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Immunological abnormalities in the tonsils of patients with IgA nephropathy: inversion in the ratio of IgA: IgG bearing lymphocytes and increased polymeric IgA synthesis

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SUMMARY

In the last few years the mucosal origin of the IgA deposited in the kidneys of patients with IgA nephropathy has been examined by several investigators. We have previously presented evidence that polymeric IgA may have a predominant role in the pathogenesis of IgA nephropathy. Taking into account that these patients often present with macroscopic haematuria following respiratory tract infections we have studied the possible existence of immunological abnormalities in the tonsils of patients with IgA nephropathy. Six patients and 13 controls suffering from chronic tonsillitis were submitted to tonsillectomy. Patients with IgA nephropathy showed a significant increase ($P < 0{\cdot}00025$) in IgA bearing lymphocytes ($14{\cdot}4 \pm 2{\cdot}3$) and a significant decrease ($P < 0{\cdot}025$) in IgG bearing lymphocytes ($20{\cdot}5 \pm 4{\cdot}6$) compared to the control group ($2{\cdot}9 \pm 1{\cdot}4$ and $31{\cdot}6 \pm 3{\cdot}6$, respectively). After 7 days of culture with pokeweed mitogen the percentage of tonsillar cells producing polymeric IgA was significantly higher in the patients than in the controls ($66{\cdot}5 \pm 12{\cdot}6$ *vs* $33{\cdot}4 \pm 10{\cdot}3$; $P < 0{\cdot}005$). These results also suggest a mucosal origin for the IgA deposited in the kidneys of these patients. Our data are consistent with the existence of an immunoregulatory dysfunction in the secretory immune system of patients with IgA nephropathy.

Keywords tonsils IgA nephropathy polymeric IgA immune abnormalities

INTRODUCTION

We have recently described the existence of high levels of polymeric IgA, partially as immune complexes, in the serum and kidneys of patients with IgA nephropathy (Lopez-Trascasa *et al.*, 1980; Egido *et al.*, 1980; Sancho *et al.*, 1982), as well as increased rates of polymeric IgA synthesis by circulating lymphoid cells after polyclonal stimulation *in vitro* (Egido *et al.*, 1982).

Since these patients often present with macroscopic haematuria following upper respiratory tract infections, the mucosal origin of the IgA deposited in the kidney has been examined by several investigators (reviewed in Egido *et al.*, 1983b). Although controversy exists concerning the IgA1 or IgA2 classes, most authors agree that the renal IgA is polymeric in this disease (Egido *et al.*, 1983b). It has also been established that there is a decrease in the number of episodes of macroscopic haematuria in patients with IgA nephropathy following tonsillectomy (Vialtel *et al.*, 1981).

In this paper, we have studied tonsils from patients with IgA nephropathy who presented with

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bouts of gross haematuria coincidentally with upper respiratory tract infection. The tonsils of these patients produced more polymeric IgA, after polyclonal stimulation *in vitro*, than did those of control subjects. Furthermore, an inversion in the percentage of IgA *vs* IgG bearing tonsillar lymphocytes was observed in these patients compared to the controls.

MATERIALS AND METHODS

Subjects. Six patients (four males and two females; mean age 19 ± 12 years, range 7–31) with IgA nephropathy diagnosed by kidney biopsy were studied. The criteria adopted were the presence of IgA in the glomerular mesangium, with or without C3 and other immunoglobulins, and the absence of clinical or biochemical evidence of liver disease, systemic lupus erythematosus, Henoch–Schönlein syndrome, or other systemic disease. Tonsillectomy was performed in these patients because of the coincidental occurrence of episodes of macroscopic haematuria following upper respiratory tract infections. All of them had chronic tonsillitis and the operation was done at least 2 months after an acute episode. The control group included 15 subjects undergoing routine tonsillectomy, age matched as far as possible with the patients.

Cell preparation. Within 1 h of the operation, cell suspensions were obtained from tonsillar stroma by teasing with scissors in Hank's balanced salt solution (HBSS) as previously described (Janossy *et al.*, 1976). Cell suspensions were centrifuged at 1,500 r/min for 5 min and the pellet treated with NH_4Cl (0·9%) to eliminate the red cells. Afterwards, the remaining cells were washed intensively with HBSS and once with RPMI 1640 medium. Finally, the cells were kept at a concentration of 1×10^7 cells/ml in RPMI 1640 supplemented with 2 mM glutamine, 50 μg/ml streptomycin, and 100 u/ml penicillin until use.

Peripheral mononuclear cell suspensions were obtained from fresh heparinized blood (drawn from patients and controls on the same day as the tonsillectomy) by standard Hypaque-Ficoll gradient centrifugation (Pharmacia Fine Chemicals, Uppsala, Sweden) using the conditions previously published (Egido *et al.*, 1983a).

Immunoglobulin bearing lymphocyte assay. IgG, IgA and IgM bearing lymphocytes from the tonsils and blood of patients and healthy controls were measured by the immunobead solid phase method (Bio-Rad laboratories, Richmond, California, USA). Monocytes were previously removed by adherence to polysterene plates (Nunc, Denmark) at 37°C for 30 min. The non-adherent fraction was washed twice with physiological buffered saline (PBS), 0·15 M containing 0·1% sodium azide, and adjusted to 1×10^7 cells/ml. One tenth of a millilitre of the cell suspension and 0·05 ml of the Immunobead reagent (μm sized hydrophilic particles covalently bound to highly purified rabbit anti-human IgG, IgA, IgM and anti-heavy and light chains) were added to small tubes. After centrifugation at 150*g* for 3 min, incubation was performed at 4°C for 10 min to allow rosette formation. The cells were resuspended, stained with 0·03% toluidine blue dye and read microscopically. Rosettes were considered positive if three or more beads were attached to a cell.

Cell culture. Isolated mononuclear cells either from blood or tonsils at a concentration of 2×10^6 cells/ml in RPMI 1640 supplemented with 10% decomplemented fetal calf serum, 2 mM glutamine, 50 μg/ml streptomycin, and 100 u/ml penicillin were incubated in the presence or absence of 10 μl/ml of pokeweed mitogen (PWM) at 37°C in a 5% CO_2 humidified atmosphere in 13 × 10 mm plastic culture tubes (Falcon Plastics, Oxnard, California). This amount of PWM had been found to produce a maximal response in previous experiments. After 7 days of culture, the percentages of cells with cytoplasmic immunoglobulin were determined by the method of Moretta *et al.* (1977).

The measurement of the affinity of the secretory component (SC) for polymeric IgA in cells and in supernates was based upon the methods of Brandtzaeg (1974) and Crago & Mestecky (1979), using published modifications (Lopez-Trascasa *et al.*, 1980; Egido *et al.*, 1980; Sancho *et al.*, 1982). To examine cells for the presence of cytoplasmic receptors for SC, cells were first fixed with a 95% ethanol and 5% acetic acid solution for 1 h at room temperature. After washing, the cells were incubated with purified SC in a moist chamber at room temperature and again washed with PBS. As shown previously (Egido *et al.*, 1980; Sancho *et al.*, 1982), the optimal conditions for SC binding were used (150 μg/ml of SC and 30 min of incubation time). Slides were then incubated with

rhodamine labelled anti-SC (Atlantic antibodies, Scarborough, Massachusetts, USA), washed again and further incubated with F $(ab')_2$ fragments of monospecific antibodies to human IgA, IgG and IgM conjugated to fluorescein isothiocyanate (Cappel Laboratories, Cochranville, Pennsylvania, USA). Two hundred cells were counted on the slides by two independent observers.

RESULTS

There were no significant differences in the percentage of IgG and IgM bearing peripheral blood lymphocytes between the controls and the patients with IgA nephropathy (Table 1). However, there was an increase in the number of IgA bearing lymphocytes in the patients compared to the controls. As shown in Fig. 1 the differences in the percentage of immunoglobulin bearing lymphocytes were more marked in tonsillar tissue. Thus, patients with IgA nephropathy showed a significant increase ($P < 0{\cdot}00025$) in the number of IgA bearing lymphocytes ($14{\cdot}4 \pm 2{\cdot}3$, mean $\pm$ s.d.) and a significant

Table 1. Percentage of surface Ig positive peripheral blood lymphocytes in controls and patients with IgA nephropathy

Group	IgG	IgA	IgM	Total
Control ($n=7$)	$29{\cdot}3 \pm 9{\cdot}4$*	$1{\cdot}25 \pm 0{\cdot}22$	$15{\cdot}8 \pm 2{\cdot}6$	$43{\cdot}0 \pm 3{\cdot}6$
IgA nephropathy ($n=5$)	$34{\cdot}1 \pm 2{\cdot}5$ n.s.	$1{\cdot}70 \pm 0{\cdot}35$ $P < 0{\cdot}05$†	$14{\cdot}3 \pm 3{\cdot}2$ n.s.	$42{\cdot}7 \pm 3{\cdot}6$ n.s.

* Mean $\pm$ s.d.; † Student's *t*-test.

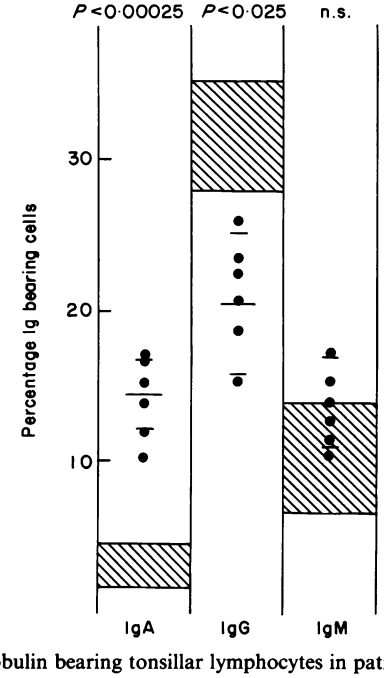

Fig. 1. Percentage of immunoglobulin bearing tonsillar lymphocytes in patients ($n=6$) and controls ($n=15$). Shaded area represents mean $\pm$ 1 s.d. of the control group. Student's *t*-test.

Table 2. Percentages of cells from tonsils with cytoplasmic immunoglobulins after 7 days of culture

	IgA	IgG	IgM
Controls ($n=13$)	23·7±5*	24·6±6	15·4±3
Patients ($n=6$)	30·1±5	11·2±1	41·0±2
	n.s.	$P<0.0025$†	$P<0.0025$

* Mean±s.d.; † Student's *t*-test.

decrease ($P<0.025$) in the number of IgG bearing lymphocytes (20·5±4·6) compared to the control group (2·9±1·4 and 31·6±3·6, respectively). No change in the number of IgM bearing lymphocytes was observed (Fig. 1).

There was no correlation between the number of IgA bearing lymphocytes in tonsils and the number of IgA bearing peripheral blood lymphocytes, nor with IgA serum levels.

Table 2 shows the percentages of cells from the tonsils of controls and patients in which cytoplasmic immunoglobulin was detected after 7 days of culture with PWM. Although there was an increase in IgA secreting cells it did not reach statistical significance. In contrast, there was a significant decrease in IgG secreting cells and a significant increase in IgM secreting cells. Interestingly, the percentage of tonsillar cells binding simultaneously SC and IgA (that is, producing polymeric IgA) was significantly higher in patients (66·5±12·6) than in controls (33·4±10·3) ($P<0.005$) (Fig. 2). No differences were found in the binding of SC/IgM or SC/IgG between patients and controls. In the patients, there was no difference in the percentage of tonsillar and peripheral blood lymphocytes producing polymeric IgA (66·5±1·2 *vs* 64·3±10·7, respectively). No such comparison could be done in the control group, due to the small number of subjects in whom these studies were performed.

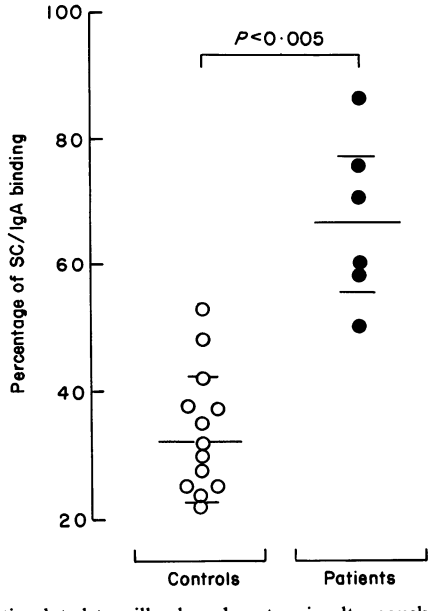

Fig. 2. Percentages of PWM stimulated tonsillar lymphocytes simultaneously binding SC and IgA (that is, producing polymeric IgA). Bars represent mean±1 s.d. Student's *t*-test.

DISCUSSION

The results of the present study indicate that tonsils from patients with IgA nephropathy have an increased percentage of IgA bearing lymphocytes in relation to the control group. Furthermore, tonsillar lymphocytes from these patients produce more polymeric IgA than do the tonsillar lymphocytes of control subjects.

Our research was initiated because of the observation that patients with this disease often have macroscopic haematuria following upper respiratory tract infections. This association probably indicates that the source of high serum levels of polymeric IgA, often observed in these patients (Lopez-Trascasa *et al.*, 1980), as well as the polymeric IgA deposited in the mesangium, was from the external secretions. In this context, less than 10% of IgA plasma cells in normal human bone marrow are capable of SC binding (Hijmans, Schut & Hulsing-Hesselink, 1971; Radl *et al.*, 1974). In contrast around 50% of plasma cells in the intestinal mucosa and PWM stimulated peripheral blood lymphocytes cultured for 7 days bind it (Brandtzaeg, 1973; Crago *et al.*, 1979). Our present results, by showing that tonsillar lymphocytes synthesize a significant amount of polymeric IgA, extend previous similar data on the peripheral blood lymphocytes of such patients (Egido *et al.*, 1982) and further support a mucosal origin for kidney IgA.

The tonsils are an important organ of contact with foreign micro-organism and, hence, are a site of clonal expansion and cellular differentiation (Brandtzaeg, Surjan & Berdal, 1978). Whether there is increased polymeric IgA synthesis after viral or other infections in patients with IgA nephropathy is not known. Recently it has been demonstrated that the antibodies eluted from renal tissues of patients with IgA nephropathy specifically bind to the nuclear regions of tonsillar cells suggesting that antibodies to tonsillar antigens might constitute a proportion of the deposited IgA immune complexes (Tomino *et al.*, 1983).

The inversion in the percentage of IgA *vs* IgG bearing tonsillar lymphocytes compared to that observed in control subjects also merits comment. The increased percentage in IgA bearing tonsillar lymphocytes together with the increased production of polymeric IgA complement the abnormalities in the regulation of IgA synthesis observed in the peripheral blood mononuclear cells of these patients (Egido *et al.*, 1983a; Egido, Blasco & Sancho, 1983). The simultaneous occurrence of a diminution in the percentage of IgG bearing tonsillar lymphocytes at rest, as well as that observed in the percentage of cytoplasmic IgG after 7 days of culture, parallel the simultaneous induction of antigen specific IgA helper T cells and IgG suppressor T cells in the murine Peyer's patch after antigen challenge (Kawanishi & Strober, 1983). It is not known whether a similar differential, isotype specific immunoregulation occurs in human tonsillar tissue. It remains to be established whether a dysfunction of T cell switching or a dysfunction of B cells (Endoh *et al.*, 1983) can explain the immunological alterations observed in the tonsils of these patients.

While this work was in progress, Bene *et al.* (1983) published the results of an immunohistomorphometrical analysis of tonsillar plasma cells from seven patients with IgA nephropathy. Their data was in some respects similar to ours in that they observed an increase in the percentage of IgA and a decrease in the percentage of IgG secreting cells, as well as an increase in the number of dimeric IgA secreting cells determined by staining both for cytoplasmic IgA and J chain.

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