Various Human Hematopoietic Growth Factors (Interleukin-3, GM-CSF, G-CSF) Stimulate Clonal Growth of Nonhematopoietic Tumor Cells

By Wolfgang E. Berdel, Susanne Danhauser-Riedl, Gabriele Steinhauser, and Elliott F. Winton

We have studied the effect of recombinant human hematopoietic growth factors (interleukin-3 [rhIL-3], granulocyte-macrophage colony-stimulating factor [rhGM-CSF], and granulocyte CSF [rhG-CSF]) on the clonal growth of human colon adenocarcinoma cell lines HTB-38, CCL 187, and WiDr (CCL 218). The factors stimulated clonal growth of HTB-38 and CCL 187 in a capillary modification of the human tumor clonogenic assay in agar up to twofold. There were dose-response correlations over a range of 1 to 10,000 U/mL for rhIL-3, rhGM-CSF, and rhG-CSF. Incubation with neutralizing monoclonal antibodies abolished the stimulation of clonal growth by rhGM-CSF. The WiDr cell line was nonresponsive to rhIL-3 and rhGM-CSF. These results represent the first evidence that a variety of hematopoietic growth factors can stimulate the growth of clonogenic cells of some nonhematopoietic malignant cell lines in vitro.

INTERLEUKIN-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte CSF (G-CSF) and macrophage CSF are glycoproteins existing in various species that regulate the growth and differentiation of hematopoietic progenitor cells. The genes of these factors have been molecularly cloned, and recombinant human (rh) factors are available. Some of those factors, such as rhGM-CSF and rhG-CSF, are currently being studied in clinical trials to accelerate reconstitution of bone marrow function after cytotoxic, myelosuppressive chemotherapy in patients with malignant tumors. This report describes the effects of rhIL-3, rhGM-CSF, and rhG-CSF on the clonal growth of human colon adenocarcinoma cell lines HTB-38, WiDr (CCL 218), and CCL 187.

MATERIALS AND METHODS

HTB-38, WiDr (CCL 218), and CCL 187 were purchased from American Type Culture Collection (Rockville, MD). Cell culture techniques have been recently described. The identity of the cell lines was confirmed by karyotype analysis before and after the experiments. RhIL-3 (purified from Escherichia coli, batch no. 1039-86, 8.1 x 10^8 U/mg protein) and rhGM-CSF (purified from E coli, batch nos. CDO1 PO24-DE27 and DO19 D2-701, 10.6 x 10^8 U/mg) were kind gifts from Dr S.C. Clark and Dr G. Wong, Genetics Institute, Cambridge, MA. RhG-CSF (purified from E coli, batch no. 10050, 2 x 10^8 U/mg protein) was purchased from Amgen Corp, Thousand Oaks, CA.

The human tumor clonogenic assay (HTCA) was performed as previously described with the following modification: glass capillaries (1.38 x 126 mm) were sonicated for 30 minutes in doubly distilled water, dried, and sterilized at 180°C. The capillary incubation mixture consisted of 300 μL cell suspension (2.5 x 10^6 cells/mL in double-enriched Connaught Medical Research Laboratories 1066 medium [for ingredients, see Berdel et al5], 705 μL RPMI 1640 medium (GIBCO, no. 220, Glasgow, Scotland), 225 μL heat-inactivated horse serum (GIBCO, no. 220-6350), and 270 μL of a 3% agar (Agar-Noble, no. 0142-01, Difco Laboratories, Detroit) diluted (1:3) in RPMI 1640 medium at 37°C. Fetal bovine serum (FBS) instead of horse serum was used where indicated. The cells were preincubated with the factors added to RPMI 1640 medium for 30 minutes at room temperature (agar cooling period) and then continuously exposed to the final concentrations of the factors indicated during the complete assay period after the addition to the capillary incubation mixture. Seventy-five microliters of the capillary incubation mixture was injected into each capillary with micropipettes. This system was evaluated before and after an incubation period of ten days at pH 7.2, 37°C, 5% CO₂, and high humidity as described. Numbers of colonies and clusters are depicted with SD for triplicate cultures.

Purified monoclonal mouse anti-rhGM-CSF antibody (Ab) was a kind gift from Dr B. Fagg, Sandoz, Ltd, Basel, Switzerland. The specificity and neutralizing capacity of the Ab was determined in normal human hematopoietic progenitor cells and chronic myelogenous leukemia. For the experiments outlined in Fig 2 the Ab was preincubated with the factor (CSF) at excess neutralizing capacities of Ab:CSF (2:1) and after reciprocal dilutions of the Ab from Ab:CSF (1:1 to 1:10⁶) as indicated in Fig 2 for 30 minutes at room temperature. Then the cells were added and the Ab/factor/ cells were added to the capillary incubation mixture after 30 minutes' incubation at room temperature (agar cooling period) at a final factor concentration of 1,000 U/mL for rhGM-CSF. Then the HTCA was performed as described and values expressed as percentages of the controls ±SD. Controls are represented by colony counts of the tumor cells alone. Parallel experiments contained the tumor cells and rhGM-CSF at 1,000 U/mL without antibody (no Ab, see Fig 2) or the anti-GM-CSF antibody alone.

RESULTS

To assess clonal growth of the human colon adenocarcinoma cell lines HTB-38, WiDr (CCL 218), and CCL 187 under the influence of hematopoietic growth factors, we have incubated increasing concentrations of rhIL-3, rhGM-CSF, and rhG-CSF with the cells in a modified HTCA. RhGM-CSF stimulated clonal growth of HTB-38 up to twofold with dose-response correlations (Fig 1). This was
CSF AND TUMOR CELL GROWTH

For HTB-38, 23.7 ± 4.4 ± HTCA twofold. There was some saturation of this activity at doses when compared with controls. 

P in the controls per capillary containing 4 x 10⁶ cells were 33.8 ± 4.4 for HTB-38, 23.7 ± 2.5 for CCL 187, and 50.7 ± 3.2 for the WiDr (CCL 218) cell line. *P < .05, unpaired t test (two tailed), when compared with controls.

reproducible in six different experiments. Approximately 10⁻⁸ to 10⁻⁴ mol/L concentrations increased clonal growth twofold. There was some saturation of this activity at doses >10⁷ U/mL. Experiments done with 2% instead of the 20% horse serum usually present in the HTCA yielded even better results (20% horse serum, 1,000 U/mL: 197.3 ± 6.1% of the controls; 2% horse serum, 1,000 U/mL: 241.9 ± 16.5% of the controls). However, complete removal of serum from the

HTCA did not allow colony growth in the control or in the factor-incubated cultures, which indicates the additional presence of other material in the serum being necessary for clonal growth of the HTB-38 cell line. Experiments with boiled factor (100°C, 15 minutes) revealed a rather high heat stability of rhGM-CSF (1,000 U/mL heated rhGM-CSF, 183.3% ± 4.6% of the controls, ν 1,000 U/mL nonheated rhGM-CSF, 198.2% ± 6.3% of the controls). Experiments done with 20% FBS instead of horse serum also showed stimulation of HTB-38 clonal growth by the CSF (Table 1), although this was less pronounced. However, the morphology of the cell growth in FBS was different and allowed us to clearly distinguish clusters. Thus, size distribution studies were performed, and they showed increased numbers of clusters and colonies under the influence of the CSF (see Table 1).

High specificity of the stimulation of the clonal growth of HTB-38 by rhGM-CSF was demonstrated by experiments incubating the factor with specific neutralizing Abs before and during the HTCA (Fig 2). Neutralizing Abs to rhGM-CSF completely abolished its ability to stimulate clonal growth of HTB-38. This neutralizing capacity of the Ab could be shown to be dependent from a reciprocal dilution of the Ab in the assay. Control experiments in the absence of rhGM-CSF have not revealed stimulation or inhibition of HTB-38 clonal growth by the anti-GM-CSF Ab alone (details not shown).

In addition to rhGM-CSF, rhIL-3 and rhG-CSF significantly stimulated the clonal growth of HTB-38, with dose-response correlations as shown in Tables 1 and 2. Approximately 10⁻⁴ to 10⁻³ mol/L concentrations of rhIL-3 increased the clonal growth of HTB-38 twofold. There was some saturation of its activity at doses >10⁴ U/mL. Combinations of rhIL-3 and rhGM-CSF at concentrations of 500 U/mL of each factor showed additive or in some experiments less than additive stimulation of the clonal growth of the HTB-38 cell line (500 U/mL rhGM-CSF plus 500 U/mL rhIL-3, 177.5% ± 5.1% of the controls, ν 1,000 U/mL rhGM-CSF, 197.3% ± 6.1%, and 1,000 U/mL rhIL-3, 215.0% ± 2.1% of the controls). Neutralizing monoclonal Abs for rhIL-3 and rhG-CSF, however, were not yet available in our laboratory to prove further specificity of the effects of these factors. Polyclonal rabbit anti–IL-3 Ab could not be used since it stimulated clonal growth of the tumor cells in the absence of rhIL-3.

Table 1. Size Distribution Studies of HTB-38 Clusters and Colonies Under the Influence of rhGM-CSF and rhIL-3

<table>
<thead>
<tr>
<th>U/mL</th>
<th>Clusters</th>
<th>SD (%)</th>
<th>Percentage of Controls</th>
<th>Colonies</th>
<th>SD (%)</th>
<th>Percentage of Controls</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>101.0</td>
<td>5.6</td>
<td>100.0</td>
<td>40.0</td>
<td>5.0</td>
<td>100.0</td>
</tr>
<tr>
<td>rhGM-CSF</td>
<td>10</td>
<td>100.0</td>
<td>2.8</td>
<td>99.0</td>
<td>46.0</td>
<td>9.6</td>
</tr>
<tr>
<td>100</td>
<td>108.0</td>
<td>7.9</td>
<td>106.9</td>
<td>48.7</td>
<td>6.3</td>
<td>121.7</td>
</tr>
<tr>
<td>1,000</td>
<td>113.0</td>
<td>6.3</td>
<td>111.9</td>
<td>59.7</td>
<td>18.7</td>
<td>149.2</td>
</tr>
<tr>
<td>rhIL-3</td>
<td>10</td>
<td>99.0</td>
<td>2.9</td>
<td>98.0</td>
<td>49.0</td>
<td>3.5</td>
</tr>
<tr>
<td>100</td>
<td>106.5</td>
<td>6.0</td>
<td>105.5</td>
<td>57.0</td>
<td>7.7</td>
<td>142.5</td>
</tr>
<tr>
<td>1,000</td>
<td>118.0</td>
<td>4.8</td>
<td>116.8</td>
<td>65.7</td>
<td>7.5</td>
<td>164.2</td>
</tr>
</tbody>
</table>

*Means of duplicates.
†Means of triplicates.

The values are given as total numbers of clusters and colonies, with SD in percentages, as well as in percentages of controls. Experiments were done in the presence of FBS instead of horse serum.
and Table 2). Furthermore, rhG-CSF was rather ineffective in the clonal growth of WiDr cell line. The growth-stimulating effects of most factors occurred with concentrations of CSF that result in colony formation by normal hematopoietic progenitor cells. In this series of experiments we have not finally addressed the question whether the CSFs recruit originally nonclonogenic cells to clonal growth or increase the size of some preexisting small clusters that were present in both the control and the CSF-incubated cultures. However, in some experiments we counted colonies and clusters of the HTB-38 cell line in the controls as well as in the rhGM-CSF- and rhIL-3-incubated cultures and also observed an increase in the numbers of clusters in the CSF-incubated cultures over the controls, which seems to argue in favor of recruitment of originally nonclonogenic cells by the CSFs. Experiments with FBS instead of horse serum and with lower serum concentration showed, however, that stimulation of clonal growth by the CSFs was most pronounced under suboptimal growth conditions.

Further screening for responsiveness to hematopoietic growth factors by using other tumor cell lines and freshly explanted tumor material seems indicated. In this respect, it is interesting that we have determined a human bladder carcinoma cell line as being responsive to the stimulatory capacity of rhGM-CSF (1,000 U/mL rhGM-CSF, 140.6% ± 7.6% of the controls; 1,000 U/mL rhGM-CSF plus an anti-GM-CSF Ab dilution of 1:1, 98.1% ± 6.7% of the controls) and rhIL-3 (1,000 U/mL rhIL-3, 195.2% ± 14.7% of the controls), whereas a human fibrosarcoma cell line and a human glioblastoma cell line were nonresponsive. In recent studies with some human lung cancer cell lines others could not demonstrate an effect of IL-1 and GM-CSF on the clonal growth of these cell lines.7 However, so far the lymphokine IL-1 is the only lymphohematopoietic growth factor that has been shown to exert cytotoxicity to some8–11 but growth-stimulating effects to other1213 tumor cell lines in vitro. On the other hand, IL-3, GM-CSF, and G-CSF have been demonstrated to stimulate the growth and clonal growth of some malignant hematopoietic cell types such as blasts of acute leukemias and lymphomas.14–19 Receptors for IL-3 and GM-CSF are present on some leukemic cell lines,20–23 and furthermore, GM-CSF receptors have recently been reported to be present on cells of a small cell lung cancer cell line.24 Earlier studies describe tumor cell lines releasing stimulating factors for mixed hematopoietic colonies,2526 G-CSF,27 or factors similar to G-CSF28 into their supernatant. Further studies on the binding sites of HTB-38 and WiDr cells for some of the hematopoietic growth factors used are underway in our laboratory. Finally, CSF-producing tumor cells may be facilitated in the metastatic process in vivo.29

Further studies with tumor cells responsive and nonresponsive to hematopoietic growth factors could yield more insight in the biology and growth-controlling mechanisms of neoplasia. More specifically, the clinical studies applying some of these factors for bone marrow recovery after chemotherapy or radiation of solid tumors may not be without hazard since this may potentially lead to growth stimulation of remaining tumor tissues. On the other hand, different sequential administration of factors such as before cytotoxic treatment may render neoplastic cells more susceptible to

### DISCUSSION

These results represent evidence that various hematopoietic growth factors can stimulate the clonal growth of some malignant nonhematopoietic cells in vitro. The growth-stimulating effects of most factors occurred with concentrations of CSF that result in colony formation by normal hematopoietic progenitor cells. In this series of experiments we have not finally addressed the question whether the CSFs recruit originally nonclonogenic cells to clonal growth or increase the size of some preexisting small clusters that were present in both the control and the CSF-incubated cultures. However, in some experiments we counted colonies and clusters of the HTB-38 cell line in the controls as well as in the rhGM-CSF- and rhIL-3-incubated cultures and also observed an increase in the numbers of clusters in the CSF-incubated cultures over the controls, which seems to argue in favor of recruitment of originally nonclonogenic cells by the CSFs. Experiments with FBS instead of horse serum and with lower serum concentration showed, however, that stimulation of clonal growth by the CSFs was most pronounced under suboptimal growth conditions.

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### Table 2. Stimulation of Clonal Growth of Two Human Colon Adenocarcinoma Cell Lines by Recombinant Human Hematopoietic Growth Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Concentration (U/mL)</th>
<th>Colonies (Percentage of Controls ± SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HTB-38</td>
</tr>
<tr>
<td>rhIL-3</td>
<td>1</td>
<td>116.6 ± 7.4†</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>155.8 ± 9.1†</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>189.8 ± 13.0†</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>217.0 ± 7.9†</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>222.8 ± 3.9†</td>
</tr>
<tr>
<td>rhG-CSF</td>
<td>1</td>
<td>93.5 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>101.1 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>132.6 ± 7.5†</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>154.3 ± 4.4†</td>
</tr>
</tbody>
</table>

*The values are given as percentages of the controls ± SD of triplicates. The controls are represented by the colony counts of the tumor cells alone. Number of colonies in the controls per capillary containing 4 × 10^3 cells were 32.0 ± 6.3 for HTB-38 and 31.3 ± 2.5 for CCL 187.

†P < .05, unpaired t test (two tailed), when compared with the controls.
therapy in a setting in which potential damage to the bone
marrow is counteracted by autologous bone marrow trans-
plantation. However, cell lines can acquire altered properties
such as changes in growth requirements, and thus before
final conclusions can be drawn, the concerns and specula-
tions mentioned earlier have to be further studied by in vivo
experiments.

ACKNOWLEDGMENT

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REFERENCES

1. Clark SC, Kamen R: The human hematopoietic colony-
317:940, 1987
3. Antman K, Griffin J, Elias A, Socinski M, Whitley M, Ryan
L, Cannistra S, Oette D, Frei E, Schnipper L: Use of rGM-CSF to
ameliorate chemotherapy induced myelosuppression in sarcoma
patients. Blood 70:129, 1987 (suppl 1) (abstr)
4. Gabrilove J, Jakubowski A, Fain K, Scher H, Grous J,
Sternberg C, Yagoda A, Clarkson B, Moore MAS, Bonilla MA,
I/II study of rhG-CSF in cancer patients at risk for chemotherapy-
induced neutropenia. Blood 70:135, 1987 (suppl 1) (abstr)
5. Berdel WE, Schick HD, Fink U, Reichert A, Ulm K, Rastetter
J: Cytotoxicity of the alkyl-linked lipoidal amine 4-aminomethyl-
1-[2,3-(di-n-decloyxy)-n-propyl]-4-phenylpiperedine (CP-46,665) in
6. Maurer HR, Ali-Osman F: Tumor stem cell cloning in agar-
containing capillaries. Naturwissenschaften 68:381, 1981
8. Onozaki K, Matsushima K, Aggarwal BB, Oppenheim JJ:
Human interleukin-1 is a cytotoxic factor for several tumor cell lines.
J Immunol 135:3962, 1985
9. Lachman LB, Dinarello CA, Llansa ND, Fidler IH: Natural
and recombinant human interleukin 1-beta is cytotoxic for human
10. Gaffney EV, Tsai SC: Lymphocyte-activating and growth-
inhibitory activities for several sources of native and recombinant
11. Bertoglio JH, Rimsky L, Kleinerman ES, Lachman LB:
B-cell line-derived interleukin 1 is cytotoxic for melanoma cells and
promotes the proliferation of an astrocytoma cell line. Lymphokine
Res 6:83, 1987
12. Lachman LB, Brown DC, Dinarello CA: Growth-promoting
effect of recombinant interleukin 1 and tumor necrosis factor for a
13. Hamburger AW, Lurie KA, Condon ME: Stimulation of
anchorage-independent growth of human tumor cells by interleukin
14. Tomonaga M, Golde DW, Gasson JC: Biosynthetic (recombi-
nant) human granulocyte-macrophage colony-stimulating factor:
Effects on normal bone marrow and leukemia cell lines. Blood 67:31,
1986
15. Griffin JD, Young D, Herrmann F, Wiper D, Wagner K,
Sabbath KD: Effects of recombinant human GM-CSF on prolifera-
tion of clonogenic cells in acute myeloblastic leukemia. Blood
67:1448, 1986
McCulloch EA: Synergism between recombinant growth factors,
GM-CSF and G-CSF, acting on the blast cells of acute myeloblastic
17. Vellenga E, Young DC, Wagner K, Wiper D, Ostapovicz D,
Griffin JD: The effects of GM-CSF and G-CSF in promoting
growth of clonogenic cells in acute myeloblastic leukemia. Blood
69:1771, 1987
18. Delwel R, Dorssers L, Touw I, Wagemaker G, Löwenberg B:
Human recombinant multilineage colony stimulating factor (inter-
leukin-3): Stimulator of acute myelocytic leukemia progenitor cells
interleukin-3 induced proliferation of normal and leukemic human B
cell precursors. Blood 70:190, 1987 (suppl 1) (abstr)
20. Walker F, Burgess AW: Specific binding of radiiodinated
granulocyte-macrophage colony-stimulating factor to hematopoietic
cells. EMBO J 4:933, 1985
21. Park LS, Friend D, Gillis S, Urdal DL: Characterization of
the cell surface receptor for human granulocyte/macrophage colony-
22. Gasson JC, Kaufman SE, Weisbart RH, Tomonaga M,
Golde DW: High-affinity binding of granulocyte-macrophage colo-
n-stimulating factor to normal and leukemic human myeloid cells.
Proc Natl Acad Sci USA 83:669, 1986
23. Mufson RA, Gesner TF, Turner K, Norton C, Yang Y-C,
Clark S: Characterization of IL-3 receptors on human acute
myelogenous leukemia cell line KG-1. Blood 70:181, 1987 (suppl 1)
(abstr)
24. Baldwin GC, DiPersio J, Kaufman SE, Quan SG, Golde DW,
25. Myers CD, Katz FE, Joshi G, Millar JL: A cell line secreting
stimulating factors for CFU-GEMM culture. Blood 64:152, 1984
26. Welte K, Platzer E, Lu L, Gabrilove JL, Levi E, Mertels-
mann R, Moore MAS: Purification and biochemical characteriza-
tion of human pluripotent hematopoietic colony-stimulating factor.
Proc Natl Acad Sci USA 82:1526, 1985
27. Nagata S, Tsuchiya M, Asano S, Kaziro Y, Yamazaki T,
Yamamoto O, Hirata Y, Kubota N, Oheda M, Nomura H, Ono M:
Molecular cloning and expression of cDNA for human granulocyte
28. Lilly MB, Devlin PE, Devlin JH, Rado TA: Production of
granulocyte colony-stimulating factor by a human melanoma cell
B, Bons L, Prodi G, Nanni P: Are colony-stimulating factor-
producing cells facilitated in the metastatic process? Anticancer Res
7:695, 1987
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