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Different colony-stimulating factors are detected by the "interleukin-3"-dependent cell lines FDC-PI and 32D cl-23

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Different Colony-Stimulating Factors Are Detected by the “Interleukin-3”–Dependent Cell Lines FDC-Pl and 32D cl-23

By Andrew J. Hapel, Hilary S. Warren, and David A. Hume

The cell lines FDC-Pl and 32D cl-23 have previously been used as unique indicators for the growth-promoting activity of interleukin-3. We show that FDC-Pl cells respond to granulocyte/macrophage colony-stimulating factor (GM-CSF, CSF-2) as well as to interleukin-3. In keeping with this finding, FDC-Pl cells express the macrophage-specific marker, F4/80. FDC-Pl cells do not, however, respond to macrophage CSF (M-CSF, CSF-1). In contrast, 32D cl-23 cells do not respond to GM-CSF and lack F4/80. Instead, 32D cl-23 cells respond to an as yet undefined factor in conditioned medium (CM) from the primate T cell line, MLA-144, and CM from mitogen-stimulated human lymphocytes (HLCM). 32D cl-23 cells are Lyt-1–. Both FDC-Pl and 32D cl-23 cells consume interleukin-3, but only FDC-Pl cells consume GM-CSF. Similarly, 32D cl-23, but not FDC-Pl, cells consume 32D cl-23 growth factor from MLA-144 CM and HLCM. Interleukin-3-dependent cell lines must therefore concurrently express different functional cell surface receptors for a variety of biochemically distinct growth factors.

MATERIALS AND METHODS

Factor-producing cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum. Conditioned media (CM) were collected from the primate T cell line, MLA-144,11 and the murine WEHI-3B cell lines as spinner cultures reached 106 cells per mL. L929 conditioned medium was collected from confluent monolayer cultures.12 Cells were removed by centrifugation and CM filtered through 0.2-μ Millipore sterilization filters. Purified interleukin-3 (IL-3) was made by published methods13 that involve sequential chromatography on DEAE cellulose at pH 7.2, phenyl-Sepharose, hydroxyapatite, and high-pressure liquid chromatography (HPLC) using a Syncopal C-18 reverse phase column (250 x 4.1 mm), eluted with an acetonitrile gradient in trifluoroacetic acid, pH 2.0. IL-3 activity detected by FDC-Pl and 32D cl-23 cell growth eluted at 37.2% acetonitrile. Active samples were pooled and extensively dialyzed against RPMI 1640. Purified granulocyte/macrophage colony-stimulating factor (CSF-2),10 granulocyte CSF,14 and multi-CSF (interleukin-3)15 were provided by Drs Metcalf, Nicola, Cutler, Johnson, and Burgess of the Walter and Eliza Hall Institute of Medical Research, Melbourne. GM-CSF was prepared by sequential chromatography of postendotoxin lung conditioned medium (which does not contain IL-3 activity) using calcium phosphate gel. DEAE cellulose, concanavalin A-Sepharose, and Ultrogel AcA 44. This resulted in approximately a 2,000-fold purification.10 G-CSF was purified by salting out chromatography, chromatography on phenyl-Sepharose, and gel filtration on Bio-Gel P-60 l mol/L acetic acid.14 These procedures separate GM-CSF from G-CSF activity. Human lymphocyte CSF was made by previously published methods.15 Briefly, human tonsil lymphocytes were incubated in phytahemagglutinin (PHA) for one hour at 37 °C, washed, and incubated in serum-free Delbecco’s modified Eagle’s medium (DMEM) overnight at 37 °C. The preparation was free of residual mitogen. Interleukin-2 activity was measured in a proliferation assay using Con-A–activated cells.15 The preparation supported growth of colonies of human bone marrow cells (CFU-C) in agar and in liquid culture (Hapel et al, unpublished observations). The FDC-Pl and 32D cl-23 cell lines were maintained in RPMI 1640 supplemented with 30% CM from WEHI-3B cells. Microaassays using these cells were performed by incubating serial twofold dilutions of factor with 2 x 106 cells in 100 μL of RPMI (+ 10% fetal bovine serum) overnight at 37 °C. Cultures were pulsed the next day using 0.5 μCi [3H]thymidine for six hours before harvesting...
cells onto glass fiber filters and determining incorporated label by scintillation counting.

RESULTS

Phenotypic Characteristics of FDC-PI and 32D cl-23 Cells

Purified interleukin-3 maintains the growth of cell lines that differ markedly in phenotype. The 32D cl-23 line expresses the T lymphocyte-associated marker Lyt-1. FDC-PI cells do not possess this antigen (unpublished) and have previously been reported to express phenotypic characteristics of granulocytes. We have used the macrophage-specific monoclonal antibody F4/80 to further discriminate between the two lines by indirect immunofluorescence. FDC-PI is F4/80+ and 32D cl-23 is not. F4/80 antigen is also present on the WEHI-3 myelomonocytic cell line that produces interleukin-3. Previous reports by Whetton and Dexter similarly indicate the presence of macrophage, but not lymphocyte, markers on FDC cell lines.

Growth of FDC-PI Cells in CSF-2

The data in Fig 1 confirm that both FDC-PI and 32D cl-23 respond to purified interleukin-3 from WEHI-3 conditioned medium. Both also respond to a purified factor with multiple colony-stimulating activities (multi-CSF) that is derived from pokeweed mitogen spleen conditioned medium. However, in contrast to previous reports, the sensitivity of the two cell lines to purified factors and the slopes of the dose–response curves differ markedly (Fig 1). Interleukin-3/multi-CSF is able to induce mixed colonies of granulocyte and macrophages in soft agar. As FDC-PI cells express F4/80 antigen, indicating a relationship to the monocyte series, we formed the hypothesis that their response to interleukin-3/multi-CSF may be related to the granulocyte-macrophage colony-stimulating activity of this factor. As shown in Fig 1, FDC-PI cells respond to CSF-2 purified from postendotoxin lung conditioned medium. The dose–response curve parallels that of interleukin-3. The response to CSF-2 is specific in that neither purified granulocyte colony-stimulating factor nor L cell conditioned medium (a source of macrophage colony-stimulating factor CSF-1) maintained growth of FDC-PI. FDC-PI cells thus differ from purified primary bone marrow-derived macrophages, which appear to respond preferentially to CSF-1 and CSF-2. These observations contradict the claim that FDC-PI is exclusively an interleukin-3–dependent cell line.

Growth of 32D cl-23 Cells in MLA and HLCM

Unlike FDC-PI, 32D cl-23 cells do not respond to CSF-2 (Fig 1). This result correlates with the observation that the majority of CSF-2 in mouse lymphokine preparations can be separated from 32D cl-23 growth activity. Neither FDC-PI nor bone marrow-derived macrophages (unpublished) respond to human sources of CSF-2, such as mitogen-stimulated lymphocyte conditioned medium or giant cell tumor (GCT) conditioned medium (Fig 1). In contrast to their inability

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![Fig 1. Growth of 32D cl-23 (A) and FDC-PI (B) cells in different purified factors and conditioned media. Indicator cells were seeded at 2 × 10⁶ in microtiter wells containing serial twofold dilutions of factor in RPMI 1640 supplemented with 10% fetal calf serum. Cultures were incubated overnight at 37 °C in a humidified 5% CO₂ atmosphere and pulsed for six hours with 0.5 μCi [³H]thymidine. Four separate preparations of MLA-144 CM and HLCM gave similar results. Assays using purified factors were reproduced at least ten times. (قرار) Interleukin-3/multi-CSF; (C) CSF-2 (GM-CSF); (O) L929 CM (M-CSF, CSF-1); (A) HLCM; (B) MLA-144 CM; (V) G-CSF. Prime (') denotes purified factor.](image-url)
to maintain FDC-PI cells, conditioned media from human (mitogen-stimulated tonsillar lymphocytes) and primate (MLA-144 T lymphocyte line) lymphoid sources contain significant 32D cl-23 growth factor activity (Fig 1). The dose–response curves for these factors parallel that for interleukin-3 with a slope of less than one.

Consumption of Different Growth Factors by FDC-Pl and 32D Cl-23 Cells

The results presented above demonstrate that two interleukin-3–dependent cell lines differ markedly in their growth factor requirements. Iscove et al., in purifying burst-promoting activity (interleukin-3) from WEHI-3B CM, comment that it remains possible that there are several very closely related, but separate, hemopoietic growth factors that copurify. To test whether the two lines were responding to different factors in CM from various sources, we attempted to specifically deplete FDC-Pl and 32D cl-23 growth factors by absorption. Both FDC-Pl and 32D cl-23 depleted WEHI-3 CM of growth factor activity for either cell line. FDC-Pl cells depleted culture media of purified CSF-2, but did not deplete 32D cl-23 growth factor from human tonsil CM. Conversely, 32D cl-23 depleted human tonsil CM of its own growth factor activity, but failed to deplete FDC-Pl growth factor from purified CSF-2 (Figs 2 and 3). Human tonsil CM contains T cell growth factor or interleukin-2. The absorption of growth factor activity from human tonsil

Fig 2. Depletion of 32D cl-23 growth activity from WEHI-3B CM, MLA-144 CM, and HLCM. FDC-Pl or 32D cl-23 cells (10^6/mL) were incubated for 16 hours at 37°C in factor-containing medium. The level of factor used was estimated at between 1 and 5 ED50/mL. Control cultures contained cells alone or factor alone. Supernatants were filtered using 0.2 μm disposable filters (Microflow 25, Flow Filtration Systems) and assayed for 32D cl-23 growth activity. (A) WEHI-3B CM; (B) HLCM; (C) MLA-144 CM. (—–) Factor control; (—–) factor depleted using FDC-Pl cells; (—–) factor depleted using 32D Cl-23 cells; (—–) background ± 3 SD. The results obtained using purified interleukin-3 from WEHI-3B CM, or purified multi-CSF from PWMS CM, were indistinguishable from those obtained with crude WEHI-3B CM, indicating that WEHI-3B CM contains only one factor active in these assays.

Discussion

F4/80 antigen has been shown to be a differentiation antigen of mononuclear phagocytes. In cultures of CSF-1-stimulated bone marrow, F4/80 is apparently absent from pluripotent stem cells but is expressed on nonadherent macrophage progenitors. The expression of F4/80 on the FDC-Pl cell line, which also has features of granulocytes, and on WEHI-3 myelomonocytic cells, suggests that the marker is expressed during differentiation of myelomonocytic hemopoietic cells prior to the bifurcation between the monocyte and myeloid lineages. Both CSF-2 and interleukin-3 induce the appearance of nonadherent F4/80+ cells in cultures of adult bone marrow (unpublished), and mature F4/80+ monocytes respond to M-CSF (CSF-1). FDC-Pl cells do not respond to CSF-1 (Fig 1). Further experiments will be directed toward ascertaining whether FDC-Pl cells can be induced to express CSF-1 receptors. Previous reports have indicated that the presence of other macrophage-specific markers on FDC lines is not always associated with responsiveness to GM-CSF or M-CSF. A comparison of several FDC lines with
different properties may therefore be a useful means of establishing a developmental lineage for these cells.

An examination of the previous literature on interleukin-3–dependent cell lines reveals that the choice of lines for particular studies has been fortuitous. 32D cl-23 was used to separate CSF-2 and interleukin-3 from mouse lymphokine preparations. FDC-Pl was used in the purification of interleukin-3 from WEHI-3 conditioned medium, which does not contain CSF-2 (D. Metcalf, Walter and Eliza Hall Institute for Medical Research, Melbourne, October 1983, personal communication). WEHI-3, rather than the lymphocyte line EL-4, which does produce CSF-2, was used as a source of mRNA in the recent cloning of cDNA for interleukin-3.

We have not yet characterized the human/primate lymphokine that induces growth of 32D cl-23 cells (Fig 1). Its failure to act on FDC-Pl cells suggests two important facts. First, the human/primate analogs of interleukin-3 and CSF-2, if they exist in our lymphokine preparations, do not interact with the mouse receptors for these factors. Second, 32D cl-23, like FDC-Pl, respond to at least two different growth factors (both of which are probably present in mouse lymphokine preparations) and cannot be used individually as a specific bioassay for interleukin-3.

The presence of Lyt-1 antigens on 32D cl-23 cells might suggest the involvement of interleukin-2 (T cell growth factor), but as human interleukin-2 interacts with mouse T cells, and mouse interleukin-2 is not active on 32D cl-23 cells, interleukin-2 is an unlikely candidate. Furthermore, 32D cl-23 cells do not specifically degrade interleukin-2. Alternatively, 32D cl-23 cells have been shown to give rise to mast cell/basophil colonies in soft agar, and human lymphokine preparations contain a factor that induces proliferation and differentiation of mast cells. Purified interleukin-3 has been shown to have mast cell growth factor (P cell-stimulating factor; histamine-producing cell-stimulating factor) activity, but this does not preclude a role for other factors in mast cell differentiation. Further delineation of the function of human/primate 32D cl-23 growth factor awaits its purification and molecular cloning of the mouse analog.

NOTE ADDED IN PROOF

It has been found that 32D cl-23 cells respond to recombinant human interleukin-2 (provided by J. Farrar, Hoffman la Roche, Nutley, NJ) and to four interleukin-2 species purified from HLCM by chromatofocusing (A.J. Hapel, unpublished observations).

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