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T cell regulation of thyroglobulin autoantibody IgG subclasses in Hashimoto's thyroiditis

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SUMMARY

Microsomal and thyroglobulin (Tg) antibodies in patients with autoimmune thyroid disease are usually predominantly of subclasses IgG1 and/or IgG4 and the distribution pattern is characteristic for the serum of an individual. We have studied the role of T cells in synthesis of total IgG and Tg antibody IgG subclasses (measured by ELISA) in cultures of peripheral blood lymphocytes (PBL) from Hashimoto patients. Unfractionated PBL incubated with the T dependent activator pokeweed mitogen (PWM) synthesized IgG of all four IgG subclasses in the proportions 69% IgG1, 20% IgG2, 8% IgG3 and 3% IgG4; these values are similar to the proportions of the subclasses in serum. In contrast, the IgG subclass of Tg antibody was predominantly IgG1 in one patient, approximately equal proportions of IgG1 and IgG4 in four patients, and almost completely restricted to IgG4 in one patient; these patterns were similar to the subclass distribution of the autoantibodies in the individual patients' serum. B cells incubated alone secreted little Tg antibody but the response could be restored to the original levels and proportions of IgG1 and/or IgG4 Tg antibody by the addition of T cells either from the same individual or from another donor. Further, removal of suppressor T cells had little effect on the proportions of IgG1 and IgG4 Tg antibody although the total amounts of Tg antibody of both subclasses were sometimes increased. Our studies indicate that T cells are required in this *in vitro* system to elicit Tg antibody synthesis and to control the magnitude of the antibody response. However, the characteristic IgG subclass distribution of Tg antibody in an individual is determined at the level of the B cell.

Keywords Hashimoto's disease IgG subclasses Tg antibody T cell regulation-subclass restriction

INTRODUCTION

Autoantibodies to the thyroid microsomal antigen and thyroglobulin (Tg) in patients with autoimmune thyroid disease are usually of IgG class, and our observation that these autoantibodies are predominantly of subclasses IgG1 and/or IgG4 (Thompson *et al.*, 1983; Parkes *et al.*, 1984) was recently confirmed by Davies *et al.* (1986). We have investigated the contribution made by IgG subclasses to microsomal or Tg antibodies, or both, in Graves' patients undergoing different forms of therapy and in Hashimoto patients before and after pregnancy as well as in the natural course of the disease, over intervals of up to 4·5 years. Despite considerable fluctuations in serum levels of

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thyroid autoantibodies, the relative proportions of the IgG subclasses remained unchanged and this suggests that the IgG subclass distribution of microsomal or Tg antibody is characteristic for an individual patient and may be regarded as a 'fingerprint' of the individual's autoimmune response to a particular autoantigen (McLachlan *et al.*, 1987).

Synthesis of thyroid autoantibodies by cultures of lymphocytes stimulated with the lectin pokeweed mitogen (PWM) is dependent on the activity of T lymphocytes (Beall & Kruger, 1980; McLachlan *et al.*, 1980) and on the basis of animal models of autoimmune thyroiditis (Kromer *et al.*, 1985) it seems likely that autoantibody synthesis *in vivo* in man is also T dependent. Consequently we have studied synthesis of Tg antibody and total IgG by Hashimoto peripheral blood lymphocytes (PBL) cultured with PWM to assess the role of T cells in the activation of B cells secreting Tg antibodies of subclasses IgG1 and IgG4.

MATERIALS AND METHODS

Analysis of IgG class Tg antibody and Tg antibody IgG subclasses. Sera and culture supernatants were analysed in duplicate for the presence of IgG class Tg antibodies by an ELISA technique (McLachlan *et al.*, 1982); the results obtained for optical density readings (OD, 492 nm) have been expressed as an ELISA index as follows:

$$\text{Index} = \frac{\text{OD of test sample}}{\text{OD of a standard Hashimoto serum}}$$

The Hashimoto serum had a haemagglutination titre of 1:5120 and at a dilution of 1:400 gave OD readings of $1{\cdot}32 \pm 0{\cdot}09$ (mean $\pm$ s.e.m. in 56 determinations).

The relative proportions of Tg antibodies of different IgG subclasses was measured by a previously described ELISA technique (Thompson *et al.*, 1983; Parkes *et al.*, 1984) using the following murine monoclonal antibodies specific for human IgG subclasses 1–4: NL16, GOM2, ZG4 & RJ4 respectively (Unipath Ltd, Bedford. UK). The results are presented as the OD 492 nm obtained for IgG subclasses 1–4 and as the percentage contribution made by subclasses 1–4 to the total OD given by all four IgG subclasses.

Analysis of IgG subclasses of total IgG. IgG subclasses of total IgG were measured by an ELISA technique in which monoclonal antibodies to human IgG 1–4 (purified by ion exchange chromatography) were coated to ELISA plates (P. Bird pers. comm.). The monoclonal antibodies were the same as those used to analyse the IgG subclass proportions of Tg antibodies. Duplicate culture supernatants were assayed in a series of doubling dilutions (1:2 to 1:128); with PWM-stimulated cultures it was sometimes necessary to use an initial dilution of up to 1:20 followed by doubling dilutions. Individual IgG subclass concentrations were calculated from a standard curve obtained using human serum previously calibrated for all four IgG subclasses. The results are presented as ng per 5×10^5 cells and as the % contribution made by each IgG subclass to the sum of values obtained for all four IgG subclasses. Measurements of total IgG of all subclasses were also made by another ELISA (Atherton *et al.*, 1985) and these values were significantly correlated with the totals obtained by summing the results for the four IgG subclasses (regression coefficient $r = 0{\cdot}76$, $n = 32$, $P < 0{\cdot}001$).

Patients studied. Peripheral blood (60–120 ml) was obtained from seven Hashimto patients (six women, one man, mean age 54 years, range 49 to 61 years). All Hashimoto patients were euthyroid on thyroxine and six had Tg antibody titres of $> 1:10{,}000$ by tanned red cell haemagglutination tests; one patient (P.G.) had a Tg antibody titre of 1:400. In addition, blood was obtained from a 61-year-old woman with Graves' disease (treated with 160 mg/day propranolol) who had a Tg antibody titre of 1:3200 and from a control donor (a woman aged 20 years) whose serum was negative for thyroid autoantibodies.

The IgG subclass distribution of Tg antibodies present in serum from the patients studied is shown in Table 1. In two patients Tg antibody was predominantly IgG1 (E.B. and P.G.) and in one patient Tg antibody was virtually restricted to IgG4 (C.D.). In the remaining patients Tg antibody

Table 1. IgG subclass distribution of Tg antibody present in serum of 7 Hashimoto patients and one patient (J.S.) with Graves' disease

Patient	% contribution by each IgG subclass to Tg antibody in serum				Tg antibody ELISA index*
	% IgG1	% IgG2	% IgG3	% IgG4	
Predominantly IgG1					
E.B.	73	6	6	14	1·73
P.G.	96	1	0	2	0·27
IgG1, IgG4 & IgG2					
I.P.	33	14	4	51	1·64
M.F.	40	5	1	55	1·64
J.L.	43	7	2	49	1·72
J.W.	24	16	3	57	0·79
J.S.	36	6	5	53	0·55
Predominantly IgG4					
C.D.	10	3	2	85	1·31

Results are given as the % contribution made by each IgG subclass for serum diluted 1:1000 except in the case of patients P.G. and J.S. (who had lower titres of Tg antibody as indicated by the Index) where a dilution of 1:100 was used.

* Serum dilution 1:1000.

was predominantly IgG4 and IgG1 with a smaller contribution from IgG2 and very low levels of IgG3 Tg antibody.

Lymphocyte isolation and culture. Lymphocytes were isolated from the peripheral blood by density gradient centrifugation (Böyum, 1976). Fractions enriched for B cells were obtained by removal of cells forming rosettes with neuraminidase treated sheep erythrocytes; subsequent lysis of the erythrocytes in the rosetting fraction provided the T cell fraction (Weiner, Bianco & Nussenzweig, 1973). Some T cells were treated with mitomycin C (MMC, Sigma Chemical Co., London, UK) to remove suppressor T cells (Siegal & Siegal, 1977). All cell fractions were extensively washed and resuspended in culture medium consisting of RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin (all from Gibco Ltd, Paisley, U.K.).

Replicate cultures (4–6) containing 1 ml samples of unfractionated PBL at 5×10^5 cells/ml were incubated in round-bottomed tubes in the presence or absence of PWM (3 μl/ml, Grand Island, Gibco Ltd, Paisley, UK) for 14 days and in some cases for 7 days. In addition, duplicate or quadruplicate cultures were set up containing B cells alone ($2{\cdot}5 \times 10^5$ cells/ml) or in the presence of an equal number of untreated or MMC treated T cells. After incubation at 37°C in an atmosphere of 5% CO_2, cultures were harvested by pooling duplicate pairs of cultures and centrifuging at 400 ***g*** to separate the supernatant from the pellet. This procedure was adopted to provide sufficent supernatant for analysis of all parameters while ensuring the optimal culture conditions obtained using 5×10^5 cells per tube.

Statistical analysis. The results have been expressed as the mean of duplicates or the mean $\pm$ s.e.m. where three or more values were available. The significance of differences was determined by Student's *t*-test.

RESULTS

The levels of subclasses 1–4 (total IgG) synthesized by PBL and combinations of B+T cells from four Hashimoto patients are given in Table 2. Compared with the small amounts of IgG subclasses in supernatants from unstimulated PBL of three of these patients, PWM induced considerable

Table 2. Synthesis of subclasses of total IgG by cultures of Hashimoto PBL, B+T cells, B+T MMC cells and B cells cultured with PWM for 14 days; data for unstimulated PBL and B+T cells for some patients are also given

			ng synthesized per 10^5 cells				% contribution to total IgG			
	Donor	PWM	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
PBL	I.P.	–	55	116	2	24	16	69	1	14
	J.L.	–	592	760	16	96	40	52	1	7
	J.W.	–	333	124	18	25	67	25	4	5
	Mean ± s.e.m.						41 ± 15	49 ± 13	2 ± 1	9 ± 3
	I.P.	+	4,159	1,152	367	129	71	20	7	2
	J.L.	+	6,125	4,190	770	613	52	36	7	5
	J.W.	+	8,544	1,043	976	406	78	10	9	4
	E.B.	+	16,770	2,960	1,710	480	77	13	8	2
	Mean ± s.e.m.						69 ± 6	20 ± 6	8 ± 1	3 ± 1
B+T	I.P.	+	1,602	1,750	318	43	43	47	9	1
cells	J.L.	+	8,320	1,880	931	310	74	15	9	3
	J.W.	+	43,392	1,640	2,208	1,492	89	3	5	3
	E.B.	+	48,960	3,140	3,150	480	88	6	6	1
	Mean ± s.e.m.						73 ± 11	18 ± 10	7 ± 1	4 ± 1
B+T	I.P.	+	2,389	1,475	591	125	52	33	13	3
MMC	J.L.	+	17,920	7,854	1,494	820	63	29	5	3
cells	J.W.	+	18,538	1,996	1,864	1,684	76	10	8	7
	E.B.	+	64,990	7,950	3,910	570	84	10	5	1
	Mean ± s.e.m.						68 ± 7	21 ± 1	8 ± 2	4 ± 1
B	J.L.	+	540	1,920	144	24	21	73	6	1
cells	E.B.	+	728	706	306	48	41	40	17	3
B+T	E.B.	–	163	606	29	48	18	70	4	8
cells	J.W.	–	247	116	14	144	52	30	3	16

Results are expressed as ng synthesized per 10^5 cells (mean of duplicate cultures) and as the % contribution made to total IgG by each IgG subclass.

increases in all four subclasses, particularly in IgG1 and to a lesser extent in IgG2. The magnitude of the response varied from one individual to another but the relative contributions made by each IgG subclass to total IgG were similar in cultures of the four patients. Higher levels of IgG subclasses were seen in some cultures of B+MMC treated T cells compared with unfractionated PBL (patients E.B. and J.L.) but the proportions of the four subclasses were essentially similar in cultures of PBL, B+T cells and B+T MMC cells. Cultures of B cells with PWM or B+T cells in medium only produced low levels of all four IgG subclasses (comparable with those of unstimulated PBL), indicating that T cells and PWM are required to induce synthesis of all IgG subclasses.

PBL from five Hashimoto patients with high circulating levels of serum antibodies synthesized readily detectable amounts of IgG class Tg antibody when cultured in the presence but not the absence of PWM (Table 3). As anticipated from previous studies (Beall & Kruger, 1980; McLachlan *et al.*, 1980), B cells cultured alone produced only low levels of IgG class Tg antibody but the addition of autologous T cells restored this response to levels comparable with those of unfractionated PBL. Tg antibody synthesis was significantly enhanced in cultures of B+T MMC cells compared with B+T cells in cultures from patients M.F. and J.L. (Table 3).

Synthesis of Tg antibody of subclasses IgG1 and IgG4 by lymphoid suspensions from three Hashimoto patients is illustrated in Fig. 1; the contributions made by IgG2 and IgG3 to Tg antibody in these cultures were negligible and have therefore been omitted. Tg antibody secreted by

Table 3. Synthesis of Tg antibody (all IgG subclasses) by cultures of unfractionated PBL, B cells and B + T cell fractions from five Hashimoto patients.

Lymphoid suspensions	PWM	Tg antibody synthesis (ELISA index) Patient E.B.	Patient M.F.	Patient J.L.	Patient J.W.	Patient I.P.
14 Day culture interval						
Unfractionated PBL	−	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00	0·00 ± 0·00	0·08 ± 0·00
	+	1·88 ± 0·02	1·52 ± 0·19	0·93 ± 0·09	0·85 ± 0·02	1·40 ± 0·02
B cells	+	0·25 ± 0·00*	0·37 ± 0·08*	0·18	ND	ND
B + T cells	+	1·77	1·12 ± 0·04	1·06 ± 0·03	1·20 ± 0·02	1·38 ± 0·07
B + TMMC cells	+	2·22	1·71 ± 0·03†	1·88 ± 0·11†	1·02 ± 0·05	1·34 ± 0·06
7 Day culture interval						
Unfractionated PBL	+	1·29 ± 0·12‡	0·85 ± 0·08‡	0·76 ± 0·09	0·68 ± 0·03‡	1·53 ± 0·02
B + T cells	+	ND	0·76 ± 0·08‡	0·95 ± 0·08‡	0·84 ± 0·03‡	1·42 ± 0·01
B + TMMC cells	+	ND	0·97 ± 0·13‡	1·45 ± 0·07†	0·74 ± 0·03‡	1·24 ± 0·10

Results are given as the mean of duplicate or mean ± s.e.m. of triplicate cultures incubated for 14 or 7 days in the presence of pokeweed mitogen (PWM); data are also given for unfractionated PBL cultured without PWM.
ND, Not Determined.
* Values significantly lower than those obtained for unfractionated PBL, $P < 0.001$.
† Values significantly greater than those obtained for cultures of B + T cells $P < 0.05$.
‡ Values significantly lower than those obtained for 14 day cultures $P < 0.05$.

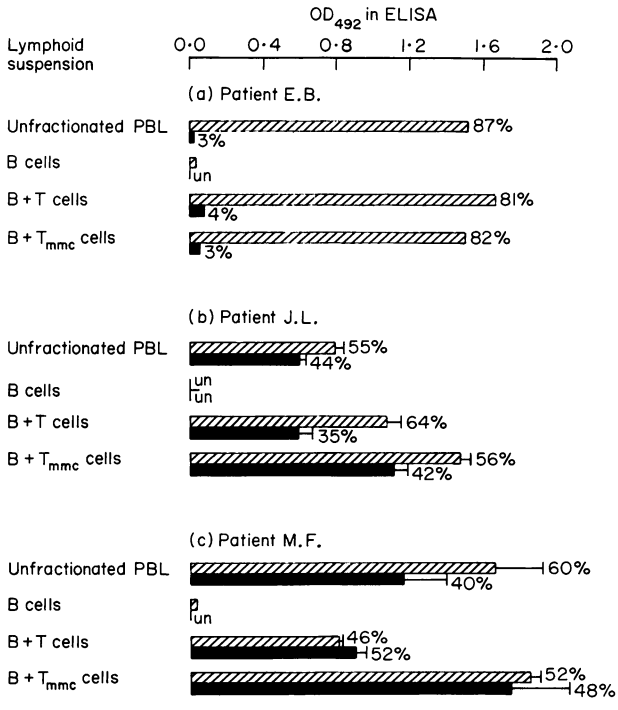

Fig. 1. Synthesis of IgG1 (▨) and IgG4 (■) Tg antibody by PWM stimulated 14 day cultures of unfractionated PBL or combinations of B cells with autologous or mitomycin C (MMC) treated T cells from three Hashimoto patients. The results are given as the mean of duplicate or mean + s.e.m. of triplicate OD 492 readings obtained in the ELISA. The contributions made by subclasses IgG1 and IgG4 expressed as a percentage are also shown; these values do not always add up to 100% because of the small contributions from IgG2 and Ig3. un, undetectable.

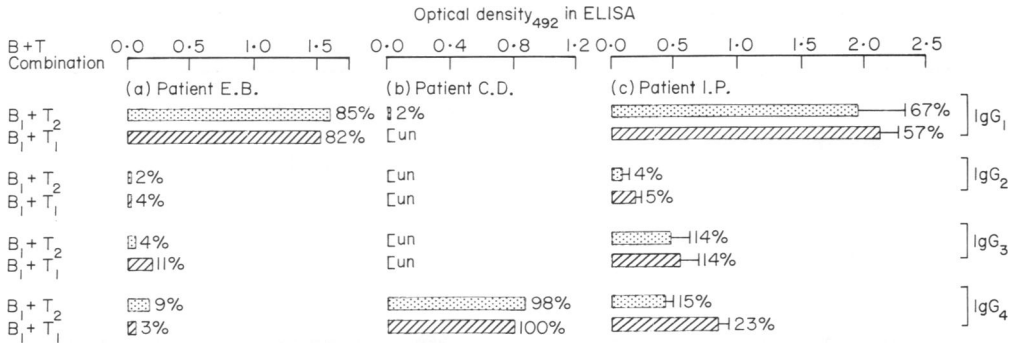

Fig. 2. Synthesis of Tg antibody of all four IgG subclasses in 14 day cultures of Hashimoto B cells incubated in the presence of PWM with MMC treated autologous T cells (B1 + T1) or MMC treated T cells from a different donor (B1 + T2). In the 'cross-over' study, B cells from patients E.B., C.D. and I.P. were cultured with T cells from a normal donor, a Hashimoto patient and a Graves' patient respectively. Results are given as the mean of duplicate or the mean ± s.e.m. of triplicate OD measurements and as the percentage contribution made by each IgG subclass to the sum of the OD values. un, undetectable.

PBL from patient E.B. in response to PWM was almost entirely IgG1; her B cells incubated alone produced very small amounts of IgG1 Tg antibody but the addition of untreated or MMC treated autologous T cells restored Tg antibody synthesis to levels similar to those observed in cultures of unfractionated PBL (Fig. 1a). In contrast, PBL from patients J.L. and M.F. secreted both IgG1 and IgG4 Tg antibody; their B cells alone did not produce detectable amounts of Tg antibody of either subclass but in the presence of autologous T cells, IgG1 and IgG4 Tg antibody was synthesized (Fig. 1b and c).

IgG class Tg antibody synthesis was also investigated in cultures of B cells reconstituted with autologous T cells or with T cells from a different donor. In the case of B cells from Hashimoto patients who had high circulating titres of Tg antibody (E.B., I.P., and C.D., Table 1), Tg antibody synthesis was detectable in cultures of B cells combined with T cells from either source (data not shown). However, Tg antibody synthesis was undetectable in cultures containing B cells from patients J.S. and P.G. who (as shown in Table 1) had lower levels of serum Tg antibody. Further, Tg antibody was undetectable in cultures of normal B cells incubated with either autologous T cells or Hashimoto T cells and these results are in accordance with earlier studies (Beall & Kruger, 1980; McLachlan *et al.*, 1980).

The Tg antibody IgG subclass distribution was studied in cultures of Hashimoto B cells reconstituted with MMC treated autologous T cells or T cells from another donor (Fig. 2). B lymphocytes from patient E.B. secreted Tg antibody which was almost entirely IgG1 whether the B cells were cultured with T cells from a normal donor or with her own T cells (Fig. 2a). In contrast, Tg antibody synthesized by B cells from patient C.D. was virtually restricted to IgG4 (Fig. 2b) in agreement with her serum Tg antibody subclass distribution (Table 1). This subclass pattern was observed both with autologous T cells and with T cells from Hashimoto patient P.G. whose Tg antibody was 96% IgG1. B cells from patient I.P. cultured with autologous T cells produced Tg antibody which was mainly IgG1 with smaller amounts of IgG4 and IgG3 and low levels of IgG2; similar values were obtained for her B cells cultured with MMC treated T cells from Graves' patient J.S. (Fig. 2c).

Kinetic studies of IgG class Tg antibody synthesis showed that higher levels of Tg antibody tended to be synthesized after 14 days than after 7 days incubation; this difference was statistically significant for cultures from patients M.F., J.L. and J.W. (Table 3). Further, increased levels of Tg antibody of subclasses IgG1 and IgG4 were detected after 14 days compared with 7 days culture as shown for lymphocytes from patient J.W. in Fig. 3a. In the same supernatants, increased values for all four total IgG subclasses were observed after the longer culture interval (Fig. 3b). Similar observations were made for total IgG subclasses in culture supernatants from a normal donor as

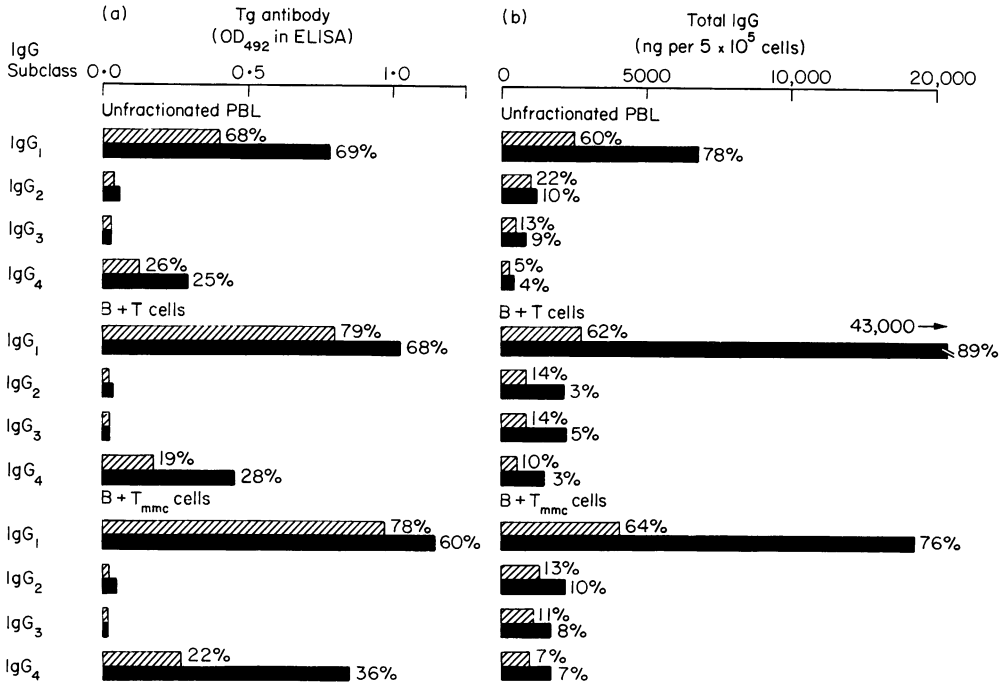

Fig. 3. Comparison of (a) Tg antibody IgG subclasses and (b) total IgG subclasses synthesized by lymphocytes from Hashimoto patient J.W. cultured for 7 days (▨) or 14 days (■). Results are given as the mean of duplicate supernatants for unfractionated PBL, B+T cells and B+T MMC cells. The % contribution made by each IgG subclass to total IgG, as well as the % contribution of the major Tg antibody IgG subclasses, is also shown.

well as for increased IgG1 and IgG4 Tg antibody in cultures from patients M.F., J.L. and I.P. (data not shown).

In general the IgG subclass distribution of Tg antibody synthesized in culture was consistent with the pattern observed for Tg antibody present in serum. However, most cultures showed evidence of 'skewing' towards IgG1 and away from IgG4 and IgG2. For example, Tg antibody secreted by lymphocytes from patients I.P. and J.W. had a considerably higher proportion of IgG1 and lower proportions of IgG4 and particularly IgG2 (Fig. 2c and Fig. 3) than might have been expected from their serum Tg antibody subclass distribution (Table 1). This 'skewing' was also seen in the mean values obtained for Tg antibody synthesized in culture by PBL from the four Hashimoto patients whose Tg antibody was predominantly IgG1 and IgG4: $59 \pm 4\%$ compared with $35 \pm 4\%$ IgG1 Tg antibody in culture and serum respectively ($P < 0{\cdot}05$) and $36 \pm 4\%$ compared with $53 \pm 2\%$ IgG4 Tg antibody in culture and serum respectively ($P < 0{\cdot}05$).

DISCUSSION

Hashimoto PBL cultured with PWM synthesized Tg antibodies which were predominantly IgG1 and/or IgG4 in accordance with the IgG subclass distribution of Tg antibodies present in the patient's serum and these observations are in agreement with our earlier studies (Thompson *et al.*, 1983). In contrast, the proportions of total IgG subclasses stimulated by PWM in cultures of Hashimoto PBL were 69% IgG1, 20% IgG2, 8% IgG3 and 3% IgG4. These results are similar to those obtained by others for PWM-stimulated PBL from normal individuals and they reflect the proportions of IgG subclasses in serum (Mayumi *et al.*, 1983; Ferrante *et al.*, 1986). Therefore the paucity of Tg antibodies of subclasses IgG2 and IgG3 cannot be attributed to the inability of

the lymphocytes to secrete these subclasses *in vitro* in response to PWM. However, the 'skewing' of Tg antibody subclasses towards IgG1 and away from IgG4 and IgG2 could be associated with the effects of PWM as observed by Mayumi *et al.* (1983) for total IgG subclass synthesis.

Our studies of Tg antibody synthesis by lymphocytes separated into fractions depleted of T cells demonstrated that T cells are required to induce synthesis of both IgG1 and IgG4 Tg antibody by Hashimoto B cells cultured with PWM. These findings differ from the conclusions reached by Davies *et al.* (1986) using PWM-stimulated synthesis of total IgG as a model system for thyroid autoantibody synthesis. In their studies, secretion of IgG1 (but not IgG2, 3 or 4) was detectable and synthesis of this subclass was under T cell control. Since production of total IgG4 did not appear to be regulated by T cells, Davies *et al.* (1986) suggested that IgG4 thyroid autoantibodies might also be independent of T cell control. However, in our system, IgG4 Tg antibody was detectable in culture supernatants from patients with serum Tg antibodies of this subclass; further, total IgG2, IgG3 and IgG4 were also detectable (agreeing with the findings of other workers as already discussed) and PWM-induced synthesis of all four IgG subclasses appeared to be T cell dependent. The discrepancy between our observations and those of Davies and co-workers (1986) may be related to the difference in assay techniques for total IgG subclasses. In addition, the difference may have arisen because of the shorter time interval (7 days) employed by Davies *et al.* (1986) since our investigations indicate that increased amounts of total Tg antibody and IgG1 and IgG4 Tg antibody, as well as the levels of total IgG of all four subclasses, were synthesized after 14 days compared with 7 days.

The factors involved in IgG subclass restriction are not fully understood. Antibodies to protein antigens are often predominantly of subclasses IgG1 and IgG4 whereas antibodies to polysaccharides are usually IgG2 (Barrett & Ayoub, 1986). This suggests a role for antigen in subclass restriction which may involve selective recombinations between the genes coding for the variable regions and the constant regions of the immunoglobulin heavy chains. Studies in mice have shown that subclass 'switching' is regulated by factors derived from T cells, for example BCDF γ which induces a switch from antibodies of subclass IgG3 to IgG1 in spleen B cells stimulated with lipopolysaccharide (Isakson *et al.*, 1982). In the case of IgA and IgE, regulation by different subsets of T cells has been demonstrated; for example, Tα cells from peripheral blood preferentially induce synthesis of IgA rather than IgG by human B cells (Endoh *et al.*, 1981). However, such clear cut control mechanisms involving T cells or T cell factors have not yet been shown to regulate IgG subclass secretion in man.

In the present study using PWM, no obvious effects of T cells on the contributions made by IgG subclasses to total IgG or Tg antibody were observed. For example, T cells from other individuals provided comparable T cell help for Tg antibody synthesis of subclasses IgG1 and IgG4, even when the T cells were obtained from an individual with a totally different serum Tg antibody IgG subclass distribution. Further, co-cultures of B cells with T cells pre-treated with mitomycin C to prevent the development of suppressor T cells sometimes secreted increased amounts of IgG1 and IgG4 Tg antibody although the percentage contribution made by these subclasses to Tg antibody was comparable with that secreted by cultures containing untreated T cells. Similarly, the proportions of total IgG subclasses synthesized by Hashimoto PBL were comparable in cultures of unfractionated PBL and B cells reconstituted with untreated or MMC treated T cells (in agreement with studies by Mayumi *et al.*, 1983). It might be argued that these results would be anticipated since PWM is likely to elicit responses only in the B memory cell population. However, we have shown elsewhere that the contributions made by IgG subclasses to thyroid microsomal or Tg antibodies, or both, secreted spontaneously by Hashimoto thyroid lymphocytes (a population which is activated *in vivo*) were unaffected in the presence of concentrations of PWM which significantly inhibited this spontaneous autoantibody synthesis (McLachlan *et al.*, 1985). It might have been preferable to use autoantigen (Tg) rather than PWM to stimulate Tg antibody synthesis. However, stimulation with Tg occurs only intermittently in cultures of PBL (McLachlan *et al.*, 1983) even using macrophages or dendritic cells pulsed with Tg (Weiss, De Bernardo & Davies, 1982; Farrant *et al.*, 1986).

In conclusion, our studies indicate that T cells are required *in vitro* to induce Tg antibody synthesis and to regulate autoantibody levels by PWM-stimulated B cells, the IgG subclass

distribution of Tg antibody (which is characteristic for an individual Hashimoto patient) is determined at the level of the B cell.

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