Clin. exp. Immunol. (1989) **78**, 378–382

Increase of IgA-specific switch T cells in patients with IgA nephropathy

H. SAKAI, M. MIYAZAKI, M. ENDOH & Y. NOMOTO *Division of Nephrology and Metabolism, Department of Internal Medicine, School of Medicine, Tokai University, Isehara City, Japan*

(Accepted for publication 17 July 1989)

SUMMARY

Enumeration and functional analysis of $CD4^+$ T cells with receptors for the Fc portion of IgA (i.e. Tα4 cells) in the peripheral blood of patients with IgA nephropathy, their relatives and age-matched controls were performed to elucidate polyclonal activation of IgA production in this disease. Enumeration of Tα4 cells was performed by a fluorescence activated cell sorter, and functional analysis was carried out by separation of Tα4 cells, and IgM-, IgA- and IgG-bearing lymphocytes using panning methods followed by cultures of these cells for 7 days with pokeweed mitogen. There was a significant increase in the amount of peripheral blood Tα4 cells in patients with IgA nephropathy and their relatives. Tα4 cells specifically enhanced the switch of IgM-bearing cells to IgA-bearing cells, and this switch activity was inhibited by addition of human myeloma IgA. It is suggested that Tα4 cells may be responsible for polyclonal activation of IgA production in IgA nephropathy.

Keywords IgA nephropathy switch T cells CD4 IgA

INTRODUCTION

IgA nephropathy is characterized by preponderant deposition of IgA in the mesangial areas of the glomeruli. Increase of *in vivo* and *in vitro* synthesis of IgA in patients with this disease and in some of their relatives has been reported by several investigators (review by Sakai, 1989). It has been demonstrated that IgA antibodies which increased in these patients and in their relatives were polyclonal, because antigenic stimulation induced an increase of not only specific IgA antibody against the antigen but also parallel increase of non-specific IgA antibodies (Sakai *et al.*, 1987). There are reports of a decrease of IgA-specific suppressor T cell activity (Sakai, Nomoto & Arimori, 1979) and of an increase of IgA-specific helper T cells (Sakai *et al.*, 1982) in patients with IgA nephropathy. These changes in suppressor and helper T cells, however, were not sufficient to explain the polyclonal activation of IgA synthesis in these patients, because it is not yet known whether the changes in such T cells were the cause or the result of the increased activity of IgA producing B cells.

The aim of this study was to elucidate the mechanism for induction of the polyclonal activation of IgA synthesis in patients with IgA nephropathy and their relatives. We have previously reported that IgA-specific helper T cells in human peripheral blood had receptors for the Fc portion of IgA (FcαR) and the majority of the T cells with FcαR had CD4 antigens on their cell surface (i.e. Tα4 cells) (Suga *et al.*, 1985). The results of this study indicated that Tα4 cells were IgA-specific switch T cells which specifically converted IgM-producing B cells to IgA-producing B cells.

Correspondence: H. Sakai, M.D., Division of Nephrology and Metabolism, Department of Internal Medicine, School of Medicine, Tokai University, Isehara City, Kanagawa-ken 259-11, Japan.

MATERIALS AND METHODS

Patients and relatives

Fifteen patients with IgA nephropathy and 28 relatives were examined. Ages of the patients ranged from 22 to 36 years and of the relatives from 18 to 56. Ten patients with chronic proliferative glomerulonephritis without glomerular deposition of IgA and 15 age-matched healthy adults served as controls. These patients were examined prior to biopsy and the results were evaluated after the diagnosis of renal biopsy specimens using light microscopy, electron microscopy and immunofluorescent staining. Patients with systemic diseases such as lupus erythematosus, diabetes mellitus or liver cirrhosis were excluded. Patients who showed any signs of infection within 1 month prior to the study were also excluded. None of the patients showed serum creatinine levels in excess of 1·5 mg/dl, and none of them was treated before the study. None of the relatives showed either proteinuria or microscopic haematuria at the time of the study. No abnormal lymphocyte counts were observed in the peripheral blood of the patients and their relatives as well as the healthy adults.

Enumeration of Tα4 cells

Peripheral blood Tα4 cells were enumerated by a double-staining technique using a fluorescence activated cell sorter (FACStar, Becton Dickinson, Oxnard, CA). Briefly, peripheral blood mononuclear cells were collected by Ficoll-Hypaque density gradient centrifugation. T cell-enriched suspensions were obtained by rosette formation of mononuclear cells with sheep red blood cells (SRBC) followed by centrifugation over a Ficoll-Hypaque gradient and then by lysis of SRBC with ice-cold ammonium chloride Tris lysing buffer (Boyle, 1968). The cells were washed three times with RPMI 1640 (GIBCO, Grand Island, NY) in each procedure. The viability of the cells was more than 99% as determined by 1% trypan blue. $CD3^+$ cells in this fraction were 65 to 88% (mean 82·8%), surface immunoglobulin-positive cells were less than 1%, and OKM^+ cells were 3 to 15% (mean 11·4%). IgA myeloma protein was purified from serum samples of a patient with IgA myeloma. After precipitation with sodium sulphate, the myeloma protein was purified by ion-exchange chromatography on DEAE cellulose followed by gel filtration on Sephadex G-200 (Pharmacia Fine Chemicals, Piscataway, NJ). The purified protein was IgA1 (lambda), which was free of contaminating IgG, IgM and other serum proteins, as determined by Ouchterlony analysis and immunoelectrophoresis. The purified IgA was then conjugated with fluorescein isothiocyanate (FITC) by the method of Kawamura (1977) (fluorescein:protein ratio 1·8). The T cell-enriched populations were adjusted to 4×10^6 cells/ml in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Then 0·5 ml of the cell suspension was incubated with 0·1 ml of 1:10 diluted phycoerythrin (PE)-conjugated anti-CD4 monoclonal antibodies (Orthomune-4, lot number T4P001, Ortho Pharmaceuticals, Raritan, NJ) and FITC-conjugated human IgA myeloma protein at 4°C for 30 min. The stained cells were enumerated by FACStar, using 200 mW of 488 nm argon laser light. Fluorescence emission passed through a 50/50 beam splitter. A 575-nm band pass-filter was used to detect green (FITC) fluorescence. Dead cells, erythrocytes, and platelets were exluded from analysis by setting an appropriate threshold trigger on the low forward light scatter parameter. Monocytes and large cells were also electronically gated out. One-hundred thousand cells were analysed in the two-colour experiments.

Separation of Tα4 cells and immunoglobulin-bearing cells (Ig^+ cells)

Tα4 cells as well as IgM-, IgA- or IgG-bearing cells were separated by a panning technique described by Kawanishi, Saltzman & Strober (1983). Briefly, mononuclear cells were obtained from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation, and were suspended at a concentration of 1×10^7 cells/ml of 10% heat-inactivated fetal calf serum (FCS) (GIBCO) in RPMI 1640. Adhesive cells were eliminated by incubation in plain plastic Petri dishes (Corning 25010, Iwaki Glass, Tokyo, Japan) at 37°C for 30 min in 5% CO_2–air. The percentage of $CD3^+$ cells in the non-adhesive cell fraction was 63 to 84% (mean 74·2%), that of Ig^+ cells was 16 to 28% (mean 22·3%), and that of OKM^+ cells less than 1%. The non-adhesive cells were then applied successively to three types of plastic Petri dishes which were coated with the $F(ab')_2$ portion of goat anti-human μ, α or γ heavy chain antibodies (lot numbers 02-05-02, 01-03-01, or 00-08-04, respectively, Tago, Burlingame, CA). The cells were incubated in each Petri dish at 37°C for 30 min in 5% CO_2–air, and the Petri dishes were rinsed three times with 10% FCS in RPMI 1640 after the transfer of cells to the next Petri dish. The adhesive cells on each Petri dish were scraped off by rubber spatulas, and then washed three times with RPMI 1640. Aliquots of cells from each type of Petri dish were examined for viability by 1% trypan blue, and stained by FITC-conjugated $F(ab')_2$ portion of goat anti-human μ, α or γ heavy chain antibodies (lot numbers 23179, 23094, 23104, respectively, Cappel Laboratories, Cochranville, PA) to count IgM-, IgA- or IgG-bearing cells, respectively. The percentages of IgM-bearing cells from anti-IgM antibody-coated Petri dishes were 32 to 40%, but those of IgA- and IgG-bearing cells were less than 1%. The non-adhesive cells from incubation in these three types of Petri dishes were passed through a nylon reticulum column to remove remaining B cells. The T cell-rich fraction was then incubated in Petri dishes coated with monoclonal anti-CD4 antibody (OKT4, lot number T404, Ortho Pharmaceuticals) at 37°C for 30 min in 5% CO_2–air. The cells adhering to these Petri dishes were collected by rubber spatulas, and were washed three times with RPMI 1640. The $CD4^+$ cell fraction was incubated in Petri dishes coated with human myeloma IgA1 (λ) at 37°C for 30 min in 5% CO_2–air. The adhesive cells were collected by rubber spatulas, washed three times with RPMI 1640, and then suspended at a concentration of 2×10^6 cells/ml in 10% FCS in RPMI 1640. The adhesive cells were assumed to be rich in $Fc\alpha R^+$, $CD4^+$ cells, while non-adhesive cells were supposed to be rich in $Fc\alpha R^-$, $CD4^+$ cells. Enumeration of Tα4 cells in these cell fractions showed that there were 28 to 44% of Tα4 cells in the adhesive cell fraction but only less than 1% of such cells in the non-adhesive cell fraction.

Culture of Tα4 cells with IgM-, IgA- or IgG-bearing cells

Cells (1×10^6) from the $Fc\alpha R^+$, $CD4^+$ cell-rich fraction were cultured with 1×10^6 cells of IgM-, IgA- or IgG-bearing cell-rich fractions with or without addition of 10 μl of pokeweed mitogen (PWM) (GIBCO). As controls, cells from the $Fc\alpha R^-$, $CD4^+$ cell-rich fraction were also cultured with IgM-, IgA- or IgG-bearing cell-rich fractions. After 7 days of culture at 37°C in 5% CO_2–air, aliquots of the cells were fixed on microscope slides by Cytospin (Cytospin 2, Shandon Southern Products, Astmoor, England) at 200 rev/min for 5 min, and then stained with the FITC-conjugated $F(ab')_2$ portion of goat anti-human μ, α or γ heavy chain antibodies at 4°C overnight to count cytoplasmic IgM-, IgA- or IgG-positive cells, respectively, using an incident-type fluorescent microscope (Zeiss, Model 9901). The rest of the cultured cells were washed three times with ice-cold 1% BSA in PBS, and then stained with the FITC-conjugated $F(ab')_2$ portion of goat anti-human μ,, α or γ heavy chain antibodies to count cell-surface IgM-, IgA-, or IgG-bearing cells using a fluorescent microscope.

Statistical analysis

The Mann–Whitney U-test was adopted for the analysis of unpaired samples. Values of $P<0.05$ were considered significant.

RESULTS

Amount of Tα4 cells in patients, their relatives and controls

The percentages of peripheral blood Tα4 cells were significantly increased in patients with IgA nephropathy and in their relatives

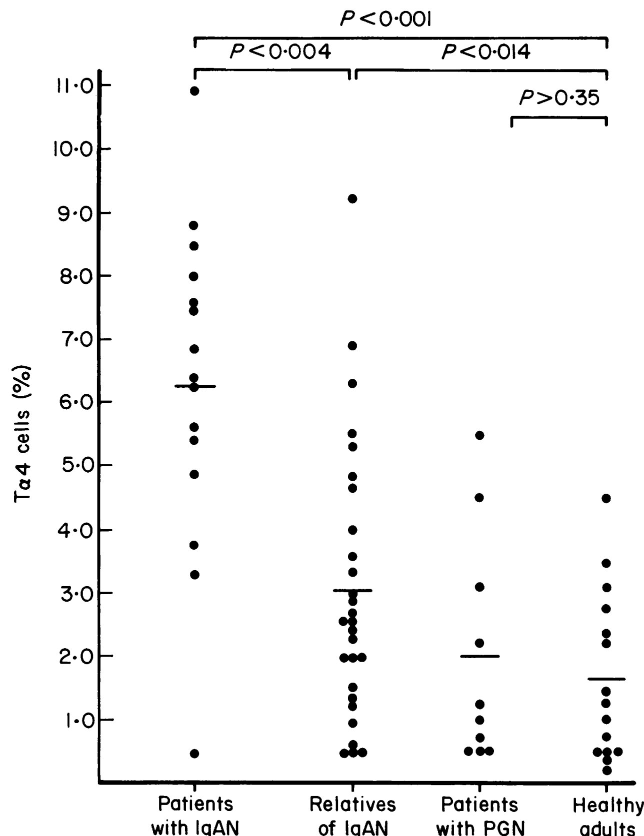

Fig. 1. Amount of Tα4 cells in patients, their relatives and controls. Percentages of Tα4 cells in the T cell-rich fraction of peripheral blood samples (ordinate) from patients with IgA nephropathy (IgAN) ($n = 15$), their relatives ($n = 28$) patients with chronic proliferative glomerulonephritis (PGN) ($n = 10$), and healthy adults ($n = 15$) (abscissa) were determined by FACStar, as described in Materials and Methods.

compared with patients with chronic proliferative glomerulonephritis and healthy adults, as shown in Fig. 1. There was no significant correlation between the amount of Tα4 cells and serum levels of IgA in the patients and their relatives. There were no significant changes in the percentages of $CD3^+$ cells or $Fc\alpha R^+$, $CD8^+$ cells (i.e. Tα8 cells) in either patients with IgA nephropathy or their relatives. The mean values of the percentages of $CD3^+$ cells in patients, relatives and controls were, respectively, 5·2%, 4·2% and 4·6% ($P > 0·30$), and those of Tα8 cells were 5·8%, 4·7% and 4·0%, respectively ($P > 0·10$).

IgA-specific switch activity of Tα4 cells

Table 1 indicates that the culture of IgM-bearing cells with Tα4 cells yielded a large amount of IgA-bearing cells after 7 days, while that with the other type of T cells did not show such deviation in the isotypes of cell-surface immunoglobulins.

Dose effect of Tα4 cells on IgA-specific switch activity

Table 2 indicates that there was a dose-dependent effect of Tα4 cells on the increase of *in vitro* IgA production which was associated with the increase of both cell-surface IgA-positive cells and cytoplasmic IgA-positive cells. The IgA-specific switch activity was observed in cultures with and without pokeweed mitogen (PWM), although the presence of PWM enhanced such switch activity.

Table 1. IgA-specific switch activity of Tα4 cells

	Cells in culture*		Surface markers after 7 days†		
	T cells	B cells	sIgM+	sIgA+	sIgG+
Healthy adults (n=3)	FcαR+, CD4+	sIgM+	4·6– 7·0	20·2–24·2	1·4– 2·8
		sIgA+	2·1– 4·5	17·8–26·5	2·0– 4·5
		sIgG+	1·5– 2·2	0– 0·8	18·2–28·0
	FcαR−, CD4+	sIgM+	19·3–26·0	0·4– 1·2	2·2– 3·8
		sIgA+	2·3– 4·7	16·6–22·9	2·0– 4·7
		sIgG+	2·0– 5·8	0·2– 0·6	22·4–27·6
Patients (n=3)	FcαR+, CD4+	sIgM+	5·2– 6·4	20·4–27·5	1·0– 3·2
		sIgA+	2·1– 4·8	18·2–24·6	2·5– 4·4
		sIgG+	2·6– 4·1	0– 0·2	20·2–28·6
	FcαR−, CD4+	sIgM+	22·5–30·8	0– 0·4	1·2– 2·7
		sIgA+	2·3– 5·6	16·0–24·0	1·4– 4·7
		sIgG+	3·0– 5·2	0– 0·8	21·5–29·4

* With 10 μl of pokeweed mitogen, obtained from three healthy adults or from three patients with IgA nephropathy.

† Range.

$sIgM^+$, $sIgA^+$, $sIgG^+$, cell surface IgM-, IgA- and IgG-bearing cells respectively.

$Fc\alpha R^+$, $Fc\alpha R^-$, cells with and without receptors for the Fc portion of IgA, respectively.

Table 2. Dose effect of Tα4 cells on IgA-specific switch activity

Cells in culture			Surface markers after 7 days (%)*				
PWM	Tα4 cells	sIgM+ cells	CD3+	sIgM+	sIgA+	sIgG+	cIgA+
+	—	1 × 10⁶	14·3	7·2	5·3	2·7	0·9
+	0·2 × 10⁶	1 × 10⁶	22·1	9·6	17·3	3·7	3·3
+	0·5 × 10⁶	1 × 10⁶	32·6	11·3	20·8	2·1	6·7
+	1·0 × 10⁶	1 × 10⁶	50·4	10·1	21·6	3·0	6·7
−	—	1 × 10⁶	12·4	6·0	5·4	1·0	0
−	0·2 × 10⁶	1 × 10⁶	21·4	6·4	7·2	1·9	3·4
−	0·5 × 10⁶	1 × 10⁶	30·0	8·9	14·2	2·8	5·0
−	1·0 × 10⁶	1 × 10⁶	46·9	7·5	13·2	2·1	4·2

PWM, pokeweed mitogen, obtained from a healthy adult.

* Mean values.

Effect of IgA on switch activity of Tα4 cells

Table 3 shows that the *in vitro* addition of IgA to the cultures of Tα4 cells with IgM-bearing cells inhibited the IgA-specific switch activity. Such inhibitory effects were observed in both cell-surface IgA-positive cells and cytoplasmic IgA-positive cells. In contrast, *in vitro* addition of IgA to the cultures of IgM-bearing cells alone slightly enhanced the formation of cell-surface IgA-positive cells but not of cytoplasmic IgA-positive cells.

Comparison of cells from patients and controls

Table 4 indicates that there was no significant difference in IgA-specific switch activity among cultures of T and B cells obtained from patients with IgA nephropathy or healthy adults.

Table 3. Effect of IgA on switch activity of Tα4 cells

Cells in culture			Surface markers after 7 days (%)*				
IgA (μg)†	Tα4 cells	sIgM$^+$ cells	sIgM$^+$	sIgA$^+$	sIgG$^+$	cIgA$^+$	cIgM$^+$
0	1 × 10^6	1 × 10^6	7·3	23·9	11·3	13·4	7·6
			8·3	25·3	10·0	8·9	7·7
160	1 × 10^6	1 × 10^6	7·9	15·0	8·0	2·2	6·3
			7·3	12·8	10·7	2·0	7·7
800	1 × 10^6	1 × 10^6	8·0	5·5	8·3	1·5	4·8
			8·3	6·6	11·5	1·7	6·8
0	0	1 × 10^6	6·0	3·5	14·3	2·5	4·2
			7·5	3·9	13·3	2·2	4·3
160	0	1 × 10^6	8·8	3·3	11·4	2·6	4·5
			8·0	5·9	13·4	2·1	2·4
800	0	1 × 10^6	7·5	12·5	14·5	2·1	4·5
			8·0	15·7	12·0	2·0	4·6

* Mean values.
† Human myeloma protein.

Table 4. Comparison of cells from patients and controls

Cells in culture		Surface markers after 7 days (%)*				
Source of Tα4 cells	Source of sIgM$^+$ cells	sIgM$^+$	sIgA$^+$	sIgG$^+$	cIgA$^+$	cIgM$^+$
Control	Control	6·5	28·7	9·0	18·4	7·2
Control	Patient	7·3	25·7	11·0	16·2	6·6
Patient	Control	6·0	22·8	10·4	14·5	5·8
Patient	Patient	8·0	25·3	11·5	17·7	6·8

* Mean values.
sIgM$^+$, sIgA$^+$, sIgG$^+$, cell surface IgM-, IgA-, and IgG-bearing cells, respectively.
cIgA$^+$, cIgM^{+s}, cytoplasmic IgA- and IgM-positive cells, respectively.

DISCUSSION

Functional heterogeneity of CD4$^+$ T cells has been suggested and a classification of inflammatory/T_H1 and helper/T_H2 subsets is currently employed (Bottomly, 1988). It is not known, however, whether the helper/T_H2 subset itself is heterogeneous with regards to the isotype specificity of CD4$^+$ T-dependent antibodies. As far as IgA-specific helper T lymphocytes are concerned, a couple of T cell lines from murine Peyer's patches have been reported. Kiyono *et al.* (1982) showed that a murine Peyer's patch T cell clone with FcαR induced antigen-specific IgA responses. Kawanishi *et al.* (1983) also showed that a concanavalin A-induced cloned T cell line from murine Peyer's patches induced isotype-specific switching from sIgM- to sIgA-bearing B cells, although the presence of FcαR in this T cell line was not examined. The role of T cells with FcαR on IgA-specific immune response has been a matter of controversy. Hoover & Lynch (1983) as well as Yodoi *et al.* (1983) reported that murine T cells with FcαR selectively suppressed IgA production. However, Endoh *et al.* (1981) reported that FcαR$^+$ T lymphocytes in human peripheral blood specifically enhanced *in vitro* production of IgA, as observed by Kiyono *et al.* (1982) in a murine Peyer's patch T cell line. The reason for this dichotomy is presently unknown. The addition of IgA to cultures, however, may have inhibited IgA production by B cells; Hoover & Lynch (1983) and Yodoi *et al.* (1983) added IgA to their cultures, whereas Endoh *et al.* (1981) and Kiyono *et al.* (1982) used IgA-free culture media. Addition of IgA to the culture medium was not mandatory to induce FcαR, at least in human peripheral blood T cells (Millet, Panaye & Revillard, 1988).

As far as the amount of peripheral blood Tα4 cells is concerned, the majority of the patients with IgA nephropathy and some of their family members exceeded the upper limit of the range of healthy adults. Since none of these patients and their relatives showed abnormal counts of total peripheral blood lymphocytes and/or CD3$^+$ cells, the increase of the percentage of Tα4 cells in T cell-rich population seemed to reflect an increase of the absolute number of Tα4 cells in their peripheral blood.

Induction of FcαR was observed in murine T cell hybridoma (Chevailler *et al.*, 1987) and in human peripheral blood lymphocytes (Adachi *et al.*, 1983) after incubation with human monoclonal IgA or sera from patients with IgA nephropathy. These reports suggested that the abnormality of patients' Tα4 cells may be due to the induction mechanism *in vivo* rather than to the increase of precursor cells which could express FcαR in the presence of IgA. This assumption was, however, not confirmed in the present observations because of the lack of correlation between the levels of peripheral blood Tα4 cells and those of serum IgA in the patients and their relatives.

A panning method with antibody-coated Petri dishes was used to separate Tα4 cells and IgM-, IgA- and IgG-bearing cells. The purity of the cell populations which were separated by the panning method was lower than that obtained using a cell sorter (unpublished data), but the cell sorter was not used for the separation because of its low yield. Nevertheless, the conversion of sIgM$^+$ cells to sIgA$^+$ cells was observed only in cultures with FcαR$^+$, CD4$^+$ cells and not in those with FcαR$^-$, CD4$^+$ cells. The purity of each cell population may be higher than that described in Materials and Methods, because of a potential masking of cell-surface determinants following treatment with antibodies, but there is no direct evidence to support this hypothesis.

The dose effect of Tα4 cells on the conversion of sIgM$^+$ cells to sIgA$^+$ cells was more prominent in the cultures with PWM than those without PWM. However, the amount of cIgA$^+$ cells after 7 days of culture was not significantly different between cultures with and without PWM. It is likely that the addition of PWM was stimulatory but not mandatory for the switch phenomenon.

The reason for a slight increase of sIgA$^+$ cells in cultures of sIgM$^+$ cells and myeloma IgA without addition of Tα4 cells is presently unknown. It is not likely, however, that the additional IgA adhered non-specifically to cultured cells, because such enhancement was not observed in cultures with Tα4 cells.

The percentages of cIgA$^+$ cells in cultures of Tα4 cells and sIgM$^+$ cells were higher in the experiments shown in Tables 3 and 4 than in those shown in Table 2. The reason for this difference is not known. The different times of the experiments may lead to some difference in the culture condition, but the details could not be elucidated.

The role of T cells in immunoglobulin isotype is still unclear. It is presently unknown whether Tα4 cells initiated the switch of $sIgM^+$ cells to $sIgA^+$ cells or enhanced post-switch expansion of IgA-committed B cells. Recently, Benson & Strober (1988) reported that the human mucosal immune system contained individual T cells which were capable of positively regulating IgA-specific isotype differentiation at two levels of B cell development. It is not known whether some T cell populations other than Tα4 also play a role in the regulation of IgA production, or whether Tα4 cells themselves are heterogeneous in regulating IgA-specific isotype differentiation. Nevertheless, an increase of Tα4 cells in the peripheral blood of patients with IgA nephropathy and some of their relatives suggests that Tα4 cells may be responsible for polyclonal activation of IgA in this disease.

ACKNOWLEDGMENTS

This study was supported by a Grant from the Ministry of Health and Welfare, and Grant 62570300 from the Ministry of Education, Culture and Science, Japan.

REFERENCES

ADACHI, M., YODOI, J., MASUDA, T., TAKATSUKI, K. & UCHINO, H. (1983) Altered expression of lymphocyte Fcα receptor in selective IgA deficiency and IgA nephropathy. *J. Immunol.* **131**, 1246.

BENSON, E.B. & STROBER, W. (1988) Regulation of IgA secretion by T cell clones derived from the human gastrointestinal tract. *J. Immunol.* **140**, 1874.

BOTTOMLY, K. (1988) A functional dichotomy in $CD4^+$ T lymphocytes. *Immunol. Today*, **9**, 268.

BOYLE, W. (1968) An extension of the ^{51}Cr-release assay for estimation of mouse cytotoxins. *Transplantation*, **6**, 761.

CHEVAILLER, A., MONTEIRO, R.C., DAËRON, M. & LESAVRE, P. (1987) Induction of Fc receptors for IgA on murine T cell hybridoma by human monoclonal IgA and by high molecular weight IgA in IgA nephropathy. *Clin. exp. Immunol.* **67**, 114.

ENDOH, M., SAKAI, H., NOMOTO, Y., TOMINO, Y. & KANESHIGE, H. (1981) IgA-specific helper activity of Tα cells in human peripheral blood. *J. Immunol.* **127**, 2612.

HOOVER, R.G. & LYNCH, R.G. (1983) Isotype-specific suppression of IgA: suppression of IgA responses in BALB/c mice by Tα cells. *J. Immunol.* **130**, 521.

KAWAMURA, A.J. (1977) *Fluorescent Antibody Techniques and Their Application* (2nd edn) p. 44. University of Tokyo Press, Tokyo.

KAWANISHI, H., SALTZMAN, L.E. & STROBER, W. (1983) Mechanisms regulating IgA class-specific immunoglobulin production in murine gut-associated lymphoid tissues. I. T cells derived from Peyer's patches that switch sIgM B cells to sIgA B cells in vitro. *J. exp. Med.* **157**, 433.

KIYONO, H., MCGHEE, J.R., MOSTELLER, L.M., ELDRIDGE, J.H., KOOPMAN, W.H., KEARNEY, J.F. & MICHALEK, S.M. (1982) Murine Peyer's patch T cell clones. Characterization of antigen-specific helper T cells for immunoglobulin A responses. *J. exp. Med.* **156**, 1115.

MILLET, I., PANAYE, G. & REVILLARD, J.-P. (1988) Expression of receptors for IgA on mitogen-stimulated human T lymphocytes. *Eur. J. Immunol.* **18**, 621.

SAKAI, H. (1989) IgA nephropathy: current views on pathogenesis and treatment. In *International Yearbook of Nephrology 1989* (ed. by V.E. Andreucci) p. 23. Kluwer Academic Publishers, Boston.

SAKAI, H., NOMOTO, Y. & ARIMORI, S. (1979) Decrease of IgA specific suppressor T cell activity in patients with IgA nephropathy. *Clin. exp. Immunol.* **38**, 243.

SAKAI, H., ENDOH, M., TOMINO, Y. & NOMOTO, Y. (1982) Increase of IgA specific helper Tα cells in patients with IgA nephropathy. *Clin. exp. Immunol.* **50**, 77.

SAKAI, H., NOMOTO, Y., TOMINO, Y., ENDOH, M., MIURA, M. & SUGA, T. (1987) Increase of *in vitro* and *in vivo* production of polyclonal IgA in patients and their family members with IgA nephropathy. In *Recent Advances in Mucosal Immunology, Part B* (ed. by J.R. McGhee, J. Mestecky, P.L. Ogra & J. Bienenstock) p. 1507. Plenum Press, New York.

SUGA, T., ENDOH, M., SAKAI, H., MIURA, M., TOMINO, Y. & NOMOTO, Y. (1985) Tα cell subsets in human peripheral blood. *J. Immunol.* **134**, 1327.

YODOI, J., ADACHI, K., TESHIGAWARA, M., MIYAMA-INABA, T., MASUDA, T. & FRIDMAN, W.H. (1983) T cell hybridomas co-expressing Fc receptor (FcR) for different isotypes. II. IgA-induced formation of suppressive IgA binding factor(s) by a murine T hybridoma bearing FcγR and FcαR. *J. Immunol.* **131**, 303.