

## CONTINUOUS PROLIFERATION OF MURINE ANTIGEN-SPECIFIC HELPER T LYMPHOCYTES IN CULTURE\*

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The low frequency of antigen-specific helper T cells in murine spleen cells limits the analysis of their mode of action in the induction of immune responses. The establishment of cloned cell cultures of helper T cells would greatly facilitate the isolation of their antigen-binding receptor and the determination of how cell communication is effected. There have been a number of reports of the successful growth of human T lymphocytes (1, 2) and murine cytotoxic T lymphocytes in culture (3-7). Continued proliferation of T cells is dependent upon the presence of growth factors found in the culture supernates of phytohemagglutinin (PHA)<sup>1</sup>-stimulated human peripheral blood lymphocytes (1, 2) or concanavalin A (Con A)-stimulated rat spleen cells (3-6).

We have recently described the purification of a class of growth factors from the supernates of Con A-activated murine spleen cells (8-10). This class of factor stimulates a number of lymphocyte proliferative or differentiative responses in culture (8, 9), but appears to exert all effects through the proliferation of antigen- or mitogen-activated T cells (10). These murine T-cell growth factors (TCGF) are 30,000 daltons in size and are separable by charge into two components (isoelectric points [pI]: 4.3 and 4.9) which both exhibit identical biological activity (10).

In this paper, the murine TCGF is used to establish and maintain long-term cultures of antigen-specific helper T cells. A limiting-dilution technique was used to determine the frequency of helper cells at various times during growth. These T cells retain antigenic specificity in culture and exhibit helper activity with syngeneic, but not allogeneic, B cells. The cells are strictly dependent upon TCGF isolated from murine, rat, or human lymphoid cell cultures for proliferation and have been maintained in culture for >50 wk. The procedure described should be suitable for the selection and growth in culture of murine antigen-specific effector T cells from each subclass.

### Materials and Methods

*Mice.* C57BL/6J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. BALB/c mice were from the Strong Laboratory, Del Mar, Calif. C57BL/6.nu and BALB/c.nu mice were from our breeding colony at the University of California, Irvine, Calif.

*Chemicals.* Con A and methyl- $\alpha$ -D-mannoside were obtained from Calbiochem-Behring

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<sup>1</sup> *Abbreviations used in this paper:* Con A, concanavalin A; FCS, fetal calf serum; pI, isoelectric point(s); PHA, phytohemagglutinin; SRBC, HRBC, sheep and horse erythrocytes; T<sub>SRBC</sub>, T<sub>HRBC</sub>, T cells activated to SRBC or HRBC antigens; TCGF, T-cell growth factor.

Corp., American Hoechst Corp., San Diego, Calif. Ultrapure ammonium sulfate was from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.

*Preparation of Murine TCGF.* Spleen cells from the BALB/c mice were cultured at a density of  $5 \times 10^6$  cells/ml in a RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 1% fetal calf serum (FCS),  $5 \times 10^{-5}$  M 2-mercaptoethanol, 1 mM glutamine, 50 U/ml penicillin, and 50  $\mu$ g/ml streptomycin. Con A was present at a final concentration of 2  $\mu$ g/ml. These cells were cultured in Falcon 3024 tissue culture flasks (Falcon Labware, Div. of Becton, Dickinson & Co., Inc., Oxnard, Calif.) in vol of 50 ml. The cultures were incubated at 37°C for 16–18 h in a gas mixture of 7% oxygen, 10% carbon dioxide, and 83% nitrogen, and then harvested. Cells were removed by centrifugation at 2,000 *g* for 10 min, then 10,000 *g* for 20 min. For the precipitation of T-cell-growth activity, 0.1 M  $\alpha$ -methyl-D-mannoside and the  $(\text{NH}_4)_2\text{SO}_4$  was added to cell culture medium at 4°C at a concentration of 40% saturation and gently stirred until all dissolved. The solution was kept 12 h at 4°C and the precipitate was removed by centrifugation at 10,000 *g* for 20 min. The supernate was then brought to 80% saturation with ammonium sulphate, kept another 12 h at 4°C, and the precipitate collected by centrifugation as above. The precipitate was dissolved in 0.1 M ammonium bicarbonate, dialyzed against 100 vol of the same buffer, and fractionated by Sephadex G-100 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) chromatography as detailed elsewhere (9). The fractions were assayed for T-cell-replacing activity as detailed below, and the active fractions pooled, lyophilized, and stored at -70°C. The units of factor activity were calculated as detailed below.

Rat and human TCGF were prepared exactly as detailed elsewhere,<sup>2</sup> and titrated to determine units of activity as detailed below.

*Culture Medium.* All cell cultures utilized RPMI-1640 medium (Grand Island Biological Co.) supplemented with 10% heat-inactivated FCS, 1 mM glutamine, 50 U/ml penicillin, 50  $\mu$ g/ml streptomycin and  $5 \times 10^{-5}$  M 2-mercaptoethanol. TCGF was added to medium at a concentration of 10 U/ml.

*Microculture Assay for Antibody Responses.* Antibody synthesis was measured in the microculture system described elsewhere (11). Spleen cells from C57BL/6.nu or BALB/c.nu mice were distributed into microculture trays (Falcon No. 3034) in 10- $\mu$ l aliquots at cell concentrations of  $1.0 \times 10^5$  cells/well. These wells also contained 0.05% sheep or horse erythrocytes (SRBC; HRBC) as antigen, and the concentrations of TCGF or helper cells detailed in the text. Generally, 120 microcultures (two microculture trays) were regularly employed to test each helper-cell concentration. The cultures were incubated for 5 d, and the supernates from each microculture were assayed using a spot test (11).

To determine the frequency to helper T cells reactive to erythrocyte antigens, the T cells were titrated into microculture wells as detailed in the text, with the appropriate erythrocytes as antigen. After 5 d, the cultures producing antibody were assayed using the same erythrocytes in the spot test (11, 12). The fraction of nonresponding cultures was determined and plotted against the number of T cells used to provide helper T cell activity. This Poisson distribution analysis reveals that when 37% of the microcultures are nonresponsive, there is on the average a single helper cell per microculture (11).

*Unit of Factor Activity.* When Con A factor is diluted into the microculture assay system, the number of positive cultures, as determined by the presence of a lytic spot, decrease. By assaying large numbers of microcultures (i.e., 180), for the presence of anti-SRBC antibody, and determining the fraction of responding microcultures, we quantitate factor activity by defining a unit of activity as the amount of factor that produce one-third (0.33) of the maximal response. The level chosen for the definition of a unit is arbitrary, but it falls within the linear portion of the dose-response curve in both crude and purified factor preparations (8).

*Cloning by Limiting Dilution.* Proliferating T cells were seeded by dilution to a density of 1 cell/ml in complete medium supplemented with 10 U/ml factor. The cells were plated out in Microtest II wells (Falcon Labware, Div. of Becton, Dickinson & Co.), in 200- $\mu$ l aliquots, a dilution such that only one in five microwells, on the average, would be expected to receive a

<sup>2</sup> Gillis, S., K. A. Smith, and J. Watson. Biochemical and biological characterization of lymphocyte regulatory molecules. II. Purification of a class of rat and human lymphokines. Submitted for publication.

T cell. Irradiated nude peritoneal cells ( $10^4$  cells/microwell) were added as filler cells. Cultures were incubated at  $37^\circ\text{C}$  in the gas mixture described above, and the medium was replaced each 3 d. Cultures were examined microscopically for the appearance of cell clones. Approximately 10% of the seeded cells gave rise to clones (i.e., 1 microwell per 50 plated).

*Activated Helper T Cells.* BALB/c or C57BL/6J mice were irradiated (850 rad) using an x-ray source, and then injected with  $10^8$  syngeneic thymocytes together with 0.2 ml 10% SRBC ( $T_{\text{SRBC}}$ ) or 0.2 ml HRBC ( $T_{\text{HRBC}}$ ) i.v. After 7 d, spleen cells were harvested and used as the source of activated T cells.

*Fluorescent Staining of T Cells.* Cells growing in culture were harvested and incubated at  $4^\circ\text{C}$  for 15 min with a rabbit anti-mouse T-cell serum obtained from Dr. G. Gutman, University of California, Irvine. This antiserum has been extensively absorbed using spleen cells from nude mice. T cells were washed and then incubated with a fluorescein-labeled sheep anti-rabbit immunoglobulin (Pasteur Institute, Paris, France).

## Results

*Antigenic Selection and T-Cell Growth in Culture.* In previous reports, the purification and biological properties of a murine TCGF has been described (8–10). TCGF stimulates the proliferation of antigen- or mitogen-activated T cells, but not nonactivated T cells (10).<sup>2</sup> This finding has been utilized as a selection step in the establishment of T-cell lines in culture. Thymocytes were activated to SRBC or HRBC antigens in vivo (Materials and Methods), and then cultured at a density of  $10^6$  cells/ml. Cultures were supplemented with 10 U/ml murine TCGF, 0.02% SRBC or HRBC, and irradiated syngeneic nude spleen cells as fillers at a final concentration of  $10^5$  cells/ml. The TCGF was prepared as detailed above to remove residual Con A. The success of this procedure depends upon removal of Con A, thereby avoiding polyclonal T-cell activation at the initiation of cell cultures. Thus, it was expected that only the T cells activated to antigens in vivo would be stimulated to proliferation by TCGF. The culture medium was replaced three times weekly by fresh medium supplemented with 10 U/ml TCGF. Each week, cells were recultured at a density of  $10^6$  viable cells/ml with  $10^5$  irradiated filler cells. It is readily apparent in these cell cultures that considerable proliferation is occurring. After 3 wk of following the schedule outlined above, the cells were subcultured at a density of  $10^4$  cells/ml. The growth medium was supplemented with 10 U/ml TCGF, but irradiated filler cells and erythrocyte antigens were no longer added to cultures.

The growth of T cells is readily observed until a period which is generally some 5–6 wk after culture initiation. The cells then appear to undergo a crisis period, during which they grow slowly, and cultures are characterized by many dead cells and cellular debris. This period lasts several weeks before rapid cell proliferation is again apparent. The cells that continue to proliferate are strictly TCGF-dependent in growth and have been maintained as such in culture for periods of >50 wk.

*Increase in Frequency of Antigen-specific Helper Cells.* The data presented in Fig. 1 show the increase in frequency of in vivo-activated C57BL/6J  $T_{\text{SRBC}}$  cells at various times after the initiation of cell culture. After thymocytes had been injected into irradiated host mice with SRBC antigens, spleen cells were harvested 7 d later and maintained in cell culture as described above. On day 1, 14, and 28 of culture, the T cells were tested for helper activity to SRBC antigens using a limiting-dilution technique to determine the frequency of  $T_{\text{SRBC}}$  cells (11). On the 1st day of culture, it is routinely observed that in the in vivo-activated T-cell population, ~1 in 4,000 cells show helper activity for SRBC (Fig. 1A). After 14 d in culture, the frequency of  $T_{\text{SRBC}}$  cells has

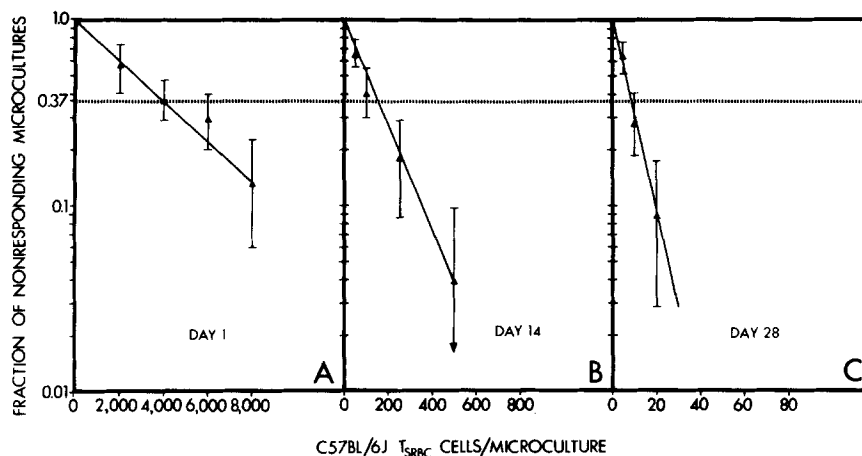


FIG. 1. Frequency of antigen-specific helper T cells during proliferation in cell culture. In vivo-activated C57BL/6J T<sub>SRBC</sub> cells were seeded in culture as detailed in Methods, and the frequency of helper T cells determined on day 1 (A), day 14 (B), and day 28 (C). Frequency determinations were made using C57BL/6.nu spleen cells and SRBC as antigen (11).

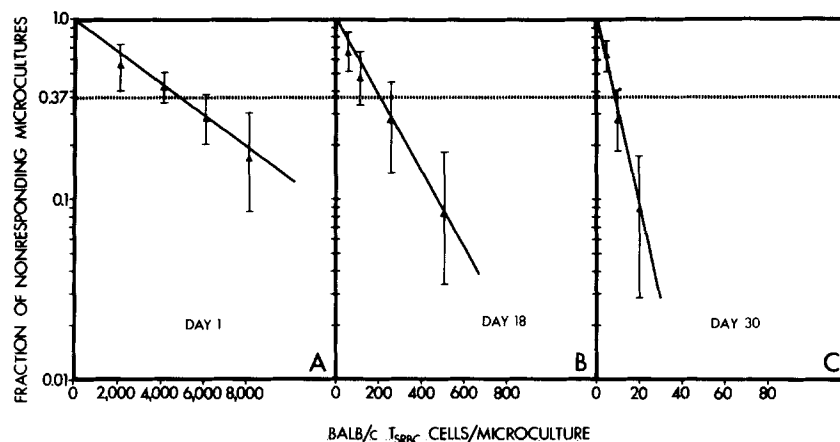


FIG. 2. Frequency of antigen-specific helper T cells during proliferation in cell culture. In vivo-activated BALB/c T<sub>SRBC</sub> cells were seeded in culture as detailed in Methods, and the frequency of helper T cells determined on day 1 (A), day 18 (B), and day 30 (C). Frequency determinations were made using BALB/c.nu spleen cells and SRBC as antigen (11).

increased to 1 in 200 cells (Fig. 1B) and by day 28, the frequency of T<sub>SRBC</sub> cells is between 1 in 10 and 1 in 20 cells (Fig. 1C). Similar data have been obtained using BALB/c thymocytes activated in vivo to SRBC or antigens, and grown in culture with TCGF (Fig. 2), or using HRBC as antigen (data not shown).

All T cells taken from culture containing TCGF are washed before assay for helper activity. There is no indication that any carry-over of TCGF to the nude spleen cultures occurs, thus the helper assay is for the T cells and not residual TCGF.

The TCGF-dependent cells growing in culture were identified as T cells by fluorescent staining using a rabbit anti-mouse Thy-1 antisera. The data presented in Table I summarize the frequency of helper cells specific for SRBC or HRBC antigens

TABLE I  
*Properties of Helper Cells in Culture\**

Strain	Antigenic specificity	Time in culture	Frequency of helper cells‡
		<i>wk</i>	
C57BL/6J	SRBC	50	1:5
C57BL/6J	HRBC	28	1:20
BALB/c	SRBC	50	1:10
BALB/c	HRBC	25	1:10

\* Cell lines are identified as T cells by fluorescent staining using a rabbit anti-mouse Thy-1 antiserum (Materials and Methods). In each cell line, 100% of the cells stained with this reagent.

‡ Frequency determinations were made at the time in culture indicated above, using syngeneic nude spleen cells as detailed in the legends to Figs. 1 and 2.

that have been maintained in culture for periods >5 mo. All cells growing in these cultures are stained with a fluorescent sheep anti-mouse T-lymphocyte serum, thus it appears that only T cells are growing in these cultures (Table I).

After cells had been growing in culture for >10 wk, various methods were used in an attempt to clone the proliferating cells. In preliminary experiments, we observed that the T cells grew poorly in soft agar supplemented with TCGF, therefore, we used a limiting-dilution procedure as detailed in Materials and Methods. Microwells were examined microscopically for growth, and ~14 d after seeding T cells at limiting dilutions (Materials and Methods), clones were transferred to large cultures. Each culture was then tested for helper activity. The properties of one such cell line, C57BL/6J T<sub>SRBC</sub>, designated HT-1 cells, are detailed below.

*Antigenic and H-2 Specificity.* The limiting-dilution procedure was used to analyze the antigenic and H-2 specificity of helper cells growing in culture. The results of a typical experiment are described in Fig. 3. Two strains of nude mice were used as sources of B cells to determine the H-2 specificity of helper activity, and antibody responses to SRBC and HRBC antigens were tested to follow antigen specificity. When a line of C57BL/6J T<sub>SRBC</sub> cells (HT-1) is assayed using C57BL/6.nu spleen cells for responses to SRBC and HRBC antigens, a helper cell frequency of 1:10 to 1:40 is observed to SRBC, but <1:1,000 to HRBC (Fig. 3A). When HT-1 cells are assayed using BALB/c.nu spleen cells for responses to SRBC and HRBC, little helper activity is observed (Fig. 3B). Thus, the helper activity of HT-1 cells is antigen specific and appears to be exerted only on syngeneic B cells.

The requirement for syngeneic B cells is observed in all cultures that possess antigen-specific helper T cells. The data presented in Fig. 4 show that helper activity of a line of C57BL/6J T<sub>SRBC</sub> cells assayed for activity to SRBC on both BALB/c.nu and C57BL/6.nu spleen cells at various times during growth in culture. During the period from 5–20 wk after initiation of cultures, the frequency of T<sub>SRBC</sub> helper cells when assayed using C57BL/6.nu spleen cells varied between 1:10 and 1:40 cells (Fig. 4). The data shown for 5 and 9 wk (Fig. 4 A and B) were obtained using the original cell cultures. At 9 wk, the cells were cloned (Materials and Methods), and the data shown for 14 and 20 wk (Fig. 4 C and D) were obtained using one clone (HT-1) derived from this parent culture. It is apparent that cloning has not altered the helper cell characteristics of the parent culture population (Fig. 4). When the same T cells

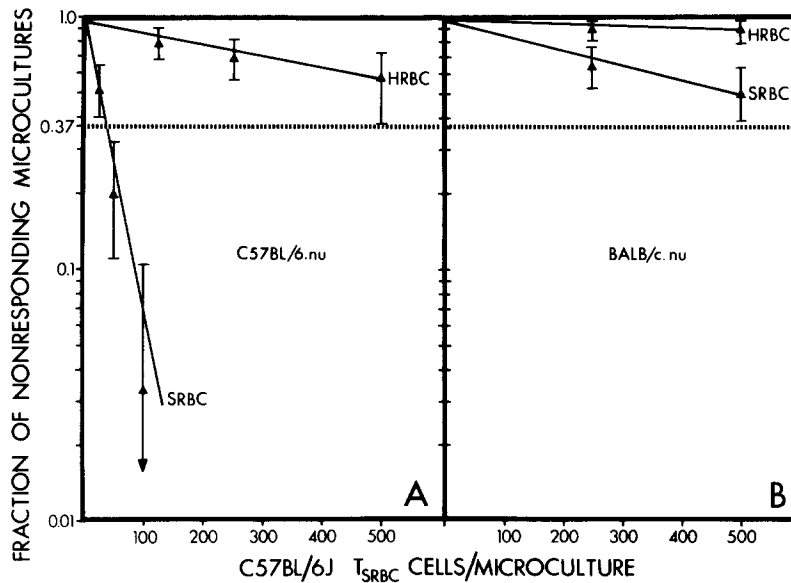


FIG. 3. Antigenic and H-2 specificity of helper T cells in culture. A line of C57BL/6J  $T_{SRBC}$  designated HT-1 was assayed for helper activity in microcultures containing (A) C57BL/6.nu, or (B) BALB/c.nu spleen cells. Microcultures contained either SRBC or HRBC as antigens. The HT-1 cells have been growing in culture for 30 wk at the time of this assay.

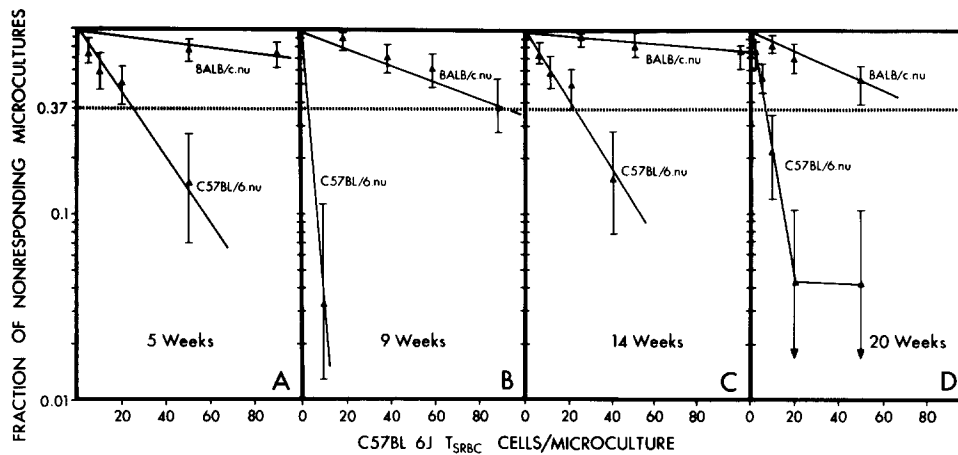


FIG. 4. Requirement for syngeneic B cells in helper cell assay. C57BL/6J  $T_{SRBC}$  cells were assayed using a limiting-dilution microculture procedure after 5 wk (A), and 9 wk (B) in culture for helper activity specific for SRBC antigens. At 9 wk, this cell line was cloned (see text) and one clone designated HT-1 assayed at 14 wk (C), and 20 wk (D) for helper activity. At each time, the helper cells were assayed both in microcultures containing C57BL/6.nu and BALB/c.nu spleen cells.

were assayed using C57BL/6.nu spleen cells, the frequency of  $T_{SRBC}$  helper cells revealed was always at least 10-fold lower (Fig. 4). Therefore, the helper activity is both antigen-specific and shows a restriction in helper activity for the B-cell source used in the antibody-induction assay.

BALB/c  $T_{SRBC}$  cells have also been cloned, and the analysis of one of these clones'

HT-2 reveals similar properties to those described for HT-1 cells (data not presented).

*Properties of T-Cell Lines.* At this time, the following properties of these various T-cell lines can be summarized:

(a) All cell lines require the presence of TCGF for continued growth. Removal of TCGF from the culture medium rapidly results in cell death. The T-cell mitogens Con A and PHA do not stimulate cell proliferation in long-term cultures, and thus appears nonmitogenic for pure populations of T cells.

(b) All cells that grow in the presence of TCGF express Thy-1 antigens. There appears to be no difference in the expression of Thy-1 antigens on cells in unselected or cloned cell culture lines.

(c) Supernates from antigen-specific helper T-cell lines do not contain antigen-specific helper factors. T cells from long-term cultures incubated in medium in the absence of TCGF, but with either Con A or PHA, do not secrete TCGF. Preliminary experiments reveal these cells secrete an antigen-nonspecific factor that stimulates immune responses to erythrocyte antigens in T-cell-depleted cultures (unpublished data). Such factors are currently being analyzed.

(d) Antigen-specific (SRBC or HRBC) helper T-cell lines derived from C57BL/6J or BALB/c mice express activity only on syngeneic or (C57BL/6J  $\times$  BALB/c)F<sub>1</sub>, spleen cells.

(e) The stability of helper activity in these cell cultures varies. Several T-cell clones (HT-1 and HT-2) have maintained helper activity for long periods. Many other cell lines have shown a gradual decline in the capacity to provide antigen-specific helper activity.

*TCGF and Cell Proliferation.* All cell lines established in culture have a strict requirement for TCGF for continuous proliferation. The data presented in the experiment of Fig. 5 A show that two cell lines, HT-1 (C57BL/6 T<sub>SRBC</sub>) and HT-2 (BALB/c T<sub>SRBC</sub>), grow with a doubling time of 20–30 h in medium supplemented with 10 U/ml murine TCGF. Cells are seeded at a density of  $1-2 \times 10^4$  cells/ml, and reach saturation density from  $1-2 \times 10^5$  cells/ml.

The data presented in the experiment of Fig. 5 B show that murine T cells proliferate in the presence of TCGF prepared from murine, human, and rat sources (10).<sup>2</sup> The preparation of rat TCGF<sup>2</sup> and human TCGF<sup>2</sup> murine lymphocytes have been detailed elsewhere. When murine T cells are grown in medium supplemented with 10 U/ml of either murine, human, or rat TCGF, the growth rates are indistinguishable.

## Discussion

Several groups have recently reported the successful growth of human (1) and murine T cells in culture (2–7, 13). A number of these cell lines retain the cytolytic effector function characteristic of mature cytotoxic T cells (1–7). The proliferation of T cells in culture requires either the presence of TCGF derived from mitogen-treated human (1), rat, or murine lymphoid cells (2–6, 13),<sup>2</sup> or repeated stimulation with irradiated allogeneic spleen cells (7).

The experiments described in this paper establish a procedure for the selection and continuous growth of antigen-specific T-helper cells in culture. Proliferation of T cells in culture is dependent upon the presence of a growth factor, TCGF. We have recently described the purification of TCGF from supernates of Con A-activated murine (10),

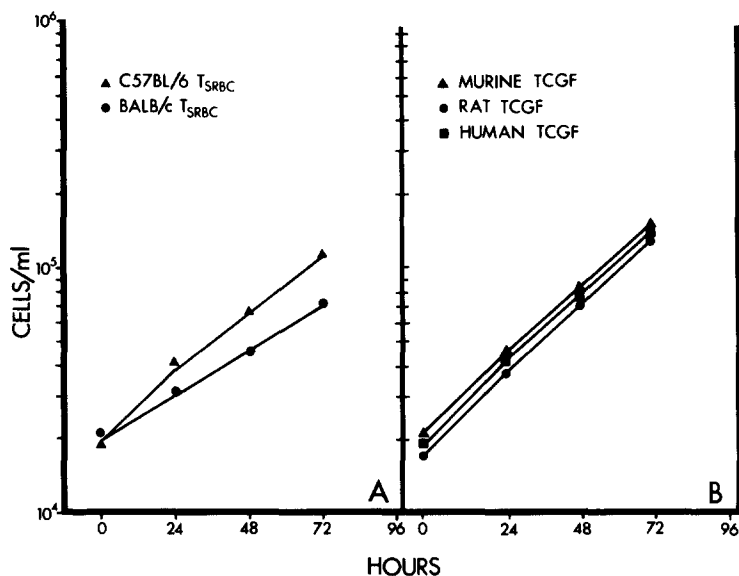


FIG. 5. Growth of cells in culture. (A) C57BL/6J  $T_{SRBC}$  and BALB/c  $T_{SRBC}$  cells were seeded at a density of  $2 \times 10^4$  cells/ml in medium supplemented with 10 U/ml murine TCGF. After 24, 48, and 72 h, the cell numbers in culture were determined. (B) C57BL/6J  $T_{SRBC}$  cells were seeded at a density of  $2 \times 10^4$  cells/ml in medium supplemented with 10 units/ml of murine ( $\blacktriangle$ ), rat ( $\bullet$ ), or human ( $\blacksquare$ ) TCGF. After 24, 48, and 72 h, the cell numbers in culture were determined.

rat,<sup>2</sup> and human<sup>2</sup> lymphoid cell cultures. Murine TCGF is contained in protein with a mol wt of 30,000 which is separable in charge into two components of pI 4.3 and pI 4.9 (10). Both isoelectrically distinct components exhibit identical biological activity (10). Rat and human TCGF is contained in protein of 15,000 daltons in size, but rat has a pI of 5.5, whereas human has a pI of 6.0–6.5.<sup>2</sup> For the murine T-cell lines we have established, TCGF from any of those species possess similar proliferative activities (Fig. 5 B).

It has also been demonstrated that TCGF will only stimulate proliferation in antigen- or mitogen-activated T cells (10).<sup>2</sup> This finding has been utilized as a selection procedure. Helper T cells were first activated *in vivo* (Materials and Methods) to SRBC or HRBC antigens, and then cultured with TCGF. Although the growth of T cells is antigen independent in continuous cultures, antigen and irradiated, syngeneic filler cells were employed with TCGF (10 U/ml) in the initial 3 wk of culture. In principle, this same selection procedure should work for any antigen to which T cells can be activated. Because TCGF has been shown to stimulate the proliferation of both cytotoxic and helper classes of murine T cells (10), it would seem likely that T cells of cytotoxic, helper, and suppressor subclasses can be selected for. It is important to stress that after antigen activation, T cells were exposed to TCGF that had been purified away from Con A, used to make TCGF (8). The presence of Con A in the initial culture period is sufficient to cause a polyclonal T-cell activation that will serve to mask any prior antigen-selection process.

Although the cell lines described here have maintained their helper activity for >50 wk after transfer of cells to continuous culture, several features of helper activity should be emphasized:



(a) The helper activity is analyzed by a limiting dilution assay utilizing microcultures of nude spleen cells (11, 12). The antigen-specific helper activity fluctuates in the continuously proliferating T-cell populations in the range of 1:5 to 1:40 cells (Figs. 1-4). These fluctuations may reflect a changing expression of helper activity as a result of variation in metabolic activity of cells at the time they are assayed, or may reflect a limitation in the B-cell response assay. In the limiting-dilution analysis to determine the frequency of antigen-specific helper T cells, when 1-40 cells are diluted into  $10^5$  nude spleen cells, the induction of antibody synthesis is only observed if interaction with the appropriate antigen-specific B cells occurs. The range of 5-40 helper T cells may represent the numbers of cells needed to ensure successful cell cooperation under these culture conditions. The microculture assay measures only cultures in which antibody to the test antigen is detected, therefore successful induction has occurred. If the burst size of antibody-forming cells were measured, it would be expected to increase as the number of helper cells is increased. The frequency analysis of helper cell activity in limiting-dilution microcultures, is a stringent means of determining specificity and the T-cell numbers required to observe responses.

(b) Many, but not all, of the T-cell lines that grow using the antigen-selection and culture procedures described above, exhibit helper activity. Because we screen only for those T-cell cultures that exhibit antigen-specific helper activity to SRBC or HRBC, T cells of other subclasses would not be detected. We have preliminary evidence that helper T cells activated to serum substances, to cell surface alloantigens and to antigens found on both SRBC and HRBC can be found in some of these T-cell cultures (Unpublished data.).

(c) Antigen-specific helper T cells show a marked preference for syngeneic B cells in the expression of biological activity (Figs. 2, 3). The antibody response of the nude B-cell population detected here is the IgM class. Although the induction of antibody responses have been tested only using BALB/c.nu and C57BL/6.nu spleen cells, it is a reasonable assumption that the preference for syngeneic B cells is an expression of H-2 restriction between helper T cells and IgM B-cell precursors (14).

In summary, a number of murine T-cell lines have been maintained in culture for periods up to 1 yr. Because they retain antigen specificity and an effector T-cell function, they provide tools for the molecular analysis of the antigen-binding receptors of T cells, and the mechanism of cell communication for the induction of immune responses.

### Summary

Murine helper T cells activated to sheep or horse erythrocyte antigens *in vivo* have been established as continuous cell lines in culture. T cells require the presence of a T-cell growth factor (TCGF) for continuous proliferation. TCGF purified from murine, rat, or human sources all stimulate murine T-cell growth. The T-cell mitogens concanavalin A and phytohemagglutinin do not stimulate cell proliferation in continuous T-cell lines. All cells that grow in the presence of TCGF express Thy-1 antigens. Helper activity of T-cell lines is both antigen specific and effective for syngeneic or F<sub>1</sub> B cells. Supernates from T-cell lines do not contain antigen-specific or nonspecific helper factors. Although several T-cell lines have shown stable helper activity for >50 wk in culture, other cell lines have shown a gradual decline in effector function. The procedure used to establish and maintain proliferation of T cells in culture should be

suitable for the selection and growth of antigen-specific effector T cells from each subclass.

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