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Production of immune interferon by an interleukin 2-independent murine T cell line

(phorbol 12-myristate 13-acetate/lymphokines)

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ABSTRACT **An interleukin 2-independent murine T cell line (BFS) was isolated that produced immune interferon after stimulation with phorbol 12-myristate 13-acetate. The BFS cell line did not produce detectable levels of interleukin 1, interleukin 2, B cell growth factor, macrophage–granulocyte colony-stimulating factor, macrophage-activating factor, or T cell replacing factor. Maximal interferon was induced 48 hr after stimulation with phorbol myristate acetate at 10–100 ng/ml. Production of interferon by phorbol myristate acetate-stimulated BFS cell cultures was synergistically increased by the addition of EL4 thymoma cell culture supernatants. BFS-derived interferon activity was sensitive to pH 2 treatment and was neutralized with antiserum to immune interferon but was resistant to heating at 56°C and to treatment with antiserum to type I interferon. In addition, the interferon activity was sensitive to trypsin but resistant to RNase. BFS-derived interferon had an apparent molecular weight of 48,000 and a pI of 5.5–6.0. Each of these properties is consistent with the conclusion that the BFS cell line produces immune interferon after stimulation with phorbol myristate acetate.**

Biochemical and biological characterization of murine α and β interferon (IFN) has been facilitated by the identification of cell lines producing these molecules (1). In contrast, antigen- or lectin-stimulated spleen cell culture supernatants have been the required source of immune IFN (IFN-γ). The use of these culture supernatants as a source of IFN-γ is limited by the expense of utilizing large numbers of animals. Such spleen cell culture supernatants also contain a plethora of other lymphokine activities, which can confuse the assignment of a biological activity to a particular lymphokine.

Several recent developments have facilitated the identification of a cell line capable of producing IFN-γ. Marcucci *et al.* (2) recently reported that a number of interleukin 2 (IL-2)-dependent murine T cell clones produced IFN-γ after stimulation with either phytohemagglutinin or concanavalin A (Con A). In addition, Nathan *et al.* (3) and Pang *et al.* (4) have reported that phorbol 12-myristate 13-acetate (PMA) either alone or in combination with mitogen or anti-T cell monoclonal antibody enhanced IFN-γ production by a human T-lymphoblast cell line and human peripheral blood T cells.

Our laboratory has routinely utilized an IL-2-dependent T cell line (CT 6) for measuring levels of IL-2 in culture supernatants. We have recently isolated a subline of CT 6 cells (BFS) that was selected by independence of IL-2 for growth and that produces IFN-γ after stimulation with PMA. In this study we report experiments that characterize the BFS cell line, describe various biochemical properties of BFS-derived IFN-γ, and examine the BFS cell line culture supernatants for the presence of other lymphokines. Additionally, we demonstrate the ability of an EL4 thymoma cell culture supernatant to synergistically enhance the production of interferon by the BFS cell line.

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MATERIALS AND METHODS

Isolation and Surface Antigen Characteristics of the BFS Cell Line. This laboratory has routinely utilized an IL-2-dependent cytotoxic T cell line (CT 6, kindly provided by J. Watson) to measure levels of IL-2. By subculturing CT 6 cells in decreasing amounts of growth factor we isolated a subline (BFS) that no longer required exogenous IL-2 to sustain cellular proliferation. The BFS cell line is maintained in RPMI 1640 medium containing 5% fetal bovine serum, penicillin at 100 μg/ml, and streptomycin at 100 units/ml. The CT 6 cell line is maintained in rat spleen cell Con A-conditioned medium as described (5). Examination of the phenotype of CT 6 and BFS cells by fluorescence-activated cell sorter analysis has shown that both cell lines are H-2^b and Thy-1.2 positive and Lyt-1.2, Lyt-2.2, and Ig negative (unpublished data).

Induction of IFN-Containing Supernatants. BFS and CT 6 cells were washed twice in RPMI 1640 medium and suspended to 1×10^6 cells per ml in RPMI medium containing 1% fetal bovine serum. For small-scale supernatant production, 1-ml cultures were plated in 24-well tissue culture plates and supplemented with either PMA (Sigma) or Con A or left as untreated controls. Cultures were incubated for 24–72 hr at 37°C in a humidified atmosphere of 5% CO_2/95% air. The culture supernatants were then collected, centrifuged to remove particulate debris, and treated with dextran-coated charcoal (WestChem Products, San Diego, CA) as described (6) to absorb the PMA. The supernatants were then dialyzed against 30 vol of RPMI medium for 18 hr at 4°C and sterilized by filtration. For generation of larger culture supernatants the BFS cells were prepared as described above and incubated as 125-ml cultures in 150-cm^2 tissue culture flasks. These culture supernatants were concentrated by ultrafiltration in an Amicon cell with a UM10 membrane, treated to remove PMA, and then dialyzed against RPMI medium.

Induction of EL4 Thymoma Culture Supernatant. EL4 thymoma cells (7) were incubated as 125-ml cultures in 150-cm^2 tissue culture flasks at 1×10^6 cells per ml in RPMI medium containing 1% fetal bovine serum and PMA at 20 ng/ml. The culture supernatant was concentrated 40-fold in an Amicon cell, treated with dextran-coated charcoal to remove PMA, and dialyzed against RPMI medium. This supernatant contained 30,000 units of IL-2 per ml but did not contain detectable IFN activity.

Treatment of BFS-Derived IFN. The following treatments were performed on BFS-derived supernatants containing 70–

Abbreviations: IFN, interferon; Con A, concanavalin A; IL, interleukin; PMA, phorbol 12-myristate 13-acetate.

250 units of IFN per ml. In order to determine the heat stability of the IFN activity, IFN-containing supernatants were incubated at 56°C for 1 hr and then immediately cooled. To examine the lability of IFN activity to pH 2 treatment, BFS culture supernatants were dialyzed against a 0.05 M glycine·HCl buffer, pH 2, for 24 hr at 4°C. A control IFN preparation was dialyzed against 0.02 M phosphate-buffered saline, pH 7.3, for 24 hr at 4°C. Both the pH 2-treated and control supernatants were then dialyzed an additional 24 hr at 4°C against RPMI medium. To determine the sensitivity of the IFN activity to trypsin, 0.7 ml of culture supernatant was treated with 10 units of bovine pancreas trypsin attached to crosslinked beaded agarose (Sigma). The trypsin-supernatant mixture was buffered with 0.01 M phosphate-buffered saline and incubated at 37°C for 1 hr with constant agitation. After incubation, the preparation was centrifuged to remove the trypsin and sterilized by filtration. As a control an identical culture supernatant was treated in the same manner with 10 units of RNase attached to crosslinked beaded agarose (Sigma). To determine the serological types of IFN present in BFS culture supernatants, the supernatants were incubated for 1 hr at 37°C with sufficient goat anti-mouse type I antiserum (Research Resources Branch, National Institute of Allergy and Infectious Diseases, Bethesda, MD) or rabbit anti-mouse IFN-γ antiserum (kindly provided by H. Johnson, University of Texas, Medical Branch, Galveston, TX) to neutralize double the units of IFN in each BFS preparation. The anti-IFN-γ antiserum does not react with IL-1, IL-2, or colony-stimulating factor (8).

IFN Assay. IFN levels were quantitated by a modification of the viral plaque reduction assay of Wagner (9). Briefly, 0.5-ml serial dilutions of culture supernatants were incubated for 16 hr at 37°C with confluent monolayers of L929 fibroblasts in 24-well tissue culture plates. The cultures were then drained, inoculated with 35–50 plaque-forming units of vesicular stomatitis virus in 0.25 ml of medium, and incubated for 90 min. Afterward, the cultures were drained again and overlaid with 0.25 ml of medium containing 1% agar. After incubation for 24 hr at 37°C, the virus plaques were visualized by staining for 4 hr with 0.03% neutral red in 0.85% saline. The IFN titer (expressed as units/ml) is defined as the reciprocal of the logarithm of the supernatant dilution resulting in a 50% plaque reduction as compared to control cultures, calculated by linear regression. This IFN assay was calibrated using World Health Organization international reference IFN standard G-002-9-04-511 (Research Resources Branch, National Institute of Allergy and Infectious Diseases).

Gel Filtration. One milliliter of a 20-fold concentrated BFS culture supernatant containing 700 units of IFN was passed over a 1.5 × 90 cm Ultrogel AcA 54 (LKB, Stockholm, Sweden) gel filtration column with reverse flow at a rate of 4 ml/hr. The column was previously equilibrated in 0.02 M phosphate-buffered saline and calibrated with blue dextran (exclusion marker), ovalbumin (M_r 43,500), myoglobin (M_r 18,800), and cytochrome *c* (M_r 12,384). One-milliliter fractions were collected in tubes containing 1% (final concentration) fetal bovine serum, sterilized by filtration, and assayed for IFN activity.

Isoelectric Focusing. BFS supernatant containing 2,500 units of IFN was dialyzed for 18 hr at 4°C against 30 vol of 0.1% polyethylene glycol in distilled H_2O. The sample was then loaded into a preparative isoelectric focusing column (LKB) in a 100 ml 5–50% sucrose gradient containing 0.1% polyethylene glycol and 2% (vol/vol) Ampholine solution (pH 3.5–10.0, LKB). The pH gradient was formed over a period of 18 hr. Three-milliliter fractions were then collected and the pH was determined. The fractions were then brought to 1% fetal bovine serum, dialyzed for 48 hr at 4°C against two changes of 30 vol of phosphate-buffered saline, and assayed for IFN activity.

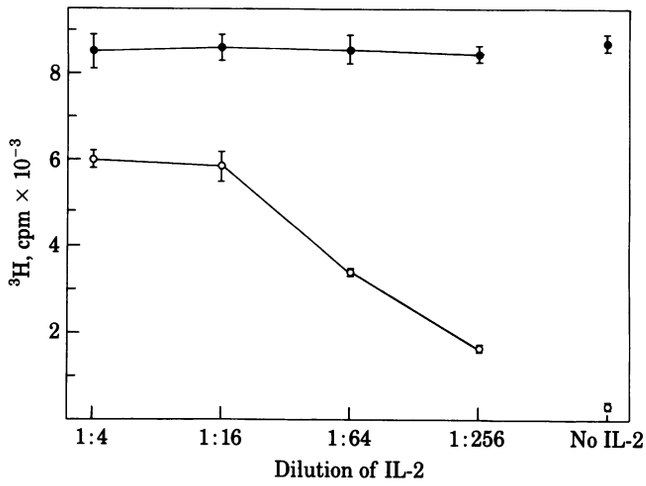

FIG. 1. IL-2-independence of the BFS cell line. BFS (●) or CT 6 (○) cells (10^4) were incubated for 16 hr with various dilutions of IL-2-containing supernatant. The cultures were treated with 0.5 μCi (1.8 × 10^4 becquerels) of [^{3}H]thymidine (1.9 Ci/mmol) for 5 hr and the amount of labeled thymidine incorporated was determined. Each value represents the mean ± SEM of triplicate samples.

RESULTS

IL-2-Independence of BFS Cell Line Growth. Fig. 1 demonstrates that the proliferation of CT 6 cells is strictly dependent upon the addition of an IL-2-containing rat spleen cell supernatant. Thus, the addition of decreasing amounts of IL-2 to CT 6 cultures led to reduced cell proliferation as determined by [^{3}H]thymidine incorporation. In contrast, the BFS cells, a subline of the CT 6 cell line, proliferated in medium alone, and the addition of a wide range of IL-2 activity had no enhancing effect on proliferation. Therefore, BFS cells are completely independent of the exogenous addition of IL-2 for maintaining maximal proliferation.

Production of IFN by BFS Cells. CT 6 and BFS cells were stimulated with selected doses of PMA and the culture supernatants were assayed for IFN content. The results in Table 1 demonstrate that treating BFS but not CT 6 cells with PMA at 10–100 ng/ml induced the production of IFN. The maximal titer of IFN in BFS supernatants has ranged between 10 and 125 units/ml per 10^6 cells in a number of experiments. Stimulation of BFS cells with Con A alone at 1–10 μg/ml did not induce IFN production, nor did it enhance PMA-stimulated IFN release (data not shown). Residual PMA present in the

Table 1. Interferon production by the BFS cell line

PMA, ng/ml	IFN, units/ml	
	BFS cells	CT 6 cells
0	<5	<5
5	<5	<5
10	7	<5
50	15	<5
100	27	<5
500	5	<5
1,000	5	<5

The IFN activity of the culture supernatants was determined by a virus plaque reduction assay. To induce IFN activity, 10^6 cells were incubated in 1 ml of medium for 48 hr with PMA at the given concentration.

Table 2. Kinetics of interferon production by PMA-stimulated BFS cells

Duration of culture, hr	IFN, units/ml	
	Control	PMA (20 ng/ml)
24	<5	<5
48	<5	39
72	<5	16

The IFN activity of the culture supernatants was determined by a virus plaque reduction assay. To induce IFN activity, 10^6 BFS cells were incubated in 1 ml of medium for the time indicated in the presence or absence of PMA.

culture supernatants did not appear to contribute to the antiviral activity because PMA-treated CT 6 cell supernatants were not active in this regard. In addition, control supernatants containing PMA but no cells also possessed no antiviral effects (data not shown).

The results in Table 2 show that maximal IFN activity was detected 48 hr after stimulation of BFS cells with PMA. By 72 hr of incubation, the level of detectable IFN activity in the culture supernatant was reduced.

Characteristics of BFS IFN. Initial studies established that pretreatment of the L929 target cells with actinomycin D at 5 μg/ml for 1 hr blocked the antiviral activity of BFS supernatants. The antiviral activity of BFS supernatants, together with the observed susceptibility of the antiviral state to actinomycin D pretreatment indicated that the BFS cell line produced IFN. Three principal types of IFN (α, β, and γ) have been defined on the basis of physicochemical and antigenic differences (10, 11). The next experiments were performed to characterize the type of IFN produced after PMA stimulation of the BFS cell line. Table 3 shows that the IFN activity produced by BFS cells was sensitive to trypsin and pH 2 treatment but not to heating at 56°C for 1 hr. In addition, treatment with rabbit anti-mouse IFN-γ antiserum eliminated its antiviral activity. In contrast, treatment of the IFN with goat anti-mouse type I antiserum, which neutralizes type I IFN (IFN-α and IFN-β) had no effect on BFS-derived IFN activity. The anti-type I IFN did, however, neutralize the antiviral activity of poly(I·C)-stimulated L929 cell supernatants. Thus, the IFN produced by BFS cells after stimulation with PMA has the characteristics of IFN-γ.

Molecular Weight and Isoelectric Point of BFS-Derived IFN-γ. Molecular weight estimates of spleen cell IFN-γ by gel filtration have ranged from 20,000 to 60,000, although most reports indicate 40,000–50,000 (11). Our results in Fig. 2 show that the IFN activity elutes from an Ultrogel AcA 54 gel filtration column with an apparent molecular weight of 48,000. The isoelectric point of BFS IFN-γ was determined to be between pH 5.5 and 6.0 (Fig. 3); this IFN exhibited considerable charge heterogeneity, consistent with the observations made with other IFNs (12).

Table 3. Characteristics of interferon produced by the BFS cell line

Treatment	IFN activity remaining, %
None	100
pH 2	10
56°C, 60 min	114
Goat anti-type I IFN antiserum	101
Normal goat serum	100
Rabbit anti-IFN-γ antiserum	4
Normal rabbit serum	100
Trypsin	0
RNase	80

Results are expressed as the % of the control supernatant activity remaining after treatment. In each experiment control interferon preparations contained 70–250 units of IFN per ml. Anti-type I IFN antiserum neutralized 100% of the antiviral activity of poly(I·C)-stimulated L929 cell supernatants containing 100 units of IFN.

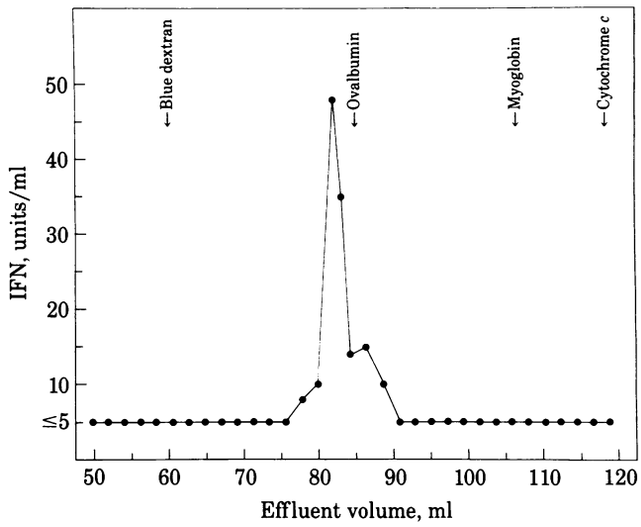

FIG. 2. Molecular weight determination of BFS interferon. IFN (750 units) was loaded onto a 1.5 × 90 cm Ultrogel AcA 54 gel filtration column and eluted with phosphate-buffered saline, pH 7.3. IFN activity was quantitated by using a virus plaque reduction technique.

Examination for Other Lymphokine Activities of BFS Supernatants. We have examined BFS supernatants for a variety of lymphokine activities in addition to IFN and have found them to be negative for IL-1, IL-2, B cell growth factor, macrophage–granulocyte colony-stimulating factor, T cell-replacing factor (unpublished data), and macrophage-activating factor (M. Meltzer, personal communication). However, IFN-γ-containing supernatants obtained from the BFS cell line do induce macrophage Ia antigen expression (unpublished data), which is consistent with previous reports demonstrating this activity for IFN-γ (13, 14).

Synergy Between PMA and EL4 Thymoma Culture Supernatants in the Induction of BFS Interferon. The titers of IFN-γ produced by the BFS cell line in response to PMA are lower than those obtained after conventional mitogen stimulation of

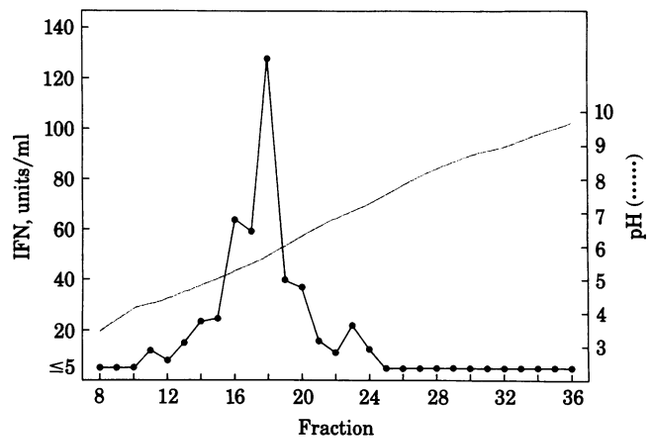

FIG. 3. Isoelectric point of BFS interferon. IFN (2,500 units) was placed into a 5–50% sucrose gradient containing 2% Ampholines (pH 3.5–10). Three-milliliter fractions were collected for pH and IFN determinations. IFN activity was quantitated by using a virus plaque reduction assay.

Table 4. Synergy between PMA and EL4 thymoma culture supernatants in the induction of BFS interferon

BFS cells	Stimulants: PMA, ng/ml	Stimulants: EL4 thymoma supernatant*	IFN, units/ml
0	0	—	<5
		1:200	<5
		1:50	<5
		1:20	<5
1×10^6	0	—	<5
		1:200	<5
		1:50	40
		1:20	216
1×10^6	40	—	32
		1:200	160
		1:50	354
		1:20	800

* Culture supernatant of EL4 thymoma cells stimulated for 48 hr with PMA at 20 ng/ml and subsequently treated with charcoal to remove residual PMA.

spleen cell cultures. Previous reports have implicated the necessity of cellular interactions in the activation of T cells for the production of IFN-γ (8, 15). In at least one instance, the cellular interaction was shown to be mediated by a soluble factor similar to IL-2 (8). Because the BFS cell line is a homogeneous population of cells obtained from a cloned cell line, the cells may not produce the factors normally involved in IFN production by mixed populations of splenic lymphocytes. In response to PMA stimulation, the EL4 thymoma cell line produces a number of lymphokines that are observed in Con A-induced spleen cell culture supernatants, including macrophage-activating factor (M. Meltzer, personal communication), IL-2, B cell growth factor, and macrophage–granulocyte colony-stimulating factor (16). We therefore examined whether culture supernatant from EL4 thymoma cells could enhance IFN production by the BFS cell line. The results in Table 4 show that EL4 thymoma culture supernatant incubated without BFS cells did not contain detectable IFN activity. The addition of EL4 culture supernatant to BFS cells (1:20, 1:50) in the absence of PMA induced moderate levels of IFN. However, the stimulation of BFS cells with PMA and EL4 thymoma culture supernatants enhanced synergistically the production of IFN by the BFS cell line. The IFN produced by the BFS cells in the presence of PMA and EL4 culture supernatant was sensitive to pH 2 treatment and resistant to heating at 56°C and to treatment with anti-type I IFN antiserum and thus had characteristics of IFN-γ (data not shown).

DISCUSSION

The results presented in this study have demonstrated the isolation of an IL-2-independent T cell line that, upon treatment with PMA, releases IFN-γ. The IFN activity is characterized as IFN-γ by its sensitivity to pH 2 treatment, neutralization with anti-IFN-γ antiserum but not with anti-type I IFN antiserum, resistance to heating at 56°C, and ability to induce macrophage Ia antigen expression. Furthermore, the molecular weight of 48,000 is similar to the molecular weight reported for spleen cell-derived IFN-γ. The pI was found to be in the range 5.5–6.0. Additional properties that are consistent with the activity's being IFN are its sensitivity to trypsin, its resistance to RNase, and the requirement for target cell protein synthesis for the induction of the antiviral state. Finally, the production of IFN-γ by the BFS cell line could be synergistically enhanced by the addition of factors present in EL4 thymoma cell culture supernatants.

One of the major difficulties in assigning a biological activity to a particular lymphokine obtained from a spleen cell supernatant is that many biochemically similar lymphokines may be present in trace, but biologically active, amounts after a number of purification steps. It is therefore advantageous to obtain a cell line that produces only a minimal number of distinct lymphokines. PMA-stimulated BFS supernatants do not contain detectable levels of a variety of other lymphokine activities including IL-1, IL-2, B cell growth factor, macrophage–granulocyte colony-stimulating factor, T cell-replacing factor, and macrophage activating factor. However, it will be important to rule out potential masking of these bioassays by IFN-γ. Thus, the existence of this cell line should facilitate further characterization of the biological and biochemical properties of murine IFN-γ.

The phenotype of the T lymphocyte producing IFN-γ during immune responses is controversial. Sonnenfeld *et al.* (17) have presented evidence that a Lyt-23^+ T lymphocyte is the source of IFN-γ production by spleen cells sensitized with bacillus Calmette–Guérin. In contrast, Landolfo *et al.* (18) have shown that both Lyt-1^+ and Lyt-123^+ spleen cells produce IFN-γ in mixed lymphocyte cultures. Marcucci *et al.* (2) reported that the IL-2-dependent T cell clones producing IFN-γ were Thy-1.2^+, Lyt-1^-, and Lyt-2^-. Thus, their cell lines had the same phenotype as the BFS cell line. This is probably explained by the observation that IL-2-dependent T cell lines tend to lose Lyt phenotypic markers upon continued culture (19). However, the parent CT 6 cell line from which BFS cells were derived was originally a cloned cytotoxic T cell line. Therefore, the origin of the BFS cell line was a T cell line that was phenotypically Lyt-1^-23^+. These data, therefore, support the concept that the Lyt-23^+ T lymphocyte is a cellular source of IFN-γ.

Controversy also exists in the published literature concerning the interaction of IL-2 and IFN-γ. Simon *et al.* (20) have shown that both IL-2 and IFN-γ are able to induce cytotoxic T lymphocytes. Farrar *et al.* (8) extended these findings by demonstrating that addition of IL-2 induces IFN-γ production in macrophage-depleted mixed lymphocyte cultures. The ability of anti-IFN-γ to inhibit the IL-2-induced cytotoxic T cell response suggested a mandatory role of IFN-γ in certain IL-2-driven primary responses. Recent data indicate that IFN-γ is capable of inducing the expression of T lymphocyte IL-2 receptors (W. Farrar, personal communication). The studies reported here concerning the ability of IL-2-containing EL4 thymoma culture supernatants to synergistically enhance the production of IFN-γ by BFS cells further suggest a complex interplay among various factors in the induction of IFN-γ. In contrast to our findings, Marcucci *et al.* (2) have reported that IL-2-dependent T cell clones produced IFN-γ only in the absence of IL-2-containing conditioned medium. The latter results suggest that a component of the conditioned medium may be negatively regulating IFN-γ production by activated T cells. Further studies with the BFS cell line at both the genetic level and with purified preparations of lymphokines should be useful in resolving this controversy concerning the relationship between IL-2 and IFN-γ and in addition should allow the examination of intracellular events involved in the induction of IFN-γ production by these factors.

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1. Kawade, Y. & Yamamoto, Y. (1981) *Methods Enzymol.* **78,** 139–143.
2. Marcucci, F., Waller, M., Kirchner, H. & Krammer, P. (1981) *Nature (London)* **291,** 79–81.
3. Nathan, I., Groopman, J. E., Quan, S. G., Bersch, N. & Golde, D. W. (1981) *Nature (London)* **292,** 842–844.
4. Pang, R. H. L., Yip, Y. K. & Vilcek, J. (1981) *Cell. Immunol.* **64,** 304–311.
5. Hilfiker, M. L., Moore, R. N. & Farrar, J. J. (1981) *J. Immunol.* **127,** 1983–1987.
6. Fuller-Farrar, J., Hilfiker, M. L., Farrar, W. L. & Farrar, J. J. (1981) *Cell. Immunol.* **58,** 156–164.
7. Farrar, J. J., Fuller-Farrar, J., Simon, P. L., Hilfiker, M. L., Stadler, B. M. & Farrar, W. L. (1980) *J. Immunol.* **125,** 2555–2558.
8. Farrar, W. L., Johnson, H. M. & Farrar, J. J. (1981) *J. Immunol.* **126,** 1120–1125.
9. Wagner, R. R. (1961) *Virology* **13,** 323–337.
10. Stewart, W. E., II (1980) *Nature (London)* **286,** 110–111.
11. Stewart, W. E., II (1979) *The Inteferon System* (Springer, New York), pp. 134–183.
12. Mogensen, K. E., Phyhala, L., Torma, E. & Cantell, K. (1974) *Acta Pathol. Microbiol. Scand. Sect. B* **82,** 305–310.
13. Oppenheim, J. J., Luger, T., Sztein, M. B. & Steeg, P. S. (1982) in *Role of Macrophages in Self Defense Mechanisms*, ed. Ishida, N. (Elsevier/North–Holland, New York), in press.
14. Steeg, P. S., Moore, R. N. & Oppenheim, J. J. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40,** 771 (abstr.).
15. Torres, B. A., Farrar, W. L. & Johnson, H. M. (1982) *J. Immunol.* **128,** 2217–2219.
16. Farrar, J. J., Benjamin, W. R., Hilfiker, M. L., Howard, M., Farrar, W. L. & Fuller-Farrar, J. (1982) *Immunol. Rev.* **63,** 129–166.
17. Sonnenfeld, G., Mandel, A. D. & Merigan, T. C. (1979) *Immunology* **36,** 883–890.
18. Landolfo, S., Kirchner, H. & Simon, M. M. (1982) *Eur. J. Immunol.* **12,** 295–299.
19. Nabholz, M. (1980) *Immunol. Rev.* **51,** 125–156.
20. Simon, P. L., Farrar, J. J. & Kind, P. D. (1979) *J. Immunol.* **122,** 127–132.