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Severe combined immunodeficient (SCID) mice: a model for investigating human thyroid autoantibody synthesis

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SUMMARY

We have studied the ability of lymphocytes from the blood, thyroid and lymph nodes of patients with autoimmune thyroid disease (AITD) to produce autoantibodies to thyroglobulin (Tg) and/or thyroid peroxidase (TPO) in SCID mice. Human IgG class Tg and/or TPO antibodies were detectable in plasma from SCID mice 7 days after transfer of 15–25 $\times 10^6$ cells/mouse and the highest levels were recorded 2–3 weeks later. In contrast, Tg and/or TPO antibodies were undetectable in recipients of lymphocytes from thyroid antibody negative controls. AITD thyroid lymphocytes produced the most antibody in recipient mice and lower levels were observed in recipients of AITD blood and lymph node lymphocytes. The amounts of Tg and/or TPO antibody detected were in accordance with the ability of thyroid and lymph node lymphocytes to secrete these autoantibodies spontaneously in culture (indicating the presence of cells activated in the patient) and with the capacity of blood lymphocytes (probably B memory cells) to secrete Tg and/or TPO antibodies in culture in response to pokeweed mitogen. Tg antibodies in plasma from SCID recipients of thyroid lymphocytes were of subclasses IgG1, IgG2 and IgG4 and the proportions closely resembled those of the donor's serum Tg antibodies. Blood lymphocytes transferred to SCID recipients were also able to produce Tg antibodies of subclasses 1, 2 and 4 but the subclass distribution varied between mice and the reason for this is not clear at present. Since SCID mice provide an environment in which B lymphocytes from patients with AITD can be activated without mitogen to secrete thyroid antibodies, this model will provide a powerful system for elucidating the mechanisms regulating the secretion of human antibodies to Tg and TPO.

Keywords B cell differentiation SCID mice thyroglobulin antibodies thyroid peroxidase antibodies IgG subclasses

INTRODUCTION

SCID mice have an autosomal dominant recessive defect that impairs the rearrangement of antigen receptor genes in both T and B lymphocyte progenitors (Bosma, Custer & Bosma, 1983; Schuler *et al.*, 1986) and they lack functional T and B cells. Because the mice are immunodeficient, human lymphoid cells adoptively transferred to these mice can survive and respond to exogenous recall antigens such as tetanus toxoid (Mosier *et al.*, 1988). In addition, recent studies have demonstrated the production of human IgM and/or IgG class mitochondrial antibodies, anti-nuclear antibodies or rheumatoid factor in SCID mice that had received lymphocytes from patients with primary biliary cirrhosis, systemic lupus erythematosus (SLE) or rheumatoid arthritis, respectively (Krams, Dorshkind & Gershwin, 1989; Duchosal *et al.*, 1990; Tighe *et al.*, 1990).

Autoimmune thyroid disease (AITD) in humans is associated with IgG class autoantibody responses to thyroid antigens including thyroglobulin, microsomal antigen/thyroid peroxidase (TPO), and the thyroid-stimulating hormone (TSH) receptor (reviewed by Rees Smith, McLachlan & Furmaniak, 1988). Lymphocytes from patients who have serum antibodies to thyroglobulin (Tg), TPO and/or the TSH receptor (but not from serum antibody-negative patients or control donors), synthesize autoantibodies to these three thyroid autoantigens in culture (Rees Smith *et al.*, 1988). Further, secretion of thyroid autoantibodies spontaneously or in response to mitogen reflects the activation status of lymphocytes from the thyroid, lymph nodes or peripheral blood (Atherton *et al.*, 1985).

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We have investigated the ability of lymphoid cells from the blood, thyroid and lymph nodes of patients with AITD to secrete antibodies to Tg and TPO in SCID mice. To provide information on the nature of the B cells secreting thyroid antibodies, the levels of Tg and/or TPO antibody produced by human lymphoid cells in SCID mice were compared with the ability of the lymphoid cells to secrete thyroid autoantibodies in culture. The distribution of Tg and TPO antibodies among the IgG subclasses provides a stable, characteristic 'fingerprint' for each individual AITD patient (McLachlan *et al.*, 1987). Therefore, the IgG subclass distribution of Tg antibodies produced in SCID mice and secreted in culture was analysed, to assess the extent to which these antibodies reflected the subclass patterns present in serum of the donor patients.

MATERIALS AND METHODS

Patients

Lymphoid suspensions were obtained from six patients with AITD, defined on the basis of the presence of high levels of autoantibodies to Tg and/or TPO (detectable by ELISA at dilutions of $\geqslant 1/1000$) (McLachlan *et al.*, 1982; Schardt *et al.*, 1982). In addition, lymphoid suspensions were obtained from two control donors, without detectable serum antibodies to Tg and/or TPO. In the AITD group, three women (patients I, III and V) and one man (patient IV) had Hashimoto's thyroiditis and were euthyroid on thyroxine; one patient with Hashimoto's thyroiditis (patient IV) had disseminated non-Hodgkin's lymphoma and was being treated with chlorambucil. Patient II (a woman) had recently been diagnosed as having Graves' disease and had been taking carbimazole (20 mg twice/day) for 4 weeks at the time of study. Patient VI was treated by thyroidectomy for an enlarged thyroid gland; histology showed that she had a non-toxic multi-nodular goitre with focal thyroiditis. On the basis of her thyroiditis and serum autoantibodies to Tg and TPO, she was included in our group of patients with AITD.

Isolation of lymphoid cells

Peripheral blood mononuclear cells (PBMC) were isolated from five patients with AITD and two control donors by density gradient centrifugation (Böyum, 1976) on Lymphoprep (Nycomed, Birmingham, UK). Lymph node lymphocytes were isolated from lymph nodes draining the thyroid gland of patient VI by mechanical disaggregation of the tissue (Atherton *et al.*, 1985). Thyroid lymphocytes were isolated from thyroid tissue of patient VI as previously described (McLachlan *et al.*, 1986) by digestion of the tissue with dispase (Boehringer-Mannheim, Mannheim, Germany), followed by incubation of the cell suspension for 18 h on tissue culture treated Petri dishes to allow thyroid cells to adhere. The non-adherent cell fraction, enriched in lymphoid cells, was then removed and centrifuged over Lymphoprep to remove debris. Cell viability, assessed using trypan blue dye exclusion (Boyse, Old & Chouroulinkov, 1964) was >95% for blood lymphocytes and >85% for lymph node and thyroid lymphocytes.

Lymphoid cells for *in vitro* studies were resuspended in RPMI 1640 containing 10% fetal calf serum supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 0·25 μg/l fungizone (all from GIBCO Life Technologies, Glasgow, UK). In some studies, suspensions were enriched for B cells by one cycle of T cell depletion using neuraminidase-treated sheep erythrocytes; such suspensions contain similar proportions of B cells (about 50%) and monocytes (about 40%) as well as some T cells (about 10%) (McLachlan *et al.*, 1983a). The T cell fractions were subsequently incubated with mitomycin C (MMC, Sigma Chemical Co., Poole, UK; 30 μg/ml at 37°C for 40 min, followed by three washes).

Lymphoid cell cultures

Lymphoid suspensions from blood, thyroid and lymph nodes were incubated at 5×10^5 cells/ml (unfractionated lymphocytes or combinations of MMC-treated T cells and B cell enriched fractions) in duplicate 1-ml aliquots in round-bottomed tubes in medium alone or in the presence of pokeweed mitogen (PWM; GIBCO Life Technologies; 0·3 or 3·0 μl/ml). In experiments investigating the effects of excess T cell help, the ratio of MMC-treated T cells (10×10^5 cells/ml) and B cell enriched fractions ($2{\cdot}5 \times 10^5$ cells/ml) ensured that the number of B cells/ml was comparable with that in unfractionated lymphoid suspensions. After incubation for 14 days at 37°C in a 5% CO_2 incubator, the culture supernatants were harvested by centrifugation at 400 ***g*** and analysed immediately or after storage at −20°C. In addition to cultures of living cells, duplicate aliquots were frozen and thawed three times to lyse the cells before culture. Supernatants from the cultures of lysed cells were used to measure the levels of thyroid autoantibodies released from (rather than synthesized by) lymphoblastoid or plasma cells present in the cell suspensions.

Lymphocyte transfer to SCID mice

SCID mice (Bosma *et al.*, 1983) were bred and maintained in isolators. Blood samples from the tail vein were used to obtain plasma which was screened for murine immunoglobulins by ELISA (described below). Mice aged 3–6 months, with murine IgG and IgM levels < 50 μg/ml, were injected intraperitoneally with human lymphoid suspensions ($15–25 \times 10^6$ cells in 300 μl RPMI/mouse). Blood samples were taken from the tail vein shortly before transfer of lymphoid cells and at weekly intervals thereafter, and the plasma separated and stored at −20°C.

Assays for autoantibodies to TPO and Tg

Autoantibodies to Tg and TPO in plasma from SCID mice were detected using highly sensitive assays (Beever *et al.*, 1989) that depend on the direct interaction between the antibodies and ^{125}I-labelled purified Tg or TPO. Reagents for the assays were obtained from RSR (Cardiff, UK). Briefly, plasma samples were diluted 1/20 in assay buffer (150 mM NaCl, 10 mM Tris-HCl, pH 7·5) and duplicate 50-μl aliquots incubated with 50 μl of ^{125}I-Tg for 18 h or with ^{125}I–TPO for 2 h. Subsequently, 50 μl of a 10% suspension of *Staphylococcus aureus* cells were added, and after further incubation for 1 h, 1 ml of assay buffer was added, and antibody-bound and free antigen separated by centrifugation (1500 ***g***, 40 min, 4°C). Assay results were expressed as U/ml of MRC research standard anti-Tg (65/93) and anti-microsome reference preparation 66/387 (National Institute of Biological Standards and Control, Hertfordshire, UK). In some experiments, because of the high mouse plasma levels of antibodies to Tg and/or TPO, it was necessary to dilute SCID plasma 1/200 prior to assay.

Synthesis of Tg antibody and TPO antibody in culture was determined (as in our previous studies) in undiluted culture supernatants by ELISA (McLachlan *et al.*, 1982; Schardt *et al.*,

1982) using plates coated with Tg or with thyroid microsomal antigen, the essential constituent of which is TPO. The results were expressed as an ELISA index, defined as the ratio of the optical density (OD) at 492 nm of the test sample to the OD of a standard Hashimoto's thyroiditis serum diluted to give OD values in both assays of approximately 1·0. Because of the limited amount of culture supernatant available, it was not possible to measure antibodies to Tg and/or TPO by direct assay as well as by ELISA.

The highly sensitive direct assay (rather than ELISA) was used to detect Tg and/or TPO autoantibodies in plasma from SCID mice, since preliminary investigations of these autoantibodies by ELISA gave inconclusive results. However, thyroid antibodies were measurable by ELISA (in subclass assays) in plasma from SCID mice which developed > 3·0 U/ml Tg and/or TPO antibody.

IgG subclass distribution of Tg antibody

The IgG subclass distribution of Tg antibody was determined by ELISA (Thompson *et al.*, 1983) using murine monoclonal antibodies to human IgG subclasses 1, 2, 3 and 4 (clones HP6012, HP6014, HP6010 and HP6011 respectively; Oxoid, Basingstoke, UK) and the results expressed as the OD at 492 nm. Internal standards in these assays included human monoclonal Tg antibody of subclasses IgG2 and IgG4 (Fukuma *et al.*, 1989) and a Hashimoto's thyroiditis serum in which Tg antibody was predominantly IgG1. In the subclass assays, culture supernatants were analysed neat; plasma from SCID mice at a dilution of 1/40 and patients' sera at dilutions of 1/100, 1/1000 and 1/10 000. Samples of lymphocyte culture supernatants and SCID mouse plasma involving the same patient's cells were analysed at the same time together with the patient's serum sample. When possible, the same ELISA plate was also used.

Reports from other laboratories of experiments on depletion of individual IgG subclasses have suggested that our approach to the analysis of thyroid autoantibody IgG subclasses may overestimate the contribution made by IgG4 and underestimate the contribution from IgG2 (Weetman *et al.*, 1989). However, the protocol we describe here enables us to establish clearly the presence or absence of a particular subclass and provides a measure of the relative contributions made by the different subclasses to Tg antibody.

Analysis of human and murine IgG and IgM in SCID mouse plasma

The levels of human IgG in SCID mouse plasma was determined by ELISA using reagents from Sigma. Briefly, ELISA plates coated with goat anti-human IgG were exposed to plasma samples diluted from 1/50 to 1/60 000. After washing, alkaline-phosphatase-conjugated goat anti-human IgG was added, the plates were washed again, substrate (disodium *p*-nitrophenyl phosphate) added and the colour read at 405 nm. IgG levels were determined by comparison with a standard curve for human IgG (Serotec, Oxford, UK). A similar technique was used to determine the levels of murine immunoglobulins. In these experiments, goat anti-mouse IgG or anti-mouse IgM (Sigma) was used to coat the plates, mouse IgG or IgM was then detected using goat anti-mouse immunoglobulin conjugated to alkaline phosphatase (Sigma). Immunoglobulin levels were quantified by reference to a standard mouse serum (Serotec).

Presentation of data

Results are given as the mean of duplicates or the mean ± s.e.m. (for three or more mice). In order to illustrate the variability in plasma antibody levels, data in some studies are shown for individual mice to which lymphocytes from the same donor were transferred.

RESULTS

Tg and/or TPO antibody production by PBMC from AITD patients

PBMC from five patients with AITD were injected into groups of SCID mice and the plasma levels of Tg and TPO antibodies and human IgG in the mice were monitored at weekly intervals. The data for all five patients are summarized in Table 1 and the results obtained using PBMC from one patient are illustrated in detail in Fig. 1. In six out of seven recipients of PBMC from patient I, Tg antibody was detectable after 7 days, maximum Tg antibody levels were attained between 2 and 4 weeks after transfer and thereafter the levels declined (Fig. 1a). Although there was considerable variability between mice injected with 15×10^6 or 25×10^6 PBMC, the highest values were observed in a mouse that received the higher dose of cells. TPO antibody was detectable in five out of seven mice and the kinetics resembled those for Tg antibody (Fig. 1b). The levels of human IgG rose rapidly in six out of seven mice and maximum values detected were seen about 4–5 weeks after transfer (Fig. 1c). In the mouse that failed to develop detectable levels of antibodies to Tg or TPO, total IgG was first measurable 4 weeks after cell transfer.

Similar results for Tg and/or TPO antibody were obtained for SCID mice injected with PBMC from patients II and IV (Table 1). PBMC from patient III only produced Tg antibody in one out of six mice and PBMC from patient V did not secrete detectable levels of Tg or TPO antibody in four SCID mice (Table 1). In experiments in which thyroid autoantibodies were detectable, higher relative levels of Tg antibody than TPO antibody were detected in SCID plasma (as shown for patient I in Fig. 1 and for patient II in Table 1). This was consistent with the ELISA data on the donors' sera which showed that Tg antibody could be detected at greater serum dilution ($1/10^5$) than TPO antibody ($1/10^4$).

Tg and/or TPO antibody and human IgG production by normal PBMC

SCID mice were injected with PBMC from two thyroid autoantibody-negative normal donors (A and B). Substantial levels of human IgG were detectable in SCID plasma obtained 8 weeks after transfer of cells from donor A to six mice, 6·2 ± 1·4 mg/ml (mean ± s.e.m.). In SCID mice that received cells from donor B, much lower but still measurable levels of human IgG were observed 7 weeks after transfer (3·0 ± 0·9 μg/ml; $n = 3$ mice). In both groups of mice antibodies to Tg and TPO were below the limits of detectability (< 0·4 U/ml for both assays).

Tg and/or TPO antibody production by AITD lymph node lymphocytes and thyroid lymphocytes

Levels of Tg antibody, TPO antibody and human IgG were monitored in SCID mice that received thyroid or lymph node lymphocytes from AITD patient VI (illustrated in Fig. 2 and summarized in Table 1). Tg antibody (Fig. 2a) and TPO antibody (Fig. 2b) rose rapidly and reached higher levels in

Table 1. Tg antibody and TPO antibody produced in SCID mice and secreted in culture by PBMC, lymph node lymphocytes or thyroid lymphocytes from patients with Hashimoto's thyroiditis or with Graves' disease (patient II)

Patient	*n*	Cells/mouse	IgG (mg/ml) in SCID plasma at week +3	Tg or TPO antibody determined	Tg/TPO antibody in SCID plasma (U/ml) at weeks:			ELISA index in culture supernatants		
					−1	+1	+3	F/T	Medium	PWM
PBMC										
I	2	15×10^6	0·63	Tg	Un.	1·1	30·0	0·03	0·03	0·57
	4	25×10^6	2·51±0·31	Tg	Un.	1·0±0·4	39·0±7·7			
II	2	25×10^6	2·22	Tg	Un.	Un.	8·2	0·08	0·07	0·12
				TPO	Un.	Un.	2·0	0·02	0·02	0·18
III	1*	15×10^6	0·003	Tg	Un.	0·8	1·4	0·01	0·01	0·29
IV	2	10×10^6	ND	Tg	Un.	1·0	2·1	0·60	0·99	0·97
V	4	20×10^6	0·03±0·00	Tg	Un.	ND	Un.	0·10	0·06	0·04
Lymph node lymphocytes										
VI	4	15×10^6	0·31±0·07	Tg	Un.	0·8±0·1	28·7±11·6	0·03	0·53	0·62
				TPO	Un.	Un	2·2±0·4	0·00	0·07	0·08
Thyroid lymphocytes										
VI	4	15×10^6	0·36±0·12	Tg	Un.	7·4±1·2	836±333	0·04	0·14	0·18
				TPO	Un.	2·6±0·6	12·7±4·1	0·00	0·00	0·00

Results are given as the mean of duplicates or mean±s.e.m. of four replicate animals or cultures. Tg/TPO antibody was measured by direct assay in SCID plasma and by ELISA in culture supernatants. Total human IgG was measured by ELISA in SCID plasma. *n*, number of mice; Un., undetectable; ND, not determined; F/T, suspension frozen and thawed before culture.

*Tg/TPO antibody undetectable in five SCID mice.

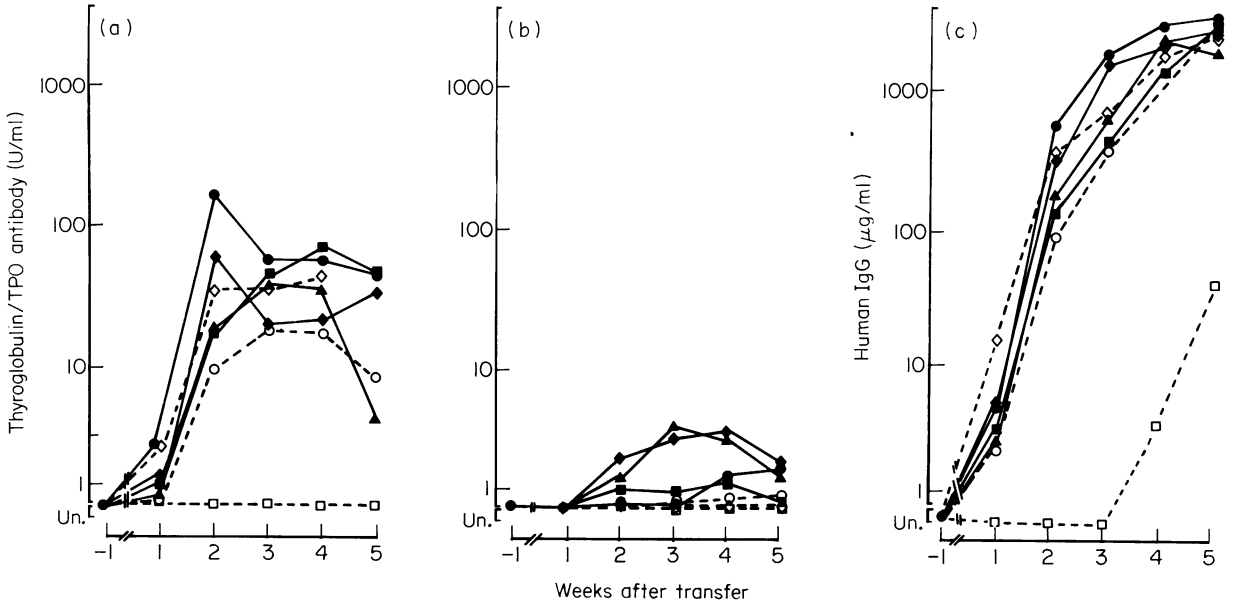

Fig. 1. Kinetics of the development of: (a) Tg antibody; (b) TPO antibody; and (c) human IgG in SCID mice injected with 15×10^6 (broken lines) and 25×10^6 (solid lines) PBMC from AITD patient I. Tg and TPO antibodies were measured by direct assay and human IgG by ELISA. Symbols are used to indicate measurements for individual mice. Un., undetectable.

recipients of thyroid lymphocytes than lymph node lymphocytes. In contrast, although human IgG was detectable a week earlier in the mice that received thyroid rather than lymph node lymphocytes, the maximum levels of human IgG attained were of similar magnitude in recipients of both types of lymphoid suspension (Fig. 2c; Table 1). Consequently, the proportion of total IgG at 3 weeks which was Tg antibody (the specific activity) was higher in SCID mice that received thyroid lymphocytes (2322 U Tg antibody/mg IgG) than lymph node lymphocytes (92·6 U Tg antibody/mg IgG). Similarly, the

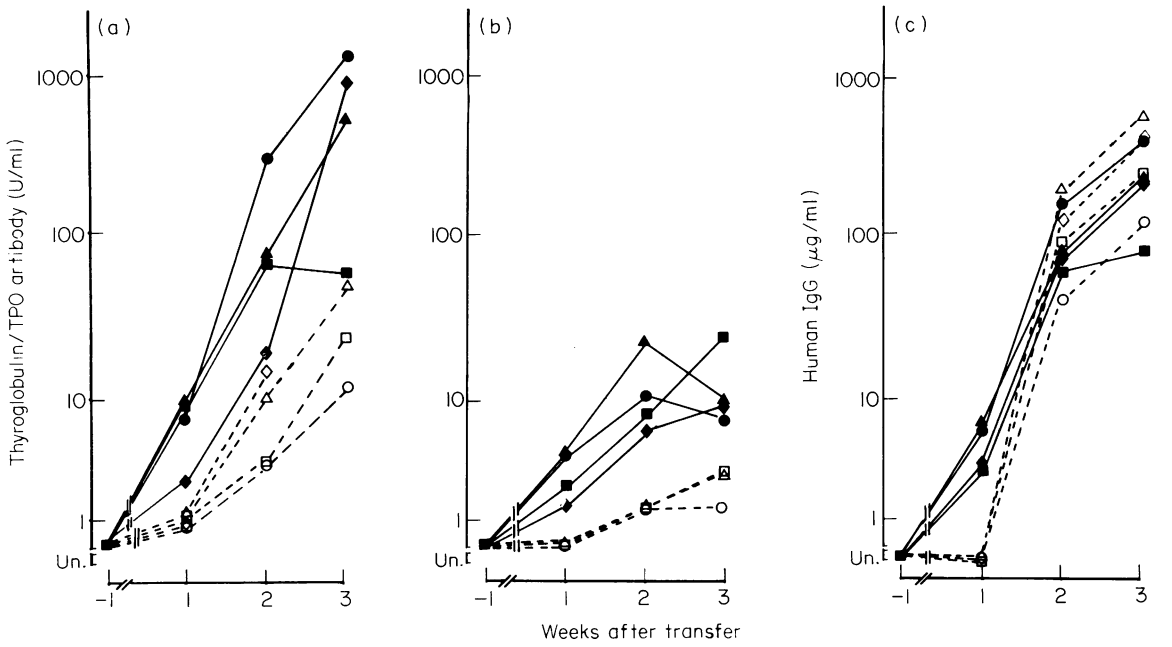

Fig. 2. Kinetics of the development of: (a) Tg antibody; (b) TPO antibody; and (c) human IgG in SCID mice injected with 15×10^6 thyroid lymphocytes (solid lines) and 15×10^6 lymph node lymphocytes (broken lines) from AITD patient VI. Tg and TPO antibodies were measured by direct assay and human IgG by ELISA. Symbols are used to indicate measurements for individual mice. Un., undetectable.

specific activities of Tg antibodies in SCID recipients of PBMC were lower than those in recipients of thyroid lymphocytes (47·6, 3·7 and 467 U/mg for patients I, II and III, respectively).

Tg and/or TPO antibody secreted in culture and produced in SCID mice by PBMC from AITD patients

The mean levels of Tg and/or TPO antibody synthesized in culture by lymphocytes from blood, thyroid and lymph nodes are given in Table 1 for comparison with the levels of the antibodies present in plasma from SCID recipients of these suspensions. PBMC from patients I, II and III produced Tg and/or TPO antibody in culture in response to stimulation with PWM but not in its absence, and in all cases lymphocytes from these patients produced detectable amounts of Tg or TPO antibody in at least one SCID mouse. Of the three patients, lymphocytes from patient I synthesized the highest levels of Tg autoantibody in response to PWM in culture and these cells also produced the greatest amounts of Tg antibody in SCID mice.

The observations made for PBMC from patients IV and V were different. PBMC from patient IV, frozen and thawed to lyse the cells prior to culture, released readily detectable amounts of Tg antibody, probably from plasma cells. Slightly higher levels of Tg antibody were secreted by living PBMC from this patient cultured in medium but there was no enhancement by PWM. In SCID mice that received lymphocytes from patient IV, Tg antibody was measurable after 1 week. In contrast, PBMC from patient V did not secrete detectable levels of Tg and/or TPO antibody in culture or in SCID mice.

Additional studies were carried out in culture, which may provide some insight into the low levels or lack of Tg and/or TPO antibody production by PBMC from AITD patients III and V. PWM-stimulated cultures of B cell enriched fractions from patient III combined with autologous MMC-treated T cells secreted more Tg antibody than unfractionated PBMC containing comparable numbers of B cells (culture supernatant ELISA index 1·72 compared with 0·29). In contrast, thyroid autoantibodies were undetectable (by ELISA or by direct assay), in cultures of unfractionated lymphocytes as well as B cell-enriched fractions combined with autologous MMC treated T cells from patient V. Similar results were obtained on other occasions using PBMC from this individual.

Tg and/or TPO antibody secreted in culture and produced in SCID mice by thyroid lymphocytes and lymph node lymphocytes

Lymph node lymphocytes from patient VI secreted Tg antibody (but not TPO antibody) spontaneously in culture (Table 1) and relatively high levels of Tg antibody were produced in SCID recipients of these lymph node lymphocytes. In addition, measurable amounts of TPO antibody were also observed in SCID plasma. The inability to detect TPO antibody synthesis in culture may reflect the lower sensitivity of the ELISA assay compared with the direct assay. The difference in the capacity for secretion of Tg antibody compared with TPO antibody *in vitro* was consistent with lower relative levels of TPO antibody compared with Tg antibody detectable in SCID mice plasma (Table 1 and Fig. 2a and b) and it was also consistent with the presence in patient VI's serum of higher relative amounts of Tg antibody compared with TPO antibody.

Thyroid lymphocytes synthesized small amounts of Tg antibody and undetectable levels of TPO antibody in culture (Table 1) and this constrasts with the very high levels of Tg antibody and lower levels of TPO antibody which developed in SCID mice (Table 1, Fig. 2a and b). However, this suspension of thyroid lymphocytes contained a high proportion of thyroid

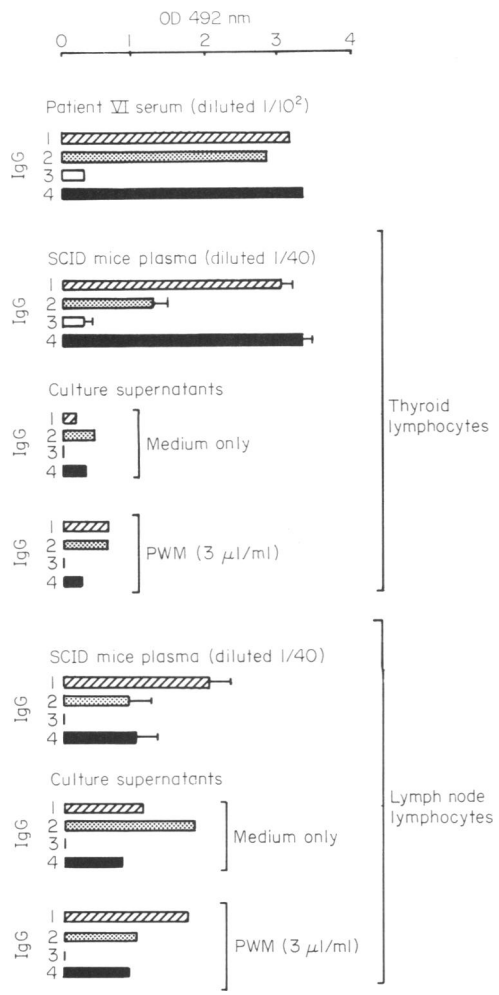

Fig. 3. IgG subclass distribution of Tg antibody present in serum, secreted in culture, and produced 3 weeks after transfer of thyroid and lymph node lymphocytes from AITD patient VI to SCID mice (15×10^6 cells/mouse). Results are expressed as the OD 492 nm in ELISA for serum diluted 1/100, for plasma (diluted 1/40) from three SCID mice (mean ± s.e.m.), and for undiluted pooled duplicate culture supernatants.

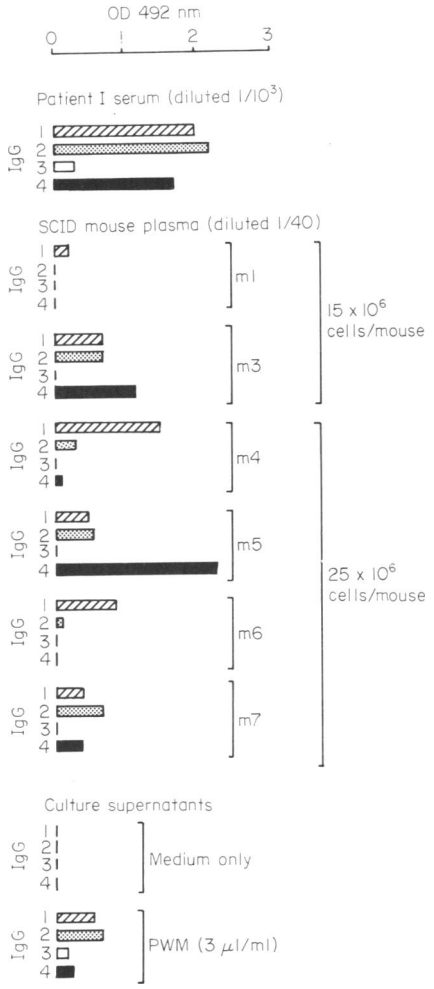

Fig. 4. IgG subclass distribution of Tg antibody present in serum, secreted in culture and produced 5 weeks after transfer of PBMC from AITD patient I to SCID mice (15×10^6 or 25×10^6 cells/mouse). Results are expressed as the OD 492 nm in ELISA for serum diluted 1/1000, for plasma (diluted 1/40) from individual SCID mice and for undiluted pooled duplicate culture supernatants.

cells (ratio of lymphoid cells:thyroid cells, 2:1). In additional studies, using the same thyroid lymphocyte suspensions subject to repeated depletion of thyroid cells by incubation for one or more days on plastic dishes, high levels of thyroid autoantibody synthesis were observed (ELISA index values of up to 1·87, compared with 0·14–1·18 for suspensions containing thyroid cells). These observations indicate that the thyroid lymphocytes had the capacity to secrete high levels of Tg and/or TPO antibody provided contaminating thyroid cells were removed to prevent antibody complexing with Tg and TPO present on or produced by the thyroid cells.

Tg antibody IgG subclasses in culture, in SCID mouse plasma and in the serum of the donor patient

The IgG subclass distribution of Tg antibody in serum was compared with the subclass pattern in SCID plasma and culture supernatants in studies involving lymphoid suspensions from patients VI, I and II. As shown in Fig. 3, serum Tg antibody of patient VI was present in IgG subclasses 1, 2 and 4, with a minor contribution from IgG3. The subclass distribution of Tg antibodies produced in SCID mice that received thyroid lymphocytes from this patient closely resembled that in the patient's serum, with little variability between individual mice. Tg antibody IgG subclasses in culture supernatants from these thyroid lymphocytes also resembled those in serum although IgG3 Tg antibody was undetectable. Similar results were obtained using lymph node lymphocytes from this patient; however, compared with Tg antibodies in serum, Tg antibody levels in SCID mouse plasma and in PWM-stimulated cultures of these lymph node lymphocytes were slightly skewed towards IgG1.

Serum Tg antibody of patient I was present at high levels in IgG subclasses 1, 2 and 4 and at a low level in subclass IgG3 and Tg antibody secreted in culture in response to PWM had a similar IgG subclass distribution (Fig. 4). However, the subclass patterns for Tg antibody in individual SCID recipients of these PBMC were extremely variable (Fig. 4); some mice had Tg

antibody predominantly of subclass IgG4 (m4) or IgG1 (m1, m4 and m6) while in others comparable contributions were made to Tg antibody by IgG1, 2 and 4 (m3 and m7). The IgG subclass distribution could not be related to either the number of cells transferred or to the amounts of total IgG class Tg antibody, which also varied from one mouse to another (Fig. 1). Further, in two SCID mice that received PBMC from patient II, Tg antibodies were of subclass IgG1 or of subclass IgG1 and IgG4; in contrast, Tg antibody in the patient's serum was of IgG subclasses 1, 2 and 4.

DISCUSSION

IgG class antibodies to Tg and/or TPO were detected in the plasma of SCID mice that received lymphocytes from thyroid antibody-positive AITD patients but not in SCID mice that received lymphocytes from antibody-negative controls. Our observations are in agreement with and extend those of others demonstrating the production of human autoantibodies in SCID mice by lymphocytes from patients with primary biliary cirrhosis, SLE and rheumatoid arthritis (Krams *et al.*, 1989; Duchosal *et al.*, 1990; Tighe *et al.*, 1990). In particular, we have compared the ability of lymphocytes extracted from different organs to produce thyroid antibodies in SCID mice and in culture. Our current observations, together with information from previous extensive investigations of lymphocyte cultures from patients with AITD (McLachlan *et al.*, 1979, 1983a, 1983b, 1983c, 1985, 1986; Beall & Kruger, 1979; McGregor *et al.*, 1979; De Bernardo & Davies, 1983; Weetman *et al.*, 1982; Atherton *et al.*, 1985), provide some insight into the nature of the B lymphocyte populations responsible for secreting Tg and/or TPO antibodies in SCID mice.

B lymphocytes capable of synthesizing IgG class autoantibodies to Tg and/or TPO in culture form a spectrum, including (i) B cells, probably B memory cells, that require activation by Epstein–Barr virus or mitogen in association with T cells before thyroid autoantibody synthesis is detectable; (ii) B cells already activated *in vivo* and capable of secreting thyroid autoantibodies when cultured without mitogen; and (iii) fully differentiated B cells, probably plasma cells, capable of releasing thyroid autoantibodies even when lysed by freezing and thawing before culture. Blood lymphocytes normally require polyclonal activation before thyroid autoantibody synthesis is detectable; thyroid lymphocytes usually contain lymphoblastoid or plasma cells already secreting Tg and/or TPO antibodies and lymph node lymphocytes frequently contain a mixture of memory cells and differentiated Tg and/or TPO antibody specific B cells (reviewed by Rees Smith *et al.*, 1988).

The ability of PBMC from three out of five AITD patients to secrete Tg and/or TPO antibodies in SCID mice was associated with the capacity of these PBMC to synthesize thyroid autoantibodies in culture in response to PWM. Consequently, SCID mice appear to provide a suitable environment in which Tg- and/or TPO-specific memory B cells, in association with autologous T cells, are able to undergo activation and differentiation into cells secreting thyroid antibodies in the absence of mitogen. SCID recipients of AITD thyroid lymphocytes, and to a lesser extent lymph node lymphocytes, developed extremely high levels of Tg antibody rapidly. Smaller amounts of TPO antibody were produced. In agreement with previous studies these thyroid and lymph node suspensions synthesized Tg antibody spontaneously in culture, reflecting the presence of *in vivo*-activated B cells (McLachlan *et al.*, 1979, 1983b, 1983c, 1985, 1986; Atherton *et al.*, 1985; Weetman *et al.*, 1982). Finally, PBMC from AITD patient IV appeared to contain plasma cells which were capable of releasing Tg antibody from cells lysed prior to culture and were able to produce Tg antibody in SCID mice.

Overall, therefore, our studies demonstrate that lymphocyte suspensions from AITD patients containing memory B cells, activated lymphoblastoid B cells or plasma cells are able to produce detectable levels of antibodies to Tg and/or TPO in SCID mice for up to 5 weeks after transfer of the lymphocyte suspensions.

The role of T cells in thyroid antibody secretion in SCID mice remains to be elucidated. However, the ability of PWM-stimulated PBMC from patient III to secrete Tg antibodies *in vitro* was clearly under the control of regulatory T cells since secretion of Tg autoantibody was markedly enhanced in cultures enriched for the patient's B cells combined with autologous MMC treated T cells (to prevent proliferation of the suppressor/cytotoxic T cell subset; Siegal & Siegal, 1977). This observation suggests that regulatory T cells may in part be responsible for the variability of Tg and/or TPO antibody levels in individual SCID recipients of PBMC from the same donor. Similar variability has been reported for rheumatoid factor production in SCID mice (Tighe *et al.*, 1990). However, the presence of regulatory T cells is unlikely to be the explanation for the absence of Tg and/or TPO antibodies in SCID mice which received PBMC from Hashimoto's thyroiditis patient V since the B cell-enriched fraction from this patient failed to secrete thyroid antibodies *in vitro* even when provided with excess T cell help. In an elegant series of experiments comparing lymphocytes from blood, spleen and lymph nodes, Callard *et al.* (1982) demonstrated that the absence of *in vitro* responses to influenza or herpes viruses by blood lymphocytes was due to the lack of appropriate recirculating B memory cells. Consequently, it seems likely that the inability of PBMC from AITD patient V to produce Tg and/or TPO antibodies in culture or in SCID mice may be attributed to the lack of the appropriate B cell precursors in the patient's blood. Similarly, the appropriate precursors are likely to be absent from the blood of thyroid autoantibody negative controls.

Tg antibody produced in SCID mice had a higher specific activity (ratio of specific antibody to total IgG) in recipients of thyroid lymphocytes than in mice that received blood or lymph node lymphocytes. These data indicate that the numbers of Tg-specific precursor B lymphocytes, relative to other non-thyroid-specific B cells, are lower in lymph nodes and blood than in the thyroid. Similarly, the specific activity of rheumatoid factor was higher in SCID recipients of rheumatoid synovial lymphocytes than mice which received PBMC (Tighe *et al.*, 1990).

The relationship between Tg autoantibodies present in patients' sera and antibody produced in SCID mice or synthesized in culture was analysed further in terms of the Tg antibody IgG subclass distribution. Tg antibody produced by thyroid lymphocytes in SCID mice and secreted spontaneously in culture closely resembled the antibody present in the donor's serum, in agreement with our previous studies of Tg and/or TPO antibodies in serum and secreted in culture by thyroid lymphocytes (McLachlan *et al.*, 1985). The IgG subclass distribution of Tg antibody synthesized by AITD PBMC cultured with PWM

usually resembles the subclass pattern in the donor's serum (Thompson *et al.*, 1983) and comparable observations were made in the present study for PBMC from patient I. Tg antibodies of all IgG subclasses (except IgG3) could be produced in SCID recipients of AITD PBMC and these results are in agreement with the observations made for mitochondrial antibodies in SCID mice which received PBMC from patients with primary biliary cirrhosis (Krams *et al.*, 1989).

However, in our studies, the IgG subclass patterns of Tg antibodies in SCID recipients of PBMC were extremely variable, with different Tg antibody IgG subclasses predominating in different recipients of the same lymphoid suspension and the reason for this is not clear at present.

In addition to the production of human autoantibodies characteristic of the donor patient, SCID recipients of lymphocytes from patients with primary biliary cirrhosis and SLE transferred to SCID mice also developed some symptoms of the autoimmune disease (Krams *et al.*, 1989; Duchosal *et al.*, 1990). The potential effects of human antibodies to Tg and/or TPO on the mouse thyroid are still under investigation. It seems unlikely, however, that murine thyroid destruction will occur in SCID recipients of lymphocytes from patients with AITD, for the following reasons: (i) human autoantibodies to human Tg do not often interact with Tg prepared from mammals other than primates (Chan *et al.*, 1987); and (ii) autoimmune thyroid destruction in humans, as in experimental murine models (Simon *et al.*, 1986), is likely to involve cytotoxic T cells in addition to autoantibody mediated damage and the necessary MHC restriction elements for human T cells are not present on mouse thyroid cells.

Whether or not autoimmune damage can be demonstrated in the murine thyroid gland, SCID mice will provide a powerful system for elucidating regulatory mechanisms involved in the secretion of human antibodies to Tg and TPO.

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