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Cellular aspects of selective IgA deficiency

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SUMMARY

Five patients with no detectable serum IgA (<20 μg/ml) and one patient with low serum IgA were compared to normal subjects. The number of circulating E-RFC was normal as was the lymphocyte DNA synthesis induced by PHA, Con A, and streptokinase–streptodornase. The patients had normal numbers of IgA-bearing lymphocytes and normal or increased numbers of B cells. Purified anti-immunoglobulin antibodies specific for IgG, IgA and IgM induced a normal lymphocyte DNA synthesis as did PWM. The patients' lymphocytes were able *in vitro* to transform into actively secreting IgA plasmocytes. This transformation was determined by counting the IgA and immunoglobulin-containing cells and then measuring the IgA and IgG secretion in the cultures. In some patients PWM was selectively suppressive in IgA B-cell transformation into IgA secreting cells; in the other patients PWM had no effect on the IgA B-cell differentiation. PWM enhanced the IgG secretion in the patients' cultures as well as IgA and IgG secretion in the normal controls.

INTRODUCTION

Selective IgA deficiency is not a homogenous syndrome. Indeed multiple aetiological factors may be responsible and the clinical symptoms may be absent or highly diversified. In some cases the defect is inherited as an autosomal dominant or recessive character and may be associated with infantile and childhood infections (Stocker, Amman & Rossi, 1968; Goldberg, Barnett & Fudenberg, 1968; Huntley & Stephenson, 1968; Soothill, Hayes & Dudgeon, 1966). In others it is associated with anti-convulsivant therapy (Aarli & Tönder, 1975). However, no causative factors can usually be detected and the defect may even be transitory (Lawton & Cooper, 1973).

These polymorphic aspects of IgA deficiency suggest that multiple pathogenic mechanisms may be involved. Previous studies reported a normal number of circulating IgA B lymphocytes in the blood of these patients contrasting with the absence of IgA-secreting plasmocytes into the mucosae (Lawton *et al.*, 1972; Buckley, 1975). It is thus suggested that the primary anomaly responsible for the IgA deficiency lies in the terminal differentiation of IgA precursor B cells into IgA plasmocytes. This might be secondary to an IgA B-cell defect or a T-cell anomaly. The latter view is supported by the observations that IgA antibody synthesis is highly dependent on a T-cell helper effect (Mitchell, Mischell & Herzenberg, 1971; Clough, Mims & Strober, 1971). In the present study we report our findings on the T- and B-cell functions in six patients, five of them having no detectable serum IgA. The T-cell status was investigated by the E rosette formation test and by the uptake of tritiated thymidine by lymphocytes stimulated with mitogens and antigens.

The B-cell function was estimated by measuring: (a) the circulating number of B cells and of IgA-bearing B cells; (b) the DNA synthesis induced in cultures stimulated with pokeweed mitogen (PWM) and anti-immunoglobulin antibodies; (c) the *in vitro* differentiation of Ig-bearing cells into Ig-containing cells; (d) the *in vitro* IgA and IgG secretion.

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MATERIALS AND METHODS

Patients (Table 1). Six patients were investigated; five had no detectable serum IgA and one had abnormally low level of this immunoglobulin. Patients D.E. and D.M. are two sisters and D.N. is the daughter of D.M. The age, sex, presenting diagnosis, and the autoimmune phenomena observed in these patients are recorded in Table 1. Blood donors with normal immunoglobulin levels were used as controls.

Methods. Concentrations of serum immunoglobulins (Ig) A, G and M were determined by single radial diffusion employing commercially available immunoplates (Quanti plates, Kallestad, Lab. Inc., Minnesota, U.S.A.). The 'low level quanti-plates' for IgA determination (Cat. No. Q 270-36) allowed the titration of 20 μg/ml IgA. IgE was measured with a solid phase radioimmunoassay (RIA) using the Phadebas kits (Pharmacia, Uppsala, Sweden).

TABLE 1. Patients with selective IgA deficiency

			Ig levels (mg/100 ml)					
Patients	Age	Sex	IgA	IgG	IgM	IgE	Autoantibodies*	Presenting disease
Mo.C.	12	M	0	2650	105	1100	—	Recurrent respiratory infections
De.M.	38	F	0	1600	80	35	TGA, CFA, M	Grave's disease, aphtous stomatitis
Se.S.	31	F	0	1850	148	40	ANF	Idiopathic thrombocytopenic purpura
Mo.L.	28	F	0	2000	365	80	TGA, CFA	Chronic diarrhoea, eczema
De.N.	8	F	0	1200	96	20	—	Recurrent respiratory infections
De.E.	40	F	69 (28)	1600	112	32	CFA, smooth muscle	None
Normal range		—	235±110	1173±261	113±53	50–150 u/ml		

* TGA = anti-thyroglobulin; CFA = anti-microsomes of the thyroid; ANF = anti-nuclear; M = anti-mitochondrial.

The methods used to identify B and T cells, and the measurement of *in vitro* lymphocyte DNA synthesis, will be summarized since they have already been described elsewhere (Delespesse *et al.*, 1976; Gausset *et al.*, 1976). Membrane fluorescence studies were conducted as described by Preud'homme & Flandrin (1974) employing FITC/swine anti-human immunoglobulin serum and rhodamine-conjugated swine antiserum specific for IgA heavy chain (Nordic Laboratories). In these assays, the monocytes were identified by the peroxydase reaction. T-cell rosette formation was performed according to Jondal, Holm & Wigzell (1972) with minor modifications: equal volumes of lymphocytes (4×10^6/ml, in Hanks's balanced salt solution, HBSS) and sheep red blood cells (4% in HBSS) were incubated at 37°C for 30 min; the mixture was centrifuged and incubated overnight in an ice bath. The percentage of lymphocytes with more than three SRBC on their surface was determined by counting 250 cells.

The lymphocyte transformation studies were performed in microplates (3040 Microtest II, Falcon, U.S.A.) employing quadruplicate 0·2 ml cultures containing 2×10^5 cells for mitogen stimulation and 6×10^5 cells for antigen or anti-immunoglobulin stimulations. The final concentration of the reagents were as follows: 1 μg/ml phytohaemagglutinin (PHA. P, Wellcome, England); 10 μg/ml Concanavalin A (Con A, Calbiochem., U.S.A.); 500 u/ml streptokinase–streptodornase (SKD, Lederle Lab., New York); 50 μg/ml a-IgA and 100 μg/ml a-IgG or a-IgM. The latter were purified rabbit anti-human immunoglobulin antibodies specific for the heavy chain determinants of IgG, IgA and IgM; they were obtained by affinity chromatography as previously described (Gausset *et al.*, 1976). Two microlitres of [^{3}H]thymidine (specific activity 10 Ci/mM) (Institute Radio-Eléments, Fleurus, Belgium) were added to the cultures 18 hr before harvesting. The duration of the culture was 3 days for mitogen-stimulated lymphocytes and 6 days when a-Ig or antigens were used as stimulants. The cells were then collected and washed on glass filter papers (943 AH, New Jersey U.S.A., Reeve Angel) using a cell harvester (Mash II, Microbiological Associates, Bethesda, U.S.A.).

The paper discs were immersed into counting vials containing 10 ml of Bray's solution and counted for 1 min in a scintillation counter.

The in vitro secretion of Ig was investigated as described by Waldman *et al.* (1974). 3×10^6 Ficoll–Hypaque purified lymphocytes were incubated for 7 days in 1 ml RPMI 1640 containing 5% foetal calf serum (FCS, GIBCO, Batch no. K-847402), 1 mM glutamine, 100 μg penicillin and 50 μg/ml streptomycin.

After 7 days the supernatants were collected and frozen at −40°C until tested by R.I.A.

The cells were washed, layered onto slides using a cytofuge, fixed and stained for intracytoplasmic fluorescence employing simultaneously FITC polyvalent anti-Ig and rhodamine-conjugated anti-IgA antisera. The number of Ig-containing cells present in 250 cells and the number of IgA-positive cells present in 100 Ig-containing cells were counted; this allowed a good

TABLE 2. RIA for IgA measurement

(1) 0·1 ml Sample
+0·1 ml sheep a-IgA (diluted in BBS with 50 μg/ml sheep IgG)
↓ 2 hr at room temperature
(2)+0·1 ml labelled IgA (diluted in BBS with 0·1% HSA)
↓ 3 hr at room temperature
(3)+0·1 ml Rabbit anti-sheep IgG
↓ overnight at 4°C
(4) Wash three times with cold BBS and count

estimation of the frequency of IgA-containing cells. The latter parameter was thus calculated and expressed as a percentage of total cells. In some assays the cultures were tested for their content in T cell-derived lymphoblasts using a modification of the E-rosette technique adapted from Smith & Haegert (1974).

The RIA used for the measurement of IgA and IgG secretion, outlined in Table 2, was a classical inhibition of double antibody method. The purified human IgA and IgG and the monospecific sheep anti-IgA and anti-IgG sera were prepared as previously reported (Gausset *et al.*, 1976). 100 μg of IgA and IgG were labelled with ^{125}I as described by Klinman & Taylor (1969); the labelled proteins were stored at 4°C in borate buffer saline (BBS) containing 0·2% human serum albumin (HSA) and 0·01% sodium azide. Sheep IgG was obtained by ammonium sulphate precipitation followed by DEAE chromatography using 0·01 M phosphate buffer, pH 8. Rabbits were immunized by three injections at 2 week intervals of 1 mg sheep IgG in complete Freund adjuvant. The rabbit anti-sheep IgG was absorbed on human IgG covalently linked to Sepharose 2B, in order to remove any cross-reaction with human IgG. It was used at a concentration precipitating in equivalence a solution of 50 μg/ml of sheep IgG. The standard curves were obtained with purified IgG and IgA diluted in the culture medium containing 5% FCS. Addition of PWM to the dilutions of the standards did not influence the results. Finally, the sheep anti-IgA or anti-IgG sera were used at a dilution allowing the precipitation of 50–60% of the labelled IgA or IgG.

RESULTS

As indicated in Table 3, normal numbers of E-RFC and IgA-bearing cells were observed in each of the patients. In two cases an increased amount of Ig-bearing cells was noted.

TABLE 3. T and B lymphocytes in selective IgA deficiency

Patients	E-RFC	Ig-bearing lymphocytes	IgA-bearing lymphocytes
Mo.C.	n.d.	18·8	1·2
De.M.	66	19·6	7
De.E.	61	n.d.	2·4
De.N.	63	30·4	7·2
Mo.L.	55	39·6	6
Se.S.	65	17·4	3·2
Normal controls (10)	58·5±8	16·7±5·7	6·1±2·9

n.d. = Not determined.

Tritiated thymidine incorporation studies

In cultures stimulated with optimal concentrations of PHA, Con A or SKD the thymidine uptake was normal except in one case. In patient M.L. (Table 4), the low lymphocyte response might be secondary to a state of malnutrition resulting from a chronic severe diarrhoea. A normal response in three patients was also noted when using suboptimal concentrations of PHA (0·1 μg/ml) and Con A (1 μg/ml) (data not indicated).

Purified anti-immunoglobulin antibodies specific for the heavy chain determinants of human IgA, IgM and IgG induced a significant [^3H]thymidine uptake in each of the investigated patients (Table 5).

TABLE 4. Lymphocyte response to PHA, Con A and SKD in IgA-deficient patients

Patients	PHA	Con A	SKD
Mo.L.	73± 8*	49± 6	n.d.
De.M.	110± 4	178±11	24± 1·9
De.N.	120± 6	99± 2	15± 0·2
De.E.	193± 7	97± 6	n.d.
Se.S.	285± 6	171± 3	60± 2
Mo.C.	192± 7	125± 4	n.d.
Normal subjects (36)	163±64	142±70	37±21

n.d. = Not determined.

* c.p.m. $\times 10^3$, mean of four replicates ± s.d.

TABLE 5. Lymphocyte response to A-Ig and PWM in IgA-deficient patients

Patients	Stimulants				
	None	a-IgA	a-IgM	a-IgG	PWM
Mo.C.	2±0·3*	n.d.	n.d.	128± 6	n.d.
De.M.	3±0·3	16± 0·4	26± 4	29± 1	85± 2
De.N.	21±0·8	30±4	n.d.	n.d.	n.d.
De.E.	23±2	36± 1	43± 5	73±15	120± 6
Se.S.	8±2	59± 0·7	124± 1	65± 2	221± 1
Mo.L.	2±0·4	7± 0·4	14± 1	21± 1	38± 0·5
Normal subjects (29)	7·2±5	21±13	26±20	56·5±34	87±41 (n = 5)

n.d. = Not determined.

* c.p.m. $\times 10^3$; mean of four replicates ± s.d.

TABLE 6. *In vitro* IgA synthesis in IgA-deficient patients

Cultures	Patients								Mean		Control subjects	
	Se.S.		Mo.L.		De.N.		De.E.					
	A*	B	A	B	A	B	A	B				
Controls	1·4	13	1·3	10·5	2·4	22	2·7	19	1·9	16·1	3·5	32±6
PWM	0·2	1·5	0·8	5·7	1·8	12	2·9	21	1·4	10	5·7	31±6
Con A		0		0				17				

* $A = \frac{\text{IgA-containing cells}}{\text{Ig-containing cells}} \times 100$ (counted); $B = \frac{\text{IgA-containing cells}}{100 \text{ cells}}$ (calculated).

A normal response was observed in the cultures stimulated with PWM even when used at different concentrations (data not indicated). Worth noting are the relatively high stimulations observed in two control cultures set up with the mitogenic batch of FCS.

In vitro differentiation of IgA precursor B cells into IgA plasmacytes (Table 6)

In the control cultures, containing 5% FCS, the patients' lymphocytes were able to transform into IgA plasmacytes. Indeed, as a mean 16·1% of the Ig-containing cells were IgA plasmacytes; this corresponded to a frequency of 1·9 Ig-containing cells for 100 cells. The corresponding mean values for the normal subjects were respectively 32·6 and 3·5. These parameters were not significantly influenced in the normal subjects by the addition of PWM to the cultures. In three out of four patients, PWM led to a reduction in the proportion of IgA cells present in the pool of Ig-containing cells; this resulted in a reduced frequency of IgA plasma cells in the first two patients.

TABLE 7. *In vitro* secretion of IgA-deficient patients

Cultures	Patients								Controls	
	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG
	Se S		De N		De M		De E			
Controls	98	2250	280	1400	110	2800	340	2000	953 (200–2070)	2870 (1200–3700)
PWM 1/1	20	2950	240	2521	0	2700	315	2350	1230 (400–2400)	3210 (1400–4100)
PWM 1/10	23	3100	260	3330			330	4150	1580 (600–2900)	3970 (2500–5900)
PWM 1/100	86	3200	290	2900			300	3800	1170 (500–2100)	3630 (1800–5200)

Results are expressed in ng/ml; mean of two replicates.

In vitro secretion of IgA and IgG (Table 7)

In the supernatant of the patients control cultures there was from 98 to 430 ng/ml IgA; corresponding values were 200–2000 ng/ml in the normal subjects' cultures. The IgG secretion was the same in both groups of subjects. PWM had a slight stimulating effect on the IgA and IgG synthesis in the normal subject cultures, the optimum dilution of the mitogen was 1/10. A similar effect of PWM was observed on the IgG secretion by the patients' lymphocytes. This was contrasting with the inhibition of IgA secretion observed in two patients and the absence of effect in two other cases. In one patient, this inhibitory effect of PWM disappeared when using increasing dilutions of PWM.

B- and T-cell triggering by FCS and PWM (Table 8)

In order to understand the relatively high secretion of Ig in the control cultures and the suppressive effect of PWM on the IgA B cells of some patients, we analysed the T- and B-cell activation induced by

TABLE 8. T and B cells response to FCS, PWM and Con A

Cultures	$\frac{\text{Blasts}}{\text{Total lymphocytes}} \times 100$	$\frac{\text{T blastocytes}}{\text{Total blasts}} \times 100$	$\frac{\text{B blasts}}{\text{Total lymphocytes}} \times 100$
Controls	18±2	42±6	8±2
PWM	40±6	70±9	10±3
Con A	35±8	88±4	4±0·5

FCS and PWM. The control cultures set up with 5% FCS contained 18% blast cells, 40% being T cell-derived as indicated by their ability to form E rosettes. PWM-stimulated cultures contained 40% lymphoblasts and 70% of these were T blasts. Thus there was between three and four times more T blasts in the cultures containing PWM than in the control cultures. On the other hand the plasmacyte content of both these cultures was not very different.

The latter finding explains the high Ig secretion observed in the control cultures and the weak stimulating effect of PWM on this secretion. The main difference between the control and the PWM-stimulated cultures was their different content in activated T cells. This might be related to the suppressive effect of PWM on the IgA B-cell differentiation observed in some of the patients' cultures.

DISCUSSION

No T-cell defect was observed in our patients: they had a normal number of circulating E-RFC and their lymphocyte responses to PHA, Con A and antigen were within the normal range. This was even the case for patient Mo.L. who was in a state of malnutrition known to impair the T-lymphocyte response (Smythe *et al.*, 1971). This data, in keeping with previous studies (Buckley, 1975), does not support the hypothesis of a T-cell deficiency as the main or the unique pathogenic mechanism responsible for the isolated absence of IgA production. This hypothesis is based mainly on animal observations of a selective deficiency in IgA antibody production in nude mice (Good & Wortis, 1971) or in thymic-deprived rodents (Clough *et al.*, 1971; Arnason, St Cyr Cole & Reyveld, 1964; Perey *et al.*, 1970). Indeed, like most patients with selective IgA deficiency, IgA-deficient athymic mice have normal numbers of IgA-bearing B lymphocytes. Further, thymus grafting of these animals results in the appearance of a normal IgA synthesis (Cooper, 1975).

In humans, such a T-cell defect might be involved in the pathogenesis of some cases of isolated IgA deficiency as indicated by the finding in some patients of a reduced number of circulating T cells (Buckley, 1975). However, most of the cases investigated in this study had no T-cell defect. Two patients had an increased number of fluorescent-positive, peroxidase-negative cells, i.e. B cells. We have no explanation for this abnormality which has been previously reported by others (Seligmann, Preud'homme & Brouet, 1973).

Purified anti-immunoglobulin antibodies specific for IgA, IgG and IgM induced a DNA synthesis in the lymphocytes cultures. These observations confirm early reports of Oppenheim, Rogentine & Terry (1969) who suggested from their data that IgA-deficient patients had normal numbers of circulating IgA-bearing lymphocytes. Our recent analysis of the human lymphocyte response to anti-immunoglobulin antibodies indicated that the induced DNA synthesis result from a mixed T and B cells proliferation and that B cells must be present in these cultures in order to obtain a response (Gausset *et al.*, 1976). Thus we interpret the present finding in IgA-deficient patients of a normal response to a-IgA as indicating a normal collaboration between IgA B cells and T lymphocytes.

Our analysis of the B-cell function, in the 7-day cultures, confirm and extend the previous findings of Lawton *et al.* (1973) that the patients lymphocytes are able to transform *in vitro* into actively secreting IgA plasma cells.

It must be pointed out that in our experimental conditions there was a high B-cell stimulation in the control culture as indicated by the high Ig secretion and the large pool of plasma cells observed in these cultures. This B-cell stimulation was related to the presence of FCS selected for its mitogenic property, indeed it was not observed in cultures set up without serum or with human AB serum (data not indicated). Adding PWM to these cultures resulted essentially in a strong T-cell stimulation with only a moderate or weak enhancement of the B-cell differentiation into plasma cells.

In the control cultures of the patients, the IgA B-cell differentiation into IgA-secreting cells was generally lower than the normal subjects. This was contrasting with a comparable IgG secretion in both groups. The above difference between the patients and the normal subjects cannot be regarded as significant owing to the small number of cases. Furthermore, in the control cultures of one normal subject there was a low IgA synthesis; in this case, however, PWM led to a 10 times increase in the IgA

secretion. In the normal subjects, the ratio of IgA plasma cells: total plasma cells was not different in the control and PWM-containing cultures. As PWM moderately increased the pool of Ig-secreting cells, the PWM-stimulated cultures contained more IgA plasma cells and secreted more IgA than the control cultures. A similar finding was observed for the IgG B cells differentiation into IgG-secreting cells. The main finding of the present work was the PWM-induced decrease in the ratio of IgA plasma cells: total plasma cells in three out of four patients. The exception corresponded to a subject who had 0·69 mg/ml IgA when tested. Thus, in most of the patients, the IgA B cells were specifically inhibited or resistant to the stimulatory effect of PWM. A striking suppressive effect of PWM was observed in two cases where the PWM-containing cultures produced much less IgA than the control culture. In two other cases PWM had no significant effect on the IgA plasma cell content of the cultures nor on the IgA secretion. Our interpretation of these findings is that the high T-cell activation induced by PWM in these patients either suppresses or is ineffective for IgA B-cell differentiation. Indeed suppressor T cells have been observed by Waldman *et al.* (1974) in cases of common variable hypogammaglobulinaemia. Alternatively, there might be a primary IgA B-cell anomaly explaining their unresponsiveness or their inhibition in the presence of highly activated T cells.

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