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Functional assessment of T and B lymphocytes in patients with selective IgM deficiency

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SUMMARY

Two patients with selective IgM deficiency were studied. Both presented with dermatitis, chronic diarrhoea, recurrent respiratory infections, failure to thrive, elevated serum IgE levels and *in vivo* impairment of antibody production. No phagocytic or complement abnormalities were found. B lymphocytes with surface IgM were present in normal or high percentage in peripheral blood, and produced normal amounts of IgM *in vitro* when co-cultured with normal T cells. Patients' T cells did not show excess suppressor function *in vitro* but had a decreased helper activity for IgM, IgG and IgA production. It is suggested that both patients have an extensive humoral immune deficiency that might be caused by the immunoregulatory T cell defect.

INTRODUCTION

Screening of serum samples from hospital patients gives a high incidence of IgM deficiency, but most can be shown to be secondary (Hobbs, 1975). Primary isolated IgM deficiency is rare (Asherson & Webster, 1980). It may be severe (less than 10% of normal values) or moderate. Patients with the disorder usually present with recurrent infections by both encapsulated bacteria and viruses (Hobbs, Milner & Watt, 1967; Yocum *et al.*, 1976; Asherson & Webster, 1980; Levitt & Cooper, 1980). Autoimmune disorders (Stoelinga, van Munster & Slooff, 1969) or atopic conditions (Hobbs, 1975) are also associated in some cases. While the cellular basis for primary hypogammaglobulinaemia and selective IgA deficiency have been thoroughly investigated (Waldmann *et al.*, 1974, 1976; De la Concha *et al.*, 1977, 1982; Siegal, Siegal & Good, 1978; Platts-Mills *et al.*, 1979), we are only aware of one case of severe isolated IgM deficiency that has been studied in detail (Levitt & Cooper, 1980).

We have investigated the cellular mechanism(s) in two patients with severe IgM deficiency by examining *in vitro* pokeweed mitogen (PWM)-induced Ig synthesis by both peripheral blood mononuclear cells (PBMC) and enriched T and B populations. Our results show that the patients' B cells are able to produce IgM *in vitro* and that the defect may involve T helper cell function.

CASE REPORTS

Case 1

Patient N.S. A 3 year old girl who was the only child of healthy second cousins. The family history

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was negative for immunodeficiency and atopic diseases. At 2 months of age she developed a severe persistent pruritic scaling erythrodermia. A skin biopsy performed at the age of 19 months was diagnosed as erythrodermic psoriasis. From the first year of life she has suffered from recurrent infections of the respiratory tract, otitis and diarrhoea with failure to thrive. Routine blood tests revealed marked eosinophilia (15–20%) and iron deficiency anaemia. The patient is being treated with commercial gammaglobulin (50 mg/Kg every 2 weeks) with marked improvement in the frequency of infections.

Case 2

Patient J.C. A 9 year old male who was the third child of healthy unrelated parents. The family history was negative for immunodeficiency but revealed atopic conditions in several members. At 3 months of age he developed atopic dermatitis. From the 2nd year of life he has suffered from chronic diarrhoea with failure to thrive, recurrent respiratory tract infections, including three well documented pneumonias, chronic sinusitis, furuncles over his trunk and extremities and severe paronychia leading to nail loss. Stool cultures grew *Giardia lamblia*, and *Staphylococcus aureus* and *Pseudomonas aeruginosa* were repeatedly isolated from skin lesions. The white cell count was normal except for a persistent eosinophilia (15–20%). An allergy work-up disclosed a marked sensitization to inhalants and food allergens.

MATERIALS AND METHODS

Serum IgG, IgA and IgM and complement components C3, C4, C5 were measured by radial immunodiffusion, IgE by radioimmunoassay (RIST, Phadebas, Pharmacia). The agglutinating titres to tetanus (Institut Pasteur), *Escherichia coli* (six common serotypes) and *Candida albicans* were detected in serum as previously reported (Webster, Efter & Aherson, 1974; Preisler, Hassenclever & Levitan, 1969). Functional neutrophil assays (NBT reduction, phagocytosis and killing of *C. albicans*, directional and random mobilities) were studied by previously described methods (Fontan *et al.*, 1976).

Cell preparation. Tonsils were obtained from subjects undergoing routine tonsillectomy. Simple cell suspensions were obtained from tonsillar stroma by teasing with forceps in Hank's balanced salt solution (HBSS) as previously described (Janossy *et al.*, 1976). PBMC were obtained from heparinized (10 units/ml) venous blood by centrifugation on Ficoll-Hypaque. T and B cell enriched populations were prepared by rosetting with SRBC as described in detail elsewhere (De la Concha *et al.*, 1977). The B cell preparation obtained from tonsils contained $\leqslant 1\%$ E rosetting cells. The T cell fraction was contaminated by $<2\%$ surface Ig (SImg) positive cells and $<2\%$ monocytes, and when cultured with PWM always failed to produce detectable Ig.

Surface markers. Phagocytic cells were identified in the same cellular preparations employed for surface marker analysis by incubation of PBMC for 1 hr with 0·8 μm latex particles. Spontaneous SRBC rosette formation: 'active' (EA) and rosettes with neuraminidase treated SRBC (En) were carried out by previously described methods (Wybran *et al.*, 1973; Janossy *et al.*, 1976). Staining of SImg was done by direct immunofluorescence (Lobo, Westervelt & Horwitz, 1975) using fluorescein and rhodamine conjugated $F(ab')_2$ fragments of specific antisera to human μ and δ chains respectively (Kent Labs.). Lymhocytes with complement receptors (EAC) were detected using fluorescinated *Salmonella typhi* and fresh human serum as a source of complement (Gelfand *et al.*, 1976). Assays to determine T cells with Fcμ (T_M) or Fcγ receptors (T_G) were performed as described previously (Moretta *et al.*, 1976). Five different monoclonal antibodies directed against membrane antigens were used: OKT3 (Ortho) and T_{101} (Hybritech Inc) are mature T cell markers (Reinherz & Schlossman, 1980; Royston *et al.*, 1980); OKT4 (Ortho) identifies helper/inducer and OKT8 (Ortho) suppressor/cytotoxic T cell subsets (Reinherz & Schlossmann, 1980), and L243 (Becton Dickinson) has specificity for a non-polymorphic determinant of HLA-DR (Lampson & Levy, 1980). Binding of monoclonal antibodies was detected by indirect immunofluorescence using fluorescein conjugated goat anti-mouse IgG (Meloy), with a Leitz microscope equipped with an IV/F epifluorescence condenser.

Culture conditions. Cells from patients and normal donors were cultured in triplicate (0·25 ml per well) in flat bottomed microplates, using 10% fetal serum in RPMI 1640 and 5% CO_2. PBMC were cultured at a cell density of 1×10^6/ml. T and B enriched populations were co-cultured at a cell density of 1×10^6/ml B enriched cells, plus graded amounts ($0{\cdot}002 \times 10^6$/ml up to 1×10^6/ml) of T cells. For Ig production, 4 μl/ml of PWM (GIBCO) was added. Concanavalin A (Con A; 10 μg/ml; Pharmacia) was added in addition to PWM in some cultures of PBCM to activate T suppressor cells. Hydrocortisone (HCO; 1×10^{-6} M; Sigma) was also added to PWM stimulated cultures where indicated.

Measurement of ^{3}H-thymidine uptake. ^{3}H-thymidine (1 μCi; 200 mCi/mmol specific activity; Radiochemical Centre, Amersham) was added to PHA (4 μg/ml), Con A (10 μg/ml), and PWM (4 μl/ml) stimulated cultures at 68 hr. Cultures were harvested 20 hr later and ^{3}H-thymidine incorporation determined by liquid scintillation spectroscopy.

Analysis of Ig synthesis. After 7 days, the concentrations of IgG, IgA and IgM in the supernatants were measured by a double antibody sandwich enzyme-linked immunosorbent assay (Engvall & Perlmann, 1971) as described in detail elsewhere (De la Concha *et al.*, 1982).

RESULTS

Both patients showed IgM values below 4 mg/dl and elevated levels of serum IgE (Table 1). The ability of antibody formation was impaired not only for IgM but also for IgG since no antibodies to *E. coli* were found and there was no secondary response to tetanus toxoid in spite of repeated immunization (Table 1). Both patients had normal values of CH50, C3, C4, C5, NBT reduction, phagocytosis and killing of *C. albicans*, and neutrophil mobility (data not shown). None showed a positive delayed cutaneous hypersensitivity response to all three tested antigens (Candida, PPD and streptokinase). Patient 1 had normal lymphocyte subpopulations (Table 2) and *in vitro* thymidine uptake after mitogen (PHA, Con A, PWM) stimulation. Patient 2 had a high percentage of circulating B lymphocytes ($SIgM^+$) and a low percentage of T lymphocytes as defined by spontaneous rosette formation or binding of monoclonal anti-T cell antibodies. The $OKT4^+$ population (helper/inducer) was also reduced (Table 2), as well as thymidine uptake in response to all three mitogens used (data not shown).

PWM-induced Ig production by PBMC during 7 days in culture was tested twice within 6 months in both patients. Patient 1 showed a very low IgM and IgA and normal IgG production. Patient 2 had no detectable IgM or IgA and very reduced IgG levels. Hydrocortisone did not increase Ig production in patients' cultures as opposed to normals. Suppression obtained by Con A addition to PWM stimulated cultures at day 0 was less marked in the patients' as compared to normal donors. One study from each patient is shown in Table 3.

Table 1. Immunoglobulin levels and antibody titres in patients' sera

	Patient 1	Patient 2
IgG*	680	900
IgA*	125	153
IgM*	<4	<4
IgE†	340	8,000
Isohaemagglutinins	1:8 (B)	1:16 (A)
Forssmann	1:16	1:16
Heterophile agglutinins	0	1:2
Candida albicans	1:10	0
Escherichia coli	0	0
Tetanus toxoid	0	0

* mg/dl; † units/ml.

Table 2. Cell surface characteristics of T and B lymphocytes*

	Patient		
	1	2	Normal range
Ta	16	15	11–34
T_N	69	41	50–87
T_{101}	58	37	55–75
OKT3	79	51	55–80
OKT4	56	32	38–57
OKT8	22	26	17–30
T_M	46	ND	40–75
T_G	23	ND	10–30
SImgM+D	6	11	2–7
SImgM	0	2	0–1
SImgD	3	1	1–4
EAC	9	7	4–12
DR	6	19	5–12

* All values are expressed in percent; † Non-phagocytic cells.
ND = not done.

When the patients' B enriched cells were co-cultured with autologous T enriched lymphocytes at 50/50 proportions, IgM production could not be detected in patient 2 and was very low in patient 1. When these same B enriched cells were co-cultured with normal T cells, production of IgM and IgG reached normal levels (Fig. 1). The T cell fraction from normal donors and patients produced no detectable Ig when cultured alone with PWM.

When graded numbers of normal T cells are added to a fixed amount of B cells in PWM stimulated cultures, Ig production increases until a maximum is reached. Higher normal T cell numbers produce a suppressor effect and Ig production decreases (Fig. 2). However, five times more patients' T cells were needed in co-cultures with tonsillar B cells to get maximum Ig production. With the T cells from patient 2, even these higher T cell numbers were not able to help normal B cells produce the amount of Ig obtained with normal T cell help. Results were similar for all three Igs tested and when repeated 6 months later gave identical results (Fig. 2).

Table 3. Ig production by PBMC

Patient No.	Cultured with								
	PWM			PWM+Con A			PWM+HCO		
	G	A	M	G	A	M	G	A	M
1	2,062	55	102	784	35	<30	2,275	<30	150
2	135	<30	<30	65	<30	<30	75	<30	<30
Normal values (Mean ± s.d.)	2,919 ±2,070	1,028 ±679	3,965 ±2,004	148 ±87	90 ±57	226 ±207	6,932 ±5,421	2,836 ±2,271	7,450 ±4,073

Data are expressed as Ig concentration (ng/ml).

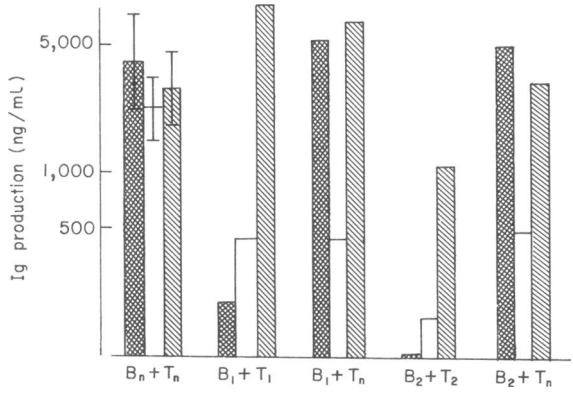

Fig. 1. Ig production by co-cultures of B enriched (1×10^6/ml) and T enriched (1×10^6/ml) cells from normal donors (Bn, Tn) and patients 1 (B_1, T_1) and 2 (B_2+T_2) stimulated with PWM (Ig A = □; IgG = ▩; IgM = ■).

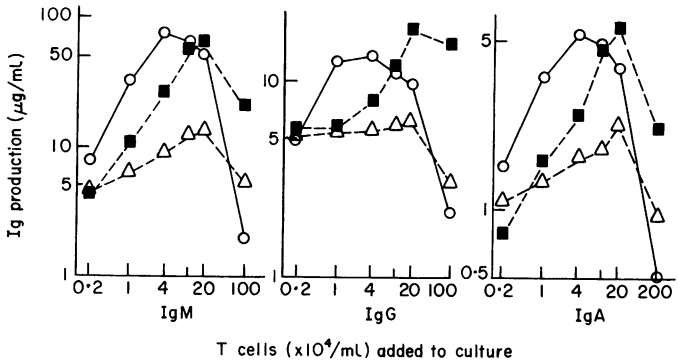

Fig. 2. Production of IgM, IgG and IgA by tonsillar B cells co-cultured with T enriched cells from patient 1 (■), patient 2 (△) or normal donors (○). Cultures were performed with 1×10^6/ml tonsillar B cells with PWM, and graded numbers of T enriched cells.

DISCUSSION

Although some subjects with primary isolated IgM deficiency are symptomless, many of those with a serum IgM of less than 10% of the normal mean present with recurrent debilitating or life threatening infections. The two patients reported here share similar features namely: dermatitis, chronic diarrhoea, recurrent respiratory tract infections, failure to thrive, eosinophilia, elevated serum IgE levels and *in vivo* impairment of antibody production. Of note is that lack of measurable serum antibody to *E. coli*, and the failure to respond to repeated (at least four) injections of tetanus toxoid. Although response to immunization with tetanus toxoid was present in other patients described in the literature, results of antibody stimulation tests revealed an absence of detectable antibody synthesis to several antigens (Stoelinga *et al.*, 1969; Record *et al.*, 1973; Yocum *et al.*, 1976). This and the good response to therapy with gammaglobulin point to a more extensive humoral immune deficiency.

B lymphocytes with SImgM were found in normal or high percentages in the peripheral blood of our patients and were able to differentiate into IgM secreting cells *in vitro*. Similar findings were obtained by Levitt & Cooper (1980) in another patient. This is not surprising in view of the normal serum IgG and IgA levels, as B cells possessing IgM are the direct progenitors of B lymphocytes producing all other Ig classes (Levitt & Cooper, 1980). A T cell defect is the likely cause of the IgM

deficiency as patients' PBMC depleted of T cell produced normal amounts of IgM *in vitro* in response to PWM when co-cultured with normal T cells.

No enhancement of Ig production was seen if HCO was added to PWM-stimulated cultures of patients' PBMC. HCO has been shown to selectively inhibit T cell suppressor function in cultures (Fauci, Pratt & Whalen, 1977). In addition, suppression appeared with lower numbers of normal T cells as compared to patients' T cells when they were co-cultured in graded amounts with normal B cells. This excludes an increased T cell suppressor activity. Tonsillar B lymphocytes produced less Ig when co-cultured with the same number of patients' T cells as compared to normal T cells, and patients B lymphocytes needed normal T cells to produce normal amounts of IgM. Therefore, the most likely explanation for the lack of IgM production is a defect in T cell helper function.

The antibody deficiency in both patients probably involves IgG as well as IgM, and possibly also IgA antibodies. Although we have not studied specific antibody production *in vitro*, it may well be that the decrease in T cell helper function observed in PWM-stimulated cultures is responsible for the antibody deficiency. The existence of regulatory abnormalities has been reported in patients with antibody deficiency and normal serum Ig levels (Saxon *et al.*, 1980). Both our patients had high levels of serum IgE, and this has been previously recorded in selective IgM deficiency (Yocum *et al.*, 1976). We have no data on *in vitro* IgE synthesis by our patients' lymphocytes and we do not know if it is associated with any immunoregulatory abnormality, as regulatory T cells for IgE are known to be isotype specific (Holt, Turner & Holt, 1981).

In conclusion two patients with selective IgM deficiency, sharing similar clinical and laboratory features were studied. Serum antibody deficiency in both, and good response to gammaglobulin therapy in one, point to the presence of an extensive humoral immune deficiency. Functional lymphocyte abnormalities *in vitro* were found to be limited to a decrease in helper T cell activity. B cells were seen to produce normal amounts of IgM when co-cultured with normal T cells. This contrasts with the studies in patients with primary hypogammaglobulinaemia and isolated IgA deficiency where a B lymphocyte differentiation defect appears to be the main cellular abnormality (De la Concha *et al.*, 1977; Cassidy, Oldham & Platts-Mills, 1979).

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