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The Effect of Sodium Cromoglycate on the Induction of Experimental IgA Nephropathy

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Mesangial IgA nephropathy was experimentally induced in ddY mice by oral and parenteral administration of the poliomyelitis vaccine (POLIO), and we then tried to investigate if IgA deposition could be prevented by the concurrent use of sodium cromoglycate (SCG), which is known to inhibit the local mucosal immune reaction. Mucosal and systemic immunity could be induced by the administration of POLIO; proteinuria, increased serum IgA levels, mesangial cell proliferation, mesangial matrix widening, mesangial deposits of IgA, and large electron dense deposits in the mesangium were observed. Concurrent administration of SCG and POLIO resulted in a significant decrease in the serum IgA level and mesangial IgA deposits. The later addition or abstinence of SCG after the 70th day did not influence the glomerular mesangial IgA deposition. But the serum IgA level was still decreased by the continuous treatment of SCG even after the 70th day. Thus, mesangial IgA nephropathy simulating IgA nephropathy in humans could be induced in ddY mice using POLIO and its induction could largely be prevented by the concurrent use of SCG. However mesangial IgA deposits already present could not be cleared by the late administration of SCG.

Key Words: Experimental IgA nephropathy, mucosal immunity, poliomyelitis vaccine, sodium cromoglycate

IgA nephropathy, first described by Berger and Hinglais in 1968, is characterized by recurrent hematuria and strong mesangial deposition of IgA. The natural course of this disease had previously been regarded to be benign and stationary (Levy *et al.* 1973; Joshua *et al.* 1977). But a few recent reports stress a significant group of patients who may develop end stage renal disease after a progressive downhill course (D'Amico *et al.* 1981; Hood *et al.* 1981; Martini *et al.* 1981; Frasca *et al.* 1982; Kobayashi *et al.* 1983).

The pathogenesis of IgA nephropathy is still obscure, and many hypotheses have been suggested such as disturbance of immune regulation (Sakai *et al.* 1979a; Sakai *et al.* 1982; Bannister *et al.* 1983; Egido *et al.* 1983a), overproduction of polymeric IgA or immune complex (Nomoto *et al.* 1979; Sakai *et al.* 1979b; Egido *et al.* 1982), a defect in the removal of abnormal immune complex by the reticuloendothelial system (Lawrence *et al.* 1983; Sato *et al.* 1983) and activation of the complement system via an alternative pathway (D'Amico *et al.* 1981; Rauterberg *et al.* 1987). Recently, an induction of experimental IgA nephropathy by passive oral immunization using a large amount of foreign protein antigen (Emancipator *et al.* 1983; Sato *et al.* 1986) has received much attention for the possible role of mucosal immunity. And Choi and Choi's experimental model in ddY mice using poliomyelitis vaccine (POLIO) demonstrated an increase of both the serum IgA level and the number of IgA-containing immunocytes in the lamina propria of the intestinal mucosa (Choi and Choi 1987).

Sodium cromoglycate (SCG) is a therapeutic agent applied to patients with asthma or food allergy. The major mechanism of action is the inhibition of mucosal immunity by stabilization of the mast cell membrane and prevention of the release of vasoactive amines (Nizami *et al.* 1977).

This experimental study is based on Choi and Choi's model (Choi and Choi, 1987). Firstly we tried to induce the IgA nephropathy by the administration of POLIO. Then the effect of SCG on the provocation of IgA nephropathy was explored.

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MATERIALS AND METHODS

Animals

Female ddY mice (5 week-old; 20gm in weight) were used.

Antigens

POLIO (Poliovax® obtained from Green Cross, Korea) was used as an antigen. Inactivation was accomplished by heating live Poliovax® in warm water (70°C) for 30 minutes. Each mouse received 0.2ml of inactivated POLIO on the first day, and two booster injections were administered on the 32nd and 60th days respectively. The animals were fed with commercial pellet mouse food.

Drugs

SCG (obtained from Sigma Chemical Co., St. Louis, USA), prepared in fresh drinking water, was given daily in the dose of 50mg/kg of body weight.

Experimental Design

The mice were separated into six groups until the 70th day. Renal biopsy (replaced by unilateral nephrectomy) was performed at the 70th day for histopathological observation. From the 71st day, each group except the normal control (group 1) was divided into two subgroups (Fig. 1) to investigate the possible change in the mesangial IgA deposition by the abstinence or addition of SCG. After the 70th day, SCG was abstained in the groups which had been treated with SCG until the 70th day (groups 2, 5, and 6) and SCG was added in the groups to which SCG had not been concurrently used until the 70th day (groups 3 and 4). All animals were sacrificed at the 100th day for histopathological observation of renal tissue and blood sampling.

From the 1st to 70th experimental day:

Group 1	Normal control	10 animals
Group 2c	SCG treated control	12 animals
Group 3c	Oral administration of live POLIO	12 animals
Group 4c	Intramuscular administration of inactivated POLIO	11 animals
Group 5c	Oral administration of live POLIO with concurrent use of SCG	12 animals
Group 6c	Intramuscular administration of inactivated POLIO with concurrent use of SCG	10 animals

From the 71st to 100th experimental day:

Group 1 Normal control

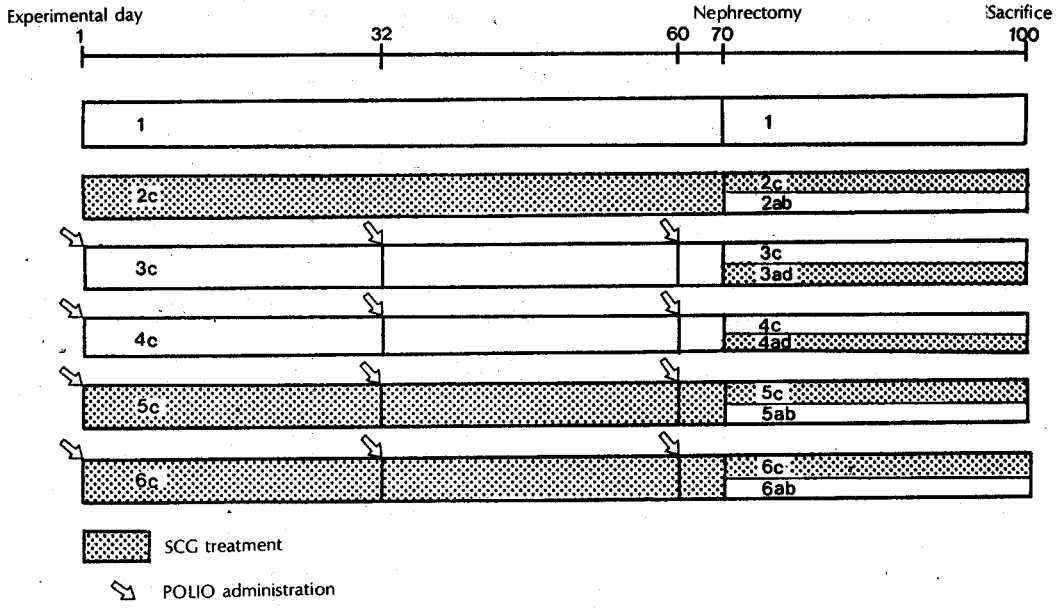

Fig. 1. Experimental groups.

Group 2c Continuous SCG treatment after the 70th day
Group 2ab Abstinence of SCG treatment after the 70th day
Group 3c No treatment after the 70th day
Group 3ad Addition of SCG treatment after the 70th day
Group 4c No treatment after the 70th day
Group 4ad Addition of SCG treatment after the 70th day
Group 5c Continuous SCG treatment after the 70th day
Group 5ab Abstinence of SCG treatment after the 70th day
Group 6c Continuous SCG treatment after the 70th day
Group 6ab Abstinence of SCG treatment after the 70th day

Urinalysis

Occult blood and protein (hema-Combistix, Ames Division, Miles Lab. Inc., Indiana) were checked.

Measurement of Serum IgA

The level of serum IgA was estimated by enzyme linked immunoabsorbent assay, using specific goat anti-mouse immunoglobulin reference serum (Sigma Chemical Co., St. Louis, USA).

Light Microscopy

Renal tissue was fixed in formalin, embedded in paraffin, sliced into thin sections, and stained with hematoxylin and eosin, periodic acid-Schiff reagent or Gomori's trichrome reagent.

Immunofluorescence

Renal tissue was frozen and cut in 2-3μm sections using cryocut (Histostat microtome, AO). It was dried in air, double-fixed in acetone and washed in phosphate buffer solution (pH 7.4). Mesangial IgA, IgM and C3 were detected by the direct immunofluorescence technique, using FITC-conjugated anti-mouse-IgA,-IgM (Sigma Chemical Co., St. Louis, USA) and -C3 (Fuji, Medicobiological Laboratory, Japan). The intensity of fluorescence was observed by fluorescent microscope (Leitz-Dialux, Leitz, West Germany) and graded as −, −/+, +, ++, and +++. A small amount of intestinal tissue taken randomly from a few animals of each group was also prepared by the same method and IgA-containing cells were counted using FITC-anti-mouse-IgA.

Table 1. Proteinuria

Group	Exp. day	Proteinuria											
1	70	+	−/+	+	+	+	−/+	NE	NE	NE	NE		
	100	−/+	−/+	++	−/+	+	+	−	+	+	−/+		
2 2c	70	−/+	+	+	−	−/+	NE	NE	NE	+	NE	NE	NE
2c (2ab)	100	+	−/+	D	D	D	+	++	−/+	(−/+	+	−/+	−)
3 3c	70	+	+	++	++	++	++	NE	NE	++	++	+	++
3c (3ad)	100	+	−/+	++	+	D	D	++	+	(++	+	+	+)
4 4c	70	++	+	+	++	+	++	++	NE	++	+	+	
4c (4ad)	100	−/+	++	D	D	D	D	D	+	(+	++	+)	
5 5c	70	+++	+	++	++	++	+	+++	NE	+	++	++	NE
5c (5ab)	100	++	++	++	D	D	D	D	+	(+	−/+	+	++)
6 6c	70	++	+	++	++	NE	NE	+++	+	+	++		
6c (6ab)	100	++	++	++	++	−/+	+	(++	+	+	+)		

Exp. day: Experimental day
NE : Not examined at the 70th day
D : Data not available due to death between the 70th & the 100th day

− : Absence
−/+ : Trace
+ : Mild
++ : Moderate
+++: Marked

Electron Microscopy

Renal tissue was fixed in 3% glutaraldehyde, post-fixed in 1% osmiumtetroxide (PBS, pH 7.4), and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and were examined and photographed by a Hitachi H-500 electron microscope (Japan).

RESULTS

Urinalysis

There was a moderate degree of proteinuria in all experimental groups but no significant difference in urinary protein levels among them (Table 1). Most

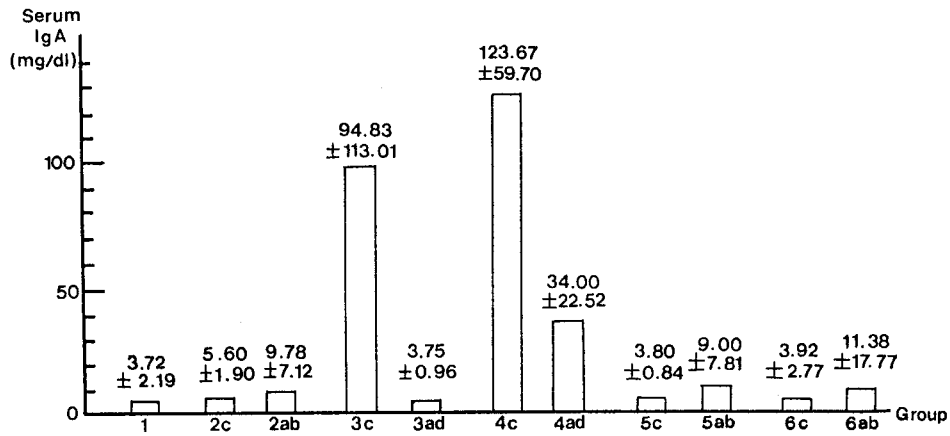

Fig. 2. Serum IgA levels.

Table 2. Hematuria

Group	Exp. day	Hematuria											
1	70	–	–	–	–	–	–	NE	NE	NE	NE		
	100	–	–	–	–	–	–	–	–	–	–		
2 2c	70	–	–	–	–	–	NE	NE	NE	–	NE	NE	NE
2c (2ab)	100	–	–	D	D	D	–	–	–	(–	–	–	–)
3 3c	70	–	–	–	–	–	–	NE	NE	–	–	–	–
3c (3ad)	100	–	–	–	–	D	D	–	–	(–	–	–	–)
4 4c	70	–	–/+	+++	–	–	–	–	NE	–	–/+	–	
4c (4ad)	100	–	–	D	D	D	D	D	–	(–	+	–)	
5 5c	70	–	–	–	–	–	–	–	NE	–	–	–	NE
5c (5ab)	100	–	–	–	D	D	D	D	–	(–	–	+	–)
6 6c	70	–	–	–	–	NE	NE	–	–	–	–		
6c (6ab)	100	–	–	–	–	–	–	(–	–	–	–)		

Exp. day: Experiemental day
NE : Not examined at the 70th day
D : Data not available due to death between the 70th & the 100th day

– : Absence
–/+ : Trace
+ : Mild
++ : Moderate
+++: Marked

mice were negative or trace for occult blood except one of group 4c at the 70th day and two of groups 4ad and 5ab at the 100th day (Table 2).

Serum IgA Level

Serum IgA levels were significantly higher in the

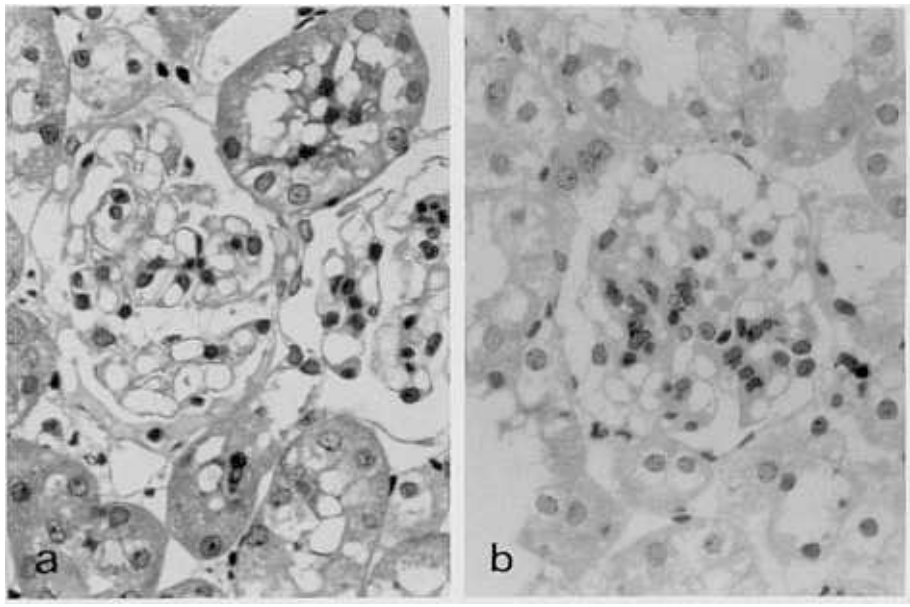

Fig. 3. Photomicrograph of renal glomerulus at the 70th day revealed a moderate degree of mesangial cell proliferation and mesangial matrix widening in the oral POLIO group (b) compared to the control group (a). (H-E, ×400)

Table 3. Light microscopic findings of the renal glomerulus (Mesangial cell proliferation)

Group	Exp. day	Mesangial cell proliferation											
1	70	–	–/+	–	–/+	–/+	–	NE	NE	NE	NE		
	100	–/+	–/+	–	–	–	+	–	–	–	–/+		
2 2c	70	–/+	–	–/+	–	–	NE	NE	NE	–	NE	NE	NE
2c (2ab)	100	–/+	–/+	D	D	D	–/+	–	–	(–/+	–/+	–/+	–)
3 3c		+		–/+	+	+	+	NE	NE		++	–/+	–/+
3c (3ad)		+		–/+	++	D	D	–/+	++		++	–/+	+)
4 4c		++		+	++	++	++	–/+	NE			+	
4c (4ad)		+		D	D	D	D	D	++			++)	
5 5c	70	–/+	+	+	+	–	+	+	NE	++	+	++	NE
5c (5ab)	100	+	++	+	D	D	D	D	+	(++	+	++	+)
6 6c		+	+	–/+	+			+	+	+	++		
6c (6ab)		+	++	+	+			(++	++	++	++)		

Exp. day: Experimental day
NE : Not examined at the 70th day.
D : Data not available due to death between the 70th & the 100th day

– : Absence
–/+ : Trace
+ : Mild
++ : Moderate
+++: Marked

POLIO administered groups (3c and 4c) than the control and higher in the parenterally administered group than the orally treated one. There was no significant difference in serum IgA levels between the POLIO groups (3c and 4c) and concurrent SCG administered groups (5c and 6c). Concurrent use of SCG after the

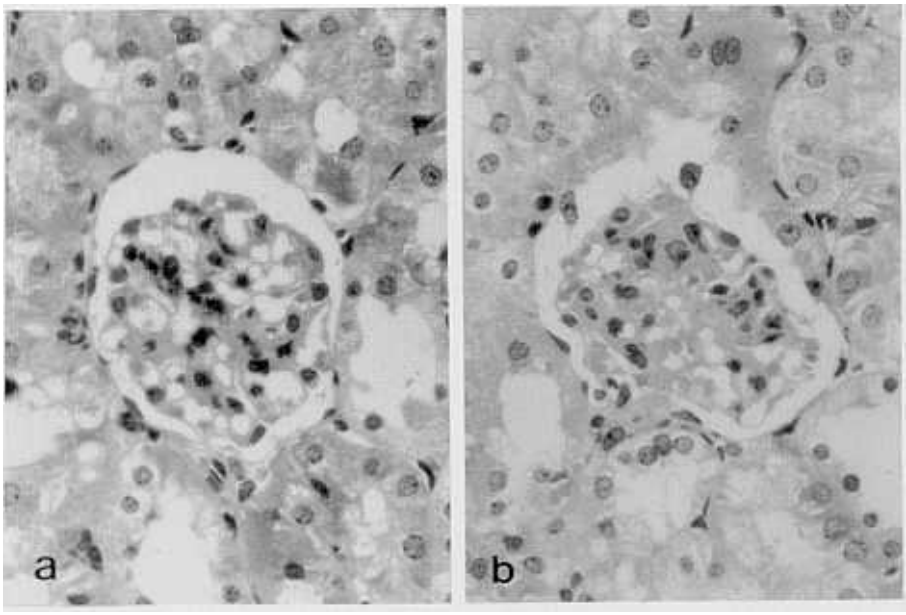

Fig. 4. Photomicrograph of renal glomerulus at the 100th day revealed similar mesangial findings between the intramuscular POLIO group (a) and intramuscular POLIO-SCG treated group (b). (H-E, ×400)

Table 4. Light microscopic findings of the renal glomerulus (Mesangial matrix widening)

Group	Exp. day	Mesangial matrix widening											
1		−	−	−/+	−/+	−/+	−	NE	NE	NE	NE		
	100	+	−	−	+	−/+	−	−/+	−	−	−/+		
2	2c	70	−	−/+	+	−/+	−	NE	NE	NE	−	NE	NE	NE
	2c (2ab)	100	+	+	D	D	D	−/+	−/+	−/+	(−/+	+	+	−/+)
3	3c	70	+	+	+	++	+	+	NE	NE	++	+++	−/+	
	3c (3ad)	100	++	+	+	+++	D	D	+	++	(+++	+++	+	
4	4c	70	++	+	+	++	++	++	−/+	NE	+	++	++	
	4c (4ad)	100	+	++	D	D	D	D	D	++	(+	++	++)	
5	5c	70	+	++	+	+	−/+	+	−/+	NE	++	+	+	NE
	5c (5ab)	100	++	+++	+	D	D	D	D	+	(+++	+	+++	++)
6	6c		+	+	−/+	++				+		+		
	6c (6ab)		++	++	++	++				++		++)		

Exp. day: Experimental day
NE : Not examined at the 70th day
D : Data not available due to death between the 70th & the 100th day

− : Absence
−/+ : Trace
+ : Mild
++ : Moderate
+++: Marked

70th day lowered the serum IgA level of groups 3ad and 4ad, but not significantly (Fig. 2).

Light Microscopy

POLIO groups (3c and 4c) showed a mild to moderate degree of mesangial cell proliferation and mesangial matrix widening (Fig. 3). The parenterally administered group was more significantly changed. In POLIO-SCG treated groups (5c and 6c), only the parenteral POLIO with concurrent SCG group (6c)

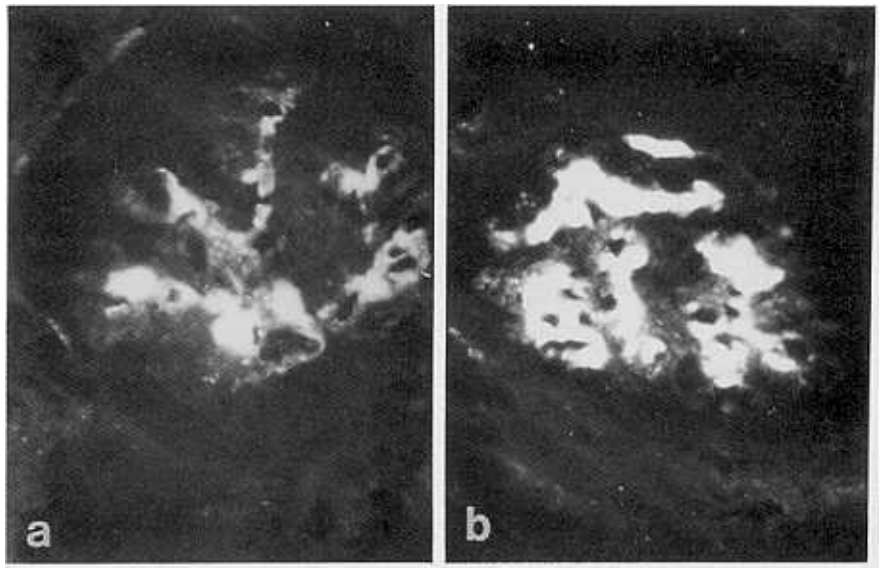

Fig. 5. The immunofluorescent localization of IgA in glomeruli from the oral POLIO group (a) and from the intramuscular POLIO group (b) at the 70th day was moderate (a) to marked (b). (×400)

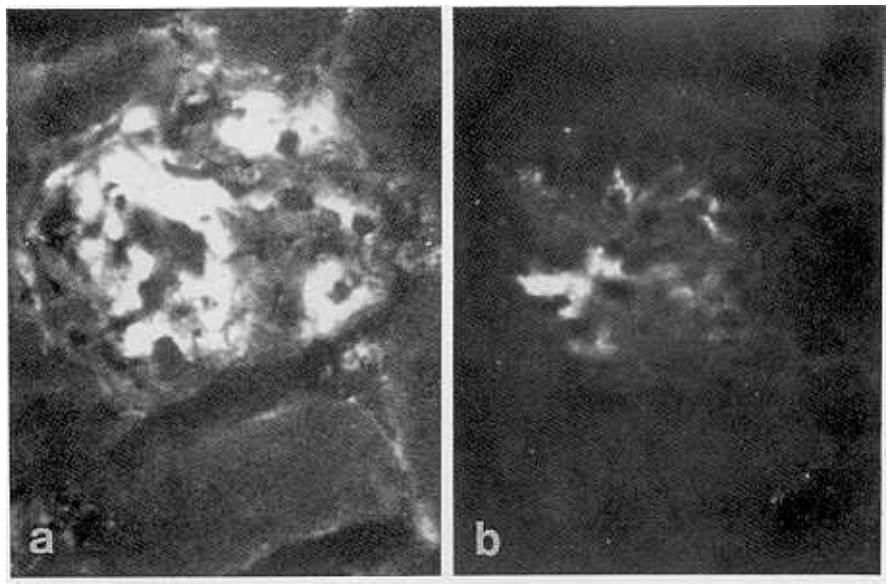

Fig. 6. The intensity of IgA deposition was less in glomeruli from the intramuscular POLIO-SCG treated group (b) than in glomeruli from the intramuscular POLIO group (a). (×400)

showed the same glomerular changes. After the 70th day, additional use of SCG in groups 3ad and 4ad resulted in no obvious difference from the POLIO groups (3c and 4c), while abstinence of SCG (groups 5ab and 6ab) led to a mild increase of the changes compared with groups 5c and 6c (Tables 3 and 5; Fig. 4). Both tubular and interstitial changes were unremarkable.

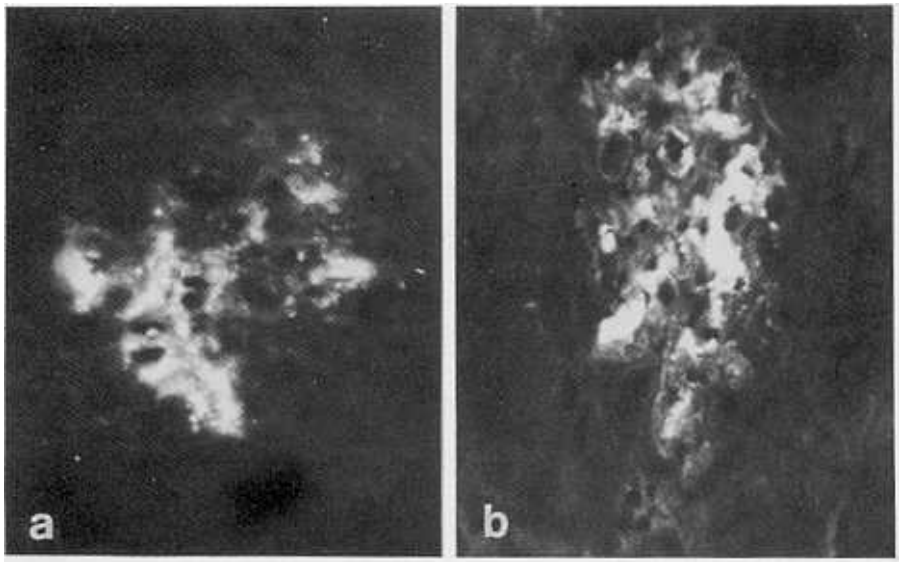

Fig. 7. The moderate to marked intensity of IgA deposition in glomeruli from the oral POLIO group at the 70th day (a) was not significantly changed after additional SCG treatment at the 100th day (b). (×400)

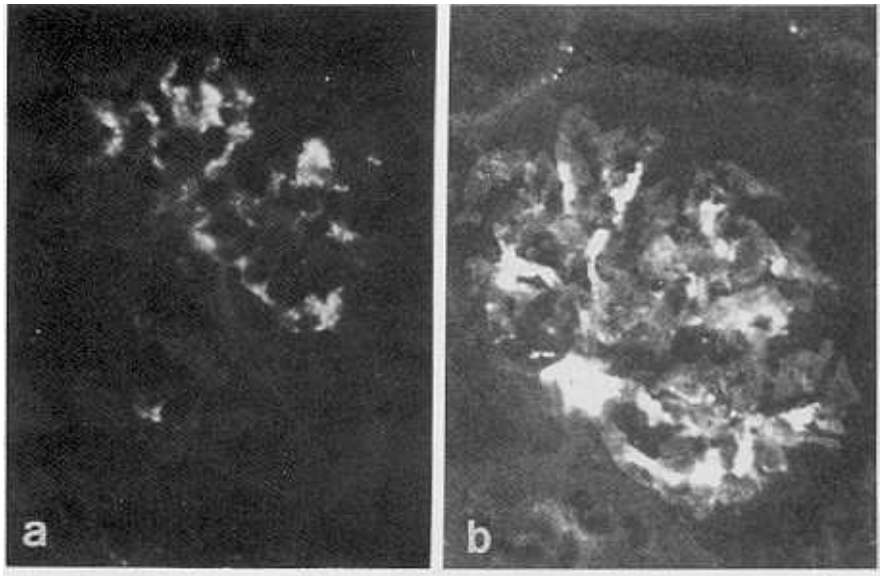

Fig. 8. The mild intensity of IgA deposition in glomeruli from the intramuscular POLIO-SCG treated group at the 70th day (a) was not significantly changed after discontinuation of SCG treatment at the 100th day (b). (×400)

Immunofluorescence

Positive findings for IgA were universally present in the POLIO groups (3c and 4c) and more marked in the parenteral POLIO group (4c) than in the oral POLIO group (3c) (Fig. 5). Mesangial IgA deposition was significantly decreased by concurrent use of SCG (groups 5c and 6c) (Fig. 6), but the later addition

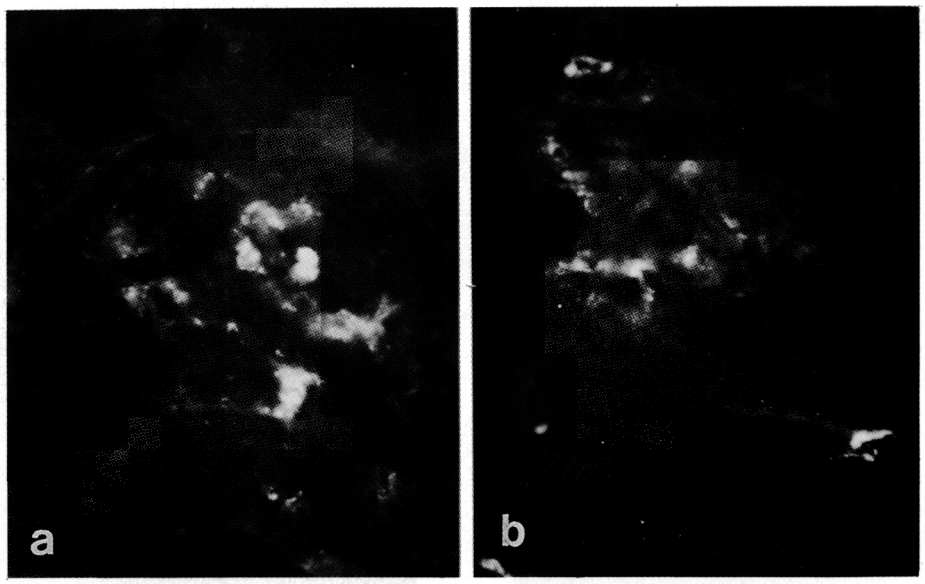

Fig. 9. The mild intensity of IgA deposition in glomeruli from the oral POLIO-SCG treated group at the 70th day (a) was decreased to a trace degree with continuous SCG treatment at the 100th day (b). (×400)

Table 5. Immunofluorescent microscopic findings of the glomerular deposits of IgA

Group	Exp. day	Glomerular mesangial deposits of IgA											
		−/+	−/+	−/+	−		−	NE	NE	NE	NE		
		+	−/+	−/+	−/+		−/+	−/+	−/+	−	−/+		
2c	70	−/+	−/+	+	−/+	+		NE	NE	−	NE	NE	NE
2c (2ab)	100	+	+	D	D	D		+	−/+	(−/+	−/+	++	+)
3		+	−/+		++	−/+		NE		+++	−/+	++	++
		++	+		+++	D		−/+		(+++	+	+	++)
				+	++	++	++	++		++	+	++	
				D	D	D	D	D		(++	−/+	++)	
		−/+	+		+++	+++	+	−/+		+	+	++	NE
		−/+	−/+		D	D	D	D		(+	++	+	++)
6			+	+	++	NE	NE	−/+	−/+		−/+		
			−/+	−/+	+	+	−/+	(−	+		−)		

Exp. day: Experimental day
NE : Not examined at the 70th day
D : Data not available due to death between the 70th & the 100th day

− : Absence
−/+ : Trace
\+ : Mild
++ : Moderate
+++: Marked

Immunofluorescence

Positive findings for IgA were universally present in the POLIO groups (3c and 4c) and more marked in the parenteral POLIO group (4c) than in the oral POLIO group (3c) (Fig. 5). Mesangial IgA deposition was significantly decreased by concurrent use of SCG (groups 5c and 6c) (Fig. 6), but the later addition

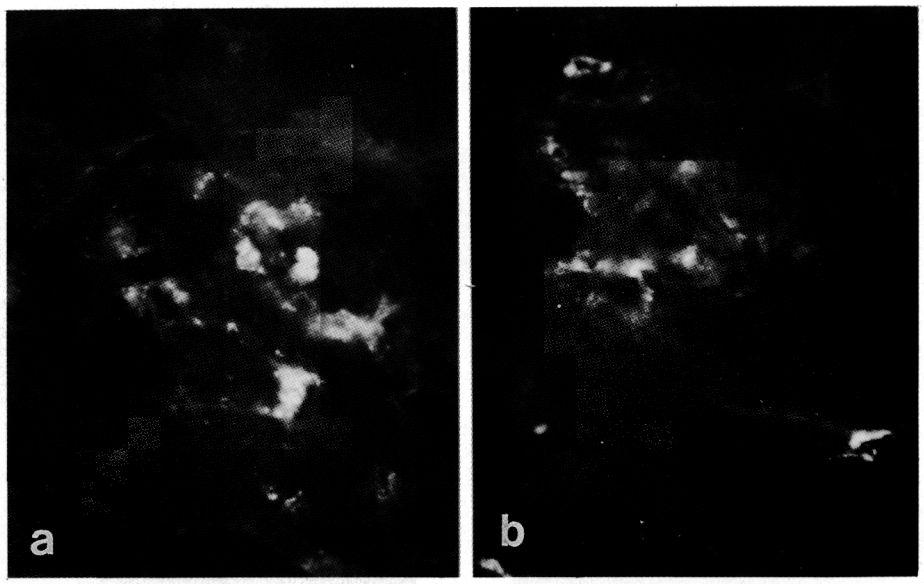

Fig. 9. *The mild intensity of IgA deposition in glomeruli from the oral POLIO-SCG treated group at the 70th day (a) was decreased to a trace degree with continuous SCG treatment at the 100th day (b). (×400)*

Table 5. Immunofluorescent microscopic findings of the glomerular deposits of IgA

Group	Exp. day	Glomerular mesangial deposits of IgA											
1	70	−/+	−/+	−/+	−	−	−	NE	NE	NE	NE		
	100	+	−/+	−/+	−/+	+	−/+	−/+	−/+	−	−/+		
2 2c	70	−/+	−/+	+	−/+	+	NE	NE	NE	−	NE	NE	NE
2c (2ab)	100	+	+	D	D	D	++	+	−/+	(−/+	−/+	++	+)
3 3c	70	+	−/+	+	++	−/+	−/+	NE	NE	+++	−/+	++	++
3c (3ad)	100	++	+	+	+++	D	D	−/+	−/+	(+++	+	+	++)
4 4c	70	++	+++	+	++	++	++	++	NE	++	+	++	
4c (4ad)	100	++	+++	D	D	D	D	D	+++	(++	−/+	++)	
5 5c	70	−/+	+	−/+	+++	+++	+	−/+	NE	+	+	++	NE
5c (5ab)	100	−/+	−/+	−	D	D	D	D	+	(+	++	+	++)
6 6c	70	+	+	+	++	NE	NE	−/+	−/+	−/+	−/+		
6c (6ab)	100	+	−/+	−/+	+	+	−/+	(−	+	−/+	−)		

Exp. day: Experimental day
NE : Not examined at the 70th day
D : Data not available due to death between the 70th & the 100th day

− : Absence
−/+ : Trace
\+ : Mild
++ : Moderate
+++: Marked

(groups 3ad and 4ad) (Fig. 7) or abstinence (groups 5ab and 6ab) (Fig. 8) of SCG did not prompt any noticeable change on the deposition (Table 5) except in the continuous SCG treated group (Fig. 9).

IgA containing cells in the mucosa of the small intestine obtained from the POLIO groups (3c and 4c) were significantly increased in number, whereas the SCG treated group showed nearly the same finding

Table 6. Immunofluorescent microscopic findings of the glomerular deposits of IgM

Group	Exp. day	Glomerular mesangial deposits of IgM											
1	70	+	+	−/+	+	+	+	NE	NE	NE	NE		
	100	+	++	+	+	+	−/+	+	+	−/+	−/+		
2 2c	70	+	+	+	+	++	NE	NE	NE	−/+	NE	NE	NE
2c (2ab)	100	−/+	−/+	D	D	D	++	++	++	(−/+	+	+	+)
3 3c	70	+	+	++	+++	+	+	NE	NE	+++	+++	+++	+++
3c (3ad)	100	++	−/+	++	+++	D	D	++	+++	(++	+++	++	++)
4 4c	70	++	+	++	++	+++	+++	+++	NE	+++	+++	+++	
4c (4ad)	100	+	+	D	D	D	D	D	+++	(++	++	++)	
5 5c	70	++	+	+	+++	+++	+++	+	NE	++	++	++	NE
5c (5ab)	100	−/+	−/+	+	D	D	D	D	+++	(++	++	++	++)
6 6c	70	+	+	++	+++	NE	NE	+	+	++	+++		
6c (6ab)	100	++	−/+	+	+	++	+	(+	+	+	++)		

Exp. day: Experimental day
NE : Not examined at the 70th day
D : Data not available due to death between the 70th & the 100th day

− : Absence
−/+ : Trace
\+ : Mild
++ : Moderate
+++: Marked

Table 7. Immunofluorescent microscopic findings of the glomerular deposits of C3

Group	Exp. day	Glomerular deposits of C3											
1	70	−/+	−	−	−	−	−	NE	NE	NE	NE		
	100	−	−	−	−	−	−	−/+	−	−	−/+		
2 2c	70	−	−	−	−	−	NE	NE	NE	−	NE	NE	NE
2c (2ab)	100	−	−	D	D	D	−	−	−	(−	−	−	−)
3 3c	70	−/+	−	−/+	−	−/+	−	NE	NE	−/+	+	+	+
3c (3ad)	100	−	−	−	−	D	D	−/+	−/+	(−/+	−	−	−)
4 4c	70	−/+	+	++	−/+	−/+	+	−	NE	−/+	−	−	
4c (4ad)	100	−	−	D	D	D	D	D	−	(−/+	+	−/+)	
5 5c	70	−	−	−	−	−	−/+	+	−	NE	−/+	−/+	−
5c (5ab)	100	−	−	−	D	D	D	D	−	(−	−	+	−)
6 6c	70	−	−/+	−	−/+	NE	NE	−/+	−	−	−	−	
6c (6ab)	100	−	−/+	−	−	−	−	−	(−	−	−	−)	

Exp. day: Experiemental day
NE : Not examined at the 70th day
D : Data not available due to death between the 70th & the 100th day

− : Absence
−/+ : Trace
\+ : Mild
++ : Moderate

as compared with the control.

The mesangial IgM deposition was moderate to severe in the POLIO groups, and significantly inhibited by the concurrent SCG. Addition or abstinence of SCG did not cause any remarkable change in this finding (Table 6).

Glomerular mesangial deposition of C3 was nearly absent or trace (Table 7). A trace amount of C3 was

Fig. 10. The trace immunofluorescent localization of C3 in glomerular mesangium and along the Bowman's capsule and the tubular basement membrane from the intramuscular POLIO group at the 70th day. (×400)

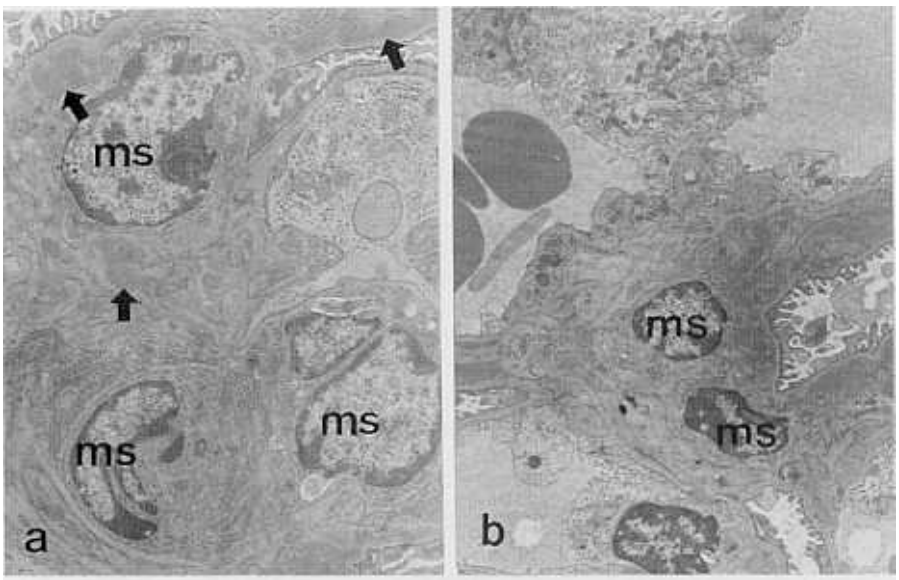

Fig. 11. The electron microscopic view of glomeruli of the intramuscular POLIO group revealed large nodular electron-dense deposits (arrows), a moderate degree of mesangial cell proliferation and mesangial matrix widening (a: ×5,250) with occasional foci of paramesangial extension (b: ×3,750).

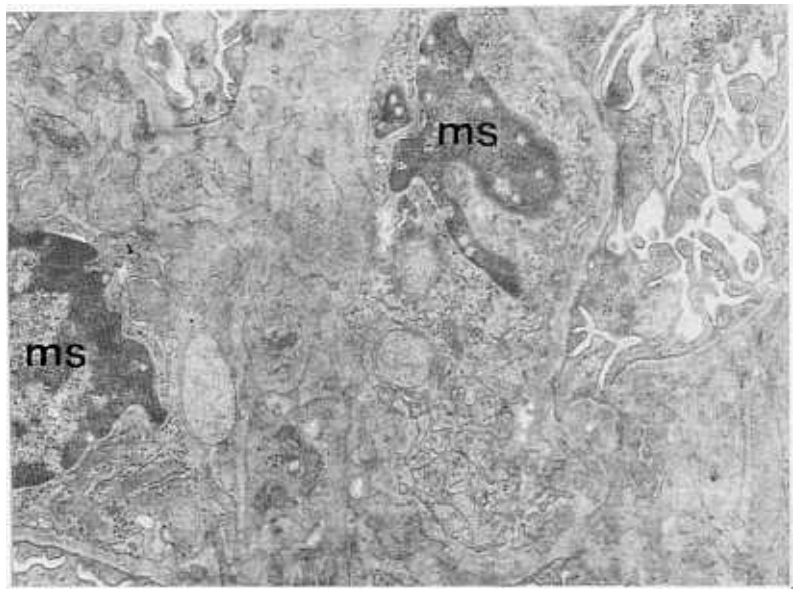

Fig. 12. The electron microscopic view of glomeruli of the intramuscular POLIO-SCG treated group revealed small rarefied deposits and numerous complex cytoplasmic processes of mesangial cells. (×9,000)

noted along the Bowman's capsule, tubular basement membrane, or interstitial vessel walls (Fig. 10).

Electron Microscopy

Both the mesangial cell proliferation and the mesangial matrix widening were observed in the POLIO groups. Large nodular electron-dense deposits were found in the mesangial area with occasional foci of paramesangial extension (Fig. 11a and b). Minimal epithelial proliferation with focal fusion of foot processes was noticed. But in the SCG treated groups (5c and 6c), both the size and the amount of the electron-dense material were significantly decreased and cytoplasmic processes of mesangial cells were prominent and complex (Fig. 12). Mesangial cell proliferation and matrix widening were also found as in the POLIO groups. The influence of addition or interruption of SCG (groups 3ad, 4ad, 5ab and 6ab) on these changes was unremarkable.

DISCUSSION

The reported incidence of IgA nephropathy varies from 2% (McCoy *et al.* 1974) to 43% (Kobayashi *et al.* 1983) of primary glomerulonephritis. In Korea, it has been reported as about 15 to 18% (Choi *et al.* 1986). It is usually discovered at the second or third decade of life, and men are two to four times more vulnerable than women (Clarkson *et al.* 1984; D'Amico *et al.* 1985; Emancipator *et al.* 1985).

The most remarkable clinical finding is gross or microscopic hematuria, but proteinuria accompanies this in 71% of the cases (Emancipator *et al.* 1985). The serum IgA level is increased in 50% of cases but its value is not usually correlated with the clinical course (Zimmerman and Burkholder 1975; Clarkson *et al.* 1980). The serum C3 level is frequently normal.

In the past, IgA nephropathy had been regarded as a relatively benign disease with excellent prognosis (Joshua *et al.* 1977). But recent reports indicate that about 10% of end stage renal disease is related to IgA nephropathy (D'Amico *et al.* 1981; Clarkson *et al.* 1984).

Morphological diversity has also been mentioned by many authors. Relatively benign changes with minimal mesangial cell proliferation and mesangial IgA deposition are the usual findings. But some cases also display glomerular sclerosis and crescent formation, co-deposition of C3, IgG, and IgM, and subendothelial or subepithelial deposition of electrondense materials (Emancipator *et al.* 1985).

IgA nephropathy has been assumed to be an immune complex type of glomerulonephritis because of many animal experiments (Rifai *et al.* 1979; Isaacs *et al.* 1981; Emancipator *et al.* 1983) and clinical detec-

tion of IgA immune complex in human IgA nephrophathy (Woodroffe *et al.* 1980; Hall *et al.* 1983; Valentijn *et al.* 1983). Because an attack of hematuria frequently follows the respiratory or intestinal infection (Egido *et al.* 1983b; Clarkson *et al.* 1984; Lozano *et al.* 1987) and IgA antibody extracted from renal tissue cross-reacts with the nuclei of tonsillar cells (Tomino *et al.* 1983), viral antigen was suspected as a causative agent. Abnormal immune response to herpes virus or hepatitis virus is reported in some patients with IgA nephropathy (Nagy *et al.* 1984; Lai *et al.* 1987). Other antigens such as bacteria (Isaacs and Miller 1982; Clarkson *et al.* 1984) or dietary antigen (Emancipator *et al.* 1983; Sancho *et al.* 1983) have also been proposed.

IgA-antibody appears on the intestinal mucosa and plays an essential role in the local defense mechanism to exogenous antigens. It is largely linked to the secretory component of the intestinal mucosa to be transported into the lumen through exocytosis (Allardyce and Bienenstock 1984). But some of the IgA is collected by the lymphatics, drained into the vein (Sato *et al.* 1987b), transported to the liver and secreted again into the intestinal lumen through bile. Intestinal secretory IgA is mainly polymeric, while serum IgA is monomeric. Thus, because the experimental IgA nephropathy can be induced only by polymeric IgA and the deposited IgA in the renal mesangium of human IgA nephropathy is identified as polymeric (Lopez-Trascasa *et al.* 1980; Bene *et al.* 1982), the causative IgA antibody seems to be of mucosal origin.

Abnormal sensitization to the tissue antigen or increased exposure to the antigen caused by enhanced permeability seems to result in overproduction of IgA (Emancipator *et al.* 1985) because of the frequent association with diseases involving mucosal surfaces rich in IgA-committed immunocytes such as celiac disease (Moorthy *et al.* 1978), Crohn's disease (Clarkson *et al.* 1984), mucous adenocarcinoma of the lung and colon (Sinniah 1982), and Sjögren's syndrome (Fujimoto *et al.* 1984). Overproduction of abnormal IgA by altered immunoregulation was also suggested (Bannister *et al.* 1983; Egido *et al.* 1983a) because of an increase in the number of IgA-containing lymphocytes in the peripheral blood of patients with IgA nephropathy (Nomoto *et al.* 1979) or their families (Sakai *et al.* 1979b) and the secretion of IgA by peripheral lymphocytes in response to polyclonal B cell activation, an increase of IgA specific helper T cells (Sakai *et al.* 1982) or a decrease of IgA specific suppressor T cells (Sakai *et al.* 1979a). Impaired clearing of abormal IgA is another possibility because IgA deposition in the glomerular mesangium with a defect in reticulophagocytic function is frequently associated with liver disease such as liver cirrhosis (Berger *et al.* 1978; Lawrence *et al.* 1983). But it is still obscure whether this disease is the by-product from the chronic overloading of polymeric IgA or the functional disturbance of Fc receptor resulting from certain genetic abnormalities (D'Amico *et al.* 1985).

Since Rifai's first experimental model in mice (Rifai *et al.* 1979), a few attempts to investigate the mechanism have been made through animal models simulating human IgA nephropathy (Gormly *et al.* 1981; Isaacs and Miller 1982, Melvin *et al.* 1983). Recently IgA deposition could be demonstrated by chronic passive oral immunization using foreign protein antigen (Emancipator *et al.* 1983; Sato *et al.* 1986). Choi and Choi (1987) were successful in the induction of IgA nephropathy in ddY mice using poliomyelitis vaccine and found an increase of IgA-committed immunocytes in the lamina propria of intestinal mucosa, suggesting a possible role of mucosal immunity in this disease.

The authors could induce an experimental model of IgA nephropathy in ddY mice using POLIO. The histologically moderate degree of mesangial cell proliferation and mesangial matrix widening was essentially matched with that of Choi and Choi.

Immunofluorescent deposition of IgA and the ultrastructural presence of large nodular electron-dense deposits were identified in the glomerular mesangium of POLIO groups, and the degree was more profound in the parenterally administered group. The number of IgA containing cells in the lamina propria of the intestinal mucosa was increased after the stimuli of POLIO.

The lower production of IgA in the orally administered group may be explained by two reasons; defective absorption of the antigen, and the relative hyporesponsiveness of systemic immunity. In man, when live POLIO is administered per os, exuberant proliferation of the virus stimulates the lymphoid tissue of the mucosa resulting in secretion of IgA (Ogra *et al.* 1968). But in mice, the absorption rate of the orally administered POLIO is poor because of failure of viral proliferation due to a defect in special attachment sites for the poliomyelitis virus.

Although the antigen is administered orally, its immune reaction is not localized in the lymphoid tissue of the intestine. Sensitized lymphocytes are drained to lymphatics, run into circulating blood, and stay in peripheral lymphoid tissues such as the spleen for a short time. Then they are finally colonized in the lamina propria of the mucosa of the gastrointestinal, upper respiratory and genitourinary tracts (Michalek

et al. 1983). While the local immunity is established in this way, systemic immune systems fall into a relative hyporesponsive state (Chase 1946). This phenomenon might be related to induction of suppressor T cells (Richman *et al.* 1978; Michalek *et al.* 1982), suppressor factor (Mattingly *et al.* 1980), immune complex (Andre *et al.* 1975), anti-idiotypic antibodies (Kagnoff 1978), suppression of helper T cells (Kiyono *et al.* 1982) or inhibition of IgA committed pre-B cells (Crabbe *et al.* 1969).

Mesangial IgM deposition was also substantial in POLIO groups. But this finding does not attenuate the importance of IgA deposition. The 70th and 100th experimental days correspond to the 16th and 20th week of age of ddY mice respectively, when ddY mice usually have spontaneous IgM deposition. But IgA deposition is very unusual in normal ddY mice (Imai *et al.* 1985). Moreover, IgM tends to be nonspeifically co-deposited with other components of immune material in many cases of glomerulonephritis.

A moderate to severe degree of proteinuria was detected in all experimental groups but hematuria was found only in three mice. Hematuria is usually a predominant finding in human IgA nephropathy. The relative infrequency of hematuria in our mice may be partly related to the discrepancy between the timing of urine collection and the hematuric attack. But experimental IgA nephropathy in mice seems to be not entirely the same as the human counterpart (Gormly *et al.* 1981; Emancipator *et al.* 1983; Melvin *et al.* 1983; Sato *et al.* 1986). The role of C3 in the occurrence of hematuria (Rifai *et al.* 1979; Isaacs *et al.* 1981; Emancipator *et al.* 1985; Emancipator and Lamm 1987) may also be associated because all three hematuric mice showed more than a mild degree of co-deposition of C3.

SCG is effective in the treatment of bronchial asthma and food allergy. Its major mechanism of action is the suppression of mucosal immunity by stabilization of the cell membrane in presensitized mast cells and prevention of the release of vasoactive amines. Like Sato *et al.* (1987a & 1987b), we tried to observe the preventive effect of SCG on the induction of experimental IgA nephropathy, and identified a significant decrease of mesangial IgA deposition by the concurrent use of SCG. IgA-containing cells in the intestinal mucosa were also decreased during the SCG treatment. These findings are related to the membrane stabilizing effect of SCG that inhibits the release of vasoactive amines. Now that the vascular permeability is decreased, exposure to antigen is diminished, circulatory IgA release is inhibited, and mesangial deposition of the immune complex is decreased.

The effect of SCG was more remarkable in the parenterally administered group than in the orally administered one. Relatively poor absorption of the drug, and the limited metabolic pathway to only three organs; the intestinal mucosa, liver and urinary tract, may be associated with the less significant change in the orally treated groups.

Deposition of immune complex in the mesangium depends on its type and molecular size as well as serum concentration (Mauer *et al.* 1972 & 1974). A small sized immune complex of about 300,000 to 500,000 dalton is deposited along the capillary wall, whereas a larger one of more than 1,000,000 dalton is deposited in the mesangial region (Rifai and Millard 1985). The polyvalency of polymeric IgA renders the IgA immune complex large enough (Clarkson *et al.* 1984; Rifai 1987). Moreover, IgA antibody, in comparison to IgG, is relatively able to form an immune complex with no regard to the ratio of the antigen-antibody (Rifai *et al.* 1979). Thus, the clearance of mesangial deposit seems to be far more difficult in IgA nephropathy so that later use of SCG after the 70th day failed to influence the IgA complex already deposited in the mesangium.

From these results, it can be regarded that mucosal immunity participates in the pathogenesis of IgA nephropathy. Further basic studies to clarify the nature of IgA nephropathy must be made with respect to further progress in the treatment and prevention of IgA nephropathy.

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