Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6613–6617, September 1986
Medical Sciences

Interferon γ and granulocyte/macrophage colony-stimulating factor inhibit growth and induce antigens characteristic of myeloid differentiation in small-cell lung cancer cell lines

MICHAEL R. RUFF*, WILLIAM L. FARRAR†, AND CANDACE B. PERT‡§

*Cellular Immunology Section, Laboratory of Microbiology and Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, MD 20892; †Laboratory of Molecular Immunoregulation, Biological Response Modifier Program, Division of Cancer Treatment, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701; and ‡Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892

Communicated by Pedro Cuatrecasas, April 18, 1986

ABSTRACT **The expression of several macrophage and hemopoietic cell surface markers recently described on small-cell lung cancer (SCLC) cell lines was studied by use of flow cytometry. The antigens Leu-M3, Leu-7, and HLA-DR were examined for their modulation by human interferon γ and granulocyte/macrophage colony-stimulating factor (GM-CSF). Both of these lymphokines generally induced enhanced expression of hemopoietic markers in several SCLC lines. A differential response to these two hormones was observed, in that qualitative and quantitative differences in marker modulation among the tested cell lines were apparent. In addition to regulating the antigenic phenotype of these cells, both interferon γ and GM-CSF had antiproliferative effects on SCLC lines as determined by [^{3}H]thymidine incorporation and clonal growth in agar. These results suggest that interferon γ and GM-CSF promote a differentiation process in SCLC cell lines that has characteristics in common with myeloid differentiation. These findings support the theory that SCLC tumors are hemopoietic cells that arise from macrophages or their precursors and suggest new therapeutic modalities for the treatment of lung cancer.**

Lung cancer remains the leading cause of cancer death in the United States, and it is anticipated that over 100,000 new cases will be diagnosed this year. For clinical and biological purposes, lung cancer has been divided into two major types: non-small-cell lung cancer, which includes lung adenocarcinoma and squamous carcinoma, as well as several other minor histological groupings, and small-cell lung cancer (SCLC). SCLC, which accounts for 25% of all lung cancers, is a rapidly progressing, highly metastatic disease with poor prognosis whose histological origin is controversial (compare refs. 1 and 2). SCLC tumors usually express certain biochemical features suggesting relatedness to neuropeptide-secreting cells, characteristics that, in general, distinguish them from non-SCLC (3, 4).

We have described a series of monocyte-specific surface antigens present on SCLC lines and tumors and have proposed that SCLC tumors are derived from macrophages or their precursors (5). Subsequently these results have been extended and additional monocyte-related markers (6, 7, 39) and activities (8) have been reported for these cells.

Among the hormones that have been well-studied for their effects on monocyte function, including effects on growth and differentiation, are interferon γ (IFN-γ) and colony-stimulating factors (CSFs). IFN-γ has been shown to induce enhanced expression of HLA-DR (Ia) antigen (9, 10), Fc receptor, and other markers (11, 12) in monocytes, as well as inhibition of proliferation in select myeloid cell lines (12–14). IFN-γ has also been shown to induce HLA and β_2-microglobulin expression in several SCLC lines (15). CSFs are a functional group of distinct lymphokines considered to have a primary role in the regulation of hemopoiesis (reviewed in ref. 16). One of these, granulocyte/macrophage (GM)-CSF (17), specifically stimulates bone marrow progenitors that ultimately give rise to cells of granulocyte, macrophage, or erythroid lineage. CSFs also have differentiative (11, 16, 18, 19) effects on macrophages and antiproliferative effects on some myeloid cell lines (12, 18, 20). Previous findings, based on lineage-specific expression of myeloid-associated surface determinants, have suggested that SCLC may be related to cells of myeloid derivation (5). These studies also suggested the possibility that SCLC may evince biological responses to lymphokines, such as the interferons or CSFs, that have previously been shown to regulate myelopoiesis.

In the present study, human IFN-γ and GM-CSF were tested for possible effects on surface antigen expression and on growth in several SCLC cell lines. We examined as differentiation markers the expression of several antigens shared in common between macrophages and SCLC lines or tumors. These include the macrophage-specific antigens recognized by the monoclonal antibodies OKM1 (5) and Leu-M3 (6); the natural killer marker Leu-7, present on macrophages (21), SCLC (6, 39), and other cells; and the type II histocompatibility antigen HLA-DR, present on mature macrophages as well as other, nonhemopoietic, cells but generally not present on SCLC cells (5, 15). Our results indicate that both IFN-γ- and GM-CSF-treated SCLC continuous culture cells modulated the expression of some of these markers. Additionally, both of these factors had antiproliferative activity on SCLC cells. These observations are suggestive of a modulatory effect of IFN-γ and GM-CSF on SCLC cells characteristic of myeloid differentiation.

MATERIALS AND METHODS

Cell Culture Lines. The SCLC lines employed were SHP-77, a gift of E. Fisher (22); NCI H-69 (23), obtained from the American Type Culture Collection; and lines NCI H-60, H-209, and H-446, gifts of A. Gazdar (24). These cell lines were grown at 37°C in a 5% CO_2/air atmosphere in RPMI 1640 medium (GIBCO) with 10% fetal bovine serum, penicillin (50 units/ml), and streptomycin (50 μg/ml). These lines were found to be free of mycoplasma.

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: IFN-γ, interferon γ; CSF, colony-stimulating factor; GM-CSF, granulocyte/macrophage CSF; SCLC, small-cell lung cancer.

§To whom correspondence should be addressed at: Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, Building 10, Room 3N256, 9000 Rockville Pike, Bethesda, MD 20892.

Materials. Recombinant human IFN-γ used in the antigen-induction experiments was the gift of Bio-Gen (Boston). Human IFN-γ used in the cell-growth experiments was a partially purified (1.5×10^6 units/mg) preparation (Meloy Laboratories, Springfield, VA). Human recombinant GM-CSF, used in the antigen-induction experiments, was the gift of D. Rennick, DNAX, Palo Alto, CA. Human GM-CSF, used in the cell-growth experiments, was a partially purified preparation (Genzyme, Norwalk, CT) free of interleukin-2, interleukin-3, and IFN-γ activity and was dialyzed against growth medium and sterilized by filtration prior to use. Anti-Leu-7, anti-Leu-M3, and anti-HLA-DR were purchased from Becton Dickinson. OKM1 was obtained from Ortho Diagnostics.

Antigen Induction and Cytofluorometric Analysis. SCLC cell lines were cultured in RPMI 1640 medium with 10% fetal bovine serum for 4 days in the presence or absence of either recombinant human IFN-γ or GM-CSF at various concentrations. For cytofluorometric analysis, cells were washed three times in RPMI 1640 and incubated with saturating amounts of the appropriate antibody or a control monoclonal antibody for 1 hr at 4°C. After two washes, cells were incubated in fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Cappel Laboratories, Cochranville, PA). After two more washes, cells were analyzed on an Ortho Cytofluorograph System-50 with an argon ion laser at 488 nm. Live cells were measured. Information was stored on an Ortho 2150 computer system. For analysis of specific antibody fluorescence, emission was measured in relative units (channel number). A comparison of fluorescence intensity was made by using the mean channel number for different histograms. The percentage of antigen-determinant-positive cells was calculated against a background of nonspecific labeling with normal IgG (1–3%).

Antiproliferative Assays. Two methods were employed to evaluate the effects of IFN-γ and GM-CSF on the growth of SCLC continuous culture lines. Cells were plated in growth medium at 10,000 cells per 0.2 ml in 96-well flat-bottomed microtiter trays (Falcon) containing various concentrations of IFN-γ or GM-CSF and were cultured for 5 days. DNA synthesis in the cultures was determined by addition of [*methyl*-^{3}H]thymidine (0.5 μCi per well; 1 Ci = 37 GBq) for the final 8 hr of culture. Cells were harvested onto glass-fiber filter strips and washed, and incorporated radioactivity was determined by liquid scintillation counting in Ultrafluor (National Diagnostics). Cell growth was also evaluated by assay of clonal growth in agar. In this method, cells obtained from stock cultures were treated with trypsin (0.1%) in order to generate largely single-cell suspensions. Cells (10^2–10^4) were then washed and admixed with 0.3% agar (Difco), containing IFN-γ, GM-CSF, or medium only (control) to a final concentration as indicated, and plated over a base layer of prehardened agar (0.5%) in 6-well tissue culture trays (Costar). Colonies of >50 cells were enumerated at 14–21 days.

RESULTS

Effect of Recombinant Human IFN-γ on SCLC Antigenic Phenotype. To determine whether IFN-γ can modulate the antigenic phenotype of SCLC lines, cultures were treated with and without IFN-γ at two concentrations, 500 and 2000 units/ml, for 4 days in growth medium. This time interval was chosen on the basis of previous reports indicating that IFN-γ effects on antigen expression required several days of exposure to become pronounced (14). This was also true for the current studies (data not shown). Flow cytometry was used to quantitate the binding of a panel of monoclonal antibodies that recognized antigens recently described on these cells. Addition of IFN-γ to cultures of SCLC lines resulted in altered expression of several hemopoietic markers on these cells (Table 1).

Table 1. Antigen expression in SCLC lines stimulated with IFN-γ

		% positive cells*						
		HLA-DR		Leu-M3		Leu-7		MFI†
Cell line	IFN	500	2000	500	2000	500	2000	(Leu-7)
SHP-77	−	0	0	0	0	13	10	290
	+	25	37	27	34	24	37	690
H-69	−	0	1	0	0	46	43	320
	+	28	43	12	26	49	60	640
H-209	−	1	2	12	10	28	17	280
	+	37	48	16	22	42	64	690
H-60	−	0	1	0	2	36	22	240
	+	26	37	24	36	54	72	660

SCLC cultures were incubated without (−) or with (+) recombinant IFN-γ (at 500 or 2000 units/ml, as indicated) for 4 days prior to cytofluorometric analysis of antigenic markers.

*Only viable cells were counted; at least 2×10^6 cells were analyzed for each determination; background was subtracted as described in *Materials and Methods*.

†Mean fluorescence index (channel marker) for Leu-7; values are shown for cells that were untreated or treated with IFN-γ at 500 units/ml.

The effect of IFN-γ was to induce, or enhance, the expression of HLA-DR, Leu-M3, and Leu-7 on SCLC cells, and the magnitude of these responses was dependent on the concentration of IFN-γ employed. Higher percentages of positive cells were recorded for 2000 units of IFN-γ per ml than for 500 units per ml. Thus, HLA-DR, which was not detected on cells of any of four lines in the absence of IFN-γ, was induced to a level of 25–37% positive cells at 500 units of IFN-γ per ml and 37–48% at 2000 units per ml. Similarly, Leu-M3, which was only detected at low levels in one line (H-209), could be induced so that 12–27% of the cells were positive at 500 units of IFN-γ per ml, whereas 2000 units per ml yielded expression on 22–36% of the cells. The H-209 line was marginally increased in its expression of this marker at the lower IFN-γ concentration; however, 2000 units per ml doubled the percentage of marker-positive cells to 22%. In contrast to HLA-DR and Leu-M3, the Leu-7 antigen can readily be detected on uninduced SCLC lines (6, 39) and is expressed on a substantial portion of the cell population. Nevertheless, IFN-γ treatment resulted generally in a significant increase in marker-bearing cells, and the higher interferon dose yielded the greatest percentage of reactive cells. We also examined the expression of the OKM1 antigen on these cell lines. This antigen was expressed at low (2–12%) levels in these cell lines, and this level was not substantially altered by treatment with IFN-γ at either concentration (data not shown). The effect of IFN-γ treatment on these cultures was not only to induce a greater fraction of marker-bearing cells but also to increase the antigen density on individual cells. This was demonstrated by the elevated mean fluorescence intensity, a measure of antigen density, reported in Table 1 for the Leu-7 marker on cells treated with IFN-γ at 500 units/ml. The H-69 cell population, a comparatively high expressor of Leu-7 in the uninduced state (43–46%), did not significantly change in the presence of IFN-γ at 500 units/ml (46–49%). These cells were, however, responsive to IFN-γ as an inducer, in that mean fluorescence intensity doubled (Table 1), indicating a 2-fold increase in antigen density.

Effect of Recombinant Human GM-CSF on SCLC Antigenic Phenotype. Using similar methods, we examined the effect of GM-CSF on the same panel of markers. In these experiments, cells were treated for 4 days with or without recombinant human GM-CSF at 100 or 500 units/ml and then analyzed by flow cytometry. The results (Table 2) show that

Table 2. Antigen expression in SCLC lines stimulated with GM-CSF

		% positive cells*					
		HLA-DR		Leu-M3	Leu-7		MFI†
Cell line	GM-CSF	100	500	100	100	500	(Leu-7)
SHP-77	−	0	0	0	13	10	170
	+	4	5	14	67	72	240
H-69	−	0	0	0	46	52	180
	+	3	3	8	92	87	290
H-209	−	2	ND	12	28	ND	220
	+	1		14	86		380
H-60	−	1	0	2	36	30	170
	+	5	3	12	78	82	350

SCLC cultures were incubated without (−) or with (+) recombinant GM-CSF (at 100 or 500 units/ml, as indicated) for 4 days prior to cytofluorometric analysis. ND, not done.
*See Table 1.
†Mean fluorescence index for Leu-7; values are shown for untreated cells and for cells treated with GM-CSF at 100 units/ml.

GM-CSF also modulated the expression of several antigenic markers. Specifically, although the HLA-DR antigen was not detectable on any of the lines, treatment with GM-CSF at 100 units/ml did result in a very slight expression of this antigen in three of four lines (0–1% to 3–5%). GM-CSF at 500 units/ml did not result in greater expression of HLA-DR. One line, H-209, did not respond to this treatment. Although the percentages of positive cells were marginal, we have observed this trend in repeated experiments and interpret these observations as indicating a low fraction of HLA-DR-inducible cells. The marker Leu-M3, in general not detectable on these cells, was induced by GM-CSF in three of four lines (12–14%). The line H-209 already expresses this marker and little or no enhancement of Leu-M3 was observed. Leu-7, variably present in uninduced cells (28–46%), was substantially increased in four of four lines (67–92%) by treatment with GM-CSF at 100 units/ml. These levels appear to be maximal responses, since little elevation above them was obtained with GM-CSF at 500 units/ml (72–87%). The OKM1 marker was only modulated significantly for one of four SCLC lines, H-69, and only at the highest GM-CSF concentration (3 to 15%, data not shown), suggesting that this marker is not substantially modulated by GM-CSF in SCLC continuous lines. As was observed with IFN-γ treatment, Leu-7 induction by GM-CSF was reflected both in greater percentages of positive cells and in greater mean fluorescence intensity.

Effect of IFN-γ on Proliferation of SCLC Lines. Antiproliferative effects on various monocytic cell lines have been described for IFN-γ (12–14) as well as CSFs (10, 18). We therefore tested several SCLC lines for possible growth effects by IFN-γ. Because several of the SCLC lines grew as suspensions of tight cell aggregates (24), we did not find direct cell enumeration, even after trypsinization, to be a reliable indicator of cell number. We therefore utilized two other methods in order to make this determination. In the first type of experiment, the extent of DNA synthesis in treated cultures was assessed by [^{3}H]thymidine incorporation (Fig. 1). Cultures of SCLC cells treated with IFN-γ for 5 days showed reduced incorporation of [^{3}H]thymidine, and the reduction was dose-dependent, suggesting that IFN-γ has antiproliferative activity on these cells. Cells tested showed a range of responsiveness, with lines H-209 and SHP-77 having a >50% inhibition of thymidine incorporation at 5000 units of IFN per ml, while lines H-446 and H-69 showed <50% inhibition at that concentration of IFN-γ. However, partial responses for all cell lines could be detected at much lower IFN-γ levels, in the range of 150–300 units/ml. A

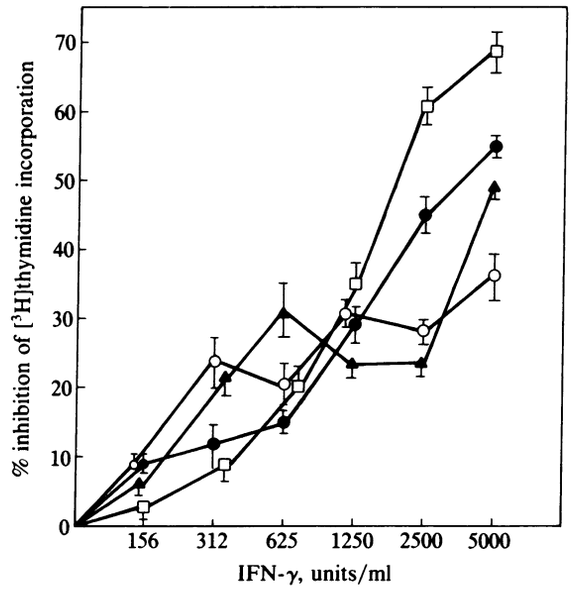

FIG. 1. [^{3}H]Thymidine incorporation by several SCLC continuous culture lines treated with various concentrations of IFN-γ for 5 days. DNA synthesis was quantitated by incubation with [^{3}H]thymidine for the final 8 hr of culture. % inhibition = [1 − (cpm, experimental/cpm, control)] × 100. Values are the mean and SEM of triplicate determinations. Control cultures incorporated 70,000–100,000 cpm. ●, SHP-77; ○, H-69; ▲, H-446; □, H-209.

neutralizing polyclonal antibody to IFN-γ (Meloy Laboratories, Springfield, VA) was able to block the inhibitory effect of 1000 units of IFN-γ per ml by >90% in these assays (data not shown). Cell viability as assessed by trypan blue exclusion was indistinguishable between controls (>85%) and IFN-γ (5000 units/ml)-treated cultures, indicating that IFN-γ was not directly cytotoxic to these cells. In view of the potential limitations of [^{3}H]thymidine incorporation as an indicator of cell growth, we also tested the effects of IFN-γ in an agar clonal assay. Results (Table 3) of an expanded survey indicate that all five SCLC lines showed responsiveness to IFN-γ, most with EC_{50} (concentration required for half-maximal effect) values in the range of 1000 units/ml. No complete responses were observed even with IFN-γ at 10^4 units/ml. At 1000 units/ml, responses ranged from 38% to 70% inhibition of agar colonies.

Effect of GM-CSF on Proliferation of SCLC Lines. Fig. 2 shows that treatment with GM-CSF reduced [^{3}H]thymidine incorporation by four SCLC lines. Cultures incubated for 5 days with GM-CSF showed diminished [^{3}H]thymidine incorporation in a dose-dependent fashion. Lines SHP-77 and H-446 appeared comparable in their sensitivity to the growth

Table 3. Inhibition of SCLC clonal growth in agar by IFN-γ

	cfu* per 10^3 cells plated		
Cell line	No IFN-γ	10^3 units/ml	10^4 units/ml
SHP-77	86, 78	46, 40 (48%)	33, 23 (66%)
H-69	60, 45	28, 23 (51%)	22, 18 (62%)
H-446	92, 65	32, 35 (58%)	19, 24 (73%)
H-209	37, 46	28, 23 (38%)	12, 15 (67%)
H-60	18, 16	6, 10 (53%)	6, 3 (74%)

IFN-γ (amount as indicated) was added to cultures at their inception and colonies were enumerated at 14–21 days. Values in parentheses are the percent inhibition of colony formation, calculated as [1 − (average cfu, experimental/average cfu, control)] × 100.
*Colony-forming units; values are given for duplicate determinations.

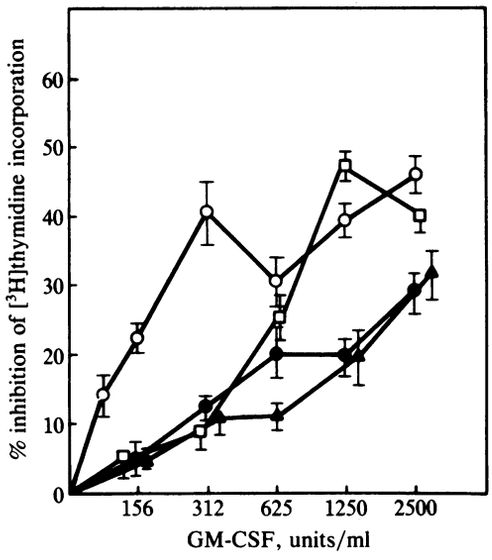

FIG. 2. [^{3}H]Thymidine incorporation by several SCLC continuous culture lines treated with various concentrations of GM-CSF. Other details were as for Fig. 1. ●, SHP-77; ○, H-69; ▲, H-446; □, H-209.

effects of GM-CSF, in that 30–35% inhibition was detected at the highest concentration tested, 2500 units/ml. One cell line, H-69, had initial sensitivity to the growth effects of GM-CSF at comparatively lower concentrations; however, the inhibition did not exceed 40–45%. This may represent a maximal response, since the curves appear to approach a plateau. No IFN activity could be detected in these culture fluids by a viral cytopathic effect assay, nor did anti-IFN-γ antibody prevent GM-CSF inhibition of [^{3}H]thymidine incorporation, indicating that the effect of GM-CSF was not indirectly mediated via release of IFNs (25). Cell viability was similar for control and GM-CSF-treated cultures. These data suggesting that GM-CSF inhibits growth of SCLC lines were verified by clonal assays in agar. Results (Table 4) indicate that GM-CSF can inhibit SCLC colony growth in agar. The EC_{50} for this effect is ≈1000 units/ml for each of the lines tested.

DISCUSSION

Our results indicate that both IFN-γ and GM-CSF can differentially modulate (enhance) the expression of Leu-7, Leu-M3, and HLA-DR (Tables 1 and 2), three SCLC antigens commonly found on macrophages. Of these, Leu-M3 is restricted to cells of myeloid derivation and has been considered to be a differentiation marker for mononuclear phagocytes (26). Additionally, both of these hormones have antiproliferative effects on several SCLC lines. Enhanced expression of HLA-DR and Leu-M3 on monocytes has been associated with a differentiation process because these antigens are normally absent, or present at low levels, on immature myeloid cells or their precursors. Leu-7 expression may also follow this theoretical paradigm. Enhanced macrophage marker expression has, in some instances, been coupled to concomitant growth inhibition. In some experimental *in vivo* models, leukemogenesis can be inhibited by treatment with hormones such as CSF (27, 28) or IFN-γ (29, 30), although IFN-γ species also have multiple effects on nonhemopoietic cells (31–33). For these reasons, we examined these lymphokines for possible antiproliferative effects on SCLC lines.

Table 4. Inhibition of SCLC clonal growth in agar by GM-CSF

Cell line	cfu* per 10^3 cells plated	
	No GM-CSF	10^3 units/ml
SHP-77	132, 103	61, 45 (55%)
H-446	33, 27	9, 10 (67%)
H-209	72, 67	32, 36 (50%)

GM-CSF was added to cultures at their inception and colonies were enumerated at 14–21 days. Values in parentheses are the percent inhibition of colony formation, calculated as in the legend to Table 3.

*Colony-forming units are given for duplicate determinations.

The results (Figs. 1 and 2, Tables 3 and 4) reveal some growth-inhibitory activity for both IFN-γ and GM-CSF. These effects are only partial, consistent with the possibility that these cell populations are heterogeneous in their responsiveness, a result also suggested by the flow-cytometry results (Tables 1 and 2). Another feature of these results is the relatively high concentrations required to achieve appreciable growth inhibition with these agents. The level of responsiveness, however, is comparable to other *in vitro* studies utilizing transformed monocytic cell lines (14, 18), myeloid leukemias (40), or normal macrophages (19). Thus, if either IFN-γ or GM-CSF is acting to promote differentiation of SCLC cells in a manner analogous to normal monocyte development, it does not appear that these treatment conditions are sufficient to elicit the entire spectrum of changes resulting in a mature, nondividing macrophage.

Our results suggest that SCLC tumors could be candidates for therapeutic modalities that incorporate new approaches. If SCLC tumors *in vivo* are also responsive to lymphokines, then inhibition of tumor growth through induction of terminal differentiation should be possible. Also, *ex vivo* antigen induction might be used to remove residual tumor cells (e.g., by cell sorting) prior to autologous bone marrow reconstitution (41). The gene products for several of the CSFs (34–36, 40) have recently been cloned, creating possibilities for such further studies.

It has been thought that only hemopoietic cells express receptors for GM-CSF (17), and this view has recently been reiterated (34). Our results indicate that SCLC cells respond to GM-CSF, and a reasonable mechanism would involve specific receptors for this hormone. The presence of CSF receptors on SCLC cells would be consistent with our proposal that SCLC tumors arise from macrophage precursors during the repair of damaged lung tissue, such as occurs in heavy smokers (5). Alternatively, the distribution of CSF receptors may be broader than currently realized, and therefore these results may have relevance to other neoplasias not currently considered to be derived from hemopoietic precursors. Nevertheless, our findings describe a biological response to IFN-γ and GM-CSF of treated SCLC continuous culture lines that has characteristics shared in common with myeloid differentiation.

Concerning the putative histological cell of origin for SCLC, it has been noted that although SCLC cells typically express such epithelial cell characteristics as desmosomes and keratin intermediate filaments (1, 2), such features do not typify hemopoietic cells. In this regard reports of Porvaznik and MacVittie (37), describing gap junctions in normal macrophage colonies, and Zauli *et al.* (38), describing coexpression of vimentin and keratin in a human granulocyte cell line, provide a basis by which to reconcile these previously disparate features of SCLC and macrophage cell biology.

We thank Dr. S. Mergenhagen for his support of these studies, Dr. D. H. Pluznik for introducing M.R.R. to experimental hematology, and Mrs. Sharon Morgan for typing the manuscript.

1. Gazdar, A. F., Bunn, P. A., Jr., Minna, J. D. & Baylin, S. B. (1985) *Science* **229**, 679.

2. Ruff, M. R. & Pert, C. B. (1985) *Science* **229,** 680.
3. Moody, T. W., Pert, C. B., Gazdar, A. F., Carney, D. N. & Minna, J. D. (1981) *Science* **214,** 1246–1248.
4. Gazdar, A. F., Carney, D. N., Nau, M. M. & Minna, J. D. (1985) *Cancer Res.* **45,** 2924–2930.
5. Ruff, M. R. & Pert, C. B. (1984) *Science* **225,** 1034–1036.
6. Bunn, P. A., Jr., Linnoila, I., Minna, J. D., Carney, D. & Gazdar, A. F. (1985) *Blood* **65,** 764–768.
7. Patterson, B. J., Robinson, J. B. & Pinkerton, P. H. (1985) *Am. J. Clin. Pathol.* **84,** 159–165.
8. Ruff, M. R., Schiffmann, E., Terranova, V. & Pert, C. B. (1985) *Clin. Immunol. Immunopathol.* **37,** 387–396.
9. Becker, S. (1984) *J. Immunol.* **132,** 1249–1254.
10. Steeg, P. S., Moore, R. N., Johnson, H. M. & Oppenheim, J. J. (1982) *J. Exp. Med.* **156,** 1780–1793.
11. Warren, M. K. & Vogel, S. N. (1985) *J. Immunol.* **134,** 982–989.
12. Harris, P. E., Ralph, P., Litcofsky, P. & Moore, M. S. (1985) *Cancer Res.* **45,** 9–13.
13. Klimpel, G. R., Fleischmann, W. R., Jr., & Klimpel, K. D. (1982) *J. Immunol.* **129,** 76–80.
14. Buessow, S. C. & Gillespie, G. Y. (1984) *J. Biol. Response Modif.* **3,** 653–662.
15. Doyle, A., Martin, W. J., Funa, K., Gazdar, A., Carney, D., Martin, S. E., Linnoila, I., Cuttitta, F., Mulshine, J., Bunn, P. & Minna, J. (1985) *J. Exp. Med.* **161,** 1135–1151.
16. Metcalf, D. (1984) *The Colony Stimulating Factors* (Elsevier, Amsterdam).
17. Metcalf, D. (1985) *Science* **229,** 10–22.
18. Chen, B. & Lin, H. (1984) *J. Immunol.* **132,** 2955–2960.
19. Moore, R. N., Oppenheim, J. J., Farrar, J. J., Carter, C. S., Waheed, A. & Shadduck, R. K. (1980) *J. Immunol.* **125,** 1302–1305.
20. Lotem, J. & Sachs, L. (1981) *Int. J. Cancer* **28,** 375–386.
21. Abo, T., Cooper, K. & Balch, C. M. (1982) *J. Immunol.* **129,** 1752–1757.
22. Fisher, E. R. & Paulson, J. D. (1978) *Cancer Res.* **38,** 3830–3835.
23. Gazdar, A. F., Carney, D. N., Russel, E. K., Sims, H. L., Baylin, S. B., Bunn, P. A., Jr., Guccion, J. G. & Minna, J. D. (1980) *Cancer Res.* **40,** 3502–3507.
24. Carney, D. N., Gazdar, A. F., Bepter, G., Guccion, J. G., Marangos, P. G., Moody, T. W., Zweig, M. H. & Minna, J. D. (1985) *Cancer Res.* **45,** 2913–2923.
25. Moore, R. N., Larsen, H. S., Horokov, D. N. & Rouse, B. T. (1984) *Science* **223,** 178–180.
26. Dimitriu-Bona, A., Burmester, G., Waters, S. & Winchester, R. (1983) *J. Immunol.* **130,** 145–152.
27. Ichikawa, Y. (1969) *J. Cell. Physiol.* **74,** 223–234.
28. Lotem, J. & Sachs, L. (1981) *Int. J. Cancer* **28,** 375–386.
29. Gresser, I., Bourali, C., Lévy, J. P., Fontaine-Brouty-Boyé, D. & Thomas, M. T. (1969) *Proc. Natl. Acad. Sci. USA* **63,** 51–57.
30. Salvin, S. B., Younger, J. S., Nishio, J. & Neta, R. (1975) *J. Natl. Cancer Inst.* **55,** 1233–1240.
31. Bradley, E. C. & Ruscetti, F. W. (1981) *Cancer Res.* **41,** 244–249.
32. Basham, T. Y. & Merrigan, T. C. (1983) *J. Immunol.* **130,** 1492–1497.
33. Nickoloff, B. J., Basham, T. Y., Merrigan, T. C. & Morheinn, V. B. (1985) *J. Invest. Dermatol.* **84,** 487–490.
34. Wong, G. G., Witek, J. S., Temple, P. A., Wilkens, S. M., Leary, A. C., Luxenberg, D. P., Jones, S. S., Brown, E. L., Kay, R. M., Orr, E. C., Shoemaker, C., Golde, D. W., Kaufman, R. J., Hewick, R. M., Liang, E. A. & Clarke, S. C. (1985) *Science* **228,** 810–815.
35. Kawasaki, E. S., Ladner, M. B., Wang, A. M., VanArsdell, J., Warren, M. K., Coyne, M. Y., Schweickart, V. L., Lee, M., Wilson, K. J., Boosman, A., Stanley, E. R., Ralph, P. & Mark, D. F. (1985) *Science* **230,** 291–296.
36. Nagata, S., Tsuchiya, M., Asano, S., Kaziro, Y., Yamataki, T., Yamamoto, O., Hirata, Y., Kubta, N., Oheda, M., Nomura, H. & Ono, M. (1986) *Nature (London)* **319,** 415–418.
37. Porvaznik, M. & MacVittie, T. C. (1979) *J. Cell Biol.* **82,** 555–564.
38. Zauli, D., Gobbi, M., Crespi, C., Tazzari, P. L., Miserocchi, F., Magnani, M. & Testoni, N. (1986) *Leuk. Res.* **10,** 29–33.
39. Koros, A. M. C., Weinbaum, D. L., Atchison, R. W., Lakomy, R. J. & Merrall, E. J. (1985) *J. Clin. Cancer Res.* **4,** 355–363.
40. Souza, L. M., Boone, T. C., Gabrilove, J., Lai, P. H., Zsebo, K. M., Murdock, D. C., Chazin, V. R., Bruszewski, J., Lu, H., Chen, K. K., Bavendt, J., Platzer, E., Moore, M. A. S., Mertdsmann, R. & Welte, K. (1986) *Science* **232,** 61–65.
41. Herzig, G. P. (1981) *Prog. Hematol.* **12,** 1–23.