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The primary immune response in patients with selective IgA deficiency

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SUMMARY

The primary immune response *in vivo* of 20 patients with selective IgA deficiency was studied and compared to controls. The primary cellular immune response tested by dinitrochlorobenzene (DNCB) was decreased in many patients. The primary humoral immune response was elicited by immunization with the test immunogen *Helix pomatia* haemocyanin (HPH). Using a direct ELISA technique antibodies against HPH of the IgA, IgG and IgM class were measured. Two weeks after immunization no response of IgA anti-HPH was seen except in three patients who showed a low but detectable antibody level. In spite of normal or even elevated serum IgG and IgM levels there was a significantly lower response of the IgG and IgM anti-HPH antibodies at 2 weeks after immunization as compared to the controls followed by a further decline at 6 weeks. We conclude that selective IgA deficiency is often accompanied by more general disturbances in humoral and cellular immunity to newly encountered antigens.

Keywords IgA deficiency *Helix promatia* haemocyanin dinitrochlorobenzene primary immune response immunity

INTRODUCTION

Patients with selective IgA deficiency (SIgAD) were originally defined as having a serum IgA less than 0·05 mg/ml without deficiencies of other classes of immunoglobulins; in addition they should have a normal cellular immunity and a normal humoral immune response for IgG and IgM class antibodies (Ammann & Hong, 1971).

With the use of more sophisticated techniques, functional abnormalities of IgA specific regulatory T cells were found *in vitro* (Waldmann *et al.*, 1976; Atwater & Tomasi, 1978; King, Vivian Wells & Nelson, 1979; De la Concha *et al.*, 1982). Production of the immunoglobulins IgG and IgM after *in vitro* stimulation of blood lymphocytes with pokeweed mitogen (PWM) was also found to be decreased in some patients with SIgAD (Cassidy, Oldham & Platts-Mills, 1979; De la Concha *et al.*, 1982).

All these studies have been performed *in vitro* and give no information about the immune response to primary antigens *in vivo*. However, primary test immunogens such as dinitrochlorobenzene (DNCB) and *Helix pomatia* haemocyanin (HPH) can be used for this purpose (Bleumink *et al.*, 1974; Weits *et al.*, 1978; Kallenberg, Torensma & The, 1983). In the present study we immunized 20 persons who were known to have a selective IgA deficiency with DNCB and HPH in order to assess

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their capacity for the cellular and humoral immune response. Since some patients with selective IgA deficiency present with recurrent infections we were especially interested in whether this subgroup showed a decreased response in IgG and IgM anti-HPH antibodies.

MATERIALS AND METHODS

Study group. Twenty subjects (six women) with SIgAD were studied. All individuals had a serum IgA concentration $<0{\cdot}02$ mg/ml and no deficiencies of IgG and IgM. The mean age was 24 years (range 6–62 years). Two patients were discovered during a family screening of patients with SIgAD, three patients during a routine population screening and 15 patients because they presented with clinical symptoms related to selective IgA deficiency (Ammann & Hong, 1971; Burgio *et al.*, 1980). None of the patients had taken immunosuppressive drugs.

The control sera for the measurement of specific antibody levels before and after immunization with HPH were obtained from a control group of 23 healthy volunteers (eight women). The mean age of this group was 50 years.

Measurement of immunoglobulins. Immunoglobulins were quantitated by the radial immunodiffusion technique of Mancini. WHO reference sera were used and the results compared to serum immunoglobulins in healthy children and adults (Zegers *et al.*, 1975). If the IgA level was below 0·02 mg/ml two different types of radioimmunoassays were used to determine the concentration (Stalman & Aalberse, 1977; van Munster, Nadorp & Schuurman, 1978). The results of both methods were in good accordance. Table 1 shows the mean values. The lowest detectable level with these methods is 0·002 mg/dl.

Measurement of dinitrochlorobenzene (DNCB) skin reactivity. Cell-mediated immune reactivity was measured by skin reactivity to DNCB as previously described (Bleumink *et al.*, 1974; De Gast *et al.*, 1975). In patients younger than 16 years, 0·01 ml of a 5% DNCB solution in acetone was applied topically with a silver patch tester on the volar aspect of the right arm, while in adults this was done within a polythene ring, using 0·1 ml of a 2% DNCB solution. Challenges were performed by patch testing the patients on their backs with 3, 10 and 30 μg of DNCB, 2 weeks later. The reactions were graded 1+ to 4+; 1+ reactions = erythema, 2+ = erythema plus induration, 3+ = erythema, induration and vesiculation, 4+ = idem plus ulceration. The sum of the reactions on the three concentrations was taken as a measure for skin reactivity, designated DNCB score (0–12+). Because the DNCB scores are age related, the DNCB scores of the patients were compared with the normal range, as determined for each age group (De Gast *et al.*, 1975). As controls for the average DNCB score we took the same group of patients as described by Bleumink *et al.* (1974).

Measurement of antibodies to HPH. After obtaining a blood sample, 1 mg of HPH was given subcutaneously in the deltoid region. In the control group samples were drawn after 2, 3 and 6 weeks, while in the patient group samples were obtained 2 and 6 weeks after immunization. The specific antibodies to HPH of the IgA, IgG and IgM class were quantified according to a modification of the original technique (Weits *et al.*, 1978). In brief, flat bottom microtitre plates (Dynatech M 129 A) were coated with HPH in 0·1 M carbonate pH 9·6 (5 μg/ml) for at least 2 days at 4°C. Before use the plates were thoroughly washed with 0·01 M Tris-HCl, pH 8·0, containing 0·15 M NaCl and 0·05% Tween 20. Subsequently, the plates were incubated with 100 μl serum dilutions (1:100 to 1:6,400) in 0·01 M Tris-HCl pH 8·0, containing 0·3 M NaCl, 4% BSA and 0·05% Tween 20 for 45 min at 37°C. After incubation the plates were washed three times and 0·1 ml of peroxidase labelled sheep anti-human IgG, IgM or IgA (Institut Pasteur, Nos 75051, 75061 and 75041), diluted 1:1,000 in 0·01 M Tris-HCl, containing 0·15 M NaCl, 2% BSA and 0·05% Tween 20, was added to each well. This was incubated for 45 min at 37°C and washed three times as described before. Bound enzyme activity was measured by using ortho-phenylene-diamine-di-HCl (OPD, Eastman) as chromogene. A solution was prepared by mixing 1 ml of an OPD solution in methanol (10 mg/ml) with 49 ml of a 50 mM phosphate buffer, pH 5·6. Immediately before use 75 μl 3% H_2O_2 was added and each well was filled with 0·1 ml of this solution. The enzyme reaction was left for 30 min, stopped with 0·1 ml 1N H_2SO_4 and read at 492 nm by a multiscan. Each plate contained blank incubations and a reference sample analysed in duplicated two-fold dilutions. This reference sample consisted of a pool of sera from volunteers, drawn 3 weeks after the HPH immunization.

Unknown anti-HPH concentrations were computed from the linearized titration curve obtained after log–logit transformation of the concentrations of the reference serum and the corresponding optical densities (Ritchie, Nickerson & Fuller, 1981). Antibody concentrations in the unknown sera were expressed as a percentage of the concentration of the reference sample, which was arbitrarily defined as 100 u at a dilution of 1 : 100. When the concentrations were very low, it was not possible to achieve precise results and the baseline value was drawn at 4 u.

The control sera for the measurement of specific antibody levels before and after immunization with HPH were obtained from a group of 23 healthy volunteers (eight women) with a mean age of 50 years (range 23–68 years).

Statistics. The Wilcoxon rank sum test (unpaired samples) was used for comparison of the anti-HPH antibody levels between patients and controls. The Student's *t*-test was used for comparison of the average DNCB scores. Differences with corrected *P* values $\leqslant 0{\cdot}05$ were considered significant.

RESULTS

We studied 20 patients with selective IgA deficiency. Clinical data, levels of serum IgA, IgG and IgM immunoglobulins and DNCB scores are shown in Table 1. IgA was not detectable in any of the

Table 1. Clinical data, levels of immunoglobulins and DNCB score in 20 patients with selective IgA deficiency

Patients			Immunoglobulins				DNCB	
No.	age	sex	IgA*	IgG*	IgM*	IgA†	score‡	Clinical features
1	8	M	<2	990	80	0·100	3+§	recurrent infections
2	8	M	<2	1,390	130	0·053	5+§	recurrent infections/asthma/ urticaria
3	15	M	<2	1,320	130	0·057	6+	none
4	15	M	<2	1,460	50	0·016	4+	rhinitis
5	13	M	<2	1,950	50	0·017	7+	none
6	16	F	<2	1,280	145	0·014	5+	rhinitis
7	13	F	<2	2,250	120	1·02	3+	none
8	9	M	<2	1,870	120	1·29	5+	abdominal pain
9	17	M	<2	1,320	145	0·119	9+	recurrent infections/asthma/ rhinitis
10	5	M	<2	1,320	100	1·49	7+	recurrent infections/rhinitis
11	8	F	<2	2,200	165	0·038	6+	recurrent infections
12	16	M	<2	1,630	175	0·383	4+	recurrent infections
13	60	F	<2	1,230	120	0·010	7+	recurrent infections/sprue/ pancreatic insufficiency
14	40	M	<2	2,720	180	<0·002	5+	none
15	60	M	<2	2,760	70	0·011	6+	scleroderma/Sjögren's syndrome
16	25	F	<2	2,110	220	<0·002	3+	hepatic cirrhosis/ recurrent infections
17	50	M	<2	1,290	225	0·004	11+	glomerulonephritis
18	19	M	<2	1,320	65	<0·002	11+	Crohn's disease/arthritis
19	33	F	<2	1,390	95	0·004	n.d.	arthralgia/rhinitis/ Quincke's oedema
20	21	M	<2	1,600	65	0·284	9+	recurrent infections

* Determined with RID (Mancini), concentration expressed as mg/dl.

† Determined with RIA. Concentration expressed as mg/dl.

‡ Normal range DNCB scores: 0–40 years: 6+–12+; 41–60 years: 4+–10+; ≥61 years: 2+–8+.

§ Only tested with 3γ and 10γ DNCB.

patients with the radial immunodiffusion technique of Mancini. Using two different types of radioimmunoassay, however, we were able to demonstrate low amounts of IgA in 17 patients. There were no deficiencies in the serum IgG and IgM concentrations. Seven patients showed elevated IgG levels and two patients elevated IgM levels. *In vitro* studies of cellular immune response, including E rosette forming cells and lymphocyte proliferation responses to mitogens and recall antigens, showed no abnormalities (results not shown). In this respect no T cell defect could be demonstrated.

DNCB-induced skin reactivity

Seventeen patients were evaluated (Table 1). Two patients were only tested with 3γ and 10γ DNCB (Nos 1 & 2) and one patient refused this test (No. 19). Sensitization to DNCB occurred in all patients, but in seven of the 17 patients there was a decreased DNCB score after challenge. The DNCB score in the patient group younger than 50 years was decreased compared to controls ($P \leqslant 0{\cdot}001$). In the patient group older than 50 years no significant differences were found, but only a few patients were studied (Table 2).

Table 2. Average DNCB score $\pm$ s.d. in controls and SIgAD patients

	Controls*		SIgAD	
Age	*n*	Average score	*n*	Average score
0–49 years	29	$10{\cdot}4 \pm 1{\cdot}6$	14	$6{\cdot}0 \pm 2{\cdot}4$
50–69 years	9	$6{\cdot}8 \pm 2{\cdot}7$	3	$8{\cdot}0 \pm 2{\cdot}6$

* Bleumink *et al.* (1974), *Cancer*, **33**, 913.

HPH-induced antibody response

Controls (Fig. 1). Before immunization with HPH very low or undetectable IgA and IgG anti-HPH antibodies were found. However, some controls showed low, but detectable IgM antibodies against HPH. After immunization the IgA anti-HPH levels peaked at day 14, followed by a gradual decline, whereas the IgG and IgM anti-HPH levels reached peak levels between day 14 and day 21 and remained about the same at 6 weeks after immunization.

Patients (Fig. 2). Again, very low or undetectable IgA and IgG anti-HPH levels were found before immunization with HPH, except for one patient who had an IgG anti-HPH concentration of 19 u. Analogous to the control group, low but detectable IgM antibody values were present in nine patients with SIgAD. After immunization there was either a very low or absent response of IgA anti-HPH. Three patients (Nos 12, 17 & 20) had a detectable response of IgA anti-HPH at 2 weeks, but at 6 weeks only one patient (No. 17) showed a subsequent rise, whereas the values returned to baseline in the other two patients. Only one other patient (No. 16), who did not have an IgA response at 2 weeks, showed a low but detectable response at 6 weeks.

The levels of IgG and IgM anti-HPH antibodies following immunization showed a great variability. Some patients had a normal response, others had a low or even absent response. However, compared to the control group, the levels were significantly lower both at 2 and 6 weeks ($P \leqslant 0{\cdot}05$). Concerning the kinetics of the response, there was a greater decline in the levels of IgG and IgM anti-HPH antibodies at 6 weeks after immunization compared to the control group.

No correlation was detected between the degree of hyporesponsiveness and the severity of clinical symptoms. For instance, one patient (No. 19), who did not show any antibody response to HPH at all, had only mild clinical symptoms, whereas another patient (No. 16) with a normal antibody response of the IgG and IgM class had an illness with severe recurrent upper and lower airway infections.

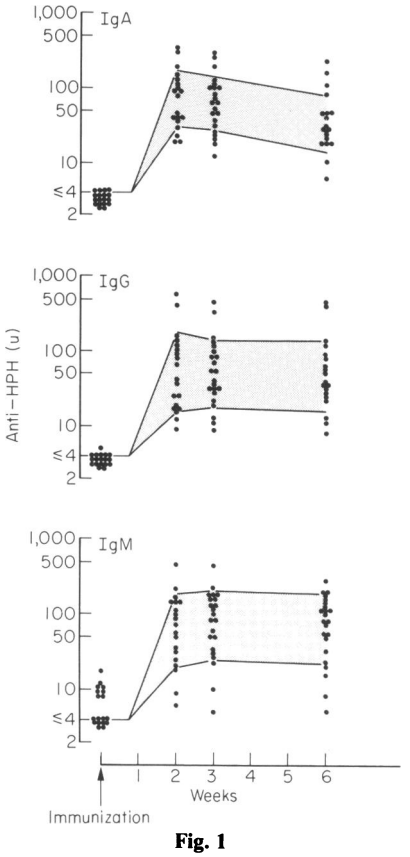

Fig. 1

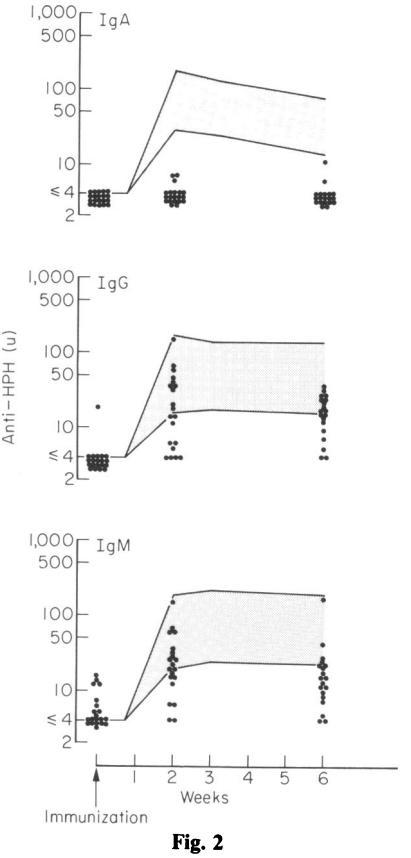

Fig. 2

Fig. 1. IgA, IgG and IgM antibody levels in 23 healthy controls before and 2, 3 and 6 weeks after immunization with 1·0 mg HPH subcutaneously. Antibody levels are expressed as a percentage of a positive reference sample. The concentration of this sample is arbitrarily defined as 100 u at a dilution of 1:100 (see Materials and Methods). The shaded area represents the normal range (±s.d.).

Fig. 2. IgA, IgG and IgM anti-HPH antibody levels in 20 patients with SIgAD before and 2 and 6 weeks after immunization with 1·0 mg HPH subcutaneously. Antibody levels are expressed in the same way as for the control group. The shaded area represents the normal range (±s.d.) of the control group.

DISCUSSION

Most *in vitro* studies of cell-mediated immune function in patients with SIgAD, including studies on lymphocyte responses to mitogens and antigens and on the number of E rosette forming cells, have failed to demonstrate a T cell defect (Delespesse *et al.*, 1976; Burgio *et al.*, 1981; De la Concha *et al.*, 1982). A functional abnormality of regulatory T cells for *in vitro* IgA synthesis has been reported by several authors (Waldmann *et al.*, 1976; Atwater & Tomasi, 1978; King *et al.*, 1979), but the relevance of this finding has been questioned (Delespesse *et al.*, 1976; De la Concha *et al.*, 1980).

Using the DNCB test with determination of the degree of skin reactivity, it is now possible to quantify the cell mediated immune response *in vivo* (Bleumink *et al.*, 1973; De Gast *et al.*, 1975).

Our results demonstrate a decreased response in seven out of 17 patients with SIgAD and a significantly lower average DNCB score in the patient group than in the controls. This suggests that there is a disturbed cellular immune response to newly encountered antigens in some patients with SIgAD.

All functional studies concerning the synthesis and secretion of IgA, IgG and IgM by peripheral

blood lymphocytes in patients with SIgAD have been performed *in vitro* by stimulation with PWM and determination of the amount of Ig in the supernatant (Wu, Lawton & Cooper, 1973; Delespesse *et al.*, 1976; Waldmann *et al.*, 1976; Atwater & Tomasi, 1978; Cassidy *et al.*, 1979; De la Concha *et al.*, 1982). In order to measure the humoral antibody response *in vivo* we used the primary test antigen, HPH. It is a strong immunogen, which elicits both a humoral and cellular immune response in all normal volunteers and it is available in pure form (Weits *et al.*, 1978; The *et al.*, 1981). We applied a more accurate modification of the original method to determine the specific antibody levels against HPH of the IgA, IgG and IgM class (Weits *et al.*, 1978), using a direct ELISA technique and a log–logit transformation of the data to compute the concentration values.

Before immunization baseline values of IgA anti-HPH and IgG anti-HPH levels were present. Low levels of IgM anti-HPH antibodies were detectable in about 40% of the patients and the controls. They probably result from prior exposure to cross-reacting determinants and do not influence the primary immune response (Kallenberg *et al.*, 1983). This possibly also applies to the one patient who had a low, but detectable pre-immunization level of IgG anti-HPH.

Following immunization the IgA response was either very low or absent in the patients with SIgAD. This was not unexpected, since several *in vitro* studies have shown that PWM-induced IgA production by peripheral blood B cells is decreased in selective IgA deficiency (Waldmann *et al.*, 1973; Atwater & Tomasi, 1978; Cassidy *et al.*, 1979; King *et al.*, 1979), although this finding has been contradicted by other authors (Wu *et al.*, 1973; Delespesse *et al.*, 1976). However, the levels of IgG and IgM class specific antibodies were also lower in the patient group than in the control group, both at 2 and 6 weeks after immunization. No correlation between the degree of hyporesponsiveness of the IgG and IgM anti-HPH antibodies and the severity of clinical symptoms was found. Concerning the kinetics of the response, there was a decline of this response at 6 weeks, which was not seen in the control group. Although the mean age of the patient group is different from that of the control group these results are comparable since no clear differences in the HPH antibody response between different age groups has been found (Weits *et al.*, 1978).

In contrast some patients with selective IgA deficiency have elevated total serum IgG and IgM (Ammann & Hong, 1971; Burgio *et al.*, 1981). We detected elevated total serum IgG levels in seven patients, while IgM levels were elevated in two patients.

In vitro studies with PWM have shown decreased IgG production in some patients with SIgAD (Cassidy *et al.*, 1979). In another study no IgM production was detected in two out of 12 patients after stimulation with PWM (De la Concha *et al.*, 1980). However, it should be noted that other investigators have not found an abnormal IgG and IgM production *in vitro* (Delespesse *et al.*, 1976; Waldmann *et al.*, 1976). A maturation defect has been suggested, leading to an inability to differentiate into cells producing normal amounts of IgA (Conley & Cooper, 1981). Our findings are consistent with the hypothesis that a more general B cell defect of varying severity is present in some patients with SIgAD (Cassidy *et al.*, 1979). However, it should be emphasized that HPH is a special antigen, which also elicits a marked delayed hypersensitivity reaction and the antibody response is dependent on T cell regulation (De Gast, The & Snijder, 1973). Abnormalities in the cellular immune response may therefore result in decreased antibody production, although in our study we were not able to correlate decreased antibody production to HPH with decreased cellular immune response to DNCB.

We conclude that selective IgA deficiency appears to be a heterogeneous syndrome, in which functional deficiencies of other classes of immunoglobulins and of cellular immunity sometimes exist. The syndrome is not so selective as originally defined, and additional more subtle defects in cell-mediated immune response and humoral antibody production may be present.

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