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Immunological investigations in individuals with selective IgA deficiency

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SUMMARY

Concentrations of IgG2, IgG4 and IgE were low in 16, 24 and 20% of 25 persons with selective IgA deficiency. Fifty-two per cent had IgD concentrations below 5 iu/ml. Trends for association between any of these parameters and the presence of clinical symptoms were not significant. All patients, except one, had normal amounts of Ig-bearing lymphocytes in the blood.

IgG1 antibodies against casein were increased in titre and frequency, whereas IgG4 antibodies were normal. Similar results were found in other sera from persons with selective IgA deficiency.

Keywords selective IgA deficiency immunoglobulin deficiencies IgG1 and IgG4 antibodies against casein

INTRODUCTION

Selective IgA deficiency (S-IgA-D), is an immunodeficiency characterized by a concentration of serum IgA $<0{\cdot}02$ mg/ml ($1{\cdot}4$ iu/ml) with normal or increased concentrations of IgM and IgG (Ammann & Hong, 1971; Hanson, Björkender & Oxelius, 1983). Subjects with S-IgA-D may be healthy or have recurrent infections and/or autoimmune diseases (Buckley, 1975). Usually serum IgA deficiency is associated with secretory IgA deficiency. Patients with S-IgA-D may also be deficient of IgG2 and/or IgG4, which may be associated with more severe recurrent infections of the respiratory tract (Oxelius *et al.*, 1981; Ugazio *et al.*, 1983).

In most studies, S-IgA-D is diagnosed by failure to detect IgA with precipitation and/or haemagglutination techniques. Studies using more sensitive techniques have shown that the IgA deficiency is often not absolute (Nadorp *et al.*, 1973; Laschinger *et al.*, 1984).

We have measured serum and secretory IgA by sensitive radioimmunoassays, the other immunoglobulin classes, the subclasses of IgG and IgG1 and IgG4 antibodies to casein in 25 persons with S-IgA-D.

MATERIALS AND METHODS

Study group. Twenty-five persons (nine female) with S-IgA-D serum IgA concentration below

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0·02 mg/ml (1·4 iu/ml) and normal or increased IgM and IgG levels were admitted to the study. The mean age was 19·4 years (range 3–60 years) (Table 1). Twenty individuals presented with clinical symptoms and five persons were discovered during family or routine screening. The primary immune responses *in vivo* of some of them have been reported (De Graef *et al.*, 1983). For some studies the persons were divided into two groups according to age: $\leqslant 16$ years, $n = 17$, (seven female) mean age: 10·4 years; above 16 years, $n = 8$, (two female) mean age: 38·5 years. It was not always possible to perform all measurements on the samples from each patient.

Sera from 49 other individuals with S-IgA-D ($21 \leqslant 16$ years, $28 > 16$ years) submitted for diagnostic screening of serum immunoglobulin concentrations were studied in addition.

The control groups for anti-casein antibody were 64 out-patients from a paediatric clinic (aged $\leqslant 16$ years), without IgA deficiency, symptoms related to IgE-mediated allergy, or gastro-intestinal complaints. Their ages were similar to those of the children with S-IgA-D. Adult controls were healthy persons, with equal number of controls in each decade of age to the S-IgA-D patients.

Methods. Low IgA concentrations were measured by an inhibition radioimmunoassay (RIA) van Munster, Nadorp & Schurman, 1978) and by a non-competitive two-site RIA (Out *et al.*, 1979), using different anti-IgA antisera and a standard serum that was calibrated against a World Health Organization (WHO) standard serum for immunoglobulins. For some analyses the mean value of IgA obtained by the two assays was taken. The conversion from iu of IgA to μg of IgA was done according to Humphrey & Batty (1974). IgG and IgM in serum were measured by radial immunodiffusion or by an immunoturbidimetric assay. Normal values have been reported (Zegers *et al.*, 1975). Subclasses of IgG were determined by radial immunodiffusion. Subclass specific antisera were obtained from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service. The results were expressed as the percentage of the concentrations in the standard serum H00-02. Normal values have been reported (van der Giessen *et al.*, 1975). IgD and IgE were measured by non-competitive two-site RIA's using class-specific anti-IgD and anti-IgE antisera (Out *et al.*, 1978; Stallman & Aalberse, 1977), and standard sera calibrated with an appropriate WHO standard serum. Saliva was collected from the parotid gland by parotid cup, in place for 15 min before stimulation during a 10-min period (Lourie, 1943). IgA in saliva was measured by the inhibition RIA (van Munster *et al.*, 1978). IgM in saliva was measured by a non-competitive two-site radioimmunoassay (Out *et al.*, 1979) or by a nephelometric immunoassay (Hyland nephelometer). IgG1 and IgG4 antibodies to antigens from food were measured as described previously (van Toorenenbergen & Aalberse, 1981). Briefly, antigens were coupled to CNBr-activated Sepharose and incubated for 16 to 20 h with appropriately diluted samples. After a wash either ^{125}I-anti-IgG1 or ^{125}I-anti-IgG4 was added and incubation was continued for another 16 to 20 h. The results were expressed as arbitrary units, relative to local standard sera, thereby making inter-assay comparisons possible. For IgG1 antibodies a serum containing a high titre of IgG1 anti-tetanus toxoid served as standard and the binding of IgG1 onto Sepharose-tetanus toxoid at various serum concentrations served as a calibration line. For IgG4 antibodies the binding of IgG4 anti-bee venom antibodies (as present in a serum from a bee keeper) onto Sepharose-bee venom was used for calibration. Antibodies to IgA were measured by the haemagglutination inhibition technique (van Loghem, 1978) and by radioimmunoassay (van Munster *et al.*, 1978). Antibodies to dsDNA were measured by the Farr assay or by immunofluorescence with the use of crithidia luciliae as substrate (Aarden, De Groot & Feltkamp, 1975). Rheumatoid factor activity was screened for with the Latex agglutination test. Autoantibodies to tissue antigens were determined by immunofluorescence (Smit *et al.*, 1980).

Lymphocyte studies. Lymphocytes were isolated from defibrinated or heparinized blood by Ficoll-Isopaque density gradient centrifugation. The number of E-rosette forming cells were determined according to Jondal, Holm and Wigzell (1972). Membrane Ig carrying lymphocytes (i.e. total Ig and Ig isotypes) were determined according to Vossen and Hijmans (1975).

RESULTS

IgA in sera

Results of IgA of the two assays were essentially the same: linear regression analysis showed that

Table 1.

Patient no.	age	sex	IgA (milli iu/ml)	IgG1 (% of standard)*	IgG2 (% of standard)*	IgG3 (% of standard)*	IgG4 (% of standard)*	IgD (iu/ml)	IgE (iu/ml)	Clinical symptoms
1	11	m	<1	50↓	50	150	170	3·4	1	rec. infections
2	4	m	<1	184↑	54	144↑	<10↓	26	190	rec. infections
3	16	f	10	110	60↓	180	75	1·4	4	rhinitis
4	15	m	11	100	130	100	60	2·0	37	rhinitis
5	13	f	12	190↑	215	128	402	2·4	5	healthy
6	11	f	15	80	41↓	80	10↓	14	150	rec. infect. upper airw., coeliac dis.
7	8	f	27	190↑	124↑	155↑	153	17	29	rec. infections
8	8	m	37	105	91	89	194↑	41	392	rec. infec./asthma/urticuria
9	15	m	40	90	100	50	60	9	500	healthy
10	8	m	68	89	131↑	85	36	2·8	3060	rec. infections
11	16	m	86	69↓	104	318↑	<5↓	0·67	70	rec. infec./asthma/rhinitis
12	16	m	270	78	218	141	147	1·8	3800	rec. infections
13	6	f	370	115	7↓	180↑	<10	0·9	<1	rec. infections
14	3	f	610	147	69	100	39	0·8	160	healthy
15	13	f	720	220↑	136	112	49	16	212	healthy
16	9	m	910	150↑	90	110↑	90	130	284	abdominal pain
17	5	m	1050	161↑	71	75	95	85	852	rec. infec./rhinitis
18	40	m	<1	107	212	262↑	91	0·37	<1	healthy
19	25	m	<1	300↑	97	330↑	<5↓	5·2	78	rec. infec./cirrhosis
20	19	m	<1	144	176	85	344	2·4	5	M. Crohn/arthritis
21	50	m	3	216↑	147	208	160	7·1	8·5	glomerulonephritis
22	33	f	3	216↑	169	62	<5↓	19	<1	arthralgia/rhinitis/ Quincke's oedema
23	60	f	7	140	135	155	<10↓	0·9	<1	rec. infec./sprue/pancreas insuff.
24	60	m	8	220↑	275↑	222	>600↑	350	3720	scleroderma/M. Sjögren
25	21	m	200	152	70↓	170	660↑	0·6	11	rec. infections

* 100% Corresponds approximately to 6·2 mg/ml (IgG1), 2·4 mg/ml (IgG2), 0·64 mg/ml (IgG3), 0·46 mg/ml (IgG4).

(↑) Increased; (↓) decreased, as compared to age-matched controls (see van der Giessen *et al.*, 1975).

IgA (two-site RIA) = 0·92 IgA (inhibition RIA) + 0·015 (expressed in log iu/l), $r = 0·93$. In five sera no IgA was detected by either assay: IgA < 1 milli iu/ml. Four of these sera contained anti-IgA antibodies. In one serum (no. 5, Table 1) IgA was not detected by two-site assay, whereas the inhibition assay showed 24 milli iu/ml; in one other serum (no. 22, Table 1) no IgA was detected by the inhibition RIA, and 6 milli iu/ml was measured by the two-site RIA. In Table 1 the clinical diagnosis and the concentrations of serum IgA and other immunoglobulins are shown. The geometric mean IgA in sera of the adults was 4·4 milli iu/ml and of the children, 53 milli iu/ml. No relation between the concentration of IgA in serum and the nature and/or severity of clinical symptoms was observed.

IgM and subclasses of IgG in sera

IgM was normal in all people (not shown); IgG was increased in 13 people, the increase being mainly due to an increase of IgG1 and/or IgG3 (Table 1). Four people (16% of the whole group, 20% of the persons with clinical symptoms) had a decreased concentration of IgG2, but IgG2 was detected in all by haemagglutination inhibition studies. Six people (24% & 30%, respectively) had a decreased IgG4 concentration; two had both IgG2 and IgG4 concentrations below the normal levels, in one of whom IgE was not detected.

Concentrations of IgG2 and/or IgG4 were low only in those persons who had clinical symptoms

Table 2. The relation between clinical symptoms and the concentrations of IgG2 and IgG4

	IgG2*		IgG4†		IgG2†	
	n	↓	*n*	↓	*n*	↓
Healthy	5	0	5	0	16	0
Clinical symptoms‡	16	4	14	6	46	11
	$P=0{\cdot}38$*		$P=0{\cdot}22$*		$P=0{\cdot}052$*	
With recurrent infections	10	3	8	5		
Without recurrent infections	11	1	11	1		
	$P=0{\cdot}33$*		$P=0{\cdot}097$*			

* Fisher's exact test.

† Data from this study pooled with those from Oxelius *et al.* (1981).

‡ The clinical symptoms of the patients from this study are mentioned in Table 1, those from Oxelius *et al.* (1981) in their paper.

(Table 1), but the difference was not statistically significant (Table 2). The combined results from this study and from that of Oxelius *et al.* (1981) suggest that such an association may be significant (Table 2). There was a trend for association of low IgG4 and recurrent infections (Table 2), but it was not statistically significant (cf. Ugazio *et al.*, 1983).

IgD in sera.

IgA-deficient persons have lower serum IgD than normals have; Mann-Whitney-U test: $P<0{\cdot}001$. Especially they have levels <5 iu/ml more frequently than normals (χ^2 analysis, $P<0{\cdot}001$), Fig. 1.

IgE in sera

No IgE was detected (IgE <1 iu/ml) in five sera (20% both from the total group and those with

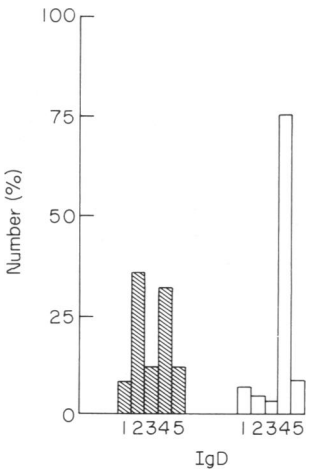

Fig. 1. The frequency distribution of the concentration of IgD in sera from S-IgA-D and controls. The IgD concentrations were divided into five groups: (1) IgD $\leq 0{\cdot}6$ iu/ml; (2) $0{\cdot}6<$ IgD $\leq 2{\cdot}5$ iu/ml; (3) $2{\cdot}5<$ IgD ≤ 5; (4) $5<$ IgD ≤ 80; (5) IgD >80 iu/ml. (□) Normals ($n=360$); (■) IgA def. ($n=25$).

clinical symptoms). In two of these no IgA was detected. Four had a concentration of IgE > 500 iu/ml. Several patients had normal IgE concentrations and the serum concentrations of IgA and IgE were significantly correlated (Spearman's rank correlation test: $\rho = 0{\cdot}49$; $P < 0{\cdot}01$). Such a relation was not observed in the additional panel of 49 other S-IgA-D sera.

IgA and IgM in saliva

No IgA was detected (inhibition RIA: IgA < 35 milli iu/ml) in 13 of 19 salivas. In three patients the concentration of IgA was higher in saliva than that in serum (patient nos 8, 23 & 25 in Table 1). The geometric mean IgM in the saliva samples was 1·03 iu/ml, which is considerably higher than in saliva samples from adult controls (mean IgM: 0·082 iu/ml; range 0·016–0·130 iu/ml, $n = 12$). These results confirm those of Brandtzaeg, Fjellanger & Gjeruldsen (1968).

Antibodies to IgA

All sera were tested for antibodies to IgA. Four of the five patients with a serum IgA below 1 milli iu/ml had class-specific antibodies to IgA. In addition, one patient (IgA = 12 milli iu/ml) had antibodies to IgA2.

Autoantibodies

One out of 23 sera tested had antiperinuclear antibodies; five of 23 had anti-smooth muscle antibodies, one of 23 had antibodies to gastric parietal cells and one of 23 to thyroid cells. No rheumatoid factor, antinuclear antibodies, anti-dsDNA or autoantibodies against erythrocyte antigens were detected (23 sera tested).

Lymphocytes

All persons had normal percentages of E-rosette-forming cells (range 46 to 85%) and surface Ig-positive lymphocytes (range 4 to 24%) except one (no. 3 in Table 1) who had 2% surface Ig-positive lymphocytes. Lymphocytes with surface IgA were < 0·3% in 16 persons, and ranged from 0·3 to 4·5% in six people.

Antibodies to casein

IgG1 antibodies to casein in sera from S-IgA-D children were higher (median 11·0 arbitrary units (A.U.)/ml (than in controls (median 4·6 A.U./ml); Mann-Whitney-U test (M-W-U): $P < 0{\cdot}016$. Also

Table 3. The IgG1 and IgG4 anti-casein antibodies in S-IgA-D persons and controls

			Age ≤ 16 years				Age > 16 years			
			IgG1 anti-casein		IgG4 anti-casein		IgG1 anti-casein		IgG4 anti-casein	
	Category	A.U./ml	S-IgA-D	Controls	S-IgA-D	Controls	S-IgA-D	Controls	S-IgA-D	Controls
(A)										
	1	neg*	3	13	5	14	0	16	3	18
	2	neg.–10	3	41	3	9	6	23	0	9
	3	10–40	8	10	1	17	2	2	5	10
	4	>40	0	0	5	24	0	0	0	4
(B)										
	1	neg	3	13	11	14	1	16	16	18
	2	neg–10	14	41	5	9	29	23	6	9
	3	10–40	18	10	6	17	5	2	9	10
	4	>40	0	0	13	24	1	0	5	4

* Negative: < 3 A.U./ml for IgG1, < 5 A.U./ml for IgG4.

(A) Results in the sera from the group from Table 1.

(B) Pooled results of the additional panel of sera from S-IgA-D persons and those of A.

the additional panel of sera from S-IgA-D children had higher IgG1 anti-casein (median 9·1 A.U./ml) than controls: M-W-U, $P < < 0{\cdot}001$. No significant differences in IgG4 anti-casein were found. IgG1 anti-casein of adults (median: 6·5 A.U./ml) were higher than those of controls (median: 3·2 A.U./ml): M-W-U, $P < 0{\cdot}001$. The same was found in the additional panel of sera from S-IgA-D adults (median 6·6 A.U./ml): M-W-U, $P < < 0{\cdot}001$, and no differences in IgG4 antibodies were found. For simplicity of presentation the scores of antibody titres were arbitrarily divided into four categories: negative, negative to 10 A.U./ml, 10 to 40 A.U./ml and >40 A.U./ml, indicated with categories 1, 2, 3, 4, respectively, and shown in Table 3.

In the paediatric group there was no increased incidence of IgG1 anti-casein in S-IgA-D persons as compared to the controls (panel A, category 1 versus categories 2+3+4; χ^2 test, no significant difference). However, as already mentioned S-IgA-D sera had higher concentrations.

In the adult group IgA-deficient persons had IgG1 anti-casein more often than the controls (panel A, category 1 versus categories 2+3+4; Fischers exact test, $P < 0{\cdot}03$), and as already mentioned they had higher titres. The titres were generally lower than in the paediatric group.

In the sera from S-IgA-D persons the higher incidence of IgG1 anti-casein is not accompanied by a high incidence of IgG4 anti-casein (Table 4). In controls no such discordance between IgG1 and IgG4 antibody responses was found. No relation was observed between the detection of IgG1 anti-casein in the sera and clinical symptoms.

Table 4. The comparison of positive and negative scores for IgG1 and IgG4 anti-casein antibodies in sera from S-IgA-D individuals

	Age ⩽ 16 years		Age > 16 years	
	Positive	Negative	Positive	Negative
IgG1 anti-casein	32	3	35	1
IgG4 anti-casein	24	11	20	16
	χ^2 test: $P < 0{\cdot}025$		χ^2 test: $P < 0{\cdot}001$	

DISCUSSION

There is a continuous range of serum IgA concentrations between the limit of detection in our sensitive assays (1 milli iu/ml) and the level at which a patient is regarded as having IgA deficiency (1·4 iu/ml). IgA concentration may vary in the sera of one individual (Laschinger *et al.*, 1984). In sera from five patients (20%) no IgA was detected (IgA < 1 milli iu/ml, < 14 ng/ml); four of these had antibodies to IgA.

No significant association between the concentration of serum IgA and nature and/or severity of symptoms was observed, but more patients are needed to confirm whether such a relationship exists. No association was found between the serum concentration of IgA and surface IgA on blood lymphocytes. However, interpretation of data on the latter tests is insecure because several polyclonal anti-IgA antisera were used in more than one laboratory.

No IgA was detected in saliva from the two patients in whom serum IgA was not detected, who were studied; the concentrations of IgM were 1·3 and 4·0 iu/ml (nos 1 & 19 in Table 1). In the three saliva samples available from the healthy persons with S-IgA-D, no IgA was detected and the concentrations of IgM were 0·2, 2·6 and 1·0 iu/ml (nos 5, 9 & 15 in Table 1). IgM concentrations in the saliva samples in which IgA was present were similar. These results in saliva did not reveal a relation between IgA and/or IgM in saliva and clinical symptoms.

Low IgG2 concentrations were not significantly associated with clinical symptoms, but, when our results were pooled with those of Oxelius *et al.* (1981) the combined results suggest that such an

association may exist (Table 2). It should be realised, however, that both in Oxelius's (1981) and in our study S-IgA-D patients were not taken randomly from a population of S-IgA-D persons, which may introduce bias in the statistical evaluation. There was no significant association between clinical symptoms and low IgG4 concentrations, but our results show the same tendency as those from Ugazio *et al.* (1983) who reported that decreased IgG2 and IgG4 concentrations were associated with recurrent infections. Other mechanisms may also play a role in the development of clinical symptoms in S-IgA-D, which require further studies.

IgD was detected in all sera. The lowest concentration observed (0·37 iu/ml) using this assay would not have been detected in earlier studies in which radial immunodiffusion was used (Buckley & Fiscus, 1975; van der Giessen *et al.*, 1976). Our results confirm earlier findings that in IgA-deficient persons IgD concentrations < 5 iu per ml occur more frequently than in normals (Buckley & Fiscus, 1975; van der Giessen *et al.*, 1976). The incidence of HLA-B8 was increased in normal individuals with low serum IgD concentrations (Fraser & Schur, 1981), and in blood donors having IgA deficiency (Oen, Petty, Schroeder, 1982; Hammarström & Smith, 1983), particularly those lacking IgA (Heikkilä *et al.*, 1984). It will be of interest to study whether the combination HLA-B8, IgA deficiency and low IgD is a factor at risk for developing clinical symptoms. In this study HLA typing was not performed.

In accordance with earlier results serum IgE < 1 iu/ml was more frequent in S-IgA-D persons than in normals (Polmar *et al.*, 1972). The observed association between the concentrations of IgA and IgE in sera from our first group (Table 1) was, however, not confirmed in the additional panel of sera from individuals with S-IgA-D.

Antibodies to food antigens. It has been suggested that secretory IgA at the intestinal mucosa may be important in protecting individuals against food antigens (Walker & Isselbacher, 1977). Buckley and Dees (1969), Barret *et al.* (1979) and Cunningham-Rundles *et al.* (1978) have reported the increased incidences and raised titres of such antibodies in S-IgA-D but they did not measure antibodies of the different subclasses of IgG. Complement activating and precipitating functions of different IgG subclasses differ (Aalberse & van der Zee, 1983).

In our S-IgA-D sera only IgG1 antibodies to casein are increased. IgG4 anti-casein was similar in S-IgA-D and controls. These findings may explain the occurrence of milk precipitins in sera of patients with S-IgA-D and their association with clinical symptoms (Cunningham-Rundles *et al.*, 1978; 1981; van der Woude *et al.*, 1983). Many sera of S-IgA-D patients have precipitating antibodies against bovine IgM (Barret *et al.*, 1979). Therefore, it would be worthwhile extending the studies of IgG1 and IgG4 antibodies to this antigen.

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