Induction of Myeloid Colony-stimulating Activity in Murine Monocyte Tumor Cell Lines by Macrophage Activators and in a T-Cell Line by Concanavalin A

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ABSTRACT

Certain fibrosarcoma lines in culture and the WEHI-3 myelomonocytic leukemia cell line have previously been shown to secrete myeloid colony-stimulating activity (CSA) spontaneously. We describe here other hematopoietic tumor cell lines in which CSA is either produced constitutively or inducible by immunostimulators. CSA production in macrophage and monocyte tumor lines is induced by lipopolysaccharide, zymosan, Mycobacterium strain Bacillus Calmette-Guerin, tuberculin purified protein-derivative preparation from mycobacteria, and dextran sulfate. Myeloma, mastocytoma, and T-lymphoma lines do not produce CSA with or without these agents. In contrast, the T-lymphocyte mitogen concanavalin A (but not phytohemagglutinin) induces CSA synthesis in one of seven T-lymphomas tested. In most cases induction of CSA is correlated with conditions of cell growth inhibition by the immunomodulators. However, other drugs that cause cytostasis or cytotoxicity do not lead to CSA production. Leukemic cells thus may retain sensitivity to normal regulatory events with resultant effects on host hematopoietic cell functions.

INTRODUCTION

Elevated numbers of granulocytes and macrophages are associated with immunological reactions and inflammatory conditions. Marrow and blood monocytes have been identified as producers of CSA, necessary for the development of granulocytes and macrophages in vitro (6, 8, 25). Bacterial LPS stimulates macrophages to increased CSA production (8, 10, 35). However, CSA synthesis is also induced during T-lymphocyte-dependent mitogen or antigen stimulation of spleen or blood cells (7, 22, 26, 37, 38).

We have described constitutive CSA production in the mouse myelomonocyte tumor cell line WEHI-3 (30) and induction of CSA in a macrophage tumor cell line PUS-1.8 by LPS, BCG, PPD, yeast zymosan, and phorbol myristate (29). This study investigates the induction of CSA in other myocyte-macrophage tumor cell lines by macrophage-activating agents and in T-lymphomas by T-lymphocyte mitogens.

MATERIALS AND METHODS

Murine Tumor Cell Lines. Monocyte and macrophage tumor cell lines are described in Ref. 32, except for Abelson leukemia virus-induced line RAW264 (33). T-lymphoma lines EL4, RBL-5, BW5147, and S49; myelomas P3 and MOPC315; mastocytoma P815; lymphoma P388; and Abelson line R8 are described in Ref. 31. Rauscher leukemia virus line RBL-3 and chemically induced leukemia L1210 (39) were obtained from K. Chang (NIH, Bethesda, Md.); fibrosarcoma L929 (5) was obtained from B. Williams (Sloan-Kettering Institute, Rye, N. Y.); bone marrow fibroblast JLSV9 and Rauscher leukemia virus-infected JLSV9-RLV (9) were obtained from A. Demsey (Sloan-Kettering Institute); Abelson lymphoma RAW309.1 was obtained from W. Raschke (Salk Institute for Biological Studies, San Diego, Calif.); F22.WC-2 T-lymphoma was obtained from T. Watanabe (Osaka University Hospital, Osaka, Japan); mesothelioma BALTNMS201, T-lymphomas P1798 and BAL-ENTL5, and Abelson lymphomas ABPL1 and ABPL2 were obtained from M. Potter (National Cancer Institute, Bethesda, Md.).

Substances Tested for Induction of CSA. Dialedy latex beads 0.81 µm in diameter, PHA P, and LPS (Salmonella typhosa W0901; Difco Laboratories, Detroit, Mich.); PPD (gift from Dr. A. Gray, Merck Sharp & Dohme, West Point, Pa.); BCG (Tice strain; University of Illinois, Chicago, Ill.); zymosan and dextran sulfate (M.W., 500,000; Sigma Chemical Co., St. Louis, Mo.); and Con A (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) were dissolved or suspended in phosphate-buffered saline, pH 7.4 (8 g NaCl, 0.2 g KCl, 1.15 g NaHPO₄, 0.2 g KH₂PO₄, 0.1 g CaCl₂, and 0.1 g MgCl₂·6H₂O per liter), at 20 to 100 times the final concentration.

Assay for CSA (3). Mouse bone marrow cells (7.5 × 10⁶) were suspended in 1 ml 0.3% Difco agar culture medium containing enriched McCoy's medium plus 10% fetal calf serum. Medium conditioned by cell lines (0.1 ml) was placed on the bottom of Petri dishes prior to addition of agar cell suspensions. Cultures were incubated in 5% CO₂-humidified air, and colonies (>40 cells/aggregate) and clusters (3 to 40 cells/aggregate) were scored after 5 to 7 days. Under these conditions a maximum of 100 to 200 colonies of granulocytes and macrophages can be obtained with optimal concentrations of CSA. For intracellular CSA, cell pellets were suspended in phosphate-buffered saline to the original volume and lysed by 2 cycles of freeze-thaw.

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3 The abbreviations used are: CSA, myeloid colony-stimulating activity; LPS, lipopolysaccharide; BCG, Mycobacterium strain Bacillus Calmette-Guerin; PPD, tuberculin purified protein derivative; PHA, phytohemagglutinin; Con A, concanavalin A; T-cell, thymus-derived lymphocyte; B-cell, bone marrow-derived lymphocyte.

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additional CSA was detected in producing lines following further cycles of freeze-thaw.

RESULTS

Spontaneous Production of CSA by Tumor Cell Lines. A number of murine cell lines adapted to culture were investigated for spontaneous production of CSA. Besides the previously described L-cell fibrosarcoma (2) and myelomonocytic leukemia WEHI-3 (30), 2 lymphoid lines secreted CSA constitutively (Table 1). Large amounts of CSA were found in supernatants of RBL-3 and, to a lesser extent, of L1210 lymphoid cell lines. A fibroblastic cell line derived from normal mouse bone marrow (JLSV9) and its Moloney leukemia virus-infected counterpart, JLSV9-RLV, also produced CSA spontaneously, as described for the similar JLSV5 virus-transformed line (44). The fibroblastic lines produced only or predominately macrophage CSA. CSA from the other constitutive lines caused bone marrow colonies of granulocyte and macrophage morphology (not shown). In the CSA-secreting lines studied by us, undiluted supernatants and 1:10 dilutions in most cases produced greater than 100 colonies/7.5 x 10^4 bone marrow cells, and dilutions greater than 1:10 were required before titrating out the activity. No CSA was found in supernatants of untreated cultures of a variety of macrophage, monocyte, T-lymphoma, myeloma, or mastocytoma cell lines (Table 1); in a number of these lines tested, an inhibitor of the CSA assay was also undetected, indicating the absence of the molecule(s).

Induction of CSA by LPS and Other Macrophage Activators in Monocyte-related Lines. Since LPS will induce CSA synthesis in the macrophage line PU5-1.8 (29), this agent was tested in the other hematopoietic lines. We use the term induction to denote the stimulation of CSA production in experimental cultures when control cultures do not contain detectable activity. As shown in Table 2, LPS

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* Colonies/7.5 x 10^4 mouse bone marrow cells stimulated by 0.1 ml tumor cell line supernatant in 1-ml agar cultures. Cell line cultures were initiated at 2 x 10^4 cells/ml, and supernatants were collected when cultures reached 10^4/ml (2 to 3 days). WEHI-3, L929, L929, L929-RLV, and RBL-3 supernatants stimulated 90 to 150 colonies also when tested at 1:10 dilution. The other constitutive producers and inducible lines (described below) showed less activity when their supernatants were diluted more than 1:2.

b Mean ± S.E.
induced CSA to varying degrees in all of the monocyte and
cytokine lines except for the constitutive producer
WEHI-3. Results are shown for a 3-day incubation with LPS,
1 µg/ml. This concentration of LPS inhibits the growth of the
monocyte-macrophage lines (except P388D1) more than
50% but not of the other cells shown in Table 1 (32). In
some experiments, control WEHI-3 cultures had lower activity
(40 to 80 colonies/0.1 ml supernatants) than was shown in
Table 2, and then LPS stimulated CSA production up to
2-fold (4). Cell lysates (at 10^6 cells/ml) of control cultures of
inducible tumor cell lines neither contained CSA nor
inhibited the activity of preformed CSA (0.1 ml tested in
assay), except for slight inhibition with J774.

Production of CSA by L1210 cells was augmented by
incubation with LPS, and induction of CSA occurred in an
Abelson lymphoma line, R8. These 2 lines and RBL-3 lack T-
lymphocyte Thy-1 antigen, surface immunoglobulin, and
myeloid markers of phagocytosis and lysozyme but bear Fc
receptors and possess an alkaline phosphatase activity
characteristic of lymphoid tumors (unpublished observa-
tions). LPS did not induce CSA in a number of T-lymphoma,
myeloma, mastocytoma, or other lymphoma lines (Table 2).

The monocyte-related tumor lines were also tested with
other macrophage-active agents known to induce CSA in
PU5-1.8 (29) and to inhibit specifically growth of these
malignant tumor types (32). As shown in Chart 1, the
immunopotenti-
tors LPS, BCG, PPD, zymosan, and dextran sulfate induced
CSA in monocyte and macrophage tumor lines at concentra-
tions generally correlated with tumor cell cytostasis. The
decline in CSA activity in supernatants of several lines as
LPS concentration is increased to 10 µg/ml may be due to
inhibition of the assay by LPS (32). However, the suboptimal
activity in cultures of the most sensitive line, RAW264, at
0.1 and 1 µg LPS per ml must be due to high-dose inhibition
of CSA production, as these concentrations do not affect
the assay system. Latex beads, which are actively phago-
cytosed by the cell lines without interfering with growth
(32), did not induce CSA.

**Induction of CSA by T-Lymphocyte Mitogens.** Since
mitogen stimulation of normal T-lymphocytes induces CSA
production, these agents were tested on a number of T-
lymphoma cell lines. PHA and Con A are preferentially toxic
to murine (27) and human (28) T tumor cell lines compared
to myelomas; however, many other hematopoietic cell lines
are as sensitive as T lines (Ref. 27; unpublished observa-
tions). Con A was very effective in stimulating granulocyte-
cytokine macrophage CSA in the T-lymphoma EL4 (Table 2). Six
other T-lymphomas and other types of hematopoietic cell
lines were not induced by Con A. Dose titration (Chart 2)
shows that the threshold for CSA induction and tumor cell
growth inhibition occurred at 2 to 5 µg Con A per ml. Con
A, up to 50 µg/ml, strongly induced CSA production despite
the fact that no viable EL4 cells remained after 3 days of
incubation in this high toxic concentration.

PHA showed a toxicity curve almost identical with that of
Con A, but no CSA was induced in EL4 by this lectin.
Controls showed that PHA did not inhibit the activity of
preformed CSA or the induction of CSA in EL4 cells by Con
A. PHA did not induce CSA in any cell line tested (Table 1).

**Kinetics of CSA Production.** Chart 3 shows that small
amounts of CSA were detected in supernatants of EL4
cultures incubated for 16 hr with Con A, 10 or 30 µg/ml.
At this time 30% of EL4 cells were killed by the higher Con A
concentration. There was a great increase in supernatant
CSA during the subsequent 2 days of incubation. At 30 µg
Con A per ml, viable cells decreased to 5% of initial
numbers at Day 2 and 0% at Day 3, and total live and dead

**Chart 1. Induction of CSA and inhibition of growth in macrophage cell
lines by macrophage activators.** Cultures were initiated at 2 × 10^5 cells/ml
plus final concentrations of LPS, PPD, zymosan (Z), and dextran sulfate (DS)
in µg/ml or BCG in bacteria/ml as shown. After 3 days of incubation, viable
tumor cells were counted (percentage of growth inhibition equals 100 times
the increase in experimental-increase in control culture), and supernatants
were tested for CSA (colonies stimulated per 7.5 × 10^5 bone marrow cells
plated in agar with 0.1 ml cell line supernatant). For several lines tested the
product induced was granulocyte-macrophage CSA.
Induction of Monocyte and T-Lymphoma Cell Lines

Stimulation of CSA by dextran sulfate in bone marrow cells, probably macrophages, has been described by Gronowicz et al. (14). In addition to these agents and BCG, PPD, and zymosan used in the present paper, we previously showed that phorbol myristate induces CSA in the monocyte line PU5-1.8 (29) and in normal adherent bone marrow cells (unpublished observations). Phorbol myristate is a potent stimulator of enzyme release and other activities of granulocytes (11, 34), induces plasminogen activator production in macrophages (43), and is a T-lymphocyte mitogen (42). Other macrophage properties stimulated by activators in the macrophage tumor cell lines include antibody-dependent cellular immunity against erythrocyte (33) and tumor targets,4 production of T-lymphocyte-activating factor (21, 24), prostaglandin release (Ref. 20; E. Rietschel, personal communication), β-glucuronidase, elastase, plasminogen activator, collagenase, endogenous pyrogen, and factors required for induction of murine T killer cells (16), murine antibody-producing cells,5 and human B-lymphocytes (17) not replaceable by mercaptoethanol. A summary of macrophage properties retained by murine and human monocyte-related cell lines is shown in Table 3.

The constitutive production of CSA by the least differentiated myelomonocytic cell, WEHI-3, but not by other lines with more properties of mature macrophages (33) deserves comment. Normal, immature myeloid cells or their progenitors are dependent on CSA for growth. There must be stringent controls to prevent self-stimulation of the progenitor by premature synthesis of CSA, which is a function of the mature cells of the monocyte branch of the myeloid series. Production of CSA associated with murine (24) and human (13) myelomonocytic leukemias suggests that 1 path to leukemogenesis in this early cell type is the inappropriate activation of genes for the molecule that stimulates its own growth. There is evidence that the murine WEHI-3 tumor (24) and WEHI-3 culture line cells (4) are still dependent on CSA for growth. A granulocyte inhibitor of CSA production blocks WEHI-3 colony formation in agar but does not block colony formation of other tumor lines, and this growth inhibition is overcome by adding an external source of CSA or by stimulating endogenous synthesis of CSA (4).

Induction of CSA in spleen and peripheral blood cells by Con A and PHA (and perhaps by pokeweed mitogen) is a property of T-lymphocytes (26, 27). However, there is evidence for separate subsets of T-cells responding to PHA and Con A, in terms of induction of DNA synthesis (41) and immune functions of helper, suppressor, and killer cells. The induction of CSA in the T-cell line EL4 by Con A but not by PHA suggests that these cells belong to a Con A-responsive, PHA-unresponsive subset and that other T lines may be found to be activated by the other T mitogens. Pokeweed mitogen did not induce CSA in EL4 cells. However, adherent cells are required for activation of some normal T-cell

**DISCUSSION**

Several facts are apparent from this study of CSA induction in different types of hematopoietic tumor cell lines. (a) Only cell type-specific agents (macrophage activators for monocyte-related lines; Con A for the T line) can induce CSA production. (b) All inductions of CSA are accompanied by drug effects strong enough to block tumor cell proliferation. Other inhibitors of cell growth [dibutyryl cyclic adenosine 5'-phosphate, high levels of thymidine, dimethyl sulfide (29), PHA, and Con A in most cell lines] do not induce CSA. (c) The macrophage lines differ in sensitivity to inducing agents, presumably because of differences in quantity or affinity of specific receptors for each kind of activating molecule. (d) The inducible T-cell line EL4 must share with the other T lines binding sites for PHA and Con A leading to toxicity but has a unique Con A-binding site for functional activation of CSA production.

The LPS induction of CSA in the monocyte-related tumor lines described here is similar to the results of experiments of Eaves and Bruce (10) with normal mouse macrophages and of Ruscelli and Chervenick (35) with human monocytes in the dose titration and kinetics requiring several days for maximal stimulation.

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Table 3

General properties of monocyte-related tumor cell lines in culture

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<th>Strain</th>
<th>Etiology</th>
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a Lysozyme synthesis and predominate secretion (30).
b Phagocytosis of zymosan and latex beads; P815 has slight ingestive activity against latex beads (32).
c Granulocyte-monocyte CSA, constitutive in WEHI-3 and inducible in the other macrophage lines by LPS, BCG, PPD, zymosan, or dextran sulfate (Chart 1).
d Antibody-dependent phagocytosis or lysis of sheep RBC and tumor targets (33).
e Plasminogen activator, collagenase, elastase (J. Hamilton and Z. Werb, personal communication).
g Prostaglandin E production, especially in response to LPS or CSA (20).
h Mouse strain: C, BALB/c.
j Ascites preparations are very active in lysis of RBC targets (Ref. 33; unpublished observations).
k Latex bead phagocytosis and antibody-dependent tumor lysis by PU5-1.8 line is stimulated by preincubation with LPS or PPD.
l Stimulated further by preincubation with LPS or PPD (33).
m NT, not tested.
* One (EL4) of 7 T-lymphomas produces CSA in response to Con A (Table 2).

functions, and this is being tested with the cell line. Other functions of T-cell lines have been described, such as inhibition (12, 40) and stimulation (T. Watanabe, personal communication) of in vitro antibody production, but the present experiments are the first examples of an induced function in a lymphoid cell line.

In addition to macrophage and T-cell sources of CSA, there are suggestions that B-lymphocytes can produce myeloid CSA. Since LPS-induced B-cell mitogenesis is correlated with CSA production by mouse spleen cultures in kinetics, genetics, and dose response, Apte et al. (1) have implied that B-cells are the source of CSA. However, spleen macrophages produce CSA in response to LPS, and no attempt was made to exclude this possibility. We find that spleen cell production of CSA in response to LPS is macrophage dependent, whereas LPS-induced proliferation of B-lymphocytes is independent of macrophages, implying that B-cells are not a major contributor to LPS-induced CSA (unpublished observations).

What uses can be made of these hematopoietic tumor line systems? Due to their homogeneity, they are ideally suited for biochemical analysis of induction and regulation, from surface membrane receptors for external stimuli to cytoplasmic transduction of activating signals to nuclear events. For example, LPS at CSA-inducing concentrations stimulates intracellular cyclic adenosine 5'-phosphate levels and phosphorylation of nonhistone nuclear protein in the J774 macrophage line, similar to that described for antiimmunoglobulin activation of rabbit B-lymphocytes (Ref. 18 and Footnote 6). Study of macrophage lines differing in expression of receptors for microbial immunostimulating agents (Chart 1) and differing in functional properties of antibody-dependent cellular cytotoxicity to RBC and tumor targets will aid investigations of similar diversity in normal macrophage subsets. Defining tumor cell retention of sensitivity to physiological controls, as in this paper or in the inhibition of growth and cancer accompanying stimulation of differentiated properties in myeloblastic leukemia cell lines by CSA (15, 19), is a continuing goal in these studies.

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REFERENCES


Y. Nishizawa, T. Kishimoto, H. Kikutani, Y. Yamamura, and P. Ralph, manuscript in preparation.
Induction of Monocyte and T-Lymphoma Cell Lines


Induction of Myeloid Colony-stimulating Activity in Murine Monocyte Tumor Cell Lines by Macrophage Activators and in a T-Cell Line by Concanavalin A

Peter Ralph, Hal E. Broxmeyer, Malcolm A. S. Moore, et al.


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