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Helper and suppressor activities of an autoreactive mouse thyroglobulin-specific T-cell clone

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SUMMARY

An autoreactive T-cell clone, MTg9B3, specific for mouse thyroglobulin, has been characterized. This clone has properties consistent with it being an autoreactive T helper cell. It is I-A restricted, with a surface phenotype of Thy 1^+, L3T4$^+$, Ly2$^-$, and produces a number of non-specific lymphokine activities following specific triggering with antigen. Furthermore, antigen-stimulated clone cells were capable of providing bystander help in SRBC-specific antibody responses. Surprisingly, MTg9B3 cells had a profound suppressive effect on thyroglobulin autoantibody responses *in vitro*.

INTRODUCTION

The existence of autoreactive T helper cells specific for mouse thyroglobulin (MTg) in normal mice has been suggested by a number of experiments (Charreire, 1982; Rose *et al.*, 1981; Romball & Weigle, 1984). However, these have provided only indirect evidence. Autoreactive T-cell lines specific for MTg have been described recently (Champion *et al.*, 1985; Maron *et al.*, 1983). A clone from one of these lines has now been produced and shown to have properties consistent with it being a true autoreactive T helper cell, although paradoxically it has a suppressive effect on Tg autoantibody secretion by primed B cells *in vitro*.

MATERIALS AND METHODS

Mice

Male CBA/Ca and (CBA × BALB/c)F_1 mice were obtained from the MRC Laboratories, Mill Hill, London.

Antigens

Mouse and rat thyroglobulins were prepared from pooled thyroids and pig and human thyroglobulin from individual thyroids as previously described (Champion *et al.*, 1985). Sheep red blood cells (Tissue Culture Services, Slough, Berks) were washed three times in phosphate-buffered saline (PBS) before use.

Abbreviations: Br-MRBC, bromelain-treated mouse red blood cells; IL-2, interleukin-2; IL-3, interleukin-3; PBS, phosphate-buffered saline; PFC, plaque-forming cell; SRBC, sheep red blood cell; Tg, thyroglobulin; TRF, T-cell replacing factor.

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Interleukin-2 (IL-2)

Concanavalin A-stimulated rat spleen cell supernatants prepared as previously described (Champion *et al.*, 1985), with α-methyl mannoside (20 mg/ml) added to bind free mitogen, were used as a source of IL-2. Although this is only an IL-2 containing supernatant, for simplicity it will be referred to as IL-2.

T-cell lines

The IL-2-dependent cell line CTLL-16 originally produced by M. Schreier (Sandoz Ltd, Basel, Switzerland) was maintained in RPMI-1640/5% FCS containing 18% (v/v) IL-2. The interleukin-3 (IL-3)-dependent cell line AD3, originally obtained from Dr T. M. Dexter (Christie Hospital, Holt Radium Institute, Manchester), was maintained in RPMI-1640/10% FCS together with IL-3-containing supernatant from the WEHI-3b cell line. MTg-specific T-cell lines were produced and maintained as previously described (Champion *et al.*, 1985). The cloned cells used in this report, MTg9B3, were obtained from one of these lines (MTg9) by limiting dilution. An average of 0·5 line cells per flat-bottomed microtitre well was cultured with 5×10^5 irradiated (2000 rads) syngeneic spleen cells in the presence of MTg (50 μg/ml) and IL-2 [18% (v/v)] in a total volume of 200 μl DMEM/10% FCS. Cloning plates were screened for cell growth after 7 days, and positive wells were expanded by repeated antigenic stimulation as for the parent lines but in the presence of IL-2. Thus far, only the one clone has shown the stable growth characteristic required to produce usable numbers of cells.

All culture media used contained 10^{-5} M 2-mercaptoethanol, 2 mM L-glutamine, 100 U/ml benzylpenicillin and 100 μg/ml streptomycin sulphate. Non-essential amino acids (Flow Laboratories, Irving, Ayrshire) and sodium pyruvate were also added to the DMEM/10% FCS used to culture MTg9B3 cells.

Proliferation assays
The proliferative responses of MTg9B3 cells were measured using the incorporation of ^{125}I-deoxyuridine as previously described (Champion *et al.*, 1985).

Production of cell-free supernatants of MTg9B3 cultures
Cloned cells (2×10^4) were cultured in multiple replicate flat-bottomed microtitre wells with 5×10^5 irradiated syngeneic spleen cells in a total volume of 200 μl DMEM/10% FCS. Stimulated cells received MTg at 50 μg/ml final concentration. After different periods of culture, replicate wells were pooled, centrifuged and the cell-free supernatant stored at −20° in several aliquots for subsequent assays.

IL-2 assays
CTLL-16 cells (10^4) were cultured with appropriate concentrations of test supernatant in triplicate 200 μl cultures in flat-bottomed microtitre wells. After 24 hr, cultures were pulsed for 4–6 hr with 0·5 μCi ^{125}I-deoxyuridine before harvesting on to glass fibre discs with a Titertek cell harvester (Flow Laboratories) and γ-counting. In order to assay for IL-2 inhibitory activity, supernatants were tested at 25% (v/v) for their ability to reduce the activity of a suboptimal concentration (10% v/v) of IL-2.

IL-3 assays
These were set up as for IL-2 assays but using the AD3 cell line. After 44 hr culture, wells were pulsed with 0·5 μCi [^{3}H]thymidine and harvested and counted 4 hr later.

T-cell replacing factor (TRF) assay
Spleen cells from CBA/Ca mice primed 5 days previously with 2×10^8 sheep red blood cells (SRBC) i.p. were depleted of T cells by treatment with anti-Thy 1.2 and complement. These B-cell enriched cells (2×10^6) were then cultured in 24-well plates with 10^6 SRBC in 2 ml RPMI-1640/5% FCS in the presence or absence of 25% (v/v) of supernatants from MTg9B3 cells. After 5 days culture, cells were harvested and washed before determining direct (IgM) and indirect (IgG) SRBC plaque-forming cells (PFCs) using the Cunningham modification of the Jerne plaque assay (Cunningham & Szenberg, 1968). The indirect PFCs detected are primarily IgG, since the developing rabbit anti-Ig serum used inhibits IgM PFCs. T-cell replacing factor (TRF) activity was detected as an enhanced PFC response and expressed as net PFC/10^6 B cells after subtraction of PFC values for cultures without added T-cell supernatant.

Bromelain-treated mouse red blood cell (Br–MRBC) PFC responses
Peritoneal cells (2×10^5) from normal (CBA × BALB/c)F_1 mice were cultured in 200 μl of RPMI-1640/5% FCS in microtitre wells. After 3 days of culture, cells were washed and Br–MRBC PFCs measured as previously described (Cunningham, 1974). Supernatants from MTg9B3 cell cultures were tested at various concentrations for their ability to enhance the Br–MRBC PFC response.

'Bystander' helper assay
Cultures of anti-Thy 1.2 and complement-treated SRBC-primed spleen cells (2×10^6/well) were set up as for the TRF assay, but different doses of MTg9B3 cells were also added with or without MTg at 50 μg/ml. After 5 days culture, cells were washed and SRBC–PFCs determined as described above.

Tg autoantibody secretion in vitro
Mice (CBA/Ca) were primed by intraperitoneal injection with rat Tg (50 μg) emulsified in Freund's complete adjuvant. After 8 weeks, and 7 days before use, mice were boosted with an intravenous injection of soluble Tg (50 μg). Spleen cells from these mice were prepared and treated with anti-Thy 1.2 and complement to produce a T-cell depleted population. These cells (2×10^6) were cultured in triplicate in 2 ml RPMI-1640/5% FCS in 24-well plates in the presence or absence of rat Tg and various numbers of cloned MTg9B3 cells. After 5 days, replicate wells were pooled and washed twice with balanced salt solution before resuspending in 2 ml RPMI-1640/5% FCS. Quadruplicate 200-μl aliquots were cultured for a further 7 days in flat-bottomed microtitre plates. Supernatants were then tested for Tg autoantibody by solid-phase radioassay on Tg-coated polyvinyl microplates with ^{125}I-labelled affinity-purified sheep anti-mouse F(ab′)$_2$ as the second antibody.

RESULTS

Production and characterization of the MTg-specific T-cell clone MTg9B3

Long-term T-cell lines specific for MTg were produced and

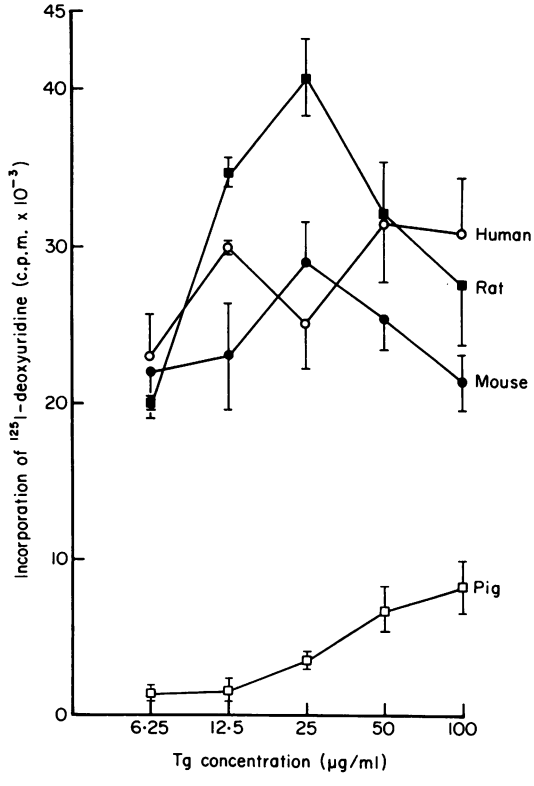

Figure 1. Proliferative responses of MTg9B3 to different thyroglobulins. MTg9B3 cells (2×10^4) were cocultured with syngeneic irradiated (2000 rads) spleen cells (5×10^5) in the presence of various concentrations of mouse (●——●), rat (■——■), human (○—○) or pig (□——□) Tg. Proliferative responses were assessed by the incorporation of ^{125}I-deoxyuridine during the final 16 hr of the 3-day culture period.

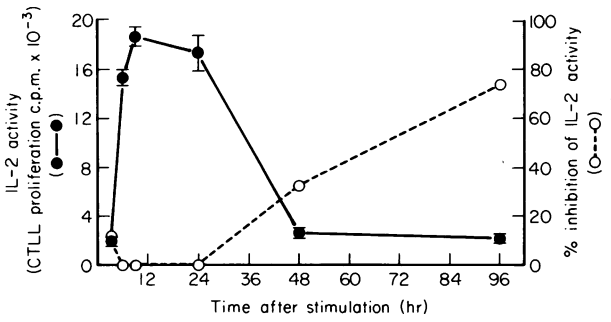

Figure 2. IL-2 and inhibitory activity in supernatants of stimulated MTg9B3 cells. Supernatants of replicate cultures of MTg9B3 cells stimulated with Tg and syngeneic irradiated spleen cells were harvested at different times following stimulation and tested (25% v/v) for IL-2 by their ability to support the proliferation of CTLL cells (●——●). Inhibitory activity (O- - - - O) of the same supernatants was tested at the same concentration by their ability to reduce the activity of a suboptimal IL-2 concentration (10% v/v). Supernatants taken at 6, 9 and 24 hr are shown to have no inhibitory activity, although they actually enhanced the CTLL response because of their IL-2 content.

maintained by repeated antigenic stimulation in the presence of antigen-presenting cells as previously described (Champion *et al.*, 1985). Cloning by limiting dilution successfully produced clones, but they proved very difficult to maintain and expand to usable numbers. However, one clone (MTg9B3) with good growth characteristics has been obtained from the parent line MTg9. This clone proliferates in response to MTg in association with I-A^k-matched antigen-presenting cells (data not shown). Like its parent line, MTg9B3 also responds strongly to rat and human Tg but only weakly to pig Tg (Fig. 1). MTg9B3 cells were found to have the phenotype $Ly1^+$, $Ly2^-$, sIg^-, $L3T4^+$ and Thy 1.2^+.

IL-2 release

Following stimulation with Tg and irradiated syngeneic spleen cells, MTg9B3 releases IL-2 into the culture medium. IL-2 activity was detectable as early as 3–6 hr after stimulation, peaked from 9 to 24 hr and always declined to background levels by 96 hr (Fig. 2). No IL-2 was detectable in the absence of Tg-stimulation (data not shown). In the light of recent reports of IL-2 inhibitory molecules (Lelchuk & Playfair, 1985; Male *et al.*, 1985), we were interested to investigate whether the decline of IL-2 activity might be partly due to the release of inhibitory molecules rather than solely to utilization by the proliferating cloned cells. It can be seen from Fig. 2 that the same supernatants obtained 48 hr and 96 hr after stimulation could indeed inhibit IL-2 activity. However, further investigation revealed that this activity was independent of antigen stimulation and was, in fact, released from the irradiated spleen cells used to present antigen. This activity did not appear to be due to prostaglandin since indomethacin had no effect on its release (data not shown). There appears to be at least some selectivity in the action of this inhibitory activity since 96 hr supernatants still had maximal levels of other lymphokine activities (see below). As yet, this activity has not been further investigated, but its presence could account for problems encountered during the growth and expansion of T-cell clones, particularly when using weak antigens such as MTg and other autoantigens.

Table 1. IL-3 activity of MTg9B3 supernatants

Source of test supernatant (25% v/v)		Proliferation of AD-3 cells (mean c.p.m. ± SE)*
—		517 ± 82
WEHI-3b†		20,404 ± 2866
MTg9B3	24 hr	47,430 ± 3942
	48 hr	54,939 ± 2504
	72 hr	60,027 ± 12,120
	96 hr	54,759 ± 4816
	144 hr	68,119 ± 4269

Supernatants of MTg9B3 cells collected 24–144 hr after stimulation were tested at 25% (v/v) final concentration for their ability to support the proliferation of the IL-3-dependent cell line AD-3.

* Proliferative responses were measured after 48 hr culture by the incorporation of [^{3}H]thymidine (c.p.m. ± SE).

† Supernatants (1% v/v) from the IL-3-producing tumour cell line, WEHI-3b, were used as a positive control.

IL-3 activity

IL-3 activity in stimulated MTg9B3 supernatants was measured with the IL-3-dependent cell line AD-3. High levels of activity were detected in all the supernatants tested (1–6 days after stimulation: Table 1).

T-cell replacing factor (TRF) activity

Supernatants taken at different times after Tg stimulation of MTg9B3 cells were assayed for TRF activity on anti-Thy 1.2/complement-treated SRBC-primed spleen cells cultured with SRBC. The results indicated that TRF activity gradually

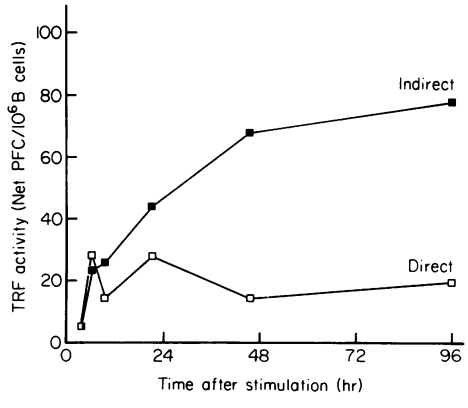

Figure 3. T-cell replacing factor (TRF) activity of MTg9B3 supernatants. Supernatants taken different times after stimulation of MTg9B3 cells with Tg were tested at 25% (v/v) for their ability to support an *in vitro* SRBC-specific PFC response of anti-Thy 1.2/complement-treated SRBC-primed spleen cells stimulated with SRBC. Direct (□——□) and indirect (■——■) PFC responses were measured and TRF activity expressed as net PFC/10^6 B cells after subtraction of PFC values for cultures lacking T-cell supernatants. Background values: direct, 4; indirect, 12 PFC/10^6 B cells.

accumulated in the medium following stimulation and was maximal from 48–96 hr (Fig. 3). The PFC responses induced by clone supernatants were primarily IgG responses (indirect PFCs). This TRF activity was clearly unrelated to IL-2 since 96 hr supernatants did not have detectable IL-2 activity (Fig. 2).

Release of a factor that enhances bromelain-treated mouse red blood cell PFC responses

Cultures of peritoneal cells from normal mice lead to the generation of PFCs specific for bromelain-treated mouse red blood cells (Br–MRBC) (Cunningham, 1974). Addition of supernatants from stimulated MTg9B3 cells to the cultures lead to a marked increase in the number of Br–MRBC PFCs (Fig. 4).

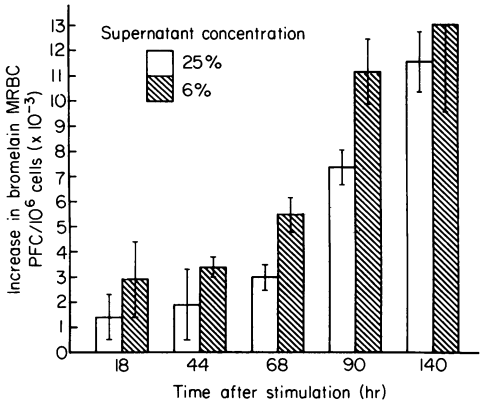

Figure 4. Enhancement of the bromelain-treated mouse red blood cell PFC response by supernatants of stimulated MTg9B3 cells. Peritoneal cells of (CBA × BALB/c)F_1 mice (2×10^5) were cultured with 25% (v/v) (open bars) or 6% (v/v) (hatched bars) supernatant from MTg9B3 cells taken at the indicated times after stimulation. Results are expressed as the increase in Br–MRBC response above that for cells cultured in medium alone (typically 3×10^3 PFC/10^6 cells).

Activity levels appeared highest after longer culture periods (90 and 140 hr). However, in all cases, supernatants tested at 6% (v/v) final concentration were more active than higher concentrations. This suggested the possibility of a competing or inhibitory activity at higher concentrations, that was masking the true level of enhancing activity in the supernatants. A direct comparison of 68 hr and 140 hr supernatants at lower concentrations [1–5% (v/v)] suggested that this might be true, since at lower concentrations the activity of the supernatants were similar (the 68 hr supernatant was actually slightly more active). However, at a concentration of over 5% (v/v), the 140 hr supernatant was almost twice as effective (data not shown). This implies that the inhibitory activity is somehow lost between 67 hr and 140 hr of culture. One possible candidate for such inhibitory activity would be γ-interferon (γIFN), since recombinant γIFN has been shown to be inhibitory in the Br–MRBC PFC response (P. R. Hutchings, P Van der Meade and A. Cooke, unpublished observations) and is relatively unstable at 37°.

Helper activity of MTg9B3

'Bystander help' (Hartmann, 1970) could be demonstrated in cocultures of MTg9B3 cells with anti-Thy 1.2/complement-treated SRBC-primed spleen cells (Fig. 5). Markedly enhanced SRBC-specific PFC responses were only observed when both the B cells and MTg9B3 were triggered with their specific antigens (SRBC and MTg, respectively). As few as 10^4 cloned cells were able to trigger a significant response. Marginal responses were sometimes observed at high T-cell numbers (10^5) in the absence of specific Tg stimulation, implying that some T cells might still be making B-cell stimulating factors at the time of assay (7 days after the last restimulation of MTg9B3 cells).

Suppressive activity of MTg9B3

We have attempted to demonstrate helper activity of MTg9B3 in an *in vitro* Tg antibody secretion system. Spleen cells from

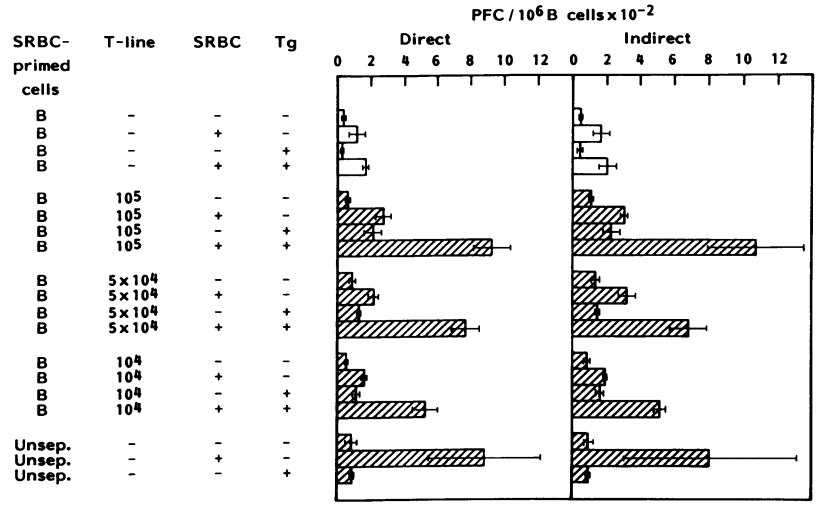

Figure 5. MTg9B3 cells give 'bystander' help for *in vitro* SRBC-specific PFC responses. Anti-Thy 1.2/complement-treated SRBC-primed spleen cells (B) were cocultured with different numbers of MTg9B3 cells in the presence or absence of SRBC and MTg as shown. The number of direct and indirect PFCs generated after 5 days of culture is expressed as PFC/10^6 B cells. The responses of unseparated primed spleen cells are also shown as a positive control.

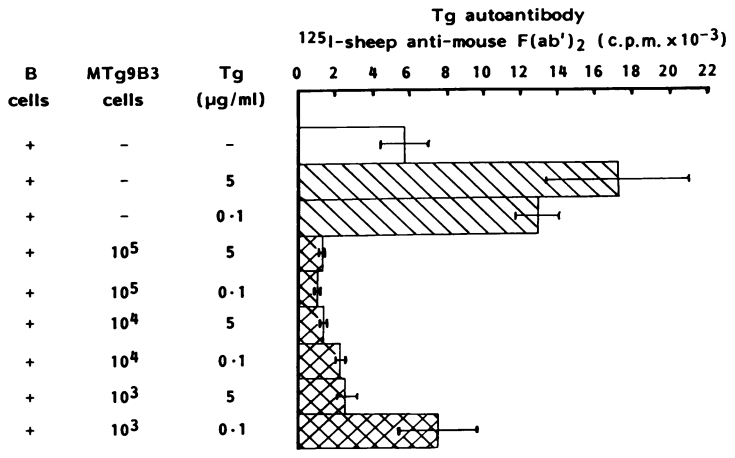

Figure 6. Inhibition of Tg antibody responses *in vitro* by MTg9B3 cells. Anti-Thy 1.2/complement-treated rat Tg primed spleen cells were cocultured with MTg9B3 cells and rat Tg at 5 and 0·1 μg/ml. After 5 days, the cells were washed to remove Tg and recultured for a further 7 days, when Tg autoantibody levels in the supernatants were measured by solid-phase radioassay. Autoantibody levels are expressed as the binding of ^{125}I-sheep anti-mouse F(ab')$_2$ (c.p.m. $\times 10^{-3}$).

mice primed and boosted with rat Tg [rat Tg strongly cross-reacts with MTg (Rose *et al.*, 1982; B. R. Champion, unpublished observation)] were treated with anti-Thy 1.2/complement before culturing in the presence or absence of rat Tg and MTg9B3 cells at different doses. Tg antibodies were detected in culture supernatants by radioassay after 12 days of culture, with cells being washed at Day 5 to remove antigen. A full report on the use of this culture system to assay for help and suppression of Tg antibody responses will be presented separately. It can be seen from Fig. 6 that MTg9B3 cells very effectively inhibited, rather than helped, Tg antibody production, even with as few as 10^3 cells. The rat Tg antibody response was also suppressed (data not shown), indicating that this effect was not specifically directed at autoantigenic epitopes on the rat Tg molecule. The cultured Tg primed cells responded *in vitro* to Tg in the absence of added T cells, implying that the anti-Thy1.2/complement treatment had not killed all the T cells since Tg is a T-dependent antigen (Esquivel, Rose & Kong, 1977).

DISCUSSION

The role of autoreactive T-helper (Th) cells in initiating autoimmune responses is unclear, although indirect evidence has indicated the existence of such cells (Charreire, 1982; Rose *et al.*, 1981; Elrehewy *et al.*, 1981; Esquivel *et al.*, 1977). Two major lines of evidence indicate that the mouse Tg-specific autoreactive T-cell clone, MTg9B3, described here is a Th cell. Firstly, following stimulation with antigen (Tg) and antigen-presenting cells, MTg9B3 cells produce the non-specific lymphokines IL-2, IL-3 and TRF, which are characteristic Th-cell products (Howard & Paul, 1983). Preliminary experiments in collaboration with Dr Anne O'Garra, MRC Laboratories, Mill Hill, London (unpublished observations) indicate that this clone also produces the B-cell stimulatory factors BSF-1 and BCGFII (Kishimoto, 1985). The presence of BCGFII might also account for the observed TRF activity (Swain *et al.*, 1983). In addition, factors produced by MTg9B3 also markedly enhanced the PFC responses of normal peritoneal cells to bromelain-treated mouse erythrocytes (Br–MRBC). Further investigations of such enhancing activities released from a long-term SJL line indicate that the factor(s) responsible is similar but not identical to BCGFII (A.-M. Varey, P. R. Hutchings, B. R. Champion, S. Marshall-Clarke and A. Cook, manuscript in preparation). Since Br–MRBC is thought to be a T-independent antigen (Cunningham, 1974), we have also tested supernatants from stimulated MTg9B3 cells for their effect on PFC responses to TNP–*Brucella abortus*—a type I T-independent antigen. The supernatants were also active in this system (data not shown). Thus, MTg9B3 can produce factors helping both T-dependent (SRBC) and T-independent antibody responses. The ability to help T-independent responses is consistent with recent reports that these responses do in fact require T cells, albeit in low numbers (Endres *et al.*, 1983; Pike & Nossal, 1984; Nossal & Pike, 1984). Alternatively, T-cell derived factors might simply make the responses to T-independent antigens more effective. A third possibility could also be considered—that clones of T-dependent B cells specific for T-independent antigens coexist with classical T-independent B cells.

In addition to, and probably as a consequence of, secreting a variety of lymphokines, MTg9B3 cells were also shown to be capable of providing 'bystander' help in cocultures with SRBC-primed T-depeleted spleen cells. Helper activity was only observed when both Tg and SRBC antigens were present. The Tg is clearly required to activate MTg9B3 cells and must, therefore, utilize antigen-presenting cells in the cultures. The role of SRBC would be to activate the primed B cells to a state in which they can respond to non-specific differentiation factors (e.g. TRF, BCGFII) produced *in situ* by the cloned cells (Melchers *et al.*, 1980).

Despite all the evidence suggesting that MTg9B3 is a clone of Th cells, the results presented in Fig. 6 indicate that these cells have a profound suppressive effect on Tg autoantibody responses *in vitro*. The mechanism of this suppressive effect remains to be elucidated, but preliminary evidence from a different Tg-specific cell line that also suppresses suggests that a cytotoxic effect may be involved. The B cells used were clearly responsive to helper signals, since a Tg-specific T-cell hybridoma was capable of markedly enhancing Tg antibody re-

sponses (B. R. Champion, P. R. Hutchings, D. C. Rayner, A. Cooke and I. M. Roitt, manuscript in preparation). Tite & Janeway (1984) have also demonstrated cytotoxic effects of Th clones, but these were generally observed with high cell numbers in the cultures. In the experiments reported here, as few as 10^3 MTg9B3 cells were capable of completely inhibiting the response to 5 μg/ml Tg. An alternative possibility is that MTg9B3 cells have a suppressor function despite having other features in common with helper cells. Bottomly and colleagues (Kim *et al.*, 1983) have recently described a rare ovalbumin-specific clone which has such properties. We are currently attempting to elucidate the mechanism of MTg9B3-mediated suppression and hope to establish how this clone of cells is capable of helping one antibody response (bystander help for SRBC PFC responses) and yet is inhibitory for another (Tg antibody responses). The ability of the T-cell clone to make cell-to-cell contact with primed B-cells expressing Tg (through specific binding to membrane antibody) and MHC class II molecules on their surface, but not with SRBC-primed B-cells, may well be a significant factor. Whether or not these surpressive properties result from an abnormal differentiation pattern in a highly selected clone, or are an artefact of the culture system, or perhaps are more typical of autoreactive T cells, also remains to be determined. Thus, although autoreactive Tg-specific T cells clearly exist (this paper and Maron *et al.*, 1983), definitive proof of the existence of normal Tg-specific autoreactive Th cells is still lacking.

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REFERENCES

Champion B.R., Varey A.M., Katz D., Cooke A. & Roitt I.M. (1985) Autoreactive T cell lines specific for mouse thyrogobulin. *Immunology* **54,** 513.

Charreire J. (1982) Syngeneic sensitization of mouse lymphocytes on monolayers of thyroid epithelial cells. II. T and B cell involvement in primary responses. *Eur. J. Immunol.* **12,** 416.

Cunningham A.J. (1974) Large numbers of cells in normal mice produce antibody to components of isologous erythrocytes. *Nature (Lond.)*, **252,** 749.

Cunningham A.J. & Szenberg A. (1968) Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology*, **14,** 599.

Elrehewy M., Kong Y.M., Giraldo A.A. & Rose N.R. (1981) Syngeneic thyroglobulin is immunogenic in good responder mice. *Eur. J. Immunol.* **11,** 146.

Endres R.O., Kushnir E., Kappler J.W., Marrack P. & Kinsky S.C. (1983) A requirement for non-specific T cell factors in antibody responses to 'T cell independent' antigen. *J. Immunol.* **130,** 781.

Esquivel P.S., Rose N.R. & Kong, Y.C.M. (1977) Induction of autoimmunity in good and poor responder mice with mouse thyroglobulin and lipopolysaccharides. *J. exp. Med.* **145,** 1250.

Hartmann K.-U. (1970) Induction of a hemolysin response *in vitro*. Interaction of cells of bone marrow origin and thymic origin. *J. exp. Med.* **132,** 1267.

Howard M. & Paul W.E. (1983) Regulation of B-cell growth and differentiation by soluble factors. *Ann. Rev. Immunol.* **1,** 307.

Kim J., Woods A., Becker-Dunn E. & Bottomly K. (1985) Distinct functional phenotypes of cloned Ia-restricted helper T cells. *J. exp. Med.* **162,** 188.

Kishimoto T. (1985) Factors affecting B-cell growth and differentiation. *Ann. Rev. Immunol.* **3,** 133.

Lelchuk R. & Playfair J.H.L. (1985) Serum IL-2 inhibitor in mice. I. Increase during infection. *Immunology*, **56,** 113.

Male D., Lelchuk R., Curry S., Pryce G. & Playfair J.H.L. (1985) Serum IL-2 inhibitor in mice. II. Molecular characteristics. Immunology, **56,** 119.

Maron R., Zerubavel R., Friedman A. & Cohen I.R. (1983) T-lymphocyte line specific for thyroglobulin produces or vaccinates against autoimmune thyroiditis in mice. *J. Immunol.* **131,** 2316.

Melchers F., Andersson J., Lernhardt W. & Schreier M.H. (1980) H-2-unrestricted polyclonal maturation without replication of small B cells induced by antigen-activated T cell help factors. *Eur. J. Immunol.* **10,** 679.

Nossal G.J.V. & Pike B.L. (1984) A reappraisal of 'T-independent' antigen. II. Studies on single, hapten-specific B cells from neonatal CBA/H or CBA/N mice fail to support classification into TI-1 and TI-2 categories. *J. Immunol.* **132,** 1696.

Pike B.L. & Nossal G.J.V. (1984) A reappraisal of 'T-independent' antigens. I. Effect of lymphokines on the response of single hapten-specific B lymphocytes. *J. Immunol.* **132,** 1687.

Romball C.G. & Weigle W.O. (1984) T-cell competence to heterologous and homologous thyroglobulins during the induction of experimental autoimmune thyroiditis. *Eur. J. Immunol.* **14,** 887.

Rose, N.R., Accavitti M., Pydyn F., Leon M.A. & Brown R.K. (1982) The use of hybridoma antibodies to probe the antigenic determinants of thyroglobulin. *Adv. exp. Biol.* **150,** 23.

Rose N.R., Kong Y.M., Okayasu I., Giraldo A.A., Beisel K. & Sundick R.S. (1981) T-cell regulation of autoimmune thyroiditis. *Immunol. Rev.* **55,** 299.

Swain S.L., Howard M., Kappler J., Marrack P., Watson J., Booth R., Wetzel G.D. & Dutton R.W. (1983) Evidence for two distinct classes of murine B cell growth factors with activities in different functional assays. *J. exp. Med.* **158,** 822.

Tite J.P. & Janeway C.A. (1984) Cloned helper T cells can kill B lymphoma cells in the presence of specific antigen: Ia restriction and cognate *vs* non-cognate interaction in cytolysis. *Eur. J. Immunol.* **14,** 878.