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IgA synthesis by peripheral blood mononuclear cells from normal and selectively IgA deficient subjects

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SUMMARY

IgA secretion and intracellular IgA synthesis by PWM-stimulated peripheral blood lymphocytes from normal and IgA deficient subjects were measured by radioimmunoassay. Cultured lymphocytes from eleven out of twelve IgA deficient subjects had impaired or undetectable IgA production. Measurement of intracellular IgA showed that the defect was more basic than simply defective secretion by IgA plasma cells. Co-culture of lymphocytes from IgA deficient and normal subjects revealed defects in both the B and T cell populations of IgA deficient subjects. In one subject the defect was in the T cells, in another the B cells, and in two others both T and B cells were defective.

INTRODUCTION

Selective IgA deficiency is defined as the absence, or a very low level, of IgA in the serum and external secretions, serum IgG and IgM levels being normal or raised. Various investigators differ in their precise definition of 'very low', (for example, Bachmann, 1965; Koistinen, 1975; Holt, Tandy & Anstee, 1977). In this study subjects were selected with a serum IgA level below 50 μg/ml.

Pokeweed mitogen (PWM) has been used in investigations of the cellular basis of IgA deficiency (Wu, Lawton & Cooper, 1973; Waldmann *et al.*, 1976a; Atwater & Tomasi, 1978). Unlike other commonly used mitogens, PWM is a polyclonal activator of human B cells, as well as T cells, *in vitro*, and consistently induces immunoglobulin production by cultured human lymphocytes (Wu, Lawton & Cooper, 1973; Waldmann *et al.*, 1974; Janossy *et al.*, 1976). Activation of B cells by PWM is subject to T cell help and suppression, and tonsillar and peripheral blood B cells have an absolute requirement for the presence of T cells or T cell products for proliferation and differentiation in response to PWM (Keightley, Cooper & Lawton, 1976; Fauci, Pratt & Whalen, 1976; Janossy *et al.*, 1977; Insel & Merler 1977; Hirano *et al.*, 1977; Gmelig-Meyling, Uyt den Haag & Ballieux, 1977). Previous work on stimulation of lymphocytes in IgA deficiency has suggested that the cellular defect lies in the secretion, rather than the intracellular synthesis, of IgA (Waldmann *et al.*, 1976a) or in the regulatory T cell populations (Waldmann *et al.*, 1976a; Atwater & Tomasi, 1978).

In the studies reported here IgA secretion and intracellular IgA synthesis were measured in PWM stimulated lymphocytes from normal subjects and from twelve subjects with varying degrees of selective IgA deficiency. In addition, B and T cells from normal and deficient subjects were co-cultured. Both B and T cell defects were detected.

MATERIALS AND METHODS

IgA deficient subjects studied. We have found that selective IgA deficient subjects, with serum IgA below 50 μg/ml, can be divided into two main groups on the basis of serum IgA level. The majority have serum IgA below 1 μg/ml and are designated

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Group 1. A smaller group has serum IgA between 10 and 50 μg/ml and is designated Group 2. Subjects with serum IgA between 1 and 10 μg/ml were rare. In this study we investigated six Group 1 subjects and six Group 2 subjects. None of the subjects was receiving immunosuppressive therapy or other specific treatment.

Low IgA subjects studied. Two subjects were investigated with serum IgA above 50 μg/ml but below 850 μg/ml (the lower limit of the normal adult serum IgA range).

Cell separation. 20–50 ml heparinized peripheral venous blood was centrifuged on a Ficoll-Hypaque cushion, density 1·077 (Böyum, 1968). The peripheral blood mononuclear cells (PBMC) thus obtained were washed three times with tissue culture medium RPMI 1640 (Gibco) containing glutamine (2 mM), penicillin (100 ug/ml) and streptomycin (100 μg/ml), referred to hereafter as the 'medium'. Washed unfractionated PBMC were then either set up in culture immediately or separated into T and non-T cell fractions. The latter cell separation was carried out using the sheep red blood cell (SRBC)-T cell rosette method (Janossy *et al.*, 1976; Brown & Greaves, 1974). Briefly, 5×10^7 PBMC were mixed with 5 ml heated absorbed human foetal serum (HFS), (56°C, 30 min), SRBC and 10 ml of a 2% suspension of five times washed SRBC in the medium. The cell suspension was centrifuged at 150 *g*, for 8 min. After 15 min at room temperature the cells were gently resuspended and then spun again. After a further 60 min at room temperature the cells were resuspended. As recommended by Brown & Greaves (1974), the SRBC were less than 1 week old, the PBMC had already been washed three times prior to incubation with the SRBC, and the rosettes were resuspended very gently. The cells were layered onto Ficoll-Hypaque (density 1·077) and centrifuged at 400 *g* for 30 min. The interfacial (non-T) cells were collected and washed three times with the medium. The SRBC-T cell pellet was collected and suspended in 2 ml ice cold NH_4Cl buffer (NH_4Cl 0·83%, $KHCO_3$ 0·1%) for 1–2 min to lyse the SRBC (Keightley *et al.*, 1976). The T cells were washed three times with the medium. On the basis that the separated cell populations failed to respond to PWM with either DNA or IgA synthesis (see Results), we regarded the cells as adequately separated into T and non-T populations. The cells were then used in co-culture experiments, being cultured as recombined autologous or allogeneic cell mixtures (see Results).

Culture system. Triplicate cell cultures were incubated at 37°C, in 5% CO_2 in air, in flat-bottomed plastic vials (Stayne, EIIR), in the medium with 10% HFS. The culture volume was 1 ml. Varying concentrations of cells and PWM (Gibco) were used (see Results).

Measurement of IgA. Low amounts of IgA in sera, culture supernatants, and cell lysates were measured by paper disc radioimmunoassay. The method was a modification of that designed for measuring IgE (Ceska & Lundkvist, 1972). Anti-human α-chain antibodies were purified from rabbit antiserum (Hoechst) using an immunoabsorbent column. Purified antibody was then coupled to 5 mm diameter paper discs (Whatman 540 filter paper) by cyanogen bromide. Approximately 200 μg antibody was coupled to 500 discs. Further antibody was labelled with ^{125}I by a modification of the chloramine T method (Klinman & Taylor, 1969), giving a specific activity of 10 μCi/μg protein. Each disc was incubated with 50 μl incubation buffer (0·05 M phosphate, 0·9% NaCl, 0·3% bovine serum albumin, 0·05% NaN_3, and 0·5% Tween 20, pH 7·9), and 50 μl IgA standard or unknown for 3 hours at room temperature. The discs were washed three times with a saline-Tween 20 solution (0·9% NaCl, 0·1% Tween 20). 100 μl ^{125}I-anti-IgA (5 ng) was then added to each disc and they were incubated overnight at room temperature. The discs were then washed four times with saline-Tween 20.

The assay range was 10–1500 ng IgA/ml. When assaying the culture supernatants, however, the lower limit of the assay range for the secreted IgA was in practice 100 ng/ml because the culture medium contained approximately 300 ng exogenous IgA/ml from the HFS (104 human cord sera were assayed for IgA and the mean IgA content, ± 1 s.d., was $3{\cdot}2 \pm 1{\cdot}6$ μg/ml).

Measurement of intracellular IgA. After removal of supernatant from the cultured cells for the assay of secreted IgA in the individual vials, the cells within each triplicate set were combined in one vial and washed twice with the medium. The washing fluid was decanted and drained from the inverted culture vials after each centrifugation. Each cell pellet was then resuspended in 4 drops 0·5% Triton X-100, incubated for 10 min at room temperature, and spun again at 1500 *g* for 15 min. 50 μl cell lysate was assayed by radioimmunoassay. The volume of each cell lysate was 0·15 ml, and this was from $1{\cdot}5 \times 10^6$ PBMC (the total number of cells in three vials at the start of the culture). The small volume, combination of cells, and removal of serum meant that in effect the limit of the assay for intracellular IgA was 1 ng/0·15 ml/$0{\cdot}5 \times 10^6$ original number PBMC.

RESULTS

Responses of normal PBMC to PWM

Source of serum in culture. Two out of three foetal calf sera were completely inhibitory for PWM-induced IgA secretion. Only two out of sixty-seven human foetal sera were completely inhibitory. Human foetal sera of low IgA content and which supported PWM-induced IgA secretion were pooled, and the pooled foetal serum was thereafter used in the culture experiments.

Optimal conditions. PBMC from nine normal subjects were cultured. In every case, when the culture conditions were optimal or fairly near to it, readily detectable IgA was secreted. The optimal culture conditions for IgA secretion were defined using five normal subjects, each of whom was studied on more than one occasion.

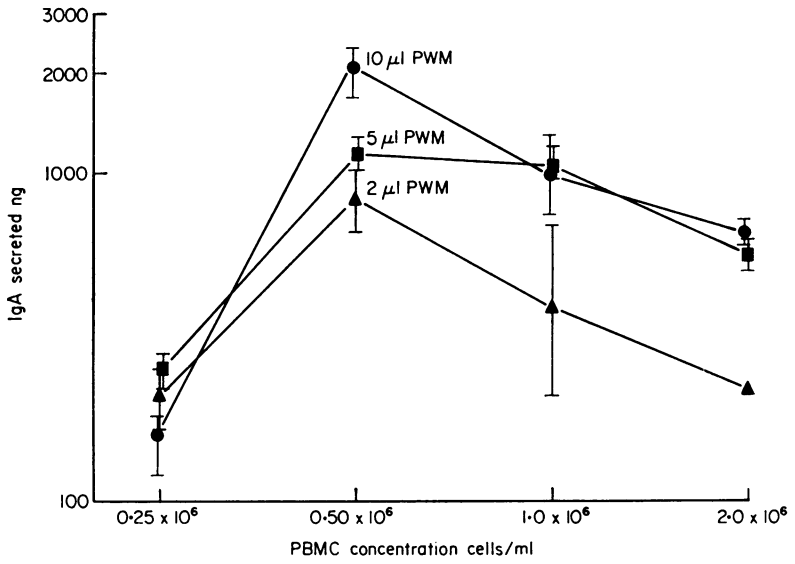

FIG. 1. Influence of cell concentration on the PWM stimulation of normal PBMC. The culture period was 7 days. The points represent mean values of triplicate determinations (± s.d.).

For IgA secretion, the optimal initial cell concentration was $0{\cdot}5 \times 10^6$ PBMC/ml. Results of a typical experiment are shown in Fig. 1, where the optimal cell concentration was the same at three different concentrations of PWM.

At $0{\cdot}5 \times 10^6$ PBMC/ml, the cells responded to PWM between 2 and 200 μl PWM/ml. The best response was in the concentration range 10–50 μl PWM/ml, for all normal subjects tested. A typical dose response curve is shown in Fig. 2.

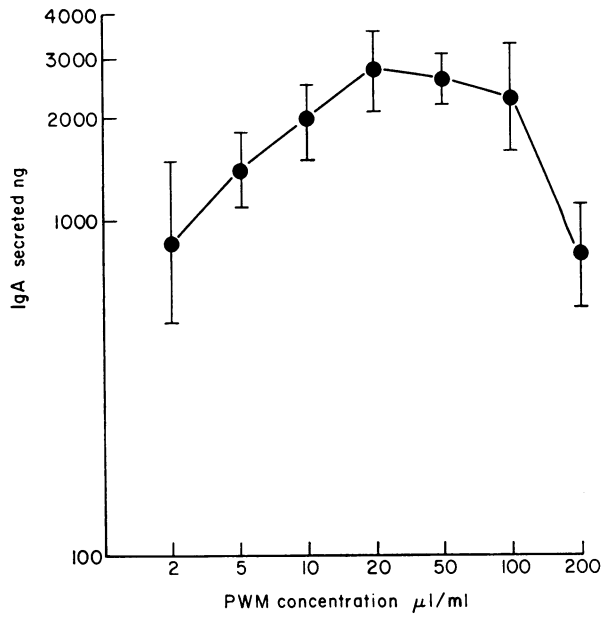

FIG. 2. Influence of mitogen concentration on the PWM stimulation of normal PBMC. The culture period was 7 days and initial cell concentration was $0{\cdot}5 \times 10^6$ PBMC/ml. Points represent mean values of triplicate determinations (± s.d.).

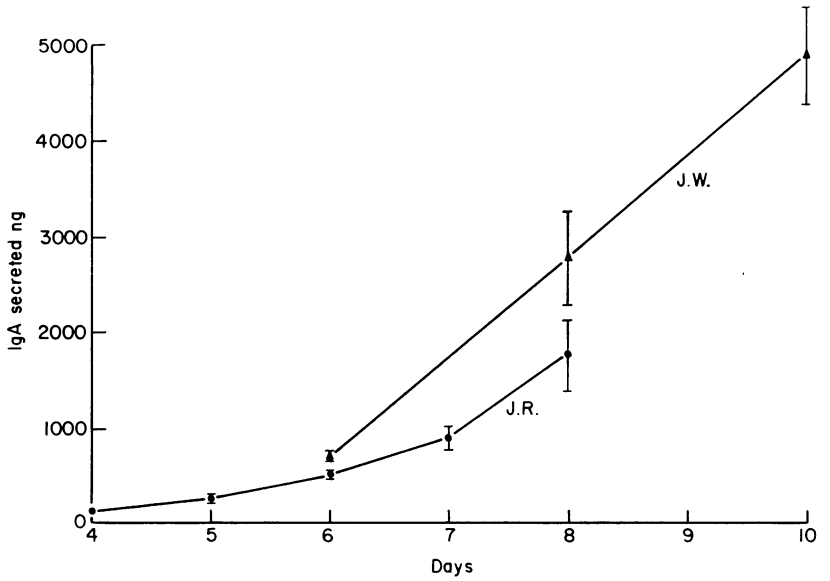

FIG. 3. Influence of culture period on the PWM stimulation of normal PBMC. Total IgA secreted is plotted against length of culture. J.W. was cultured at an initial cell concentration of $0{\cdot}72 \times 10^6$ PBMC/ml, and J.R. at an initial cell concentration of 1×10^6 PBMC/ml. PWM concentration was 10 μl/ml. The points represent mean values of triplicate determinations ($\pm$ s.d.). The various triplicates were set up at the same time, but harvested at different times.

Thus, for five normal subjects with $0{\cdot}5 \times 10^6$ PBMC/ml, 10 μl PWM, and 7 days culture, the mean IgA secretion ($\pm$1 s.d.) was 1920 ($\pm$1270) ng IgA. IgA secretion in the absence of PWM was usually 100 ng or less.

The time course of IgA secretion by PBMC from two normal subjects was studied at near optimal cell concentrations. IgA first appeared in the supernatant between days 4 and 5. The rate of secretion then was fairly steady even up to 10 days culture (Fig. 3).

The optimal cell concentration for DNA synthesis was the same as for IgA secretion, whilst the optimal PWM concentration was slightly lower. DNA synthesis peaked at about day 4, started to fall after day 5, and was virtually the same as background (no PWM) at day 7.

Response of PBMC from IgA deficient subjects to PWM

Six Group 1 subjects (serum IgA below 1 μg/ml), and six Group 2 subjects (serum IgA between 10 and 50 μg/ml) were examined (see Materials and Methods section).

The PBMC were set up in culture at $0{\cdot}25 \times 10^6$ and $0{\cdot}5 \times 10^6$ cells/ml, each cell concentration being tested with 0, 10 μl, and 50 μl PWM/ml. The culture period was 7 days.

Of the twelve subjects, eleven had an impaired or undetectable IgA secretion response to PWM (Table 1). No Group 1 PBMC produced detectable secreted IgA. However, three out of the six Group 2 PBMC produced detectable amounts of secreted IgA (> 100 ng), and one of these three (T.F.) produced normal quantities. In no instance was detectable IgA secreted when PWM was absent.

The results from the three Group 2 PBMC which secreted IgA gave no indication that the optimal conditions for PWM culture might be other than the optimal conditions defined for normal PBMC. T.F. was investigated more extensively. His cells gave normal IgA secretion/PWM concentration, and IgA secretion/cell concentration curves.

Response of PBMC of two subjects with low IgA to PWM

These subjects had lower than normal serum IgA but did not have so low an IgA level as to be defined as IgA deficient.

TABLE 1. IgA secretion by PBMC of IgA deficient subjects cultured with PWM*

	Subject	Serum IgA† (ng/ml)	*In vitro* secreted IgA (ng/ml)
Group 2	T.F.	50×10^3	2300
	M.A.	22×10^3	150
	A.S.	29×10^3	150
	I.K.	24×10^3	< 100
	W.	19×10^3	< 100
	I.C.	17×10^3	< 100
Group 1	H.S.	500	< 100
	L.M.	200	< 100
	E.W.	< 100	< 100
	R.S.	< 100	< 100
	A.H.	< 100	< 100
	G.C.	< 100	< 100

* $0{\cdot}5\times10^6$ PBMC, 10 μl PWM, cultured for 7 days.

† Sera were diluted at least ten times for the radioimmunoassay to eliminate non-specific effects, therefore the lower limit of the assay range for both serum IgA and IgA secreted *in vitro* is 100 ng IgA/ml.

N.C. had normal IgG and IgM serum levels. In December 1976 he had 29 μg IgA/ml serum, but when his PBMC were cultured in October 1977 his serum IgA level had increased to 170 μg/ml (normal adult serum IgA range is 850–4500 μg/ml). His response to PWM was normal ($0{\cdot}5\times10^6$ cells/ml, 50 μl PWM, 7 days culture—1850 ng secreted IgA).

F.D. was intermittently hypogammaglobulinaemic. At the time of culture his serum IgA was 74 μg/ml, and his serum IgG and IgM were in the low normal range. Culture of his PBMC for 7 days with PWM produced no detectable secreted IgA.

Response of PBMC from one normal and one IgA deficient subject to PWM in the presence of hydrocortisone

Published reports suggest that hydrocortisone may reduce suppression of PWM-induced immunoglobulin synthesis *in vitro* (Waldmann *et al.*, 1976b; Fauci, Pratt & Whalen, 1977), and that at least some IgA deficient subjects have excess or overactive suppressor cells (Waldmann *et al.*, 1976a; Atwater & Tomasi, 1978). The effect of hydrocortisone on IgA secretion by PWM-stimulated normal and IgA deficient PBMC was therefore studied.

The IgA deficient subject (I.C.) was in Group 2. Her PBMC did not secrete detectable IgA after PWM stimulation. Hydrocortisone, 10^{-5} M, with or without PWM, had no apparent effect on IgA secretion.

In contrast, PBMC from a normal subject (M.P.) had significantly increased IgA secretion when hydrocortisone was present in addition to PWM. Thus with $0{\cdot}5\times10^6$ cells/ml, 9 days culture, and 10 μl PWM, 5000 ng IgA were secreted, but in the presence of 10^{-5} M hydrocortisone 10,000 ng were secreted. Hydrocortisone without PWM did not cause IgA secretion.

Intracellular IgA synthesis in PWM stimulated PBMC from normal, low IgA, and IgA deficient subjects

Waldmann *et al.* (1976a) have suggested that the majority of IgA deficient subjects have a defect in their IgA plasma cells which prevents secretion of synthesized IgA. Therefore, we measured intracellular IgA in PBMC from IgA deficient, low IgA, and normal subjects after 7 days culture with PWM, and compared this with the amount of IgA secreted by these cells into the culture medium (Table 2).

Intracellular IgA correlated significantly with secreted IgA ($r = +0{\cdot}92$, $P<0{\cdot}01$) for the eight cases

TABLE 2. Intracellular IgA synthesis by PWM stimulated PBMC*

	Subject	Secreted IgA (ng)	Intracellular IgA (ng)
Normal†	M.K.	2200	75
	T.S.	3100	55
	M.P.	3400	70
	J.R.	350	8
	J.W.	1050	18
Low IgA‡	N.C.	1200	17
	F.D.	< 100	1
IgA deficient§	M.A.	150	5
	A.S.	150	2
	I.K.	< 100	2
	W.	< 100	< 1
	I.C.	< 100	< 1
	H.S.	< 100	< 1
	L.M.	< 100	< 1
	E.W.	< 100	< 1
	R.S.	< 100	< 1
	A.H.	< 100	< 1
	G.C.	< 100	< 1

* $0{\cdot}5 \times 10^6$ PBMC, 10 μl PWM, 7 days culture.
† Normal IgA range is 850–4500 μg IgA/ml serum.
‡ Low IgA is defined as 50–850 μg IgA/ml serum.
§ IgA deficient is defined as 0 to 50 μg IgA/ml serum.

where detectable IgA was secreted. In the two cases where intracellular IgA was detectable but secreted IgA was not, the amount of intracellular IgA was very low and the failure to detect secreted IgA is explicable by the lower sensitivity of the assay for secreted IgA because the culture medium contained a certain amount of exogenous IgA from the HFS (see Materials and Methods section). Thus, IgA deficient subjects who do not secrete IgA, also do not have intracellular IgA.

PWM stimulated IgA secretion by allogeneic mixtures of separated lymphocytes from normal and IgA deficient subjects

Several investigators have reported that in experiments using mixtures of lymphocytes from different normal donors, T cells can help allogeneic B cells in PWM-induced immunoglobulin synthesis (Waldmann *et al.*, 1974; Keightley *et al.*, 1976; Hirano *et al.*, 1977; Gomez de la Concha *et al.*, 1977). Accordingly, purified populations of T and non-T cells were prepared from normal and IgA deficient subjects (see Materials and Methods section).

Of the PBMC subjected to incubation with SRBC an average total of 64% were finally recovered. The average ratio of cells in the T fraction to cells in the non-T fraction, for six normal subjects, was 63:37. For six IgA deficient subjects, the average ratio of T to non-T was 66:34. There was therefore no significant difference in the absolute numbers of T cells between normal and IgA deficient subjects.

One experiment was performed with the separated and recombined autologous cell populations from one normal subject (J.W.) to test their proliferative response to PWM. It was found that the response of each separated population was very low compared with the recombined population. That is, the T cells apparently needed non-T cells, and the non-T cells needed T cells, for a normal proliferative response to PWM.

Six experiments were performed to measure IgA secretion in allogeneic mixtures of separated T and non-T cell populations from normal and IgA deficient subjects. Four IgA deficient subjects were investigated, two from Group 1, and two from Group 2.

TABLE 3. IgA secretion by allogeneic co-culture of normal and IgA deficient PBMC stimulated by PWM*

Experiment	Subjects	Cells† non-T	Cells† T	IgA secreted in response to PWM‡ (ng)	Conclusion
1	Normal M.P.	M.P.		< 100	Cross-help
	and		J.W.	< 100	
	normal J.W.	M.P.	M.P.	4800	
		M.P.	J.W.	5000	
2	Normal J.W.	J.W.		< 100	Cross-help
	and		J.W.	< 100	
	normal M.F.	M.F.		< 100	
			M.F.	< 100	
		J.W.	J.W.	280	
		J.W.	M.F.	350	
		M.F.	M.F.	2550	
		M.F.	J.W.	750	
3	Normal J.W.	J.W.		< 100	R.S. has T
	and Group 1		J.W.	< 100	and B cell
	deficient R.S.	R.S.		(n.d.)	defects
			R.S.	(n.d.)	
		J.W.	J.W.	460	
		J.W.	R.S.	< 100	
		R.S.	R.S.	< 100	
		R.S.	J.W.	< 100	
4	Normal J.W.	J.W.		< 100	E.W. has T
	and Group 1		J.W.	< 100	and B cell
	deficient E.W.				defects
		E.W.		(n.d.)	
			E.W.	(n.d.)	
		J.W.	J.W.	220	
		J.W.	E.W.	< 100	
		E.W.	E.W.	< 100	
		E.W.	J.W.	< 100	
5	Normal J.W.	J.W.		< 100	M.A. has T
	and Group 2		J.W.	< 100	cell defect
	deficient M.A.	M.A.		(n.d.)	
			M.A.	(n.d.)	
		J.W.	J.W.	500	
		J.W.	M.A.	100	
		M.A.	M.A.	< 100	
		M.A.	J.W.	220	
6	Normal M.P.	M.P.		< 100	A.S. has B
	and Group 2		M.P.	< 100	cell defect
	deficient A.S.	A.S.		(n.d.)	
			A.S.	(n.d.)	
		M.P.	M.P.	1650	
		M.P.	A.S.	1280	
		A.S.	A.S.	< 100	
		A.S.	M.P.	< 100	

* $0{\cdot}75 \times 10^6$ total PBMC, 10 μl PWM, 8 or 9 days culture.

† Non-T: T ratio was 1:1.

‡ These figures are minus the occasional background response (no PWM).

n.d. = Not done.

A slightly higher than usual total cell concentration ($0{\cdot}75 \times 10^6$ PBMC/ml) was employed in these experiments to compensate for any possible loss of viability during the cell separation procedure. A non-T to T cell ratio of 1:1 was chosen on the basis of one experiment with different ratios of recombined autologous normal cell populations, and previous reports that such a ratio is within the optimal range for PWM-stimulated immunoglobulin production (Janossy *et al.*, 1977; Gmelig-Meyling *et al.*, 1977; Gomez de la Concha *et al.*, 1977). The IgA secretion by recombined autologous normal lymphocytes was not always as great as secretion by unseparated PBMC cultured simultaneously. For example, recombined cells of one normal subject (J.W.) gave 40% of the IgA secretion by unseparated cells. The reason for this difference could not be identified and therefore we employed a longer than usual culture period (8 or 9 days instead of 7).

Purified T cells never secreted detectable IgA. Purified normal non-T cells sometimes secreted a little, but the amount was minimal and was unaffected by the presence or absence of PWM, indicating that it was due to B cells already activated for differentiation before the culture was begun.

The results of the allogeneic co-culture experiments are summarized in Table 3. The results are interpreted as follows:

Experiment 1. Normal M.P. B cells were helped equally well by either autologous M.P. T cells or allogeneic normal J.W. T cells.

Experiment 2. In autologous culture, normal M.F. PBMC responded better than did normal J.W. PBMC, further illustrating the wide range of IgA responses of PBMC from different normal individuals. In allogeneic culture, J.W. B cells were helped equally well by allogeneic M.F. T cells as by autologous T cells. However, M.F. B cells responded better with autologous T cells than with allogeneic J.W. T cells. Nevertheless, the response of the latter combination (M.F. B cells and J.W. T cells) was significant, and represented T cell help since there was no response by M.F. B cells in the absence of T cells.

Experiments 3 and 4. The T cells of the two Group 1 deficient subjects fail to help IgA synthesis by normal B cells. Their B cells are also apparently incapable of making IgA even when normal T cells are present.

Experiment 5. The T cells of Group 2 deficient subject M.A. give only slight help to the IgA synthesis by normal B cells, whereas M.A. B cells are capable of making IgA if normal T cells are present.

Experiment 6. Conversely, the other Group 2 deficient subject A.S. apparently has a B cell defect, but has T cells which give normal help to normal B cells.

DISCUSSION

There have been three main studies on the response of lymphocytes from IgA deficient subjects to PWM. Wu *et al.* (1973) tested subjects who mainly had serum IgA levels of below 30 μg/ml. They reported normal numbers of IgA containing plasma cells and normal IgA secretion after 7 days culture. Waldmann *et al.* (1976a) studied subjects with serum IgA levels below 1 μg/ml. They reported normal numbers of IgA plasma cells after 7 days culture in eleven out of fourteen cases. However, no IgA secretion (<100 ng IgA per 2×10^6 lymphocytes) was found in any of the fourteen cases. On the basis of co-culture experiments with allogeneic mixtures of unseparated peripheral blood leucocytes from normal and IgA deficient subjects, they concluded that the three IgA deficient subjects who did not produce IgA plasma cells possessed IgA specific suppressor cells in their peripheral blood. Atwater & Tomasi (1978) studied subjects with serum IgA levels below 80 μg IgA/ml and found the mean IgA secretion was 106 ng per 10^6 lymphocytes after 7 days, i.e., very low but just detectable. On the basis of a co-culture technique similar to that used by Waldmann *et al.* (1976a) and of other experiments in which normal T cells were added to unseparated lymphocytes from IgA deficient subjects, they concluded that two and possibly three defects exist in IgA deficiency: (i) excess numbers of, or overactive, suppressor cells; (ii) low numbers of, or inactive, helper T cells; (iii) unknown defect.

There appears to be a discrepancy between the results of Wu *et al.* (1973) and other workers in respect of IgA secretion by IgA deficient lymphocytes. This, as Waldmann *et al.* (1976a) have suggested, may

be due to differences in technique. Wu *et al.* (1973) used radioimmuno-electrophoresis to detect secreted IgA, a technique which is not quantitative and which may give false positive results (Hochwald, Thorbecke & Asofsky, 1961). Waldmann *et al.* (1976a) and Atwater & Tomasi (1978) used radioimmunoassay.

In the present study it was found that PBMC from IgA deficient subjects with a serum IgA level below 1 μg/ml would not secrete IgA in response to PWM. Some subjects with a serum IgA level of between 10 and 50 μg/ml would, however, secrete low but detectable IgA, and one subject (T.F.) secreted normal amounts. Apart from T.F., the results on IgA secretion by unseparated PBMC correspond with those of Waldmann *et al.* (1976a) and Atwater & Tomasi (1978). We think that a detectable IgA response to PWM *in vitro* is probably only found among those selective IgA deficient subjects with serum IgA levels in the upper range of IgA deficiency (Group 2, as defined in this report). It is unclear why T.F. produced normal amounts of IgA *in vitro*, but was IgA deficient *in vivo*. The possible role of serum suppressive factors (Nelson & Gatti, 1976) was not examined.

We found that the amount of IgA within cells cultured for 7 days correlated with the amount which had been secreted. Thus if no IgA was secreted, no intracellular IgA was found. This appears to be in contradiction to the findings of Waldmann *et al.* (1976a). Possibly the difference is due to variations in technique. We used a quantitative radioimmunoassay to assay intracellular IgA, whilst Waldmann *et al.* (1976a) used a qualitative immunofluorescence technique. If the latter method was extremely sensitive it might have detected minute quantities of IgA within B cells which had not matured to the normal plasma cell stage and therefore were not yet capable of normal IgA synthesis or IgA secretion.

The defective cells in IgA deficiency were considered to be mainly in the B cell-plasma cell series by Waldmann *et al.* (1976a) although both they and Wu *et al.* (1973) reported normal numbers of circulating IgA bearing lymphocytes. Atwater & Tomasi (1978) refer to reports that the method for measuring IgG and IgA bearing B cells may be faulty, and that even in normal people IgA B cells may be less than 1% of peripheral blood lymphocytes (Winchester *et al.*, 1975; Vitetta *et al.*, 1975; Vitetta & Uhr, 1975).

The nature of the suppressor cell found in some IgA deficient subjects (Waldmann *et al.*, 1976a; Atwater & Tomasi, 1978) was not defined, although the former implied it was a T cell on the basis of finding suppressor T cells in common variable hypogammaglobulinemia (Waldmann *et al.*, 1974, 1976b). Both Waldmann *et al.* (1976b), and Atwater & Tomasi (1978) described one IgA deficient subject with an apparent defect in the helper T cell population.

We sought the cellular defect in four IgA deficient subjects using lymphocytes separated into T and non-T populations. In both subjects with less than 1 μg IgA/ml serum, both T and non-T cell defects were found. In the two subjects with 10–50 μg IgA/ml serum, one had a T cell defect and the other had a non-T cell defect. We presume the defective cell in the non-T population was a B cell, although we did not eliminate the possibility of a defective regulatory cell in the non-T population. In the case of the T cell defects, this co-culture system could not distinguish between lack of help or excess suppression. However, the T cell defects were not associated with lack or excess of absolute numbers of T cells. Indeed, Moretta *et al.* (1977) reported that in most cases of IgA deficiency and common variable hypogammaglobulinaemia, there were normal numbers of IgM binding T cells (helper T cells in the PWM system) and IgG binding T cells (suppressor T cells in the PWM system).

We also tested the effect of hydrocortisone *in vitro* on the lymphocytes from one normal and one IgA deficient subject. Although hydrocortisone (10^{-5} M) in conjunction with PWM increased IgA synthesis by normal lymphocytes, it failed to promote any IgA synthesis by the deficient lymphocytes. McCarthy *et al.* (1978) found a similar result with one IgA deficient subject who had IgA specific suppressor cells. The negative result may be due to either, (i) a primary B cell defect rather than, or in addition to, excess T cell suppression, or (ii) a lack of helper T cells, hydrocortisone being unable to replace helper T cell function (Fauci *et al.*, 1977).

Thus, the defect in IgA deficiency is not always restricted to a single cell population. Those subjects with negligible serum IgA probably either have a more serious defect, or a combination of both T and non-T (probably B) cell defects. The primary cause of IgA deficiency is still unclear. If it is primarily defective B cells, then the defect must be at a stage before normal IgA synthesis begins; otherwise, if secretion but not synthesis were defective, easily detectable IgA would be found intracellularly.

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