

Metabolism of immunoglobulin A, lymphocyte function, and histocompatibility antigens in patients on anticonvulsants

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SUMMARY Low serum IgA levels were found in patients taking phenytoin, together with evidence of depressed T cell function. There was no correlation between the dose or the serum level. A correlation was found, however, with HL-A status, patients with a low IgA showing increased frequency of HL-A2. It is suggested that epileptic patients with HL-A2 status are likely to develop IgA deficiency when given phenytoin.

Several immunological abnormalities have been described in patients taking phenytoin. These have included depressed cellular and humoral immunity, and, in particular, low serum immunoglobulin A (IgA) (Sorrell *et al.*, 1971; Grob and Herold, 1972; Slavin *et al.*, 1974). In a detailed study cell-mediated immunity was measured *in vivo* by skin testing with ubiquitous recall antigens, and *in vitro* by measurement of lymphocyte DNA and RNA synthesis. It was found to be depressed; low IgA levels together with defective antibody production to *Salmonella typhi* and tetanus toxoid were also found (Sorrell and Forbes, 1975). It was subsequently suggested that epilepsy with constitutional characteristics might predispose to low IgA, and that the low IgA only occurs when hydantoins are given (Fontana *et al.*, 1976). We, therefore, studied a large group of patients on phenytoin and other anticonvulsants to determine their immunological status and the relation of that to their histocompatibility antigens.

Patients and methods

Eighty-three patients were studied (40 female and 43 male) whose ages ranged between 17 and 76 years with a mean age of 41 years. These patients were divided into two groups: group A consisted

of 56 patients on phenytoin plus other anticonvulsant drugs, and group B of 27 patients on anticonvulsants other than phenytoin. The duration of treatment was greater than 10 years in all patients. Drug regimes of groups A and B are shown in Table 1. Seven patients in group A and four in group B had a family history of epilepsy. No patient included suffered from neoplasia or any of the presumed autoimmune diseases.

Table 1 Drug regimes of patients in groups A and B

Drugs	Group A (56)	Group B (27)
Phenytoin	56	—
Phenobarbitone	29	11
Primidone	18	16
Carbamazepine	8	8
Sodium valproate	9	3
Clonazepam	1	1
Ethotoin	—	2

HLA TYPING

Twenty patients were typed for their histocompatibility antigens (HLA). These were all from group A, and consisted of 10 patients with low and 10 with normal serum IgA. HLA typing was carried out by a modification of the lymphocytotoxicity test using glass plates (Kissmeyer-Neilsen and Kjerbye, 1967).

SERUM IMMUNOGLOBULINS DETERMINATION

In all patients serum concentrations of IgG, IgA, and IgM were measured by the Technicon AIP system using Technicon standards.

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PERIPHERAL BLOOD LYMPHOCYTE PROTEIN SYNTHESIS

The synthesis of protein by peripheral blood lymphocytes *in vitro* was measured by the whole-blood technique (Behan *et al.*, 1976). This method estimates the uptake of tritiated leucine by peripheral blood lymphocytes on stimulation with purified phytohaemagglutinin (PHA) over a period of 22 hours. Lymphocytes from 30 patients were studied: 20 from group A (10 with low serum IgA and 10 with normal levels), and 10 from group B (normal IgA levels). Protein synthesis by lymphocytes was also measured in 35 normal healthy adult controls. A dose response curve to various concentrations of purified PHA (Burroughs Wellcome) was drawn from each patient.

SERUM LEVELS OF ANTICONVULSANTS

The serum levels of sodium phenytoin, phenobarbitone, and primidone were measured in all patients using a gas chromatography method (Goudie and Burnett, 1973).

SERUM FOLATE LEVELS

These were determined by the *Lactobacillus casei* method (Herbert, 1966).

Results

HLA TYPING

The HLA pattern is shown in Table 2. There were eight patients with HLA-A2 in those with low IgA compared to three in those with normal serum IgA.

IMMUNOGLOBULINS

The 100% mean normal adult value (MNA) was as follows: IgG—947 mg/dl, IgA—248 mg/dl, IgM—94 mg/dl, and the normal range for each was 50—175% MNA.

In both groups normal levels of IgG and IgM were found. With regard to IgA, however, a con-

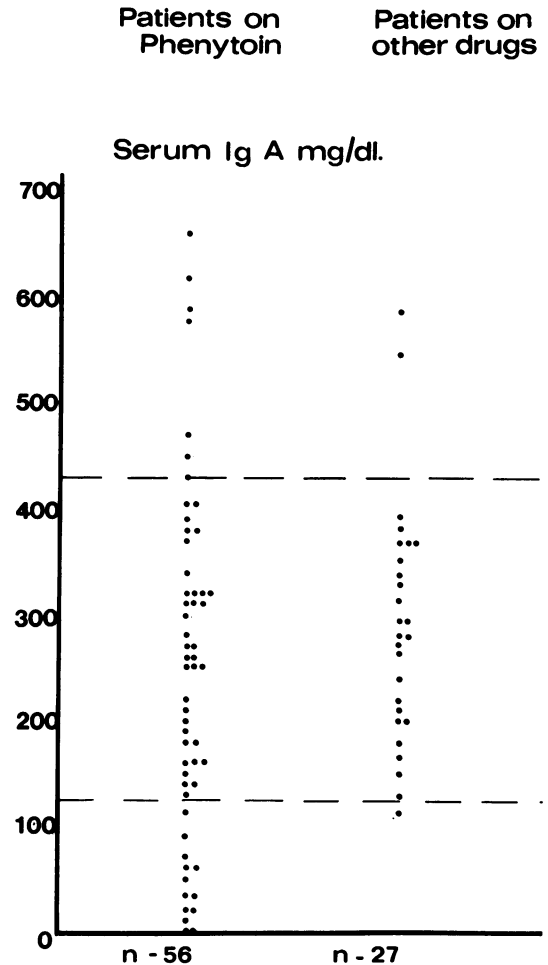


Fig. 1 Serum IgA mg/dl in groups A and B. Dotted horizontal lines indicate 50–175% MNA.

spicuous difference was seen between groups A and B (Fig. 1). In group A 13 patients (23.2%) had low IgA levels (less than 50% MNA) while in group B only one patient had a slightly reduced

Table 2 HLA typing in 20 patients on phenytoin

Patients	HLA-A		HLA-B		Patients	HLA-A		HLA-B	
	Low serum IgA group					Normal serum IgA group			
Male	2	3	7	W17	Male	2	3	7	W14
Female	2	28	8	14	Female	2	11	12	W37
Male	2	3	27	W16	Male	2	W25	W40	
Male	2	W19	14	W21	Female	1	9	7	8
Female	2		7	13	Female	1	2	8	W15
Male	2	3	5	W15	Female	1		8	W17
Male	2	9	7	W16	Male	1	29	W17	
Male	2	9	5	7	Male	3	W32	7	W35
Female	3	W26	27	W40	Female	3	W32	8	W27
Male	3	9	7	12	Male	3		7	

level of IgA (64% MNA). The findings were consistent, not differing significantly on repeated estimations. No correlation was found between serum IgA levels and phenytoin dose or serum concentration.

LYMPHOCYTE PROTEIN SYNTHESIS

Optimum stimulation was obtained at a PHA concentration of 8.33 $\mu\text{g}/\text{ml}$. Responses to PHA are expressed as the ratio of the counts/minute with PHA to counts/minute in the unstimulated cultures. A highly significant depression of protein synthesis was found in all patients from group A

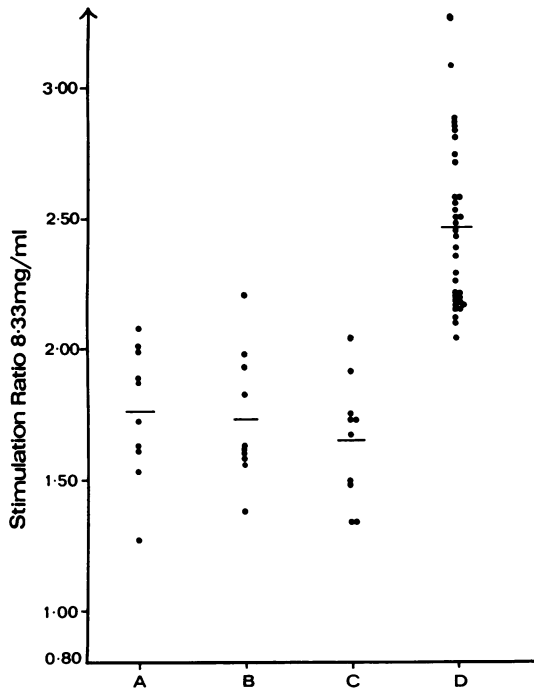


Fig. 2 Lymphocyte protein synthesis—PHA concentration of 8.33 $\mu\text{g}/\text{ml}$. A: patients with low IgA on phenytoin and other drugs ($P < 0.0001$). B: patients with normal IgA on phenytoin and other drugs ($P < 0.0001$). C: patients with normal IgA on other drugs ($P < 0.0001$). D: normal controls.

(low IgA and normal IgA) as well as in patients who were on other anticonvulsants (Fig. 2).

ANTICONVULSANT LEVELS

As stated, no correlation was found between serum IgA level and phenytoin dose. In group A, however, patients who had low serum IgA levels had lower serum primidone levels than patients with normal serum IgA, while the serum phenobarbitone derived from primidone was not different in the two groups. Four of the patients with low IgA were on phenobarbitone and these had a high serum level when compared with normal IgA group and group B patients, in spite of the fact that they were on comparable dosage (Table 3). The primidone and the phenobarbitone levels were significantly different ($P < 0.05$ for both using Student's *t* test) in the two groups of patients, but because of the small number of patients this finding is not considered to be important.

SERUM FOLATE LEVELS

Low serum folate levels were found in both groups A and B ($2.22 \mu\text{g}/\text{l} \pm 0.95 \text{ SD}$ and $3.164 \mu\text{g}/\text{l} \pm 1.567 \text{ SD}$, respectively). The lowest limit of the intermediate level between normality and deficiency is $3.2 \mu\text{g}/\text{l}$ in our laboratories. There was a significant difference between groups A and B, patients in group A having lower serum folate levels; this difference was highly significant using Student's *t* test ($P < 0.0005$).

Discussion

There is good evidence that immunological abnormalities are found in patients taking anti-convulsants. Several workers have found low serum levels of IgA and evidence of impaired T cell function as measured both in vivo by lack of cutaneous reactivity to ubiquitous recall antigens, and in vitro by impaired lymphocyte DNA and RNA synthesis (Sorrell and Forbes, 1975). The mechanisms involved in producing these immune deficiencies are unknown, but it has been suggested that genetic factors may operate (Fontana *et al.*, 1976).

Table 3 Serum anticonvulsant levels in $\mu\text{mol}/\text{l}$ in the two groups of patients

Drugs	Patients on phenytoin (group A)				On other drugs (group B)	
	Number	Low serum IgA	Number	Normal serum IgA	Number	
Phenytoin	13	43.23 \pm 23.58 SD	43	41.6 \pm 25.6 SD	—	—
Phenobarbitone	4	179.25 \pm 63.24 SD	24	118.86 \pm 45.6 SD	10	111.8 \pm 52.31 SD
Primidone	7	17.14 \pm 8.68 SD	16	32.75 \pm 19.1 SD	15	23.5 \pm 9.00 SD
(Phenobarbitone)*	7	123.00 \pm 42.19 SD	16	120.10 \pm 58.0 SD	15	118.0 \pm 60.40 SD

*Phenobarbitone derived from primidone.

Our results show a conspicuous impairment of both T cell function and IgA metabolism in patients on long-term anticonvulsant therapy. The technique used to measure T cell function was the *in vitro* measurement of peripheral blood-lymphocyte protein synthesis—this technique is a sensitive and reliable index of thymus-derived lymphocyte function (Pauly *et al.*, 1973). Impaired immune responses by this method have previously been demonstrated in patients with autoimmune diseases, drug reactions, and malignancies (Thomas *et al.*, 1975; Behan *et al.*, 1976; Simpson *et al.*, 1976).

It is possible that phenytoin causes depressed T cell function by direct action on cells. This hypothesis is supported by evidence of the action of phenytoin on normal lymphocytes in culture, where it produces depression of both DNA and RNA synthesis (Sorrell and Forbes, 1975). In our patients this effect may have been operative, although the depression was not related to phenytoin dosage or blood level. Similar depressed lymphocytic responses were also found in patients taking other anticonvulsants. A factor common to both groups was a low serum folate level; this is known to affect cellular nucleic acid synthesis (Norris and Pratt, 1974).

Nearly a quarter of the patients (23.2%) who were on phenytoin had low serum IgA levels, while only one patient from the other group (patients on other anticonvulsants) had even a slightly reduced level, and it is interesting to note that this patient was on carbamazepine, which was reported previously to cause IgA deficiency in 11% of patients (Sorrell and Forbes, 1975). In the patients affected there was no relationship between phenytoin dosage or serum level and the amount of IgA depression. The reduced serum primidone levels which were found in patients with low serum IgA have been reported previously (Slavin *et al.*, 1974), but the explanation of this is still unknown.

Low serum IgA levels have been found in patients with a variety of autoimmune disorders, including systemic lupus erythematosus (Tomasi, 1968), pernicious anaemia (Odgers and Wangel, 1968), Sjögren's syndrome (Claman *et al.*, 1966), and myasthenia gravis (Simpson *et al.*, 1976), persistent viral and fungal infections (Claman *et al.*, 1966; Hobbs, 1968), and in a variety of malignancies usually affecting the lymphoid system (Scheurlen *et al.*, 1971). IgA function is related to intact thymic function (Lancet, 1975): it is known that congenitally athymic nude mice have IgA deficiency although they have many IgA bearing B lymphocytes (Bankhurst and Warner, 1972).

This is similar to patients with IgA deficiency secondary to phenytoin therapy (Sorrell and Forbes, 1975) who also have normal numbers of IgA bearing B lymphocytes but low serum IgA levels. In animal experiments impaired immune responses, atrophy of thymic and lymphoid tissue, and increased incidence of lymphoid tumour were produced by chronic phenytoin ingestion (Kruger and Harris, 1972).

The most interesting association we found was the increased frequency of the histocompatibility antigen HLA-A2 in those patients taking phenytoin with low IgA levels as compared to those taking phenytoin but with normal serum IgA. The sample size of our patients was small, and there is a known high frequency of HLA-A2 in the west of Scotland (nearly 48%), but the difference between both groups was striking. Further studies will be needed to clarify this finding.

Our findings strongly support the hypothesis that phenytoin causes selective IgA deficiency in genetically predisposed patients.

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