Dissociation of Human Cytokine Receptor Expression and Signal Transduction

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We have examined the relationship between granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) receptor expression and signal transduction in populations of HL-60 cells differing in proliferative capacity to these cytokines. GM-CSF or IL-3 stimulation of HL-60 cells pretreated with either dimethyl sulfoxide (DMSO) or retinoic acid results in increases in proliferative response as well as both tyrosine and serine phosphorylation. In contrast, neither GM-CSF or IL-3 stimulation of parental HL-60 cells (those not treated with DMSO or retinoic acid) produced any changes in either proliferation or protein phosphorylation. Thus, although parental HL-60 cells expressed both GM-CSF and IL-3 receptors, treatment with either DMSO or retinoic acid was necessary to confer the capacity for signal transduction as assessed by both a biologic and biochemical response. Pretreatment of cells with genistein, a protein tyrosine kinase inhibitor, resulted in inhibition of GM-CSF-induced protein tyrosine phosphorylation as well as proliferation. These data show a strong correlation between cytokine-induced increases in protein phosphorylation and subsequent biologic responses. Further, this work demonstrates that cytokine receptor expression and signal transduction can be disassociated and suggests the potential for independent regulation of these two components of signal transduction.

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A NUMBER OF CYTOKINES regulate the development and function of the immune system. The principal binding proteins and associated subunits for many of the cytokine growth factors have been molecularly cloned.1-6 Surprisingly, certain structural homologies have been identified among many of these receptors.7-9 These observations have lead to the suggestion that these binding proteins constitute a new superfamily termed the “hematopoietin receptor superfamily.”10 Recently, subunits of both the human interleukin-3 (IL-3) and granulocyte macrophage colony-stimulating factor (GM-CSF) receptors have been cloned and characterized.6,10 Interestingly, whereas the receptors for human IL-3 and GM-CSF have unique a subunits of 70 and 85 Kd, respectively, they share a b subunit, a 120-Kd protein termed KH97.

In addition to the study of cytokine receptor structure, recent work has addressed the signal transduction mechanism of these receptors. Although the signal transduction pathway has yet to be elucidated for any of the cytokine receptors, it is clear that IL-2, IL-3, and GM-CSF stimulate increases in tyrosine and serine phosphorylation.10-15 Cytokine-induced increases in protein phosphorylation likely occur through activation of as yet uncharacterized protein kinases. A common feature of the “hematopoietin receptor superfamily” is the absence of homology with tyrosine kinase consensus regions in the cytoplasmic domain.9 These observations suggest that one or more protein kinases may interact with certain cytokine receptors in a quaternary complex to mediate signal transduction. How these kinases associate with the ligand binding proteins and whether the expression of signal transduction components such as protein kinases are coordinately or independently regulated with regards to receptor expression are important questions to address in understanding cytokine signal transduction processes.

We have developed a model to address the potential for independent regulation of GM-CSF receptor expression and the capacity for ligand-induced protein phosphorylation. The myelomonocytic cell line HL-60 expresses low numbers of high-affinity GM-CSF and IL-3 receptors.16-18 Interestingly, these cells do not respond to either cytokine when tested for proliferation or increases in serine or tyrosine phosphorylation. Treatment of HL-60 cells with dimethyl sulfoxide (DMSO) or retinoic acid has been shown to induce granulocytic differentiation.19 Interestingly, these agents also confer the capacity for proliferative responses to GM-CSF.20-22 This permits us to compare signal transduction in a cell population capable of cytokine-induced proliferation (HL-60 pretreated with DMSO or retinoic acid) with those that express both GM-CSF and IL-3 receptors but are incapable of a proliferative response (parental HL-60 cells). Our data demonstrate that parental HL-60 cells are incapable of signal transduction through the constitutively expressed high-affinity GM-CSF and IL-3 receptors. HL-60 cells pretreated with either DMSO or retinoic acid responded to GM-CSF as well as IL-3 with increases in both tyrosine and serine phosphorylation. The correlation of cytokine-induced proliferation and phosphorylation was further examined using the protein tyrosine kinase inhibitor, genistein. Genistein treatment of cells resulted in inhibition of cytokine-induced tyrosine phosphorylation as well as proliferation. These results suggest that one or more protein tyrosine kinases are critical in coupling the GM-CSF and IL-3 receptors to biologic responses and that expression of these signal transduction components...
can be regulated independently from expression of either receptor.

MATERIALS AND METHODS

Cell lines and growth factors. The HL-60 cell line (late passage) was cultured in RPMI 1640 (ABI, Columbia, MD), 10% fetal calf serum (FCS; HyClone, Logan, UT). To examine the effects of DMSO and retinoic acid on cell response to growth factor, cells were cultured 4 to 5 days in DMSO (1.25%) or retinoic acid (1 \mu M/L). AML-193 cells were grown in Iscove's modified Dulbecco's medium (IMDM; GIBCO, Grand Island, NY), 5% FCS, and human GM-CSF. Peripheral blood monocytes were isolated as described by Muñoz. Recombinant human IL-3 and GM-CSF obtained from the Biological Response Modifiers Program (Fredrick Cancer Research Facility, Frederick, MD) as a gift from Immunex Corp and Schering, respectively.

Proliferation assays. HL-60 cells were washed in serum-free RPMI 1640 and resuspended in RPMI 1640, 1% FCS at a concentration of 10^5/mL. Cells (0.1 mL) were added to a 96-well flat-bottomed microtiter plate (Costar, Cambridge, MA) and incubated at 37°C, 5% CO2 for 3 days with a 0.1 mL aliquot of IL-3, GM-CSF (at concentrations specified in the text), or with media (RPMI 1640, 1% FCS). All samples were performed in triplicates. Each well was pulsed with 1 \mu Ci of 3H-thymidine (New England Nuclear, Boston, MA) for 4 hours and then harvested (Skatron Semiautomatic Cell Harvester; Skatron, Inc, Sterling, VA) onto glass filter fiber paper (Filtermat; Skatron, Inc). Filter strips were dried and counted in a liquid scintillation counter (LKB, Piscataway, NJ; Model 1216).

Receptor binding assays. Recombinant human GM-CSF and IL-3 were radiolabeled using the enzymobead radioiodination reagent (Biorad, Richmond, CA) as previously described. In both cases, a fusion protein with an octapeptide containing tyrosine attached to the N-termirinus was used to aid in radiolabeling. Binding assays were performed by a phthalate oil separation method and data analyzed as previously described.

In vivo radiolabeling, preparation of cell lysates, and immunoprecipitation. Cells were washed twice in RPMI 1640 without phosphate (AB), resuspended at 40 x 10^6/mL in phosphate-free RPMI 1640 with 5% dialyzed FCS and 0.5 mg/mL of 35S-orthophosphate (New England Nuclear). This preparation was incubated 90 minutes in a shaking water bath at 37°C. Cells were stimulated with the appropriate factor for the time indicated in the text, rapidly pelleted (3,000 rpm, 5 minutes at 4°C), supernatant discarded, and resuspended in extraction buffer (1% Triton X 100, 50 mmol/L NaCl, 10 mmol/L Tris, 5 mmol/L EDTA, 30 mmol/L sodium pyrophosphate, 50 mmol/L sodium fluoride, 100 mmol/L sodium orthovanadate, and 0.1% bovine serum albumin (BSA), pH 7.6). Experiments using total cell lysates did not include BSA in the extraction buffer. Cells (40 x 10^6) were lysed by the addition of 1 mL of the extraction buffer at 0°C and incubated for a minimum of 45 minutes. Samples were subjected to a cycle of rapid freeze thawing to insure optimum release of proteins and then centrifuged at 10,000 rpm at 4°C for 20 minutes. Immunoprecipitations of phosphotyrosylproteins were performed on the clarified supernatant with PY20 (ICN, Costa Mesa, CA), a monoclonal antibody (MoAb) recognizing phosphotyrosine. All comparisons of protein phosphorylation in control versus cytokine-stimulated cells used samples containing equal amounts of counts per minute (CPM) isolated from equivalent numbers of cells.

Electrophoresis. Two-dimensional electrophoresis was performed as described previously. Samples for isoelectric focusing (IEF) were solubilized in sample buffer (9.5 mol/L urea, 2.0% NP-40, 1.6% ampholines pH 5-7, 0.4% ampholines pH 3.5-10, and 5.0% 2-mercaptoethanol) and separated in tube gels (140 x 3 mm) consisting of 4.0% acrylamide, 2.0% NP-40, 3.0% pH 3.5-10 ampholines, and 2.0% pH 5.7-10 ampholines. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (7.5% acrylamide in the resolving gel, 4.75% acrylamide in the stacking gel) was used for the second dimension. IEF gels equilibrated for 1 to 2 hours in 2 × SDS sample buffer (4.6% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.125% Tris and 0.001% Bromophenol blue, pH 6.8) were applied to the top of the slab gel and immobilized with 1.0% agarose. Samples subjected to one-dimensional SDS-PAGE were diluted with one volume of 2 × SDS sample buffer, boiled 5 minutes, centrifuged at 10,000 rpm for 1 minute, and applied to preformed wells in a 2-inch stacking gel (4.75% acrylamide). The resolving gel consisted of 7.5% acrylamide. Gels were fixed in a methanol/acetic acid/water solution (5:1:5) and visualized using autoradiography (XMAR film; Eastman Kodak, Rochester, NY) with two intensifier screens at ~80°C.

RESULTS

Effects of DMSO and retinoic acid on GM-CSF and IL-3 receptor expression. The promyelocytic cell line HL-60 has been shown by a number of investigators to express high-affinity GM-CSF receptors (20 to 200 pmol/L Kd), although in relatively low numbers. Before examining the effects of DMSO or retinoic acid on GM-CSF-induced phosphorylation, we sought to characterize the effects of these agents on expression of GM-CSF receptors. Similar to the observations of Gasson et al., culture in DMSO led to expression of 1,690 GM-CSF receptors, an almost sixfold increase over the 300 GM-CSF receptors expressed on untreated parental cells (Table 1). Treatment of HL-60 cells for 4 days in retinoic acid resulted in expression of 1,200 GM-CSF receptors, a fourfold increase over parental cells. Thus, both DMSO and retinoic acid upregulated expression of receptors for GM-CSF. Scatchard analysis of GM-CSF receptor binding was linear in both parental- and DMSO- or retinoic acid-treated cells. Furthermore, treatment with either agent did not result in a increase in the affinity of the GM-CSF receptor.

DMSO and retinoic acid also upregulated the expression of IL-3 receptors. Interestingly, IL-3 receptors were expressed in very low levels on untreated HL-60 cells.

Treatment of HL-60 cells with DMSO or retinoic acid results in increased proliferative response to both GM-CSF and IL-3.

Table 1. Effect of DMSO and Retinoic Acid Treatment of HL-60 Cells on GM-CSF and IL-3 Receptor Expression

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Human GM-CSF</th>
<th>Human IL-3</th>
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<tbody>
<tr>
<td>None</td>
<td>300 ± 140</td>
<td>35 ± 25</td>
</tr>
<tr>
<td>DMSO</td>
<td>1,690 ± 250</td>
<td>80 ± 40</td>
</tr>
<tr>
<td>Retinoic acid</td>
<td>1,200 ± 420</td>
<td>300 ± 20</td>
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HL-60 cells were cultured for 4 days in media containing 1.25% DMSO, 1 \mu M/L retinoic acid, or no additions. For assessment of GM-CSF and IL-3 receptor expression, cells (3.3 x 10^6/mL) were incubated with various concentrations of iodinated GM-CSF or IL-3 for 1 hour at 37°C and assayed for binding as described in Materials and Methods. For GM-CSF, molecules bound per cell was determined by Scatchard analysis and represents total sites per cell. For IL-3, the values reported represent specific molecules bound per cell at 1 nmol/L iodinated IL-3.
To determine if the upregulation of GM-CSF and IL-3 receptors in DMSO-treated HL-60 cells was accompanied by enhanced biologic activity, comparisons of GM-CSF- and IL-3–induced proliferative responses were performed on parental- and DMSO-treated HL-60 cells. As shown in Fig 1, DMSO-treated HL-60 cells had a 60% increase in proliferation in response to GM-CSF when compared with media controls. This is in contrast to the absence of GM-CSF–induced proliferation by parental HL-60 cells. Even more dramatic were the effects of retinoic acid on proliferative response to GM-CSF. HL-60 cells treated 4 days with retinoic acid were then assessed for proliferative responses to GM-CSF. The proliferative responses of retinoic acid–treated HL-60 cells were 255% higher than cells incubated in the absence of GM-CSF (Fig 1).

Similarly, Fig 1 shows that IL-3 did not stimulate proliferation of parental HL-60 cells (only a 5.4% increase over media controls), but that treatment of HL-60 cells with either DMSO or retinoic acid resulted in enhanced proliferation in response to IL-3 (59% and 103% over controls, respectively). Neither DMSO or retinoic acid treatment of HL-60 cells resulted in a proliferative response to IL-2.

**Tyrosine phosphorylation.** Increases in tyrosine phosphorylation in response to a number of cytokines has been previously reported by several investigators. Specifically, human GM-CSF and IL-3 have been shown to stimulate increases in phosphate incorporation on tyrosine residues of proteins of 140, 97 to 93, and 70 Kd. To evaluate potential differences in signal transduction pathways of parental HL-60 cells with those treated with DMSO, we compared the effects of stimulation with GM-CSF on changes in tyrosine phosphorylation (Fig 2A). Stimulation of parental HL-60 cells with concentrations of GM-CSF as high as 200 nmol/L resulted in no detectable changes in tyrosine phosphorylation as assessed by two-dimensional gel electrophoresis of radiolabeled phosphotyrosylproteins purified by immunoprecipitation with antiphosphotyrosine MoAb. In contrast, GM-CSF stimulation of HL-60 cells grown in 1.25% DMSO for 4 days resulted in tyrosine phosphorylation of proteins of 140, 97, and 70 Kd. A protein of 55 Kd was also phosphorylated in some, but not all, experiments. Studies were then performed to assess the effects of IL-3 stimulation of parental- and DMSO-treated HL-60 cells on tyrosine phosphorylation and results were identical to those obtained with GM-CSF. While IL-3 produced no changes in tyrosine phosphorylation of untreated HL-60 cells, cells previously treated for 4 days in DMSO also had increases in tyrosine phosphorylation of proteins of 140, 97, and 70 Kd. Western blot analysis of cell lysates with MoAbs recognizing phosphotyrosine confirmed that GM-CSF and IL-3 stimulated tyrosine phosphorylation in proteins of 140, 97, and 70 Kd. Further, phosphoamino acid analysis of these proteins indicated that approximately 50% of the phosphate incorporation was on tyrosine residues, with the remainder occurring on serine.

These data suggest that DMSO confers the capacity for GM-CSF– and IL-3–mediated stimulation of one or more tyrosine kinases involved in the cytokine signal transduction pathway.

We next examined tyrosine phosphorylation induced by GM-CSF and IL-3 in HL-60 cells pretreated for 4 days with retinoic acid. Shown in Fig 2B are the results of an experiment assessing the effects of a 4-day treatment with retinoic acid on GM-CSF–induced tyrosine phosphorylation. As previously observed, treatment of parental HL-60 cells with GM-CSF resulted in no changes in tyrosine phosphorylation (Fig 2A, upper panel), while those cultured in retinoic acid responded to GM-CSF (Fig 2B) with increases in phosphorylation of similar proteins as found after DMSO treatment (p140, p97, and p70). Similar results were observed when retinoic acid–treated HL-60 cells were stimulated with IL-3 (Fig 2B). Thus, the changes in signal transduction of GM-CSF and IL-3 was not unique to the DMSO treatment and more likely represent an event related to the enhanced proliferative responses of the DMSO- and retinoic acid–treated cells. These data show that expression of low levels of IL-3 receptors is sufficient for coupling to the tyrosine kinase signal transduction pathway, providing the cognate coupling mechanism exists. In contrast, the 300 GM-CSF receptors expressed on the untreated HL-60 cells were insufficient to mediate either proliferation or increases in protein tyrosine phosphorylation. These results suggest that treatment of HL-60 cells with DMSO or retinoic acid may upregulate certain signal transduction elements necessary for coupling of the GM-CSF and IL-3 receptors.

**Serine phosphorylation.** Phosphorylation of a 68-Kd protein has been observed in both murine and human myeloid
cell lines treated with GM-CSF, IL-3, or G-CSF.\textsuperscript{10,11,15} To
determine if GM-CSF or IL-3 increased serine phosphory-
lization in parental- or DMSO-treated HL-60 cells, we
compared phosphoproteins from orthophosphate labeled
cells using two-dimensional gel electrophoresis. As shown
in Fig 3 (upper panel), treatment of parental HL-60 cells
with GM-CSF, IL-3, or IL-2 resulted in no detectable
increases in phosphorylation of any proteins. Stimulation of
parental HL-60 cells with phorbol myristate acetate (PMA)
resulted in phosphorylation of p68 (data not shown). These
results show that parental HL-60 cells, although expressing
refractory GM-CSF receptors, contain an intact serine
kinase pathway responsive to nonreceptor-mediated stim-
uli. In contrast, increases in p68 phosphorylation were
observed in GM-CSF– and IL-3–stimulated HL-60 cells
previously cultured for 4 days in DMSO (Fig 3, lower
panel). Amino acid hydrolysis of p68 phosphorylated in
response to human GM-CSF, IL-3, and PMA has previ-
ously shown it to be phosphorylated on serine residues.\textsuperscript{15}
IL-2, a cytokine that does not increase proliferation of
DMSO- or retinoic acid-treated HL-60 cells, did not induce
p68 phosphorylation.

Figure 4 demonstrates that 4 days of pretreatment of
HL-60 cells with retinoic acid yields cells capable of
increases in p68 phosphorylation in response to GM-CSF.
Similar responses were observed with retinoic acid-treated
HL-60 cells stimulated with IL-3 (data not shown). These
data support the hypothesis that treatment of HL-60 cells
with DMSO or retinoic acid is necessary for the develop-
ment of the coupling of the GM-CSF and IL-3 receptors to
the signal transduction components necessary for both
tyrosine and serine phosphorylation.

**Correlation of protein phosphorylation and cytokine-
induced cellular responses.** Comparison of the kinetics of
GM-CSF–induced increases in tyrosine phosphorylation
with those of serine phosphorylation has suggested that
activation of putative protein tyrosine kinases occurs more rapidly then serine kinases.\textsuperscript{15} To further address the relationship between cytokine-induced increases in protein phosphorylation and subsequent biologic response, we chose to compare the effects of GM-CSF and IL-3 on tyrosine phosphorylation in a relatively immature, factor-dependent, human myeloid cell line (AML-193 cells) with those of peripheral blood monocytes, a more mature myeloid phenotype. Figure 5 shows that stimulation of either AML-193 cells or monocytes with GM-CSF or IL-3 results in increases in tyrosine phosphorylation. While the dominant phosphotyrosylproteins observed in cytokine-stimulated AML-193 cells were 140 and 97 Kd, proteins of 70 and 55 Kd were also observed in some experiments. Similarly, GM-CSF or IL-3 stimulation of monocytes resulted in tyrosine phosphorylation of p140, 97, 70, and 55 Kd. These results support the postulate that protein tyrosine phosphorylation is involved in signal transduction of human IL-3 and GM-CSF in both mature and immature myeloid phenotypes.

Thus, GM-CSF and IL-3 induction of protein tyrosine phosphorylation strongly correlates with the capacity for cells to respond biologically to these cytokines. To more rigorously address the role of protein tyrosine kinases in cellular responses to GM-CSF and IL-3, we used the protein tyrosine kinase inhibitor genistein. As evident in Fig 6, genistein pretreatment of cells inhibited GM-CSF-induced tyrosine phosphorylation in a dose-related manner. Further, this inhibition corresponded to inhibition of GM-CSF-induced proliferative responses. These results stand in strong support of the postulate that protein tyrosine kinases are critical in the signal transduction of human GM-CSF and IL-3. Further, these data suggest that the mechanism mediating DMSO- and retinoic acid-induced coupling of the these receptors may be related to upregulation of protein tyrosine kinases.

DISCUSSION

Treatment of appropriate target cells with GM-CSF or IL-3 has been shown by many investigators to increase
Our studies have shown that stimulation of HL-60 cells expressing high-affinity GM-CSF and IL-3 receptors do not produce increases in either tyrosine or serine phosphorylation (Figs 2 and 3). The absence of cytokine-induced protein phosphorylation was paralleled by an absence of biologic activity, while cells that showed cytokine-induced increases in protein phosphorylation also responded biologically (Figs 1 through 5). GM-CSF–induced tyrosine phosphorylation and proliferation were both inhibited in a dose-related manner when pretreated with genistein, a protein tyrosine kinase inhibitor (Fig 6). These results are strong evidence for the
relationship between increases in protein tyrosine phosphorylation, protein tyrosine kinases, and the biologic activity of these cytokines. Further, our data suggest that expression of cytokine receptors can be independent of expression of signal transduction elements necessary for coupling of receptor-ligand interaction to the biologic response of the cells.

Parental HL-60 cells express approximately 300 high-affinity GM-CSF receptors and 35 IL-3 receptors. Our studies have shown that concentrations of GM-CSF as high as 200 nmol/L failed to stimulate increases in either proliferative response or protein phosphorylation. One interpretation of these results could be that these cells are expressing receptor numbers below a critical threshold that leads to impaired signal transduction and subsequent absence of detectable biologic response. Although possible, this is unlikely in light of previous work by other laboratories showing that as few as 20 GM-CSF receptors expressed on AML cells were sufficient for stimulating proliferation.24,28,29 These observations show that extremely low numbers of GM-CSF receptors are sufficient to mediate biologic responses and suggest that parental HL-60 cells may be lacking protein kinases or other signal transduction components that are upregulated after treatment with DMSO or retinoic acid. Further support for this postulate comes from the work of Taetle et al, which showed that HL-60 cells transfected with the EGF receptor did not proliferate in response to EGF until pretreated with DMSO for 2 to 3 days.30 Thus, treatment with DMSO may upregulate signal transduction components critical in mediating proliferative responses through growth factor receptors that have protein tyrosine kinase activity (the EGF receptor) as well as hematopoietin receptor superfamily members (the GM-CSF and IL-3 receptors).

An interesting caveat to our data is the observation that treatment of HL-60 cells with either DMSO or retinoic acid, agents that ultimately stimulate granulocytic differentiation, results in coupling these receptors to proliferative responses. Our observations are not restricted to these initiators of granulocytic differentiation because pretreatment of HL-60 cells with vitamin D3 or PMA, agents producing monocyctic/macrophagic differentiation, also yield similar results (data not shown). This apparent paradox is well documented in the literature.20-22 DMSO-treated HL-60 cells have been shown to respond to GM-CSF by increases in colony formation, cell numbers, and tritiated thymidine incorporation.21,22 Thus, though DMSO- or retinoic acid-treated HL-60 cells are cycling towards a differentiated, postmitotic phenotype, these cells clearly maintain a transient capacity for cytokine-induced proliferative responses. DMSO or retinoic acid treatment of HL-60 cells has been shown to increase the percentage of cells in the G1 stage of the cell cycle.20 Therefore, it is possible that HL-60 cells in G1 are the factor-responsive population and that treatment with DMSO and retinoic acid results in increasing the percentage of potentially responding cells over the threshold necessary for detection in both phosphorylation and proliferation assays.

We have identified phosphotyrosylproteins of 140, 97, and 70 Kd in lysates from GM-CSF– and IL-3–treated human myeloid cell lines (Figs 2, 5, and 6).13 These observations are in relative agreement with those previously reported. In the human cell line Mo7E, Kanakura et al found the dominant phosphotyrosylproteins in either GM-CSF– or IL-3–stimulated cells to be 150, 93, 70, and 42 Kd.13 In murine cells stimulated with IL-3, a 140- to 150-Kd phosphotyrosylprotein has consistently been observed.11-13,15,27 This protein has been postulated to be a subunit of the murine IL-3 receptor.31,32

We have also compared cytokine-induced protein phosphorylation associated with peripheral blood monocytes with the more immature population of cells represented by AML-193 cells. These studies showed that similar phosphotyrosylproteins were present in GM-CSF–stimulated monocytes and AML-193 cells. Others have also reported similar results when comparing phosphotyrosylproteins in GM-CSF–stimulated neutrophils, monocytes, bone marrow, and Mo7E cells.15,33 These data suggest that, in human myeloid cells, the most immediate substrates in GM-CSF– and IL-3–induced proliferation, differentiation, or activation are the same. Thus, if substrate specificity is associated with each of these events, it may be related to events later than the rapid changes in phosphorylation examined here.

The importance of tyrosine phosphorylation in mediation of cellular growth is underscored by the prevalence of receptors for growth factors such as insulin, epidermal growth factor, and colony stimulating factor-1, which encode a tyrosine kinase. While tyrosine phosphorylation has been shown to be a very early signal in response to IL-2, IL-3, GM-CSF, and Epo, the cytoplasmic domain of these receptors does not have significant homology with known tyrosine kinases.1-6,34 These observations have lead to the suggestion that the receptors for these cytokines may couple to one or more tyrosine kinases that mediate the early events in signal transduction. Recent reports have suggested that two members of the hematopoietin receptor superfamily, the IL-2 receptor and the Epo receptor, are associated with protein tyrosine kinases.35-38 Interestingly, treatment of HL-60 cells with DMSO or retinoic acid has been shown to upregulate activity of the protooncogene c-fes and increase expression of c-HCK.39-41 The role for either of these tyrosine kinases in hematopoietic cell physiology has yet to be determined; however, it is provocative to consider that these molecules or perhaps related tyrosine kinase(s) may interact with the GM-CSF and IL-3 receptors and provide an early signal in mediation of biologic responses of these cytokines.

Lastly, in addition to differentiation-induced changes in protein tyrosine kinases, the potential role for regulation of protein phosphatase levels is also important to consider. Treatment of cells with orthovanadate, an inhibitor of protein tyrosine phosphatases, has been shown to enhance proliferative responses to GM-CSF and IL-3 as well as to increase the amount of cytokine-induced protein tyrosine phosphorylation.13 Although little is known of the role of protein phosphatases in signal transduction of hematopoietic...
etctic cytokines, these enzymes likely share an important role with protein kinases in these events.

Our data suggest that the absence of GM-CSF-induced tyrosine phosphorylation in parental HL-60 cells reflects uncoupling of the GM-CSF receptor from a putative tyrosine kinase signal transducer necessary for biologic responses. Although much work remains to be done to fully understand the interaction between the receptors for GM-CSF and IL-3 and signal transducing elements, these studies provide evidence for the importance of protein tyrosine kinases in mediating the bioactivities of human IL-3 and GM-CSF, as well as suggest the potential for the independent regulation of cytokine receptors and signal transduction elements.

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REFERENCES


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