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In vivo alteration of antibody production in patients with IgA nephropathy

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SUMMARY

Augmentation of IgA production has been postulated for the development of IgA nephropathy. An influenza HA vaccine was administered to healthy adults and patients with IgA nephropathy to elucidate if there was any *in vivo* alteration of antibody production in response to antigenic stimulation in these patients. The vaccine was administered s.c. in a dose of 350 CCA units at an interval of 4 weeks. IgG, IgA and IgM class antibodies to influenza HA antigens and three classes of rheumatoid factors (RF) were determined using a solid phase fluorescence immunoassay. The titres of IgG class antibodies to influenza HA antigens did not change significantly in either group after the vaccination. No significant differences were observed in the titres of IgG antibodies between the two groups. IgA antibodies were significantly increased only in patients in the 4th week and continued to the 8th week. The titres of IgA antibodies were always higher in patients than in controls during the study period. IgM antibodies were significantly increased stepwise in both groups to an equal degree. IgG and IgA RF were always higher in patients than in controls. IgM RF were significantly increased and higher than in controls in the 8th week in patients. It is concluded that patients with IgA nephropathy might be high responders for IgA antibody production, and that polyclonal activation might be associated with increased IgA production following *in vivo* antigenic stimulation in these patients.

Keywords IgA nephropathy *in vivo* vaccination antibody production

INTRODUCTION

IgA nephropathy (Berger's disease) is known as an entity of primary chronic glomerulonephritis (Berger & Hinglais, 1968) which is presumed to be induced by IgA dominant immune complexes (Woodroffe *et al.*, 1980; Mustonen *et al.*, 1981; Stachura, Singh & Whiteside, 1981; Tomino *et al.*, 1982; Lesavre, Digeon & Bach, 1982; Hall *et al.*, 1983; Valentijin *et al.*, 1983). Serum IgA is increased in some patients with this disorder (Nomoto, Sakai & Arimori, 1979; Woodroffe *et al.*, 1980; Stachura *et al.*, 1981). It is speculated that augmentation of IgA production *in vivo* might play a role in the development of IgA nephropathy. We have reported an increase in IgA bearing cells (Nomoto *et al.*, 1979), a decrease in IgA specific suppressor T cell activity (Sakai, Nomoto & Arimori, 1979) and an increase in IgA specific helper Tα cells (Endoh *et al.*, 1981a; Sakai *et al.*, 1982) in peripheral blood from these patients. Occasional association of autoimmune phenomena such as anti-nuclear antibody (Nomoto & Sakai, 1979), scleritis (Nomoto *et al.*, 1980; Hurault De Lig *et al.*, 1983) and autoimmune diseases (Endoh *et al.*, 1981b; Spichtin *et al.*, 1980; Jennette *et al.*, 1982; Imai *et al.*, 1983) were also observed in some patients with this disease.

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This study was performed to elucidate if there are *in vivo* alterations of antibody production in response to some antigenic stimulation in these patients. An influenza HA vaccine was administered to healthy adults and patients with IgA nephropathy, and IgG, IgA and IgM antibodies to influenza HA antigens and to rabbit IgG (rheumatoid factors; RF) were serially studied by a solid phase fluorescence immunoassay to elucidate altered antibody production in these patients.

MATERIALS AND METHODS

Patients and controls. Thirteen patients with IgA nephropathy (Berger's disease) without renal failure were studied. The mean age was 30 years (19–45). All patients were diagnosed by open renal biopsy evaluated by light microscopy, electron microscopy and immunofluorescent staining. Fourteen age matched healthy adults (medical students and medical staff) served as controls. Neither patients nor controls showed any signs of infection during the study period. This clinical trial has been conducted with the full consent of our Ethical Committee.

Immunization and clinical follow-up. Influenza HA vaccine was obtained from Takeda Chemical Industries (Osaka, Japan). The vaccine was prepared from influenza A/Kumamoto/37/79(H1N1), A/Niigata/102/81(H3N2) and B/Singapore/222/79 influenza strains. The vaccine was administered s.c. in a dose of 350 CCA units/0·5 ml at an interval of 4 weeks. Serum specimens were obtained in the 0, 4th and 8th weeks, and stored in aliquots at −70°C until assayed. Clinical and laboratory evaluations were repeated at intervals of 2 weeks. They consisted of complete urinalysis, leucocyte count and differential, haemoglobin, platelet count and serum creatinine. Levels of IgG, IgA, IgM, C3 and C4 were measured by laser nephelometry. The latex fixation test was also performed for RF.

Determination of antibodies to influenza HA. A solid phase fluorescence immunoassay (FIA) was employed to determine IgG, IgA and IgM class antibodies to influenza HA antigens. Polyacrylamide beads (Immunobeads Reagent Coupling Kit®, BIO-RAD Laboratories, Richmond, California, USA) were coupled covalently with influenza HA antigens from the same strains as used for the vaccine (Takeda Chemical Industries). Briefly, 200 mg of polyacrylamide beads (5–10 μm diameter) were mixed with 10 mg of HA antigens and 40 mg of EDAC(1-ethyl-3[3-dimethyl aminopropyl] carbodi-imide HCl) in 0·003 M phosphate buffer (pH 6·3) at 4°C overnight. Then these reagents were washed with phosphate-buffered saline (PBS, pH 7·2) three times, followed by 1·4 M NaCl-PBS three times, and finally by PBS three times. These reagents were stored in 20 ml of 0·005 M phosphate–2% bovine serum albumin (BSA) buffer at 4°C. These immunobead reagents were reconstituted at a concentration of 1 mg/ml in 0·5% Tween in PBS (PBS-Tween). Duplicate samples of 1·0 ml of immunobeads and 50 μl of sera were mixed in 17 × 100 mm plastic tubes (Nissui, Tokyo, Japan), incubated at 37°C for 2 h and kept at 4°C overnight. After incubation, each tube was centrifuged at 1,700*g* for 8 min and insoluble reagents were washed three times with PBS-Tween. Then 50 μl of FITC conjugated heavy chain specific rabbit antihuman IgG, IgA or IgM antiserum (Behring-Werke AG, Federal Republic of Germany; F/P ratio: 1·8–2·5) was added. These antisera were previously diluted five-fold with PBS, absorbed three times with mouse liver acetone powder (Sigma, St Louis, Missouri, USA) and centrifuged at 105,000*g* for 30 min prior to use. After incubation with antisera for 2 h at 37°C, immunobeads were washed three times with PBS. The precipitate was resuspended in 2·0 ml of PBS and the intensity of fluorescence in each sample was determined by a fluorescence spectrophotometer (Hitachi MPF-4, Japan) using filters at an excitation wave length of 485 nm and emission wave length of 525 nm. The value of corrected relative fluorescence indicated the value of the sample minus that of a reagent blank.

Determination of RF. FIA was also used to determine IgG, IgA and IgM RF. Polyacrylamide beads were coupled covalently with rabbit IgG fraction (Cappel Laboratories, Cochranville, Pennsylvania, USA) and blocked with BSA as described above. The amount of each class RF was expressed by the value of corrected relative fluorescence.

Statistical analysis. Serial changes of the amounts of antibodies to HA antigens and RF were analysed by Wilcoxon's test. Differences in the amounts of antibodies and RF between controls and patients were analysed by Mann–Whitney's U-test.

RESULTS

There were no significant changes either in physical conditions, urinalysis, CBC or serum creatinine levels after the immunization by influenza HA vaccine in patients with IgA nephropathy. Immunoglobulin and complement levels were almost unchanged during the study period both in healthy adults and patients with IgA nephropathy.

Figs 1–3 show serial changes in the titres of IgG, IgA and IgM antibodies to influenza HA antigens. The titres of IgG class antibodies did not change significantly during the study period in both the control and patient groups. There were no significant differences in the titres of IgG antibodies between the two groups. IgA antibodies were significantly increased only in patients with IgA nephropathy in the 4th week and continued to the 8th week after the immunization with influenza HA antigens. Furthermore, the titres of IgA antibodies were always higher in the patient group than in the control group during the study period. IgM antibodies were significantly increased stepwise both in the controls and patient groups, but there were no significant differences in the titres between the two groups.

Figs 4–6 show serial changes of IgG, IgA and IgM RF after immunization with influenza HA antigens. IgG RF showed a tendency to increase in the 4th week and decrease in the 8th week in both groups. However, IgG RF was always higher in patients with IgA nephropathy during the study period. Although IgA RF showed no significant changes after immunization with influenza HA antigens in both groups, the titres were always higher in patients with IgA nephropathy than in controls similar to the case of IgG RF. IgM RF were significantly increased and higher than controls in patients with IgA nephropathy. Latex fixation tests were negative in all persons during the study period.

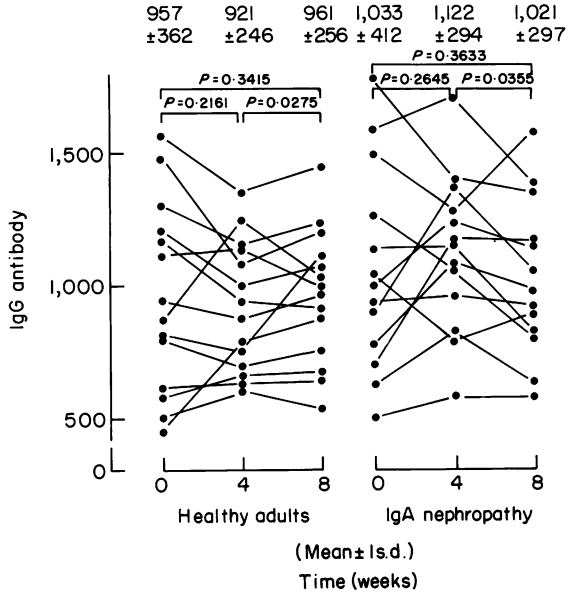

Fig. 1. IgG antibodies to influenza HA antigens are serially determined using a solid phase fluorescence immunoassay. Each value represents corrected relative fluorescence described in Methods. No significant increases in IgG antibodies were observed in either group.

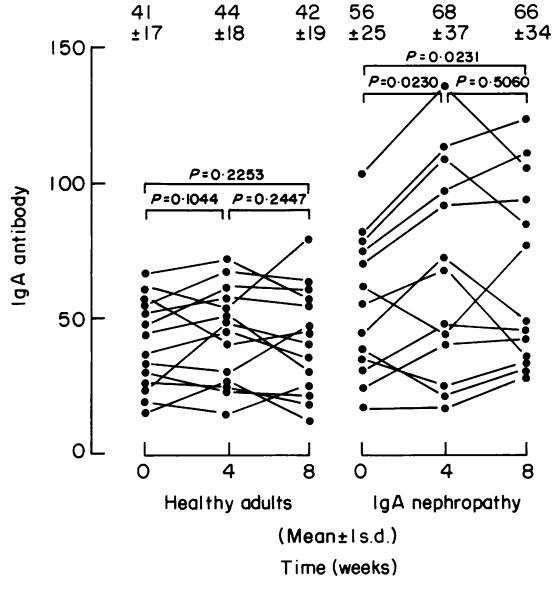

Fig. 2. Serial IgA antibodies to influenza HA antigens are shown. The titres were increased only in patients with IgA nephropathy in the 4th week and continued to the 8th week. The titres were always higher in patients than in healthy adults during the study period.

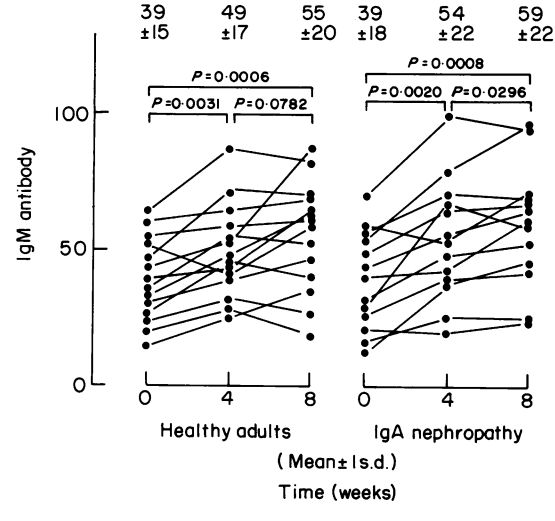

Fig. 3. Serial IgM antibodies to influenza HA antigens are shown. The titres were significantly increased stepwise to an equal degree in both groups.

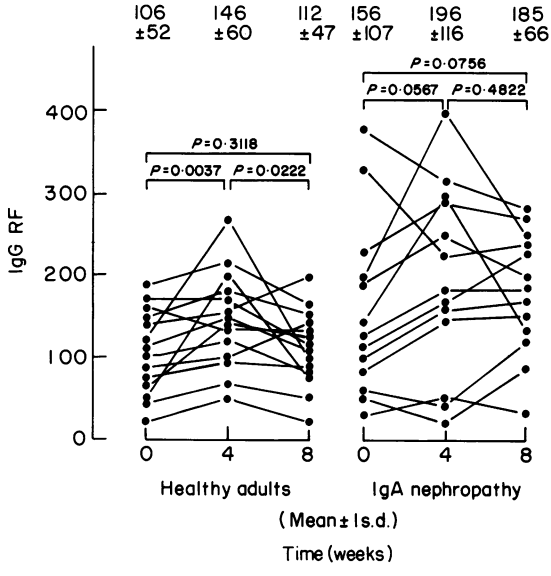

Fig. 4. RF were determined using solid phase FIA. IgG RF were increased in the 4th week and decreased in the 8th week in both groups. The titres were always higher in patients than in healthy adults.

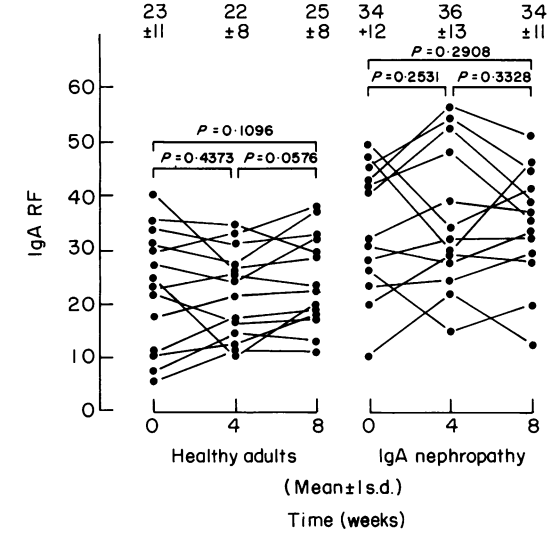

Fig. 5. Serial IgA RF are shown. IgA RF were always higher in patients with IgA nephropathy than in healthy adults.

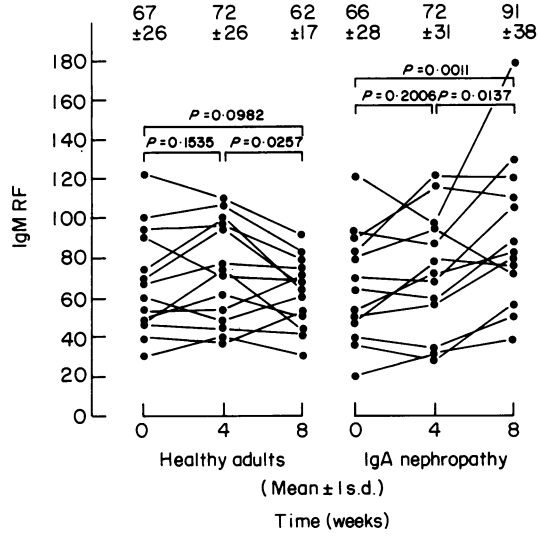

Fig. 6. Serial IgM RF are shown. IgM RF were significantly increased and higher than in healthy adults in the 8th week in patients with IgA nephropathy.

DISCUSSION

IgA nephropathy is defined by the deposition of IgA dominant immune complexes in the mesangial area of the glomeruli (Berger & Hinglais, 1968). These immune deposits have been observed in idiopathic cases (Berger's disease), systemic lupus erythematosus, Henoch–Schönlein purpura and alcoholic cirrhosis. In this study, we examined patients with idiopathic cases (Berger's disease). The pathogenic mechanism in the development of this nephropathy has not been elucidated. Patients with IgA nephropathy might have nephritogenic levels of circulating IgA immune complexes (Stachura *et al.*, 1981; Tomino *et al.*, 1982; Lesavre *et al.*, 1982; Hall *et al.*, 1983; Valentijin *et al.*, 1983). Although impaired clearance from the circulation of potentially nephritogenic IgA containing immune complexes might be involved (Sancho *et al.*, 1983), excessive IgA production in response to some antigenic exposure might also be involved in the development of IgA dominant immune complexes in these patients. Increased spontaneous IgA synthesis *in vitro* by peripheral blood mononuclear cells (Endoh *et al.*, 1983) suggests that IgA antibody production *in vivo* might be augmented in patients with IgA nephropathy. We determined the serial changes in the titres of IgG, IgA and IgM antibodies after immunization with influenza HA antigens. The vaccination of influenza HA antigens induced a significant increase in the titres of IgM class antibodies in patients and healthy adults, but IgG antibodies were not significantly increased in either group. It was suggested that only a primary response was induced in both groups in this study, since the viruses used for the preparation of these vaccines belonged to strains new to the people studied. Significant increases in IgA antibodies to influenza HA antigens were observed only in patients with IgA nephropathy. Furthermore, the titres of IgA antibodies were always higher in patients than in controls during the study period. These data suggested that patients with IgA nephropathy might be high responders for IgA antibody production. The antigens used in the vaccine contained numerous antigenic sites, and therefore the antibody production to such antigens might be polyclonal. The increased production of IgA antibody in the patients following vaccination might reflect polyclonal expansion of IgA antibodies in such patients. To further determine the polyclonal expansion of antibody production in these patients, we also studied three Ig classes of RF before and after the administration of influenza vaccines. We have previously reported the presence of anti-nuclear antibody (Nomoto & Sakai, 1979) and association with scleritis (Nomoto *et al.*, 1980) in patients

with this disorder. Association of autoimmune disease such as myasthenia gravis (Endoh *et al.*, 1981b), immunothrombocytopenia (Spichtin *et al.*, 1980; Imai *et al.*, 1983) and spondylarthropathies (Jennette *et al.*, 1982) was reported in patients with IgA nephropathy. We observed increases in titres of IgG, IgA and IgM RF in patients with IgA nephropathy after vaccination. IgG and IgA RF were always higher in patients than in controls. IgM RFs were increased in patients with IgA nephropathy after the immunization of influenza HA antigens. These results suggested that polyclonal B cell activation developed in patients with IgA nephropathy.

It is concluded that patients with IgA nephropathy might be high responders for IgA antibody production and that polyclonal activation might be associated with increased IgA production following *in vivo* antigenic stimulation in these patients.

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