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Immunological study of IgA deficiency during anticonvulsant therapy in epileptic patients

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SUMMARY

Of 10 epileptic children with IgA deficiency, one showed normal IgA synthesis and secretion on *in vitro* pokeweed mitogen (PWM) stimulated lymphocyte culture in contrast to IgA deficiency *in vivo*, two showed IgA synthesis in cytoplasm without any release of IgA into the supernatant and seven failed to synthesize IgA. Co-cultures with allogeneic T or B cells in various combinations with PWM showed intrinsic IgA–B cell defect without T cell defect in two of the second group affected at IgA secretion and in five of the third group, and intrinsic IgA-B cell defect with dysfunction of T cells in two of the third group. Thus, the IgA deficiency in these epileptic patients was demonstrated to be heterogenous.

Keywords IgA deficiency anticonvulsant T-B co-culture IgA-B cell defect

INTRODUCTION

Since the first report by Sorrell *et al.* (1971) that the level of serum IgA was low in some epileptic patients receiving phenytoin (sodium diphenylhydantoin), several investigators confirmed their findings (Grob & Herold, 1972; Slavin *et al.*, 1974; Seager *et al.* 1975; Aarli, 1976; Yabuki & Nakaya, 1976; Smith *et al.*, 1979). Not only phenytoin but also other anticonvulsants were found to induce IgA deficiency of epileptic patients (Sorrell & Forbes, 1975). Therefore, immunological studies on these patients have been carried out and depression of humoral immunity and delayed type hypersensitivity reaction was observed *in vivo* (Grob & Herold, 1972; Sorrell & Forbes, 1975; Higashi *et al.*, 1978; Andersen, Mosekilde & Hjort, 1981). Seager *et al.* (1975) reported that treated patients with low IgA had normal number of lymphocytes with surface IgA, and suggested that phenytoin causes failure of terminal differentiation of IgA–B cells. Recently, the development of abnormal suppressor T cells in a hypogammaglobulinaemic patient receiving phenytoin was also reported (Dosch, Jason & Gelfand, 1982).

Though primary IgA deficiency is relatively rare in Japan (Kanoh, Koya & Uchino, 1981), the frequency of drug-induced IgA deficiency in epileptic patients is similar to that in Caucasians (Yabuki & Nakaya, 1976). Serum immunoglobulin (Ig) concentration of 69 epileptic patients treated with anticonvulsants over 6 months was measured, and 10 of 20 children showing IgA deficiency were studied. We report our studies on synthesis and secretion of IgA and IgG by PWM stimulated co-cultures with various combinations of T cell and B cell fractions from patients, their mothers and unrelated normal donors. The results showed the heterogeneity of the disease, but an intrinsic defect of IgA–B cells without T cell abnormalities was demonstrated in most.

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MATERIALS AND METHODS

Epileptic patients with IgA deficiency studied. Among 69 epileptic patients who had been treated with anticonvulsants over 6 months, 20 had serum IgA below the lower limit of the age matched child control (Ueda *et al.*, 1977). Ten patients with IgA deficiency (EA.1–EA.10) were selected for investigation (Table 1). None had autoimmune disease or malignancy. All patients were studied at least twice for serum Ig, cell surface markers and Ig synthesis and secretion, and one of the representative results for each patient was shown in Tables 1–5.

Table 1. Clinical features of ten epileptic patients with IgA deficiency*

Case	Age (years)	Sex	Drugs used†	Classification of epilepsies	Serum Ig levels (mg/dl)‡ IgG	IgA	IgM	Salivary IgA (mg/dl)
EA.1	10	F	PB,CBZ	partial	850	8·2	239	nt
EA.2	20	F	PB,CBZ→VPA,CZP	primary generalized	1,680	<0·8	334	<0·8
EA.3	21	M	PB,PHT,CBZ,primidon	partial	1,920	<0·8	358	<0·8
EA.4	2	F	PB,PHT,CBZ→PB,VPA	secondary generalized	2,020	2·3	102	<0·8
EA.5	3	F	PB,PHT,CBZ→PB,VPA,CBZ	secondary generalized	1,360	13·0	148	1·8
EA.6	6	F	PB,PHT,CBZ	partial	1,020	6·2	120	1·4
EA.7	9	M	PB,ethosuximide	primary generalized	900	7·2	92	<0·8
EA.8	14	M	PB,CBZ	secondary generalized	1,670	3·3	76	1·4
EA.9	5	F	PB,PHT,CBZ→PB,VPA	partial	1,320	8·8	124	7·6
EA.10	5	F	PB,PHT,CBZ→PB,VPA	partial	1,240	12·6	142	4·2

* Serum IgA level was below the lower limit of age matched controls (< 2 s.d.).

† PB = phenobarbital; CBZ = carbamazepin; VPA = valproic acid; CZP = clonazepam; PHT = phenytoin.

‡ Serum IgA level of EA.1, EA.6 and EA.8 before the treatment with anticonvulsants were measured and normal.

Cell surface markers. Heparinized blood was centrifuged on Ficoll-Paque (Pharmacia) and the interface cells were washed three times with Eagle's minimum essential medium (MEM) containing 2% fetal calf serum (FCS) (Gibco). T lymphocytes were detected by E rosette formation. Active E rosette forming cells (active T) were assayed by Wybran's method. B lymphocytes were determined with fluorescein labelled rabbit anti-human Ig (IgG + IgA + IgM, total Ig), or anti-heavy chain specific (α, μ or γ) antisera (Behringwerke). EAC (erythrocyte—antibody–complement) rosette formation was also studied.

Lymphocyte cultures. Blood lymphocytes obtained as above were cultured immediately or were separated into T and non-T cell fractions for co-culture by centrifuging E rosette forming cells on Ficoll-Paque gradient. The culture medium used was RPMI 1640 with 15% FCS, 2 mM L-glutamine, 10 mM Hepes, 1·9 mg/ml $NaHCO_3$, 100 u/ml penicillin, 100 μg/ml streptomycin and 1/100 non-essential amino acid (Gibco).

Whole cell cultures consisted of 1×10^6 unfractionated lymphocytes in 1 ml RPMI 1640 containing 50 μg of PWM (Gibco) in a polypropylene tube (Falcon 3033). For co-cultures, 0·2 ml of cells (5×10^5 cells/ml) with 25 μg of PWM were set up in U bottomed microplate (Nunc) with nine combinations of T and B lymphocytes (5×10^4) from patients, healthy unrelated age matched controls or patients' mothers, who share the major histocompatibility complex haplotype with patients, besides cultures with only B cell or T cell fraction.

Assessment of Ig synthesis and secretion by cultured lymphocytes. After 6 days incubation at 37°C in humidified 5% CO_2, the supernatant fluid was collected for radioimmunoassay (RIA) in order to detect the Ig secretion. The cells were washed with phosphate buffered saline containing 2% FCS, layered onto slides using a cytocentrifuge (Shandon Elliot), fixed with cold acetone for 10 min and

then stored frozen at −20°C. The slides were stained with appropriately diluted Fluorescein labelled anti-human Ig antisera. A minimum of 1,000 cells were counted from each culture.

RIA was used for the measurement of IgA and IgG secretion into culture fluid. Human IgG and IgA purified with a combination of ammonium sulphate precipitation, ion exchange column chromatography, gel filtration and affinity column chromatography, were radiolabelled with ^{125}I (New England Nuclear) by the chloramine-T method (specific activity: 40–80 μCi/μg protein) (Klinman & Taylor, 1969).

Measurement of IgA and IgG secretion was performed with a classical inhibition of the double antibody method (Rouslahti & Seppala, 1971) and a solid phase RIA (Kishimoto & Ishizaka, 1975), respectively. The assay range by both assays was 30–2,000 ng/ml.

RESULTS

Lymphocyte surface markers

In the epileptic patients with IgA deficiency, the percentages for surface total Ig bearing lymphocytes were within the normal range for age (Table 2). Surface IgA bearing lymphocytes were normal in eight of the 10 patients, while one patient had a low percentage and another had high percentage. The percentage of E-RFC was normal in all 10 patients studied, but two out of seven had a low percentage of active T.

Table 2. Lymphocyte surface markers of treated epileptic patients

	Rosette forming cells (%)			Surface Ig bearing cells (%)			
Case	Active T	E-RFC	EAC-RFC	Total Ig	IgG	IgA	IgM
EA.1	nt	50·4	25·5	12·0	4·0	5·5	7·0
EA.2	35·4	67·0	22·9	11·1	5·0	7·5	6·0
EA.3	8·4	57·3	25·2	12·0	4·0	3·5	6·6
EA.4	5·3	51·5	19·4	16·0	2·0	2·0	10·0
EA.5	18·9	62·4	16·6	11·9	4·4	17·7	10·8
EA.6	22·3	43·7	15·3	12·6	2·5	4·1	5·3
EA.7	12·5	60·5	21·3	11·6	4·7	8·0	5·5
EA.8	nt	79·4	11·5	12·7	5·6	1·0*	5·0
EA.9	nt	41·3	9·4	13·5	5·5	3·0	9·0
EA.10	20·9	43·9	13·1	18·5	9·0	3·0	8·5
controls	22·2	54·4	26·2	13·3	9·2	5·5	6·6
(mean ± s.d.)	±8·7	±8·0	±10·4	±4·8	±4·5	±2·7	±2·8

* < control mean − 2 s.d.
nt = not tested.

Results with whole cell cultures

Results of cytoplasmic Ig, and Ig release into the culture supernatant are shown in Table 3. The lymphocytes of patient EA.1 synthesized and secreted IgA more than normal controls *in vitro*, despite IgA deficiency *in vivo*. The lymphocytes of patient EA.2 and EA.3 produced normal or increased amounts of cytoplasmic IgA, but they did not release IgA into the culture fluid. IgG secretion by the lymphocytes was normal. The lymphocytes of seven epileptic patients synthesized and secreted a small amount of IgA, but normal amounts of total Ig and IgG. Thus, the IgA deficiency in epileptic patients seems to be heterogenous.

Results using co-cultures with T and B cell fractions

When the B lymphocytes of 2 epileptic patients (EA.2 & 3) with defective IgA secretion from IgA

Table 3. PWM-induced Ig production

Case	Cytoplasmic Ig positive cell (%)		Ig secreted (ng/ml)	
	Total Ig	Ig A	Ig G	Ig A
EA.1	9·0	6·3	4,480	2,500
EA.2	12·0	3·2	2,320	< 50*
EA.3	2·4	1·3	1,760	< 100*
EA.4	2·5	0·02*	1,560	< 60*
EA.5	3·1	0·1	840	85*
EA.6	3·7	0·6	1,280	300*
EA.7	7·1	0·2*	2,300	< 60*
EA.8	5·5	0·5	2,520	40*
EA.9	3·7	0·1*	1,360	220*
EA.10	3·3	0·4	840	210*
controls	5·8† ±3·0	1·9† ±0·8	1,563‡ (250–9,781)	1,399‡ (305–6,418)

* < Control mean − 2 s.d.
†Arithmetic means ± 1 s.d. from 54 normal subjects.
‡ Geometric means (normal range derived from ± 2 s.d. of log values) from 54 normal subjects.

synthesizing cells were cultured either with autologous T cells, or with T cells from allogeneic normal donors or mothers, IgA release into supernatant was defective (Table 4). Intracytoplasmic total Ig and IgA, and IgG secretion into supernatant by these B cells were normal.

Of seven epileptic patients with defective IgA synthesis *in vitro*, the B cells of five patients (EA. 4–8) cultured with autologous T cells, T cells from allogeneic normal donors or the mothers, produced little intracellular IgA, but normal total Ig synthesis and IgG release (Table 5). These patients' T cells helped normal B cells from unrelated donors or mothers to produce and secrete IgA.

In the other two (EA.9 & 10), intracellular IgA synthesis and IgA secretion by their B cells, co-cultured with T cells from either themselves, normal subjects or mothers were low, although production of IgG was normal. However, both IgA and IgG production by allogeneic B cells from normal subjects or their mothers were generally depressed when patients' T cells were added.

Table 4. IgG and IgA secretion by B cells of treated epileptic patients, whose lymphocytes fail to secrete IgA *in vitro*, co-cultured with autologous or allogeneic T cells

	Ig secreted (ng/ml)	Patient B cell			Control B cell			Mother B cell		
		+pat.T	+cont.T	+moth.T	+pat.T	+cont.T	+moth.T	+pat.T	+cont.T	+moth.T
EA.2	Ig G	5,500	5,700	5,500	4,000	5,100	4,900	3,400	3,400	4,200
	Ig A	< 60*	< 60*	< 60*	1,920	1,930	1,980	940	720	840
EA.3	Ig G	4,900	4,800	4,800	4,400	4,600	2,700	4,400	4,300	4,600
	Ig A	< 60*	< 60*	< 60*	720	880	960	2,100	1,780	2,300

* < Control mean − 2 s.d. The normal range (geometric means ± 2 s.d. of log values) of IgG and IgA secretion in 71 control co-cultures with autologous or allogeneic combination were 3,968 ng/ml (977–10,218 ng/ml) and 2,173 ng/ml (537–8,709 ng/ml), respectively.

Table 5. Percentage cytoplasmic Ig positive cells in B cells of treated epileptic patients, co-cultured with autologous or allogeneic T cells

		Patient B cell			Control B cell			Mother B cell		
		+pat.T	+cont.T	+moth.T	+pat.T	+cont.T	+moth.T	+pat.T	+cont.T	+moth.T
EA.4	total Ig	3·5	1·5	7·5	6·0	12·0	15·0	3·0	8·0	12·2
	Ig A	0·01*	0·1*	0·3*	1·5	2·5	0·7*	1·7	1·5	3·6
EA.5	total Ig	10·0	18·2	30·9	18·5	25·0	21·4	12·0	12·2	14·0
	Ig A	0·2*	0·5*	0·8*	3·3	4·7	5·3	1·9	1·6	2·6
EA.6	total Ig	8·6	13·7	28·1	8·8	14·5	21·7	11·8	6·7	25·6
	Ig A	0·4*	0·5*	0·8*	2·7	3·4	4·5	1·9	2·0	8·8
EA.7	total Ig	10·7	20·3	20·3	8·3	15·9	10·0	4·6	13·2	7·7
	Ig A	0·2*	0·5*	0·2*	2·2	4·0	2·5	1·9	1·4	2·1
EA.8	total Ig	14·5	12·1	12·3	5·8	10·9	10·0	8·5	12·5	9·3
	Ig A	0·7*	0·7*	0·8*	3·0	5·0	2·0	5·0	5·5	2·7
EA.9	total Ig	3·4	2·3	2·1	1·0*	14·6	10·2	0·6*	12·4	5·1
	Ig A	0·3*	0·3*	0·8*	0·4*	3·8	4·0	1·0	2·5	3·2
EA.10	total Ig	4·0	2·8	6·1	1·0*	14·6	10·2	3·4	12·4	5·1
	Ig A	0·7*	0·8*	0·2*	0·7*	3·8	4·0	0·7*	2·5	3·2

* < Control mean − 2 s.d. The normal range of total Ig and IgA synthesis in 71 control co-cultures with autologous or allogeneic combinations were 18·7 ± 8·3% and 3·2 ± 1·0% (arithmetic means ± 1 s.d.), respectively.

DISCUSSION

The number of circulating B cells with surface IgA was normal in the present study except in one patient. This finding was in agreement with the results of the 10 drug-induced IgA deficiency patients of Seager *et al.* (1975), and is similar to that of most patients with selective IgA deficiency (Lawton *et al.*, 1972). The co-culture studies suggested the heterogeneity of IgA deficiency in treated epileptic patients and possibly four groups. In one patient, normal IgA synthesis and secretion *in vitro* contrasted with low IgA *in vivo*, which indicated that extrinsic factors such as the autoantibody against IgA may well be involved in this IgA deficiency (Cassidy *et al.*, 1969; Petty *et al.*, 1979). Two patients showed IgA synthesis in cytoplasm without any release of IgA into supernatant. Five patients failed to synthesize IgA (i.e., intrinsic IgA–B cell defect without T cell defect), and two patients had IgA–B cell defect and T cell which failed to help allogeneic B cells to produce both IgA and IgG. These four types of defects have already been reported in patients with primary IgA deficiency (Wu, Lawton & Cooper, 1973; Waldmann *et al.*, 1976; Cassidy, Oldham & Platts-Mills, 1979; King *et al.*, 1979). IgA suppressor T cells also occur in patients with primary IgA deficiency (Waldmann *et al.*, 1976; Atwater & Tomasi, 1978; Schwartz, 1980). The T cell dysfunction observed in two of our patients, however, was probably dysfunction of the helper T cells, and not to excess of suppressor T cells.

Our present study showed that the cellular events involved in IgA deficiency in epileptic patients were heterogenous, but were similar to those involved in primary IgA deficiency, which might suggest that some of the patients studied were not induced by anticonvulsants. Three patients were tested to have normal level of serum IgA before the treatment (Table 1), but the rest of seven were not examined. But it is very unlikely that all these seven were primary, because of the high incidence of IgA deficiency in epileptic patients (20 out of 69), in contrast to the low incidence of primary IgA deficiency in Japan (1:300–20,000) (Kanoh *et al.*, 1978). Furthermore, all 17 patients whose IgA level was examined before the treatment had normal serum IgA, and six (35%) among them showed decreased serum IgA after one year's treatment with anticonvulsants (unpublished data), which demonstrated that IgA deficiency observed in these patients were, in fact, drug-induced. At present,

it is not known why this heterogeneity was observed with regard to defects in terminal differentiation of IgA–B cell, but probably multiple factors including the kind of drugs used, the type of epilepsy and genetic factors (Shakir *et al.*, 1978) are involved in the development of the disease. The comparative study of drug-induced IgA deficiency and selective IgA deficiency will clarify the reason why the defective Ig class is IgA, not IgG or IgM, in these diseases.

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