Proc. Natl. Acad. Sci. USA
Vol. 78, No. 2, pp. 1157–1161, February 1981
Immunology

Multiple biologic activities of a cloned inducer T-cell population

(inducer T-cell clones/T-cell factors/granulocyte-macrophage colony-stimulating factors)

GARY NABEL, JOEL S. GREENBERGER, MARY ANN SAKAKEENY, AND HARVEY CANTOR

Departments of Cell Biology, Radiation Therapy, and Pathology, Harvard Medical School; and Joint Center for Radiation Therapy, Sidney Farber Cancer Institute, Boston, Massachusetts 02115

Communicated by Edward Boyse, November 3, 1980

ABSTRACT **The mouse T-cell clone Ly1$^+$2$^-$/9, belonging to the Ly1 set, displays the following functions *in vitro*: (*i*) augmentation of immunoglobulin output by B cells; (*ii*) stimulation of bone marrow cells to produce colonies composed of granulocytes, macrophages, or both; and (*iii*) proliferative stimulation of T-cell clones belonging to other Ly sets. These functions are induced by Ly1$^+$2$^-$/9 cells themselves and by supernatants of Ly1$^+$2$^-$/9 cultures and are not evinced by tested clones belonging to other Ly sets. The agent or agents responsible for colony formation and for B-cell stimulation had an apparent molecular weight of 45,000–50,000 and could not be physically separated. The T-cell stimulating agents(s) had an apparent molecular weight of 30,000 and could be separated from the agent(s) that acts upon colony formation and B cells. Thus, clone Ly1$^+$2$^-$/9 produces at least two soluble products that induce or augment activities of at least three other differently programmed cell sets.**

A portion of the T-lymphocyte population activates several other immunologic and nonimmunologic cells, and this T-cell set ("inducer") is identified by the surface phenotype Lyt-1$^+$:Lyt-23$^-$ (1). Cells of the inducer set activate B cells to secrete immunoglobulin, induce hematopoietic precursor cells to make colonies, and stimulate the multiplication of T cells belonging to other sets (1–4). To further understand the multiple functions of inducer cells, we have developed a general method for producing cloned populations of this set and of other T-cell sets (5). In this report, we compare the biologic activities of a cloned line called Ly1$^+$2$^-$/9 (Cl.Ly1$^+$2$^-$/9) with T-cell clones that express different surface phenotypes.

MATERIALS AND METHODS

Animals. T cells for cloning were all obtained from C57BL/6 mice. Cells from C57BL/6 or BALB/c mice (obtained from The Jackson Laboratory, Bar Harbor, ME) were used to produce conditioned medium (see below).

Antisera. Lyt-1.2 and Lyt-2.2 antisera, prepared as described (6), were kindly donated by F. W. Shen; monoclonal antibody against Thy-1.2 mc-α-Thy-1.2 was donated by Ed Clark; and mc-α-Lyt-1 and mc-α-Lyt-2 were donated by J. Ledbetter and L. Herzenberg. Expression of Ly1 and Ly2 by each clone was determined with both Lyt antisera and monoclonal Lyt antibodies by immunofluorescence.

Cell Culture and Cloning. Culture conditions for initiation and maintenance of cell lines have been described (5). Briefly, cells were diluted into microwells (Falcon plastic microtiter plates no. 3040) at estimated final concentrations of 100, 10, 1, and <1 cell per well. Each well contained 0.1 ml of complete conditioned medium (5) and irradiated (1500 R; 1 R = 2.58 × 10^{-4} C/kg) cell monolayers from different tissues (final concentrations 4–8 × 10^5 cells per well). Cultures were supplemented every 2 days with 30 μl of complete conditioned medium until colonies were visible (10 days–3 weeks) in wells that initially received <100 cells. Cloning efficiency was calculated by Poisson distribution (7). At least 96 wells were used to calculate each cloning efficiency. The fraction of negative wells was plotted as a function of the logarithm of initial cell number. Single cells from colonies arising at frequencies <36% with cloning efficiency >5% were regrown twice either after dilution at <1 cell per well or after single-cell micromanipulation. With the possible exception of cells mediating NK lysis, which occasionally lost activity, the characteristics of these initial cell colonies were stable and identical to those of cloned populations derived from them by single-cell micromanipulation or redilution. Separate tests of more than 12 different cell colonies showed that 3–10 daughter clones derived from each expressed the cell-surface antigens, structure, and function of the parent colony. After the initial cloning procedure, cells were expanded in cultures containing conditioned medium in the absence of irradiated cell monolayers (5). Doubling times were 24–48 hr for all cells tested. The number of cells could be increased to >10^8. At least 20 different cloned populations have been propagated for more than 8 months *in vitro*.

Frozen Storage of Cells. Cloned cells were frozen in liquid N_2 and thawed with complete recovery of growth and function by the use of a modification (5) of the method of Lionetti *et al.* (8).

Preparation of Selected Lymphoid Populations. Ly1 cells were obtained as follows. Single-cell suspensions were incubated on rabbit anti-mouse Ig-coated plates to remove Ig-positive cells. The remaining cells were then incubated (5 × 10^7 cells per ml) with α-Lyt-2.2 for 30 min at 4°C, centrifuged, and resuspended with selected rabbit serum (complement) at a predetermined optimal concentration (1:10 final dilution) at 37°C for 40 min as described (1, 5). Contamination by B cells was assessed by counting the number of Ig-secreting cells observed after incubation for 5 days at 37°C *in vitro*. In most experiments no Ig-secreting cells were detectable.

To obtain purified B cells, we suspended spleen cells in α-Lyt-1.2 plus α-Lyt-2.2 plus mc-α-Thy-1.2 at optimal lytic concentrations for 30 min at room temperature, washed them, and incubated them in a selected rabbit serum (complement) at 37°C for 40 min. To ensure depletion of all T cells, we then further incubated the remaining cells with mc-α-Thy-1.2 (final dilution 1:1000) and rabbit complement at 37°C for 30 min. These remaining cells did not secrete appreciable Ig after 4 days of culture *in vitro* unless purified T cells were added.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: CFU-c, colony-forming units–culture; Cl, clone; CSF, colony-stimulating factor; GM, granulocyte-macrophage; mc-α, monoclonal antibody.

Stimulation of Ig Secretion *in Vitro*. Cell culture conditions have been described (9). Briefly, 10^6 nonimmune B cells or mixtures of Lyl cells (2×10^5) and B cells (8×10^5) were incubated for 4 days in 0.2 ml of Dulbecco's modified Eagle's medium supplemented with 4% (vol/vol) fetal calf serum, 50 μM 2-mercaptoethanol, 2 mM glutamine, nonessential amino acids, and essential vitamins.

Enumeration of Ig-Secreting Cells. Ig-secreting cells were enumerated by a modification of a hemolytic plaque assay described elsewhere (10, 11). Twenty-five microliters of a 12% suspension of horse erythrocytes, coated with rabbit anti-mouse $F(ab')_2$, and 25 μl of lymphocytes from the cell culture were pipetted into 10×75 mm glass test tubes and mixed with 450 μl of a 0.8% solution of Sea-Plaque agarose (Maine Colloids, Rockland, ME) in a special balanced salt solution. The contents of each tube were layered on 2 ml of gelled 1% Sea Kem agarose (Maine Colloids) in a 30×10 mm petri dish. After 1 hr at 37°C in a humid atmosphere containing 5% CO_2, 0.34 ml of rabbit anti-mouse $F(ab')_2$ developing serum (final dilution 0.05 mg/ml in special balanced salt solution) was pipetted onto the petri dishes. After another hour at 37°C, the antiserum was decanted and replaced with an equal volume of reconstituted guinea pig complement (GIBCO), diluted 1:10. Zones of lysis (plaques) were enumerated 2 hr later.

Preparation of Supernatant from Cl.Ly1$^+$2$^-$/9. Cells were washed three times and resuspended at a final concentration of 5×10^5/ml in modified Eagle's medium (5) supplemented with 5 μg of human transferrin per ml, 5 μg of bovine insulin per ml, 50 μM 2-mercaptoethanol, and 2 mM glutamine. After 24 hr at 37°C in a humidified atmosphere with 10% CO_2, the cells were centrifuged at $300 \times g$ for 10 min. For sterility, supernatant was passed through a 0.22-μm Swinnex filter.

Assay of T-Cell Proliferation *in Vitro*. T-cell proliferation was determined for three cloned Thy-1$^+$ cell populations: Cl.Ly1$^-$2$^-$/11, Cl.Ly1$^-$2$^+$/16, and Cl.Ly1$^+$2$^+$/10. Cells were incubated in flat-bottom microtiter wells (Falcon 3040) in modified Eagle's medium/4% heat-inactivated fetal calf serum/50 μM 2- mercaptoethanol/2 mM glutamine at final cell concentrations of $1–10 \times 10^4$/ml with varied concentrations of supernatant in a final volume of 0.1 ml. After incubation for 24 hr at 37°C in a humidified atmosphere containing 10% CO_2, 0.5 μCi of [^{3}H]thymidine ([^{3}H]dThd) was added to each well (New England Nuclear; specific activity, 5.4 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels). Four hours later each cell culture was applied to a glass filter strip and radioactivity was measured by liquid scintillation.

Preparation of Purified Granulocyte-Macrophage (GM) Progenitor Cells. Nonadherent cells from 96-day continuous mouse bone marrow cultures were harvested as described (12, 13). Briefly, cells from a single mouse femur and tibia were cultured in 10 ml of Fisher's medium supplemented with 25% fetal calf serum and 0.1 μM hydrocortisone hemisuccinate. Nonadherent cells and medium were removed weekly and replaced wth 10 ml of fresh medium. Nonadherent cells harvested 96 days after initiation of culture were depleted of polymorphonuclear leukocytes, band forms, and metamyelocytes by density sedimentation. Cell purity was monitored by the Wright–Giemsa and ASD–chloroacetate esterase stains. We found 86–94% immature granulocytes and <1% macrophages or mast cells by morphologic and histochemical examination. The remaining cells (<14%) were mature granulocytes. This method removes adherent cells capable of endogenously producing GM colony-stimulating factor (CSF) (13).

Assay for GM Colony-Forming Units–Culture (CFU-c). Immature granulocytes retrieved from 96-day marrow cultures were transferred to petri dishes (0.5×10^5/ml) as described (14) and incubated at 37°C in 7% CO_2 for 7 days. CSF from L929 fibroblast cells (15) served as a positive control. Cl.Ly1$^+$2$^-$/9 cells ($0.5–2 \times 10^5$/ml) or cell-free supernatant (see above) was added to 1-ml agar cultures. After 7 days at 37°C, colonies of >50 cells and clusters of 10–49 cells were enumerated with a Unitron inverted microscope (14). Colonies were also removed for morphologic and histochemical examination (12).

Molecules Synthesized by Cl.Ly1$^+$2$^-$/9 Cells: Separation of Molecules Stimulating GM CFU-c and Ig Secretion from Molecules that Activate T Cells. Cl.Ly1$^+$2$^-$/9 cells were washed three times and incubated for 30 min at 2×10^6 cells per ml in methionine- and leucine-free minimal essential medium supplemented with glucose (4.5 g/liter), essential vitamins, bovine insulin (5 μg/ml), human transferrin (5 μg/ml), 2-mercaptoethanol (50 μM), and glutamine (2 mM). An equal volume of this medium containing 10 μCi of [^{35}S]methionine per ml (800 Ci/mmol, New England Nuclear) and 10 μCi of [^{3}H]leucine per ml (111 Ci/mmol, New England Nuclear) was added, and incubation was continued for 4 hr at 37°C. Modified Eagle's medium containing bovine insulin (5 μg/ml), human transferrin (5 μg/ml), 2-mercaptoethanol (50 μM), leucine (8 mM), methionine (2 mM), and glutamine (2 mM) was added, and the cells were incubated (5×10^5/ml) for an additional 20 hr at 37°C. Supernatants were concentrated by vacuum dialysis and fractionated on a column of Sephadex G-100 (35×1 cm), and 56 fractions (0.8 ml) were tested for stimulation of GM CFU-c;

Table 1. Cl.Ly1$^+$2$^-$/9 cells activate B cells to secrete Ig

Cloned cells, no. $\times 10^{-4}$/culture	No. of Ig-secreting cells after addition of:		
	Cl.Ly1$^+$2$^-$/9	Cl.Ly1$^-$2$^+$/11	Cl.Ly1$^+$2$^+$/12
0	120, 40	—	—
0.4	200, 360	204	377
3.0	2040, 1480	260	104
10.0	4000, 5260	ND	ND

Cells from Cl.Ly1$^+$2$^-$/9, Cl.Ly1$^-$2$^+$/11, or Cl.Ly1$^+$2$^+$/12 were added in increasing numbers to 10^6 B cells and incubated for 4 days. ND, not done.

Table 2. Cl.Ly1$^+$2$^-$/9 supernatant activates B cells to secrete Ig

Dilution of supernatant	No. of Ig-secreting cells		
	Cl.Ly1$^+$2$^-$/9	Cl.Ly1$^-$2$^+$/16	Medium alone
None added	120, 40	100, 60	120, 40
10^{-3}	720, 880	ND	ND
10^{-2}	3040, 3080	ND	ND
10^{-1}	5240, 4960	80, 40	40, 100

Supernatant from Cl.Ly1$^+$2$^-$/9 or Cl.Ly1$^-$2$^+$/16 or control medium was substituted for cloned cells. ND, not done.

Table 3. Cl.Ly1$^+$2$^-$/9 cells stimulate GM colony formation

Cloned cell	Cloned cells, no. $\times 10^{-4}$/culture	Colonies, no.	Clusters, no.
Medium alone	0	0, 0	0, 0
Ly1$^-$2$^-$/11	10	0, 0	0, 0
Ly1$^-$2$^+$/16	10	0, 0	0, 0
Ly1$^+$2$^-$/9	10	48, 57	60, 100
L929 CSF*	0	106, 97	>100, >100

Cells from the clones were incubated for 7 days in soft agar with 10^5 macrophage-depleted, cultured bone marrow cells.
* Positive control: L929-derived CSF.

Table 4. Cl.Ly1$^+$2$^-$/9 supernatant stimulates GM colony formation

Source of supernatant	Dilution of supernatant	Colonies, no.	Clusters, no.
Medium alone	None added	0, 0	0, 0
Ly1$^-$2$^-$/11	1:10	0, 0	0, 0
Ly1$^-$2$^+$/16	1:10	0, 0	0, 0
Ly1$^+$2$^+$/4	1:10	0, 0	0, 0
Ly1$^+$2$^-$/9	1:10	24, 24	68, 48
L929 CSF*	1:10	120, 88	100, 80

Supernatants in serum-free medium were substituted for cloned T cells.
* Positive control: L929-derived CSF.

Table 5. Activation of purified GM progenitor cells by Cl.Ly1$^+$2$^-$/9 supernatant

Source of CSF	Macrophage, %	Mixed GM, %	Granulocyte, %
Cl.Ly1$^+$2$^-$/9	77	21	2
L929 cells	95	4	1
WEHI-3 cells	60	20	20

CSF from the indicated sources was added to nonadherent, macrophage-depleted, cultured bone marrow cells. After incubation for 7 days, colonies (>200) were removed from the soft agar with a Pasteur pipette, stained with Wright–Giemsa, and classified.

activation of B cells to secrete Ig; and stimulation of T-cell proliferation.

RESULTS

By using a general method that allows continuous propagation of lymphocytes from each major T-cell set (5), we have generated and studied more than 10 Thy-1$^+$ clones of the phenotype Ly1$^+$2$^-$ typical of inducer T cells. We detail here the ability of one cloned population, Cl.Ly1$^+$2$^-$/9, to activate various target cells. This cloned line, derived from a single cell ($P \leq 0.002$; see *Materials and Methods*), has been propagated in continuous culture for >15 months, and all cells of this clone have expressed a stable profile of cell-surface components (Ig$^-$Lyb-2$^-$Thy-1$^+$Ly$^+$Ly2$^-$Ly3$^-$Ly5$^+$NK-1$^-$Qat5$^-$) and characteristic biological functions.

Cl.Ly1$^+$2$^-$/9 Cells and Cell-Free Supernatants Activate B Cells to Secrete Ig. Uncloned Ly1 cells activate B cells to secrete Ig (1). Addition of as few as 10^4 Cl.Ly1$^+$2$^-$/9 cells to 10^6 purified B cells activated Ig secretion (Table 1); 10^5 Cl.Ly1$^+$2$^-$/9 cells gave a 60-fold increase in Ig secretion as compared with background secretion. Cell-free supernatant was an effective substitute for intact Cl.Ly1$^+$2$^-$/9 cells (Tables 1 and 2).

Supernatants diluted approximately 10^{-3} stimulated B cells to secrete Ig (Table 2). Addition of higher concentrations resulted in a 50- to 70-fold increase in Ig secretion. Stimulation of Ig secretion was not accompanied by any increase in the number of B cells during the 4-day culture, according to viable cell counts and to [^{3}H]dThd incorporation in comparison with controls not exposed to supernatant. As previously noted (5), cloned T-cell populations not expressing the Ly1$^+$2$^-$ inducer phenotype did not stimulate Ig secretion (Table 1).

Cl.Ly1$^+$2$^-$ Cells and Supernatants Stimulate GM CFU-c in Semisolid Agar. Addition of 5×10^4 Cl.Ly1$^+$2$^-$/9 cells, but not of cloned cells of different Ly phenotypes, induced significant numbers of GM CFU-c both from freshly removed bone marrow cells and from purified GM CFU-c progenitor cells (Table 3). Supernatant from Cl.Ly1$^+$2$^-$/9, but not from clones of phenotype other than Ly1$^+$2$^-$, also supported colony growth (Table 4). Most colonies (≈77%) were of the homogeneous macrophage type, about 20% were mixed colonies of granulocytes and macrophages, and a few were homogeneous granulocyte populations (Table 5).

Supernatants from Cl.Ly1$^+$2$^-$/9 Cells Stimulate T-Cell Proliferation. Concanavalin A-activated mouse spleen cells secrete a factor with an apparent M_r of 30,000 that stimulates proliferation of T cells *in vitro* (16, 17). This molecule has also been

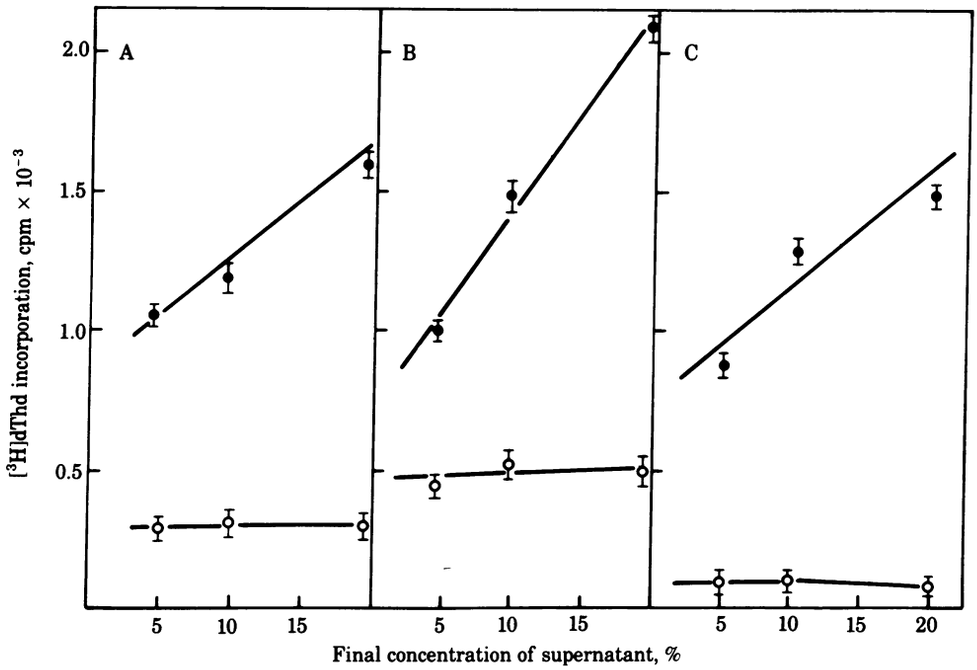

FIG. 1. Cl.Ly1$^+$2$^-$/9 cells synthesize material that stimulates proliferation of other cloned T-cell lines. Supernatants, generated in serum-free culture, were added in graded concentrations to clones of phenotype Ly1$^+$2$^+$ (*A*), Ly1$^-$2$^+$ (*B*), or Ly1$^-$2$^-$ (*C*). [^{3}H]dThd incorporation was measured after 24 hr. ●, Supernatant from Cl.Ly1$^+$2$^-$/9; ○, supernatant from Cl.Ly1$^-$2$^+$/16. Values are means ± SEM.

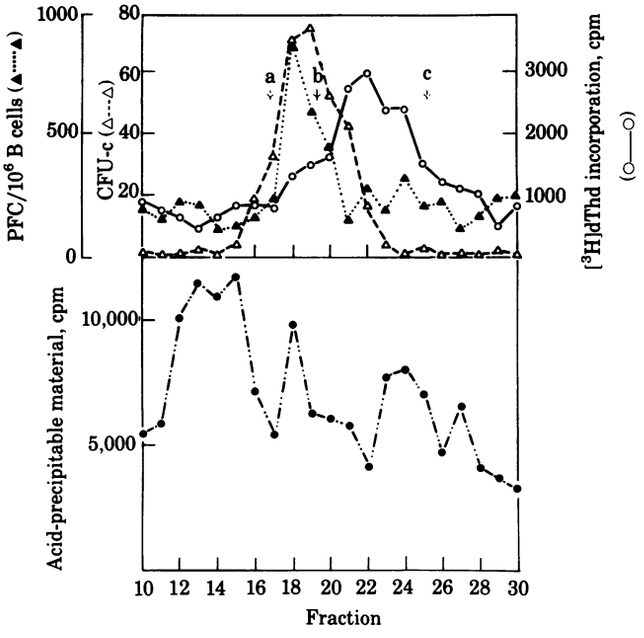

FIG. 2. Functional assays of Sephadex G-100 fractions from Cl.Ly1$^+$2$^-$/9 supernatant. (*Upper*) ▲···▲, Stimulation of Ig-secreting B cells (PFC, plaque-forming cells); △---△, stimulation of GM colony formation (colonies/10^5 cells); ○—○, stimulation of T-cell proliferation. Arrows indicate locations of the molecular weight standards: a, bovine serum albumin; b, ovalbumin; c, lysozyme. (*Lower*) Trichloroacetic acid-precipitable material from Cl.Ly1$^+$2$^-$/9 supernatant.

identified in supernatants of mixed lymphocyte culture-activated spleen cells. Fig. 1 shows assays of the ability of Cl.Ly1$^+$2$^-$/9 supernatant to induce T-cell proliferation, as determined by [^{3}H]dThd incorporation, in three different Thy-1$^+$ clones of phenotypes Ly1$^+$2$^+$, Ly1$^-$2$^+$, and Ly1$^-$2$^-$. All three indicator clones were stimulated by Cl.Ly1$^+$2$^-$/9 supernatant; none was stimulated by control supernatant of Cl.Ly1$^-$2$^+$/16 (Fig. 1) or by Ly1$^+$2$^+$ clones (data not shown). Supernatant from Cl.Ly1$^+$2$^-$/9 gave a 5- to 13-fold increase in [^{3}H]dThd uptake after 24 hr (Fig. 1). This stimulation of T-cell proliferation was mediated by molecules having an apparent M_r of 30,000, according to Sephadex G-100 chromatography (Fig. 2).

Separation of T-Cell Growth-Promoting Activity from GM CFU-c Stimulating Activity and B-Cell Stimulating Activity by Sephadex G-100 Column Chromatography. To characterize the molecular species associated with each function, we concentrated radiolabeled supernatants by vacuum dialysis and fractionated them by Sephadex G-100 column chromatography. Stimulation of T-cell proliferation was limited to several fractions with a mean apparent M_r of 30,000. In contrast, stimulation of GM CFU-c production and of B-cell Ig production was located in several fractions with an apparent M_r of 45,000–50,000. All three activities were sensitive to treatment with Pronase or trypsin.

DISCUSSION

Uncloned Ly1 T cells activate several immunologic and other cell types. We now see that the progeny of a single Ly1 clone can perform at least three of these inducer functions, synthesizing at least two factors. One of these factors has an apparent M_r of ≈30,000 and fits descriptions of interleukin II (16, 17). Previous reports that T-cell growth factor requires Ly1 cells for its production (4, 17) do not exclude synthesis by a different cell acted upon by Ly1 cells. However, the T-cell growth factor we describe is clearly synthesized by Ly1 cells.

T inducer cells circulate freely and may be thought of as sentinels which are alerted by antigens (18, 19). Such specific stimulation would be expected to prompt macrophage precursors to divide and differentiate and B cells to secrete Ig, leading to local expansion of immunologic cells and an effective inflammatory response. In the context of tumor immunology, for example, one may consider the following: inducer cells are specifically stimulated by lymphoma cells, according to [^{3}H]dThd uptake by Ly1 cells from immunized donors, yet their admixture with inoculated lymphoma cells fails to retard tumor growth (20) and may even enhance it. Admixture of T cells of the cytotoxic set, on the other hand, prevents lymphoma growth. We suggest that lymphoma cells, like malignant cells of endocrine organs (21, 22), may remain responsive to hormone-like products of inducer cells (themselves no doubt in turn subject to hormonal regulation). The lesson may be that in designing protocols for immunologic control of tumor growth, the effects of activated immunologic inducer cells intended for therapeutic purposes should first be assessed *in vitro* along the lines indicated in this report.

The two other functions we have investigated, stimulation of GM colony formation and of B cells, were not traced to separable factors; both were associated with supernatant fractions of M_r 45,000–50,000. The M_r of GM CSF purified from cultured yolk sac cells is reported to be 60,000 (23); that from cultured L cells is 70,000 (24). We find that the latter does not stimulate Ig production by B cells. Although T cells have been implicated in GM CSF production, there has been no evidence that T cells themselves synthesize any GM CSF; partially purified T cells and freshly explanted bone marrow cells used for GM CSF assay in reported studies contain adherent cells, including macrophages, that might be triggered to produce CSF (13). The GM CFU-c progenitor cells that we used, however, were purified from long-term bone marrow culture; they included fewer than 1% morphologically identifiable macrophages, and no CSF activity could be detected. Our data, therefore, substantiate the conclusion that inducer T cells synthesize CSF that induces precursor cells to differentiate and produce colonies composed mainly of macrophages. The mechanism of activation of B cells to secrete Ig, on the other hand, is not so clear. It may be that inducer cell products activate B cells directly or that they induce macrophages to synthesize a B-cell-activating factor.

Despite evidence of homogeneity of our clonal populations, it is still possible that differentiation into subsets is continuously taking place within the clonal population. Thus, it is not certain that the cells of inducer clones are uniform; the question of whether all factors produced by the clone are synthesized by all its member cells awaits a definitive answer.

We thank Dr. E. A. Boyse and J. Bard for valuable editorial comments and Alec Chessman for assistance in the experiments. This work was supported in part by Grants GM-07753, AI-12184, AI-13600, CA-25412, and CA-26785 from the National Institutes of Health.

1. Cantor, H. & Boyse, E. A. (1975) *J. Exp. Med.* **141**, 1376–1389.
2. Cantor, H. & Boyse, E. A. (1975) *J. Exp. Med.* **141**, 1390–1399.
3. Nathan, D. G., Chess, L., Hillman, D. G., Clarke, B., Breard, J., Merler, E. & Housman, D. E. (1978) *J. Exp. Med.* **147**, 324–339.
4. Wagner, H. & Rollinghoff, M. (1978) *J. Exp. Med.* **148**, 1523–1538.
5. Nabel, G., Fresno, M., Chessman, A. & Cantor, H. (1981) *Cell* **23**, 19–28.
6. Shen, F. W., Boyse, E. A. & Cantor, H. (1975) *Immunogenetics* **2**, 591–595.
7. Quintans, J. & Lefkovits, I. (1973) *Eur. J. Immunol.* **3**, 392–397.

8. Lionetti, F. J., Hunt, S. M., Mattaliano, R. J. & Valeri, C. R. (1978) *Transfusion* **18,** 685–692.
9. Mishell, R. I. & Dutton, R. W. (1967) *J. Exp. Med.* **126,** 423–442.
10. Molinaro, G. A., Maren, E. & Dray, S. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1229–1233.
11. Strelkauskas, A. J., Eby, W. E., Molinaro, G. A. & Dray, S. (1976) *Clin. Immunol. Immunopathol.* **6,** 334–340.
12. Greenberger, J. S., Davisson, P. B. & Gans, P. J. (1979) *Virology* **95,** 317–333.
13. Greenberger, J. S., Newburger, P. & Sakakeeny, M. A. (1980) *Blood* **56,** 368–379.
14. Bradley, T. R. & Metcalf, D. (1966) *Aust. J. Exp. Biol. Med. Sci.* **44,** 287–299.
15. Stanley, R. E. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2969–2973.
16. Watson, J., Gillis, S., Marbrook, J., Mochizuki, D. & Smith, K. A. (1979) *J. Exp. Med.* **150,** 849–861.
17. Shaw, J., Caplan, B., Paetkan, V., Pilarski, L. M., Delovitch, T. L. & McKenzie, I. F. (1980) *J. Immunol.* **124,** 2231–2239.
18. Cantor, H. (1979) *Annu. Rev. Med.* **30,** 269–277.
19. Huber, B., Devinsky, O., Gerson, R. K. & Cantor, H. (1976) *J. Exp. Med.* **143,** 1534–1539.
20. Leclerc, J. C. & Cantor, H. (1980) *J. Immunol.* **124,** 851–854.
21. Haran-Ghera, N., Pullar, P. & Furth, J. (1960) *Endocrinology,* **66,** 694–701.
22. Huggins, C. & Berganstal, D. M. (1952) *Cancer Res.* **12,** 134–141.
23. Johnson, G. R. & Burgess, A. W. (1978) *J. Cell Biol.* **77,** 35–47.
24. Stanley, E. R. & Heard, P. M. (1977) *J. Biol. Chem.* **252,** 4305–4312.