human serum,† IgA, lactoferrin, and components of human colostrum other than S-IgA. A single line was present on Ouchterlony analysis of the absorbed antiserum against whole colostrum obtained shortly after delivery. (This single line was thought to represent a reaction with S-IgA, since free SC is scarce in early colostrum.) A single line was also present when the antiserum was reacted against isolated S-IgA. FITC-conjugated goat anti-SC (GASC) brilliantly stained renal tubular structures as well as glomerular deposits in a variety of renal diseases. However, when this antiserum was serially diluted, glomerular staining disappeared early, while the intensity of fluorescence for tubular epithelium and casts persisted at much higher dilutions ($>1:256$). The glomerular fluorescence completely disappeared when GASC was absorbed with IgG and/or IgM moieties containing both heavy and light chains; however, there was no change in the intensity of the renal tubular or cast fluorescence. These findings were thought to reflect an antibody contaminant to a light chain determinant not removed by immunoabsorption with IgA myeloma serum. Therefore, the antiserum was further purified by passing it through a Sepharose 4B column (Pharmacia, Fine Chemicals, A.B., Uppsala) conjugated to human IgG. The resulting antiserum gave a single precipitant line with S-IgA. Immunofluorescent studies with this FITC antiserum (F/P ratio of $8{\cdot}6 \times 10^{-3}$) revealed: (1) positive

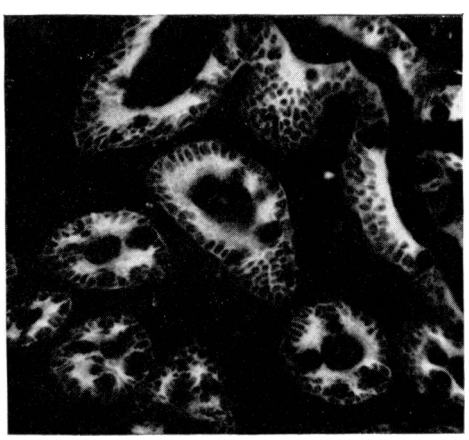

FIG. 1. An intestinal biopsy from a patient with combined immunodeficiency. Immunofluorescent staining revealed diffuse, homogeneous cytoplasmic localization of SC in villus epithelium. Note the negative nuclear shadow. (Magnification $\times 245$.)

* Dr T. B. Tomasi of Mayo Clinic, Rochester, Minnesota, analysed the goat anti-SC antiserum prior to its passage over the Sepharose 4B column. This serum was reported to contain antibodies to LF and SC.

† Normal serum levels of S-IgA are unable to be detected by this method.

staining for renal tubular epithelium and casts, as well as brilliant staining of intestinal epithelium from a patient with combined immunodeficiency and undetectable amounts of IgA (see Fig. 1); (2) negative staining on human lymph node tissues and human leucocytes; (3) negative staining for renal tubular epithelium and casts after absorption with isolated S-IgA; whereas no inhibition was observed after absorption with IgG, IgM or lactoferrin.

RESULTS

Group 1

Seventeen living-related allograft donors were evaluated by immunofluorescent and routine histological techniques. All routine sections except one were normal. There was no tissue localization of IgA, SC, lactoferrin or other proteins in sixteen of these tissues. In addition, no SC was detected by indirect immunofluorescence in tissues from seven of these patients. The tissue of one donor with interstitial fibrosis of unknown etiology had focal localization of tubular SC within a scarred area of the biopsy.

Group 2

α Chain was demonstrated in glomeruli from twenty-four patients (Table 1), in a mesangial distribution in sixteen, and in a glomerular basement membrane (Fig. 2a) or capillary loop orientation in eight. Glomerular μ chain was detected in sixteen of these patients. SC was not detected in the glomerulus of any patient.*

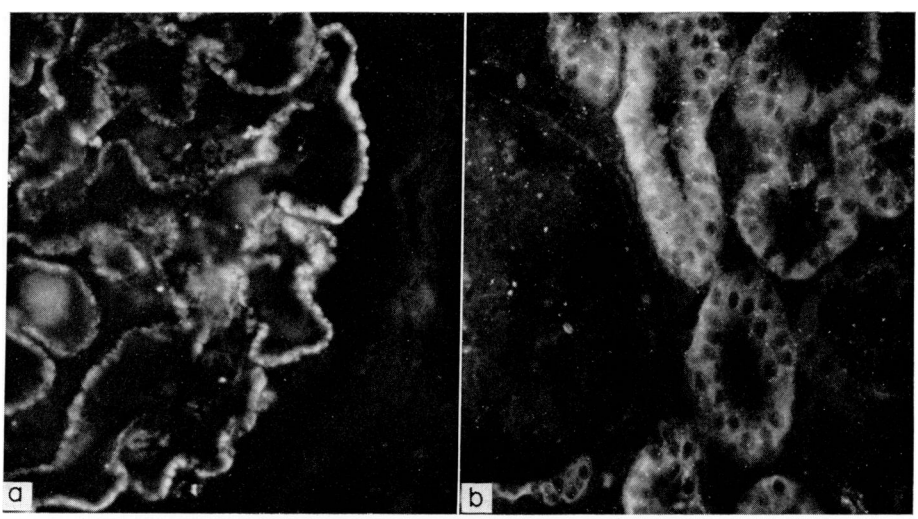

FIG. 2. Immunofluorescent micrograph of kidney tissue from a patient with a renal allograft who developed recurrent membranous nephropathy. (a) Stained for IgA (α chain): diffuse microgranular deposits are present in the glomerular basement membrane. Tubular cytoplasm, seen to the right, was negative for IgA. The patient also had IgG, IgM and C3 deposits located on the glomerular basement membrane. (Magnification $\times$ 720). (b) Stained for SC: diffuse homogeneous tubular epithelial localization was observed; glomeruli are negative. Positive staining was also seen in the epithelial lining of Bowman's space (lower left). No tubular epithelial staining was noted in this biopsy for other proteins. (Magnification $\times$ 320.)

* In a subsequent analysis of twenty-two patients with glomerular deposits of α chain we have found no evidence of glomerular localization of SC utilizing the indirect immunofluorescent technique.

In contradistinction to the findings in normal donors, SC was detected in tubular epithelium of fourteen out of twenty-four patients. Staining for SC was localized in a homogeneous pattern within the cytoplasm of the tubular cells (Fig. 2b). There was a negative nuclear shadow, and the staining was contiguous with adjacent cells. Available resolution could not distinguish intercellular staining. α Chain was not found in the tubular epithelium of any patient. Neither SC nor α chain could be demonstrated in casts. One patient with SLE and mesangial deposition of IgA also had tubular basement membrane staining for α chain. No patient had α chain localization in cells in the interstitium.

A comparison of patients with anaphylactoid purpura or recurrent haematuria who had demonstrable tubular SC with patients with these diseases who did not, revealed no differences with respect to: serum creatinine, creatinine clearance, proteinuria and glomerular and tubular histology.

Group 3

An immunopathological analysis of tissue from sixty-four patients without α chain in the glomerulus is outlined in Table 2. Twenty of these patients also had μ chain demonstrable within the glomerulus. None of these patients had glomerular SC. However, in thirty-three of sixty-four patients with a variety of renal diseases, SC was localized within the tubular epithelium; twenty-four of these patients also had SC in casts. An additional three patients had unique localization of SC in casts, but not in tubular epithelium: two with chronic glomerulonephritis and end stage kidney disease, and one patient with congenital nephrotic syndrome. Casts were found predominantly within distal tubules. The tubular epithelial and/or cast localization of SC was especially common in patients with damaged kidneys, e.g. chronic glomerulonephritis, and allograft rejection with and without cytomegaloviruria (Fig. 3). Conversely, tissue with nil or minimal histological abnormalities in the glomerulus had little or no tubular staining; SC was demonstrated in only one of seven specimens with nil lesion nephrotic syndrome, none of five patients with mild mesangial hyperplasia associated with recurrent microscopic haematuria, and one of six patients with interstitial nephritis–

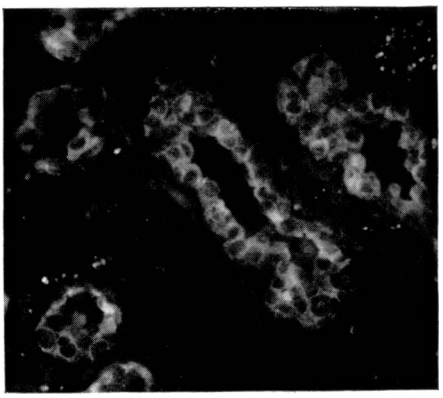

FIG. 3. Renal allograft rejection: representative section of renal tissue demonstrating diffuse immunofluorescent staining for SC in the tubular epithelium. This localization was characteristic of the cytoplasmic tubular fluorescence seen in ten out of fourteen patients with homograft rejection. (Magnification ×280.)

TABLE 2. Comparative relationships of α chain and secretory component in the human nephron. Immunopathological analysis of group 3: sixty-four patients with varied renal diseases with no glomerular deposits of α chain

Number of patients	Disease	Number of patients demonstrating localization of:			
		α Chain in:		SC in:	
		Tubular epithelia	Casts	Tubular epithelia	Casts
16	Immune-mediated renal disease*	0	1	12	8
3	Diabetes	0	2	2	2
6	Interstitial nephritis-pyelonephritis†	0	1	1	1
3	Infantile nephrotic syndrome‡	0	2	1	2
5	Idiopathic microscopic haematuria–minimal mesangitis	0	0	0	0
2	Haemolytic uraemia syndrome	0	0	0	0
1	Post-partum renal failure	0	0	1	1
5	Rejection	0	2	3	2
9	Rejection with cytomegaloviruria	0	3	7	3
5	Chronic glomerulonephritis and end-stage kidneys	0	4	3	5
2	Amyloidosis	0	0	2	2
7	Nil-lesion nephrotic syndrome	0	0	1	1
Total 64		0	15	33	27

* Specific disease and number of patients: membranoproliferative glomerulonephritis, five; idiopathic membranous nephropathy, three; nephritis with cryoglobulinaemia, one; *Staphylococcus albus* shunt nephritis, one; vasculitis, three; Goodpastures syndrome, one; systemic lupus erythematosus, one; rapidly progressive non-streptococcal nephritis, one.

† Idiopathic interstitial nephritis, three; obstructive uropathy and pyelonephritis, three.

‡ Congenital nephrotic syndrome, two; infantile glomerulonephritis with the nephrotic syndrome, one.

pyelonephritis. However, there was no correlation between the presence of SC in tubules and the occurrence of glomerular deposits of any immune protein.

Biopsy tissue from fifteen of these sixty-four patients revealed casts containing both α chain and SC; in twelve patients, however, SC was present in casts without α chain. Tubular epithelial localization of α chain was not found in a significant amount in any patient in this group. Verification of the sensitivity of the direct immunofluorescent technique was demonstrated by an analysis of an additional twenty-five patients with various forms of renal disease using the indirect immunofluorescent technique. Tubular localization for IgA was not found whereas SC could be easily demonstrated.

In comparing patients with rejection, who had demonstrable tubular SC with those who did not, no differences were shown with respect to the following: levels of serum creatinine,

creatinine clearance, protein and lysozyme excretion, number of rejection episodes, time after transplantation, light histology, and type of biopsy, i.e. nephrectomy or autopsy. A similar analysis was carried out, comparing the one patient with tubular epithelial localization of SC who had idiopathic nephrotic syndrome to the other seven in the same diagnostic category; no correlations could be made.

Scattered cells with α chain localization were often observed in the renal interstitium of tissue from patients with allograft rejection, with or without cytomegaloviruria.

Lactoferrin

No kidney localization of LF was observed in tissue from normal donors or from any patient in group 2. In biopsy specimens from group 3, scattered interstitial and tubular luminal localization of LF was occasionally seen, especially in end-stage kidney disease, rejection and pyelonephritis. However, no glomerular deposits were seen, and no definite tubular or interstitial pattern could be determined.

DISCUSSION

SC was not found in the glomeruli of eighty-eight patients with a variety of renal diseases, including twenty-four patients with glomerulonephritis associated with glomerular deposits of α chain. These results suggest two conclusions: (1) glomerular IgA lacks SC and is therefore not S-IgA; (2) glomerular deposits of immunoglobulin are not derived from the secretory immune system. Our observations are in agreement with unpublished studies cited by Lowance, Mullins & McPhaul (1973), but are in disagreement with the preliminary studies of Hyman *et al.* (1973), and the report by McCoy *et al.* (1974) who suggested that SC was present coincidentally with α chain in the glomeruli of some patients with nephritis (two out of fifteen in the latter review).

Immunopathological studies have demonstrated glomerular deposits of α chain in focal glomerulitis associated with recurrent macroscopic haematuria, anaphylactoid purpura, membraneous nephropathy and lupus erythematosus (Berger, 1969; Morel-Maroger *et al.*, 1972; Urizar *et al.*, 1968; Lowance *et al.*, 1972; Hyman *et al.*, 1973; McCoy *et al.*, 1974). Many of these studies have also shown glomerular deposition of C3. Although initial reports failed to demonstrate that IgA was able to fix complement, Day *et al.* (1973) have demonstrated that an IgA serum fraction from a patient with recurrent gross haematuria activates the alternative complement pathway in the cold. In addition, a recent immunohistochemical analysis of tissue from thirty-four patients with dermatitis herpetiformis implicated IgA with alternate complement pathway activation. In this latter study S-IgA was not found in the four skin biopsies examined (Seah *et al.*, 1973).

SC was found in the renal tubule (proximal and distal tubular epithelia and/or casts) in biopsies from fifty out of eighty-eight patients with a variety of diseases, but was not found in the normal or minimally abnormal kidney. There was no relationship between SC localization in the lower nephron and the presence or absence of glomerular deposits of α chain. In contrast to a previous report by Paronetto & Koffler (1965), α chain was rarely seen in the tubular epithelium of any patient, but was present in casts of approximately one-fourth of those tissues with tubular SC. Although the immunopathological technique utilized was not designed to localize diffusible proteins by freeze substitution methods (Post, 1965), SC was found in casts in a greater number of biopsies than α chain. The presence of SC without significant IgA in epithelial cells in diseased kidneys suggest local synthesis and/or secretion

in this site, rather than the reabsorption of free SC or S-IgA which had been filtered through an abnormally porous glomerulus; or free SC after it had been cleaved from the parent molecule within the nephron. With the exception of three patients with IgA deficiency, where SC was thought to be 'attached' to IgM, there are no reports that SC is present in human serum as free polypeptide (Thompson, 1970). In addition, Bienenstock & Tomasi (1968), were unable to find degradation products on sucrose density gradient ultracentrifugation, when labelled S-IgA was added to normal urine and incubated.

Our failure to find tubular localization of SC in kidneys that were normal or minimally abnormal is contrary to the studies of Tourville *et al.* (1969), who found positive staining for SC in tubular epithelia and lumena, using the direct immunofluorescent technique, in tissue from six surgically removed kidneys; histological and functional studies were not reported. In a more recent report (McCoy *et al.*, 1974) S-IgA was observed in tubular lumina in a number of the fifteen biopsies that had α chain glomerular deposits. In our study of 105 biopsies all thirty-three specimens which had localization of SC in casts within tubular lumina had evidence of tissue destruction; parallel functional data supported this observation. These findings indicate a very significant quantitative difference in the relative amounts of SC produced in diseased nephrons as compared to the normal nephron. Tubular production of SC may be a reflection of a cellular response to non-immune or immune injury, and may be initiated without the involvement of α chain. SC, therefore, could have an additional biological role within the kidney quite apart from its function in the transport and stabilization of S-IgA.

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REFERENCES

BERGER, J. (1969) IgA glomerular deposits in renal disease. *Transplant. Proc.* **1,** 939.

BIENENSTOCK, J. & TOMASI, T.B. (1968) Secretory IgA in normal urine. *J. clin. Invest.* **47,** 1162.

BRANDTZAEG, P. (1971) Human secretory immunoglobulins. V. Occurrence of secretory piece in human serum. *J. Immunol.* **106,** 318.

CHAPLIN, H., COHEN, S. & PRESS, E.M. (1965) Preparation and properties of the peptide chains of normal human 19S globulin (IgM). *Biochem. J.* **95,** 256.

DAY, N.K., GEIGER, H., MCLEAN, R., RESNICK, J., MICHAEL, A.F. & GOOD, R.A. (1973) The association of respiratory infection, recurrent hematuria and focal glomerulonephritis with activation of the complement system in the cold. *J. clin. Invest.* **22,** 1698.

ESKELAND, T. & BRANDTZAEG, P. (1974) Does J chain mediate the combination of 19S IgM and dimeric IgA with the secretory component rather than being necessary for their polymerization? *Immunochemistry,* **11,** supplement 161.

HYMAN, L.R., WAGNILD, J.P., BEIRME, G.J. & BURKHOLDER, P.M. (1973) Immunoglobulin A distribution in glomerular disease: analysis of immunofluorescence localization and pathogenetic significance. *Kidney Int.* **3,** 397.

JERRY, L.M., KUNKEL, H.G. & ADAMS, L. (1972) Stabilization of dissociable IgA proteins by secretory component. *J. Immunol.* **109,** 275.

LITMAN, G.W. & GOOD, R.A. (1972) Rapid purification of IgA from normal human sera. *Biochem. biophys. Acta,* **263,** 89.

LOWANCE, D.C., MULLINS, J.D. & MCPHAUL, J.J. (1973) Immunoglobulin A (IgA) associated glomerulonephritis. *Kidney Int.* **3,** 167.

MASSON, P.L., HEREMANS, J.F. & SCHONNE, E. (1969) Lactoferrin, an iron-binding protein in neutrophilic leukocytes. *J. exp. Med.* **130,** 643.

MCCOY, R.C., ABRAMOWSKY, C.R. & TISHER, C.C. (1974) IgA nephropathy. *Amer. J. Path.*, **76,** 123.

MCLEAN, R.H. & MICHAEL, A.F. (1973) Alternate pathway components—properdin and C3 proactivator—in human glomerulonephritis. *J. clin. Invest.* **52,** 634.

MESTECKY, J., KULHAVY, R. & KRAUS, F.W. (1972) Studies on human secretory immunoglobulin A. II. Subunit structure. *J. Immunol.* **108,** 738.

MICHAEL, A.F., DRUMMOND, K.N., GOOD, R.A. & VERNIER, R.L. (1966) Acute poststreptococcal glomerulonephritis: immune deposit disease. *J. clin. Invest.* **45,** 237.

MICHAEL, A.F. & MCLEAN, R.H. (1974) Evidence for activation of the alternate system in glomerulonephritis. *Advances in Nephrology.* Yearbook Publishers. (In press.)

MICHAEL, A.F., WESTBERG, N.G., FISH, A.J. & VERNIER, R.L. (1971) Studies on chronic membranoproliferative glomerulonephritis with hypocomplementemia. *J. exp. Med.* **134,** 208s.

MOREL-MAROGER, L., LEATHEM, A. & RICHET, G. (1972) Glomerular abnormalities in non-systemic diseases. *Amer. J. Med.* **53,** 170.

OUCHTERLONY, O. (1958) Diffusion gel methods for immunological analysis. *Progr. Allergy*, **5,** 1.

PARONETTO, R. & KOFFLER, D. (1965) Immunofluorescent localization of immunoglobulins, complement and fibrinogen in human disease. I. Systemic lupus erythematosus. *J. clin. Invest.* **44,** 1657.

POST, R.S. (1965) A technique for cutting thin sections from solvent-substituted, paraffin-embedded tissues. *Cryobiology*, **1,** 261.

QUERINJEAN, R., MASSON, P.L. & HEREMANS, J.F. (1971) Molecular weight, single-chain structure and amino acid composition of human lactoferrin. *Europ. J. Biochem.* **20,** 420.

RADL, J., KLEIN, F., VAN DEN BERG, P., DE BRUYN, A.M. & HIJAMS, W. (1971) Binding of secretory piece to polymeric IgA and IgM paraproteins *in vitro*. *Immunology*, **20,** 843.

SCHEIDEGGER, J.J. (1955) Une micromethode de l'immunoelectrophorese. *Int. Arch. Allergy*, **7,** 103.

SEAH, P.P., MAZAHERI, M.R., FRY, L. & MOWBRAY, J.R. (1973) Alternate-pathway complement fixation by IgA in the skin in dermatitis herpetiformis. *Lancet*, **ii,** 175.

THOMPSON, R.A. (1970) Secretory piece linked to IgM in individuals deficient in IgA. *Nature (Lond.)*, **226,** 946.

THOMPSON, R.A. & ASQUITH, P. (1970) Quantitation of exocrine IgA in human serum in health and disease. *Clin. exp. Immunol.* **7,** 491.

THOMPSON, R.A., ASQUITH, P. & COOKE, W.T. (1969) Secretory IgA in serum. *Lancet*, **ii,** 517.

TOMASI, T.B. & BIENENSTOCK, J. (1968) Secretory immunoglobulins. *Advanc. Immunol.* **9,** 1.

TOMASI, T.B., BULL, D., TOURVILLE, D., MONTS, M. & YURCHAK, A. (1969) Distribution and synthesis of human secretory components. *The Secretory Immunologic System* (ed. by D. H. Dayton, P. A. Small, R. M. Chanock, H. E. Kaufman and T. B. Tomasi), p. 41. National Institute of Child Health and Human Development, Bethesda, Maryland.

TOMASI, T.B. & GREY, H.M. (1972) Structure and function of immunoglobulin A. *Progr. Allergy*, **16,** 81.

TOMASI, T.B., TAN, E.M., SOLOMON, A. & PENDERGAST, R.S. (1965) Characteristics of an immune system common to certain external secretions. *J. exp. Med.* **121,** 101.

TOURVILLE, D.R., ADLER, R.H., BIENENSTOCK, J. & TOMASI, T.B. (1969) The human secretory immunoglobulin system: immunohistological localization of IgA secretory 'piece', and lactoferrin in normal human tissues. *J. exp. Med.* **129,** 411.

TRYGSTAD, C.W. & STIEHM, E.R. (1970) Elevated serum IgA globulin in anaphylactoid purpura. *Pediatrics*, **47,** 1023.

URIZAR, R.E., MICHAEL, A.F., SISSON, S. & VERNIER, R.L. (1968) Anaphylactoid purpura. II. Immunofluorescent and electron microscopy study of the glomerular lesions. *Lab. Invest.* **19,** 737.

VAN DE PUTTE, L.B., DE LA RIVIERE, G.B., VAN BREDA VRIESMAN, P.J.C. (1974) Recurrent or persistent hematuria. Sign of mesangial immune-complex deposition. *New Engl. J. Med.* **290,** 1165.

WALDMAN, P.H., MACH, J.P., STELLA, M.M. & ROWE, D.S. (1971) Secretory IgA in human serum. *J. Immunol.* **105,** 37.

WESTBERG, G., NAFF, G., BOYER, J. & MICHAEL, A.F. (1971) Glomerular deposition of properdin in acute and chronic glomerulonephritis with hypocomplementemia. *J. clin. Invest.* **50,** 642.