The Role of Interleukin-1 and Granulocyte-Macrophage Colony-Stimulating Factor in the Paracrine Stimulation of an In Vivo-Derived Murine Myeloid Leukemia

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**WEHI-274.3** is a cell line isolated from an in vivo-derived, murine myelomonocytic leukemia. Although the survival and growth of WEHI-274.3 cells in vitro is absolutely dependent on the addition of exogenous growth factors such as interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), or colony-stimulating factor-1, when injected into syngeneic mice the cell line is tumorigenic. Sera from normal mice contain low levels of an activity that sustains survival of WEHI-274.3 but does not stimulate growth. In contrast, sera from mice bearing the WEHI-274.3 leukemia contained levels of CSF-1 and GM-CSF that stimulated the growth of WEHI-274.3 cells. Supernatants of cultures of WEHI-274.3 cells contained an activity that stimulated 3T3 fibroblasts to release an activity that stimulated the growth of the WEHI-274.3 cells. The 3T3-stimulatory activity released by the WEHI-274.3 cells was neutralized completely with an antisemur specific for murine IL-1α, but not with antisemur specific for IL-1β. Moreover, WEHI-274.3 cells both in vitro and in vivo contained high levels of IL-1α and IL-1β mRNAs. The leukemia-stimulatory activity released by the 3T3 cells was neutralized by an antisemur specific for GM-CSF. We postulate that the IL-1α constitutively released by the WEHI-274.3 cells stimulates the production of GM-CSF from host cells such as fibroblasts or endothelial cells. A similar paracrine mechanism of growth stimulation may occur in acute myeloid leukemias in humans.

**MATERIALS AND METHODS**

**Cell lines and cell culture conditions.** The murine cell lines used in these studies have been described elsewhere: WEHI-274.3 and WEHI-274.14,1 FDC-P1,1 P388D1 WEHI-3B,1 and R6-X.2 The Swiss 3T3 cells were obtained from American Type Culture Collection (cat. no. ATCC CCL 92). The murine T-cell line referred to in Fig 1 is the p41.1 line described by Ziltener et al.3 Cells were routinely grown in RPMI 1640 supplemented with newborn calf serum (10%), 2-mercaptoethanol (5 x 10^-5 mol/L), glutamine (2.8 x 10^-3 mol/L), penicillin (100 U/mL), and streptomycin (100 μg/mL) with WEHI-3B-conditioned medium (2% of a 10-fold concentrate) as indicated.

**Antiser and cytokines.** Antimurine GM-CSF antiserum was raised in sheep against synthetic GM-CSF and specifically abrogated the growth response of FDC-P1 cells to GM-CSF, but not to IL-3 (see ref 9). The goat antimurine colony-stimulating factor-1 (CSF-1) antisemur, provided by Dr E.R. Stanley (Albert Einstein College of Medicine at Yeshiva University), was raised against L-cell-derived CSF-1 and contains no antibodies against other known CSF subclasses (data not shown; controls shown in ref 9). The synthetic GM-CSF was the gift of Dr Ian Clark-Lewis (The Biomedical Research Centre, Vancouver, Canada) and was prepared as has been described elsewhere for human GM-CSF.10 Recombinant human IL-1β was provided by Dr E. Liew (Wellcome Laboratories, Beckenham, UK). The sheep antimurine IL-1 antisemur was generously provided by Dr Stephen Poole (National Institute for Biological Standards and Control, Hertfordshire, UK) (specificity not shown).

**Preparation of conditioned media.** Cells were washed three times with fresh RPMI-1640 then cultured at 10^6 cells/mL in 10-mL tissue culture-treated Petri dishes (Falcon, New Jersey). After 3 days the medium was filtered through a Whatman GF/A glass fiber filter (Whatman, New Jersey).

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Thymidine incorporation assays. Assays were performed using the cell lines WEHI-274.3 and FDC-P1 according to the method described by Schrader and Crapper. Briefly, twofold dilutions of the test samples were prepared in 96-well microtiter trays. Triplicate 5-μL aliquots were transferred to the wells of Terasaki trays (Lux Corp, Newbury Park, CA) to which were then added 500 cells in 10 μL of culture medium. If antisera were to be added, dilutions were prepared separately in an additional 96-well microtiter tray from which further 5-μL aliquots were transferred to the Terasaki trays. In these cases, 500 cells were added in only 5 μL of culture medium. Trays were incubated at 37°C for 3 days, then pulsed for 6 hours with [3H] thymidine and harvested onto glass fiber filters. Filters were washed then counted in a liquid scintillation counter. Data were analyzed using a computer program which fits a "four parameter logistic model" to dose-response curves (ref 11 and Ziltener and Delaney [manuscript in preparation]). One unit of biologic activity was defined as the concentration of factor per milliliter required to stimulate a 50% maximal response in the assay.

The isolation of whole-cell polyadenylated RNA and its analysis using agarose-formaldehyde (1.0% agarose) gel electrophoresis was performed according to the method of Gonda et al. For all gels RNA samples of 2 μg were loaded per lane with the exception of that derived from concanavalin A-activated T cells where the aliquot was 0.2 μg. The RNA slot blot in Fig 2 was performed with 2 μg of polyadenylated RNA added per slot.

Hybridization conditions and probes. DNA fragments were labeled by random priming with random hexamers according to the method of Feinberg and Vogelstein. Hybridization with RNA filters was performed in 50% formamide, 0.1% ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 6× SSC, 1% sodium dodecyl sulfate (SDS), 15 mmol/L EDTA, and 50 mmol/L Tris HCl for 16 hours at 42°C. Filters were washed at 55°C for 2 hours in 0.1% SDS and 0.1X SSC (15 mmol/L sodium chloride, 1.5 mmol/L sodium citrate) with four changes of buffer before autoradiography.

The murine tumor necrosis factor α (TNFα) probe was the 1-kb EcoRI cDNA from pMuTNF, courtesy of Genentech Inc (San Francisco, CA). The murine IL-1α and IL-1β probes consisted of the EcoRI cDNA inserts from pMil-1α and pMil-1β, respectively, also courtesy of Genentech Inc. The β-actin probe consisted of the 1.5-kb PsI cDNA fragment from the bovine clone pBA1, courtesy of Dr Robert McMaster (Department of Medical Genetics, University of British Columbia, Vancouver, Canada).

**RESULTS**

Responsiveness of the WEHI-274.3 clone to hematopoietins. WEHI-274 is a myelomonocytic leukemia that arose in a BALB/c mouse infected with the Abelson/Moloney leukemia virus complex. However, there is no evidence that the v-ab1 gene is present in any lines derived from WEHI-274. The cell line WEHI-274.3 was one of multiple clones isolated from an in vivo-passaged, uncloned sample of the WEHI-274 leukemia and was routinely passage in 2% WEHI-3B-conditioned medium. In vitro WEHI-274.3 cells proliferated in response to exogenous IL-3, GM-CSF, and CSF-1 (L-cell-conditioned medium) (Fig 3). In the absence of exogenous hematopoietin, WEHI-274.3 died rapidly (Fig 3). This absolute dependence on exogenous growth factors for survival and proliferation was evident at low and high cell densities (≤10^6/mL), and in both liquid and semisolid culture media. The failure to survive at high cell densities was not an in vitro artifact due to exhaustion of some component of the medium or to the production of some toxic or inhibitory substance, because the addition of IL-3

![Northern blot analysis of WEHI-274.3 for monokine-specific RNAs. Whole-cell polyadenylated RNA was fractionated on a denaturing gel and analyzed by Northern blotting using an IL-1α (A), IL-1β (B), or TNFα (C) cDNA probe. Lanes: 1, concanavalin A-activated T-cell line; 2, WEHI-274.3; 3, WEHI-274.14; 4, P88D.](image)

![Analysis of IL-1 RNAs from WEHI-274.3 cells grown in vitro and in vivo. Whole cell polyadenylated RNA recovered from the spleen of an otherwise healthy mouse bearing the WEHI-274.3 leukemia as an ascitic neoplasm, from the spleen of a control mouse, and from WEHI-274.3 cells cultured in vitro were analyzed by RNA slot-blot analysis using probes for IL-1α and IL-1β.](image)
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Fig 3. Response of WEHI-274.3 to auto-conditioned medium and exogenous cytokines. Responses of WEHI-274.3 cells to L-cell-conditioned medium (CSF-1) (10X concentrate) (●), synthetic GM-CSF (3 µg/mL) (△), and auto-conditioned medium (■) were measured with a 6-hour thymidine incorporation assay after a 3-day incubation. A dilution of 1 represents the starting concentration (indicated above); subsequent points represent consecutive 1:2 dilutions of the stimulating agent. Each point represents the mean of triplicate estimations of thymidine incorporation (counts per minute); bars indicate standard errors of the mean (SEM). In the presence of medium alone, the incorporation of WEHI-274.3 cells was 581 cpm (SEM = 60).

even to cultures plated at 10⁶ cells/mL and where more than 90% of the cells had died resulted in the vigorous proliferation of surviving cells.

WEHI-274.3 induces growth factor production in vivo. This failure of WEHI-274.3 cells to survive in vitro in the absence of growth factors contrasted with the ability of those cells to survive and grow in syngeneic BALB/c mice in vivo, as few as 100 cells injected intravenously giving rise to lethal leukemias. Thus, it was possible that leukemic mice contained circulating factors that could support the survival and growth of WEHI-274.3 cells and account for their tumorigenicity. While sera from normal BALB/c mice maintained the survival of WEHI-274 cells, they stimulated only modest thymidine incorporation by the WEHI-274.3 clone (Fig 4A). In contrast, however, the sera from leukemic mice maintained survival of WEHI-274.3 cells and stimulated proliferation. When inocula of 10⁶ cells were injected intravenously into 10 × BALB/c mice, all mice developed a systemic disease with the mean latency of disease being 21.2 (±0.8) days. Sera taken from these leukemic mice strongly stimulated thymidine incorporation by WEHI-274.3 cells in liquid cultures (Fig 4A), with an activity of 24 U/mL. Similar stimulatory activity (4.1 U/mL) was present in the ascites fluid taken from mice bearing the WEHI-274.3 cells as an intraperitoneal neoplasm (Fig 4A).

Most of the WEHI-274.3 stimulating activity present in the ascites fluid taken from mice bearing the WEHI-274.3 cells as an intraperitoneal neoplasm could be inhibited by a goat antImurine CSF-1 (Fig 4B). In control experiments this antiserum did not affect IL-3-mediated stimulation of WEHI-274.3 cells, while completely abolishing LCCM (L-cell-conditioned medium)-mediated stimulation, at the dilution of 1:180 used here (data not shown). While sheep antImurine GM-CSF antiserum only marginally inhibited the stimulatory activity (Fig 4B), when the anti–GM-CSF and anti-CSF-1 antisera were added in combination all of the WEHI-274.3 stimulatory activity present in the ascites fluid was abolished (Fig 4B). In control experiments, the sheep anti–GM-CSF antiserum did not affect IL-3-mediated stimulation of the WEHI-274.3 line at the dilu-

Fig 4. Presence of WEHI-274.3 stimulatory activity in serum and ascites from mice bearing the WEHI-274.3 leukemia. Responses of WEHI-274.3 cells to (A) serum from a control mouse (■), serum from a mouse bearing the WEHI-274.3 leukemia 3 weeks after intravenous injection of 10⁶ cells (△), and ascites from a mouse bearing the WEHI-274.3 leukemia 3 weeks after intraperitoneal injection of 10⁶ cells (△) were measured with a 6-hour thymidine incorporation assay after a 3-day incubation. Also shown (B) are the effects of sheep anti–GM-CSF antiserum (1:45 constant) (○), goat anti-CSF-1 antiserum (1:180 constant) (●), and a mixture of both antisera (●) on the response of WEHI-274.3 cells to the ascites fluid. The starting concentrations were 1:16 of the serum or ascites for (A) and 1:12 of the ascites for (B). Subsequent dilutions and statistical analyses are as for Fig 3. In the presence of medium alone, the incorporation of WEHI-274.3 cells was 1,433 cpm (SEM = 160) for (A) and 1,385 cpm (SEM = 424) for (B).
tion of 1:45 used here while completely abolishing GM-CSF-mediated stimulation (data not shown). Similarly, the ability of IL-3 to stimulate WEHI-274.3 was not affected by a combination of the anti-GM-CSF and anti-CSF-1 antisera at the concentrations indicated (data not shown).

Thus, the WEHI-274.3 stimulatory activity present in the ascites fluid taken from mice bearing the WEHI-274.3 cells as an intraperitoneal neoplasm was comprised predominantly of CSF-1, with a minor component being GM-CSF.

WEHI-274.3 cells induce the release of GM-CSF by 3T3 cells in vitro. These observations suggested that the WEHI-274.3 cells were stimulating the mice to release a leukemia-stimulating activity. To explore this possibility, an in vitro model was constructed. When the WEHI-274.3 cells were added to monolayers of fibroblast (Swiss 3T3) cells, they adhered strongly to the monolayer, survived, and proliferated rapidly. Medium conditioned by cultures containing both 3T3 cells and WEHI-274.3 cells contained readily detectable amounts of an activity that stimulated the growth of WEHI-274.3 cells, while medium conditioned by 3T3 cells alone had significantly less activity (data not shown).

To determine whether cell-cell contact between 3T3 and WEHI-274.3 cells was required for the survival and growth of the WEHI-274.3 cells cocultured with the 3T3 cells, we asked whether WEHI-274.3 cells released a soluble factor that is able to stimulate 3T3 cells to release a growth factor or factors. Medium was collected from cultures in which washed WEHI-274.3 cells were cultured at 10^6 cells/mL in medium alone for 3 days. As shown before (Figs 3 and 5B), this WEHI-274.3-conditioned medium had no activity to support the survival or growth of WEHI-274.3 cells. A mixture of 3T3-conditioned medium and WEHI-274.3 auto-conditioned medium (ACM) had only marginally more leukemia-stimulating activity than 3T3-conditioned medium alone (Fig 5B), demonstrating the absence of a significant synergy between factors contained in these media. However, when this WEHI-274.3 ACM was added to cultures of 3T3 cells, the culture medium harvested on day 3 contained a 10-fold higher concentration of WEHI-274.3 stimulating activity (Fig 5B).

A significant component of the WEHI-274.3 stimulating activity released by the 3T3 cell in response to WEHI-274.3 ACM could be inhibited by a sheep antimurine GM-CSF antiserum (Fig 6A). In control experiments this antiserum did not affect IL-3–mediated stimulation of the WEHI-274.3 line, while completely abolishing GM-CSF–mediated stimulation, at the dilution of 1:45 used here (data not shown). Part of the stimulatory activity was marginally, but reproducibly inhibited with goat antimurine CSF-1 (Fig 6A). In control experiments this antiserum did not affect IL-3–mediated stimulation of WEHI-274.3 cells, while completely abolishing LCCM–mediated stimulation, at the dilution of 1:200 used here (data not shown). However, when the anti–GM-CSF and anti–CSF-1 antisera were added in combination much of the WEHI-274.3 stimulatory activity was abolished (Fig 6A). Again, this effect is specific because in control experiments the ability of IL-3 to stimulate WEHI-274.3 was not affected by this combination of antisera at these concentrations.

The identity of a component of the leukemia-stimulating activity released by the 3T3 cells in response to WEHI-274.3 ACM as GM-CSF was confirmed further by the ability of this culture medium to stimulate the growth of the cell line FDC-P1 (Fig 5A), which is responsive to both GM-CSF and IL-3, but not to CSF-1. The culture medium...
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Fig 6. WEHI-274.3 cells respond to GM-CSF released by 3T3 cells in vitro. The responses of WEHI-274.3 cells to culture medium (40%) (△) of unstimulated 3T3 cells (B), or 3T3 cells grown in 20% WEHI-274.3 auto-conditioned medium (A) were measured with a 6-hour thymidine incorporation assay after a 3-day incubation. The effects of sheep anti-GM-CSF antiserum (1:45 constant) (△), goat anti-CSF-1 antiserum (1:200 constant) (□), and a mixture of both antisera (●) are shown. A dilution of 1 represents the starting concentration (indicated above); subsequent dilutions and statistical analyses are as for Fig 3. In the presence of medium alone, the incorporation of WEHI-274.3 cells was 593 cpm (SEM = 191).

was unable to stimulate the growth and survival of the IL-3-responsive cell line R6-X7 indicating that the 3T3-derived activity was not IL-3 (data not shown). Moreover, this FDC-P1 stimulatory activity could be abolished using an affinity-purified sheep antimurine GM-CSF antiserum (Fig 5A). In control experiments this antiserum did not affect IL-3-mediated stimulation of the FDC-P1 line at the dilution of 1:45 used here. In most experiments the FDC-P1 cells responded only minimally to the culture medium of 3T3 cells grown in medium alone, to WEHI-274.3 ACM, or to a combination of these culture media (Fig 5A). However, in some experiments the FDC-P1 cells demonstrated a somewhat greater response to 3T3 ACM, but the level of thymidine incorporation only reached 4,000 cpm. These experiments indicated that the 3T3 cells were releasing GM-CSF in response to a factor(s) produced by the WEHI-274.3 cells.

Whereas most of the WEHI-274.3 stimulating activity that was released from the 3T3 cells when stimulated with WEHI-274.3 ACM was inhibited by antisera specific for GM-CSF and to a much lesser extent by antisera specific for CSF-1, both anti-GM-CSF and anti-CSF-1 antisera were equally effective in inhibiting the WEHI-274.3 stimulating activity released by unstimulated 3T3 cells (Fig 6B). When used in combination these two antisera almost completely abolished the WEHI-274.3 stimulating activity released by the unstimulated 3T3 cells. Because the inhibitory effect of the two antibodies combined is greater than the sum of the inhibitory effects of each antibody separately, it is possible that a synergism exists between the effects of GM-CSF and CSF-1 on proliferation of the WEHI-274.3 cells. Thus, most of the increased WEHI-274.3 stimulatory activity released by the 3T3 cells when grown in the presence of WEHI-274.3 ACM can be attributed to the increased production of GM-CSF, although CSF-1 was also involved.

WEHI-274.3 cells release IL-1α. Medium from cultures of 3T3 cells stimulated with recombinant human IL-1β (rhIL-1) showed increased titers of an activity that stimulated thymidine incorporation both by WEHI-274.3 cells (Fig 7B) and by FDC-P1 (Fig 7A). Inhibition studies using the anti-GM-CSF and anti-CSF-1 antisera showed that this activity on the WEHI-274.3 cells again was predominantly GM-CSF with a minor component due to CSF-1 (data not shown). The activity on FDC-P1 cells could be completely abolished with the anti-GM-CSF antiserum (Fig 7A).

Hence, in terms of their abilities to induce 3T3 cells to release activities that stimulate WEHI-274.3 and FDC-P1 cells, rhIL-1 and WEHI-274.3 ACM were indistinguishable. In keeping with this observation, the activity secreted by WEHI-274.3 cells stimulating the release of GM-CSF and from 3T3 cells could be accounted for entirely by IL-1α. Thus, sheep antimurine IL-1α antiserum was able to abolish the release of GM-CSF by 3T3 cells stimulated with WEHI-274.3 ACM. However, this inhibition was not evident with pre-immune serum or with a neutralizing sheep antimurine IL-1β antiserum (Fig 8). In control experiments these antisera did not affect GM-CSF-mediated proliferation of FDC-P1 or WEHI-274.3 cells at the dilutions of antisera used here (data not shown). However, there was no evidence that the IL-1α produced by the WEHI-274.3 cells acted in an autocrine manner on those cells. Thus, exogenous rhIL-1 was unable to stimulate proliferation (Fig 7B) or maintain the viability (data not shown) of WEHI-274.3 cells.

Northern blot analysis of the WEHI-274.3 clone. The finding that the 3T3 stimulating activity released by WEHI-274.3 cells could be neutralized entirely with anti-IL-1α antiserum is in agreement with the observation that whole-cell polyadenylated RNA from WEHI-274.3 cells contained RNA species that hybridized with an IL-1α probe (Fig 1B).
Fig 7. Activity released by WEHI-274.3 cells can be mimicked by the IL-1. The responses of FDC-P1 (A) and WEHI-274.3 (B) cells to 3T3-conditioned medium (40%) (○), human recombinant IL-1β (40 ng/mL) (△), a mixture of 3T3-conditioned medium (40%) and IL-1 (40 ng/mL) (■), and conditioned medium of 3T3 cells grown in the presence of 100 ng/mL IL-1 (40%) (●) were measured with a 6-hour thymidine incorporation assay after a 3-day incubation. The effect of sheep antimurine GM-CSF (1:45 constant) on the response of FDC-P1 to the conditioned medium of 3T3 cells grown in 100 ng/mL IL-1 (40%) is shown (△), although obscured by the symbol for human recombinant IL-1β (both fail to induce thymidine incorporation by FDC-P1 cells). A dilution of 1 represents the starting concentration (indicated above); subsequent dilutions and statistical analyses are as for Fig 3. In the presence of medium alone, the incorporation of FDC-P1 cells was 15 cpm (SEM = 4) for (A), and the incorporation of WEHI-274.3 cells was 646 cpm (SEM = 96) for (B).

The level of IL-1α-specific RNAs in WEHI-274.3 (lane 2) was significantly lower than that in P388D (lane 4), with P388D being a known source of IL-1α and IL-1β bioactivities and mRNAs. The Northern blot was stripped and reprobed with an IL-1β probe (Fig 1A). The level of IL-1β-specific RNA accumulation in WEHI-274.3 (lane 2) was comparable with that in P388D (lane 4) (relative to β-actin RNA levels, data not shown).

Hybridization of a separate Northern blot with a TNFα probe demonstrated the accumulation of very low levels of TNFα RNAs in WEHI-274.3 cells (Fig 1C). The level of TNFα-specific RNA accumulation was significantly lower in WEHI-274.3 (lane 2) than that observed in P388D (lane 4), and vastly lower than that observed in an activated T-cell line (lane 1) (relative to β-actin RNA levels, data not shown).

To exclude the possibility that the expression of the IL-1α and IL-1β genes observed in vitro did not reflect constitutive, pathologic activation of these genes but rather induction of the genes by artifactual stimuli in the culture system such as endotoxin or contact with plastic, we examined RNA recovered from spleens of otherwise healthy mice bearing the WEHI-274.3 leukemia as an ascitic neoplasm. The spleens were collected on ice, the tissue disrupted by passage through a sieve, then the RNA extracted immediately as described. RNA slot-blot analysis showed similar results to those seen with in vitro cells, ie, readily detectable levels of IL-1α and IL-1β RNAs (Fig 2). These results indicated that expression of the IL-1α and IL-1β genes was constitutive both in vitro and in vivo.

Fig 8. WEHI-274.3 cells release IL-1α. The response of WEHI-274.3 cells to culture medium (40%) of 3T3 cells grown in the presence of 20% WEHI-274.3 auto-conditioned medium (○) is shown. The effects of sheep anti-IL-1α antiseraum (1:1,000 constant) (□), sheep anti-IL-1β antiseraum (1:1,000 constant) (●), and pre-immune serum (1:1,000) (■) are shown. A dilution of 1 represents the starting concentration (indicated above); subsequent dilutions and statistical analyses are as for Fig 3. In the presence of medium alone, the incorporation of WEHI-274.3 cells was 13 cpm (SEM = 4).

DISCUSSION

Here we have described the paracrine stimulation of growth of an in vivo-derived murine myelomonocytic leukemia. This indirect mechanism of auto-stimulation appeared to operate both in vitro and in vivo and was mediated by the constitutive production of IL-1α by the leukemic line with the resultant release of GM-CSF from bystander cells.

Several lines of evidence have suggested that human myeloid leukemic cells may produce IL-1 or TNFα constitutively. Thus, Griffin et al have reported the expression of IL-1β mRNAs in 10 of 17 purified human acute myeloid leukemia (AML) samples. Similarly, in 13 of 13 cases of human AMLs studied by Cozzolino et al, both intracytoplasmic IL-1α and IL-1β could be detected by immuno-
fluorescence. In some cases, on first examination it appears that the endogenously produced IL-1 may participate in autocrine stimulation of the leukemic cells. Thus, in the study of Cozzolino et al\textsuperscript{22} the basal proliferation of 10 of 10 AMLs could be inhibited by both anti-IL-1α and anti-IL-1β antisera and most could be stimulated by exogenous IL-1α or IL-1β. Similarly, Sakai et al\textsuperscript{10} have described the inhibition of the basal proliferation of crudely purified human M7 AML cells by an anti-IL-1β antibody. However, in only one of nine cases of human AML reported by Delwel et al\textsuperscript{2} could anti-IL-1 antisera suppress spontaneous DNA synthesis.

However, the production of IL-1 by such myeloid leukemic cells may not be surprising because their normal, nonmalignant counterparts can produce IL-1. Thus, the physiologic, inducible production of IL-1 mRNA or protein by normal human peripheral monocytes\textsuperscript{23-25} and by murine resident peritoneal\textsuperscript{26} or bone marrow-derived\textsuperscript{37} macrophages in response to stimuli such as lipopolysaccharide (LPS), TNFα, GM-CSF, CSF-1, or IL-1 itself is well established. These same stimuli also induce the production of TNFα.\textsuperscript{25,27-29} In attempting to assign a pathologic significance to the production of IL-1 by human AML cells in the examples cited above, we must consider the possibility that the production of IL-1 may not be truly constitutive and an intrinsic element of the pathogenesis of the disease, but results from stimulation of transcription or mRNA accumulation induced by the processes used to isolate the cells. Thus, the study of Kaufman et al\textsuperscript{30} demonstrating that GM-CSF mRNA was detected in freshly isolated mononuclear cells in only 1 of 48 AMLs, but in 5 out of 10 cases following in vitro depletion of T cells and monocytes, highlights the observation that in vitro manipulation can result in accumulation of mRNA not present in vivo. It is possible that a similar phenomenon may be operating in the artificial induction and detection of IL-1 mRNAs and bioactivities.

While some of the examples of IL-1 production by human myeloid leukemic cells in vitro may be due to artifactual gene activation in the process of cell purification, others may reflect primary pathological activations of IL-1 genes. Analogous to the physiologic paracrine stimulation of normal myeloid cells mediated by IL-1, the IL-1 released by AML cells could potentially participate in a similar paracrine mode of pathologic growth stimulation of those AML cells by involvement of adjacent, nonmalignant cells. Certainly, macrophase-derived\textsuperscript{31,32} or recombiant IL-1 has been demonstrated to induce the production of GM-CSF, CSF-1, and G-CSF from human endothelial cells,\textsuperscript{33-38} fibroblasts,\textsuperscript{33,38,39} and murine bone marrow-derived stromal lines.\textsuperscript{40,41} The release of similar activities has been described after stimulation of these adherent cells with TNFα.\textsuperscript{35,41-45} Moreover, in parallel with the in vitro findings of our study, cell culture supernatants of AML cells constitutively releasing IL-1 are able to stimulate the release of GM-CSF and G-CSF by human endothelial cells\textsuperscript{34,46,47} and could be inhibited with an anti-IL-1 antisem. In some of the examples from the literature where it has been postulated that the in vitro growth behaviors of human AML cells are via the autocrine effect of IL-1, it is possible that paracrine or indirect phenomena are involved. This confusion stems from the fact that the human leukemic samples in these studies are only crudely purified and have a low level of contamination with nonleukemic cells. As such the response of crudely purified leukemic cells to endogenously produced or exogenously added IL-1 may indeed be occurring via the release of other growth factors from contaminating cells. Thus, while several of the examples cited above suggest the involvement of IL-1 in the pathology of many human AMLs at the level of direct autocrine stimulation, a degree of caution is warranted in their interpretation.

In the study reported here, analysis of RNA extracted immediately postmortem from spleens of otherwise healthy mice bearing the WEHI-274.3 leukemia as an ascitic tumor demonstrated that the IL-1α and IL-1β RNAs were indeed present in vivo (Fig 2) and thus do not reflect artifactual gene activations in the process of cell purification. Moreover, the leukemic sample used is a cloned cell line so that the question of whether specific activities derive from a minor contaminating cell population does not exist. The paracrine mechanism of growth stimulation described here is likely to be pathologic in nature, rather than merely reflecting a normal, physiologic monokine secretion, because the accumulations of IL-1α and IL-1β mRNAs in the WEHI-274.3 cells in vitro and in vivo and the release of IL-1α bioactivity appeared constitutive rather than inducible. In all physiologic cases described to date, IL-1 accumulation in myelomonocytic cells and the release of bioactive IL-1 appear to be inducible.\textsuperscript{22,27}

The IL-1-like bioactivity released by the WEHI-274.3 cells could be abolished completely with an antiserum specific for murine IL-1α alone (Fig 8), despite the demonstration of significant accumulations of both IL-1α and IL-1β mRNAs in vitro (Fig 1) and in vivo (Fig 2). However, this observation is not unusual because the constitutive accumulation of specific IL-1 mRNAs is not always accompanied by the constitutive release of IL-1 bioactivity. In the study of Griffin et al\textsuperscript{39} of four AML samples producing IL-1β mRNAs, only three were releasing significant IL-1 bioactivities as measured by a D10.G4.1 proliferation assay.\textsuperscript{48} Similarly, the presence of cytoplasmic IL-1 need not be associated with the release of IL-1 bioactivity. While Cozzolino et al\textsuperscript{22} found that cytoplasmic IL-1 protein was demonstrable by immunofluorescence in all 13 of 13 human AMLs they examined, in only 10 of 13 cases was IL-1 bioactivity released by the cell. Thus, aside from the documented regulation of IL-1 mRNA accumulation at the levels of gene transcription and message stability,\textsuperscript{23} it appears that the expression of at least human IL-1 bioactivity is subject to significant translational and posttranslational regulation. In some of the examples from the literature discussed previously it is possible that the autogeneously produced IL-1 may enhance the response of the leukemic cells to cytokines from other sources,\textsuperscript{49,50} both in vitro and in vivo. However, there was no evidence that the IL-1α bioactivity released by WEHI-274.3 cells acts on those cells.

Our observations on the WEHI-274.3 cell line suggest...
that the paracrine mode of growth stimulation demonstrated in vitro also occurs in vivo. Thus, in vivo the induction of leukemia disease by the WEHI-274.3 cell line is accompanied by the constitutive accumulation of IL-1α mRNAs in tissues bearing the tumor (Fig 2), and the release of a WEHI-274.3 stimulatory activity into the circulation by the host (Fig 4A). The source of this stimulatory activity in vivo probably is not limited to fibroblasts, the in vitro source demonstrated in this study, but perhaps includes other cell types known to produce bioactivities and responsive to exogenous CSFs, studies currently poorly documented in the literature. However, the question of whether the paracrine mechanism of autoregulation described here represents a primary oncogenic event rather than a secondary mechanism of tumor progression remains to be resolved. We are currently exploring methods by which to interrupt these autoregulatory circuits in vivo using immunologic and molecular genetic techniques.

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PARACRINE STIMULATION OF A MYELOID LEUKEMIA


The role of interleukin-1 and granulocyte-macrophage colony-stimulating factor in the paracrine stimulation of an in vivo-derived murine myeloid leukemia

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