EXPERIMENTAL IgA NEPHROPATHY*

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Immunological injury mediated by immune complexes appears to play a major role in human glomerulonephritis (1). Evidence for the pathogenetic role of immune complexes has been derived from studies in both humans and in experimental animals (2). These studies have focused primarily on the role of IgG in nephritis because deposits of this immunoglobulin predominate in the affected glomeruli. In contrast, certain human nephritides such as Berger's Disease (3, 4) and Henoch-Schoenlein purpura (5, 6) are characterized by the predominance of IgA deposits in the affected glomeruli. Although this finding suggests an association between IgA and the development of glomerulonephritis, no direct evidence has been presented for a causal role of IgA immune complexes in the pathogenesis of these nephropathies. In fact, an experimental model for the study of IgA-associated glomerulonephritis has not been described to date. The paucity of IgA in normal sera together with the difficulty of obtaining enough IgA with specificity for a single antigen, may account for the lack of such an experimental model.

In this study, we describe an animal model for IgA immune complex nephropathy, using IgA from MOPC-315 myeloma (7) which possesses specific antigenic reactivity for the dinitrophenol (DNP)[1] hapten (8) and bovine serum albumin (BSA) conjugated with DNP (DNP-BSA) as the antigen. Immune complexes formed either in vitro or in vivo with polymeric IgA, but not monomeric IgA, induced glomerulonephritic changes in mice. Our data also indicates that although immune complexes fix C3, the participation of this component of complement was not essential for the induction of renal injury.

Materials and Methods

Animals. The inbred mice used in these experiments were 2- to 3-mo-old female BALB/c weighing 20–25 g. All mice were obtained from Charles River Breeding Laboratories, Wilmington, Mass.

Antigens. DNP-BSA was prepared by the reaction of 2,4-dinitrobenzene sulfonic acid (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.) with BSA (Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) at room temperature under

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[1] *Abbreviations used in this paper:* DNP, dinitrophenol; BSA, bovine serum albumin; DNP_{12}-BSA or DNP_{33}-BSA, dinitrophenylated bovine serum albumin containing either 12 or 33 DNP groups per molecule of BSA; DNP-HSA, dinitrophenylated human serum albumin; GARG, goat antiserum specific for rabbit gamma globulins; PBS, phosphate-buffered saline, pH 7.2; PAS, periodic acid-Schiff reagent.

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alkaline conditions as described by Eisen et al. (9). The degree of derivatization was controlled by the duration of the reaction; a 3-h and 22-h reaction yielded DNP_{12}-BSA and DNP_{33}-BSA respectively. The number of DNP groups per molecule of conjugated BSA was calculated from absorbancy at 360 nm (Molar extinction coefficient for ε-DNP lysine: 17,530) and from the dry weight of protein or from estimation of protein concentration by absorption at 280 nm.

Source of Antibody. Murine plasmacytoma MOPC-315 (obtained through the courtesy of Dr. M. Potter, National Institutes of Health, Bethesda, Md.) was maintained in serial transplants by subcutaneous passage in female BALB/c mice. Ascites were collected by paracentesis of mice which had been inoculated i.p. with 5×10^5 viable tumor cells 2-3 wk earlier. Pooled ascites were allowed to stand for 48 h at 4°C then centrifuged at 12,000 *g* to remove insoluble residues. Clarified ascitic fluid was stored at −20°C. The anti-DNP content of the ascitic fluid was determined by the quantitative precipitin reaction described by Farah et al. (10).

Purification of IgA Anti-DNP. Pooled MOPC-315 ascitic fluid was dialyzed against Tris-saline buffer (0.01 M Tris-0.15 M NaCl, pH 7.4) at 4°C overnight and applied to a DNP-L-lysine-Sepharose (Sepharose, Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) immunoabsorbent column prepared by the method of Rockey et al. (11). The MOPC-315 protein specifically bound to the immunoabsorbent column. Bound antibody was recovered by elution with 10 mM 2,4-*n*-ε-DNP-L-lysine (Sigma Chemical Co., St. Louis, Mo.) in saline. The protein containing eluates were pooled, dialyzed repeatedly against Tris-saline buffer at 4°C for 3 d, and then concentrated in an Amicon ultrafiltration unit (Amicon Corp., Scientific Sys. Div., Lexington, Mass.) equipped with UM-10 a Diaflo membrane. The concentrated eluates contained only IgA as determined by immunoelectrophoresis with rabbit anti-whole mouse serum and anti-mouse IgA (Meloy Laboratories, Inc., Springfield, Va.). Purified IgA concentration was determined from absorbance at 280 nm using an extinction coefficient ($E_{1cm}^{1mg/ml}$) = 1.44 and mol wt of 160,000 (12). The multimeric IgA recovered was fractionated into monomeric, dimeric, and higher polymeric forms by gel chromatography on 1.5 × 100 cm column of Ultrogel AcA-34 (LKB, Rockville, Md.).

Antisera. Specific anti-mouse IgA was prepared by injecting rabbits s.c. with 1 mg purified dimeric MOPC-315 IgA in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). The animals were reinjected twice at 4-wk intervals with 2 mg of purified IgA in incomplete Freund's adjuvant and bled 1 wk after the last injection. The pooled serum was tested for specificity by immunoelectrophoresis against mouse serum.

Goat antiserum specific for rabbit gamma globulins (GARG) was produced by i.m. injection of 5 mg rabbit gamma globulin (13) in complete Freund's adjuvant and the animal was bled 2 wk later. The antiserum was shown to be monospecific for rabbit IgG by immunoelectrophoresis.

Radioiodination. DNP-protein conjugate (DNP_{33}-BSA) was iodinated with ^{125}I by the chloramine-T method (14).

Radioassay of IgA Anti-DNP. A constant amount (4.5 μg) of ^{125}I-DNP-BSA was added to twofold serial dilutions of experimental mouse sera prepared in duplicate. Tris-saline buffer containing 20% fetal calf serum was used as the diluent. After incubation at 37°C for 1 h, rabbit anti-mouse IgA (50 μl) was added and the tubes were held at 4°C for 2 h. To enhance precipitation, GARG (75 μl) was added in excess and allowed to incubate for an additional 4 h. The pellets obtained by centrifugation at 12,000 *g* for 20 min at 4°C were washed twice with cold phosphate-buffered saline (PBS, pH 7.2) and counted in an automatic gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Control tubes containing only ^{125}I-DNP_{33}-BSA with rabbit anti-mouse IgA and GARG were used to determine nonspecific precipitation. The IgG content of experimental sera was evaluated from a standard curve obtained with pooled MOPC-315 reference serum. The concentration of anti-DNP immunoglobulins in the reference serum was determined by the quantitative precipitin curve described by Farah et al. (10) and a modified Farr assay (15) using tritium-labeled 2,4-*n*-ε-DNP-L-lysine. Dilutions of experimental sera yielding values within the linear range of the standard curve (30-70%) were used to calculate the IgA concentration.

Measurement of Hematuria. Mice were induced to urinate by stroking the lower part of their dorsa. The presence of an abnormal number of erythrocytes in urine of mice was detected with an orthotolidine reagent strip (Hemastix; Ames Co., Elkhart, Ind.). The estimation of hematuria as small, moderate, or large was based on the instructions accompanying the reagent strips.

Immunofluorescence Study. Mouse renal tissue was snap-frozen in *n*-hexane, and cut with a cryostat into 4-μm sections. The sections were air dried, fixed in acetone for 10 min at 22°C and washed twice with PBS and fluorescein-labeled antiserum was applied. Antisera used were rabbit anti-mouse IgA, IgM, IgG (Meloy Laboratories, Inc.), rabbit anti-mouse C3 (courtesy of Dr. A. Gabrielsen, New York State Health Department, Albany, N. Y.) and rabbit anti-mouse fibrin (N. L. Cappel Laboratories Inc., Cochranville, Pa.). Slides were incubated for 30 min at 37°C in a humid chamber, washed three times with PBS and counterstained for 20 s with Eriochrome Black (Difco Laboratories) diluted with PBS (1:50). After washing twice, the sections were mounted with PBS-buffered glycerol. Examination was made by Leitz Ortholux microscope (E. Leitz, Inc., Rockleigh, N. J.) equipped with Ploem-type vertical illuminator and a xenon 150-W light source. Filters used were: primary K 480, interference Kp 500, and a secondary K 510-nm filter.

The indirect immunofluorescent technique was used for detecting deposition of antigen with the use of rabbit anti-BSA or anti-DNP (courtesy of Dr. W. Clem, University of Florida, Gainesville, Fla.) and fluorescein-labeled GARG (N. L. Cappel Laboratories, Inc.).

Histologic Examination. A portion of each renal specimen was fixed in Vande Grift's solution for 8 h and kept in 80% ethanol until processed. Tissues were paraffin embedded, sectioned, and stained with periodic acid-Schiff reagent (PAS).

Complement Depletion of Mice. Decomplementation of mice was carried out by the i.p. injection of 10 U of cobra venom factor (Cordis Laboratories Inc., Miami, Fla.) as described by Cochrane et al. (16).

Results

Effect of Preformed IgA Immune Complexes. Varying amounts of DNP_{33}-BSA (0.25–3.00 mg) were added to a constant amount of MOPC-315 ascitic fluid containing 1.7 mg of anti-DNP IgA. The antigen-antibody precipitates were allowed to stand at 22°C for 10–15 min before injection. The precipitates were kept in homogeneous suspension by aspiration and ejection using a tuberculin syringe with a 27-gauge needle. The immune complexes formed at different antigen-antibody ratios were adjusted to a vol of 0.5 ml with PBS and injected i.v. into groups of four mice each. Controls received either antigen or antibody alone. The results are summarized in Table I. 11 of the 24 mice receiving the immune complexes developed hematuria which was detectable 2 h after injection and lasted 4–6 h. Immune complexes prepared between three- and sevenfold antigen excess were most effective in producing hematuria.

TABLE I

Induction of Hematuria in Normal BALB/c Mice Given Preformed DNP_{33}-BSA-IgA Complexes Prepared with MOPC-315 Ascitic Fluid

Antigen	Antibody	Equivalence ratio*	Number of mice with hematuria			
			Negative	Small	Moderate	Large
mg	*mg*					
0.25	1.70	0.8	2	2	—	—
0.50	1.70	1.6	4	—	—	—
1.00	1.70	3.2	1	1	—	2
1.50	1.70	4.7	2	—	—	2
2.00	1.70	6.3	0	—	2	2
3.00	1.70	9.5	4	—	—	—
2.00	—	—	4	—	—	—
—	1.70	—	4	—	—	—

* Multiple of antigen concentration at equivalence as determined by precipitin curve.

TABLE II

Induction of Hematuria and Renal Deposition of IgA Immune Complexes Following Injection of DNP_{12}-BSA-IgA Complexes Prepared In Vitro in Normal BALB/c Mice

Antigen	Antibody	Number of mice with hematuria			Immunofluorescence*	
		Negative	Small	Moderate	IgA	C3
mg	*mg*					
2.0	4.0	1	—	2	3+	2+
2.0	3.0	—	4	—	3+	2+
2.0	1.5	4	—	—	3+	2+
2.0	0.5	4	—	—	2+	1+
2.0	—	4	—	—	0	0
—	3.0	4	—	—	0	0

* Immunofluorescence was graded on a scale of 0–3+.

Preformed immune complexes prepared with the lower substitution antigen, DNP_{12}-BSA and varying amounts of purified IgA were examined for their potential to induce hematuria or to localize in the kidneys (Table II). Hematuria was induced only in mice that received complexes preformed with the higher amounts of antibody (3 or 4 mg). However, immune complexes were detected by immunofluorescence in the glomeruli of all animals receiving the immune complexes, regardless of the amount of antibody. No hematuria or glomerular localization of IgA occurred in the control animals.

The mice that received immune complexes prepared with DNP_{12}-BSA were killed 36 h after injection. Histological examination using PAS staining of the renal tissues of experimental animals revealed morphological changes that were similar in all animals, even in those that manifested no hematuria. These morphological changes consisted of widening of the mesangial area in 1–2% of the glomeruli and prominent increase in intraluminal PAS-positive material in ~15% of the glomeruli (Fig. 1 A). Increase in mesangial cells or neutrophilic infiltration were seldom seen. In general, the light microscopy of these renal biopsies exhibited only mild histological alterations in the affected glomeruli. Sections from control animals that received antigen or antibody alone (Fig. 1 B) were similar to those of normal mice.

Immunofluorescent microscopy demonstrated localization of IgA in the area of the glomerular mesangium and capillary lumen (Fig. 1 C) of all glomeruli. Staining for C3 showed a similar distribution but with a lesser intensity. In contrast to the focal changes seen with PAS staining, the glomerular immunofluorescent pattern consisted of generalized-diffuse granular deposits.

Immunofluorescent staining of specimens from control mice that received either IgA or antigen alone, with anti-mouse IgA was negative. IgG was absent from the glomeruli of either experimental or control mice. Staining with anti-mouse IgM resulted in reactions of varying of intensity (between trace and 2+) in both experimental and in normal mice. Minimal staining for C3, between negative and trace, mainly in a peritubular pattern, was observed in the control group.

Effect of IgA Immune Complexes Formed In Vivo. In an attempt to determine whether the mode of formation of IgA immune complexes would influence their capacity to induce renal injury, complexes were formed in vivo by two methods. The first consisted of injecting i.v. 1 or 2 mg of DNP_{12}-BSA into two groups of mice bearing

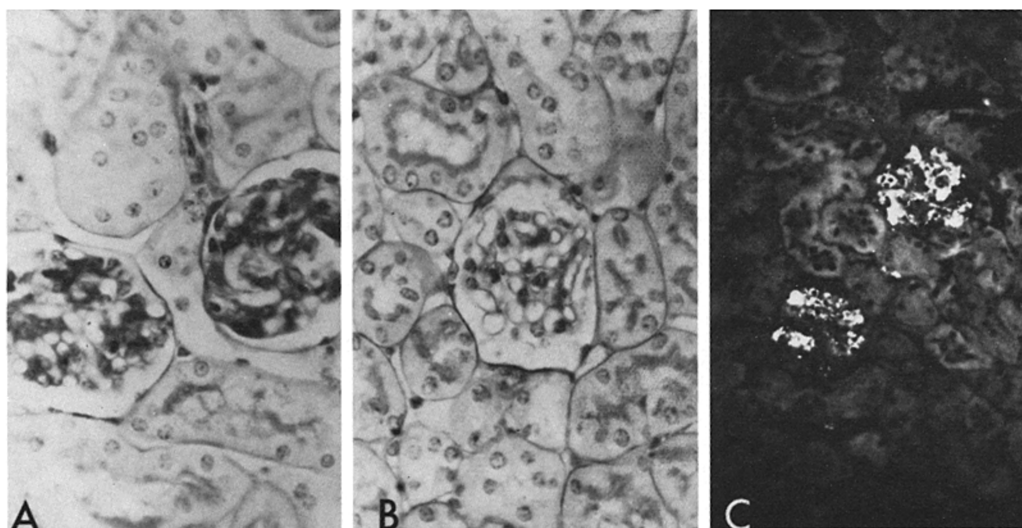

FIG. 1. (A) Photomicrograph of a glomerulus from a mouse given one injection of DNP_{12}-BSA-IgA complexes, 2 mg DNP_{12}-BSA and 3 mg IgA, prepared in vitro, and killed after 36 h. Hypertrophy of mesangial area with PAS-positive deposits. × 450. (B) Photomicrograph of a glomerulus from a control mouse, that received 2 mg DNP_{33}-BSA, stained with PAS. × 450. (C) Fluorescent photomicrograph of glomeruli from a mouse that was injected with preformed DNP_{12}-BSA-IgA complexes (2 mg DNP_{12}-BSA and 3 mg IgA). Kidney stained with anti-mouse IgA. Generalized-diffuse deposition of IgA. × 275.

MOPC-315 myeloma. The first group received 1 mg and the second group 2 mg of DNP_{12}-BSA. The level of circulating IgA anti-DNP in the sera of these animals, which fluctuated from day to day, was determined by radioimmunoassay immediately before the administration of antigen. Each animal was given an initial (0 h) dose of antigen followed by a similar dose at 60, 84, and 132 h thereafter. Mice were killed 12 h after the fourth dose of antigen.

Histologic changes detected in these mice were variable. Animals with high levels of circulating IgA that received 2 mg of antigen exhibited a mild endothelial and mesangial proliferative response with some neutrophilic infiltration. The glomerular basement membrane was usually intact. Mice with low levels of IgA that received 1 mg of antigen were mildly affected. Mesangial proliferative response in these mice was focal and minimal. The characteristic abnormality detected in all these mice was the presence of glomerular PAS-positive deposits. Examination of 100–200 glomeruli from each mouse revealed a linear relationship with a high degree of correlation ($r = 0.90$; $P < 0.0001$) between the percentage of glomeruli with PAS-positive deposits and the level of circulating IgA in tumor-bearing mice that received 1 mg of antigen. Animals that received 2 mg of antigen also manifested a linear relationship ($r = 0.98$; $P = 0.02$) with a higher percentage of glomerular involvement (Fig. 2). However, the intraglomerular distribution of these deposits was variable. Animals with high levels of circulating IgA had a diffuse pattern, with heavy mesangial and capillary PAS-positive deposits, whereas mice with low serum IgA levels manifested only segmental deposits.

The immunofluorescent pattern was essentially identical in all cases. Despite the fact that <40% of the glomeruli contained PAS-staining material, all the glomeruli

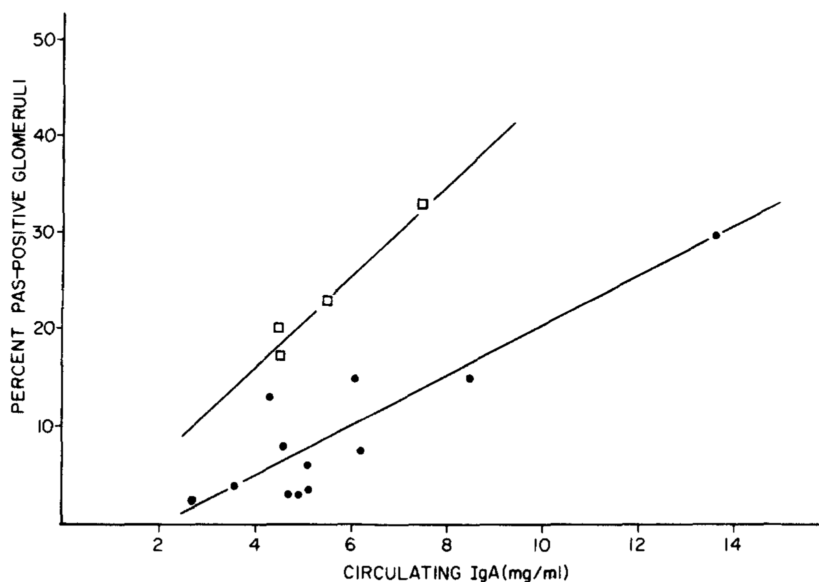

FIG. 2. Effect of DNP_{12}-BSA-IgA complexes formed in vivo. IgA levels and the development of glomerular changes in tumor-bearing mice that were given 1 (●) or 2 (□) mg DNP_{12}-BSA at 0-, 60-, 84-, and 132-h intervals. Mice were sacrificed 12 h after the last injection.

contained IgA deposits as determined by immunofluorescent staining. The intraglomerular pattern was diffuse with mesangial and intraluminal deposits (Fig. 3A). No extraglomerular deposits were observed in any of the renal sections. The pattern resulting from immunohistological staining for BSA or DNP (Fig. 3B) using the indirect-immunofluorescent technique, was found to be similar to the pattern observed for IgA. Staining for C3 revealed a diffuse intraglomerular pattern (Fig. 3C), which was present in all glomeruli, similar to IgA immune deposition. It should be noted that none of the histologic or immunopathologic changes described above were encountered in tumor-bearing control mice, even those with levels of IgA as high as 16 mg/ml.

To determine the rate of deposition and disappearance of antigen-antibody complexes in the kidneys, an alternate approach for the formation of IgA immune complexes in vivo was used. 2-mg quantities of DNP_{12}-BSA were administered i.v. to four groups of four normal mice each. All 16 animals were injected i.v. with 5 mg of IgA anti-DNP, 10–15 min after administration of the antigen. Control mice received either antigen or antibody alone in the same amounts. The procedure was repeated after 24 h and the different groups were killed 36, 48, 72, and 96 h after the first injection (Table III). Morphologic changes in mice sacrificed at 36 h showed a generalized-diffuse pattern of PAS-positive deposits. These deposits were mainly intraluminal and mesangial in distribution. This intraglomerular pattern became more focal at 48 h. At the end of 72 h, the PAS-positive deposits became less discrete. All the glomeruli appeared normal at the end of 96 h.

Immunofluorescent staining for IgA and C3 revealed the characteristic generalized-diffuse pattern of immune deposits similar to those shown in Fig. 3. However, this pattern changed with time; the immune deposits observed at 36 h in the mesangium

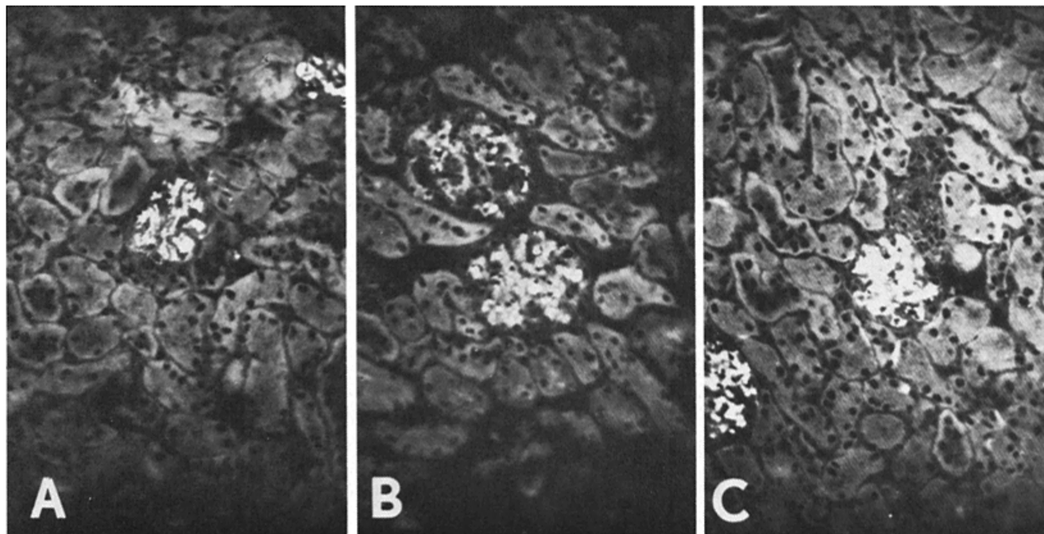

FIG. 3. (A) Fluorescent photomicrograph of glomeruli from a tumor-bearing mouse, with 3 mg/ml of circulating anti-DNP IgA, that received three doses of 1 mg DNP_{12}-BSA over a 132-h period. The mouse was killed 12 h after the last injection. Kidney stained with rabbit anti-mouse IgA. Generalized diffuse deposition of host IgA. × 275. (B) Demonstrates deposits of BSA in the glomeruli by indirect immunofluorescent staining. × 275. (C) Reveals the presence of C3 deposits in a granular diffuse pattern. × 275.

and capillary lumina became fine mesangial granules with little fluorescence at the end of 96 h. Deposits of C3 were similar to those of IgA but the C3 deposits were only seen after 48 h after the first injection rather than at 36 h, as was encountered with IgA deposits. There was no C3 detectable by immunofluorescence at the end of 96 h.

Results of immunofluorescent staining for fibrin in the glomeruli of mice in which IgA immune complexes were formed in vivo by either of the two methods described above, were negative. However, staining for IgG deposits yielded different results. Examination of kidneys obtained from the two experimental groups of tumor-bearing animals revealed the presence of IgG in large amounts, in a pattern similar to that of IgA, whereas no IgG staining could be detected in the renal tissue of the normal mice in which IgA immune complexes were formed in vivo by the injection of DNP_{12}-BSA followed by injection of IgA-anti-DNP.

Effect of C3 on Localization of IgA Immune Complexes. The role of complement, especially C3, in renal deposition of IgA immune complexes was evaluated by the induction of these complexes in vivo in two groups of mice. In one group, C3 was depleted by the administration of cobra venom factor. Animals in the other group served as controls with normal complement. Immune complexes were induced by the i.v. injection of 2 mg of DNP_{12}-BSA 15 min before challenge with 4 mg of purified IgA through the same route. Other control mice received either antigen or antibody in the same amounts. The animals were sacrificed after 48 h and the tissues were examined by immunofluorescent microscopy (Table IV). As noted, depletion of C3 had no effect on the deposition of IgA complexes in the kidneys of experimental animals.

Immunofluorescent examination revealed a typical generalized-diffuse pattern of

TABLE III

Time Course of Deposition and Disappearance of DNP_{12}-BSA-IgA Complexes Formed In Vivo in BALB/c Mice

Number of mice	Time sacrificed	Antigen	Antibody	Immunofluorescence		Histologic changes*
				IgA	C3	
	h	*mg*	*mg*			
4	36	2.0	5.0	3+	0	Present
4	48	2.0	5.0	3+	2+	Present
4	72	2.0	5.0	3+	2+	Present
4	96	2.0	5.0	2+	1+	Absent
4	48	2.0	—	0	0	Absent
4	48	—	5.0	0	0	Absent

* PAS deposits or mesangial widening.

TABLE IV

Effect of Treatment with Cobra Venom Factor on the Glomerular Deposition of IgA-DNP_{12}-BSA Complexes Formed In Vivo in Normal BALB/c Mice

Number of mice	Treated	Antigen	Antibody	Immunofluorescence		
				IgA	C3	BSA
		mg	*mg*			
3	Yes	2.0	4.0	3+	0	2+
3	No	2.0	4.0	3+	2+	2+
3	No	—	4.0	0	0	0
3	No	2.0	—	0	0	0

IgA localized in the glomeruli of both decomplemented mice (Fig. 4 A) and normal mice (illustration not shown). However, C3 could only be detected in the kidneys of those mice with normal complement (Fig. 4 B) and not in those that were complement depleted (Fig. 4 C). Control animals that received antigen or antibody alone did not show any IgA or C3 deposits.

Effect of the Polymerism of IgA on the Localization and Clearance of Immune Complexes. Because the IgA produced in the ascitic fluid of the myeloma is 70–80% polymeric and 20–30% monomeric, we investigated the capacity of complexes prepared with different forms of IgA to deposit in the kidneys. Purified multimeric IgA was separated into monomeric and polymeric forms on Ultrogel AcA-34. Preformed monomeric and multimeric IgA immune complexes were prepared in vitro with DNP_{12}-BSA. The immune complexes were administered i.v. at 0 and 24 h to mice that were killed 48 h after the first injection. Immune complexes prepared with monomeric IgA failed to deposit in the kidneys of these mice (Table V). In contrast, the injection of immune complexes prepared with a similar concentration of multimeric IgA and yielding the same antigen-to-antibody mass ratio resulted in glomerular deposition of the immune complexes.

The glomerular deposition of immune complexes formed in vivo with different forms of purified IgA was also examined. Immune complexes were formed in vivo by administering 2 mg of DNP_{12}-BSA to normal mice that were challenged later with

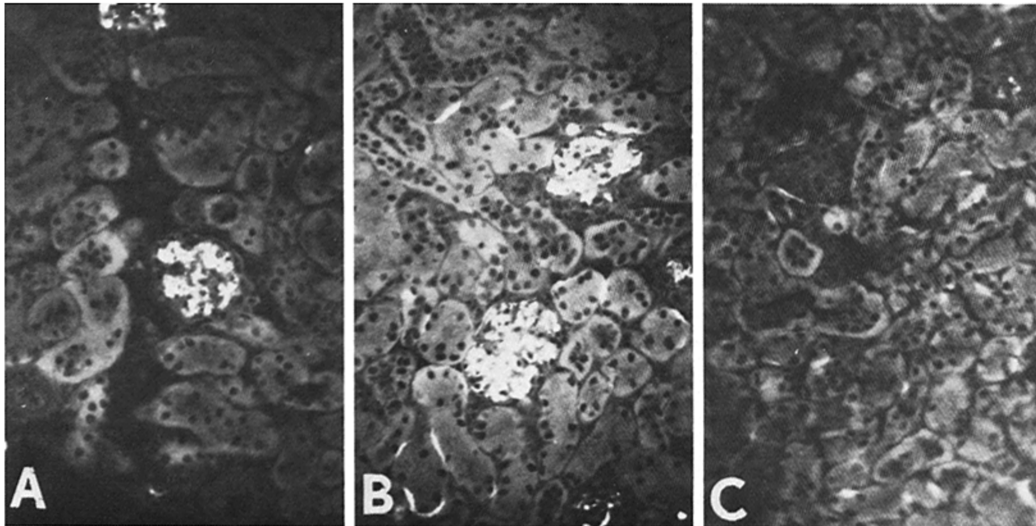

FIG. 4. (A) Fluorescent photomicrograph showing IgA deposits in glomerulus of a complement-depleted animal in which DNP_{12}-BSA-IgA complexes were formed in vivo. (B) Deposits of C3 in glomeruli of complement nondepleted mouse. (C) Absence of C3 deposits in glomerulus of complement-depleted mouse. × 275.

TABLE V

Glomerular Deposition in Kidneys of Normal Mice of Immune Complexes Formed In Vitro or In Vivo with Multimeric or Monomeric IgA and DNP_{12}-BSA

Preparation of immune complexes	Number of mice	IgA form	Antibody	Antigen	Immunofluorescence	
					IgA	C3
			mg	*mg*		
Complexes preformed in vitro	3	Monomeric	1.15	0.5	0	0
	3	Monomeric	1.15	1.0	0	0
	3	Monomeric	1.15	2.0	0	0
	3	Multimeric*	1.0	2.0	3+	2+
Complexes formed in vivo‡	3	Monomeric	2.0	2.0	0	0
	3	Polymeric	4.0	2.0	3+	2+
	3	Multimeric*	5.0	2.0	3+	2+

* Multimeric is a mixture of monomeric and polymeric.
‡ Mice injected with DNP_{12}-BSA followed in 10 min by injection of IgA.

either multimeric (a mixture of polymeric and monomeric), polymeric, or monomeric IgA. This procedure was repeated at 24 h and renal tissues were obtained at the end of 48 h. Immunofluorescence microscopy demonstrated the localization of IgA only in the kidneys of mice that received multimeric or polymeric IgA (Table V), but not in animals that received monomeric IgA. Glomeruli containing multimeric or polymeric IgA immune complexes showed deposits of C3 in a distribution identical to that of IgA.

Discussion

To establish an animal model for IgA nephritis, we utilized a mouse myeloma IgA with anti-DNP specificity (8). Immune complexes of DNP-BSA and IgA were formed both in vitro and in vivo. The capacity of these complexes to produce renal disease was first established by showing that the IgA immune complexes induced hematuria, and that a certain minimal level of antigen and antibody were required.

IgA immune complexes formed in vitro or in vivo produced histological and immunopathological renal changes. The morphological changes consisted of PAS-positive deposits in the capillary loops and mesangium accompanied in some cases by a mild proliferative cellular response in the mesangial area. These changes were classified as mild focal glomerulonephritis. In contrast, immunohistological staining invariably showed dense, granular IgA deposits in a generalized-diffuse pattern. The differences in the degree of histological changes encountered between PAS and immunohistological staining in this experimental model has also been observed in human IgA nephropathy (4, 17). In the latter, morphological changes include an increase in mesangial matrix and the presence of PAS-positive deposits in some of the glomeruli. In most of the reported cases, the changes are characterized as focal glomerulonephritis but are mild and not distinctive. However, immunofluorescent staining shows granular deposits of IgA in all glomeruli. These deposits are localized mainly in the mesangium and capillary loops and are accompanied sometimes by deposits of C3 and IgG.

The amount of antibody and antigen in circulation, but not the antigen-antibody ratio, appears to be critical in the production of experimental IgA nephropathy. Tumor-bearing mice making high amounts of antibody showed a higher percentage of glomerular involvement. The concentration of antigen also appears to be a factor related to the development of nephritis. Experimental IgA nephropathy occurs in antibody excess as seen in some of the tumor-bearing mice that had high levels of IgA anti-DNP as well as in far antigen excess. This suggests that the ratio of the immune reactants in this system is not as critical as the amount of IgA. To localize in the kidneys and induce nephritis in the mouse, IgG immune complexes need to be in moderate antigen excess at a mass ratio of antigen to antibody of ~0.3 (18). In contrast, IgA immune complexes were nephritogenic at 14 times this ratio.

Proof that a disease is a result of deposition of immune complexes requires demonstrating that the immunoglobulin in the glomeruli represents antibody combined with antigen. Although this remains enigmatic for human IgA nephropathy, because no antigen has been defined, it has been accomplished in this experimental model where we showed that the specific antigen, DNP-BSA, is deposited along with IgA. Persistence of these complexes in the renal tissue may determine the extent of the inflammatory reaction. Rapid catabolism of these deposits is usually accompanied by the disappearance of C3 and rapid recovery without histologic damage. On the other hand, persistence of these complexes may trigger an IgG antibody response which upon reaction with the deposited IgA complexes may produce further histopathological changes. Support for this hypothesis comes from our observation that the tumor-bearing mice which received DNP_{12}-BSA over a period of 6 d had deposits of IgG along with the IgA deposits in their glomeruli. Such a mechanism may explain the simultaneous presence of IgA and IgG in renal biopsies of some patients with Berger's Disease and Henoch-Schoenlein purpura.

The presence of C3 in glomerular lesions has been reported in most cases of human

IgA nephropathy (19). In these reports, activation of complement has been suggested to occur through the alternate pathway because properdin has been detected along with C3 and in the absence of C4. Although the role of complement in IgA-associated human nephropathy is still speculative, the present findings suggest that in the mouse model of IgA nephropathy, complement is not essential for the induction of glomerular disease. This conclusion is based on two findings: mice that received antigen first followed by IgA shortly thereafter failed to show complement deposits in their glomeruli when sacrificed 12 h after the second injection. This occurred despite the fact that these glomeruli exhibited characteristic histologic changes of nephritis and contained IgA deposits at that time. Secondly, animals that were decomplemented with cobra venom factor showed no complement deposition but showed glomerular IgA deposits comparable to normal animals that received the same type of immune complexes.

Konig and his co-workers (20) reported that DNP antigens, dinitrophenylated-human serum albumin (DNP-HSA), activate C3 in vitro via the alternate pathway. This activation depends on the degree of DNP-substitution, with antigens having 32 or more DNP residues effecting complement activation, whereas antigens with <19 DNP residues producing little or no complement activation at concentrations up to 10 mg DNP-HSA/ml. This observation raises the possibility that the finding of C3 deposits in association with immune-complex deposits in the glomeruli of the experimental animals in our study may be secondary to the polyanionic effect of DNP antigens. This appears to be unlikely because controls receiving either high- or low-substituted antigen alone showed no complement deposition and because complement deposition with immune complexes occurred to the same extent in the glomeruli of mice that received either IgA immune complexes formed with low-substituted antigen (DNP_{12}-BSA) as well as high-substituted antigen (DNP_{33}-BSA) or in mice that had immune complexes induced in vivo with these antigens.

The multimeric IgA used in this study consisted of 70–80% polymeric and 20–30% monomeric IgA. It was important therefore, to determine which of the two fractions was responsible for the observed pathogenetic effect. The failure of monomeric IgA immune complexes prepared in vitro or induced in vivo to localize in kidneys represents another peculiarity of the IgA system that differentiates it from IgG. In the IgG system nonprecipitating antibody in preformed immune complexes can induce nephritis (21). In contrast to monomeric IgA, purified polymeric IgA immune complexes were capable of renal deposition. The deposition pattern of polymeric IgA immune complexes was similar to the one observed with the multimeric IgA, suggesting that the polymeric form of IgA is essential for nephritogenicity.

The recognized forms of human IgA nephropathy, Berger's disease and Henoch-Schoenlein purpura, usually follow upper respiratory infections (22, 23). This association suggests that a polymeric IgA response resulting in the formation of circulating IgA immune complexes with the infecting agent may play a role in the pathogenesis of these diseases (24). The observation that polymeric IgA is necessary for immune deposition raises the possibility of the involvement of secretory IgA in the pathogenesis of some of the human nephropathies.

Summary

An animal model for IgA immune complex nephritis was developed. IgA immune complexes formed in vitro with an IgA anti-dinitrophenyl (DNP) derived from

MOPC-315 plasmacytoma, and dinitrophenylated bovine serum albumin (DNP-BSA) produced mild focal glomerulonephritis in mice. Similar, but more severe pathological changes were produced with complexes formed in vivo either in normal mice or MOPC-315 tumor-bearing mice. In contrast to the focal nature of the PAS-positive glomerular lesions observed by light microscopy, immunofluorescent examination revealed IgA deposits in all glomeruli. This discrepancy between immunofluorescent and histopathologic findings as well as the distribution of the immune complexes within the affected glomeruli, are some of the features which bear resemblance between this experimental model and human IgA nephropathy.

Fixation of complement by DNP-BSA-IgA immune complexes, formed in vitro or in vivo, was shown to occur in the glomeruli of mice with IgA immune complex nephropathy. The pattern of C3 glomerular deposits was similar to that of IgA. However, complement proved to be nonessential for complex deposition. This conclusion is based on the observation that decomplemented mice, although showing no deposition of C3 in their glomerulus, developed glomerular immunohistological changes similar to those observed in experimental mice that were not decomplemented.

Polymeric IgA was observed to be critical for renal deposition of complexes and induction of nephritic histological changes. In contrast, monomeric IgA immune complexes failed to produce glomerular deposits. This finding raises the possibility that secretory IgA, which is predominantly polymeric, may play a role in human IgA-associated glomerulonephritis.

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