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Experimental IgA Nephropathy Induced by Oral Administration of Dextran

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ABSTRACT

Attempts were made to produce experimental IgA nephropathy by oral administration for 18 weeks of dextran having a molecular weight of 10,000, 70,000, and 500,000 to 6-week old female BALB/c mice. It was observed that in three out of eight cases belonging to the group given dextran having a molecular weight of 70,000 and in three out of seven cases belonging to the group given dextran having a molecular weight of 500,000, deposition of IgA on the mesangium was demonstrated by immunofluorescence direct method. Furthermore, deposition of IgG and IgM was observed in not only the groups given dextran but also in the control group, but no significant difference in deposition pattern could be demonstrated between the groups. C3 was observed only in one case belonging to the group given dextran having a molecular weight of 10,000. The serum IgA value showed a higher value in the cases with IgA deposition than in the control group. Serum C3 value was higher in the IgA deposition cases in the group given dextran having a molecular weight of 500,000 than in the control group. These results suggest that it is possible to produce experimental IgA nephropathy by continual oral administration of carbohydrate antigen of bacterial origin and that bacterial infection is involved in the development of IgA nephropathy.

IgA nephropathy is a disease entity proposed by Berger[1] in 1968 and with the dissemination of the immunofluorescent technique, the proportion occupied by IgA nephropathy among renal diseases has increased. It was assumed at the early stage that the prognosis of this disease entity was satisfactory, but it has become known that one out of about four cases leads to renal failure. The elucidation of its etiology and establishment of its treatment method are matters of urgency. In IgA nephropathy following the clinical manifestation of upper respiratory infection and intestinal infection, macrohematuria is observed and in view of reports on the effect of tonsillectomy[17], bacterial or viral infection has been suspected as the cause[4,9,11,15,16], but the etiology is yet to be confirmed.

The first attempt to produce experimental IgA nephropathy was made by Rifai et al[12] in 1979. Subsequently, Emancipator et al[2] successfully produced experimental IgA nephropathy in mice by oral immunization of protein. Furthermore, Isaacs et al[6] were able to produce experimental IgA nephropathy in mice by intravenous immunization of dextran. This model is particularly interesting in relation to infection which is speculated to be one of the factors of the pathogenic pattern in human IgA nephropathy in view of the bacterial origin of dextran. We were successful in producing experimental IgA nephropathy not by intravenous but by oral administration of dextran. A report of the findings with some discussion will be presented.

MATERIALS AND METHODS

Dextran having a molecular weight of 10,000, 70,000, and 500,000 was each dissolved in tap water to a concentration of 1 mg/ml, which were provided daily ad libitum to 6-week old female BALB/c mice. Six mice were assigned to the group given dextran (Sigma) having a molecular weight of 10,000, eight mice to the group given dextran having a molecular weight of 70,000, seven mice to the group given dextran having a molecular weight of 500,000, and as controls seven mice were given tap water only ad libitum. At the 18th week after the oral administration was begun, the mice were sacrificed under ether anesthesia and using the kidney and blood obtained from these mice the following studies were conducted.

1. Studies by immunofluorescent technique

The renal tissues were rapidly frozen by n-hexane and then cut at 4 μm in a cryostat. after drying the cryostat sections on slide glass for 2-3 hr, they were washed with PBS. After adding to each, FITC-labelled goat anti-mouse IgG (Cappel, 10-fold dilution), goat anti-mouse IgA (Cappel, 10-fold dilution), goat anti-mouse IgM (Cappel, 10-fold dilution), and goat anti-mouse C_3 (Cappel, 10-fold dilution), they were incubated in a moist chamber at 37°C for 40 min. After washing with PBS for three times, they were covered with glycerin and then subjected to microscopy.

2. Studies by light microscopy

After fixing the renal tissues in 10% neutral formalin, they were dehydrated with ethanol, embedded in paraffin, and then stained with hematoxylin and eosin and PAS stain. They were then subjected to light microscopy.

3. Studies by electron microscopy

After fixing the renal tissues in 2% glutaraldehyde, they were postfixed in 1% OsO_4 and dehydrated with ethanol. They were then subjected to epon embedding and electron microscopy according to routine procedure.

4. Determination of serum IgA and C_3 value

Determination of serum IgA and C_3 value was made by SRID method. Briefly, serum IgA value was determined by partigen plate manufactured by Meloy Co. and serum C_3 value by partigen plate which we fabricated using goat anti-mouse C_3 (Cappel Co.). The standard curve was established using the value of the pooled control serum as 100%.

RESULTS

1. Findings of immunofluorescent technique

Table 1. IF Findings in Mice

	NO	IgG	IgA	IgM	C_3
Dextran (M.W. 10,000)	1	+	−	+	+
	2	±	−	+	−
	3	±	−	+	−
	4	±	−	−	−
	5	±	−	+	−
	6	+	−	+	−
Dextran (M.W. 70,000)	1	+	−	+	±
	2	+	−	+	−
	3	+	−	+	−
	4	+	+	+	−
	5	±	+	±	−
	6	±	±	+	−
	7	+	±	±	−
	8	±	+	+	−
Dextran (M.W. 500,000)	1	−	−	+	−
	2	±	+	+	±
	3	±	−	+	−
	4	±	+	±	−
	5	−	+	+	−
	6	+	−	±	−
	7	+	−	+	−
Control	1	±	−	±	−
	2	+	−	+	−
	3	±	−	+	−
	4	+	−	+	−
	5	±	−	+	−
	6	+	−	+	−
	7	±	−	+	−

The findings of immunofluorescent technique are summarized in Table 1. Fluorescence intensity was classified into (+), (±) and (−) and only (+) was regarded to be positive. In three out of eight cases belonging to the group given dextran having a molecular weight of 70,000 and in three out of seven cases belonging to the group given dextran having a molecular weight of 500,000, granular deposition of IgA was observed in the mesangium area (Fig. 1). No

difference in the pattern and level of IgA deposition could be observed between the group given dextran having a molecular weight of 70,000 and that of 500,000. On the other hand, deposition of IgG and IgM was observed in not only the groups given dextran having a molecular weight of 70,000 and 500,000, but also in the group given dextran having a molecular weight of 10,000 and in the control group. Furthermore, no significant difference in their frequency could be observed between the groups. C_3 was observed in only one case belonging to the group given dextan having a molecular weight of 10,000.

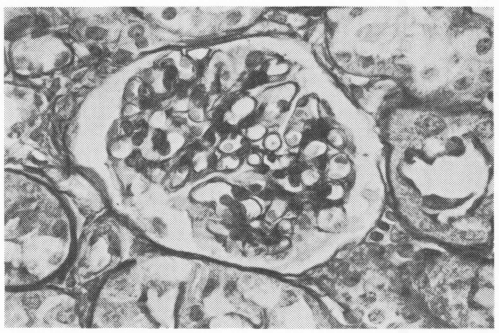

Fig. 2. Light microscopy shows slight increase of the mesangial cells and mesangial matrix. (× 440 PAS)

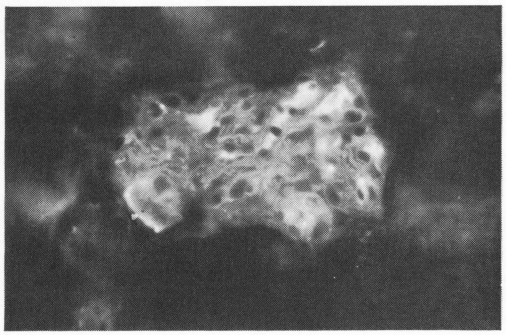

Fig. 1. Immunofluorescence shows granular deposits of IgA in the mesangium (the section was incubated with FITC-labelled anti-mouse IgA)

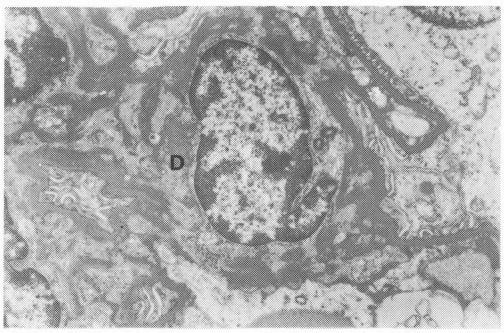

Fig. 3. Electron microscopy shows electron dense deposits (D) in the mesangial matrix (× 5000)

2. Light microscopic findings

Light microscopically, no remarkable changes could be observed in each group, but in the cases with IgA deposition in the glomerulus the proliferation of the mesangium tended to be stronger than in control group (Fig. 2).

3. Electron microscopic findings

In cases in which IgA deposition could be observed in the glomerulus, electron dense deposits could be seen in the mesangium area (Fig. 3). There were no other remarkable findings.

4. Serum IgA and C_3 value

As shown in Fig. 4 and Fig. 5, serum IgA and C_3 value were examined according to IgA deposition group and non-deposition group. In comparison with the control group, the serum IgA value of the IgA deposition group in the group given dextran having a molecular weight of 70,000 and those of the IgA deposition group and of the non-IgA deposition group in the group given dextran having a molecular weight of 500,000 were significantly higher at the 2%, 0.1% and 1% level, respectively. In comparison with the control group, the serum C_3 value of the non-IgA deposition group in the group given dextran having a molecular weight of 10,000 and in the group given dextran having a molecular weight of 70,000 and of the IgA deposition group and non-IgA deposition group in the group given dextran having a molecular weight of 500,000 was significantly higher at the 5%, 5%, 0.1% and 0.1% level, respectively.

5. Urinalysis fundings

Urinalysis was conducted with the test tape

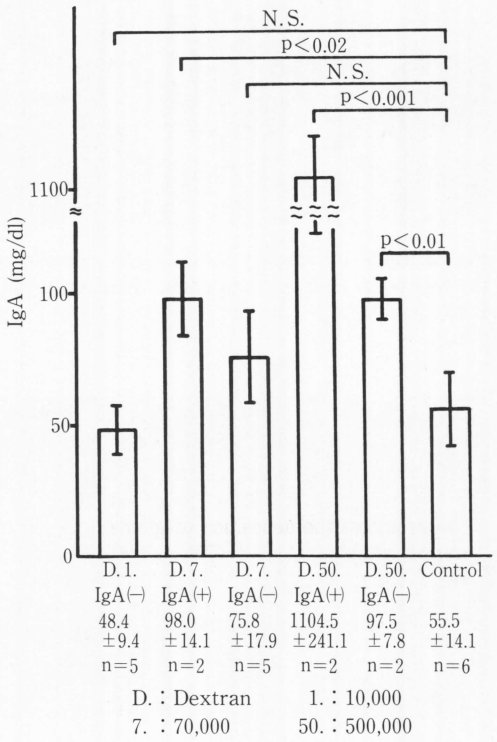

Fig. 4. Serum IgA values in mice

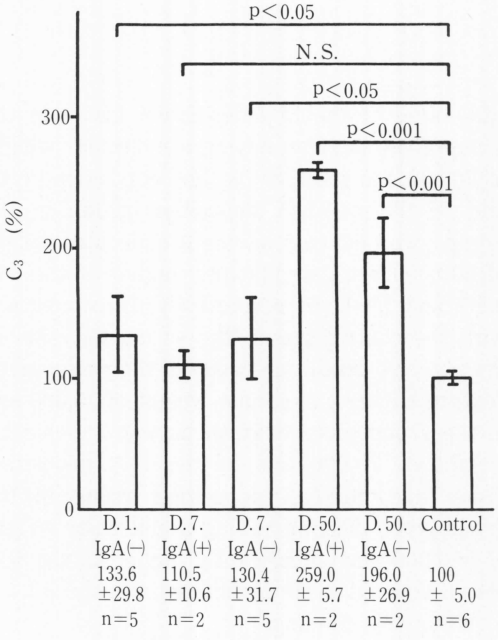

Fig. 5. Serum C_3 values in mice

method [URO-LABSTIX-Ⅲ(AMES)]. During the entire course, protein ranged from trace to (+) and occult blood was (−) with no significant difference between the groups.

DISCUSSION

As the frequency of IgA nephropathy is high and as not a few cases lead to renal failure, the elucidation of its developmental mechanism and establishment of its treatment method are urgently required. For their resolution, experimental models are of importance. To date a number of models of experimental IgA nephropathy have been reported. In 1979 Rifai et al[12] attempted to produce nephritis with the use of various immune complexes and were first to discover that only immune complex of polymer IgA deposited on the glomerulus. This finding suggested that IgA originates from the secretory system. Emancipator et al[2] by long term oral immunization of protein observed IgA deposition on the glomerulus, which implies that IgA is of intestinal origin. Furthermore, through bile duct ligation[3,10] and production of experimental liver cirrhosis[5] it has been observed that IgA deposits on the glomerulus. This is considered to result from excretory disorder of IgA having secretory component to the bile duct and the reduced handling of IgA immune complex in the liver. Furthermore, an interesting report was published by Isaacs et al[6] in 1981. By intraperitoneal and intravenous administration of dextran of different molecular weight and electrical charge in mice, they have observed deposition of IgA and C_3 in the glomerulus independent of molecular weight and electrical charge. Dextran originates from Leuconostoc mesenteroides strain B512 (95% alpha-1,6- and 5% alpha -1,3- glycosidic linkages) and is a type of carbohydrate. This model is the first report of experimental IgA nephropathy produced by carbohydrate antigen. As for the property of carbohydrate of inducing nephritis, Shibata[13,14] has observed in "nephritogenoside", a nephritis induction substance which he produced, that nephritis induction property is located in the sugar chain. This suggests that similar to protein antigen dextran must also possess nephritis induction property. At the same time, there is also a possibility that IgA antibody appears in the blood against carbohydrate antigen in the bac-

teria. It is therefore considered possible that nephritis can be induced by continued administration of dextran.

We therefore attempted to produce experimental IgA nephropathy by oral administration of dextran and observed deposition of IgA in the glomerulus of mice belonging to the group given dextran having a molecular weight of 70,000 and that of 500,000. Furthermore, a significant elevation in serum IgA and C_3 values was observed in cases with IgA deposition.

In general, protein antigen administered orally stimulates lymphocytes including IgA producing precursor cells present in the gut-associated lymphoreticular tissue (GALT) particularly around Peyer's patches. These stimulated lymphocytes enter the corporeal circulation via the mesenteric lymph nodes and thoracic duct and then reach the lamina propria mucosae of the upper respiratory tract and intestinal duct, and the external endocrine tissue of the lacrimal gland, salivary gland, and mammary gland to produce IgA antibodies[7]. In the present model, it was observed that similar to protein antigen, carbohydrate antigen could by oral immunization produce IgA antibody in mice and bring about IgA deposition in the glomerulus. It is, however, necessary to determine whether IgA deposited in the glomerulus is IgA immune complex or aggregated IgA through assay of serum immune complex and elution study of the renal tissue.

Next, a discussion will be made on the differences from the experimental model of Isaacs et al[6] who used dextran as in our model, though the method of administration mutually differs. First, in our method IgA deposition could not be demonstrated in mice given dextran having a molecular weight of 10,000, second, C_3 was negative in almost all of the cases, and third, deposition of IgG and IgM could be observed in also normal mice. On the first problem, it is considered that further study should be made on antigen concentration and period of administration. With regard to the second problem, since similar results were obtained by Emancipator et al[2] who used the same strain of mice, it is considered that studies should be made on the difference in the mouse strain. With regard to the third problem, Markham et al[8] have observed deposition of immunoglobulin in also normal mice, which is regarded to be similar to that in also BALB/c female mice. Thus, it is speculated that strain difference is large. However, as IgA was negative in all control cases, it is regarded that IgA deposition in the glomerulus by the administration of dextran is of significance. Though there are a number of differences as described earlier, it is considered that our method is a useful model of experimental IgA nephropathy and the involvement of bacterial infection in the development of IgA nephropathy is suggested.

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