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Decrease of IgA-specific suppressor T cell activity in patients with IgA nephropathy

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SUMMARY

The activity of IgA-specific suppressor T cells was lower in eight patients with IgA nephropathy than in six patients with chronic proliferative glomerulonephritis without glomerular deposition of IgA, two patients with acute glomerulonephritis, or five healthy adult controls. It was determined by the quantitation of immunoglobulins produced from pokeweed mitogen-stimulated B cells cultured with the T cell supernatant (TCS) obtained from concanavalin A-stimulated T cells. Results from a study on an identical twin sister with IgA nephropathy suggested that the decreased activity of IgA-specific suppressor T cells might not be a cause but a result of increased IgA-bearing lymphocytes and serum IgA in patients with IgA nephropathy.

INTRODUCTION

IgA nephropathy is characterized by preponderant mesangial deposition of IgA detected by immunofluorescent staining (Berger, 1969). Although the pathogenesis of this disorder is obscure (Lowance, Mullins & McPhaul, 1977), IgA-bearing peripheral blood lymphocytes are selectively increased in such patients (Nomoto, Sakai & Arimori, 1979), as is serum IgA occasionally (Nomoto, Sakai & Arimori, 1979; Whitworth, 1976). The increase in IgA-bearing blood lymphocytes has also been observed in some family members of patients with IgA nephropathy (Sakai *et al.*, 1979), suggesting that some familial factors might be involved in the development of IgA nephropathy. We determined the activity of IgA-specific suppressor T cells in order to investigate the mechanism of the increase of IgA-bearing lymphocytes and serum IgA in patients with IgA nephropathy.

MATERIALS AND METHODS

Individuals studied. Sixteen patients with glomerulonephritis and five healthy adults were examined. All patients were diagnosed by renal biopsies evaluated by light microscopy, electron microscopy and immunofluorescent staining. Among the sixteen patients, there were eight patients with IgA nephropathy, six patients with chronic proliferative glomerulonephritis without glomerular deposition of IgA, and two patients with acute glomerulonephritis. Among the eight patients with IgA nephropathy, one had an identical twin, as confirmed by the identical HLA types (AW19, AW24, B7 and BW4 0·1) and negative MLC reactions (one way and two ways). Skin allografts were not performed. Repeated urinalysis showed no abnormality in the sister.

Assay systems. The activity of IgA-specific suppressor T cells was determined by the method of Waldmann *et al.* (1974) with minor modifications. Blood lymphocytes were obtained by Ficoll-Hypaque gradient centrifugation (Parker, Schreinemachers & Meuwissen, 1972). Separation of T and B cells was performed twice by E-rosette formation followed by Ficoll-Hypaque gradient centrifugations (IUIS Report, 1975). 95% of the separated T cells formed E-rosettes, and 65–70% of the B cell rich population bore immunoglobulins and none formed E-rosettes. Both T and B cell rich populations were washed six times: immunoglobulin was not detected in the supernatants from the last wash by the radioimmunoassay test described

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below. The T cell rich population was suspended in RPMI 1640 (GIBCO, Grand Island, New York) at a concentration of 2×10^6 cells/ml. Ten μg/ml of concanavalin A (Con A) (GIBCO) was added, and the culture was incubated at 37°C in 5% CO_2-air for 24 hr. The B cell rich population was suspended in 10% foetal calf serum (GIBCO) in RPMI 1640 at a concentration of 2×10^6 cells/ml, and maintained at 37°C in 5% CO_2-air for 24 hr. Con A-stimulated T cells were centrifuged, and the supernatant was incubated with an equal volume of wet Sephadex G-75 at 4°C for 1 hr to absorb Con A (Rich & Pierce, 1974). This absorption was repeated three times. One ml of the absorbed T cell supernatant (TCS) was mixed with one ml of either autologous or allogeneic B cell suspension at a concentration of 2×10^6 cells/ml in 10% foetal calf serum in RPMI 1640. In parallel studies, various amounts of Con A-stimulated T cells obtained from two healthy adults were washed and co-cultured with 2×10^6 of autologous B cells in 2 ml of 10% foetal calf serum in RPMI 1640.

Ten μl/ml of pokeweed mitogen (PWM) (GIBCO) was added to all cultures, and the cultures were incubated at 37°C in 5% CO_2-air for six days. The cultures were then centrifuged at 400 *g*, and the supernatants were stored at −20°C for the assay of immunoglobulins.

IgG, IgA and IgM in the supernatants was measured by the method of Salmon *et al.* (1969). In brief, one ml of purified human IgG, IgA or IgM (1 mg/ml, produced in our laboratory from sera of patients with myeloma) was incubated at room temperature for 18 hr in polystyrene test tubes (Falcon 2054, Oxnard, California). After washing three times with phosphate buffered saline (PBS) (0·01 M, pH 7·3), the tubes were filled with 10% bovine serum albumin (SIGMA, St. Louis Missouri) in PBS and were incubated at room temperature for 18 hr. All solutions contained 0·1% sodium azide unless indicated otherwise. After washing three times with PBS the tubes were coated with antihuman IgG IgA or IgM antibodies by incubation with one ml of 1:10 diluted heavy chain-specific anti human IgG IgA or IgM antisera from rabbits (Behringwerke, Marburg, Germany) at room temperature for 24 hr (the specificity of these antisera was examined by immunoelectrophoresis using purified human IgG IgA and IgM). The tubes were washed five times with PBS, air-dried, and stored at 4°C until use. Test samples or standard amounts of immunoglobulins were placed in the tubes, and at least 50,000 cpm of ^{125}I-labelled human IgG, IgA or IgM (specific activity 8–10 μC/μg, produced in our laboratory) were added. After washing three times with PBS, the radioactivity attached to the tubes was measured by a γ-counter (Aloca, Model JDC-755). The amount of IgG, IgA and IgM was calculated by the standard curves prepared at the same time as the assay. The specificity of the solid phase radioimmunoassay system used in this study was evaluated by negative controls including different classes of immunoglobulins. The degree of the interference by different classes of immunoglobulins was less than 5%.

The degree of suppressor T cell activity was calculated as follows:

$$\text{Degree of suppressor T cell activity observed in patient's B cells} = \frac{\text{Amount of IgG, IgA or IgM in cultures of patient's B cells and control TCS} - \text{Amount of IgG, IgA or IgM in cultures of patient's B cells and patient's own TCS}}{\text{Amount of IgG, IgA or IgM in cultures of patient's B cell and control TCS}}$$

The significance of differences among the patients was evaluated by the Student's *t*-test.

RESULTS

Table 1 depicts the effect of various numbers of Con A-stimulated T cells on PWM-induced immunoglobulin production in autologous B cells. There was a significant ($P<0·01$) decrease in the production of IgG, IgA and IgM when the ratios of T to B cells were more than 1:2.

Table 2 shows the amounts of immunoglobulins produced in various combinations of cultures prepared for a twin sister with IgA nephropathy. Con A-stimulated T cells, as well as TCS, obtained from the patient showed decreased suppression of IgA production in autologous and allogeneic B cells. Her T cells and TCS, however, demonstrated normal suppressive activity on the production of IgG and IgM in autologous and allogeneic B cells. Her B cells showed an increased IgA production, as reflected in the cultures with T cells or TCS obtained from her healthy sister or control adults. In contrast to the patient, her sister did not show a significant decrease in suppressor T cell activities.

Table 3 shows the effect of Con A-stimulated TCS on PWM-induced immunoglobulin production in autologous and control B cells obtained from the patients and the controls. TCS obtained from patients with IgA nephropathy showed a decreased suppressive activity on IgA synthesis in autologous and allogeneic B cells ($P<0·001$), while there was no significant changes in the suppression of IgG and IgM synthesis. Some patients with glomerulonephritis other than IgA nephropathy also showed decreased activity of IgA-specific suppressor T cells. However, these patients showed a generalized decrease in suppressor T cell activity, reflected by a decrease in the suppression of IgG, IgA and IgM production.

TABLE 1. Effect of various numbers of Con A-stimulated T cells on PWM-induced immunoglobulin production in autologous B cells

	Amount of Con A-stimulated T cells ($\times 10^6$ cells)	Amount of PWM-stimulated B cells ($\times 10^6$ cells)	IgG (ng/2$\times 10^6$ cells)	IgA (ng/2$\times 10^6$ cells)	IgM (ng/2$\times 10^6$ cells)
Experiment 1	0	2·0	>2,500	>2,500	2,200
	0·25	2·0	910	1,010	750
	0·5	2·0	880	810	910
	1·0	2·0	590	540	480
	2·0	2·0	430	420	380
Experiment 2	0	2·0	>2,500	2,180	2,010
	0·25	2·0	760	880	940
	0·5	2·0	430	760	660
	1·0	2·0	380	520	440
	2·0	2·0	360	560	460

Each value represents a mean value of triplicate samples.

TABLE 2. Suppressor activity of T cells and T cell supernatant (TCS) in a patient with IgA nephropathy and her identical twin sister

Donor of B cells	Donor of T cells	Donor of TCS	IgG (ng/2$\times 10^6$ cells)	IgA (ng/2$\times 10^6$ cells)	IgM (ng/2$\times 10^6$ cells)
Patient	Patient	—	300	>2,500	320
Patient	—	Patient	260	>2,500	410
Patient	Sister	—	520	750	390
Patient	—	Sister	250	1,030	140
Patient	Control	—	340	1,210	340
Patient	—	Control	880	2,010	910
Sister	Sister	—	260	400	280
Sister	—	Sister	450	520	480
Sister	Patient	—	250	>2,500	380
Sister	—	Patient	170	n.d.	420
Sister	Control	—	740	520	430
Sister	—	Control	1,550	630	550
Control	Control	—	620	710	550
Control	—	Control	n.d.	630	590
Control	Patient	—	150	2,010	340
Control	—	Patient	20	>2,500	450
Control	Sister	—	1 550	490	410
Control	—	Sister	460	740	710

n.d. = Not done.

Fig. 1 demonstrates the relationship among the activity of IgA-specific suppressor T cells and amounts of IgA-bearing peripheral blood lymphocytes or serum IgA in patients with various types of glomerulonephritis. The activity of the IgA-specific suppressor T cells was significantly decreased in patients with increased amounts of IgA-bearing lymphocytes ($P<0·001$) or serum IgA ($P<0·005$), respectively.

TABLE 3. Effect of Con A-stimulated T cell supernatant on PWM-induced immunoglobulin production in autologous and control B cells

	Donor of T cell supernatant							
	IgA nephropathy (n = 8)		Proliferative GN (n = 6)		Acute GN (n = 2)		Controls (n = 5)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Autologous B cells								
IgG (ng/2 × 10^6 cells)	280–1,400	660	240–960	480	420–780	600	240–880	560
IgA (ng/2 × 10^6 cells)	480–> 2,500	1,440*	440–1,110	640	400–1,840	1,120	470–630	520
IgM (ng/2 × 10^6 cells)	420–1,020	690	390–840	560	390–660	525	380–590	410
Control B cells								
IgG (ng/2 × 10^6 cells)	320–1,010	720	340–880	620	560–840	700	360–780	480
IgA (ng/2 × 10^6 cells)	520–> 2,500	1,380*	340–1,210	690	460–1,480	970	440–620	540
IgM (ng/2 × 10^6 cells)	400–880	640	420–780	540	440–780	610	420–580	480

*$P < 0.001$

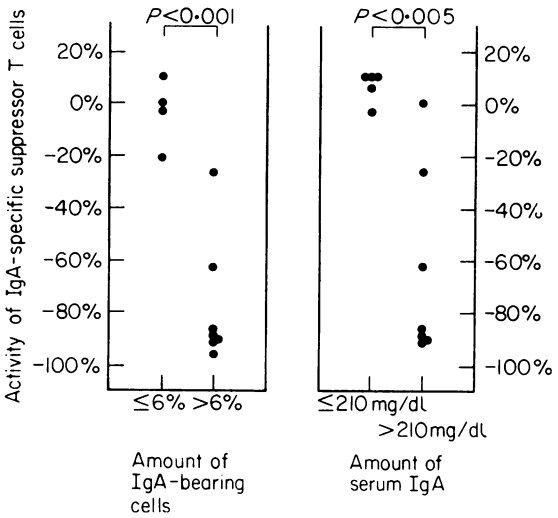

FIG. 1. Relationship between the activity of IgA-specific suppressor T cells and the amount of IgA-bearing lymphocytes and serum IgA.

DISCUSSION

It has been a matter of controversy whether suppressor T cells play a key role in the development of immunoglobulinopathies in humans. An increase in IgA-specific suppressor T cell activity has been reported by Waldmann *et al.* (1974) in patients with selective IgA deficiency. However, Cassidy, Oldham & Platts-Mills (1979) did not observe altered suppressor T cell activity in the same disease.

Increase in IgA-bearing peripheral blood lymphocytes associated with an occasional increase of serum IgA occurs in patients with IgA nephropathy (Nomoto, Sakai & Arimori, 1979). The activity of IgA-specific suppressor T cells has been determined to investigate the mechanism of the increase of IgA-bearing lymphocytes and serum IgA in patients with IgA nephropathy.

The results obtained from the study on the effect of Con A-stimulated T cells on *in vitro* production of immunoglobulins from PWM-stimulated human blood B cells indicated that the suppression of immunoglobulin synthesis was observed in co-cultures of autologous T and B cells. The T and B cells used in this study were not 100% pure, and thus it is feasible that the residual T cells in the B cell rich population might affect the synthesis of immunoglobulins from B cells. However, there were no E-rosette forming cells in the B cell rich population. It is likely that the majority of suppressor T cells is E-rosette forming cells, because the Con A-stimulated E-rosette forming cell population inhibits *in vitro* synthesis of immunoglobulins from the PWM-stimulated B cell rich population (Haynes & Fauci, 1977; Schwartz *et al.*, 1977). The amounts of immunoglbulins synthesized by B cells observed in this study were less than those reported by other laboratories (Waldmann, *et al.*, 1974; Cassidy, Oldham & Platts-Mills, 1979), perhaps because of different culture conditions such as different batches of foetal calf serum.

The results from the study on the twin sister showed that the activity of IgA-specific suppressor T cells was decreased only in the patient with IgA nephropathy. It is premature to conclude that the decrease in IgA-specific suppressor T cell activity is controlled genetically, because the activity of IgA-specific suppressor T cells in her identical twin sister showed no significant changes.

The suppressor activity of T cell supernatant (TCS) from Con A-stimulated T cells paralleled that of Con A-stimulated T cells themselves. Therefore, TCS was used for the studies on unrelated patients. Although the activity of Con A-induced suppressor T cells in humans is not affected by histocompatibility (Schwartz *et al.*, 1977), Con A-stimulated T cells were not cultured with allogeneic B cells, because cells in the T cell rich population might stimulate allogeneic T cells followed by the release of non-specific mitogenic factors.

In patients with IgA nephropathy, the activity of IgA-specific suppressor T cells was significantly decreased, while that of IgG and IgM-specific suppressor T cells remained unchanged. In some patients with glomerulonephritis other than IgA nephropathy, there was a generalized decrease of IgG, IgA and IgM-specific suppressor T cell activities. The decrease in the activity of IgA-specific suppressor T cells in patients with IgA nephropathy might not be a cause but a result of the increase in IgA-bearing lymphocytes and serum IgA in such patients, because a healthy sister of an identical twin with IgA nephropathy did not show decreased activity of suppressor T cells which are considered to be controlled genetically (Tada, Taniguchi & Okumura, 1977).

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