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Cross-reactivity of IgA antibodies between renal mesangial areas and nuclei of tonsillar cells in patients with IgA nephropathy

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SUMMARY

A study on autoradiographical analysis of antigenic sites in patients with IgA nephropathy is described. Renal biopsy specimens were obtained from patients with IgA nephropathy. These specimens were treated with citrate buffer (pH 3·2) and the 'eluate' was neutralized by sodium hydroxide. The 'eluate' was labelled with ^{125}iodine by the chloramine-T method. ^{125}I-labelled eluate was then applied to the tonsillar cells obtained from the same and other patients with IgA nephropathy as well as to those with other glomerular diseases. The tonsillar cells were dipped into the emulsion (NBT-2) and then examined with a light microscope. It was demonstrated that the antibodies eluted from renal tissues of patients with IgA nephropathy specificially bound with the nuclear regions of tonsillar cells. The binding of eluted antibodies and tonsillar cells was completely inhibited by the addition of anti-human IgA antisera, but not inhibited by human IgA myeloma proteins. The eluted antibodies bound with tonsillar cells from the same patients, but only 10% of them bound with the tonsillar cells obtained from other patients with IgA nephropathy. It is concluded that IgA antibodies deposited in glomeruli specificially bind with tonsillar cells obtained from patients with IgA nephropathy and these antibodies show some heterogeneity among those patients.

INTRODUCTION

IgA nephropathy is assumed to be an immune complex-mediated glomerulonephritis (Berger, 1969; McCoy, Abramowsky & Tisher, 1974; Shirai *et al.*, 1978). However, the antigenic substances involved in this disease are still obscure. It has been postulated that IgA may play a role in the pathogenesis and development of this disease (Lowance, Mullins & McPhaul, 1973). IgA nephropathy is frequently preceeded by an episode of upper respiratory or gastrointestinal infection which is presumed to have a viral aetiology. Finlayson *et al.* (1975) has reported an increase of nasal IgA concentrations in some patients with IgA nephropathy. Recently, we suggested that the increase of IgA and total proteins in pharyngeal washings from patients with IgA nephropathy might be associated with local infections (Tomino *et al.*, 1982b). Moreover, we have reported that IgA antibodies obtained from renal tissues in IgA nephropathy are specificially recombined with mesangial areas of these patients although these antibodies did not show any anti-mesangial activity (Tomino *et al.*, 1982a). It was suggested that several different antigenic substances may be

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composed of such immune complexes since these antibodies show some heterogeneity among patients with IgA nephropathy. The purpose of the present study was to determine whether IgA antibodies eluted from renal biopsy specimens specificially bind with tonsillar cells in patients with IgA nephropathy. The results of this study suggested that some antigenic sites might exist in the epithelial cells of upper respiratory tracts in some patients with IgA nephropathy.

MATERIALS AND METHODS

Patients. Eight patients with IgA nephropathy, three patients with other glomerular diseases and four healthy adults were examined. IgA nephropathy is characterized by a mesangial deposition of IgA in renal biopsy specimens by immunofluorescent staining. Patients with IgA nephropathy whose biopsy specimens stained predominantly for IgA in mesangial areas were included in this study after the exclusion of patients with systemic lupus erythematosus, Henoch–Schoenlein purpura, liver cirrhosis or other systemic diseases. Patients who had tonsillitis or pharyngitis within 2 months prior to the study were excluded. Among three patients with other glomerular diseases, there were two patients with chronic proliferative glomerulonephritis (PGN) and one patient with minimal change nephrotic syndrome (MCNS).

Elution procedure. Modified open renal biopsy was performed as described previously (Kawamura *et al.*, 1980). Elution procedures were also performed as previously (Tomino *et al.*, 1982a). In brief, open renal biopsy specimens were treated with citrate buffer (pH 3·2) and the 'eluate' was neutralized by sodium hydroxide. Approximately 80 glomeruli were observed in the 10 serial sections placed on a single glass slide. Although the mean of total proteins in the 'eluate' from six patients with IgA nephropathy was 5·7 μg/ml as measured by Lowry *et al.*'s method (1951), that of IgA in the 'eluate' was 1·9 μg/ml as measured using a laser nephelometer.

Autoradiographical analysis

(a) *Direct method.* The 'eluate' was labelled with ^{125}iodine by the chloramine-T method (McConahey & Dixon, 1966). Specific activity of this radiolabelled proteins was $1{\cdot}4 \times 10^5$ c.p.m./μg protein. Tonsillar cells were obtained from patients and healthy adults by scraping using a swab and then suspended in 1·0 ml of isotonic saline (pH 7·2). These cells were then centrifuged in a Shandon cytocentrifuge (Shandon Instruments, Pennsylvania, USA) onto clean glass slides using one to two drops of cell suspension per slide. These cells were fixed with cold ethanol (95%) for 30 min and then stored at −20°C. For the exclusion of non-specific binding, slides of either patients' cells or normal cells were previously incubated with the 'eluate' obtained from glomerular diseases other than IgA nephropathy at 4°C overnight in a moist chamber. These cells were washed with phosphate-buffered isotonic saline (PBS) (pH 7·2). The optimum incubation periods were determined by preliminary experiments.

Tonsillar cells were incubated at 4°C overnight with ^{125}I-labelled 'eluate' obtained from same and/or other patients with IgA nephropathy. Because of the small quantity of the biopsy specimens, these 20 combinations were randomly selected from 64 combinations (i.e. 8 × 8) among eight patients with IgA nephropathy. ^{125}I-labelled eluates from four patients with IgA nephropathy were applied to tonsillar cells from three patients with other glomerular diseases. ^{125}I-labelled eluates from six patients with IgA nephropathy were incubated with tonsillar cells from four healthy adults (medical staff) without glomerular diseases. In parallel studies, ^{125}I-labelled eluates were incubated with various amounts of anti-human IgA, IgM and IgG antisera or human IgA myeloma proteins (prepared in our laboratory) prior to incubation with tonsillar cells. These cells were dipped in emulsion (NBT-2, Eastman Kodak Company, New York., USA) at 43°C in a water bath and then exposed at room temperature for 7 days in a complete dark box as described by Messier & Leblond (1957). After exposure, these cells were developed with Konodol X® (Konishiroku Photo Ind. Co., Tokyo, Japan) at room temperature for 5 min and then fixed with Unifix® (Konishiroku Photo Ind. Co., Ltd., Tokyo, Japan) at room temperature for 10 min. Finally, these cells were stained with methylene blue for several seconds and then examined by a light microscope. 'Positive' cells

indicated cells which possessed intracellular grains determined by light microscopy. All samples were secreened by two observers who independently counted the cells.

Indirect method. Anti-human IgA antisera were labelled with ^{125}iodine by the chloramine-T method (McConahey & Dixon, 1966). Anti-human IgA antisera (heavy chain specific) were obtained from Behringwerke AG, Marburg-Lahn, West Germany (lot No. 2093AB). Tonsillar cells were incubated at 4°C overnight with the non-radiolabelled 'eluate' obtained from same and/or other patients with IgA nephropathy. The cells were washed with PBS and then incubated with ^{125}I-labelled anti-human IgA antisera at 4°C overnight. The cells were examined as described above.

Immunofluorescent studies. Immunofluorescent staining was performed as previously described (Tomino *et al.*, 1981). In brief, tonsillar cells were incubated at 4°C overnight with 'eluate' obtained from same and/or other patients with IgA nephropathy. The 'eluate' was concentrated five times by 5-minicon® (Amicon Corp. Lexington, Massachusetts, USA). These cells were washed with PBS and then stained with FITC labelled anti-human IgA, IgM and IgG antisera (heavy chain specific) (Behringwerke AG, Marburg-Lahn, West Germany, F/P molar ratios ranged from 1·8 to 2·9) at 4°C overnight. In parallel studies, the cells were directly stained with FITC labelled anti-human IgA, IgM and IgG antisera at 4°C overnight as controls. The cells were examined with a Zeiss Orthoflux microscope(Model 9902; Carl Zeiss, Inc. New York, New York, USA).

RESULTS

Autoradiographical analysis

Direct method. During incubation of the ^{125}I-labelled 'eluate' with the tonsillar cells from the same patient with IgA nephropathy, the 'eluate' was bound with nuclear regions of tonsillar cells in seven out of eight combinations (87·5%) (Table 1, Fig. 1). During incubation of the ^{125}I-labelled 'eluate' with the tonsillar cells from other patients with IgA nephropathy, the 'eluate' was bound with the nuclear regions of tonsillar cells in two out of 12 combinations (16·7%) (Table 1). The binding was completely inhibited by the addition of anti-human IgA (heavy chain specific) antisera. The binding was not inhibited by the addition of anti-human IgG and IgM (heavy chain specific) antisera. Human IgA myeloma proteins did not inhibit the binding of ^{125}I-labelled eluates with tonsillar cells.

^{125}I-labelled 'eluate' was not bound with the tonsillar cells obtained from six combinations with PGN and MCNS, and nine combinations with healthy adults (Fig. 1).

Indirect method. During incubation of the 'eluate' and ^{125}I-labelled anti-human IgA antisera (heavy chain specific) with the tonsillar cells from the same patient with IgA nephropathy, the 'eluate' was bound with the nuclear regions of tonsillar cells in two out of three combinations. During incubation of the 'eluate' and ^{125}I-labelled anti-human IgA antisera with the tonsillar cells from the other patients with IgA nephropathy, the 'eluate' was bound with the nuclear regions of the tonsillar cells in one out of two combinations, although it was not bound with the tonsillar cells obtained from healthy adults.

Immunofluorescent studies. The 'eluate' obtained from patients with IgA nephropathy was not

Table 1. The incidence of positive binding with tonsillar cells and the eluate in IgA nephropathy

	Number of combinations	Positive cases (%)
Bound with tonsillar cells obtained from		
same patients with IgA nephropathy	8	7 (87·5)
other patients with IgA nephropathy	12	2 (16·7)
patients with other glomerular diseases	6	0 (0)
healthy adults without glomerular diseases	9	0 (0)

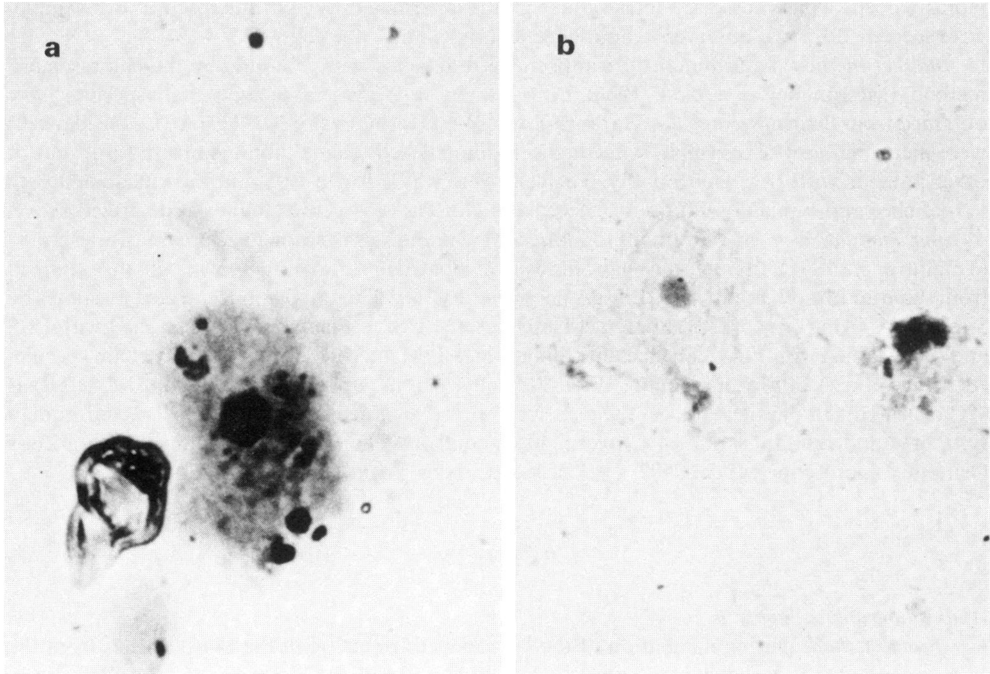

Fig. 1. (a) ^{125}I-labelled eluate obtained from a patient with IgA nephropathy was bound with the nuclear regions of the patient's own tonsillar cells. (b) ^{125}I-labelled eluate obtained from a patient with IgA nephropathy was not bound with tonsillar cells obtained from a healthy adult.

detected in tonsillar cells obtained from the same and/or other patients with this disease by immunofluorescence.

The 'eluate' obtained from patients with IgA nephropathy was not detected in tonsillar cells from healthy adults. The deposition of IgA, IgG and IgM was not observed in the tonsillar cells obtained from patients with IgA nephropathy and healthy adults.

DISCUSSION

The results obtained from this study showed that antibodies eluted from renal tissues of patients with IgA nephropathy specificially bound with the nuclear regions of tonsillar cells as determined by autoradiographical analysis. In addition to the direct method of autoradiographical analysis, such specific binding was confirmed by the indirect method. Non-specific binding of IgA antibodies to tonsillar cells does not appear likely because such binding was not inhibited by human IgA myeloma proteins. These results indicated that there is a common antigenic sites in both mesangial areas and nuclear regions of tonsillar cells in patients with IgA nephropathy.

Although eluted antibodies bound with tonsillar cells obtained from the same patients, they reacted with only two out of 12 combinations from other patients with IgA nephropathy. Moreover, the binding of eluted antibodies and the tonsillar cells was completely inhibited by the addition of anti-human IgA antisera although these bindings were not inhibited by the addition of anti-human IgG and/or IgM antisera and human IgA myeloma proteins. It is indicated that these IgA antibodies specificially bind in subgroups of patients with IgA nephropathy, but do not ubiquitously in all patients. It is therefore suggested that there is some antigenic heterogeneity among these patients. The eluted antibodies, however, might be specific in patients with IgA nephropathy since they did not bind with tonsillar cells obtained from patients with glomerular

diseases other than IgA nephropathy and healthy adults. There was no significant relationship between the intensity of the antibodies bound with tonsillar cells and the histopathological changes in patients with IgA nephropathy. However, these IgA antibodies were not detected on tonsillar cells by immunofluorescence in this study. It is postulated that the amounts of these antibodies might too small to be detected by immunofluorescent staining. The renal tissues were obtained by modified open renal biopsy in our clinic. Such modified open renal biopsy is a routine procedure in our clinic, since this procedure is safe enough to stop bleeding in open site. In addition, this procedure yield sufficient amount of tissues for analysis of various pathological tests. More than 550 cases were operated by using this procedure, and none of them has shown any signs of serious complications.

Although IgA nephropathy is presumed to be an immune complex-mediated glomerulonephritis (Berger, 1969; McCoy *et al.*, 1974; Shirai *et al.*, 1978), the antigenic substances of this disease are still obscure. It is presently unknown whether the eluted antibodies composed of soluble antigen–antibody complexes deposited in the glomeruli or some *in situ* antigenic sites exist in the glomerular mesangium of patients with IgA nephropathy. Clinically, development and exacerbation of IgA nephropathy are frequently preceeded by episodes of upper respiratory and/or gastrointestinal infections. An increase of pharyngeal and/or nasal IgA concentrations was observed in patients with IgA nephropathy as described by Finlayson *et al.* (1975), Whitworth *et al.* (1976) and the authors (Tomino *et al.*, 1982b). Conley, Cooper & Michael (1980) and the authors (1981) have reported that IgA1 was observed in glomeruli from patients with IgA nephropathy. Although IgA2 is considered to be produced in the bronchial and gastrointestinal mucosa as described by André, André & Fargier (1978), IgA1 was also detected in pharygeal washings obtained from patients with IgA nephropathy as described previously (Tomino *et al.*, 1982b). The IgA1 observed in pharyngeal washings may be exudated from the mucosal membranes in the upper respiratory tracts and/or oral cavity (Tomino *et al.*, 1982b).

It is postulated that the lesions of antigenic stimulation in IgA nephropathy may be located in the upper respiratory tract and/or oral cavity. It is suggested that the application of autoradiographical analysis is useful for exploring the antigenic sites in IgA nephropathy. Further studies are warranted to determine whether the cross-reactivity of IgA antibodies between renal tissues and tonsillar cells is a specific phenomenon in patients with IgA nephropathy.

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