A Somatic Cell Genetic System for Dissecting Hemopoietic Cytokine Signal Transduction*

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Somatic cell genetics has proven to be a powerful tool for the dissection of cytokine signal transduction pathways. Here we describe a system in which interleukin-6 (IL-6) signaling may be dissected using myeloid leukemic M1 cells. We utilized two properties of M1 cell differentiation to isolate IL-6-unresponsive mutants. First, M1 differentiation is associated with cessation of cell division. Second, differentiated M1 cells migrate rapidly and form dispersed colonies in agar. Mutant clones that are unresponsive to IL-6 are, therefore, large and compact as compared with clones derived from IL-6-responsive wild type M1 cells. Following spontaneous or chemically induced mutagenesis and selection in a high dose of IL-6, we isolated 27 M1 clones unresponsive to IL-6. Three harbored mutations that acted in a dominant manner, whereas 24 contained recessive mutations. gp130, an IL-6 receptor component, was affected in many mutant clones. We show that these clones display IL-6 and leukemia inhibitory factor receptors with reduced binding affinities and express gp130 at reduced levels. The IL-6-unresponsive phenotype of these mutant clones was fully rescued by the transfection of exogenous gp130 DNA. Therefore, this approach targets components of the IL-6 signaling pathway and may be suitable to study signaling from a variety of cytokines.

Genetics has been successfully used to identify and analyze components of signal transduction pathways in a number of systems, including chemotaxis in bacteria (1), the response to the mating pheromone in yeast (2), the development of the Drosophila melanogaster visual system (3) and nematode vulva (4), and the increase in gene transcription in response to interferon (IFN) in mammalian somatic cells (5).

The study of interferon signaling is of particular interest because it demonstrates that mammalian somatic cells may be used for a genetic analysis of signal transduction. Genetic dissection of interferon signal transduction led to the demonstration that Janus tyrosine kinases (JAKs) and signal transducers and activators of transcription (STATs) are key elements in the interferon signal transduction pathway (9, 23). Subsequent biochemical studies have also implicated these molecules in the signal transduction pathways of cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), LIF, oncostatin-M (OSM), and IL-6 (6).

Clearly, a somatic cell genetic system that would enable dissection of the signal transduction events controlling cytokine-induced hemopoietic differentiation would be valuable. The murine myeloid leukemic cell line M1 represents a useful model of normal macrophage differentiation and provides the starting point for establishing such a system. Like normal hemopoietic progenitor cells, M1 cells express the receptor tyrosine kinase flt3/flk2 (7), the cell surface marker CD34 (8), and the transcription factors Scl (9) and Myc (10). Upon induction of differentiation by a range of cytokines including LIF (11), OSM (12), IL-6 (13), G-CSF (14), GM-CSF (9, 15), IL-11 (16), and thrombopoietin (17), these primitive markers are lost, and there is a concomitant increase in the expression of genes characteristic of mature macrophages. Among the proteins expressed by differentiating M1 cells are the transcription factors Myb (18) and Fos (19), Fc receptor types I and II (20), the IL-4 receptor a-chain (21), the receptor for complement component C3b (20), and lysozyme (22). Additionally, like primary macrophages, differentiated M1 cells are vacuolated, phagocytic, capable of extensive movement (23), and dependent on macrophage colony-stimulating factor for survival (24).

In the experiments described, we demonstrate that M1 cells provide the basis for a somatic cell genetic approach to dissect the signal transduction pathways used by cytokines to stimulate macrophage differentiation. Two spontaneous and 25 ICR-191-induced IL-6-unresponsive clones of M1 cells were selected in agar based on their ability to form tightly packed colonies, rather than small clusters of dispersed cells, in the presence of high concentrations of this cytokine. These lines were at least 2 orders of magnitude less responsive to IL-6 than wild type M1 cells. Somatic cell fusion experiments demonstrated that the majority of the mutations were recessive. Furthermore, among the recessive cell lines, one group appears to lack functional gp130, consistent with the important role that this receptor component plays in IL-6 signal transduction.

EXPERIMENTAL PROCEDURES

Cytokines—Recombinant murine LIF was produced in Escherichia coli and purified as described previously (25). Purified recombinant human OSM was purchased from PeproTech Inc. (Rocky Hill, NJ). Recombinant G-CSF and GM-CSF were produced in E. coli, whereas recombinant murine thrombopoietin was produced in Chinese hamster ovary cells. Recombinant murine IL-6 was a kind gift from Dr. Simpson.
and R. Moritz (Joint Protein Structure Laboratory of the Ludwig Institute for Cancer Research and The Walter and Eliza Hall Institute, Melbourne, Australia). All recombinant cytokines were purified to homogeneity prior to use.

**Cells and Cell Culture**—M1 cells were originally isolated from a spleen nodule observed in subsequent bone marrow cultures from SL mice (23). The clone used in this study was obtained from Dr. M. Hozumi and has been in culture at The Walter and Eliza Hall Institute for ~15 years. M1 cells were routinely maintained by weekly passage in DME medium containing 10% (v/v) fetal calf serum (FCS) (D10 medium). Cytokine-unresponsive M1 cell clones were passaged in a similar manner. M1 cells that had been transfected with DNA encoding neomycin phosphotransferase or puromycin N-acetyltransferase gene were passaged in the presence of 1.2 mg/ml of Geneticin or 40 µg/ml puromycin, respectively.

To quantitate the capacity of M1 cells to differentiate in response to cytokines, 300 cells were cultured in 35-mm Petri dishes containing 1 ml of DME medium supplemented with 20% (v/v) FCS, 0.3% (w/v) agar, and 0.1 ml of serial dilutions of IL-6, LIF, OFS, GM-CSF, GM-CSF, GM-CSF, GM-CSF, GM-CSF, and GM-CSF, respectively. To determine the suitability of the M1 cell line as a substitute for IL-6 in subsequent experiments. Neomycin-resistant M1 cells were resuspended at 1.6 × 10^6 cells/ml in D10 medium. ICR-191 was added to a concentration of 5 µg/ml, and the cells were incubated at 37 °C for 2 h, after which they were washed twice in D10 medium. Cells were then resuspended at 10^6 cells/ml in D10 medium containing 1 mg/ml Geneticin and allowed to recover at 37 °C for 2 days. Cells were then either subjected to another round of mutagenesis performed in an identical manner or cultured, as described below, to select for cytokine-unresponsive mutants.

Untreated or ICR-191-treated neomycin-resistant M1 cells were cultured in agar at a concentration of 5 × 10^4 per ml in the presence of 100 ng/ml IL-6 or LIF. After 7 days of culture, colonies with an undifferentiated phenotype (i.e., tightly packed and devoid of a corona of migrating cells) were picked and suspended in 1 ml of D10 medium. After 4–6 days of culture, the phenotype of the cells was reassessed by culture in agar in the presence of cytokine.

**Dominance Studies Using Cell Fusion**—Somatic cell hybrids of M1 cell lines were generated by fusing puromycin-resistant wild type M1 cells with neomycin-resistant mutant lines. Briefly, 2 × 10^6 cells of each of the lines to be fused were combined in a 10-ml tube, washed twice in PBS, resuspended in 200 µl of PBS, and placed in a 0.2-mm electroporation cuvette (Bio-Rad). The cells in the cuvette were then pelleted by centrifugation at 452 × g for 5 min and electroporated (290 V; 960 microfarads). The cells were incubated on ice for 10 min and resuspended in 50 ml of D10 medium containing the relevant drugs for selection of hybrids (1.2 mg/ml Geneticin and 40 µg/ml puromycin). Cells were placed in tissue culture flasks and, after 10 days, pools of hybrid cells were analyzed further.

**Radiolabeling of Cytokines and Binding Studies**—Radiolabeling of IL-6 and LIF was performed using a modification of the iodine monochloride method (26), as described previously (27). Binding studies were performed essentially as described (27). Scatchard analyses of saturation binding isotherms were performed using the computer program LIGAND (28).

**Western Blotting**—Protein was extracted from ~1 × 10^7 cells from wild type and mutant cell lines using kinase assay lysis buffer (1% (v/v) Triton X-100, 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 2 mM EDTA, and 0.1% (w/v) phenylmethylsulfonyl fluoride). The protein was diluted into reducing sample buffer (0.5 M Tris-HCl, pH 6.8, 3.3% (v/v) SDS, bromophenol blue, 10 mM dithiothreitol) heated to 95 °C and loaded onto 14–15% (v/v) polyacrylamide Tris/glycine precast gels (Bio-Rad). The proteins were electrophoresed at 150 V in SDS running buffer (26 µl Tris-HCl, 192 µm glycine, 0.1% (w/v) SDS) and transferred onto polyvinylidene difluoride membranes (Miron Separations Inc., Westminster, MA) in Western buffer (21 mM Tris-HCl, 150 mM glycine, 20% (v/v) methanol) at 100 V for 90 min. Membranes were blocked in PBS containing 5% (w/v) skim milk powder and 5% (v/v) Tween 20 (Sigma) for 1 h and incubated with 2 µg/ml rabbit anti-gp130 polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA). The membranes were washed in PBS containing 0.1% (v/v) Tween 20 before the addition of 75 ng/ml peroxidase-conjugated goat anti-rabbit secondary antibodies (Bio-Rad). The membranes were once again washed in PBS containing 0.1% (v/v) Tween 20 prior to the addition of enhanced chemiluminescence reagents (Pierce).

**RESULTS**

**Selection of Spontaneously Occurring Cytokine-unresponsive M1 Cells**—To determine the suitability of the M1 cell line as a somatic cell genetic tool, we first assessed the frequency of spontaneous cytokine-unresponsiveness. M1 cells were cultured again in the presence of increasing doses of IL-6. In the absence of a stimulus, wild type M1 cells form tightly packed colonies containing several hundred cells. Colonies grown in the presence of IL-6 were smaller and more dispersed than those grown in its absence, reflecting the differentiation of blast cells into postmitotic macrophages capable of extensive migration through agar (Fig. 1 A). Further, as the concentration of IL-6 in cultures was increased, the number of clones present after 7 days was reduced (Fig. 1A). Indeed, at high concentrations of IL-6, clonal suppression is dramatic with no colonies...
observed after culturing 300 M1 cells in the presence of concentrations of IL-6 greater than 2 ng/ml. In a larger experiment, 2 × 10^7 M1 cells were cultured in agar in the presence of 100 ng/ml IL-6. Six colonies with an undifferentiated phenotype were observed, and two of these (UR4 and UR5A) appeared to have a stable phenotype, failing to show any signs of differentiation when recultured in the presence or absence of IL-6. The frequency of spontaneous IL-6-unresponsive M1 cells was therefore estimated to be ~1 in 10^7 cells (Table I). The low frequency of spontaneous IL-6 unresponsiveness suggested that it might be feasible to treat M1 cells with a mutagen and select those mutants that failed to respond to IL-6.

**Generation of a Panel of IL-6-unresponsive M1 Cell Mutants**—To obtain additional IL-6-unresponsive M1 clones, a neomycin-resistant derivative of wild type M1 cells (M1.neo) was subjected to mutagenesis with ICR-191, the mutagen used successfully in the experiments of Pellegrini et al. (30). In an initial experiment, 47 pools of 5 × 10^5 cells were subjected to three sequential rounds of mutagenesis. After each round, cells from the pools were cultured in agar containing IL-6. Large, tightly packed colonies containing undifferentiated M1 cells were picked and retested for the stability of their phenotype. After the first round of mutagenesis, 14 pools gave rise to large, compact colonies composed of cells that were stably unresponsive to IL-6. This frequency increased after the second round of mutagenesis with 26 pools containing stable IL-6-unresponsive cells, whereas after the third round, all the pools contained such cells. This experiment provides a minimum estimate of the frequency of IL-6-unresponsive cells of 1 in 3 × 10^6 cells after one round of mutagenesis, 1 in 9 × 10^5 cells after two rounds of mutagenesis, and 1 in 2.5 × 10^5 after three rounds of mutagenesis, which in each case is higher than the spontaneous frequency of 1 in 10^7 (Table I).

**Characterization of the Growth of Mutant M1 Cells in Agar**—Twenty-four unresponsive M1 cell lines (UR4, UR6, UR12, UR14–18 UR20–21, UR23–29, UR31, UR39–43, and UR45) were selected for further analysis. The capacity of increasing doses of IL-6 to induce differentiation of each cell line was investigated in agar. In the presence of as little as 0.5 ng/ml IL-6, wild type M1 cells produced fewer colonies than when grown in the absence of IL-6. Moreover, those colonies that were generated exhibited a dispersed morphology. In contrast, none of the M1 cell mutants showed any evidence of differentiation or clonal suppression in agar at IL-6 concentrations of up to 256 ng/ml (Fig. 1; data not shown). Unexpectedly, examination of unstimulated cultures revealed that many of the mutant M1 clones formed smaller colonies than their wild type counterparts (Fig. 2). Further, for some mutants, the size of colonies increased in the presence of IL-6 although the morphology of colonies remained compact, in contrast to the dispersed morphology of IL-6-stimulated wild type M1 cells (data not shown).

**Expression of Macrophage Markers by Mutant M1 Cells in Response to IL-6**—Since mutants were selected for their ability to proliferate in the presence of IL-6 and their inability to migrate through agar, we thought it of interest to determine whether other responses to IL-6 remained intact in these cells. Each cell line was cultured in the presence or absence of 50 ng/ml IL-6 for 4 days, after which their morphology was examined and their expression of Fc receptors, a marker of macrophage differentiation, was measured by flow cytometry. Upon treatment of wild type M1 cells with IL-6, expression of Fc receptors increased dramatically (Fig. 3A), and the cells exhibited the morphology of macrophages, displaying an increased cytoplasmic/nuclear volume ratio, membrane ruffling, and vacuoles (Fig. 4, A and B). Although the basal level of Fc receptor expression varied somewhat between mutant cell lines, there was little or no evidence of increased Fc receptor expression or morphological differentiation in response to IL-6 (Figs. 3B–D and 4C–F). The lack of response of mutant cells to IL-6 did not appear to represent a change in the kinetics of response since even prolonged culture of cells in IL-6, up to 2 weeks, failed to result in any detectable macrophage differentiation (data not shown).

**IL-6-unresponsive M1 Cells Harbor Primarily Recessive Mutations**—To determine whether the mutations that gave rise to the spontaneous and ICR-191-induced cytokine-unresponsive M1 cells were dominant or recessive, somatic cell hybrids were

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**TABLE I**

| Mutagen     | No. Pools | Cells/pool | Frequency of IL-6 unresponsiveness |
|-------------|-----------|------------|-----------------------------------|
| ICR-191 round 1 | 47        | 2.0 × 10^7 | <1 in 1 × 10^7                   |
| ICR-191 round 2 | 47        | 1.0 × 10^6 | >1 in 3 × 10^6                   |
| ICR-191 round 3 | 47        | 2.5 × 10^5 | >1 in 2.5 × 10^5                 |

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**FIG. 1.** Mutant M1 cells do not differentiate or undergo clonal suppression in response to IL-6. 300 cells from wild type (A) or mutant M1 cell clones UR15 (B), UR21 (C), UR24 (D), UR40 (E), and UR45 (F) were cultured for 7 days in agar with the indicated concentrations of IL-6. The percentage of colonies increased in the presence of IL-6 although the morphology of colonies remained compact, in contrast to the dispersed morphology of IL-6-stimulated wild type M1 cells (data not shown).
generated between neomycin-resistant mutant cell lines and a puromycin-resistant derivative of the cytokine-responsive wild type M1 cell line (M1.puro<sup>+</sup>). Pools of these hybrids were then tested for their ability to differentiate in response to IL-6.

Control experiments in which only puromycin-resistant or neomycin-resistant cells were used in the fusion failed to yield cells that were resistant to both drugs. In contrast, if puromycin- and neomycin-resistant cells were fused, hybrids resistant to both drugs were found at a frequency of between 1 in 10<sup>4</sup> and 1 in 10<sup>5</sup> (data not shown).

As expected, somatic cell hybrids generated by fusion of the IL-6-responsive M1.neo<sup>+</sup> and M1.puro<sup>+</sup> clones retained the ability to differentiate in response to IL-6 (Fig. 5A). In contrast, hybrids generated by fusion of the IL-6-responsive M1.puro<sup>+</sup> line with the spontaneous cytokine-unresponsive M1 derivatives, UR4 and UR5A, or the ICR-191-induced mutant, UR15, either failed to differentiate in response to cytokine or exhibited a severely reduced ability to differentiate when compared with wild type cells (Fig. 5B). This observation suggested that the mutations that led to the cytokine-unresponsive phenotype in these cell lines act in a dominant or semidominant manner. The reverse appeared to be true for the remaining ICR-191-induced cytokine-unresponsive M1 cells. Hybrids between M1.puro<sup>+</sup> and UR6, UR12, UR14, UR16-UR21, UR23-UR29, UR31, UR39-UR43, or UR45 differentiated normally in response to IL-6 (Fig. 5, C–F). Similar results were obtained when somatic cell hybrids were assessed for their response to IL-6 by the capacity to increase Fc receptor expression (data not shown).

A Subset of IL-6-unresponsive M1 Cells Have a Defect in the IL-6 Receptor Component gp130—Among the proteins that have been implicated in IL-6 signal transduction, the receptor component gp130 plays a critical role. In reconstitution experiments in Ba/F3 cells, the IL-6 receptor<sub>H9251</sub>-chain binds IL-6 with low affinity. This binary complex then interacts with gp130 to generate a high affinity receptor capable of transducing a proliferative signal. gp130 also forms part of the high affinity receptors for cardiotrophin, LIF, ciliary neurotrophic factor, and IL-11.

If, as expected, gp130 is required for IL-6-mediated macrophage differentiation of M1 cells, gp130 might be among the genes mutated to yield IL-6-unresponsive M1 cells. We examined wild type M1 cells and each of the IL-6-unresponsive cell lines for the expression of a functional gp130 protein by per-
forming saturation binding experiments using $^{125}$I-LIF and $^{125}$I-IL-6. In wild type M1 cells, Scatchard analysis of saturation binding isotherms revealed the presence of $\sim 300–1,000$ high affinity LIF receptors ($K_D = 50–200$ pM) and $1,000–3,000$ IL-6 receptors with a lower affinity than expected from the literature ($K_D = 3–10$ nM; Fig. 6A). In many of the mutant cell lines examined (UR5, UR6, UR12, UR15, UR20, UR23, UR26, UR27, UR28, UR29, and UR40), approximately normal numbers of LIF and IL-6 receptors were detected, and these were of wild type affinity (Fig. 6B). In a subset of cell lines (UR24, UR31, UR39, UR41, UR42), however, normal total numbers of LIF receptors were detected, and there was a marked reduction in the number of high affinity receptors with a concomitant increase in the number of low affinity receptors (Fig. 6D). In other cell lines (UR4, UR14, UR16, UR17, UR18, UR21, UR25, UR43, UR45), high affinity LIF receptors were not detectable (Fig. 6C). In cases in which the number of high affinity LIF receptors were low or undetectable, specific binding of IL-6 could not be detected, suggesting a marked reduction in the number or affinity of functional IL-6 receptors (Fig. 6, C and D).

Given the importance of gp130 in the generation of high affinity LIF and IL-6 receptors, we measured the amount of this protein expressed at the surface of each clone by flow cytometry using monoclonal anti-mouse gp130 antibodies. In general, gp130 expression, as judged by FACS, correlated very well with the number of high affinity LIF receptors detected on the various mutants (Figs. 6 and 7). gp130 was readily detectable on wild type, IL-6-responsive M1 cells and was expressed at comparable levels on the surface of IL-6-unresponsive UR15 cells, which also expressed normal numbers of high affinity LIF and IL-6 receptors. Lower gp130 expression was observed by FACS on UR24 and UR40, and these cells expressed reduced, but still detectable, numbers of high affinity LIF receptors. Finally, neither gp130 expression nor high affinity LIF and receptors were low or undetectable, specific binding of IL-6 could not be detected, suggesting a marked reduction in the number or affinity of functional IL-6 receptors (Fig. 6, C and D).

FIG. 5. Response of somatic cell hybrids to IL-6. 300 somatic cell hybrids from IL-6-responsive M1.puro' cells fused to either IL-6-responsive M1.neo' cells (A) or neomycin-resistant IL-6-unresponsive mutant clones UR15 (B), UR21 (C), UR24 (D), UR40 (E), and UR45 (F) were cultured in agar with the indicated concentration of IL-6. After 7 days the number of colonies (open circle) and the percentage of colonies with a dispersed phenotype (closed circle) were determined.

FIG. 6. A subset of IL-6-unresponsive mutant M1 cell clones do not express high affinity LIF and IL-6 receptors. IL-6 and LIF receptor components were examined by Scatchard analysis on wild type (A) and IL-6-unresponsive mutant M1 cell clones UR15 (B), UR21 (C), and UR24 (D) using $^{125}$I-LIF (open dots) and $^{125}$I-IL-6 (closed dots).
IL-6 receptors could be detected on UR21 and UR45 cells (Figs. 6 and 7). The FACS results were confirmed by Western blotting, although the latter appeared a less sensitive measure of gp130 expression (data not shown).

Rescue of Cytokine Unresponsiveness in Mutant Cells by gp130 Expression—To confirm that the reduction in gp130 expression might be the underlying cause of IL-6 unresponsiveness in specific mutants, we sought to complement the defect by transfection of a vector capable of driving expression of epitope-tagged gp130. Stable transfectants were obtained by co-transfection of FLAG-tagged gp130 and a plasmid conferring resistance to puromycin. Expression of FLAG-tagged gp130 was confirmed by FACS analysis in all cases (data not shown). Unlike the mutant cells from which they were derived, UR21, UR24, UR39, and UR45 cells expressing FLAG-tagged gp130 bound IL-6 and LIF with an affinity indistinguishable from that of wild type M1 cells, and moreover, they differentiated normally in response to IL-6 and LIF in agar (Fig. 8) and in liquid culture. In contrast, although transfected UR12, UR15, and UR40 cells expressed high levels of FLAG-tagged gp130 in addition to endogenous gp130, little or no evidence of complementation of IL-6 responsiveness was observed in agar (data not shown). Similarly, these cells did not exhibit characteristic features of macrophage differentiation, including an increase in Fc receptor expression, when cultured in IL-6 (data not shown).

DISCUSSION

Somatic cell genetics is a powerful tool by which cellular processes may be investigated. Previously, the genes required for interferon signal transduction have been investigated using a specially engineered fibroblast cell line capable of producing hypoxanthine-guanine phosphoribosyltransferase in response to type I and type II interferons (33). Following mutagenesis of this cell line, positive and negative selection allowed the identification of IFN-unresponsive mutants. In the first experiment in which complementation was successful, a cDNA library was transfected, and clones that regained responsiveness to IFN were isolated. Using this strategy, Tyk2, a previously isolated cytoplasmic tyrosine kinase and a member of the Janus kinase family, was shown to be essential for IFN signaling. In subsequent experiments, complementation of the defect was achieved using candidate genes, including the other members of the JAK family, the STATs, and receptor components (9, 23, 33).

In this study, rather than producing a cell line in which the promoter of a cytokine-responsive gene drives expression of a selectable marker, we have taken advantage of an existing biological response, terminal macrophage differentiation, in M1 cells. As with the studies of Stark, Kerr and colleagues, we found that the spontaneous rate of cytokine unresponsiveness to be very low and have demonstrated that it was possible to dramatically elevate this rate following multiple rounds of treatment with the frameshift mutagen ICR-191. Consistent with the expected effects of ICR-191 in inducing loss of function mutations, most of the mutations in the IL-6-unresponsive lines arising from ICR-191 treatment behaved in a recessive manner. In contrast, the two spontaneous IL-6-unresponsive cell lines that were isolated in the initial optimization of the system and one of the mutants isolated following ICR-191 treatment contained mutations that acted in a dominant man-

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**FIG. 7.** Analysis of gp130 expression in wild type and mutant M1 cell clones at the protein level. Wild type (A) and mutant M1 cell clones UR15 (B), UR21 (C), UR24 (D), UR40 (E), and UR45 (F) were examined for expression of gp130 (gray) by flow cytometry using an anti-gp130 monoclonal antibody and compared with an isotype-matched control antibody (black).

**FIG. 8.** Complementation of mutant M1 cell clones by transfection with epitope-tagged gp130. Wild type M1 cells (A and B, open squares), the IL-6-unresponsive M1 cell clone UR21 (C and D, open circles), and UR21 cells expressing FLAG-tagged gp130 (C and D, filled circles) were analyzed for IL-6 and LIF receptor binding affinity using Scatchard analysis (A and C) and for the capacity to form differentiated colonies in agar (B and D).
ner. These might be loss of function mutations that act in a dominant-negative manner, or alternatively, mutations that result in overproduction of a negative regulator, such as SOCS1 or SOCS3, which have been shown to be capable of inhibiting IL-6 signal transduction in M1 cells (17).

To demonstrate the utility of the M1 cell system for identifying genes important in cytokine signal transduction, we determined whether any of the mutant cell lines lacked the crucial IL-6 receptor component gp130. Together with the IL-6 receptor α-chain and the IL-6 ligand, gp130 forms part of the IL-6 receptor complex in which two gp130 molecules are required for activation of signaling (29). Flow cytometry and Western blot analysis revealed that gp130 expression was reduced or undetectable in 14 out of 27 mutants (UR4, UR14, UR16, UR17, UR18, UR21, UR24, UR25, UR31, UR39, UR41, UR42, UR43, UR45). The transfection of wild type FLAG-tagged gp130 restored the IL-6-responses of these gp130-deficient cell lines; however, it should be stated that final proof that the causative mutation lies in gp130 will require sequencing of the gp130 gene in these cell lines. Indeed, mutations in other genes might indirectly be responsible for reduced expression of gp130, and it is conceivable that overexpression of gp130 might also compensate for such defects. For example, the IFN-unresponsive U1 cell line had severely impaired IFN-β signal transduction. Finally, where mutation in the genes of known components of the signal transduction pathway cannot explain the unresponsive phenotype, it may be possible to identify the defective gene by complementation with cDNAs from a library, as occurred with Tyk2 and IFN signal transduction (31).

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