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Antibodies to colony-stimulating factors block Lewis lung carcinoma cell stimulation of immune-suppressive bone marrow cells*

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Summary. Progressive growth of metastatic Lewis lung carcinoma (LLC) tumors results in a concurrent stimulation of myelopoiesis and the appearance of immune-suppressive bone marrow cells. The present study has shown that normal bone marrow cells could be induced to become immune-suppressive by 3 days of culture with supernatants of cloned metastatic LLC-LN7 variant cells. The capacity of the LLC-LN7 supernatants to stimulate the appearance of suppressor cells was directly proportional to the concentration of supernatant used in the bone marrow culture. When adoptively transferred with a LLC-LN7 tumor inoculum, the supernatant-induced suppressor bone marrow cells increased the rate of appearance of palpable tumors and the frequency of tumor establishment. The LLC-LN7 supernatants containing suppressor-cell-inducing activity also had colony-stimulating factor (CSF) activity. The CSF activity produced by the LLC-LN7 cells could be diminished with neutralizing antibodies to either granulocyte/monocyte(GM-) CSF or to interleukin-3 (IL-3). Likewise, the suppressor-inducing activity in the LLC-LN7 supernatants was diminished by pretreatment with anti-GM-CSF or anti-IL-3. The combination of anti-GM-CSF and anti-IL-3 completely neutralized all suppressor-inducing activity produced by the LLC-LN7 cells. These results suggest that the secretion of IL-3 and GM-CSF by LLC-LN7 tumor cells is a mechanism by which the tumors stimulate myelopoiesis and induce normal bone marrow cells to become immune-suppressive. Bone marrow cells that are induced to become immune-suppressive by culture with LLC-LN7 supernatants can, in turn, facilitate the establishment of tumor in vivo.

Key words: Lewis lung carcinoma – GM-CSF – IL-3 – Bone marrow – Immune suppressor

Introduction

Tumor cells are vulnerable to destruction by immune effector cells such as macrophages, natural killer (NK) cells, lymphokine-activated killer cells and cytotoxic T lymphocytes [3, 14, 19, 21]. However, tumor induction of immune-suppressor cells can facilitate immune escape [1, 9, 16, 25]. These tumor-induced immune-suppressor cells have classically been characterized to be Thy-1$^+$ T lymphocytes or adherent macrophages. More recently, tumors have been shown to stimulate myelopoiesis and, consequently, the appearance of bone-marrow-derived immune-suppressor cells [7, 24, 27, 30]. While in some instances these myelopoiesis-associated suppressor cells resemble bone-marrow-derived macrophages [7], other studies have shown these suppressor cells to be immature null cells lacking markers typical of mature macrophages or lymphocytes [24, 27, 30].

Myelopoiesis and the appearance of bone-marrow-derived immune-suppressor cells can be directly induced by colony-stimulating factors (CSFs) [7, 9, 17, 24, 31]. Many tumor types including ovarian, breast, pancreatic and lung tumors produce CSF activities and stimulate myelopoiesis in vivo [5, 7, 15, 18, 24, 27]. There are four well-characterized CSFs that influence the development of bone marrow progenitor cells. Generally, IL-3 acts on pluripotent stem cells, GM-CSF acts on bipotential stem cells to produce monocytes and granulocytes, G-CSF induces granulocyte colony formation, and CSF-1 induces monocyte colony formation [4, 8, 13]. These CSFs can also directly regulate immunity [6, 7, 12, 20, 22, 26]. In addition, the stimulation of myelopoiesis by CSFs can induce the appearance of myelopoiesis-associated immune-suppressor cells [11, 24, 31]. Murine GM-CSF and IL-3 can each induce bone marrow cells to become immune-suppressive during in vitro culture. When combined, GM-CSF plus IL-3 synergize to induce a greater level of immune-suppressor activity [31].

In the Lewis lung carcinoma (LLC) tumor model, we have shown that the progressive growth of metastatic LLC variant tumors results in myelopoiesis, which has been

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characterized by increases in the number of granulocyte/macrophage progenitor cells in the bone marrow and spleen, and increases in the proportion of immature monocytic cells in the peripheral blood [27, 30]. The myelopoietic stimulation in the LLC tumrr bearers coincided with a progressive decline in T-cell competence and with the appearance of a population of myelopoiesis-associated immune-suppressor cells, first in the bone marrow and then in the spleen. These bone-marrow-derived suppressor cells resembled immature cells of the monocyte lineage as they were nylon-wool-nonadherent, Thy-1$^-$, IgM$^-$, MG1.2$^-$, asialoGM1$^-$, sensitive to treatment with the monocyte toxin L-leucine methyl ester, and mediated their suppression through a pathway that was not dependent on prostaglandins [27]. The results of the present study suggest that LLC cells stimulate both myelopoiesis and the appearance of bone-marrow-derived immune-suppressor cells by secretion of GM-CSF and IL-3.

Materials and methods

Mice. Male C57Bl/6 mice, 6–8 weeks old, were used for all studies. The mice were obtained from Harlan Sprague Dawley (Indianapolis, Ind.) and then housed at the Hines V. A. animal research facility.

Medium. The culture medium used was RPMI-1640 containing 100 U/ml penicillin, 100 µg/ml streptomycin, 0.02 M HEPES buffer solution, 0.05 mM 2-mercaptoethanol, 2 mM L-glutamine and 10% low-endotoxin fetal bovine serum (Sigma Chemical Co., St. Louis, Mo).

Tumor cells. Cloned metastatic LLC-LN7 variants had been isolated from lung nodules of mice bearing subcutaneously implanted parental LLC tumors as previously described [28].

LLC-LN7 supernatant. Supernatants of LLC-LN7 cells were prepared by culturing 1×10^6 cells/ml for 24 h. Cultures were then centrifuged, supernatants were collected and filtered. In select experiments, aliquots of culture medium or LLC-LN7 supernatants were incubated for 2 h at 37° C with anti-(mouse GM-CSF) antiserum at a 10^{-4} dilution and/or with monoclonal anti-(mouse IL-3) antibody at a dilution of 1 : 200. The goat anti-(mouse GM-CSF) antiserum was kindly provided by Dr. J. Schreurs, while rat hybridoma supernatant containing anti-(mouse IL-3) antibody was generously provided by Dr. J. Abrams, both of DNAX Research Institute of Molecular and Cellular Biology.

Soft-agar colony-forming assay. Into each 35×10-mm tissue-culture dish, were placed 7.5×10^4 bone marrow cells in 1 ml semisolid supplemented RPMI-1640 medium containing 20% fetal bovine serum, 0.3% agar (Bacto Agar; Difco laboratories, Detroit, Mich.), and various concentrations of culture supernatant from LLC-LN7 cells. In some studies, the media or supernatants were first preincubated for 2 h with anti-IL-3 and/or anti-GM-CSF antibodies as described above and then used at a 20% concentration in the colony-forming assay. The colonies (>50 cells) were counted after 6 days of culture.

In vitro induction of immune-suppressor cells. Normal bone marrow cells were cultured for 3 days at a concentration of 4×10^6 cells/ml in medium alone or in various concentrations of supernatants of LLC-LN7 cells. In some studies, the medium or supernatants were first preincubated for 2 h with anti-IL-3 and/or anti-GM-CSF antibodies, and then added at a supernatant or medium concentration of 20% to bone marrow cells for 3 days of culture.

Assay for immune-suppressor cell activity. Cultured bone marrow cells and fresh bone marrow cells were washed, irradiated with 2500 R and adjusted to an equal number of viable cells/ml. Suppressor activity of these bone marrow cells was measured as we have previously described [31] by the capacity to suppress normal splenic T cell blastogenesis to the mitogen concanavalin A (ConA). Briefly, normal spleen cells were incubated at 37° C with irradiated suppressor cells and ConA for 3 days in flat-bottom microtiter plates. Each assay was conducted in triplicate, irradiated suppressor cells being mixed with 2×10^5 normal responder spleen cells at a ratio of 1 : 0.25 or, in select experiments, also at 1 : 0.06. ConA was used at the optimal concentrations of 4 µg/ml or 2 µg/ml or, in some experiments, also at the suboptimal concentration of 1 µg/ml. For the last 18 h of culture, 1 µCi [^{3}H]thymidine was added to each well. Cells were sedimented and the incorporated [^{3}H]thymidine was counted in a Beckman liquid scintillation counter.

Fractionation of bone marrow suppressor cells. Following culture with either medium or LLC-LN7 supernatants, bone marrow cells were fractionated into subpopulations by their adherence to nylon wool, or by their sensitivities to various treatments, and then assessed for their capacity to inhibit T cell blastogenesis to ConA. Nylon-wool-nonadherent bone marrow cells were obtained by incubating 5×10^7 bone marrow cells for 1 h in 6 ml nylon-wool columns at 37° C. After this time, nonadherent cells were eluted by washing with warmed medium. Cells of the macrophage lineage were depleted from nylon-wool-nonadherent bone marrow cells by treatment with the lysosomotropic agent, L-leucine methyl ester (LeuOMe), as previously described [23]. After nylon-wool fractionation of the bone marrow cells, the nonadherent cells were resuspended to 5×10^6 cells/ml in 5 mM LeuOMe or RPMI-1640 medium and incubated for 1 h at 22° C. The cells were washed twice with Hank's balanced salt solution and resuspended in culture medium. T lymphocytes or NK cells were depleted from the cultured bone marrow cells by treatment with complement (low-tox-M rabbit complement, Cedarlane Laboratories, Hornby, Ontario, Canada) and Thy1.2 antibody (Becton-Dickinson, Mountain View, Calif.), or asialoGM1 antibody (Waco Chemicals USA Inc., Dallas Tex.). Bone marrow cells, 5×10^6, were mixed with either 1 µg Thy1.2 antibody for 45 min at 4° C, or with 600 µg asialoGM1 antibody for 30 min at 37° C for 30 min at room temperature. After the incubation with antibodies, cells were washed and incubated with 1 ml complement at a final dilution of 1 : 12 for 45 min at 37° C. The remaining cells were then washed, irradiated and used in the suppressor assay. The results shown are those for which the effectiveness of the cell-depletion procedures was assured by parallel treatment of normal spleen cells and measurement of the following: depletion of LeuOMe-sensitive monocytic cells or Thy1^{+1} T lymphocytes, by the elimination of blastogenic responsiveness of the remaining spleen cells to ConA; for NK cells, by the elimination of cytolytic reactivity against the NK-sensitive YAC-1 target cells.

Adoptive transfer experiments. Groups of 10 C57Bl/6 mice received a dorsal subcutaneous injection of 1×10^5 LLC-LN7 tumor cells, a concentration that results in establishment of tumors in approximately 20% of the mice. With this tumor inoculum were also mixed 1×10^6 irradiated (2500 R) bone marrow cells, which had been preincubated for 3 days with either medium or with LLC-LN7 supernatant. The mice were monitored during the subsequent 4 weeks for the appearance of palpable tumors.

Analysis of data. The Student's *t*-test was used to determine the significance of the differences between values in assays measuring suppressor activity and in soft-agar colony-forming assays. All data were expressed as means of triplicates ± SD. All assays were repeated at least three times.

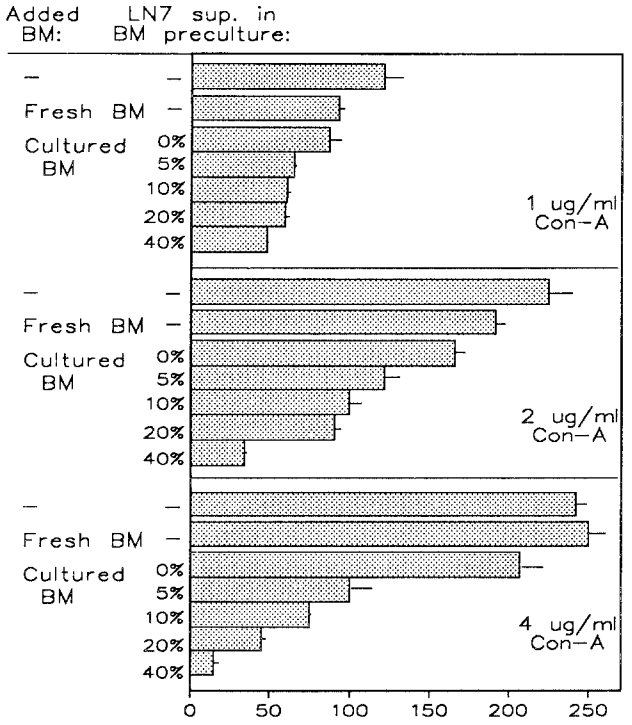

Fig. 1. Induction of immune-suppressive bone marrow (*BM*) cells by culture with supernatants of Lewis lung carcinoma LLC-LN7 cells. Normal bone marrow cells were cultured for 3 days with medium or with LLC-LN7 culture supernatants diluted in medium to different concentrations. These cultured bone marrow cells and fresh bone marrow cells were then washed, irradiated and their effects on the blastogenic responses of normal spleen cells to optimal and suboptimal concentrations of concanavalin A (*ConA*) were assessed. Values are mean cpm ± SD of triplicates

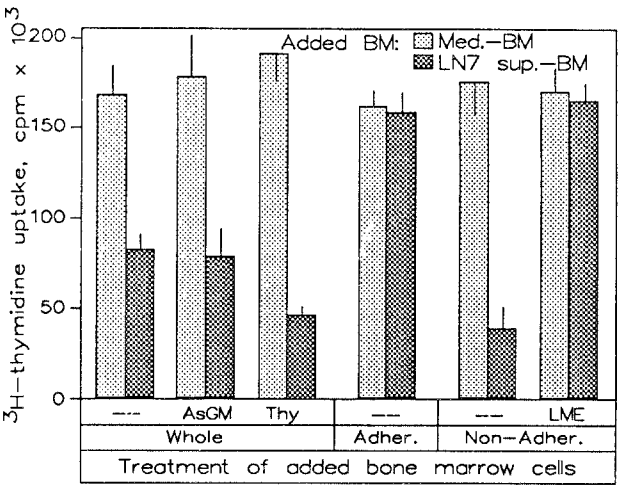

Fig. 2. Characterization of bone marrow suppressor cells. After culture with either medium or LLC-LN7 supernatant, bone marrow cells were either unfractionated (*Whole*) or fractionated into nylon-wool-adherent (*Adher.*) or nonadherent (*Non-Adher.*) cells. Cell populations were treated with control medium, L-leucine methyl ester, or complement plus either asialoGM1 (*AsGM*) or Thy1.2 (*Thy*) antibodies. The remaining cells were then washed, irradiated and mixed with 4 μg/ml ConA plus normal spleen cells at a spleen: bone marrow ratio of 1 : 0.25. Values are mean radioactivity (cpm) ± SD of triplicates

Results

Dose-dependent capacity of LLC-LN7 supernatants to induce bone marrow suppressor cells

The capacities of different concentrations of supernatants (5%–40%) from LLC-LN7 cells to induce normal bone marrow cells to become immune-suppressive were compared (Fig. 1). Freshly isolated bone marrow cells were minimally suppressive to T cell activity while slightly more suppressor activity was apparent for bone marrow cells that had been precultured with medium. However, bone marrow cells that had been precultured with LLC-LN7 supernatants were more suppressive to T cell blastogenesis ($P < 0.01$). The extent of bone marrow immune-suppressor activity was directly proportional to the concentration of LLC-LN7 supernatant used in the bone marrow preculture. T cell blastogenesis was inhibited by at least 50%, even when the LLC-LN7 supernatant used to generate the bone marrow suppressor cells was as little as 5%. In addition, these levels of suppressor activity were apparent regardless of whether T cell blastogenesis was measured at the optimal doses of 4 μg/ml and 2 μg/ml ConA, or at the suboptimal dose of 1 μg/ml ConA.

These bone marrow cells, which had been cultured with either medium or LLC-LN7 supernatant, were then subjected to various treatments to identify the characteristics of the suppressor cells. Figure 2 shows the suppressive effect of these treated bone marrow cells on normal spleen cell blastogenesis. The bone marrow cells that had been precultured with LLC-LN7 supernatants were insensitive to depletion with complement and either Thy1.2 or asialoGM1 antibodies. This excludes the possibility that the bone marrow suppressor cells were T lymphocytes or NK cells. When the bone marrow cells precultured with LLC-LN7 supernatant were fractionated by their adherence to nylon wool, there was a complete loss of suppressor activity in the adherent cell fraction and a corresponding enrichment of suppressor activity in the nonadherent cell fraction ($P < 0.05$). Finally, the nylon-wool-nonadherent bone marrow cells were treated with LeuOMe to determine if the suppressor bone marrow cells might be of the monocyte lineage. Treatment with LeuOMe ablated the suppressive activity of the nonadherent bone marrow cells that had been precultured with LLC-LN7 supernatant.

In-vitro-generated bone marrow suppressor cells facilitate establishment of LLC-LN7 tumors

The possibility was evaluated that the immune-suppressive bone marrow cells generated during in vitro culture with LLC-LN7 supernatant would facilitate the establishment of LLC-LN7 tumors in vivo. Mice were injected with a dose of LLC-LN7 tumor cells to yield approximately a 20% tumor establishment frequency. The admixture of the tumor inoculum with bone marrow cells that had been preincubated in LLC-LN7 supernatant resulted in an earlier appearance of palpable tumors than when medium-precultured bone marrow cells were mixed with the LLC-LN7

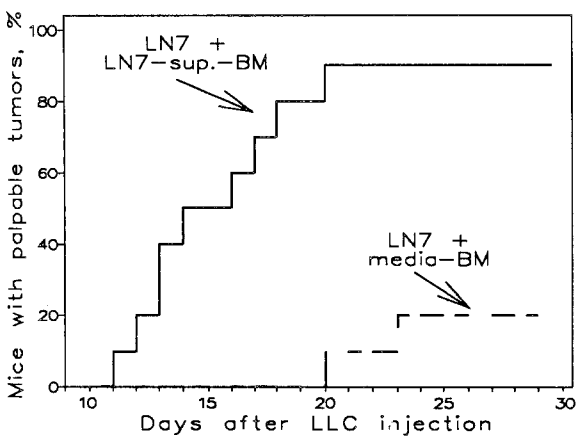

Fig. 3. Effects of bone marrow cells preincubated in LLC-LN7 supernatant on tumor establishment. Mice were injected with a low dose (10^5) of LLC-LN7 cells plus 10^6 irradiated bone marrow cells, which had been preincubated for 3 days with either medium (– – – –) or with LLC-LN7 supernatant (————). Data shown are when palpable tumors first became apparent

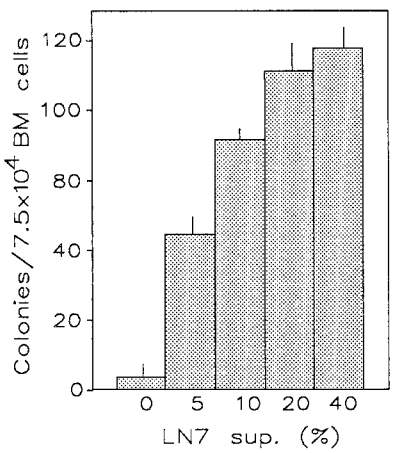

Fig. 4. Production of colony-stimulating factor (CSF) activities by cloned LLC-LN7 cells. Into each 35-mm dish were seeded 7.5×4 femoral bone marrow cells from normal mice together with medium or LLC-LN7 culture supernatants diluted in medium to different concentrations. After 6 days, the number of colonies that grew was enumerated. Values are mean colonies/dish ± SD of triplicates

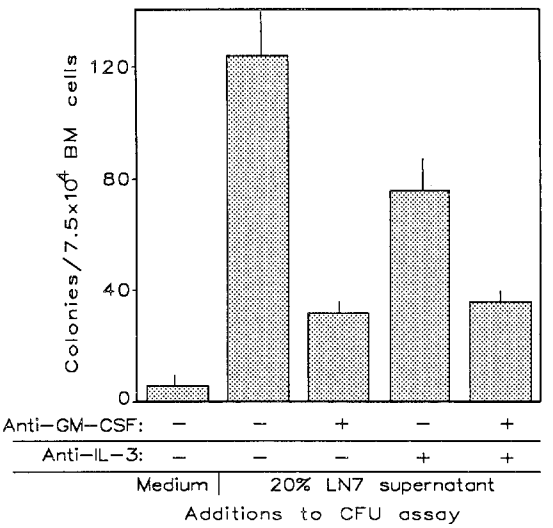

Fig. 5. Effects of anti-GM-CSF and anti-IL-3 antibodies on CSF activities in supernatants of LLC-LN7 cells. Medium or LLC-LN7 supernatants were preincubated for 2 h with diluent, or with antibodies to GM-CSF and/or to interleukin-3 (*IL-3*). These samples were then diluted to 20% and added into 35-mm dishes with 7.5×4 femoral bone marrow cells from normal mice. After 6 days, the number of colonies that grew was enumerated (*CFU assay*). Values are mean colonies/dish ± SD of triplicates

inoculum (Fig. 3). In addition, the bone marrow cells precultured with LLC-LN7 supernatant increased the tumor establishment frequency to 90% as opposed to the 20% establishment frequency in mice implanted with LLC-LN7 cells plus medium-precultured bone marrow cells.

CSF activities secreted by LLC-LN7 cells

The same LLC-LN7 supernatants as were used above to induce the appearance of bone marrow immune-suppressor cells were tested for their capacity to support growth of normal bone marrow cells into colonies in soft agar (Fig. 4). In the absence of added LLC-LN7 supernatant, bone marrow cells were unable to grow into colonies. In the presence of LLC-LN7 supernatants, bone marrow cell growth into colonies was supported. As the concentration of LLC-LN7 supernatant in the soft agar increased, a greater number of colonies formed. Most of the colonies whose growth was supported by the LLC-LN7 supernatants were composed of both granulocytes and monocytes (>75%), regardless of the concentration of LLC-LN7 supernatant used.

Since the medium conditioned by LLC-LN7 cells stimulated growth of mainly granulocytic/monocytic colonies, the possibility was considered that the CSF activity produced by the LLC-LN7 cells was GM-CSF and/or IL-3. Therefore, culture supernatants were first preincubated with neutralizing anti-GM-CSF antiserum and/or monoclonal anti-IL-3 antibodies, and then used as a CSF source in soft-agar colony-forming assays (Fig. 5). Each of the antibodies reduced the CSF activity of the LLC-LN7 supernatant ($P < 0.01$) with the anti-GM-CSF having a greater inhibitory effect than the anti-IL-3 ($P < 0.02$). However, the combination of the antibodies did not reduce the CSF activity in the LLC-LN7 supernatant below the level of inhibition by the anti-GM-CSF antibodies alone. This suggested that the LLC-LN7 cells secreted both GM-CSF and IL-3, and that the growth-supporting activity of the GM-CSF in the supernatant was augmented by the presence of IL-3.

Neutralization of suppressor-cell-inducing activity of LLC-LN7 cells by antibodies to GM-CSF and IL-3

Neutralizing antibodies to GM-CSF and to IL-3 were used to determine if the inducer of suppressor cells produced by

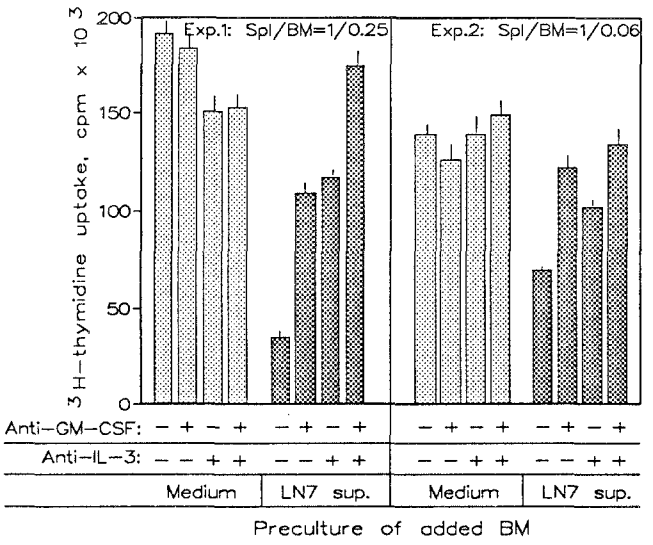

Fig. 6. Effects of anti-GM-CSF and anti-IL-3 antibodies on suppressor-inducing activities in supernatants of LLC-LN7 cells. Medium or LLC-LN7 supernatants were preincubated for 2 h with diluent, or with antibodies to GM-CSF and/or to IL-3. These samples were then diluted to 20% and tested for the capacity to induce bone marrow suppressor cells during 3 days of culture. The cultured bone marrow cells were then washed, irradiated and mixed with 4 μg/ml ConA plus normal spleen cells at a spleen: bone marrow ratio of either 1 : 0.25 or 1 : 0.06. Values are mean radioactivity (cpm) ± SD of triplicates

LLC-LN7 cells could be GM-CSF and/or IL-3 (Fig. 6). Medium conditioned by LLC-LN7 cells and treated only with diluent induced normal bone marrow cells to become suppressive to normal T cell blastogenesis. This suppressive activity was apparent at a spleen to bone marrow cell ratio of 1:0.25 in one series of experiments and even as low as 1:0.06 in a second series of experiments. In contrast, treatment of LLC-LN7 cell supernatant with either anti-GM-CSF or anti-IL-3 significantly (P <0.01) diminished the suppressor-inducing activity. When combined, the anti-GM-CSF and anti-IL-3 ablated the suppressor-inducing activity, suggesting that the LLC-LN7 cells induced suppressor bone marrow cells by secretion of both GM-CSF and IL-3.

Discussion

Progressive growth of metastatic LLC variant tumors in host mice has previously been shown to stimulate myelopoiesis and to concurrently induce the appearance of immune-suppressive cells first in the bone marrow and then in the spleen [27, 30]. The results of the present study suggest that the suppressor-inducing activity secreted by metastatic LLC-LN7 variant cells is the combined effect of GM-CSF and IL-3. The data that support this conclusion include the following: (a) LLC-LN7 supernatants induced suppressor cells in a dose-dependent manner, which paralleled the level of growth-supporting activity for granulocytic/monocytic progenitor cells, (b) both the CSF activity and the suppressor-inducing activity produced by LLC-LN7 cells could be diminished by antibodies to GM-CSF or to IL-3, (c) the combination of anti-GM-CSF and anti-IL-3 ablated all suppressor-cell-inducing activity secreted by the LLC-LN7 cells. Thus, these results suggest that both the myelopoietic stimulation and the appearance of bone-marrow-derived immune-suppressor cells in LLC tumor bearers are induced by tumor secretion of GM-CSF and IL-3. That GM-CSF and IL-3 produced by the LLC-LN7 cells can induce bone marrow suppressor cells is supported by our prior studies showing that normal bone marrow cells could be induced to become immune-suppressive by culture with recombinant GM-CSF and recombinant IL-3 [31].

The results of antibody neutralization studies described in this report are currently being confirmed by studies to demonstrate directly production of GM-CSF and IL-3 by the LLC-LN7 cells. However, the suggestion of this report that LLC-LN7 tumor cells secrete CSFs is in agreement with results of others showing secretion of CSF activities by a variety of tumor types. GM-CSF was previously shown to be secreted by melanoma, hepatoma, myeloma and mammary tumor cells [7, 20, 24]. CSF-1 was produced by human myeloma cells [15] while G-CSF was produced by human pancreatic carcinoma cells [5]. Whether tumor cells secrete IL-3 has not been as extensively examined.

In prior studies, we showed that the bone marrow and splenic suppressor cells, which appear in LLC-bearing mice during periods of myelopoietic stimulation, were distinct from T lymphocytes, granulocytes, NK cells or mature macrophages, but resembled immature cells of the monocyte lineage [27, 30]. The present study evaluated some of the characteristics of the LLC-LN7-supernatant-induced suppressor cells and found that they also were distinct from T lymphocytes, NK cells or mature macrophages. These results suggest that the in-vitro-generated bone marrow suppressor cells were similar to the suppressor cells that appeared in the bone marrow and spleen of mice with metastatic LLC tumors. While such myelopoiesis-associated immune-suppressor cells have not been extensively studied in tumor models, they have been well documented in various non-tumor systems where myelopoiesis is stimulated. For example, myelopoiesis-associated immune-suppressor cells have been described in newborns, and in animals following treatment with total lymphoid irradiation, high-dose cyclophosphamide, ^{89}Sr, or linoleic acid [2, 10, 11, 29]. These suppressor cells have frequently been characterized as immature null cells lacking markers typical of mature macrophages or lymphocytes.

Several studies have evaluated the possibility that the CSFs produced by tumor cells could lead to suppression of immune competence. For example, murine myeloma, hepatoma and mammary carcinoma cell lines that secreted GM-CSF were capable of inducing suppressor spleen cells [24]. GM-CSF-containing supernatants of murine mammary tumor cells induced bone marrow macrophages to become immune-suppressive [7]. The results of these above studies [7, 24], in which correlations were shown between GM-CSF secretion and induction of suppressor cells, were extended by our study showing that neutralization of GM-CSF inhibits the suppressor-inducing activity secreted by the tumor cells. Thus, our study shows not only a correlation between tumor secretion of CSF activity and

induction of suppressor cells, but also identifies a suppressor-inducer to be GM-CSF.

Few studies have been conducted to evaluate whether tumor cells secrete IL-3 or whether IL-3 can influence suppressor cell induction. In a prior study, we showed that recombinant IL-3 induces normal bone marrow cells to become immune-suppressive and that this suppressor-inducing activity is synergistically augmented with recombinant GM-CSF. The present study shows that both the CSF and suppressor-inducing activities produced by LLC-LN7 cells are diminished by neutralization with antibody to IL-3. These results are novel as they suggest tumor cell secretion of both IL-3 and GM-CSF, and demonstrate that secretion of these CSFs is a mechanism by which tumors stimulate the growth of myeloid progenitor cells and induce immune suppressive bone marrow cells.

Tumor-induced immune-suppressor cells have long been shown to facilitate immune escape by tumor cells [16]. However, the suppressor cells used in such studies have typically been characterized as T lymphocytes. Our study showed that the suppressor bone marrow cells induced by LLC-LN7 supernatants increased the rate of appearance of palpable tumors and the frequency of tumor establishment. While we have previously shown that recombinant GM-CSF and IL-3 induce normal bone marrow cells to become immune-suppressive [31], we have not yet conducted studies to show that the bone marrow suppressor cells that are induced by the recombinant CSFs can facilitate tumor establishment in vivo. However, on the basis of our presently described and prior [27, 30] studies, it is reasonable to suggest that LLC-LN7 cell secretion of GM-CSF and IL-3 in vivo stimulates myelopoiesis and induces the appearance of bone-marrow-derived immune-suppressor cells. Bone marrow-derived suppressor cells may, in turn, facilitate the establishment of tumor. Studies are currently being conducted to measure production of GM-CSF and IL-3 in vivo by progressively growing LLC-LN7 tumors.

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