Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4670–4674, June 1990
Cell Biology

Low-affinity placenta-derived receptors for human granulocyte–macrophage colony-stimulating factor can deliver a proliferative signal to murine hemopoietic cells

(growth factors/ligand binding/cell lines/receptors)

DONALD METCALF*, NICOS A. NICOLA, DAVID P. GEARING†, AND NICHOLAS M. GOUGH

The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital 3050, Victoria, Australia

Contributed by Donald Metcalf, March 26, 1990

ABSTRACT **Retrovirally mediated introduction of a cDNA encoding a placenta-derived low-affinity receptor for human granulocyte–macrophage colony-stimulating factor (GM-CSF) into murine FDC-P1 hemopoietic cells allowed these cells to proliferate when stimulated by human GM-CSF. The expressed human receptors on cloned lines were of low affinity (K_d = 4–6 nM), were internalized, and did not interact with endogenous GM-CSF receptors. Concentrations of human GM-CSF of 6.5–13 nM were required to stimulate 50% maximal colony formation versus a concentration of murine GM-CSF of 6 pM; this difference is comparable with the difference in relative affinities of the human and murine receptors for their respective ligands. If maintained in murine GM-CSF, cells able to bind or respond to human GM-CSF were rapidly lost due to transcriptional inactivation of the inserted cDNA. The observations indicate that low-affinity receptors for human GM-CSF can deliver a proliferative signal in appropriate cells and that the signaling mechanisms are not species-specific.**

Granulocyte–macrophage colony-stimulating factor (GM-CSF) is a growth factor regulating the proliferation, differentiation, and functional activity of neutrophils, macrophages, and eosinophils (1–3). GM-CSF has also been reported to have proliferative activity for endothelial cells (4), fibroblasts (5), osteoblasts (6), and placental cells (7).

On hemopoietic and nonhemopoietic cells, both high- and low-affinity membrane GM-CSF receptors have been described with equilibrium dissociation constants (K_d) of 10–50 pM and 1–5 nM, respectively (8–11). Because most of the biological effects of GM-CSF are observed at picomolar concentrations (1) and high-affinity receptors are preferentially internalized (N.A.N. and L. Peterson, unpublished results), it is not clear whether low-affinity receptors are biologically functional. In the murine system at 37°C (12) and the human system at 4°C or 37°C (11, 13, 14), multipotential CSF (interleukin 3) can down-modulate GM-CSF receptors on some types of hemopoietic cells, but it is not clear whether this is mediated by different receptor subclasses or by receptor–receptor interactions (10, 11, 13).

We have cloned a low-affinity receptor for human GM-CSF (hGM-CSF) from placental cells (K_d of 5 nM) (10). We now show that when this receptor is introduced into a murine GM-CSF (mGM-CSF)-dependent hemopoietic cell line (FDC-P1) by using a retroviral vector, it retains its low-affinity phenotype but can nevertheless transmit the biological signals required for cell proliferation.

MATERIALS AND METHODS

Production and Selection of hGM-CSF Receptor Retrovirus. A 1.7-kilobase pair *Xho* I fragment containing the insert cDNA of pGMR29 (10) was inserted into the *Xho* I site of pJZen2(SVNeo), a derivative of the retroviral vector pMPZen (15) in which a multiple cloning site has replaced the single *Xho* I site of pMPZen and a 1.7-kilobase pair neomycin-resistance expression cassette was inserted into the *Cla* I site (16). ψ2 packaging cells (17) were electroporated with pJZen2(SVNeo)-hGM-R DNA as previously described (15), and transfectants were selected 2 days later by using G418 (Geneticin, Sigma) at 400 μg/ml. G418-resistant ψ2 clones were selected for high surface expression of the hGM-CSF receptor by binding of ^{125}I-labeled hGM-CSF (^{125}I-hGM-CSF). Retroviral titers of receptor-positive ψ2 clones were tested by Polybrene-mediated infection of NIH 3T3 fibroblasts (18). The clone selected for further work (ψ2-GMR) had a titer of 1.2×10^4 viral particles per ml.

Derivation of Infected FDC-P1 Cell Lines. Adherent ψ2-GMR cells (3×10^6 per 75-cm^2 flask) were irradiated (35 grays) and cocultured with 10^6 FDC-P1 cells (19) in 20 ml of Dulbecco's modified Eagle's medium (DMEM) with 10% (vol/vol) fetal calf serum (FCS) and 10% (vol/vol) pokeweed mitogen-stimulated spleen cell conditioned medium. Washed supernatant cells from 48-hr cocultures were cultured in agar medium at a density of 300 cells per ml with either mGM-CSF (10^3 units/ml), hGM-CSF (6×10^3 units/ml), or a combination of both. After a 3-day incubation, clones developing in mGM-CSF had achieved 50–100 cells in size. In contrast, fewer clones had developed in cultures stimulated by hGM-CSF; these were dispersed in morphology and most contained only 10–30 cells. Individual clones growing in cultures stimulated by hGM-CSF were removed with a micropipette, and cloned cell lines were established and maintained in 1-ml cultures of DMEM with 20% FCS containing either hGM-CSF (6×10^5 units/ml) (12 lines) or hGM-CSF (6×10^3 units/ml) plus mGM-CSF (10^3 units/ml) (36 lines). Subcloning of individual cell lines was performed by growing colonies in agar medium cultures of 200 cells per ml, removing the individual colonies after 7 days of incubation, and continuing culture of these colonies in suspension.

Agar Cultures. These were performed in 35-mm plastic Petri dishes (Nunc) using 1 ml of agar medium (DMEM with final concentrations of 20% FCS and 0.3% agar) (1) and 300 cultured cells.

The stimuli used for colony formation were purified recombinant mGM-CSF (50 units/ml = 12 pM) or purified recombinant hGM-CSF (50 units/ml = 36 pM) produced as nonglycosylated derivatives in *Escherichia coli*. These were included as 0.1-ml volumes during preparation of the agar

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GM-CSF, granulocyte–macrophage colony-stimulating factor; mGM-CSF and hGM-CSF, murine and human GM-CSF, respectively; hGM-R-FD, FDC-P1 cells transfected with hGM-CSF receptor retrovirus; FCS, fetal calf serum.

*To whom reprint requests should be addressed.
†Present address: Immunex Corporation, 51 University Street, Seattle, WA 98101.

cultures, and serial 2-fold dilutions were performed by using 5% FCS in 0.95% saline. Mean cell numbers in 7-day colonies were determined by pooling 30–50 sequential colonies.

Iodination and Binding Procedures. Purified mGM-CSF and hGM-CSF were radiodinated by the modified iodine monochloride method described (20), yielding ^{125}I-hGM-CSF with a specific radioactivity of 30,000 cpm/ng and a binding ability of 100% and ^{125}I-mGM-CSF of specific radioactivity 120,000 cpm/ng and binding ability of 40–50%.

Saturation binding of ^{125}I-hGM-CSF to FDC-P1 cells transfected with hGM-CSF receptor retrovirus (hGM-R-FD cells) and Scatchard transformations were performed as described (10) with incubation times of 1 hr at 20°C. Cross-modulation of hGM-CSF receptors by mGM-CSF and of multipotential CSF or mGM-CSF receptors by hGM-CSF was performed as described (12) with preincubation times of 30 min at 37°C followed by binding times of 3 hr at 0°C. Receptor internalization studies were performed as described in ref. 20 at the indicated concentrations of ^{125}I-hGM-CSF. The data were analyzed by curve fitting the experimental points as described (20).

Southern and Northern Blots. Ten-microgram aliquots of high molecular weight genomic DNA were digested with *Pst* I, electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose. Conditions for hybridization and washing were as described (10). The hybridization probe was the gel-purified 786-base-pair *Kpn* I–*Eco*RI fragment spanning the 3′ end of the hGM-CSF receptor coding region (10) radiolabeled to a specific activity of ≈ 2–4×10^8 cpm/μg by nick-translation and included in the hybridization mixtures at $\approx 2 \times 10^7$ cpm/ml. Total cytoplasmic RNA (from $\approx 5 \times 10^5$ cells), prepared essentially as described (21), was electrophoresed, transferred to nitrocellulose filters, and hybridized as described (10). The hybridization probe was as above, but it was radiolabeled to a specific activity of $>10^9$ cpm/μg by random-priming (22) and was included in the hybridization mixture at $\approx 5 \times 10^7$ cpm/ml.

RESULTS

The FDC-P1 cell line used in this laboratory does not proliferate in cultures containing hGM-CSF at 10^6 units/ml, and no cells survive in such cultures. From four separate cocultivation experiments with $\psi 2$ cells producing the hGM-CSF receptor retrovirus, 0.3–1% of FDC-P1 cells were able to proliferate clonally in agar cultures stimulated by hGM-CSF. From individual colonies, cloned cell lines (hGM-R-FD lines) were developed and maintained by using either a high concentration of hGM-CSF or a mixture of mGM-CSF with a lower concentration of hGM-CSF.

Southern blot analysis of DNA from 19 such hGM-R-FD lines (Fig. 1*a*, lanes 2–20) revealed the presence of a single viral integration in each clone, except clone 57 in which two integrations were evident (lane 19). With the exception of clones 50 and 52, which came from the same cocultivation experiments and are therefore potentially siblings (Fig. 1, lanes 13 and 14), the viral integration sites differed in each cloned line, as revealed by the different sizes of hybridizing DNA fragments, confirming the independent clonal origin of each line.

Differences Between Cell Lines Maintained in hGM-CSF Versus mGM-CSF Plus hGM-CSF. At 25–39 days after the establishment of the cloned lines, a comparative analysis of 19 of these lines, chosen at random, revealed distinctive differences between the two types of lines in their behavior in clonal cultures (Fig. 2).

The parental FDC-P1 cell line usually exhibits a cloning efficiency in agar medium of 60–100% when stimulated by mGM-CSF; it forms large, tight colonies. hGM-R-FD lines maintained in hGM-CSF usually exhibited lower clonogenic potential (42 ± 17%; mean ± SD), and total colony numbers were similar in cultures stimulated by hGM-CSF or mGM-CSF (Fig. 2). The colonies characteristically had an irregular shape or were wholly dispersed, and maximal colony size was relatively small. Colony size was typically 2–4 times larger in parallel cultures stimulated by mGM-CSF. (For nine cell lines, mean colony size with mGM-CSF was 530 ± 340 cells versus 240 ± 110 with hGM-CSF.)

When hGM-R-FD cell lines maintained in mGM-CSF plus hGM-CSF were stimulated by hGM-CSF, the frequency of clonogenic cells was even lower (15 ± 15%) than that of lines maintained in hGM-CSF alone, although the morphology of the colonies was similar. With increasing duration of maintenance in the mixture of mGM-CSF plus hGM-CSF, a progressive fall occurred in the frequency of clonogenic cells

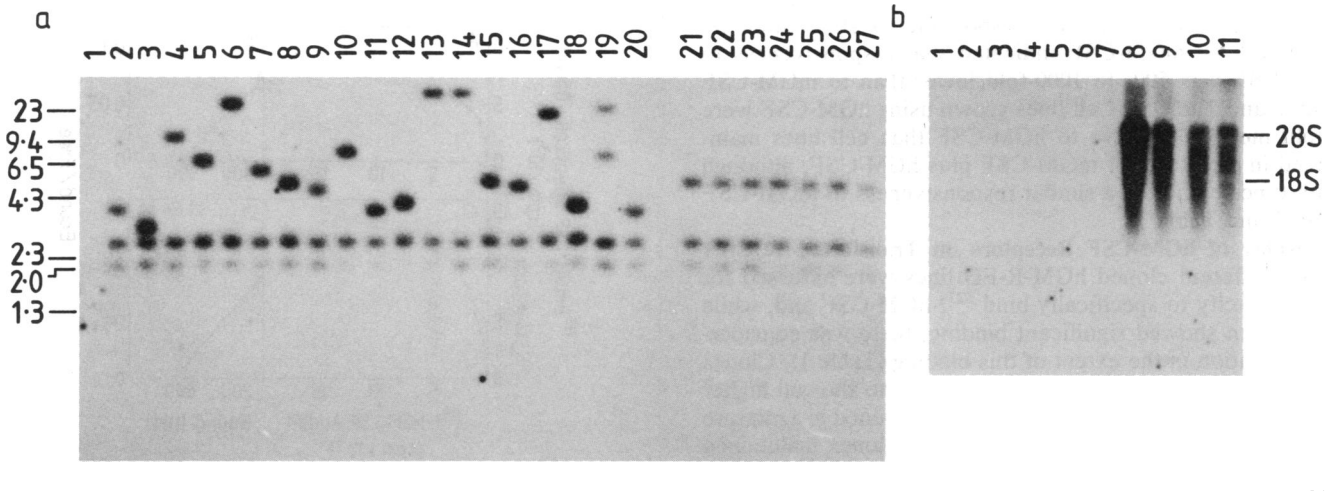

FIG. 1. Viral integration and transcripts in hGM-R-FD cells. (*a*) DNA from FDC-P1 cells (lane 1); hGM-R-FD clones 1, 6, 8, 10, 11, 13, 21, 24, 33, 34, 49, 50, 52, 53, 54, 55, 56, 57, and 58 (lanes 2–20); and hGM-R-FD clone 21 and subclones 21.13, 21.15, 21.17, 21.21, 21.22, and 21.23 (lanes 21–27) was digested with *Pst* I and probed for hGM-CSF receptor sequences. Note the common 2.5-kilobase-pair fragment derived from within the hGM-CSF receptor viral construct. (*b*) Total cytoplasmic RNA from FDC-P1 cells (lane 1) and hGM-R-FD subclones 21.13, 21.15, 21.17, 21.21, 21.22, 21.23, 21.7, 21.8, 21.10, and 21.11 (lanes 2–11) was probed for viral hGM-CSF receptor transcripts. The major species is 5.5 kilobases in length and corresponds to the full-length unspliced viral transcript. Primary lines were generated from three different cocultivation experiments: experiment 1, lines 1–13; experiment 2, lines 21–34; experiment 3, lines 49–58. Lines from experiments 1 and 2 were established in mGM-CSF plus hGM-CSF, and lines from experiment 3 were established in hGM-CSF. Subclones 21.13–21.23 were established and maintained in mGM-CSF, and subclones 21.7–21.11 were established and maintained in hGM-CSF.

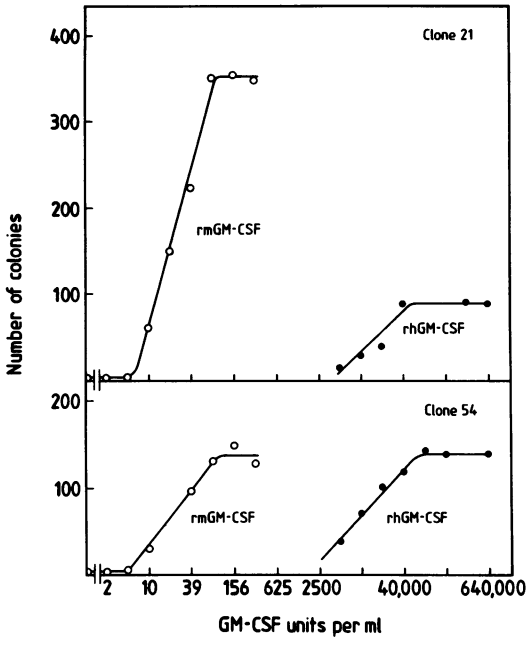

FIG. 2. Responsiveness of cells from cloned hGM-R-FD cell lines to proliferative stimulation by recombinant (r) mGM-CSF or hGM-CSF. Lines maintained in hGM-CSF exhibit a similar content of clonogenic cells with both stimuli (e.g., clone 54), whereas in lines maintained in mGM-CSF plus hGM-CSF (e.g., clone 21) murine-responsive clonogenic cells are more frequent than human-responsive cells. For both types of cell lines, clonogenic cells are less responsive to hGM-CSF than to mGM-CSF.

responsive to hGM-CSF alone. In sharp contrast, when cells of these lines were stimulated by mGM-CSF, the frequency of clonogenic cells was much higher (96 ± 21%). The morphology and size of these colonies resembled those of parental FDC-P1 cells and there was a more than 10-fold difference between the size of colonies stimulated by mGM-CSF or hGM-CSF. (For 10 cell lines, the mean colony size with mGM-CSF was 1900 ± 880 cells versus 170 ± 130 with hGM-CSF.)

Responsiveness to GM-CSF of Lines Maintained in hGM-CSF Versus hGM-CSF Plus mGM-CSF. The dose–response curves of hGM-R-FD lines responding to stimulation by hGM-CSF or mGM-CSF indicated that responsiveness to hGM-CSF was 500- to 1000-fold lower than to mGM-CSF (Fig. 2 and Table 1). Cell lines grown using hGM-CSF were 2-fold more responsive to hGM-CSF than cell lines maintained in a mixture of mGM-CSF plus hGM-CSF, although both types exhibited a similar responsiveness to mGM-CSF (Fig. 2 and Table 1).

Display of hGM-CSF Receptors on Transfected FDC-P1 Cells. Different cloned hGM-R-FD lines were assessed for their capacity to specifically bind ^{125}I-hGM-CSF and, while all of them showed significant binding, there was considerable variation in the extent of this binding (Table 1). Clones maintained continuously in hGM-CSF alone showed higher average levels of binding than clones maintained in a mixture of hGM-CSF and mGM-CSF (Table 1). Clones maintained exclusively in mGM-CSF ultimately lost all capacity to bind ^{125}I-hGM-CSF (data not shown).

Despite the variation in binding of ^{125}I-hGM-CSF to different hGM-R-FD clones, saturation binding analysis and Scatchard transformation of the binding data showed similar binding affinities (slopes of the Scatchard transformation) (K_d = 4–6 nM) for all clones examined (Fig. 3). This binding affinity was of a single low-affinity class and was the same as for receptors on human placental membranes, transfected

Table 1. Quantitative responsiveness of cloned hGM-R-FD cell lines to stimulation by mGM-CSF or hGM-CSF and their capacity to bind hGM-CSF

Line	Units needed to stimulate 50% maximal colonies		^{125}I-hGM-CSF bound per 10^6 cells, cpm
	mGM-CSF	hGM-CSF	
FDC-P1	15	—	0
	Maintained in mGM-CSF plus hGM-CSF		
1	30	10,000	12,000
21	20	20,000	2,200
33	30	10,000	7,700
34	30	18,000	6,700
11	30	20,000	17,200
6	15	20,000	7,600
24	20	20,000	14,100
13	15	—	6,900
10	40	40,000	15,800
8	15	40,000	600
	Maintained in hGM-CSF alone		
54	20	10,000	9,300
53	20	10,000	9,000
58	7	7,000	11,800
49	20	10,000	73,400
50	15	<5,000	14,200
55	15	10,000	12,200
56	60	30,000	10,100
52	50	7,000	29,600
57	20	8,000	15,700

Three hundred cells were added to duplicate cultures containing increasing 2-fold concentrations of mGM-CSF or hGM-CSF. Colony counts were performed on day 7, and the GM-CSF concentration stimulating 50% maximal colony numbers was determined from each titration curve. Binding of ^{125}I-hGM-CSF was determined in parallel by using 1 × 10^6 cells per point.

COS-7 cells (10), and retrovirally-infected ψ2 clones (data not shown).

The binding of hGM-CSF at concentrations up to 65 nM to the transfected receptor in hGM-R-FD clones for 30 min at 37°C did not affect the subsequent binding of ^{125}I-mGM-CSF to the native mGM-CSF receptor coexpressed on these cells. Similarly, the binding of mGM-CSF or murine multipotential

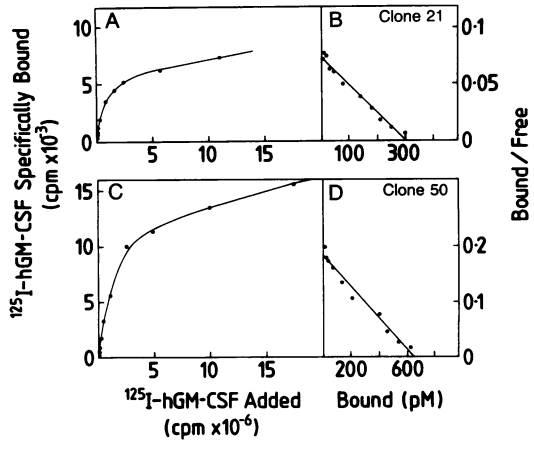

FIG. 3. Saturation binding analysis and Scatchard transformation of ^{125}I-hGM-CSF binding to hGM-R-FD cell clones. (*A* and *B*) Clone 21 was maintained in hGM-CSF plus mGM-CSF (2 × 10^6 cells per point). (*C* and *D*) Clone 50 was maintained in hGM-CSF only (0.8 × 10^6 cells per point). (*A* and *C*) Specific binding curves with increasing amounts of ^{125}I-hGM-CSF added. (*B* and *D*) Scatchard transformations. The Scatchard transformations gave K_d = 4 nM, 4000 receptors per cell for clone 21 and K_d = 6 nM, 20,000 receptors per cell for clone 50.

CSF at concentrations up to 30 nM to their native receptors on hGM-R-FD cells at 37°C did not affect the subsequent binding of ^{125}I-hGM-CSF to the transfected hGM-CSF receptor (data not shown).

When ^{125}I-hGM-CSF was incubated with hGM-R-FD cells at 37°C, it rapidly associated with cell surface GM-CSF receptors and was then slowly internalized into the cells. The binding and internalization kinetics were essentially identical for hGM-R-FD clones maintained in mGM-CSF or hGM-CSF (Fig. 4). However, the internalization rate of occupied hGM-CSF receptors (k_e) was slower in hGM-R-FD cells (k_e = 0.0042 min^{-1}) than in human HL60 cells (k_e = 0.061 min^{-1}) and was slower than the internalization rate of occupied mGM-CSF receptors on FDC-P1 cells (k_e = 0.056 min^{-1}) (ref. 20; N.A.N., unpublished results).

Evolution of Recloned Sublines. Analysis of colonies grown from hGM-R-FD cell lines using mGM-CSF plus hGM-CSF showed them to contain a major population of cells responsive only to mGM-CSF and a minor population responsive to hGM-CSF; this latter population rapidly diminished if colonies were grown for 1 week with only mGM-CSF. Colonies grown from cell lines maintained with hGM-CSF alone retained a stable content of clonogenic cells responding to either type of GM-CSF.

Cells from 9 sublines derived from mGM-CSF-stimulated lines, when maintained exclusively in mGM-CSF, usually formed colonies only when stimulated by mGM-CSF (cloning efficiency = 68 ± 24%), and the colonies formed were of uniformly large size. Cells from 13 sublines, derived from mGM-CSF plus hGM-CSF-stimulated cultures, but then maintained in hGM-CSF, formed relatively small numbers of colonies (cloning efficiency = 37 ± 25%) of medium size in cultures stimulated by hGM-CSF or mGM-CSF, with maintenance of the characteristic 2- to 4-fold size difference between the two types of colonies.

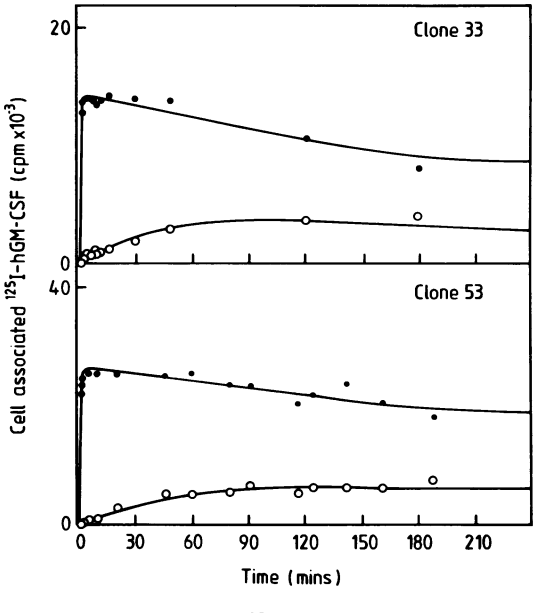

FIG. 4. Internalization of ^{125}I-hGM-CSF bound to hGM-R-FD clone 33 cells (*Upper*) or hGM-R-FD clone 53 cells (*Lower*) at 37°C. The former cells had been maintained in a mixture of mGM-CSF and hGM-CSF, whereas the latter had been maintained in hGM-CSF only. The curves show the variation with time after addition of ^{125}I-hGM-CSF of cell-surface-associated (●) and internalized (○) radioactivity, determined as described (20). The lines through the experimental points (means of duplicate tubes) were fitted by computer as described (20). For clone 33, 1.9 × 10^6 cells were used per point and the ^{125}I-hGM-CSF concentration was 13 nM; for clone 53, 1.8 × 10^6 cells were used per point and the ^{125}I-hGM-CSF concentration was 13 nM.

In tests on six cloned sublines derived from hGM-CSF-stimulated lines and maintained in hGM-CSF, 50 ± 26% of the clonogenic cells were able to form colonies in cultures containing G418 at 800 ng/ml. In contrast, clonogenic cells from six cloned sublines maintained in mGM-CSF were uniformly unable to form colonies in the presence of G418. This suggested that transcription of the inserted neomycin-resistance gene was not maintained when cells were stimulated solely by mGM-CSF.

Southern blot analysis of DNA from a series of mGM-CSF-responsive and hGM-CSF-responsive sublines of primary line 21 revealed that in all subclones both the content and context of the hGM-R viral integration were maintained (e.g., Fig. 1*a*, lanes 21–27). This confirms the common derivation of these lines, despite their divergent biological properties, and suggests that the lack of both hGM-CSF responsiveness and display of cell surface hGM-CSF receptors was not due to loss of the hGM-R construct.

RNA from six sublines maintained with mGM-CSF contained no detectable hGM-CSF receptor viral transcripts (Fig. 1*b*, lanes 2–7) compared with abundant hGM-CSF receptor viral transcripts evident in sublines maintained with hGM-CSF (Fig. 1*b*, lanes 8–11 and data not shown), suggesting that the alteration in such cells was at the transcriptional or immediately posttranscriptional level.

DISCUSSION

The existence of both low- and high-affinity forms of hGM-CSF receptors on hemopoietic cells and the existence of GM-CSF receptors on nonhemopoietic cells have raised the questions of (*i*) whether both affinity forms of the receptor are able to transmit the same biological signals, (*ii*) the structural relationships of one to the other, and (*iii*) whether or not nonhemopoietic GM-CSF receptors are related to, and use the same signaling pathways as, hemopoietic GM-CSF receptors.

After retrovirally mediated transfection of murine hemopoietic FDC-P1 cells with a cDNA encoding the low-affinity human placental GM-CSF receptor, cell-surface hGM-CSF receptors were displayed at a level of 10^4–10^5 per cell as a single binding class of low affinity (K_d = 4–6 nM). Despite careful analysis, no high-affinity binding was detected, yet the transfected cells could internalize the hGM-CSF receptor (at a rate 10 times slower than that for endogenous mGM-CSF receptors) and acquired the capacity to proliferate in response to hGM-CSF. The quantitative responsiveness of hGM-R-FD cells to hGM-CSF was 500- to 1000-fold lower than that to mGM-CSF, but determination of the binding constants suggest that occupied hGM-CSF or mGM-CSF receptors might be equally efficient in transducing proliferative signals in murine FDC-P1 cells. First, the transfected hGM-CSF receptor binds hGM-CSF with a 100-fold lower affinity (K_d = 5 nM) than the endogenous high-affinity receptor binds mGM-CSF (K_d = 50 pM) (8). Second, as we have demonstrated elsewhere (20), the 10-fold slower internalization rate of occupied hGM-CSF receptors compared to occupied mGM-CSF receptors at 37°C implies that the apparent steady-state affinity constants will differ by an additional factor of 10.

The behavior of cloned hGM-R-FD cell lines differed depending on whether they were maintained in hGM-CSF alone or a mixture of hGM-CSF and mGM-CSF. The former cell lines maintained a stable phenotype with equal clonogenicity in hGM-CSF or mGM-CSF, but clonogenicity was significantly lower than for cell lines maintained in mGM-CSF. The cell lines maintained in a mixture of mGM-CSF and a low concentration of hGM-CSF showed a progressive loss of cells able to respond to stimulation by hGM-CSF.

The behavior of cell lines maintained in hGM-CSF was not influenced by the site of viral integration. The selection pressure exerted by culture with hGM-CSF alone maintained a constant level of expression of both of the viral genes (hGM-CSF-R and the neomycin-resistance gene), receptor expression, and quantitative responsiveness to hGM-CSF. However the low clonogenicity and colony size exhibited by these cells suggested the continuous generation within lines maintained in hGM-CSF of progeny cells that had lost responsiveness to hGM-CSF. In the absence of any other stimulus, these cells irreversibly lost proliferative potential and could not be rescued by subsequent culture in mGM-CSF. This phenomenon was confirmed by analysis of subcloned hGM-R-FD lines maintained in the presence of only mGM-CSF. Such subcloned lines maintained their viral inserts but expression of both hGM-CSF receptor and the neomycin-resistance genes declined progressively to zero with increasing time in culture. The reasons for the relatively high frequency at which the viral genes were turned off are unclear, but this clearly did not depend on viral integration site. Indeed, suppression of retroviral expression has been observed in a variety of different cells, including murine hemopoietic cells (23–25) and the simian virus 40 early region promoter, used in the present construct to drive the neomycin-resistance gene, has been shown to be particularly sensitive to trans- and cis-acting negative regulatory factors (23–27).

The observation that the human placental GM-CSF receptor stimulated the proliferation of murine hemopoietic FDC-P1 cells lends support to the suggestion that this receptor subunit may form a component of the hGM-CSF receptor on hemopoietic cells (10). It also demonstrates that a low-affinity GM-CSF receptor of nonhemopoietic origin can transduce proliferative signals and be internalized in a hemopoietic cell, in a ligand-dependent manner, in the absence of any high-affinity binding component. Indeed, since biological responsiveness of the transfected FDC-P1 cells was almost the same to mGM-CSF or hGM-CSF on a receptor-occupied basis, this further suggests that the signaling components of both hGM-CSF and mGM-CSF receptors may be highly conserved and that the main function of high-affinity subunits might be solely to increase the rate of receptor internalization and to increase the responsiveness of hemopoietic cells to low external concentrations of GM-CSF. Alternate interpretations of the data involving interaction or cointernalization of the introduced hGM-CSF receptor with endogenous mGM-CSF or multipotential CSF receptors were not supported by the present experiments. Despite the conservation of signaling between human and mGM-CSF receptors, there is no functional conservation in the GM-CSF-binding domain, and there was no conversion of the hGM-CSF receptor to a high-affinity receptor by interaction with the mouse cells that displayed high-affinity mGM-CSF receptors. The relationship of the cloned low-affinity hGM-CSF receptor to high-affinity GM-CSF receptors remains unclear. It is still possible that high-affinity GM-CSF receptors are unrelated to the low-affinity hGM-CSF receptor. However, if, as we have postulated (10), the low-affinity subunit can be converted to a high-affinity receptor by interaction with an "adaptor" subunit, then this interaction seems not to occur across these species.

Recombinant mGM-CSF and hGM-CSF were kind gifts of Dr. J. Cebon, Ludwig Institute for Cancer Research, Melbourne. We acknowledge the excellent technical assistance of D. Cary, L. Di Rago, and J. King. This work was supported by National Institutes of Health Grant CA-22556, the Anti-Cancer Council of Victoria, the National Health and Medical Research Council, Canberra, the J. D. & L. Harris Trust Fund, and AMRAD Corporation, Melbourne.

1. Metcalf, D. (1984) *The Hemopoietic Colony Stimulating Factors* (Elsevier, Amsterdam).
2. Gough, N. M. & Nicola, N. A. (1989) in *Colony-Stimulating Factors: Molecular and Cellular Biology*, eds. Dexter, T. M., Garland, J. M. & Testa, N. G. (Dekker, New York), pp. 111–153.
3. Morstyn, G., Lieschke, G. J., Sheridan, W., Layton, J. & Cebon, J. (1989) *Trends Pharmacol. Sci.* **10,** 154–159.
4. Bussolino, F., Wang, J. M., Defilippi, P., Turrini, F., Sanavio, F., Edgell, C.-J. S., Aglietta, M., Arese, P. & Mantovani, A. (1989) *Nature (London)* **337,** 471–473.
5. Dedhar, S., Gaboury, L., Galloway, P. & Eaves, C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9253–9257.
6. Evans, D. B., Bunning, R. A. D. & Russell, R. G. G. (1989) *Biochem. Biophys. Res. Commun.* **160,** 588–595.
7. Wegman, T. G., Athanassakis, I., Guilbert, L., Branch, D., Dy, M., Menu, E. & Chaonat, G. (1989) *Transplant. Proc.* **21,** 566–568.
8. Walker, F. & Burgess, A. W. (1985) *EMBO J.* **4,** 933–939.
9. Gasson, J. C., Kauffman, S. E., Weisbart, R. H., Tomonaga, M. & Golde, D. W. (1986) *Proc. Natl. Acad. Sci USA* **83,** 669–673.
10. Gearing, D. P., King, J. A., Gough, N. M. & Nicola, N. A. (1989) *EMBO J.* **8,** 3667–3676.
11. Elliot, M. J., Vadas, M. A., Eglinton, J. M., Park, L. S., To, L. B., Cleland, L. G., Clark, S. C. & Lopez, A. F. (1989) *Blood* **74,** 2349–2359.
12. Walker, F., Nicola, N. A., Metcalf, D. & Burgess, A. W. (1985) *Cell* **43,** 269–276.
13. Lopez, A. F., Eglinton, J. M., Gillis, D., Park, L. S., Clark, S. & Vadas, M. A. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7022–7026.
14. Park, L. S., Friend, D., Price, V., Anderson, D., Singer, J., Prickett, K. S. & Urdal, D. L. (1989) *J. Biol. Chem.* **264,** 5420–5427.
15. Johnson, G. R., Gonda, T. J., Metcalf, D., Hariharan, I. K. & Cory, S. (1989) *EMBO J.* **8,** 441–448.
16. Bernard, O., Reid, H. H. & Bartlett, P. F. (1989) *J. Neurosci. Res.* **24,** 9–20.
17. Mann, R., Mulligan, R. C. & Baltimore, D. (1983) *Cell* **33,** 153–159.
18. Cepko, C. L., Roberts, B. E. & Mulligan, R. C. (1984) *Cell* **37,** 1053–1062.
19. Dexter, T. M., Garland, J., Scott, D., Scolnick, E. & Metcalf, D. (1980) *J. Exp. Med.* **152,** 1036–1047.
20. Nicola, N. A., Peterson, L., Hilton, D. J. & Metcalf, D. (1988) *Growth Factors* **1,** 41–49.
21. Gough, N. M. (1988) *Anal. Biochem.* **173,** 93–95.
22. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
23. Chang, J. M. W., Wager-Smith, K., Tsao, T. Y., Tigges, J. H., Vaishnav, S. & Caskey, C. T. (1987) *Mol. Cell. Biol.* **7,** 854–863.
24. Magli, M.-C., Dick, J. E., Huszar, D., Bernstein, A. & Phillips, R. A. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 789–793.
25. Emerman, M. & Temin, H. M. (1984) *Cell* **39,** 459–467.
26. Gorman, C. M., Rigby, P. W. S. & Lane, D. P. (1985) *Cell* **42,** 519–526.
27. Williams, D. A., Orkin, S. H. & Mulligan, R. C. (1986) *Proc. Natl. Acad. Sci USA* **83,** 2566–2570.